Published in final edited form as:

Dev Neuropsychol. 2011 May ; 36(4): 429–452. doi:10.1080/87565641.2010.550178.

Neural Systems of Threat Processing in Adolescents: Role of Pubertal Maturation and Relation to Measures of Negative Affect

Erika E. Forbes,

Department of Psychiatry and Psychology, University of Pittsburgh

Mary L. Phillips,

Department of Psychiatry, University of Pittsburgh

Neal D. Ryan, and

Department of Psychiatry, University of Pittsburgh

Ronald E. Dahl

Departments of Psychiatry and Pediatrics, University of Pittsburgh

Abstract

Adolescence ushers in dramatic social and affective changes and increased vulnerability for affective disorders. Yet, little is known about the effects of pubertal maturation on neural systems of social threat processing. We examined adolescents' brain function to social stimuli in relation to pubertal maturation, depressive symptoms, and real-world subjective negative affect. Compared with pre/early adolescents, mid/late adolescents exhibited less amygdala reactivity to emotionally neutral faces relative to non-face stimuli; less ventrolateral prefrontal cortex (VLPFC) reactivity to fearful faces relative to non-face stimuli, neutral faces, or angry faces; and more VLPFC reactivity to angry relative to neutral faces. Amygdala and VLPFC reactivity were correlated with negative affect and depressive symptoms. Threat-processing changes during puberty may facilitate changes in social behavior and negative affect.

The onset of adolescence ushers in a dramatic set of social and affective changes that are part of normal developmental processes. These changes include increased depth of peer relationships, concern about social status, and interest in romantic relationships (Davey et al., 2008). A subset of these changes in early adolescence appear to be directly linked to the biology of puberty, including the development of romantic interests and increases in sensation-seeking (Dahl & Spear, 2004; Steinberg, et al., 2008). Adolescents' social context is also important to the risky behavior typical of this developmental period: many behaviors associated with negative health outcomes such as substance use, accidents, or sexually transmitted diseases, often occur within the increasingly salient peer social context (Gardner & Steinberg, 2005; Steinberg, 2005). Thus, the processing of social stimuli could take on new importance during adolescence, and changes in processing social stimuli could be linked to pubertal maturation.

Threat processing is inherent in the social exploration that accompanies the movement toward new friendships, romantic relationships, and peer groups during adolescence. These behaviors are valued by adolescents but are high-cost pursuits because failures in the social domain (e.g., social rejection) are among the most painful (and feared) experiences of adolescence. Correspondingly, changes in reactivity to some types of social threats could be

adaptive for adolescents. For example, reduced sensitivity to social signals of indifference combined with lowered fear of social rejection could enhance motivation to meet a new peer or interact with members of an unfamiliar social group. As a result, altered processing of such social signals—in the form of reduced affective reactivity or changes in perceptual processing—could promote social exploration and the expansion of peer relationships.

One potential set of changes relevant to processing social stimuli could include alterations in the reactivity of threat-related brain systems. This contention is suggested by developmental research within social and affective neuroscience. A recent review of social cognition during adolescence (Blakemore, 2008) noted evidence of developmental declines in some measures of processing social stimuli (e.g., a pubertal dip in encoding faces (Diamond, Carey, & Back, 1983)). And a triadic model of the neurobiology of adolescent development proposes that the social behavior typical of adolescence is facilitated by low reactivity in both threat-related brain systems and self-regulatory brain systems (Ernst, Pine, & Hardin, 2006). In contrast, some recent studies have indicated that adolescents might exhibit enhanced rather than reduced reactivity of threat-related brain systems during affect regulation (Hare, et al., 2008) or processing of threat in general (Quevedo, Benning, Gunnar, & Dahl, 2009). These studies either have not considered the role of pubertal maturation or have not examined social threats specifically, however. Because social stimuli may represent a uniquely important category of potential threats for effective functioning during adolescence, it is valuable to investigate the engagement of threat-related brain circuits during puberty to understand the neural correlates of adolescent social development.

Functional changes in brain regions critical to processing social threats – such as the amygdala and related prefrontal areas such as the ventrolateral prefrontal cortex (VLPFC) – could facilitate the type of bold social behavior that is both typical and adaptive during adolescence. The amygdala plays a key role in detecting threat and ambiguity (Whalen, et al., 2001), and it is particularly important in processing social threat (Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002) and affective features of social stimuli (Haxby, Hoffman, & Gobbini, 2002). The VLPFC appears to serve a key regulatory function for amygdala reactivity during contexts of social threat (e.g., Brown et al., 2006; Monk et al., 2008) and contributes to voluntary processes of affect regulation (Phillips, Ladouceur, & Drevets, 2008). In addition, the VLPFC seems to respond to affective stimuli in tandem with the amygdala (e.g., Blair et al., 2008). Human faces are optimal stimuli for engaging these threat-related regions. In terms of the threatening content of facial expressions, human faces expressing angry affect are considered unambiguous threat signals, while expressions of neutral or fearful affect are considered ambiguous because they might not convey direct threat to the person viewing the expression (Whalen et al., 2001). Developmentally, neutral facial expressions can be a salient stimulus for eliciting threat-related brain function in young people (Thomas, Drevets, Whalen, et al., 2001).

Navigating emotionally high-stakes social challenges represents not only a core dimension of normal adolescent development, but it is also relevant to understanding clinically relevant questions about vulnerability to experiences of negative affect such as depressed mood (Allen & Badcock, 2003). Adolescence involves changes in the experience of negative affect and depression, both of which are relevant to the neural processing of social threat. Levels of subjective negative affect (Larson, Moneta, Richards, & Wilson, 2002) and depression (Angold & Costello, 2006; Seeley & Lewinsohn, 2008) both increase during adolescence, and subclinical depression has been linked to pubertal maturation (Graber, 2008). In addition, depression is postulated to involve enhanced sensitivity to perceived social threat (Allen & Badcock, 2003) and tendency to perceive ambiguous stimuli as threatening (Nunn, Mathews, & Trower, 1997). Accordingly, neuroimaging studies have reported greater amygdala reactivity in association with negative affect (Costafreda,

Brammer, David, & Fu, 2008; Davidson, 2002) and greater or unusual reactivity of threat-related brain circuits in both adults and young people with depression (Phillips, Drevets, Rauch, & Lane, 2003; Thomas, Drevets, Dahl, et al., 2001). Because investigations of threat-related brain function must be grounded in the relation of brain function to behavior, it is critical to examine the association of adolescents' reactivity to social threat stimuli with their actual experience of negative affect and depression, so that possible pubertal maturation influences can be understood in the context of behavior in natural environments.

The current study investigated the influence of pubertal maturation—defined as stage of physical development—on threat-related brain function in psychiatrically healthy young people. To isolate the role of pubertal maturation and to avoid the confounding of age and pubertal development that can occur in many studies of adolescent development, we recruited a sample of adolescents who were within a narrow age range, but likely to exhibit varying levels of pubertal development, as measured by physical examination of sexual maturation. Participants completed a well-validated fMRI threat-processing task (Hariri, Bookheimer, & Mazziotta, 2000; Hariri, Tessitore, et al., 2002) that employs faces with affective expressions as stimuli, includes both ambiguous and unambiguous threat stimuli, and reliably elicits amygdala and VLPFC reactivity in adults. Based on the role of the amygdala and the VLPFC in brain function in response to social stimuli with affective content, we examined whether mid/late pubertal adolescents would differ from pre/early pubertal adolescents in amygdala and VLPFC reactivity to ambiguous and unambiguous social threats. To place threat-related brain function in the context of affective experience, we also examined whether threat-related brain function in response to social threat was correlated with subjective negative affect measured in real-world settings and with depressive symptoms. Because negative affect and depression are likely to be associated with threat processing, we hypothesized that greater amygdala and VLPFC reactivity to social threat, regardless of ambiguity, would be associated with higher negative affect and higher depressive symptoms.

Method

Participants

Adolescents were part of a study of pubertal influences on normal affective development, and they were recruited from the community through advertisements, flyers, and demographically targeted phone lists. All adolescents were recruited to be in a narrow age range—specifically, 11-13 years—but to vary in pubertal development, so that the effects of pubertal development could be examined with minimal confounding by age. Girls were recruited to be 11-12 years old ($M=11.49$, $SD=.60$), and boys to be 12-13 years old ($M=12.39$, $SD=.59$), based on epidemiologic findings that girls in the United States undergo puberty earlier than boys (Herman-Giddens, et al., 1997; Karpati, Rubin, Kieszak, Marcus, & Troiano, 2002; Wu, Mendola, & Buck, 2002), and with the intention of maximizing developmental variability within groups of girls and boys. Adolescents were free of current and lifetime psychiatric disorders, did not have braces, and had no history of head injury, serious medical illness, psychotropic medication use, alcohol use, or illicit drug use.

All participants provided informed consent according to the guidelines of the University of Pittsburgh Institutional Review Board. The final sample for the study included 76 healthy normal subjects, including 27 who were pre/early pubertal (70% female; age $M=11.80$, $SD=.72$; 85% European American) and 49 mid/late pubertal (43% female; $M=12.46$, $SD=.86$; 69% European American). The original sample of adolescents included 126 participants. A total of 9 adolescent participants did not complete the threat task. Participants were excluded for excessive head movement during the scan ($n=18$), having siblings in the study ($n=10$), medical or psychiatric conditions ($n=3$), missing physical examination data ($n=7$), or

withdrawal from the study ($n=2$). Siblings were excluded to eliminate the possible confound of within-family similarity in brain function.

Materials

Psychiatric health—Parents or guardians of adolescent participants reported on adolescents' history of psychiatric disorder during a phone screen. If the parent or guardian endorsed “any mental or behavioral health issues,” staff asked questions about specific Axis I disorders based on items from the KSADS-PL (Kaufman, et al., 1997).

Pubertal development—Adolescents underwent physical examination by a trained research nurse to determine stage of pubertal development with criteria specified by Marshall and Tanner (Marshall & Tanner, 1968). Consistent with our ongoing approach to examining affective aspects of pubertal development (Forbes, Williamson, Ryan, & Dahl, 2004), and given the non-continuous nature of Tanner scale data, we classified participants categorically as *pre/early adolescent* if they were Tanner stage 1 or 2 for breast/genital development and as *mid/late adolescent* if they were Tanner stage 3, 4, or 5 for breast/genital development. Because breast and genital development reflect changes in levels of gonadal steroids, which influence neural development and affect-related brain function during puberty, we focused on this Tanner scale.

Threat processing—The fMRI threat reactivity paradigm is a block-design, face-processing paradigm used in dozens of studies of affective processes (Hariri, et al., 2000; Hariri, Mattay, et al., 2002). The paradigm contains four blocks of a perceptual face processing task interleaved with five blocks of a sensorimotor control task. During the face task, subjects view a trio of faces and select one of two faces (bottom) that is identical to the target face (top). Each face processing block contained 6 novel exemplars of one type of affective facial expression—angry, fearful, or neutral—presented for 2 sec each, with 3 images of each sex. Type of affect was consistent within block, and two blocks of neutral faces alternated with two blocks of affective faces (one with angry faces, one with fearful faces) with order counterbalanced across participants. This allowed us to easily separate ambiguous and unambiguous social threat stimuli. All face stimuli are derived from a standard set of pictures of facial affect (<http://www.macbrain.org/resources.htm>). Within each face block, the interstimulus interval (ISI) varied (i.e., jittered) between 2, 4, and 6 s (mean ISI = 4). During the sensorimotor control task, subjects viewed a set of 3 geometric shapes (circles, vertical ellipses, and horizontal ellipses) and selected one of the two shapes (in the bottom row) that matched the target shape (in the top row). Each control block consisted of six different images, each presented twice in a pseudorandom order for 2 sec. Subject performance (accuracy and reaction time) was monitored during all scans and did not differ by pubertal maturation group.

Subjective negative affect in natural settings—The child version of the Positive and Negative Affectivity Scale (PANAS-C; Laurent, et al., 1999), a mood questionnaire with good psychometric properties, was used to assess subjective negative affect by cell phone EMA in natural settings. For each PANAS-C item (e.g., *upset*) participants rated their experience “at the moment the phone rang” using a 5-point scale (1=*very slightly or not at all*, 5=*extremely*). All 20 items were administered once per day, and a subset of 8 items (4 reflecting NA) was administered at all other calls to reduce time burden on participants. Because we have not found that mean NA score within day is excessively variable, mean NA across the weekend was computed for use in regressions. Two participants did not complete the EMA procedure and were not included in related analyses.

Depressive symptoms—Adolescents completed the Mood and Feelings Questionnaire (MFQ; Angold & Costello, 2006), a psychometrically sound self-report and parent-report measure of depressive symptoms that has been used widely in research on adolescent depression. Total self-report score was included in analyses. Two participants did not complete the MFQ, and they were thus excluded from related analyses.

Procedure

Each participant completed an fMRI scan on a Tuesday or Wednesday and underwent a 4-day EMA protocol on the weekend before or after the scan. EMA was completed after the scan 79% of the time with a mean of 4.2 days between scan and EMA ($SD = 8.4$).

fMRI acquisition, processing, and analysis—Each participant was scanned using a Siemens 3T Allegra scanner. BOLD functional images were acquired with a gradient echo planar imaging (EPI) sequence and covered 34 axial slices (3mm thick) beginning at the cerebral vertex and encompassing the entire cerebrum and the majority of the cerebellum ($TR/TE = 2000/25$ ms, $FOV = 20$ cm, matrix = 64×64). All scanning parameters were selected to optimize the quality of the BOLD signal while maintaining a sufficient number of slices to acquire whole-brain data. Before the collection of fMRI data for each participant, we acquired a reference EPI scan that we visually inspected for artifacts (for example, ghosting) as well as for good signal across the entire volume of acquisition, including the striatum. The fMRI data from all participants included in this study were cleared of such problems.

Whole-brain image analysis was completed using SPM2 (<http://www.fil.ion.ucl.ac.uk/spm>). For each scan, images for each participant were realigned to the first volume in the time series to correct for head motion. Data sets were then selected for their high quality (scan stability), as demonstrated by small motion correction (< 4 mm). Realigned images were spatially normalized into a standard stereotactic space (Montreal Neurological Institute template) using a 12-parameter affine model. These normalized images were then smoothed to minimize noise and residual difference in gyral anatomy with a 6mm full-width at half-maximum Gaussian filter. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean.

Preprocessed data sets were then analyzed using second-level random effect models that account for both scan-to-scan and participant-to-participant variability to determine task-specific regional responses. For each participant and scan, predetermined condition effects (i.e., main effects of task) at each voxel were calculated using a t -statistic, producing a statistical image for each of the 3 contrasts of interest: (1) neutral > control; (2) fearful > control; and (3) angry > control. Individual contrast images were then used to determine mean task-related amygdala and VLPFC reactivity using within-group t -tests, which were thresholded at a voxel level of $p < .05$ and an extent of at least 10 contiguous voxels. Group results were then masked across the volume of the amygdala or VLPFC using the WFU PickAtlas Tool (v2.4). The region of interest (ROI) mask was created as a single mask using PickAtlas, with amygdala and BA47 as the selected anatomical regions, and the entire mask dilated once in 2 dimensions. Furthermore, to restrict analyses to the regions exhibiting BOLD response to the task (rather than the entire amygdala and VLPFC), anatomical masks for each condition (e.g., neutral faces > shapes) were further masked with a functional image for that condition, limited, as defined by the main effects of condition within the entire sample (e.g., neutral faces > shapes). Finally, ROI analyses were corrected for multiple comparisons by thresholding using the false discovery rate across activation clusters of interest within the ROIs.

EMA Protocol—Participants received 12 phone calls at consistent intervals over four days, including two weekend days and two weekdays (Friday-Monday). EMA was conducted via cell phone from Friday afternoon to Monday evening for all participants. Monday's calls occurred after school hours. Calls occurred within the same 3-hour time window for all participants. Participants received calls twice on Friday (4-7 pm and 7-10 pm), four times each on Saturday and Sunday (11 am-1 pm, 1-4 pm, 4-7 pm, and 7-10 pm), and twice on Monday (4-7 pm and 7-10 pm). EMA was conducted primarily during the weekend so that calls would not interfere with school participation and because participants would have more freedom to choose their activities and companions. Monday evening calls allowed us to include assessment of mood and behavior during a weeknight in addition to during the weekend. Phone calls were conducted by trained research assistants who used a script developed for the study. The content of the calls included questions about mood, activities, and companions at the moment of the phone call ("when the phone rang"). Analyses for the current study focus on mean subjective negative affect across the 4-day EMA using all available data on NA items. One participant did not complete the EMA protocol and thus was excluded from related analyses.

Data analyses

Main effects of task were examined for each developmental group using a within-group *t* test. Pubertal maturation effects were examined using one-way analyses of covariance (ANCOVAs) focused on developmental group differences in amygdala and VLPFC reactivity, with age and sex as covariates. Each task condition (neutral, angry, or fearful faces) was analyzed separately. Associations between task-related amygdala or VLPFC reactivity and subjective NA or depressive symptoms were tested using regression analyses in SPM. For all analyses, a threshold of $p < .05$ and an extent of 10 voxels were applied.

Results

Preliminary Findings

Analyses of variance to examine possible group differences indicated that pre/early and mid/late pubertal adolescent groups did not differ in task behavior, mean subjective negative affect in natural environments, or self-reported depressive symptoms ($F_s = 1.56-3.38$, $ps > .05$). Because the pre/early pubertal group was slightly younger than the mid/late pubertal group ($M = 11.80$, $SD = .72$ and $M = 12.46$, $SD = .85$, respectively; $F(1,74) = 11.57$, $p < .005$) and had a larger proportion of girls (.70 and .43, respectively; $X^2 = 5.29$, $p < .05$), age and sex were included as covariates in between-group analyses.

Overall Threat Reactivity

Within-group *t* tests to examine the main effects of task indicated that the social threat task elicited amygdala reactivity in both developmental groups during all conditions, and it elicited VLPFC reactivity in the mid/late pubertal group (Table 1). Additional whole-brain analyses indicated that in general, the task elicited activation in the ventral visual stream and prefrontal cortex in both developmental groups (Supplementary Table 1).

Pubertal Development and Threat Reactivity

Regression analyses to examine group differences based on pubertal development for each contrast indicated that mid/late pubertal adolescents exhibited less amygdala reactivity than pre/early adolescents while viewing neutral faces and less VLPFC reactivity than pre/early adolescents while viewing fearful faces (Table 2; Figure 1). The groups did not differ while viewing angry faces, and the pre/early pubertal group did not exhibit less reactivity than the mid/late group during any of the task conditions.

To follow up these findings by directly testing group differences between affective conditions, we computed 3 additional contrasts: (1) fearful > angry; (2) fearful > neutral; and (3) neutral > angry. Regression analyses conducted in the same way as those for the original 3 contrasts indicated that the mid/late pubertal group exhibited less VLPFC reactivity than the pre/early pubertal group for the fearful > angry, fearful > neutral, and neutral > angry contrasts (Table 2; Figure 1).

Threat Reactivity in Relation to Subjective Negative Affect and Depressive Symptoms

Regression analyses to examine the relation of threat-related brain function and self-reported affect (i.e., negative mood and depressive symptoms) conducted within the entire sample indicated that adolescents' subjective negative affect in natural environments was associated with greater VLPFC reactivity while viewing fearful faces (Table 3; Figure 3). Adolescents' depressive symptoms were associated with (1) greater VLPFC reactivity while viewing neutral faces and (2) greater amygdala reactivity while viewing angry faces (Table 3; Figure 3).

Because within-group *t* tests indicated that the mid/late pubertal adolescents exhibited task-related VLPFC reactivity while the pre/early pubertal group did not (Table 1), it is possible that regressions including both groups together did not detect associations with VLPFC reactivity. To address this issue, regression analyses for negative affect and depressive symptoms were also conducted within just the mid/late pubertal group. These analyses indicated that depressive symptoms were correlated with VLPFC reactivity while viewing angry faces ($t = 2.94, p < .005$, 12 voxels, Talairach coordinates: 24, 31, -7). All other regression analyses within the mid/late group yielded nonsignificant results.

Discussion

The current study examined neural systems of threat processing and measures of negative affect in relation to pubertal maturation. Our findings indicate that pubertal maturation is associated with changes in neural reactivity to some types of social stimuli. Mid/late pubertal adolescents exhibited less amygdala reactivity to neutral faces relative to control stimuli and less VLPFC reactivity to fearful facial expressions relative to control stimuli compared with pre/early pubertal adolescents. When affective conditions were compared directly, the mid/late pubertal group exhibited less VLPFC reactivity to fearful relative to neutral faces and to fearful relative to angry faces, and more VLPFC reactivity to angry relative to neutral faces. Because both groups were in the same narrow age range, and age was adjusted in analyses, these results indicate that pubertal maturation rather than age-related experience (e.g., being in middle school vs. high school) accounts for these differences. In addition, threat-related brain activity explained 5-6% of variance in subjective negative affect in natural environments and depressive symptoms, indicating that these measures of neural activity in laboratory settings are relevant to real-world measures of affective experience and to clinically relevant mood ratings.

Our findings are somewhat consistent with the triadic model of affect-related adolescent brain function (Ernst et al., 2006) to explain the high rates of risk-taking behavior during adolescence. As in that model, mid/late pubertal adolescents exhibited less amygdala reactivity than their less-mature peers when viewing neutral faces relative to non-face control stimuli. Similarly, and in line with findings that the VLPFC and amygdala show similar reactivity to social threat (Blair et al., 2007), mid/late pubertal adolescents exhibited less VLPFC reactivity than their less-mature peers when viewing fearful faces relative to control stimuli. We note, however, that we only addressed one part of the triadic model: the reactivity of threat-related brain systems. Future studies will be needed to address the

development of reward-related and control-related brain systems in combination with threat-related brain systems to test this model more fully.

However, because developmental differences in threat-related brain function were not evident during viewing of unambiguous social threats (i.e., angry faces vs. control stimuli), our findings do not suggest an overall decrease in threat reactivity with pubertal maturation. Instead, our findings provide support for the claim that threat-related brain systems' response to ambiguously—but not unambiguously—threatening social stimuli decreases with pubertal maturation, while response to clear, direct threats does not decrease. Analyses directly comparing affective conditions further supported this claim: compared with pre/early adolescents, mid/late adolescents exhibited less reactivity to ambiguous relative to unambiguous social threats (i.e., fearful faces vs. angry faces) and more reactivity to unambiguous relative to ambiguous social threats (i.e., angry faces vs. neutral faces).

Ambiguous and unambiguous social threats could present different challenges to social exploration in adolescents. Processing of ambiguous social threat—such as facial displays of neutral affect, which do not convey direct threat but could signal danger to someone viewing the display—could be under greater pubertal influence than unambiguous social threats. In contrast, processing of unambiguous social threat—such as facial displays of anger, which convey more direct danger to the viewer—could remain more constant across adolescent development because of the survival value of responding appropriately to explicit threat. Clearly, future studies are needed to test this claim longitudinally and more comprehensively, with a variety of threatening stimuli, social and otherwise. Similarly, it will be important for future studies to examine amygdala reactivity related to affect reactivity *and* affect regulation, as our study addressed the former and a previous study with opposite findings (Hare et al., 2004) addressed the latter.

Another interpretation of our findings is that they reflect maturational changes in cognitive rather than affective processes. For example, pubertal maturation could be associated with reduced perceptual or cognitive processing of certain types of social stimuli. Social stimuli conveying direct or unambiguous threat might be perceived similarly regardless of pubertal maturation, whereas social stimuli conveying ambiguous threat might receive less perceptual weight. This possible change in processing ambiguous social threats could reflect a change in classifying social stimuli into salience categories. Cognitively, pubertal maturation might influence processing of ambiguous social threats by reducing attention to or reducing the value ascribed to such threats.

It is interesting to speculate that reduced reactivity to ambiguous social stimuli during adolescence could perform an adaptive, facilitative role for some types of bold social behaviors. Specifically, in novel or ambiguous social situations, adolescents might be more likely to explore potentially threatening social domains—such as approaching an unfamiliar peer or potential romantic partner, trying to join a popular group—in part because of a developmental shift toward less threat-related brain response in the absence of overt or unambiguous threat signals. This could help to embolden behaviors toward widening social networks and taking advantage of opportunities to develop new friendships, peer groups, and romantic partners.

We have previously described pubertal influences associated with reduced *reward*-related brain function and interpreted these contributing to increased reward-seeking behaviors to compensate for low neural reactivity (Forbes et al., under review). In the case of low threat-related brain function, the same interpretation is also possible: because potential threats (i.e., ambiguous social signals) may become less potent with pubertal maturation, enhanced threat-seeking or risk-taking behavior may occur to compensate for low reactivity. After all,

risky behavior is likely to result in reward or threat, so that pubertal development could lead to greater inclination to approach novel or ambiguous stimuli in general. This could result in decreased reactivity in both brain systems mediating reward-related behavior and brain systems mediating threat-related behavior, so that the two systems show parallel changes with pubertal maturation.

The findings of a correlation between threat-related brain function and subjective experiences of mood and depressive symptoms raise some additional questions. Whereas brain function to ambiguous social threats decreased with development, high levels of threat-related brain function to both ambiguous and unambiguous social threats was associated with higher subjective negative affect and depressive symptoms. One might have predicted that more advanced pubertal maturation would be associated with higher levels of negative affect and/or depressive symptoms, since previous studies have reported that negative affect and depressive symptoms both appear to increase during the early high school years (Larson, et al., 2002) and Angold and colleagues reported an increase in depression associated with elevations of pubertal hormones (Angold & Costello, 2006). It is unclear why this study failed to find the expected developmental shift in negative affect. One possibility in a psychiatrically healthy population, however, is that the increase in VLPFC reactivity with negative mood served a regulatory function, compensating for increased affective reactivity that occurred with pubertal development.

Despite the lack of a maturational shift in negative affect, the correlation between amygdala reactivity and depressive symptoms bears some resemblance to reports of enhanced amygdala reactivity in adults and young people with clinical depression (Drevets, 2000; Thomas, Drevets, Dahl, et al., 2001). In the current study, participants were psychiatrically healthy, and their level of depression was below the clinical range. The association of depressive symptoms with amygdala response to social threat in the current sample, while not a strong effect, was similar to the association reported in clinical samples. This suggests that normal individual differences in depressed mood are associated with greater response of threat-related brain systems and that this greater response is especially evident during clear social threats.

An early fMRI study from this research group found increased amygdala reactivity to neutral faces in young people relative to adults (Thomas, Drevets, Whalen, et al., 2001). While we were not able to compare amygdala response to neutral social stimuli in adolescents and adults, we found that the less mature pubertal development group showed a greater response during this ambiguous social threat condition. Thus, it may be that early pubertal adolescents show a particularly elevated response to neutral social stimuli relative to adults. With pubertal maturation and greater social experience, development may tend to lead adolescents to perceive and treat neutral expressions as less and less ambiguous. That is, both pubertal maturation and age-related increases in experience interacting with others in varied social contexts could change the relevance of neutral facial expressions as potential threats, making them appear less threatening.

Our findings of VLPFC reactivity during ambiguous social contexts in relation to both pubertal maturation and affective experience have potential relevance to affect regulation research. VLPFC was activated less in mid/late pubertal adolescents than in pre/early pubertal adolescents, and it was activated more in those with higher levels of subjective negative affect and depressive symptoms. Given that the VLPFC seems to play an important role in affect regulation generally (Phillips, et al., 2008) and in the functional regulation of the amygdala specifically (Monk, et al., 2008), and given that amygdala and VLPFC findings were in the same direction in the current study, both greater affective reactivity and greater affect regulation appear to occur in those who are high in negative affect

characteristics. Because affect regulation can be conceptualized as occurring throughout the process of affective reactivity (Thompson, Lewis, & Calkins, 2008), it is likely that VLPFC reactivity would occur in concert with amygdala reactivity. In the case of subjective negative affect and depressive symptoms, perhaps there is a greater need for regulation of threat-related brain circuits—as well as a greater reactivity in those circuits—in adolescents who experience higher levels of fear, anger, and dysphoria. Alternatively, there is evidence that the VLPFC is functionally correlated with the amygdala during the processing of negative stimuli (e.g., Blair, et al., 2007). From this perspective, both brain areas contribute to affective reactivity to threat, and the current findings indicate that both areas are more reactive in adolescents who are high in subjective negative affect.

This study's limitations include its cross sectional design, focus on social threat rather than other types of threat, and lack of an adult comparison group. As a result of these, we are not able to draw general conclusions about the development of threat processing across the lifespan, and we cannot address intraindividual changes in threat processing. However, the participants in the current study are scheduled to return for a second fMRI assessment two years after their original assessment, and we anticipate examining the longitudinal development of their brain function once that round of assessment is completed. We did not collect self-report measures of perception of social threat in ambiguous vs. unambiguous facial expression stimuli, and as a result, we are not able to determine whether pubertal maturation was related to changes in the overt perception of ambiguous social threats or whether threat-related brain function was associated with perception of social stimuli as threatening. Finally, some of our null findings for developmental effects could have been influenced by power to detect those findings, as when one group failed to show activation in a particular condition (e.g., pre/early pubertal group's VLPFC activation during neutral faces).

In sum, the current study contributes to our knowledge of pubertal influences on social threat processing. By employing a large sample of adolescents with a combination of low variability in age and high variability in pubertal development, this study contributes to understanding pubertal influences on emotional development within a framework of developmental, affective, and social neuroscience. These results suggest that some aspects of affective reactivity and regulation change during pubertal maturation in ways that may inform not only our understanding of normative increases in adolescent risk-taking and social exploration, but also some aspects of adolescent vulnerability to experience negative affect that may lead to depression. Clearly, this work represents an early step in this line of investigation. More studies—particularly longitudinal studies—are needed to help elucidate the normative as well as pathologic changes in these systems, which can ultimately inform early intervention strategies aimed at key intervals of development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by NIH R01 DA018910 (Ronald E. Dahl, PI), K01 MH74769 (Erika E. Forbes, PI), and a NARSAD Young Investigator Award (Erika E. Forbes PI). We thank Donna Moyles and Alex Johnston for assistance with data processing and analysis, Jill Tarr for study coordination, Kara Deal for assistance with references, and Samantha Sciarillo and Kelsey Ronan for careful help with preparing figures. We are also grateful to the adolescents and families who participated in the study.

References

- Allen NB, Badcock PBT. The social risk hypothesis of depressed mood: Evolutionary, psychosocial, and neurobiological perspectives. *Psychological Bulletin*. 2003; 129(6):1–28.
- Angold A, Costello EJ. Puberty and depression. *Child and Adolescent Psychiatric Clinics of North America*. 2006; 15(4):919–937. [PubMed: 16952768]
- Angold A, Erkanli A, Silberg J, Eaves L, Costello EJ. Depression scale scores in 8-17-year-olds: effects of age and gender. *Journal of Child Psychology and Psychiatry*. 2002; 43(8):1052–1063. [PubMed: 12455926]
- Blair KS, Smith BW, Mitchell DG, Morton J, Vythilingam M, Pessoa L, et al. Modulation of emotion by cognition and cognition by emotion. *Neuroimage*. 2007; 35(1):430–440. [PubMed: 17239620]
- Blakemore SJ. The social brain in adolescence. *Nature Reviews, Neuroscience*. 2008; 9:267–277.
- Costafreda SG, Brammer MJ, David AS, Fu CH. Predictors of amygdala activation during the processing of emotional stimuli: a meta-analysis of 385 PET and fMRI studies. *Brain Research Reviews*. 2008; 58(1):57–70. [PubMed: 18076995]
- Dahl RE, Spear LP. Adolescent brain development. *Annals of the New York Academy of Sciences*. 2004; 1021:1–22. [PubMed: 15251869]
- Davidson RJ. Anxiety and affective style: role of prefrontal cortex and amygdala. *Biological Psychiatry*. 2002; 51(1):68–80. [PubMed: 11801232]
- Diamond R, Carey S, Back KJ. Genetic influences on the development of spatial skills during early adolescence. *Cognition*. 1983; 13(2):167–185. [PubMed: 6682743]
- Drevets, WC. Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. *Progress in Brain Research; Proceedings of the 21st International Summer school of Brain Research, held at the Royal Netherlands Academy of Sciences; Amsterdam, the Netherlands*. 23-27 August 1999; 2000.
- Ernst M, Pine DS, Hardin M. Triadic model of the neurobiology of motivated behavior in adolescence. *Psychol Med*. 2006; 36(3):299–312. [PubMed: 16472412]
- Forbes EE, Williamson DE, Ryan ND, Dahl RE. Positive and negative affect in depression: Influence of sex and puberty. *Annals of the New York Academy of Sciences*. 2004; 1021:341–347. [PubMed: 15251907]
- Gardner M, Steinberg L. Peer influence on risk taking, risk preference, and risky decision making in adolescence and adulthood: An experimental study. *Developmental Psychology*. 2005; 41(4):625–635. [PubMed: 16060809]
- Graber, JA. Pubertal and neuroendocrine development and risk for depression. In: Allen, NB.; Sheeber, LB., editors. *Adolescent Emotional Development and the Emergence of Depressive Disorders*. New York: Cambridge University Press; 2008.
- Hare TA, Tottenham N, Galvan A, Voss HU, Glover GH, Casey BJ. Biological substrates of emotional reactivity and regulation in adolescence during an emotional go-nogo task. *Biological Psychiatry*. 2008; 63(10):927–934. [PubMed: 18452757]
- Hariri AR, Bookheimer SY, Mazziotta JC. Modulating emotional responses: effects of a neocortical network on the limbic system. *Neuroreport*. 2000; 11(1):43–48. [PubMed: 10683827]
- Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, et al. Serotonin transporter genetic variation and the response of the human amygdala. *Science*. 2002; 297(5580):400–403. [PubMed: 12130784]
- Hariri AR, Tessitore A, Mattay VS, Fera F, Weinberger DR. The amygdala response to emotional stimuli: a comparison of faces and scenes. *Neuroimage*. 2002; 17(1):317–323. [PubMed: 12482086]
- Haxby JV, Hoffman EA, Gobbini MI. Human neural systems for face recognition and social communication. *Biological Psychiatry*. 2002; 51(1):59–67. [PubMed: 11801231]
- Herman-Giddens ME, Slora EJ, Wasserman RC, Bourdony CJ, Bhapkar MV, Koch GG, et al. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Research in Office Settings network. *Pediatrics*. 1997; 99(4):505–512. [PubMed: 9093289]

- Karpati AM, Rubin CH, Kieszak SM, Marcus M, Troiano RP. Pubertal stage assessment in American boys: the 1988-1994 Third National Health and Nutrition Examination Survey. *Adolescent Health*. 2002; 30(3):205–212.
- Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): Initial reliability and validity data. *Journal of the American Academy of Child and Adolescent Psychiatry*. 1997; 36(7):980–988. [PubMed: 9204677]
- Larson RW, Moneta G, Richards MH, Wilson S. Continuity, stability, and change in daily emotional experience across adolescence. *Child Development*. 2002; 73(4):1151–1165. [PubMed: 12146740]
- Laurent J, Catanzaro SJ, Joiner TE Jr, Rudolph KD, Potter KI, Lambert S, et al. A measure of positive and negative affect for children: Scale development and preliminary validation. *Psychological Assessment*. 1999; 11(3):326–338.
- Marshall WA, Tanner JM. Growth and physiological development during adolescence. *Annual Review of Medicine*. 1968; 19(283-300)
- Monk CS, Telzer EH, Mogg K, Bradley BP, Mai X, Louro HM, et al. Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder. *Archives of General Psychiatry*. 2008; 65(5):568–576. [PubMed: 18458208]
- Nunn JD, Mathews A, Trower P. Selective processing of concern-related information in depression. *British Journal of Clinical Psychology*. 1997; 36(Pt 4):489–503. [PubMed: 9403142]
- Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception II: Implications for major psychiatric disorders. *Biological Psychiatry*. 2003; 54(5):515–528. [PubMed: 12946880]
- Phillips ML, Ladouceur CD, Drevets WC. A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder. *Mol Psychiatry*. 2008; 13(9):829, 833–857. [PubMed: 18574483]
- Quevedo KM, Benning SD, Gunnar MR, Dahl RE. The onset of puberty: Effects on the psychophysiology of defensive and appetitive motivation. *Development and Psychopathology*. 2009; 21:27–45. [PubMed: 19144221]
- Seeley, JR.; Lewinsohn, PM. Epidemiology of mood disorders during adolescence: Implications for lifetime risk. In: Allen, NB.; Sheeber, LB., editors. *Adolescent Emotional Development and the Emergence of Depressive Disorders*. New York: Cambridge University Press; 2008.
- Sergerie K, Chochol C, Armony JL. The role of the amygdala in emotional processing: A quantitative meta-analysis of functional neuroimaging studies. *Neuroscience and Biobehavioral Reviews*. 2008; 32(4):811–830. [PubMed: 18316124]
- Steinberg L. Cognitive and affective development in adolescence. *Trends in Cognitive Science*. 2005; 9(2):69–74.
- Steinberg L, Albert D, Cauffman E, Banich M, Graham S, Woolard J. Age differences in sensation seeking and impulsivity as indexed by behavior and self-report: Evidence for a dual systems model. *Developmental Psychology*. 2008; 44(6):1764–1778. [PubMed: 18999337]
- Thomas KM, Drevets WC, Dahl RE, Ryan ND, Birmaher B, Eccard CH, et al. Amygdala response to fearful faces in anxious and depressed children. *Archives of General Psychiatry*. 2001; 58(11): 1057–1063. [PubMed: 11695953]
- Thomas KM, Drevets WC, Whalen PJ, Eccard CH, Dahl RE, Ryan ND, et al. Amygdala response to facial expressions in children and adults. *Biological Psychiatry*. 2001; 49(4):309–316. [PubMed: 11239901]
- Thompson RA, Lewis MD, Calkins SD. Reassessing emotion regulation. *Child Development Perspectives*. 2008; 2(3):124–131.
- Whalen PJ, Shin LM, McInerney SC, Fischer H, Wright CI, Rauch SL. A functional MRI study of human amygdala responses to facial expressions of fear versus anger. *Emotion*. 2001; 1(1):70–83. [PubMed: 12894812]
- Wu T, Mendola P, Buck GM. Ethnic differences in the presence of secondary sex characteristics and menarche among US girls: the Third National Health and Nutrition Examination Survey, 1988-1994. *Pediatrics*. 2002; 110:752–757. [PubMed: 12359790]

Figure 1a

Figure 1b

Figure 1c

Figure 1d

Figure 1e

Figure 1.

Regions where the mid/late pubertal adolescent group exhibited less reactivity in threat-related brain areas to ambiguous social stimuli than the pre/early adolescent group. Statistical parametric maps and scatterplots depict group differences in BOLD response in (a) the amygdala during processing of neutral faces; (b) ventrolateral prefrontal cortex (VLPFC) during processing of fearful faces; (c) VLPFC during processing of fearful faces contrasted with neutral faces; (d) VLPFC during processing of fearful faces contrasted with angry faces; and (e) VLPFC during processing of neutral faces contrasted with angry faces. BOLD response values are in arbitrary units.

Figure 2a

Figure 2b

Figure 2c

Figure 2.

Association between threat-related brain function and subjective negative affect and depressive symptoms. Statistical parametric maps depict regression results and scatterplots depict association of BOLD response with individual scores for (a) subjective negative affect and ventrolateral prefrontal cortex (VLPFC) function during processing of fearful faces; (b) depressive symptoms and VLPFC function during processing of neutral faces; and (c) depressive symptoms and amygdala function during processing of angry faces. BOLD response values are in arbitrary units. Regression lines and R^2 values are shown separately for the pre/early adolescent and mid/late adolescent groups.

Table 1
Within-Group Main Effects in the Amygdala and Ventrolateral Prefrontal Cortex (VLPFC) during Task Conditions, by Region of Interest

Region	Hemisphere	Talairach coordinates of maximum voxel in cluster			Cluster size	t	p <
		x	y	z			
Neutral > Shapes							
Pre/Early Adolescent							
Amygdala	R	22	-6	-11	44	6.47	0.001
Mid/Late Adolescent							
Amygdala	R	24	-3	-12	42	6.84	0.001
VLPFC (BA 47)	R	26	30	-12	19	3.49	0.005
Fear > Shapes							
Pre/Early Adolescent							
Amygdala	R	24	-3	-12	40	5.00	0.001
Mid/Late Adolescent							
Amygdala	R	22	-6	-11	48	4.71	0.001
VLPFC (BA 47)	L	-44	27	-10	31	3.30	0.005
Anger > Shapes							
Pre/Early Adolescent							
Amygdala	R	24	-10	-10	35	3.90	0.001
Mid/Late Adolescent							
Amygdala	R	26	1	-15	48	5.94	0.001
VLPFC (BA 47)	L	-42	27	-10	18	2.60	0.01

Note: PFC = prefrontal cortex; BA = Brodmann Area. Results are from region of interest (ROI) analyses focusing on the amygdala, fusiform gyrus, and prefrontal cortex. df = 26 for pre/early adolescent group and 48 for mid/late adolescent group. Significant clusters in each analysis are listed in descending order of t score. Developmental groups were determined by sexual maturation, with Tanner breast/genital score of 1-2 for the pre/early adolescent group and 3-5 for the mid/late adolescent group.

Table 2
Greater Amygdala and Ventrolateral Prefrontal Cortex (VLPFC) Reactivity to Social Threat in Pre/Early than Mid/Late Pubertal Adolescents, by Task Condition

Contrast and Region	Hemisphere	Talairach coordinates of maximum voxel in cluster			Cluster Size	t	p<
		x	y	z			
Neutral Faces > Shapes							
Amygdala	L	-32	-5	-18	29	2.94	0.005
Angry Faces > Shapes							
Fearful Faces > Shapes							
No suprathreshold clusters							
Fearful Faces > Neutral Faces							
VLPFC (BA47)	L	-40	31	2	27	2.58	0.01
Fearful Faces > Angry Faces							
VLPFC (BA47)	L	-51	18	1	19	2.11	0.05
Neutral Faces > Angry Faces							
VLPFC (BA47)	L	-44	17	-1	107	3.63	0.001
Neutral Faces > Fearful Faces							
VLPFC (BA47)	L	-44	17	-3	25	2.51	0.01

Note: PFC = prefrontal cortex; BA = Brodmann Area. df = 70 for each model, *p* values were corrected using false discovery rate. Adolescent groups were determined by physical development, with Tanner breast/genital score of 1-2 for the pre/early adolescent group and 3-5 for the mid/late adolescent group. All ANCOVAs included sex, age, and race as covariates. Contrasts of pairs of affective conditions were included to directly test group differences for different types of social threat stimuli. There were no clusters for which mid/late pubertal adolescents exhibited more reactivity than pre/early pubertal adolescents.

Table 3
Association of Adolescents' Social Threat Processing with Affective Experience: Subjective Negative Affect in Natural Environments and Depressive Symptoms

Condition and Region	Talairach coordinates of maximum voxel in cluster			Cluster size	t	p <
	x	y	z			
Subjective Negative Affect						
Neutral Faces > Shapes						
No suprathreshold clusters						
Fearful Faces > Shapes						
VLPFC (BA47)	-48	29	-12	26	2.02	.05
Angry Faces > Shapes						
No suprathreshold clusters						
Depressive Symptoms						
Neutral Faces > Shapes						
VLPFC (BA47)	-46	37	-4	13	2.18	.05
Fearful Faces > Shapes						
No suprathreshold clusters						
Angry Faces > Shapes						
Amygdala	32	-6	-11	40	2.45	.01
Amygdala	-30	-8	-10	13	2.38	.05

Note: VLPC = ventrolateral prefrontal cortex; BA = Brodmann Area. Results are from region of interest (ROI) analyses of regressions focusing on functional regions of interest from a face-processing paradigm. df = 74 for negative affect and 73 for depressive symptoms. *p* values were corrected using false discovery rate.