Sex-specific developmental changes in amygdala responses to affective faces

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It is hypothesized that adolescent development involves a redistribution of cerebral functions from lower subcortical structures to higher regions of the prefrontal cortex to provide greater self-control over emotional behavior. We further hypothesized that this redistribution is likely to be moderated by sex-specific hormonal changes. To examine developmental sex differences in affective processing, 19 children and adolescents underwent fMRI while viewing photographs of faces expressing fear. Males and females differed in the pattern of their amygdala *vs* prefrontal activation during adolescent maturation. With age, females showed a progressive increase in prefrontal relative to amygdala activation in the left hemisphere, whereas males failed to show a significant age related difference. There appear to be sex differences in the functional maturation of affect-related prefrontal–amygdala circuits during adolescence. *NeuroReport* 12:427–433 © 2001 Lippincott Williams & Wilkins.

Key words: Adolescence; Affect; Amygdala; Development; Dorsolateral prefrontal cortex; Emotion; Face perception; Fear; fMRI; Neuroimaging; Sex differences

INTRODUCTION

Emotional experience is regulated by an integrated functional system that includes the neocortex and numerous subcortical limbic nuclei. Of these structures, the amygdala has consistently emerged as one of the most critical for ascribing emotional significance to stimuli and influencing affective responsiveness and emotional learning [1,2]. Neuroimaging studies of adults have shown that the amygdala often produces increased activation during the perception of fearful facial expressions [3,4]. Furthermore, a recent fMRI study by Hariri and colleagues [5] suggests that the prefrontal cortex, particularly on the right, may provide humans with the capacity to modulate emotional responses by attenuating activity within the amygdalae. In contrast to the growing literature on the neurobehavioral processing of affect in adults, there is relatively little information available regarding the development of emotional circuits during maturation from childhood through the adolescent years. The transitional period of puberty involves significant changes in physical and cognitive functioning, which are paralleled by equally striking transformations in affective processing. Normal adolescent development involves a shift from characteristically childlike emotional reactions toward greater self-regulation, social awareness, and the capacity for voluntary modification of emotional displays. During adolescent maturation, there is a progressive increase in myelinated axonal projections to the prefrontal lobes [6–8], consistent with evidence that the prefrontal lobes are generally among the latest cerebral structures to reach full development [9]. Recent neuroimaging studies have demonstrated that maturation during the adolescent period is mirrored by age-related increases in functional activation within the frontal lobes [10]. These findings suggest that adolescent maturation may involve a developmental transition within the brain whereby executive control is transferred from immature subcortical systems to frontal lobe cortical networks characteristic of the adult brain, particularly within the left prefrontal cortex.

Our understanding of the development of the adolescent brain is complicated by the fluctuations of reproductive hormones that may result in sexually dimorphic cerebral structure and function [11]. Structural neuroimaging studies have shown that by early adolescence, the brains of males and females show significant morphological differences. In particular, adolescent females have disproportionately larger volumes of the hippocampus, pallidum, and caudate but have significantly smaller amygdala volumes compared to males [6], while males show significantly greater growth of the left amygdala relative to females during adolescence [12]. By adulthood, females appear to have a significantly larger percentage of gray matter within the dorsolateral prefrontal cortex relative to males [13]. It is likely that such structural dimorphism is manifested in differences in behavior between the sexes. One of the most well documented sex differences in behavior is the consistent finding that males display more frequent and severe aggressive behavior than females, particularly during the adolescent and young adult years [14]. Thus, a comprehen-

sive model of frontal-subcortical development of affective processing must account for the sex differences observed in emotional behavior and brain structure.

In general, while maturation affords the individual greater control over emotional behavior, the neurobiological processes that underlie this regulatory capacity and their developmental sequence are not fully understood. One possibility is that with maturational development, the prefrontal cortex acquires a greater capacity to modulate the activity of the subcortical circuits involved in emotional processing. While this hypothesis appears to be supported in a recent study of adults [5], it has not been examined developmentally. To test this hypothesis, children and adolescents were presented with a facial affect perception task while undergoing fMRI. We hypothesized that chronological age would be associated with a progressive increase in frontal modulation of amygdala activity as evidenced by relatively increased activation within the dorsolateral prefrontal cortex (DLPFC) and decreased activation within the amygdala. Furthermore, given the known behavioral and morphometric differences within the amygdala of males and females, we expected that these effects would be moderated by sex.

MATERIALS AND METHODS

Subjects: Participants included 19 healthy children and adolescent volunteers (13 right- and six left-handed by self-report), ranging in age from 9 to 17 years (mean ($\pm$s.d.) 13.5 ± 2.1). The sample included nine males and 10 females, all of whom were provided monetary compensation for participation. The subjects had no known history of psychiatric illness or severe medical problems, and all had normal visual acuity. All subjects and their parents or guardian(s) provided written informed consent prior to participation in the study.

Imaging methods: Functional neuroimaging data were collected on a 1.5 T GE Signa MRI scanner (General Electric Systems, Milwaukee, WI) equipped with a whole-body echo-planar imaging system (Advanced NMR, Inc., Wilmington, MA) and a quadrature head coil. BOLD contrast images were acquired using an echo-planar gradient echo pulse sequence (TR = 3 s, TE = 40 ms. For functional imaging, 50 sequential images were collected in each of 12 axial slices of 6 mm thickness, with a 64×128 acquisition matrix, and an in-plane resolution of 3×3 mm. Head movement was restricted by comfortable placement of foam padding around the head.

Fearful face activation paradigm: Visual stimuli consisted of six fearful faces selected from the stimulus set of Ekman and Friesen [15]. Face stimuli were generated on a Macintosh computer and were projected onto a translucent screen placed at the subject's feet using a magnetically shielded LCD video projector. The screen was visible via a mirror mounted to the head coil. Each 150 s scanning sequence consisted of five alternating 30 s stimulus/rest periods. The experimental paradigm has been used previously and is described in greater detail elsewhere [16]. During baseline and rest periods, subjects were asked to visually fixate on a small white circle located in the center of the screen. Each stimulus period presented three face photographs. In order to assess participation, all subjects were asked to report the affect displayed following each scanning session.

Image processing and analysis: All images were corrected for in-plane and translational motion [17]. Matched T1 axial images were inspected to determine the single slice for each subject that included the largest area of both amygdala. Regions of interest (ROIs) for each amygdala were selected with reference to an anatomic atlas [18]. Each ROI was comprised of four pixels, each pixel 3×3 mm, sampled from one axial slice, and placements were made based on gyral boundaries and structural landmarks visible on MR images. The amygdala ROI's were placed in medial aspects of the amygdala on an axial slice that included the subcallosal area (Brodmann's area 25) and the inferior regions of the middle and superior temporal gyrus (see Fig. 1, left). Two ROI's were placed in the dorsolateral prefrontal cortex (Brodmann's areas 46 and 9), localized anterior to the cingulate cortex at the approximate level of the genu of the corpus collosum (see Fig. 1, right).

Measures of signal intensity were derived by averaging the MR signal measured in all pixels in each ROI for each time point during the task activation period. The MR signal

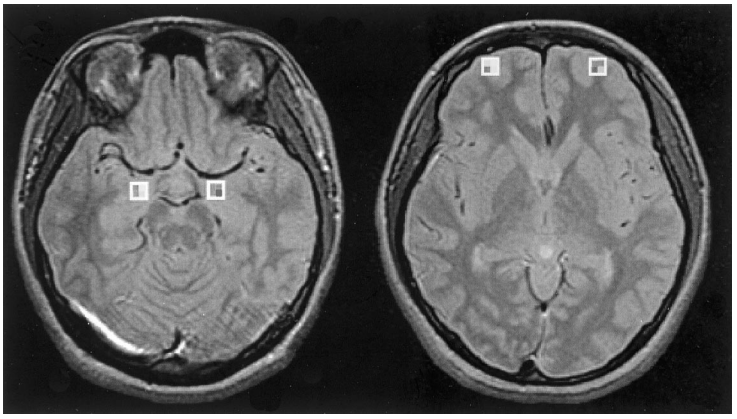

Fig. 1. Axial slices acquired in a 14-year-old female subject illustrating relative changes in signal intensity during the viewing of fearful facial affect. Left: activation of the left and right amygdala. Right: activation in left and right dorsolateral prefrontal cortex (DLPFC) regions of interest.

was then normalized to each subject's baseline average, derived from the mean of the first seven images, and converted into a metric representing the percent change in MR signal from baseline. Signal responses were averaged for the two activation periods for each ROI. To determine the relationship between developmental maturation and amygdala activity, the mean increase in MR signal during the viewing of the fearful faces was correlated with age for each ROI separately using a Pearson product-moment correlation.

RESULTS

Age and amygdala activation: Figure 2 presents the scatterplots showing the relationship between chronological age and the percentage change in MR signal for the left and right amygdala separately. As evident from Fig. 2a, chronological age and BOLD signal change were negatively associated, indicating a decrease in functional activation within the left amygdala as age increased from late childhood through adolescence ($r = -0.45$, $p = 0.05$). In contrast, it is clear from the scatterplot in Fig. 2b that there was no significant linear relationship between chronological age and MR signal change within the right amygdala ($r = -0.04$, $p = 0.89$).

Gender effects: The relationship between age and amygdala activation was further explored by conducting separate analyses by gender. For male participants, there was no significant association between chronological age and signal intensity within either the left ($r = -0.39$, $p = 0.30$) or right ($r = -0.04$, $p = 0.93$) amygdala. In contrast, female participants demonstrated a significant negative correlation

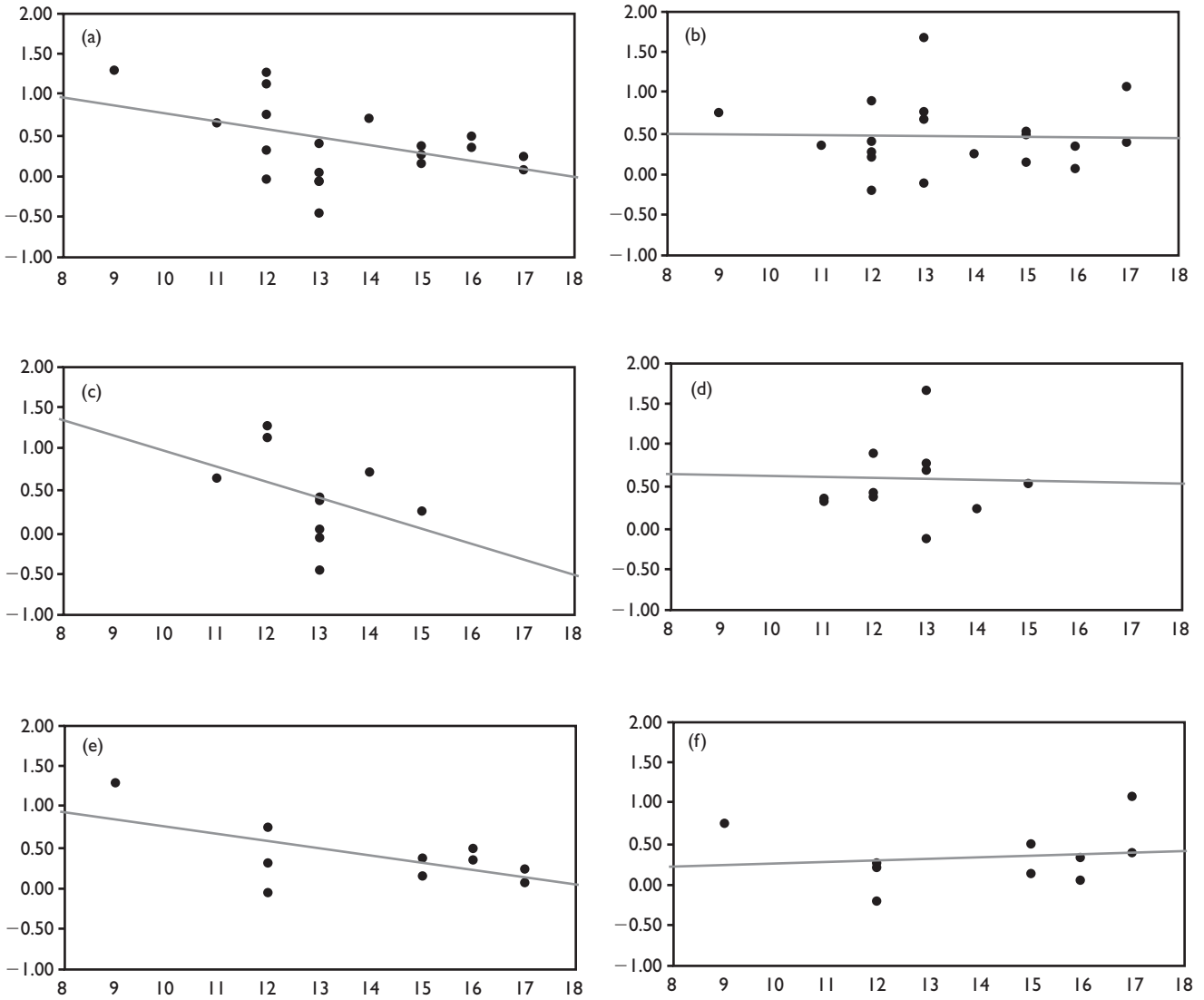

Fig. 2. Correlations between chronological age and normalized signal intensity for the amygdala. Scatter-plots for the total sample ($n = 19$) show a significant correlation between age and activation in the (**a**) left amygdala ($r = -0.45$, $p = 0.05$), but not for the (**b**) right amygdala ($r = -0.04$, ns). When examined separately by sex, males did not demonstrate a significant correlation between age and signal intensity in either the (**c**) left ($r = -0.39$, ns) or (**d**) right amygdala ($r = -0.04$, ns), but females showed a significant correlation for the (**e**) left ($r = -0.63$, $p = 0.05$), but not (**f**) right amygdala ($r = 0.12$, ns).

between chronological age and signal intensity within the left ($r = -0.63$, $p = 0.05$), but not the right ($r = 0.12$, $p = 0.75$) amygdala. When the magnitudes of the correlations were compared across gender using Fisher's r-to-z transformation, the two groups did not differ significantly for either the right or the left amygdala.

Age and DLPFC activation: The correlation plots between chronological age and DLPFC activation are presented in Fig. 3. When DLPFC activation was considered for each hemisphere individually, there was no significant relationship between chronological age and left ($r = 0.28$, $p = 0.25$) or right ($r = 0.17$, $p = 0.48$) prefrontal cortical activation.

Gender effects: When male subjects were analyzed independently, there emerged a significant negative correlation between age and signal intensity within the left DLPFC ($r = -0.67$, $p = 0.05$). Activation within the right prefrontal cortex was not significantly associated with age in the sample of males ($r = 0.49$, $p = 0.18$). In contrast, female subjects showed a non-significant trend toward greater left DLPFC with age ($r = 0.54$, $p = 0.11$), while no significant association was evident within the right DLPFC ($r = 0.13$, $p = 0.72$). Correlations between chronological age and DLPFC signal intensity were significantly different in magnitude between males and females on the left ($z = 2.54$, $p = 0.01$), but not on the right ($z = 0.73$, $p = 0.47$).

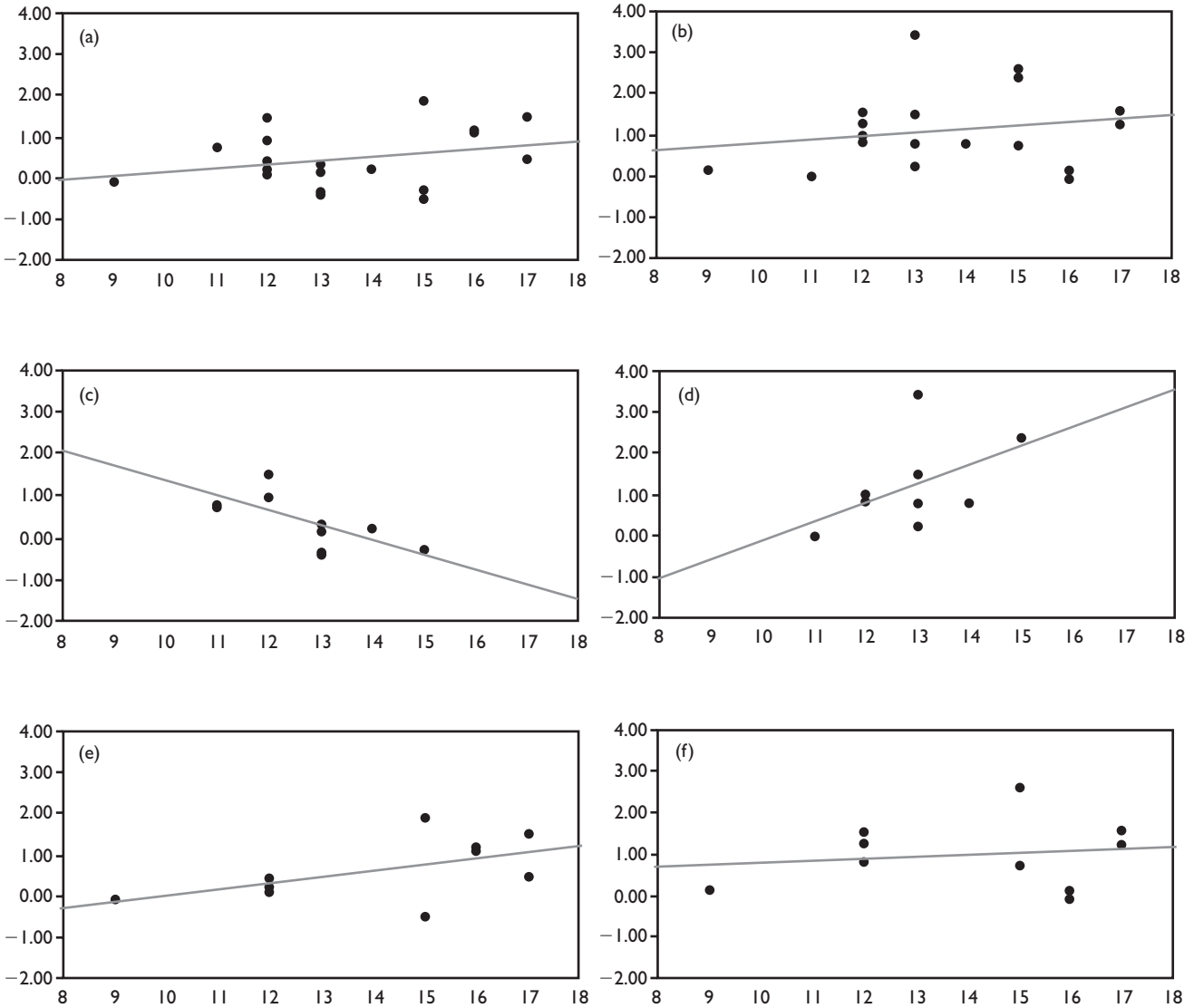

Fig. 3. Correlations between chronological age and normalized signal intensity for the DLPFC. Data for the combined sample ($n = 19$) did not reveal a significant correlation between age and activation in the (**a**) left DLPFC ($r = 0.28$, ns) or (**b**) right DLPFC ($r = 0.17$, ns). However, when examined separately by sex, males demonstrated a significant correlation between age and signal intensity in the (**c**) left ($r = -0.67$, $p = 0.05$), but not the (**d**) right DLPFC ($r = 0.49$, ns). Females, in contrast, showed a non-significant trend toward increased activation of the DLPFC with age for the (**e**) left ($r = 0.54$, $p = 0.11$), but not the (**f**) right ($r = 0.13$, ns).

Age and DLPFC–amygdala difference: To examine the relationship between frontal and amygdala activity during adolescent brain development, we subtracted the normalized signal intensity of the amygdala from the signal intensity of the ipsilateral DLPFC of each hemisphere to yield a difference score. As evident in Fig. 4a, the left DLPFC–left amygdala difference score for the total sample correlated significantly with chronological age ($r = 0.56$, $p = 0.01$), indicating a progressive age-related disparity between relatively greater activation within the left prefrontal region and decreased activation within the left amygdala over the adolescent years. In contrast, Fig. 4b shows that the right DLPFC–right amygdala difference score was not significantly related to chronological age ($r = 0.16$, $p = 0.50$).

Gender effects: We evaluated the relationship between age and the DLPFC–amygdala difference scores separately by gender. In the sample of males, the difference between DLPFC and amygdala did not correlate significantly with age for either the left ($r = s-0.43$, $p = 0.25$) or the right ($r = 0.40$, $p = 0.29$) hemisphere. Similar analyses for the females, in contrast, yielded a significant association between chronological age and the DLPFC-amygdala difference score for the left ($r = 0.73$, $p = 0.02$) but not the right ($r = 0.08$, $p = 0.83$) hemisphere. Again, comparison of the magnitude of correlations obtained for males and females revealed a significant difference between genders on the left ($z = 2.50$, $p = 0.01$) but not on the right ($z = 0.62$, $p = 0.54$).

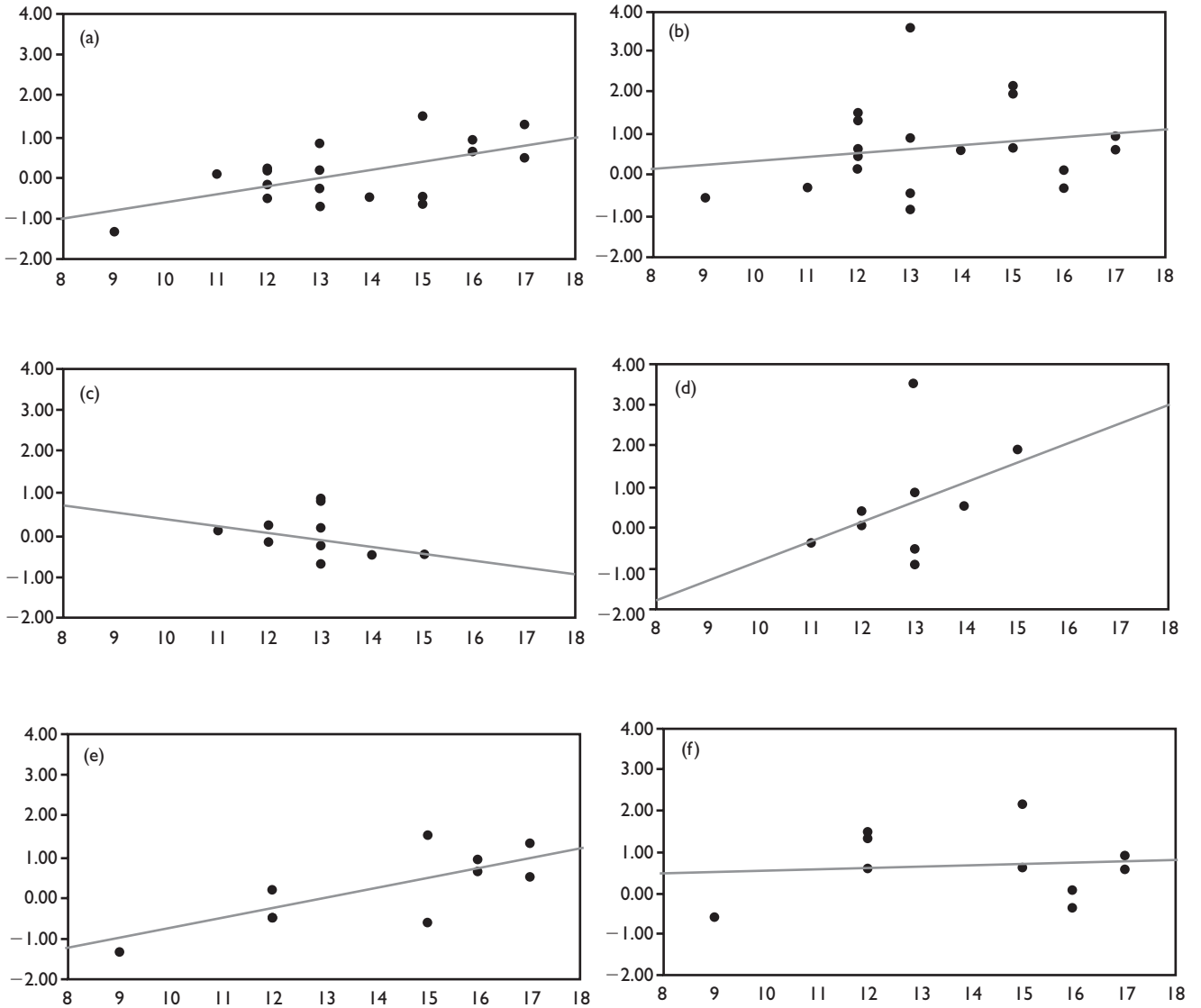

Fig. 4. Correlations between chronological age and the difference score between the ipsilateral DLPFC-amygdala signal intensity. Overall, for the combined sample ($n = 19$), there was a significant correlation between the DLPFC–amygdala difference on the (**a**) left ($r = 0.56$, $p = 0.01$), but not for the (**b**) right ($r = 0.16$, ns). For males considered as a group, there was no significant correlation between age and DLPFC–amygdala difference scores for the (**c**) left ($r = -0.43$, ns) or the (**d**) right ($r = 0.40$, ns). Females, in contrast, showed a significant correlation between the DLPFC-amygdala difference score on the (**e**) left ($r = 0.73$, $p = 0.02$), but not the (**f**) right ($r = 0.08$, ns).

DISCUSSION

The present results suggest that there are functional changes within the amygdala and DLPFC during the perception of affective facial stimuli that correlate with maturational development during adolescence. In our child and adolescent sample as a whole, greater chronological age was associated with decreased functional activation of the left amygdala during the viewing of photographs of faces expressing fearful affect. Further analyses revealed that this relationship reached statistical significance only for the females. In contrast, activation within the right amygdala was not linearly related to chronological age for either sex. These findings complement other studies of adolescents [16] and adults [3,4] that find activation within the amygdala in response to fearful faces. Our results further suggest that maturational development is associated with a decline in left amygdala responsiveness to fearful affective expressions. These findings are consistent with our initial hypothesis that age-related maturation would be associated with progressively greater modulation of amygdala activation by the prefrontal cortex.

While we expected that the modulation of amygdala activation would result in greater activation within the prefrontal region for the sample as a whole [5], we were also interested in examining the potential moderating effects of gender on the development of these affect-related circuits. We found that males and females demonstrated significantly different trajectories of left DLPFC responsiveness over the adolescent period. Although females demonstrated a non-significant trend toward greater left DLPFC activation with increasing age, the trajectory for the males was reversed, with reduced left DLPFC signal intensity associated with greater age. The difference in the observed trajectories between the males and females was significant and suggests that adolescent maturation may involve sexually dimorphic development of prefrontal cortex-amygdala circuits involved in affective processing. The sexually dissociated trajectories in functional activity that we observed are likely to be related to the responsiveness of these structures to sex-specific hormones during adolescent development [6,12].

As we have recently reported [19], the identification of facial affect requires the ability to extract visuospatial and figural information, as well as the ability to concentrate, attend, and recall affective categories presented. The challenge paradigms in the current study are therefore dependent on both emotional and cognitive processing, making it impossible to isolate a single component function that may be responsible for the activation differences observed. However, studies describing the neurobiologic correlates of emotional processing have highlighted attentional components including orienting, response choice and sustained attention suggesting that the differences in affective processing seen in the current study may in part be due to differences in attentional capacity or strategy between males and females. This interpretation is supported by recent studies that have reported sex differences in visual attention, vigilance and boredom [20,21].

It has been hypothesized that maturation into adulthood involves a progressive frontalization of cognitive and emotional regulation [10]. This perspective suggests that as the adolescent child develops, the prefrontal lobes gain progressively greater inhibitory control over emotional responses involving the amygdala and other limbic structures [5,19,22]. Our data suggest, however, that developmental redistribution of cerebral functions may occur differently for males and females. With age, the relative activation of the amygdala within the female sample became progressively lower than that of the DLPFC. This relationship was reversed in the males, indicating that greater age was associated with a trend toward less prefrontal relative to amygdala activation. The present findings suggest that during adolescence, males and females demonstrate divergent neurobiological strategies in the processing of fearful facial affect.

The left-lateralized nature of the maturational change is also noteworthy, as it raises the possibility of differential affective functioning of the amygdalae. Our findings are also in accord with electroencephalographic studies of frontal asymmetry patterns that find left frontal hypoactivation to be associated with negative affect or withdrawal related emotion [23] and relative increased left frontal activation to be associated with positive or approach related emotions and a reduced risk of psychopathology [24]. Other functional neuroimaging studies have found greater left amygdala responsiveness during facial perception and encoding tasks [25], particularly those involving affective processes [26,27]. Studies using PET have shown increased left amygdala metabolism in family history positive depressive patients when tested during a euthymic state [28]. There is also some evidence that affective disorders may involve a disinhibition of the left amygdala by dysfunctional modulatory systems [19,28]. Thus, negative affective processing is often associated with increased left amygdala activation, and reduced left prefrontal activation.

Given that our results are preliminary and were obtained with a relatively small sample, conclusions based on these findings must be viewed as tentative until replicated with larger groups of subjects. Future studies would benefit from the inclusion a comparison group of adults so that the trajectory of amygdala response may be examined beyond the adolescent years. Secondly, functional imaging studies have consistently shown that the amygdala rapidly habituates to affective stimuli, resulting in reduced BOLD signal in studies that employ a blocked stimulus presentation paradigm [3,29]. As our study included a blocked presentation, we may have minimized our ability to detect amygdala activation, and future studies may benefit from the use of event related designs. Another potential limitation was that the ROIs used in the present study were limited to four pixels selected from a single coronal slice for each region. It is therefore possible that some regions that are critical for emotional regulation and processing were not adequately sampled. We believe, however, that the sampling of individual ROIs within each individual is the most anatomically correct approach given the age related differences in brain sizes across our sample. Finally, our challenge task was designed specifically to activate the amygdala and not the DLPFC. Future studies should use multiple tasks that separately activate amygdala and DLPFC in order to provide converging evidence of developmental changes in the activation of each region.

CONCLUSION

The present data suggest that the left amygdala responds to affective photographs of fearful facial stimuli in children and adolescents, but further suggests that the amount of activation decreases across the adolescent maturational period. Moreover, the decrease in amygdala activity was moderated by sex, with only females showing a significant decline over the adolescent period. In addition, over the adolescent period, there is a sex-dependent change in the degree of DLPFC activity, with females showing a progressive increase, and males a progressive decrease in left prefrontal signal intensity. Overall, females show a trend toward greater responsiveness of the prefrontal lobes relative to the amygdala with maturation, while males demonstrate the reverse pattern with age. These findings support a developmental model whereby cerebral maturation is associated with progressively greater control over emotional behavior via prefrontal cortical systems that modulate lower limbic responses, but further suggest that the rate of development of this affective system and the ultimate expression of emotional behavior may be significantly influenced by sex-specific developmental factors.

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