# The association between early life stress and prefrontal cortex activation during implicit emotion regulation is moderated by sex in early adolescence

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## Abstract

Early life stress (ELS) is a significant risk factor for the emergence of internalizing problems in adolescence. Beginning in adolescence, females are twice as likely as males to experience internalizing disorders. The present study was designed to examine sex differences in the association between ELS and internalizing problems in early pubertal adolescents, and whether and how corticolimbic function and connectivity may underlie these associations. Fifty-nine early pubertal males and 78 early pubertal females, ages 9–13 years (all Tanner Stage 3 or below) underwent functional magnetic resonance imaging as they performed an emotion label task that robustly interrogates corticolimbic function. Participants were also interviewed about their experience of ELS. Females exhibited a positive association between ELS and internalizing problems, whereas males exhibited no such association. Whole-brain and amygdala region of interest analyses indicated that whereas females exhibited a positive association between ELS and the ventrolateral prefrontal cortex during implicit emotion regulation, males showed no such association. Activation in these regions was positively associated with internalizing problems in females but not males; however, activation in these regions did not mediate the association between ELS and internalizing problems. Finally, both boys and girls exhibited an association between ELS and increased negative connectivity between the right ventrolateral prefrontal cortex and bilateral amygdala. Using a carefully characterized sample of early pubertal adolescents, the current study highlights important sex differences in the development of corticolimbic circuitry during a critical period of brain development. These sex differences may play a significant role in subsequent risk for internalizing problems.

The experience of early life stress (ELS) is associated with mental health difficulties such as depression and anxiety (Andersen & Teicher, 2008; Heim & Nemeroff, 2001; Lupien, McEwen, Gunnar, & Heim, 2009; Teicher & Samson, 2016). Exposure to ELS accounts for nearly one-third of all mood and anxiety disorders in the United States, underscoring the critical role of ELS as a risk factor for the onset of internalizing psychopathology (Green et al., 2010; McLaughlin et al., 2012). ELS has been theorized to confer risk for the emergence of psychopathology through several stress-related neurobiological pathways. Specifically, ELS may affect functioning of the hypothalamic–pituitary–adrenal axis and con- sequently influence the development of neural structures with high densities of corticotropin-releasing factor neurons, including portions of the prefrontal cortex (PFC) and amygdala (Heim et al., 2002; Lupien et al., 2009; Malter Cohen, Tottenham, & Casey, 2013; Nemeroff, 2004). ELS-induced alterations in the development of neural regions involved in this corticolimbic stress regulatory system may contribute to risk for psychopathology (for a review, see Heim & Binder, 2012; Lupien et al., 2009).

For instance, researchers have found that adults with a history of ELS exhibit both heightened amygdala response and positive functional connectivity, or temporal correlation, between right ventrolateral PFC (right vlPFC) and amygdala while labeling threatening (i.e., fearful and angry) faces, compared to adults without histories of adversity (Taylor, Eisenberger, Saxbe, Lehman, & Lieberman, 2006). Negative functional connectivity between PFC and amygdala during the processing of threat-related stimuli is typically found in adults and is posited to reflect adaptive emotion regulation by the PFC (Gee, Humphreys, et al., 2013). In contrast, the positive functional connectivity of PFC and amygdala in adults with ELS suggests that stress leads to atypical emotion regulation, presumably through ineffective PFC regulation of amygdala responses (Taylor et al., 2006). It is important to note, however, that most of the studies that have found altered

corticolimbic function following exposure to ELS have been conducted with adult samples and have used retrospective reports of ELS obtained many years after exposure to stress (Burghy et al., 2012; Dannlowski et al., 2012; Fan et al., 2014; Heim & Binder, 2012; Herringa et al., 2013, 2016; Taylor et al., 2006; van Harmelen et al., 2013, 2014). Thus, it is difficult to assess whether these neurobiological changes are a direct consequence of having experienced adversity early in development or, alternatively, are a signature of adult-onset psychopathologies.

Given the significant consequences of exposure to ELS, combined with the growing recognition that many of these effects are not observed until adolescence (Gee & Casey, 2015; Kessler et al., 2005; Lee et al., 2014), researchers have begun to focus on elucidating the psychological and neurobiological consequences of ELS during this developmental period (see Tottenham & Galván, 2016, for a review). Investigators have posited that exposure to ELS alters the development of neural structures and connections that underlie emotion processing and regulation, including the PFC and amygdala, that, in turn, increases adolescents' risk for developing internalizing disorders (Burghy et al., 2012; Eiland & Romeo, 2013; Maughan & Cicchetti, 2002; Pechtel & Pizzagalli, 2011; Tottenham et al., 2010). This is particularly salient in adolescence when these neural systems are undergoing dramatic reorganization (Casey, Jones, & Hare, 2008). Supporting this formulation, researchers have found that children and adolescents who have experienced high levels of ELS exhibit heightened activation to emotionally evocative faces and images in brain regions that are involved in processing salient stimuli (i.e., amygdala, anterior insula, dorsal anterior cingulate cortex; Garrett et al., 2012; Gee, Gabard-Durnam, et al., 2013; Marusak, Martin, Etkin, & Thomason, 2014; McCrory et al., 2013; McLaughlin, Peverill, Gold, Alves, & Sheridan, 2015; Suzuki et al., 2014; Tottenham, Hare, Millner, et al., 2011). Fewer investigators have examined how ELS may affect PFC regulation of the processing of emotional stimuli.

The PFC may be particularly sensitive to exposure to ELS given its high density of glucocorticoid receptors and its protracted development into adulthood (Giedd, 2004; McEwen & Morrison, 2013; Pechtel & Pizzagalli, 2011; Sanchez, Young, Plotsky, & Insel, 2000; Teicher et al., 2003). For example, McLaughlin et al. (2015) used a cognitive reappraisal task to examine functional magnetic resonance imaging (fMRI) responses of 13- to 19-year-old participants as they attempted to explicitly regulate their affective responses to emotionally salient images. Although previously maltreated adolescents showed the typical increased response in regions involved in processing salient stimuli to viewing negative images, they also showed increased activation in the superior frontal gyrus and frontal pole when downregulating their negative affect to a negative image, relative to nonmaltreated adolescents. Similarly, Marusak et al. (2014) found that adolescents exposed to childhood trauma exhibited elevated dorsolateral PFC (dlPFC) activation when performing an emotional conflict task, suggesting early adverse experiences

may have important consequences on the development of the PFC in adolescence.

In addition to the effects of ELS on activation to salient stimuli in the amygdala and the PFC, researchers have documented that ELS affects the functional connectivity between these two structures (Burghy et al., 2012; Gee, Gabard-Durnam, et al., 2013; Marusak et al., 2014; Wolf & Herringa, 2016). Using task-based connectivity analyses, researchers have found that adolescents exposed to ELS show stronger negative amygdala-PFC connectivity, a more mature, adult-like pattern of connectivity, while viewing negative stimuli (Gee, Gabard-Durnam, et al., 2013; Wolf & Herringa, 2016). These findings may reflect adaptive functioning in the face of adversity: children exposed to ELS may develop more adultlike patterns of connectivity at an earlier age in order to deal more effectively with environmental adversity. Gee, Gabard-Durnam, et al. (2013) showed that among previously institutionalized children and adolescents, having a negative pattern of amygdala–medial PFC connectivity was associated with lower separation anxiety, indicating that this adultlike pattern may be protective following experiences of early adversity. However, this pattern may also represent a premature end to a sensitive period for the development of this circuit. The long-term consequences of this earlier maturation are unclear, as are the developmental trajectories of the effects of ELS on this circuitry from childhood to adulthood.

Although researchers have now demonstrated that ELS affects the development of corticolimbic circuitry in childhood and adolescence, they have not examined possible sex differences in this association. ELS has, among females, been found to be a particularly important risk factor in the development of major depressive disorder (Ge, Conger, & Elder, 2001; Ge, Lorenz, Conger, Elder, & Simons, 1994; Rudolph & Flynn, 2007; Weiss, Longhurt, & Mazure, 1999). Females also differ from males in their perceptions of stressful life events (Raffaelli et al., 2016), in their biological response to both acute and chronic stressors, and in their neural responses to negative stimuli (Bourke, Harrell, & Neigh, 2012; Kajantie & Phillips, 2006; for reviews, see Bangasser & Valentino, 2014; Novais, Monteiro, Roque, Correia-Neves, & Sousa, 2016; Ordaz & Luna, 2012; Stevens & Hamann, 2012). Given striking sex differences in the incidence of internalizing disorders, including evidence that females are twice as likely as males to develop major depressive disorder in adolescence and adulthood (Hankin & Abramson, 1999), there may be sex-specific mechanisms, including sex-specific effects on corticolimbic circuitry, through which ELS contributes to vulnerability for internalizing problems in adolescence (Teicher et al., 2003).

In this context, the age at which sex differences in internalizing disorders become most pronounced corresponds to the complex developmental period of puberty; moreover, there is evidence that pubertal status is a stronger predictor of the onset of depression than is chronological age (Angold, Costello, & Worthman, 1998; Hayward, Gotlib, Schraedley, &

Litt, 1999; Oldehinkel, Verhulst, & Ormel, 2011). Thus, puberty is a critically important period to study in order to understand neurobiological differences between males and females that may underlie sex differences in internalizing problems. It is important to note, however, that males and females differ significantly in pubertal timing; females typically experience the onset of puberty 1.5 years earlier than do males (Negriff & Susman, 2011). These sex differences in pubertal timing mean that age-matched samples of adolescent males and females are almost certain to be confounded by sex differences in pubertal stage. Similarly, given the difficulties inherent in recruiting and studying high-risk samples such as adolescents who have been exposed to maltreatment or who have experienced institutional care, many studies are simply not sufficiently powered to detect sex differences in the neurobiological effects of ELS during the narrow developmental window of early puberty during which sex differences in internalizing problems begin to emerge. Significant puberty-related changes occur in corticolimbic circuitry in adolescence (Peters, Jolles, Duijvenvoorde, Crone, & Peper, 2015; Spielberg, Forbes, et al., 2014; Spielberg, Olino, Forbes, & Dahl, 2014). Therefore, it is critical that we examine sex differences in corticolimbic development in carefully characterized samples of adolescent males and females, matched for pubertal development.

The present study was designed to address these issues by examining how ELS differentially affects male and female corticolimbic activation and connectivity during early puberty, a period in which sex differences in rates of internalizing problems begin to emerge. We also examined how corticolimbic circuitry is associated with internalizing disorders in adolescence. Given significant differences between males and females in pubertal timing (Negriff & Susman, 2011) and the documented impact of pubertal stage on corticolimbic circuitry (Peters et al., 2015; Spielberg, Forbes, et al., 2014; Spielberg, Olino, et al., 2014), in this study we used a puberty-matched sample of early adolescent boys and girls. We examined fMRI activation as boys and girls labeled emotion faces compared to matching emotion faces. Previous findings using this task suggest that labeling versus matching emotional face stimuli recruits implicit emotion regulation processes and elicits strong activation in the PFC, particularly in the vlPFC (Gee et al., 2012; Lieberman et al., 2007; Taylor et al., 2006). This design allows us to examine emerging differences in the effects of ELS on corticolimbic circuitry during a period of development in which sex differences in internalizing problems are beginning to emerge. Based on evidence of sex differences in rates of internalizing symptoms in this age group, we hypothesized that early pubertal females will show a stronger association between exposure to ELS and internalizing problems than will their male counterparts. We predicted further that the association between ELS and internalizing problems in females would be mediated by increased amygdala activation and PFC activation during implicit emotion regulation. Finally, we expected to find greater negative connectivity between the amygdala and PFC in individuals who were exposed to more severe ELS and, further, that this negative connectivity would mediate the association between ELS and internalizing problems. We also explored sex differences in patterns of PFC-amygdala connectivity, but made no directional hypotheses.

## Method

### Participants and procedure

A total of 137 participants met criteria for inclusion in this study and analyses: 59 early pubertal males and 78 early pubertal females, ages 9–13 years ($M = 11.42$, $SD = 1.08$). An additional 39 participants were excluded from the analyses due to excessive motion ($n = 35$) or incomplete scans ($n = 4$). The participants were part of a larger study examining the effects of ELS on brain structure and function across early adolescence. After completing a brief phone interview to determine eligibility, participants were invited to the laboratory to complete parental consent and child assent forms. In this session, parents and children also completed interviews and questionnaires assessing their experience of ELS and measures of their cognitive and emotional functioning. Males and females were matched on pubertal development based on self-report Tanner stage and exposure to ELS (see Table 1 for descriptive statistics by child sex). Participants were recruited from the San Francisco Bay area through a combination of print and online advertisements. Exclusion criteria included: (a) self-reported Tanner pubertal stage >3 and the experience of menarche in female participants, (b) nonfluent English speakers, (c) contraindications to scan (e.g., metal implants, braces), (d) history of major neurological disorder or illness, and (e) intellectual delay or learning difficulties. Upon confirming eligibility, participants were invited to return within 1 month to complete the fMRI portion of the study. This study was approved by the Stanford University Institutional Review Board and all participants were compensated for their participation in the study.

### Measures

*ELS.* We assessed levels of ELS severity and the impact of early life stressors using a modified version of Traumatic Events Screening Inventory for Children (Ribbe, 1996). In this interview, we assessed 30+ types of stressful life experiences. For each type of ELS endorsed, interviewers followed up with general and specific probes in order to gather detailed information about the severity of the experience and the child's perceived severity of the stressor (e.g., relationship of persons involved, duration of experience, and consequences of experience). A panel of three coders, blind to the child's subjective severity ratings and reactions and behaviors during the interview, rated the objective severity of each type of stressor based on a modified version of the UCLA Life Stress Interview coding system (Rudolph & Hammen, 1999; Rudolph et al., 2000). Coders made objec-

**Table 1.** *Descriptive statistics for males and females*

| | Males | Females | $t$ or $\chi^2$ | df | p |
|---|---|---|---|---|---|
| Age, M (SD) | 12.018 (0.925) | 11.146 (1.033) | 5.115 | 135 | <.001 |
| Tanner development, M (SD) | | | | | |
|    Hair | 1.881 (0.672) | 1.872 (0.795) | 0.074 | 135 | .941 |
|    Breast | 2.000 (0.615) | 2.083 (0.787) | −0.672 | 135 | .503 |
| Medication use | 17% | 13% | 0.532 | 1 | .627 |
| Handedness (right dominant) | 88% | 81% | 0.865 | 1 | .510 |
| Race/ethnicity | | | 3.859 | 1 | .570 |
|    White/Caucasian | 44% | 40% | | | |
|    African American | 9% | 9% | | | |
|    Hispanic | 13% | 9% | | | |
|    Asian | 12% | 11% | | | |
|    Native American | 2% | 0% | | | |
|    Pacific Islander | 0% | 0% | | | |
|    Other | 18% | 31% | | | |
|    No response | 2% | 0% | | | |
| Family income | | | 9.787 | 1 | .550 |
|    <$5000 | 0% | 1% | | | |
|    $5001–$10,000 | 0% | 3% | | | |
|    $10,001–$15,000 | 0% | 1% | | | |
|    $15,001–$25,000 | 3% | 4% | | | |
|    $25,001–$35,000 | 3% | 1% | | | |
|    $35,001–$50,000 | 5% | 3% | | | |
|    $50,001–$75,000 | 9% | 8% | | | |
|    $75,001–$100,000 | 12% | 11% | | | |
|    $100,001–$150,000 | 24% | 26% | | | |
|    $150.001+ | 34% | 33% | | | |
|    No response | 10% | 9% | | | |
| ELS severity, M (SD) | 6.864 (5.265) | 5.987 (4.768) | 1.019 | 135 | .310 |
| YSR internalizing problems, M (SD) | 11.220 (8.413) | 13.150 (10.276) | −1.168 | 134 | .245 |

*Note:* ELS, early life stress; YSR, Youth Self-Report.

tive severity ratings on a 5-point scale (0 = *nonevent or no impact*, e.g., witnessed debris from car crash; 4 = *extremely severe impact*, e.g., experienced sexual abuse), interclass correlation = 0.99. For each child, objective ratings for each type of ELS endorsed were summed to create an index of the cumulative severity of ELS.

*Pubertal status.* Pubertal stage was determined using self-reported Tanner staging (Marshall & Tanner, 1969, 1970). Using schematic drawings of two secondary sex characteristics (pubic hair and breast/testes development), each participant reported on his/her developmental stage on a scale of 1 to 5. A Tanner staging of 1 signifies that no pubertal development has begun, and a staging of 5 signifies that adult levels of pubertal maturation have been achieved. To be included in this study, all participants rated themselves at Stage 3 or below on measures of both pubic hair and breast/testes development. Tanner staging scores have been found to correlate with physicians' physical examinations of pubertal development (Coleman & Coleman, 2002; Slora et al., 2009; see Shirtcliff, Dahl, & Pollak, 2009).

*Internalizing problems.* Participants completed the Youth Self-Report (YSR; Achenbach, 1991; Achenbach & Res-

corla, 2001) measure, which assesses a broad array of behavioral problems in children and adolescents. For this study we examined total scores on the internalizing problems subscale of the YSR. One male participant did not complete this questionnaire.

### MRI

*Implicit emotion regulation fMRI task.* We used a modified emotion label task to examine the neural correlates of implicit emotion regulation (see Hariri, Bookheimer, & Mazziotta, 2000; Lieberman et al., 2007). This task was designed based on the formulation that linguistic processing of an emotional expression (e.g., labeling an emotional face) requires greater downregulation of regions responsible for processing salient information than does perceptual processing of the same emotional expression (e.g., matching emotional faces), potentially serving as a marker of the neural correlates of implicit emotion regulation (Lieberman et al., 2007). This task requires participants to identify, or label, a target emotional expression (emotion label) or to match a target emotional expression to two other emotional expressions (emotion match). We also included a sensorimotor control condition in which participants were required to match a target shape

to two other shapes (shape match). For the label conditions, participants were instructed to make a button press to indicate whether the appropriate label for the target figure was located on the bottom left or the bottom right of the screen. For all match conditions, participants were instructed to make a button press to indicate whether the target figure at the top of the screen was more similar to the figure below it on the left or on the right. Each face/label set was presented on the screen for 5000 ms, and each block of stimuli consisted of 10 trials. All task blocks were interspersed with rest blocks (lasting 15000 ms) in which participants were instructed to focus on a fixation cross in the middle of the screen. Each run consisted of one block of each task condition (positive label, positive match, negative label, negative match, ans shape match) as well as interspersed rest blocks. Presentation of conditions was randomized across participants, and all participants completed two complete runs of the task.

The task was presented using E-Prime software Version 2.0. A total of 50 trials were divided across two emotion conditions (positive and negative) and two response conditions (match and label) in addition to the control shape match condition. The positive and negative emotional expressions were facial displays from the NimStim picture set (Tottenham et al., 2009). Ten actors (5 females, 5 males) were displayed showing positive or negative emotional expressions. Positive emotional expression blocks included both high and low arousal positive displays including happy, excited, surprised, and calm facial expressions. Negative emotional expression blocks included both high and low arousal negative displays including sad, angry, and fearful facial expressions. To optimize power in testing our hypotheses, our analyses focused on the contrast of all emotion label relative to all emotion match conditions.

*MRI acquisition.* Participants who met eligibility criteria completed an fMRI scan. All scans were conducted on a 3 Tesla GE whole-body scanner (GE Healthcare Systems, Milwaukee, Wisconsin). Foam padding was used to minimize head movement. Two T2*-sensitive gradient echo-planar pulse sequences were used for functional imaging (repetition time = 2000 ms, echo time = 30 ms, flip angle = $77°$, matrix size = $70 \times 70$, 43 axial slices, field of view = 22.4 cm, 3 mm thick), each run lasting 5 min and 54 s. An automated high-order shimming procedure was used to reduce $B_0$ inhomogeneity. Additional high-resolution structural images were acquired with an axial 3D FSPGR sequence with T1 contrast (repetition time = 6.0 ms, echo time = 2 ms, flip angle = $12°$, matrix size = $256 \times 256$, 186 axial slices, field of view = 23 cm, 0.9 mm) for spatial registration.

*fMRI data analysis.* Analyses were conducted in FSL Version 6.0.0 (FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl), using FEAT, the fMRI expert analysis tool. The first four volumes of each participant's functional scan were discarded to allow for stabilization of longitudinal magnetization. The remaining images were preprocessed using stan-

dardized procedures, including motion correction to the mean image using motion correction FMRIB's linear image registration tool MCFLIRT (Jenkinson, Bannister, Brady, & Smith, 2002), slice-timing correction using Fourier space–time series phase shifting, spatial smoothing using a Gaussian kernel of full width half maximum 5 mm, grand-mean intensity normalization of the entire four-dimensional data set by a single multiplicative factor, and high-pass temporal filtering (Gaussian-weighted least squares straight line fitting, with $\sigma = 50.0$ s). Functional data were linearly registered to a common stereotaxic space by first registering the in-plane T2 image to the T1-weighted structural image (6 degrees of freedom), transforming the T1-weighted structural image to the MNI152 T1 brain, and then applying that deformation matrix and resampling to 2-mm resolution onto the functional images using FMRIB's linear image registration tool FLIRT (12 *df*; Jenkinson et al., 2002; Jenkinson & Smith, 2001). All fMRI analyses were therefore performed in MNI space at 2 mm$^3$ resolution.

Time series statistical analysis was conducted using full information at maximum likelihood with local autocorrelation correction (Woolrich, Ripley, Brady, & Smith, 2001). The voxelwise general linear model included regressors for each block condition (positive/match, positive/label, negative/match, negative/label, and shape match) as well as their temporal derivatives. Twelve motion correction parameters, as well as an indicator function to identify volumes as having excessive motion according to framewise displacement of 0.9 mm, were included as covariates of noninterest. Participants with absolute motion >3 mm or >20% of volumes with framewise displacement >0.9 mm were excluded from the analyses ($n = 35$). Both within-subject runs were combined in a fixed-effects model for each participant, which averaged the contrast estimates over runs within participant by setting the random effects variance to zero in FMRIB's local analysis of mixed effects (FLAME; Beckmann, Jenkinson, & Smith, 2003; Woolrich, 2008; Woolrich, Behrens, Beckmann, Jenkinson, & Smith, 2004). All participants were then combined in a higher level mixed-effects model to investigate within- and between-group differences. Prior to thresholding, we used a binarized gray matter mask (obtained from https://canlabweb.colorado.edu/wiki/doku.php/help/core/brain_masks). Higher level group analyses were conducted using FLAME Stage 1 (Beckmann et al., 2003; Woolrich, 2008; Woolrich et al., 2004). Given significant age differences between males and females, we included age as a covariate in all analyses.

*Whole-brain analyses: Effects of sex and ELS.* We examined the effects of sex and ELS severity on blood oxygen level dependent response during contrasts of interest in whole-brain analyses. Statistical images were thresholded using clusters determined by $Z > 2.3$ and a corrected cluster significance threshold of $\alpha = 0.05$ (Worsley, 2001).

*Region of interest (ROI) analysis of amygdala.* We also examined amygdala activation during implicit emotion regulation

using a ROI analysis. A bilateral amygdala ROI was created using the Harvard–Oxford subcortical Atlas in FSL (25% threshold).

*Psychophysiological interaction (PPI) analyses to assess PFC-amygdala functional connectivity.* We used PPI analyses to examine functional connectivity during implicit emotion regulation between the bilateral amygdala ROI and the two PFC regions that showed a significant interaction of ELS and sex (Friston et al., 1997; O'Reilly, Woolrich, Behrens, Smith, & Johansen-Berg, 2012). This analysis identifies regions in the brain that are correlated most strongly with the significant prefrontal ROIs during emotion label relative to emotion match conditions. For each PFC ROI, the general linear model analysis was conducted in FSL and included regressors for each task condition, the ROI time series, and the interaction of the task contrast (emotion label vs. emotion match) and the ROI time series. Again, both within-subject runs were combined in a fixed-effects model for each participant, which averaged the contrast estimates over runs within participant by focusing the random effects variance to zero in FLAME (Beckmann et al., 2003; Woolrich, 2008; Woolrich et al., 2004). All participants were then combined in a higher level mixed effects model to investigate connectivity patterns across both groups. Higher level group analyses were conducted using FLAME Stage 1 (Beckmann et al., 2003; Woolrich, 2008; Woolrich et al., 2004). Statistic images were thresholded using clusters determined by $Z > 2.3$ and a corrected cluster significance threshold of $\alpha = 0.05$ (Worsley, 2001). We then extracted parameter estimates from the bilateral amygdala ROI (as defined above) and used linear regression in SPSS (Version 23) to examine the effect of sex, ELS, and the Sex × ELS interaction on connectivity patterns between bilateral amygdala and the two PFC regions that showed a significant interaction effect with sex and ELS severity, after covarying for chronological age. We used simple slope analyses to probe significant interactions (Aiken & West, 1991).

### Demographics and behavioral analyses

All statistical analyses reported below were conducted with SPSS (Version 23) using two-tailed tests ($\alpha = 0.05$). Independent samples $t$ tests or $\chi^2$ tests were used to compare males and females on demographic variables, exposure to ELS severity, and scores on the YSR internalizing problems subscale. We conducted analyses of covariance to compare task performance between males and females, after covarying for chronological age. Finally, we conducted correlational analyses to test whether ELS severity was associated with task performance.

### Mediation analyses

Based on our findings (described below), we tested whether blood oxygen level dependent responses to emotion label relative to match in regions that showed a significant interaction between ELS severity and sex mediated the association between ELS severity and YSR internalizing scores in females. To do this, we used a single-step nonparametric resampling procedure (1,000 samples with replacement) for testing indirect effects (Hayes, 2013). Mediation is supported when the indirect effect is statistically significant. To assess the indirect effect, we calculated 95% confidence intervals (CIs) for coefficients; if the CI does not include zero, the indirect effect is considered to be statistically significant.

## Results

### Demographic and clinical characteristics

Participant demographic and clinical characteristics are presented in Table 1. As shown in this table, boys and girls did not differ significantly in Tanner stage, medication use, handedness, race/ethnicity, or family income. As expected, however, given that we matched boys and girls on pubertal status, the two sexes did differ in chronological age, $t$ (135) = 5.115, $p < .001$.

### Sex differences in the effects of ELS on internalizing problems

There were no significant sex differences in ELS severity or in internalizing problems in our sample (see Table 1). In examining the contributions of sex and ELS severity to self-reported internalizing problems after controlling for chronological age, a linear regression indicated that sex moderated the association between ELS severity and internalizing problems, $B = 0.903$, $SE = 0.306$, $t$ (131) = 2.953, $p = .004$. Post hoc simple slopes analyses within each sex controlling for age indicated that ELS was significantly associated with internalizing problems in females, $r$ (75) = .476, $p < .001$, but not in males, $r$ (55) = .079, $p = .559$.

### fMRI task behavioral performance

We conducted analyses of covariance to compare task performance between males and females, controlling for age. Males and females did not differ on task accuracy or response time (RT; Table 2). ELS severity was associated with longer RT to match emotions, $r$ (134) = .174, $p = .042$. ELS severity was not associated with RT to label emotions, $r$ (134) = .069, $p = .425$, or with task accuracy ($|rs| < .048$, $ps > .576$).

### Whole-brain analysis: Main effect of ELS

We conducted whole-brain correlational analyses to examine the effect of ELS severity, controlling for the effects of chronological age, on brain regions involved in implicit emotion regulation (i.e., labeling emotion faces vs. matching emotion faces). These analyses yielded a significant cluster that was negatively correlated with ELS severity: right lateral occipital cortex/precuneus cortex ($k = 1,088$; peak voxel: $x = 18$, $y = -72$, $z = 42$; $Z = 3.95$).

**Table 2.** *Task performance*

| | Males | Females | |
|---|---|---|---|
| Response time, *M* (*SD*) | | | |
| Emotion label | 1847.172 (276.215) | 1857.447 (260.699) | *F* (1, 134) = 0.092, *p* = .762 |
| Emotion match | 1950.548 (315.084) | 2001.075 (295.768) | *F* (1, 134) = 0.174, *p* = .678 |
| Accuracy (% correct) | | | |
| Emotion label accuracy | 72.966 (15.246) | 72.308 (12.714) | *F* (1, 134) = 0.102, *p* = .750 |
| Emotion match accuracy | 83.517 (14.74) | 83.75 (14.83) | *F* (1, 134) = 0.576, *p* = .449 |

We also conducted a linear regression to examine the effect of ELS severity, controlling for the effects of chronological age, on activation in a bilateral amygdala ROI during implicit emotion regulation. There was no association between ELS severity and bilateral amygdala activation, *r* (134) = −.049, *p* = .575.

### Whole-brain analysis: Main effect of sex

We conducted whole-brain *t* tests to examine sex differences, controlling for the effects of chronological age, in regions involved in labeling emotion faces versus matching emotion faces. Direct comparisons between males and females yielded one significant cluster in the left lingual gyrus in which males showed significantly greater activation than did females (*k* = 580; peak voxel: *x* = −12, *y* = −70, *z* = −6; *Z* = 3.38). There were no clusters in which females showed greater activation than did males.

We also conducted an analysis of covariance to examine sex differences in activation in a bilateral amygdala ROI, controlling for the effects of chronological age. There were no significant differences between males and females in amygdala activation when labeling emotion faces relative to matching emotion faces, *F* (1, 134) = 0.034, *p* = .855.

### Whole-brain analysis: Interaction of ELS and sex

Finally, we conducted a whole-brain interaction model to examine whether the linear association between ELS severity and brain regions involved in labeling emotion faces versus matching emotion faces differed by sex, controlling for the effects of chronological age. This interaction model yielded three significant clusters (Figure 1). Compared with males, females showed a significantly greater association between ELS and activation in left vlPFC (*k* = 606; peak voxel: *x* = −36, *y* = 48, *z* = −4; *Z* = 3.8), right dlPFC/vlPFC (*k* = 784; peak voxel: *x* = 44, *y* = 44, *z* = 26; *Z* = 4.1) and bilateral intracalcarine cortex (*k* = 1,999; peak voxel: *x* = −18, *y* = −72, *z* = 12; *Z* = 4.18).

### ROI analysis of amygdala: Interaction of ELS and sex

We also conducted a linear regression to examine the interaction between ELS severity and sex in predicting bilateral amygdala ROI response to labeling emotion faces relative to matching emotion faces. This interaction was not significant, *B* = 0.488, *SE* = 1.147, *t* (132) = 0.426, *p* = .671.

### Associations between brain regions and internalizing symptoms

We next examined the association between self-reported internalizing problems and parameter estimates extracted from the ROIs defined by the interaction of ELS and sex, including left vlPFC, right dlPFC/vlPFC, and bilateral intracalcarine cortex, separately for males and females (Figure 2). Females showed a positive association between all three ROIs and YSR internalizing problems total score, such that greater activation in left vlPFC, right dlPFC/vlPFC, and intracalcarine cortex was associated with greater internalizing problems, left vlPFC: *r* (78) = .237, *p* = .037; right dlPFC/vlPFC: *r* (78) = .232, *p* = .041; intracalcarine cortex: *r* (78) = .260, *p* = .021. In contrast, males showed a positive association between internalizing problems and activation only in intracalcarine cortex, intracalcarine cortex: *r* (58) = .320, *p* = .014; there was no significant relation in males between internalizing problems and activation in left vlPFC or in right dlPFC/vlPFC, left vlPFC: *r* (58) = −.070, *p* = .601; right dlPFC/vlPFC: *r* (58) = .141, *p* = .293.

### Mediation analysis: Testing whether brain activation mediates the association between ELS severity and internalizing symptoms in females

Because there was a significant positive association between activation during implicit emotion regulation in left vlPFC, right dlPFC/vlPFC, and intracalcarine cortex and internalizing symptoms in females only, we tested whether activation in these regions mediated ELS severity and internalizing symptoms. Activation during implicit emotion regulation in these three regions did not mediate the association between ELS and internalizing problems in females; that is, the indirect effect did not differ significantly from zero, left vlPFC: point estimate = 0.010 (0.018), 95% CI [−0.011, 0.070]; right dlPFC/vlPFC: point estimate = 0.025 (0.034), 95% CI [−0.023, 0.117]; intracalcarine cortex: point estimate = 0.076 (0.081), 95% CI [−0.057, 0.270].
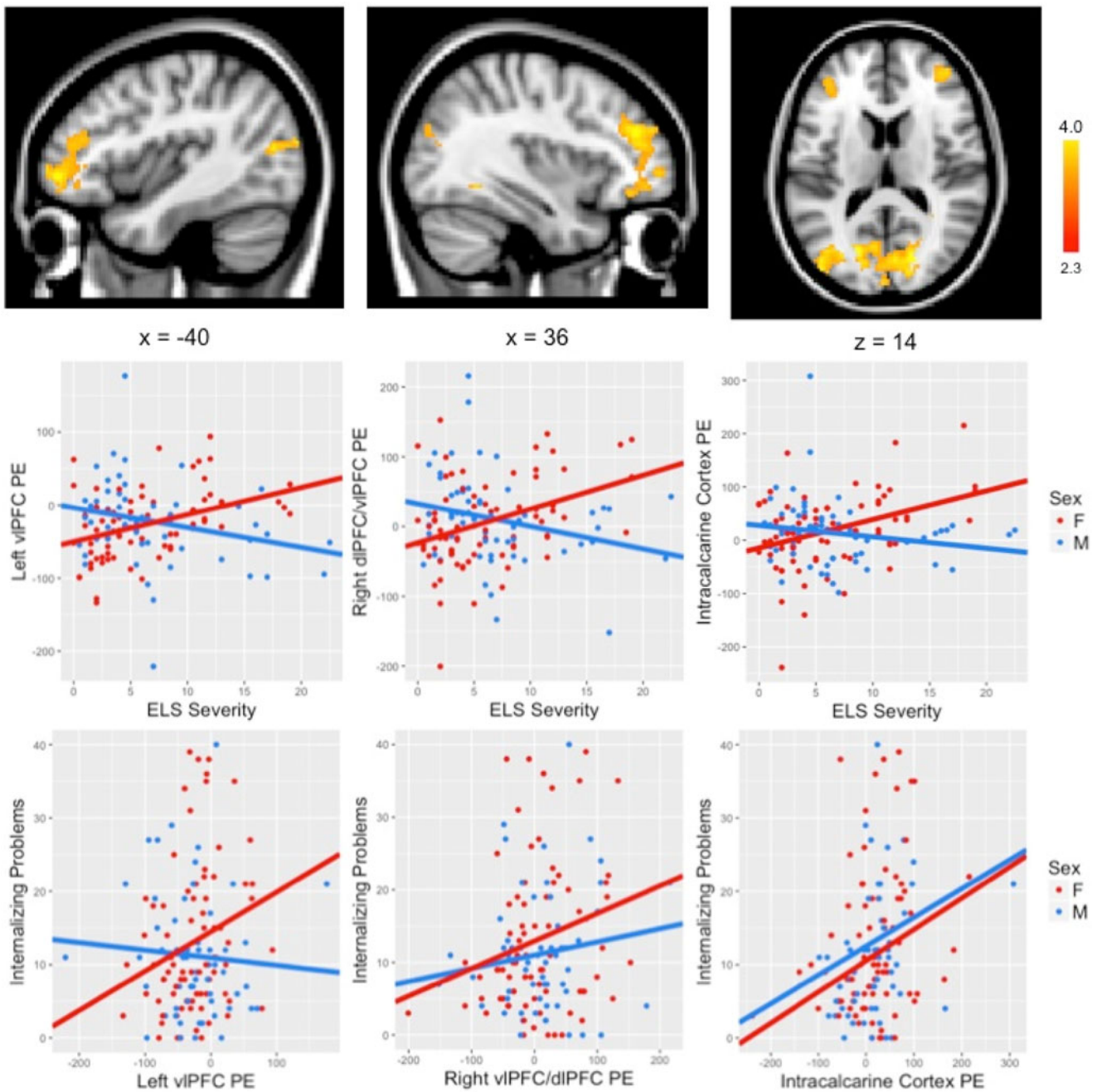
**Figure 1.** (Color online) A whole-brain interaction model examining whether the linear association between early life stress severity and brain regions involved in labeling emotion faces versus matching emotion faces differed by sex yielded three significant clusters in the left vlPFC, right dlPFC/vlPFC, and bilateral intracalcarine cortex. Activation maps are thresholded at $Z > 2.3$ and corrected for multiple comparisons using a cluster-based $p < .05$. MNI coordinates are indicated for slice distance (mm). Parameter estimates (showing the amount of signal change measured in arbitrary units) of blood oxygen level dependent signal response were extracted from each significant cluster and plotted in the bar graph. Parameter estimates were also related to internalizing problems in males and females separately. vlPFC, ventrolateral prefrontal cortex; dlPFC, dorsolateral prefrontal cortex.

*PPI analysis to assess PFC-amygdala functional connectivity*

We also conducted PPI analyses to examine task-dependent functional connectivity between the two prefrontal regions that showed a significant interaction of ELS severity and

sex and the bilateral amygdala ROI. We tested the main effects of ELS severity and sex and their interaction on the strength of these connections during emotion label relative to emotion match conditions. The main effects of ELS and sex, and the ELS × Sex interaction, on the strength of connectivity between the left vlPFC and bilateral amygdala were not
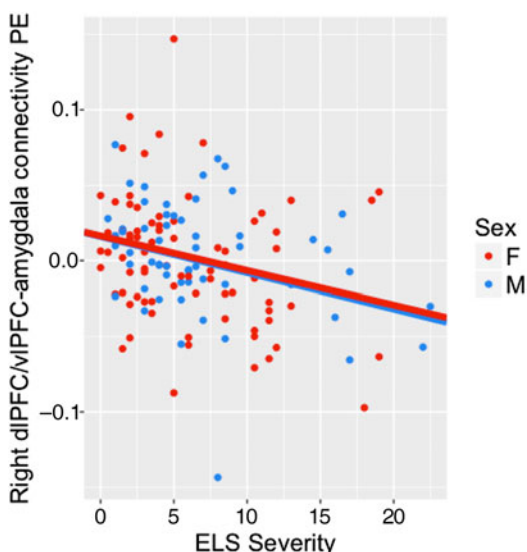
**Figure 2.** (Color online) Strength of connectivity between right the dlPFC/vlPFC and bilateral amygdala was negatively associated with early life stress severity across males and females. vlPFC, ventrolateral prefrontal cortex; dlPFC, dorsolateral prefrontal cortex.

significant. There was a significant main effect of ELS severity on the strength of connectivity between the right dlPFC/vlPFC and bilateral amygdala, $B = -0.002$, $SE = 0.001$, $t(132) = -2.352$, $p = .020$, such that greater ELS severity is associated with more negative amygdala-PFC connectivity for emotion label relative to emotion match conditions (Figure 2).

### Associations between PFC-amygdala functional connectivity and internalizing symptoms

Functional connectivity between the bilateral amygdala and left vlPFC and right dlPFC/vlPFC was not associated with internalizing symptoms across all participants, left vlPFC: $r(133) = -.138$, $p = .110$; right dlPFC/vlPFC: $r(133) = -.084$, $p = .334$, or in each sex separately, males: left vlPFC: $r(58) = -.038$, $p = .776$, right dlPFC/vlPFC: $r(58) = .162$, $p = .225$; females: left vlPFC: $r(78) = -.181$, $p = .112$, right dlPFC/vlPFC: $r(78) = -.210$, $p = .065$. Given null associations between functional connectivity and internalizing symptoms, we did not conduct follow-up mediation analyses.

### Discussion

In a sample of puberty-matched early adolescent males and females, we documented differential associations between ELS severity and internalizing problems. Among girls, there was a significant association between ELS severity and internalizing problems, whereas among boys there was no such association. In attempting to understand the neurobiological bases of emerging sex differences in the effects of ELS on internalizing problems, we conducted whole-brain and a priori ROI analyses of brain activation while participants were per-

forming an emotion label task designed to measure implicit emotion regulation. We found a significant interaction of ELS severity and sex in the left vlPFC, right dlPFC/vlPFC, and bilateral intracalcarine cortex. Among girls, there was a positive association between ELS severity and activation in these regions during implicit emotion regulation, and as above, among boys there was no association between ELS severity and activation in these regions. Moreover, greater activation in these three regions was associated with higher levels of internalizing problems in girls. Nonetheless, this activation did not statistically mediate the association between ELS severity and internalizing problems. We also found a significant effect of ELS severity on the right lateral occipital/precuneus cortex and a significant main effect of sex in the left lingual gyrus during emotion label relative to emotion match conditions. Contrary to our hypothesis, ROI analyses yielded no significant main effects or interactions of ELS severity and sex on amygdala response during emotion label relative to emotion match conditions. When we examined patterns of PFC-amygdala functional connectivity, we found greater negative association between ELS severity and connectivity in both sexes combined between the right dlPFC/vlPFC and bilateral amygdala during implicit emotion regulation; this did not mediate the relation between ELS and internalizing symptoms.

The vlPFC is largely involved in cognitive responses to negative emotions, including cognitive reappraisal and emotion regulation (Ochsner, Silvers, & Buhle, 2012) and thus plays a significant role in modulating negative affect (Forbes, Phillips, Silk, Ryan, & Dahl, 2011; Phan et al., 2005). Although there are no known direct anatomical connections between the vlPFC and the amygdala, the vlPFC is posited to modulate amygdala response through activation in the medial PFC (Pessoa, 2010; Pessoa, Kastner, & Ungerleider, 2002; Silvers et al., 2016). Cognitive reappraisal has been associated with a negative correlation between vlPFC and amygdala activation (Silvers et al., 2016). The vlPFC does, however, share direct anatomical connections with the dlPFC; research suggests that both of these regions support executive function and inhibitory processes (Pessoa, 2010; Pessoa et al., 2002). Given our finding that greater vlPFC activation is associated with higher levels of ELS severity in early pubertal females, several possible interpretations can be generated. For example, heightened PFC activation in response to affective stimuli has been interpreted as reflecting immaturities in regulating an affective response (see Pfeifer & Blakemore, 2012, for a review of this literature in adolescence). In this context, it is plausible that early pubertal females require additional neural engagement to label the facial emotion and, in turn, to down-regulate amygdala response. Given the nonsignificant results of our mediation analyses, however, it is not clear how increased activation in these brain regions during this implicit emotion regulation task gives rise to heightened internalizing symptoms in early pubertal females. It is possible that increased neural recruitment at this point in early puberty contributes to maladaptive cognitive processes and risk for disor-

der in the future (i.e., late puberty when first depressive epi-
sodes commonly occur and sex differences in depressive
symptoms peak); it is important that this possibility be exam-
ined longitudinally in future research.

One additional theoretical explanation for our finding that
greater exposure to ELS is associated with greater recruitment
of PFC in early pubertal females is the stress acceleration hy-
pothesis. Integrating evidence from both animal and human
studies of exposure to early adversity, the stress acceleration
hypothesis posits that experiencing ELS leads to a faster mat-
uration of neural circuits involved in emotional functioning,
primarily the corticolimbic circuit. Callaghan and Tottenham
(2016) argue that in the face of high levels of stress, acceler-
ated development of neural circuitry involved in emotion
functioning is adaptive in the short term, as it facilitates ear-
lier independence from a potentially unstable or harmful
environment. Gee, Humphreys, et al. (2013) found a more
mature pattern of amygdala-mPFC functional connectivity
(i.e., more negative connectivity) in previously institutional-
ized children and adolescents relative to never-institutional-
ized children and adolescents, which was in turn associated
with reduced separation anxiety in the previously institution-
alized group only. However, this accelerated development
could have later consequences, and researchers have yet to ex-
plore this possibility longitudinally. Increasing age in adoles-
cence has been associated with greater vlPFC activation both
in an emotion regulation context and during passive viewing
of fearful faces (McRae et al., 2012; Yurgelun-Todd & Kill-
gore, 2006; see Forbes et al., 2011). Given typical develop-
mental increases in the recruitment of the dlPFC and vlPFC
(Cohen-Gilbert & Thomas, 2013; Somerville, Hare, & Casey,
2011; Tottenham, Hare, & Casey, 2011) in support of emo-
tion regulation, the increased recruitment of left vlPFC and
right dlPFC/vlPFC that we found in our sample of early ado-
lescent females who were exposed to high levels of ELS may
represent a more developmentally mature pattern of neural
function. Gee, Gabard-Durnam, et al. (2013) found height-
ened activation in the amygdala and in prefrontal and superior
temporal gyrus when viewing fear faces in a sample of pre-
viously institutionalized children and adolescents, but no dif-
ference in mPFC activation between children with and with-
out a history of adversity. When we examined functional
connectivity of prefrontal ROIs that exhibited significant
interactions of sex and ELS severity, we also found that
ELS severity was associated with greater negative connectiv-
ity of the right dlPFC/vlPFC and bilateral amygdala for
emotion label relative to emotion match conditions. Although
sex did not moderate this association, we believe that this is
nonetheless an important demonstration of the stress accelera-
tion hypothesis.

Contrary to our hypothesis, we did not find significant ef-
fects of stress, sex, or their interaction on amygdala activation
during implicit emotion regulation. Activation in the amyg-
dala increases in response to salient (both positive and
negative) stimuli in the environment; amygdala activation is
posited to serve as a motivating signal to guide learning
and memory for emotional material. Our nonsignificant find-
ings for the effect of ELS on amygdala activation during im-
plicit emotion regulation stand in contrast to a large body of
work suggesting that individuals exposed to ELS show
heightened amygdala reactivity to emotion face stimuli (in-
cluding angry, happy, fearful, sad, and neutral faces; Garrett
et al., 2012; Gee, Gabard-Durnam, et al., 2013; Marusak
et al., 2014; McCrory et al., 2013; Suzuki et al., 2014; Totten-
ham, Hare, Millner, et al., 2011), as well as to negative images
(McLaughlin et al., 2015). In understanding these discrepant
findings, it is instructive to note that because of the block de-
sign of this task, we combined a range of emotional face stim-
uli, including happy, surprised, sad, angry, and fearful faces.
The block design of the task prevents us from being able to
assess reliably amygdala activation to each emotion. It is pos-
sible that the effects of ELS on amygdala activation are selec-
tive to fearful or angry faces or specific to task demands (i.e.,
passively attending to facial stimuli or performing a cognitive
task involving emotional face stimuli). Future research should
investigate the specificity of this effect across a range of emo-
tional faces and task conditions.

Similarly, the majority of existing work examining the ef-
fects of ELS on amygdala activation to threat-related stimuli
in adolescence uses an extreme-group approach to examine
brain function in individuals exposed to severe and homoge-
nous forms of ELS, including early institutional care and se-
vere maltreatment, relative to healthy controls (McLaughlin
et al., 2015; Mueller et al., 2010; Tottenham, Hare, Millner,
et al., 2011). Less work has focused on the neurobiologi-
cal consequences of more commonly experienced forms of
ELS such as those reported by the young participants in
this study, including witnessing an injury or an accident
and moving homes. It is possible that heightened amygdala
reactivity as a consequence of ELS occurs only after exposure
to extreme forms of early adversity or to specific types of
ELS, such as exposure to severe threat or harmful input
from caregivers early in development (Humphreys & Zeanah,
2015; McLaughlin, Sheridan, & Lambert, 2014; Teicher &
Samson, 2016). The measure of ELS severity that we used
in this study includes both more severe forms of ELS such
as physical and sexual abuse, as well as less severe stressors.
Thus, heightened amygdala reactivity may occur only after
exposure to specific types of extreme ELS, a possibility that
should be examined more explicitly in future research.

In summary, this is the first study to carefully assess sex
differences in corticolimbic activation and connectivity dur-
ing implicit emotion regulation in a unique sample of early
pubertal youth as a function of exposure to ELS severity. In
addition to the strengths of this investigation, there are three
important limitations of this investigation. First, although
we used careful coding systems to evaluate the cumulative ef-
fects of objectively rated ELS severity on corticolimbic devel-
opment, we use the term ELS broadly to include multiple
forms of adverse childhood experiences, ranging from mal-
treatment and neglect to residential moves and exposure to
marital disagreements. As we noted above, researchers have

suggested that different forms of early adverse experiences have different psychobiological consequences (Humphreys & Zeanah, 2015; McLaughlin & Sheridan, 2016; Teicher & Samson, 2016; Teicher, Samson, Anderson, & Ohashi, 2016). Future research should examine the differential effects of types of stress exposure, including exposure to threat and neglect, on neural functioning. Second, we did not examine effects of the timing of ELS severity or its chronicity in this study. Researchers have developed coding systems to describe more fully experiences of maltreatment; one notable example of this is the Maltreatment Classification System (Barnett, Manly, & Cicchetti, 1993). Given our use of child-reported stressors and objectively rated severity of stressors (as opposed to obtaining both child and parent reports, or reports from Child Protective Services for more severe stressors), we did not have sufficient details in our stress assessment to explore specific and differential effects of onset, chronicity, or developmental timing. It will be important in future research to elucidate how exposure to ELS at different points in development, as well as chronicity of adverse experiences, differentially affect corticolimbic circuitry, given that this system is still maturing through adulthood. In the present study, we defined ELS as any adverse experience prior to participating in the study, when adolescents were in early pubertal development. There is evidence in both human and rodent models, however, to suggest that the effects of adverse experiences on different neural systems depends on the

individual's developmental stage at the time of exposure (Andersen & Teicher, 2008; Andersen et al., 2008; Lupien et al., 2009; Pechtel, Lyons-Ruth, Anderson, & Teicher, 2014). Third, it is important to mention the limitations of cross-sectional investigations such as the present study, which precludes our ability to draw causal inferences. It will be important in future research to understand how corticolimbic development across puberty differs in males and females, particularly with respect to its temporal relation to ELS and risk for internalizing problems. Elucidating deviations from normative neural development in individuals who are exposed to ELS will lead to a better understanding of the etiology of internalizing problems and will highlight potential ways to intervene in the association between ELS and internalizing problems.

Despite these limitations, this study documents the effects of a wide range of ELS exposure on corticolimbic function in early puberty, and elucidates how sex moderates the effects of ELS on corticolimbic function. These results are important in refining models describing the impact of ELS on development, and highlight the need to study homogenous samples of well-characterized individuals at specific points in development. Understanding how ELS affects psychobiological development over time will facilitate the generation of a model describing the emergence of internalizing disorders, and will help to identify specific points or sensitive periods at which intervention may be most effective.

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