Maternal Protection in Childhood is Associated with Amygdala Reactivity and Structural Connectivity in Adulthood

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ABSTRACT

Recently, we reported that variability in early-life caregiving experiences maps onto individual differences in threat-related brain function. Specifically, we found that greater familial affective responsiveness is associated with increased amygdala reactivity to interpersonal threat, particularly in adolescents having experienced relatively low recent stress. Here, we conceptually replicate and extend on our previous work to provide further evidence that subtle variability in specific features of early caregiving shapes structural and functional connectivity between the amygdala and medial prefrontal cortex (mPFC) in a cohort of 312 young adult volunteers. Multiple regression analyses revealed that participants who reported higher maternal but not paternal protection exhibited increased amygdala reactivity to explicit signals of interpersonal threat (i.e., angry facial expressions) but not implicit signals of broad environmental threat (i.e., fearful facial expressions). While amygdala functional connectivity with regulatory regions of the mPFC was not significantly associated with maternal protection, participants who reported higher maternal protection exhibited relatively decreased structural integrity of the uncinate fasciculus (UF), a white matter tract connecting these same brain regions. The observed associations were independent of the potential confounding influences of participant sex, socioeconomic status, and self-reported childhood trauma. There were no significant associations between structural or functional brain measures and either maternal or paternal care ratings. These findings suggest that an over controlling parenting style in mothers during childhood is associated with functional and structural alterations of brain regions involved in generating and regulating responses to threat in young adulthood.

INTRODUCTION

A rich history of literature details the widespread effects of early caregiving on child psychosocial development (Belsky & de Haan, 2011; Callaghan & Tottenham, 2015; Cicchetti & Curtis, 2015; Tottenham, 2017). Seminal early work revealed the formative and lasting impacts of parenting style and attachment on socioemotional development over time across species (Bowlby, 1958; Ainsworth, 1969; Harlow, 1961; Harlow & Zimmermann 1959; Lorenz, 1935). As a natural extension of these findings, there is a large body of research linking stressful early environments, such as those marked by trauma, abuse, and neglect, with similar outcomes in humans. Children exposed to early life adversity have poorer outcomes in terms of social, emotional, and behavioral development (reviewed in Tottenham, 2017). More recent neuroimaging research (McCrory et al., 2010; Tottenham, 2014) has described parallel effects in brain with children exposed to trauma, abuse, and neglect in early life exhibiting maladaptive alterations in the structure and function of brain regions supporting emotional behaviors, particularly the amygdala and regulatory regions of the medial prefrontal cortex (mPFC).

Taken together, the above research suggests that many of the effects of early caregiving may be determined by the shaping of brain circuits supporting emotional behaviors and psychological well-being (Belsky & de Haan, 2011; Burghy et al., 2012; Callaghan & Tottenham, 2015; Cicchetti & Curtis, 2015). However, the majority of this research has focused nearly exclusively on extremes of early life adversity such as trauma, abuse, neglect, and institutionalization (e.g., Gee et al., 2013; Malter Cohen et al., 2013; Herringa et al., 2013; McLaughlin et al., 2015; Pechtel et al., 2014; Sheridan et al, 2012; Tottenham et al., 2010;

Tottenham, 2012). With few exceptions (Farber et al., 2018; Romund et al., 2016; Tan et al., 2014; Whittle et al., 2009; Tottenham, 2017), there is little work to date investigating potential impacts of normative variability in early caregiving on the development of these brain circuits in the absence of such harsh early life environments.

Recently, we examined associations between variability in caregiving and threat-related amygdala reactivity in a cohort of 232 adolescents (Farber et al., 2018). In this work, we modeled the childhood caregiving environment using the general functioning and affective responsiveness scales of the Family Assessment Device (FAD). Our analyses revealed that greater familial affective responsiveness (i.e., the appropriate expression and recognition of emotion through warmth, care, and affection) is associated with increased amygdala reactivity to explicit, interpersonal threat but not implicit, environmental threat as conveyed by angry and fearful facial expressions, respectively. This association is robust to the potential influence of participant sex, age, broad familial risk for depression, and early life stress, as well as contemporaneous symptoms of depression and anxiety. Moreover, this association is moderated by the experience of recent stressful life events wherein higher affective responsiveness was associated with higher amygdala reactivity in participants reporting low but not high recent stress. In contrast, there were no significant associations between amygdala reactivity and the FAD scale for general family functioning (e.g., “We don’t get along well together”).

This work suggests that adolescents who report less stressful environments outside of the home and home environments marked by greater affective responsiveness exhibit increased amygdala reactivity to interpersonal threat. We hypothesized that this paradoxical association may reflect increased associative learning following less-frequent, more unpredictable experiences of threat or conflict. We further speculated that our observed associations among better familial affective responsiveness, less stress, and higher amygdala reactivity suggest a mechanism through which parental overprotection may manifest as psychosocial dysfunction.

While this prior work extends the literature on the impact of caregiving extremes on behaviorally-relevant brain function, the data available were not ideal for assessing caregiving in fine detail as the FAD does not provide information on family structure or parent-of-origin effects. Additionally, the FAD does not generate indices of the extent to which caregivers were permissive or controlling, two facets of particular importance in shaping the early caregiving environment (Parker, 1983). Parental overprotection, defined as "restrictive and controlling parenting" has been associated with later psychopathology including depression and anxiety disorders (Thomasgard & Metz, 1993). In contrast, families marked by high parental care and low parental overprotection have been described as having "optimal bonding," with children from such families reporting less distress, better general well-being, and better social support (Canetti et al., 1997).

In the present study, we sought to conceptually replicate our prior finding in adolescents and further extend upon it by capturing more detailed aspects of early caregiving experiences as

well as additional features of corticolimbic circuit integrity using data from 312 young adult volunteers who completed the Duke Neurogenetics Study. First, we expand upon broadband family functioning to parse the specific dimensions of (a) care and (b) control/protection—for mothers and fathers independently. Second, we broaden our neuroimaging analyses beyond threat-related amygdala reactivity to also examine both functional and structural connectivity of the amygdala with regulatory regions of the mPFC. Early parenting style was indexed by the Parental Bonding Instrument (PBI; Parker et al., 1979), which examines paternal and maternal caregiving separately along the dimensions of care and protection. Amygdala reactivity to explicit, interpersonal and implicit, environmental threat as communicated by angry and fearful facial expressions, respectively, was measured using task-based functional magnetic resonance imaging (fMRI). Seed-based amygdala functional connectivity with the mPFC was modeled using general psychophysiological interaction (gPPI). The mPFC was targeted because of its important reciprocal connections with the amygdala supporting the integration and regulation of threat-related processing. Accordingly, structural connectivity between these regions was assessed using diffusion weighted imaging-based fractional anisotropy (FA) estimates of white matter microstructural integrity of the uncinate fasciculus (UF), a major structural pathway between the amygdala and mPFC.

Building on our previous work, we hypothesized that higher parental care (both maternal and paternal) experienced during childhood (i.e., before 18 years of age) would be associated with increased amygdala reactivity to angry but not fearful facial expressions in young adulthood (i.e., 18-22 years of age). Extending this primary hypothesis, we explored the following related

questions: (1) are maternal and paternal care and protection differentially associated with amygdala reactivity, (2) are parental care and protection associated with functional connectivity between the amygdala and mPFC, and (3) are parental care and protection associated with structural connectivity between the amygdala and mPFC?

MATERIALS & METHODS

Participants

Study participants included a subset of individuals (*N* = 312) having completed the Duke Neurogenetics Study (DNS), which was designed to identify biomarkers of risk for psychopathology amongst young adult university students. The present analyses focus on a substantially smaller subsample of the full DNS sample (*N* = 1332) because the measure of caregiving was added to the DNS protocol during the final year of data collection. All procedures were approved by the Duke University Medical Center Institutional Review Board all and participants provided informed consent before study initiation. Participants in the present DNS subsample (a) were free of cancer, stroke, diabetes, chronic kidney or liver disease, hypertension, or psychotic symptoms; (b) were not actively using psychotropic, glucocorticoid, or hypolipidemic medication; and (c) met quality control for MRI data as described below. In addition to a formal clinical screening for past and current mental illness, all participants provided extensive self-report measures related to behavior and life experiences. All participants further completed a neuroimaging protocol on one of two research-dedicated GE MR750 3T scanners equipped with high-power high-duty-cycle 50-mT/m gradients at 200 T/m/s

slew rate, and an eight-channel head coil for parallel imaging at high bandwidth up to 1MHz at the Duke-UNC Brain Imaging and Analysis Center.

Self-Report Measures

Parental care and protection were assessed using the Parental Bonding Instrument (PBI), a 40-item scale used across a variety of research contexts with acceptable validity and reliability (Parker et al., 1979; Parker et al., 1983). The PBI consists of two separate scales for each parent—care and protection—and participants rate the extent to which each item corresponds with the attitudes and behaviors of their parents "when [they] were growing up." Scores range from 1 ("very like") to 4 ("very unlike") with higher scores reflecting higher parental care/protection. In addition, the PBI generates separate maternal and paternal quadrants based on the rater's maternal and paternal care and protection scores. The parenting style represented by each quadrant are labeled as "weak" (low care, low protection), "affectionless control" (low care, high protection), "autonomy support" (high care, low protection), and "affective constraint" (high care, high protection). However, we focus on dimensional indices of care and protection rather than categorical quadrant placements to better model subtle variability in parenting style onto brain continuously rather than categorically. Early life adversity was assessed using the Childhood Trauma Questionnaire (CTQ), a widely-used measure of trauma and early life adversity (Bernstein et al., 2003). We used CTQ Total Scores as a covariate in our primary analyses to identify variance in brain function and structure attributable to parenting style above and beyond that associated with childhood trauma. Socioeconomic status (SES) was assessed using The MacArthur Scale of Subjective Social Status,

which was developed to capture the common sense of social status across SES indicators by presenting a "social ladder" and asking individuals to place an "X" on the rung on which they feel they stand (Adler & Stewart, 2007).

Amygdala Reactivity Task

Our experimental protocol consisted of four task blocks interleaved with five control blocks. The four task blocks consisted of one block each with fearful, angry, surprised, or neutral facial expressions presented in a pseudorandom order across participants. During task blocks, participants viewed a trio of faces and selected one of two faces (bottom) identical to a target face (top). Each task block consisted of six images, balanced for gender, all of which were derived from a standard set of pictures of facial affect (Ekman & Friesen, 1976). During control blocks, participants viewed a trio of simple geometric shapes (circles and vertical and horizontal ellipses) and selected one of two shapes (bottom) identical to a target shape (top). Each control block consisted of six different shape trios. All blocks are preceded by a brief instruction ("Match Faces" or "Match Shapes") that lasted 2 s. In the task blocks, each of the six face trios was presented for 4 s with a variable interstimulus interval (ISI) of 2-6 s (mean = 4 s) for a total block length of 48 s. A variable ISI was used to minimize expectancy effects and resulting habituation and maximize amygdala reactivity throughout the paradigm. In the control blocks, each of the six shape trios was presented for 4 s with a fixed ISI of 2 s for a total block length of 36 s. Total task time was 390 s.

BOLD fMRI Data Acquisition

A semi-automated high-order shimming program was used to ensure global field homogeneity. A series of 34 interleaved axial functional slices aligned with the anterior commissure-posterior commissure plane were acquired using an inverse-spiral pulse sequence to reduce susceptibility artifacts (TR/TE/flip angle=2000 ms/30 ms/60; FOV=240mm; 3.75×3.75×4mm voxels; interslice skip=0). Four initial radiofrequency excitations were performed (and discarded) to achieve steady-state equilibrium. To allow for spatial registration of each participant's data to a standard coordinate system, high-resolution three-dimensional structural images were acquired in 34 axial slices coplanar with the functional scans (TR/TE/flip angle=7.7 s/3.0 ms/12; voxel size=0.9×0.9×4mm; FOV=240mm, interslice skip=0).

BOLD fMRI Data Pre-Processing

Anatomical images for each participant were skull-stripped, intensity-normalized, and nonlinearly warped to a study-specific average template in Montreal Neurological Institute (MNI) standard stereotactic space using ANTs (Klein et al., 2009). BOLD time-series for each participant were processed in AFNI (Cox, 1996). Images for each participant were despiked, slice-time-corrected, realigned to the first volume in the time series to correct for head motion, coregistered to the anatomical image using FSL's Boundary Based Registration (Greve & Fischl, 2009), spatially normalized into MNI space using the non-linear warp from the anatomical image, resampled to 2mm isotropic voxels, and smoothed to minimize noise and residual difference in gyral anatomy with a Gaussian filter set at 6-mm full-width at half-maximum. All transformations were concatenated so that a single interpolation was performed. Voxel-wise signal intensities were scaled to yield a time series mean of 100 for each voxel. Volumes

exceeding 0.5mm frame-wise displacement (FD) or 2.5 standardized DVARS (Nichols, 2017; Power et al., 2014) were censored from the subsequent GLM analyses.

fMRI Quality Control

Quality control criteria for inclusion of a participant's imaging data were: >5 volumes for each condition of interest retained after censoring for FD and DVARS and sufficient temporal signal-to-noise ratio (SNR) within the bilateral amygdala, defined as greater than 3 standard deviations below the mean of this value across participants. The amygdala was defined using a high-resolution template generated from 168 Human Connectome Project datasets (Tyszka et al. 2016). Since we did not have *a priori* predictions regarding hemispheric differences, and to reduce the number of statistical tests, the extracted values were averaged across left and right hemispheres for further statistical analyses. Additionally, data were only included in further analyses if the participant demonstrated sufficient engagement with the task, defined as achieving at least 75% accuracy during the face matching condition.

BOLD fMRI Data Analysis

Following preprocessing, the AFNI program 3dREMLfit (Cox, 1996) was used to fit general linear models for first-level fMRI data analyses. To obtain parameter estimates for each task block, we modeled only the respective block (convolved with the canonical hemodynamic response function) along with the adjacent half of the preceding and following control blocks, and a first order polynomial regressor to account for low frequency noise. This allowed for the estimation of the individual task block parameters while minimizing the influence of adjacent task blocks as

well as low frequency noise across the entire run. Based on our prior work, the contrasts of interest for the current analyses were angry facial expressions > shapes and fearful facial expressions > shapes.

Psychophysiophysiological Interactions

Task-modulated functional connectivity was estimated using the generalized psychophysiological interaction (gPPI) toolbox (McLaren et al., 2012) in SPM12. Following preprocessing, deconvolved time courses averaged across the amygdala (tc1) and mPFC (tc2) were extracted, and entered into first-level statistical models, which also included a psychological task regressor as well as all interaction terms (tc1*tc2, tc1*task, tc2*task and tc1*tc2*task). Individual beta images corresponding to the three-way interaction term (tc1*tc2*task) were then used in a second-level random effects model accounting for scan-to-scan and participant-to-participant variability to determine mPFC activation that varies as a function of amygdala reactivity and experimental condition using one-sample *t*-tests with a voxel-level statistical threshold of $p < 0.05$, FWE corrected for multiple comparisons across the entire brain and a cluster threshold of 10 contiguous voxels (Gorka et al., 2015). The mPFC was anatomically defined as Brodmann Areas 10, 11, 12, 24, 25, and 32 using SPM12.

Diffusion Weighted Imaging

Following an ASSET calibration scan, two 2-min 50-s diffusion weighted imaging acquisitions were collected, providing full brain coverage with 2-mm isotropic resolution and 15 diffusion weighted directions (10-s repetition time, 84.9 ms echo time, b value 1,000 s/mm2, 240 mm

field of view, 90° flip angle, 128×128 acquisition matrix, slice thickness=2 mm). Diffusion weighted images were processed according to the protocol developed by the Enhancing Neuro Imaging Genetics Through Meta-Analysis consortium (Jahanshad et al., 2013 or http://enigma.ini.usc.edu/protocols/dti-protocols/). In brief, raw diffusion weighted images underwent eddy current correction and linear registration to the non-diffusion weighted image in order to correct for head motion. These images were skull-stripped and diffusion tensor models were fit at each voxel using FMRIB's Diffusion Toolbox (FDT; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT) and the resulting two FA maps were linearly registered to each other and then averaged. Average FA images from all subjects were non-linearly registered to the ENIGMA-DTI target FA map, a minimal deformation target calculated across a large number of individuals (Jahanshad et al., 2013). The images were then processed using the tract-based spatial statistics (TBSS) analytic method (Smith et al., 2006) modified to project individual FA values onto the ENIGMA-DTI skeleton. Following the extraction of the skeletonized white matter and projection of individual FA values, left and right UF pathways of interest, defined using the Johns Hopkins University White Matter Tractography Atlas (Mori et al., 2005), were binarized to extract mean FA values each participant. Since we again did not have *a priori* predictions regarding hemispheric differences, and to reduce the number of statistical tests, the extracted FA values were averaged across left and right hemispheres for further statistical analyses (d'Arbeloff, 2018; Kim et al., 2018a).

Statistical Analyses

Mean individual contrast-related BOLD parameter estimates from functional clusters were entered into second-level analyses in SPSS, version 25 (IBM, Armonk, NY). To test our primary hypothesis, we ran a linear multiple regression analysis including all four PBI dimensions as predictor variables (maternal care, maternal protection, paternal care, paternal protection), extracted BOLD values for the contrast of angry facial expressions greater than shapes averaged across hemispheres as the sole outcome variable; and age, sex, SES, and CTQ score as covariates. To probe the specificity of any association, we conducted post hoc analyses by duplicating our initial model with outcome variable of extracted BOLD values for the contrast of fearful expressions greater than shapes.

After finding a significant association specifically between maternal protection and amygdala reactivity to angry facial expression, we focused subsequent analyses on this PBI dimension exclusively to reduce inflated false positives due to multiple tests. To this end, analyses testing our secondary hypotheses were conducted as simple, bivariate correlations between (1) maternal protection and extracted amygdala-mPFC gPPI values and (2) maternal protection and extracted FA values for the uncinate fasciculus. We then duplicated significant models using partial correlation analyses in SPSS with SES and CTQ Total scores as covariates to determine if maternal protection is associated with UF microstructural integrity above and beyond childhood trauma and socioeconomic status.

RESULTS

Participant Characteristics

Data were available from a maximum of 312 participants (170 women). Sample distributions and descriptive statistics for each PBI subscale score as well as SES and CTQ Total scores are detailed in Supplemental Table 1. The subsample (n = 168) of participants included in structural connectivity analyses did not significantly differ from the full sample (N = 312) on sex, age, race, CTQ Total, SES, or PBI subscale scores with the exception of maternal care (t = -2.40, p = 0.004). Zero-order correlations among all self-report measures are reported in Supplemental Table 2.

Caregiving and Amygdala Reactivity

Consistent with our prior work, first-level analyses revealed robust bilateral amygdala reactivity to angry and fearful facial expressions across participants (e.g., Kim et al., 2018b; Swartz et al., 2017; Swartz et al., 2015; Nikolova et al., 2014; Prather et al., 2013). Linear regression analyses using extracted BOLD parameter estimates from clusters exhibiting main effects of expression revealed a significant negative correlation between PBI maternal protection scores and amygdala reactivity to angry facial expressions (*Std. B* = 0.195, p = 0.009; Figure 1). There were no significant correlations between amygdala reactivity to angry facial expressions and paternal protection or paternal or maternal care (paternal protection: *Std. B* = -0.036, p = 0.613; maternal care: *Std. B* = 0.119, p = 0.091; paternal care: *Std. B* = -0.093, p = 0.167). The association between maternal protection and amygdala reactivity to angry facial expressions remained significant when controlling for age, sex, SES, and CTQ Total scores (*Std. B* = 0.181, p = 0.015). There were no significant correlations between any PBI subscales and amygdala reactivity to fearful facial expressions (maternal care: r = -0.030, p = 0.598; maternal protection: r = -0.013, p = 0.825; paternal care: r = -0.115, p = 0.063; paternal protection: r = -0.055, p =

0.333;). Given the specificity of this association to maternal protection, all subsequent analyses focused on this PBI scale to limit multiple comparisons. Full regression statistics for this primary analysis, including and excluding covariates, are reported in Supplemental Table 3.

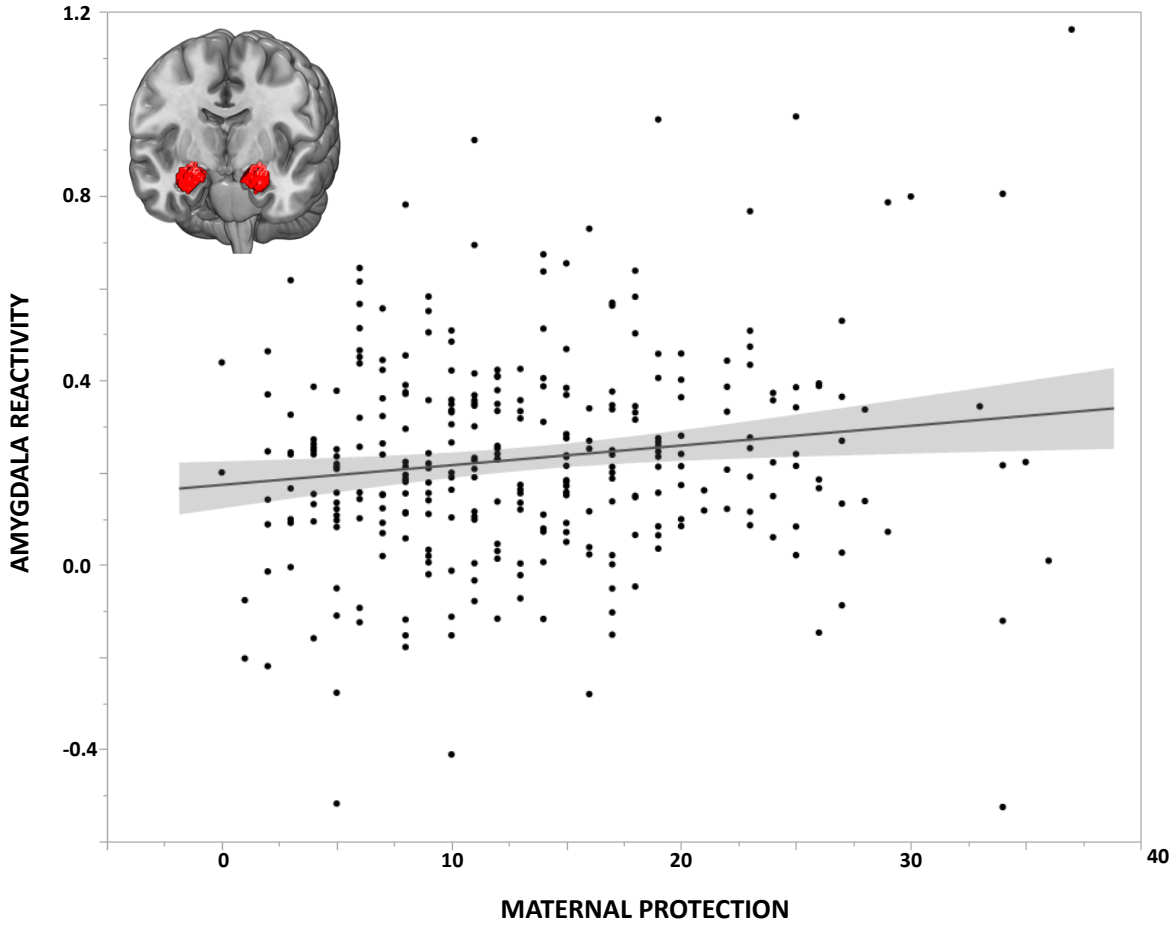

Figure 1. Maternal Protection and Amygdala Reactivity. Parental Bonding Instrument (PBI) maternal protection scores are positively associated with mean bilateral amygdala reactivity to interpersonal threat as indexed by angry facial expressions (*Std. B* = 0.195, *p* = 0.009; *N* = 312).

Maternal Protection and Amygdala Functional Connectivity

Extending our primary finding, we next tested for an association between maternal protection and amygdala-mPFC functional connectivity when processing angry facial expressions. We

extracted mean gPPI values across our mPFC mask generating a single value indicating the strength of task-modulated functional connectivity between the amygdala and mPFC for each participant. Bivariate correlation analysis in SPSS revealed no significant correlation between maternal protection and amygdala-mPFC functional connectivity (r = -0.009, p = 0.873).

Maternal Protection and Amygdala Structural Connectivity

We next explored associations between maternal protection and amygdala-prefrontal structural connectivity. We extracted FA values such that each individual subject had a single value representing the white matter microstructural integrity of the UF, averaged across left and right hemispheres. Bivariate correlation analysis in SPSS revealed a significant negative correlation between maternal protection and UF FA (r = -0.166, p = 0.031; Figure 2). This association remained significant when controlling for sex and SES (*Std. B* = -0.156, p = 0.042). However, when controlling for sex, SES, and CTQ total scores, this association was reduced to a trend-level (*Std. B* = -0.146, p = 0.066), suggesting that CTQ absorbs some of the variance captured by maternal protection on this phenotype.

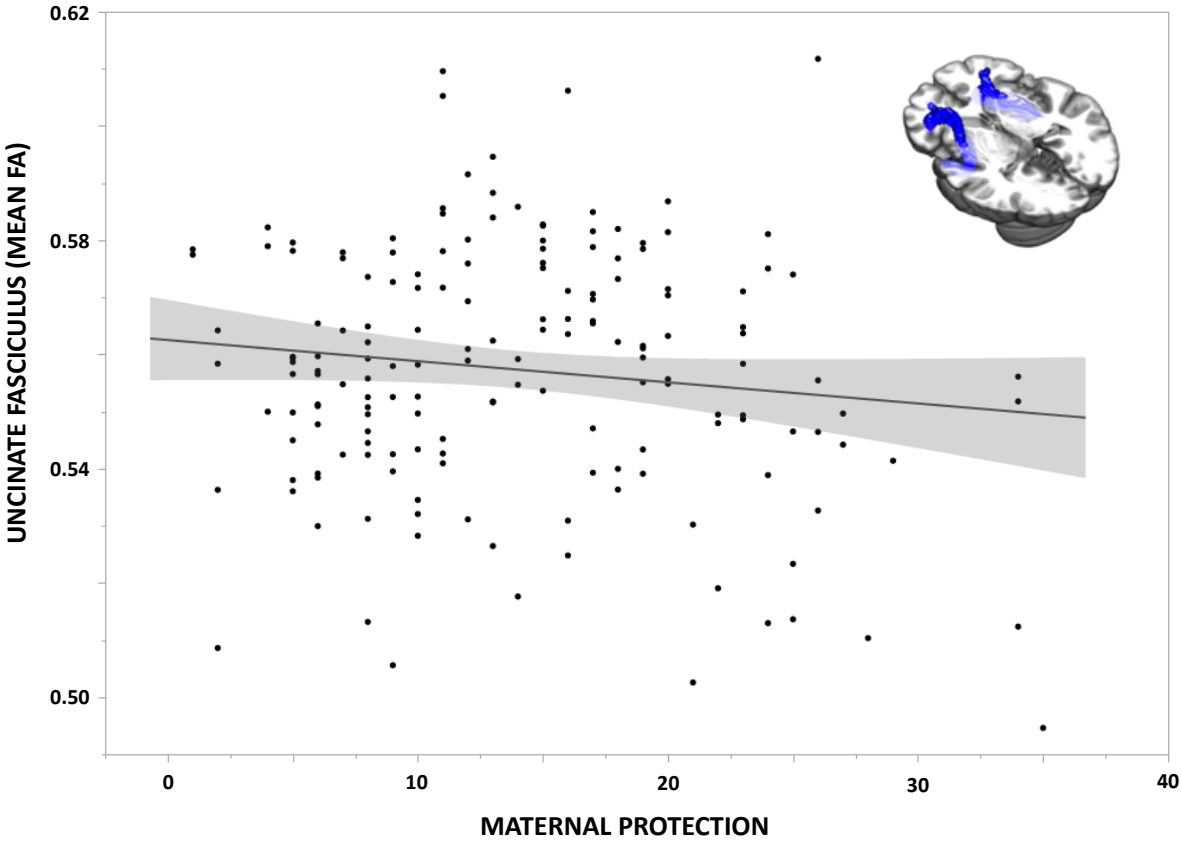

Figure 2. Maternal Protection and Amygdala Structural Connectivity. Paternal Bonding Inventory (PBI) maternal protection scores are negatively correlated with mean bilateral fractional anisotropy of the uncinate fasciculus (r = -0.166, p = 0.031; n = 168).

DISCUSSION

The results of our study provide further evidence that normative variation in caregiving during childhood is associated with later behaviorally-relevant neural function and structure. First, we conceptually replicate our previous work linking greater familial affective responsiveness with amygdala reactivity. We found that higher maternal protection but not paternal protection or maternal or paternal care is associated with increased amygdala reactivity to interpersonal threat in the form of angry facial expressions. Second, we were able to expand these findings into structural and functional connectivity between the amygdala and regulatory regions of the

mPFC. Here we found that higher maternal protection is associated with decreased structural integrity of the uncinate fasciculus, a central white matter tract connecting the amygdala and mPFC. Contrary to our hypothesis, maternal protection was not significantly associated with functional connectivity between the amygdala and mPFC when processing angry facial expressions.

Our current findings are relevant for research on the buffering effects of parental presence. For example, maternal presence has been associated with suppression of amygdala reactivity in childhood but not adolescence (Gee et al., 2014). Thus, amygdala hyperactivity and poor microstructural integrity of the uncinate fasciculus may reflect a childhood marked by overprotectiveness and, possibly, prolonged and ultimately maladaptive maternal buffering. It is interesting to speculate that children who are relatively sheltered, particularly by their maternal caregiver, may not have sufficient opportunity to experience stress and subsequently fail to fully develop important structural pathways between the amygdala and mPFC. This impact on structure may then emerge as amygdala hyperactivity to interpersonal threat cues possibly in combination with insufficient prefrontal regulation. Such speculation, of course, cannot be directly tested in our cross-sectional data but requires longitudinal data ideally incorporating objective measurements of early upbringing.

Nevertheless, there is support for this speculation in both basic and clinical research. As discussed in our previous work (Farber et al., 2018), animal research illustrates that physiological hypersensitivity to threat, via behavioral freezing or amygdala reactivity, is most

exacerbated when aversive stimuli are infrequent and unpredictable (Fanselow & Tighe, 1988; Bouton ,2007). Clinical studies have reported similar patterns. For example, only young adults who report lower levels of family conflict in early life exhibit increased cortisol reactivity to an acute laboratory stressor (Andreotti et al., 2015). Our results are further consistent with the Stress Inoculation Hypothesis, which states that brief intermittent stress exposure early in life induces the development of subsequent stress resilience (Rutter, 1993; Masten, 2001; Parker et al., 2006). Such resilience is, at least in part, reflected in higher structural integrity of pathways between the amygdala and mPFC, which are associated with enhanced emotion regulation (Lee et al., 2012) and decreased risk for stress-related disorders including depression and anxiety (Etkin & Wager, 2007; Koenigs & Grafman, 2009; Murray, Wise, & Drevets, 2011; Tottenham, 2017). Thus, young adults who report higher overprotection by maternal caregivers may have experienced too little stress during childhood, without which they were unable to fully develop the neural architecture for stress resilience.

Our work, of course, is not without limitations. First, we relied on self-report measures of parenting; therefore, our findings may be subject to reporting bias and are likely more representative of the perception of events rather than of objective events. Second, our sample is not population representative; our participants had relatively high IQ, were highly educated, and came from relatively high SES households. Future studies in more diverse samples are necessary to evaluate the extent to which our current findings are present more broadly. This extension may be especially important in cohorts of individuals raised in lower SES environments with chronic stressors wherein increased parental protection may promote more

adaptive functioning. Likewise, future work should examine these patterns in more diverse family structures. While we limited our present analyses to adolescents raised in two-parent, one maternal and one paternal caregiver households, it is important to explore effects of normative caregiving in individuals raised in single-parent households, two-parent same-sex households, and other familial configurations. Third, our fMRI task precludes examination of amygdala-mPFC functional connectivity during explicit emotion regulation. Thus, we cannot provide functional results in parallel with our structural results. Lastly, a growing number of studies report poor test-retest reliability of amygdala reactivity during tasks using emotional facial expressions as stimuli, including the task used in our protocol (Lois et al., Psychophysiology 2018; Nord et al., Neuroimage 2017; Lipp et al., Neuroimage 2014; Sauder et al., Psychophysiology 2013; Plitcha et al., Neuroimage 2012). Thus, task-elicited functional activation in *a priori* regions of interest may not be well suited for individual differences research. That said, we have conceptually replicated our earlier associations between amygdala reactivity and normative variability in caregiving.

These limitations notwithstanding, our current findings further extend the literature on the brain effects of caregiving extremes to more subtle, normative variability. Our findings suggest that overprotective maternal caregiving is associated with increased amygdala reactivity to explicit signals of interpersonal threat and decreased microstructural integrity of a pathway between the amygdala and mPFC supporting emotion integration and regulation. These observations may be timely as there is an ongoing cultural narrative surrounding the notion of "helicopter parenting," with some arguing that overprotective parenting is harmful to the child

and calling for “free range parenting” and “rewilding” (Flynn, 2018; Prichep, 2018; ”Rewilding”, 2018). To borrow from research on the importance of risky play for children, our work suggests it may be ideal for caregivers to keep children “as safe as necessary,” not “as safe as possible” (Brussoni et al., 2012).

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