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Title:

PARADOXICAL ASSOCIATIONS BETWEEN HIGHER FAMILIAL WARMTH, LOWER STRESS, AND INCREASED AMYGDALA REACTIVITY TO INTERPERSONAL THREAT

Short Title:
Familial Warmth and Amygdala Reactivity to Threat

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Abstract

Studies of early life extremes such as trauma, abuse, and neglect highlight the critical importance of quality caregiving in the normal development of brain circuits supporting emotional behavior and mental health. The impact of normative variability in early life caregiving on biobehavioral processes, however, is poorly understood. Here, we provide initial evidence that even subtle variability in normative early life caregiving may shape threat-related brain function and, potentially, associated psychopathology in adolescence. Specifically, we report that greater familial warmth is associated with heightened amygdala reactivity specifically to interpersonal threat, particularly in adolescents having experienced relatively low recent stress. These findings extend the literature on the effects of early life caregiving extremes on brain function to subtle, normative variability, but suggest that presumably protective factors are associated with increased risk-related amygdala reactivity. We consider these paradoxical associations with regard to studies of basic associative threat learning as well as effects of parental overprotection on psychological developmental.

Significance Statement

Behavioral research illustrates powerful effects of caregiving on psychological development. However, the biological mediators of such effects have almost exclusively been examined using caregiving extremes such as trauma, abuse, or neglect. Here, we provide evidence that even subtle, normative variability in caregiving is associated with how the brain processes threat during adolescence. Specifically, we find that higher familial warmth is associated with greater amygdala reactivity to interpersonal threat, particularly in adolescents having experienced relatively low stress. Our results suggest that while even normative variability in caregiving shapes brain function, factors typically considered protective are paradoxically associated with risk-related amygdala reactivity. These patterns are, nevertheless, supported by studies of associative threat learning and effects of parental overprotection on psychological development.

Introduction

A wealth of empirical research demonstrates that the quality of caregiving in early life predicts later psychosocial outcomes, in part, by shaping the development of brain circuits supporting emotional behavior (1-3). However, much of this research has focused on caregiving extremes, such as trauma, abuse, and neglect (1-8). Such a focus ignores the potential impact of normative variability in caregiving on neurodevelopment and behavior. A rich literature demonstrates lasting positive effects of normative caregiving including secure attachment and positive parenting (i.e., high care, low control) on behavioral development (9-13). Despite this literature, comparatively little is known about the potential impact of normative variability in caregiving on the underlying brain circuitry implicated in both normal and abnormal behavior (1-8).

Here, we seek to address this research gap by examining how normative variability in family functioning affects individual differences in threat-related amygdala reactivity in a large cohort of adolescents aged 12-15 years. We focus on threat-related amygdala reactivity because of converging evidence that this specific aspect of brain function is not only affected by extremes of early life caregiving (14-22) but also represents a biomarker of future risk for psychopathology (23-25). As the extant literature presents a nearly exclusive focus on the effects of maternal caregiving (4, 6-8, 12, 15, 25-28), we examine holistic family functioning to help inform more general effects of the caregiving environment on behaviorally and clinically relevant brain function.

Our analyses centered on data available for a subset of participants having completed the Teen Alcohol Outcomes Study, originally developed to examine the impact of familial loading for depression on the risk to develop alcohol use disorders. Study participants were characterized as either “high risk” (having a first- and second-degree relative with major depressive disorder; MDD) or “low risk” (having no first-degree and minimal second-degree relatives (< 20%) with MDD) (29). The broader caregiving environment was indexed through the lens of family functioning as measured by the general functioning and affective responsiveness subscales of the Family Assessment Device (FAD). Amygdala reactivity to explicit, interpersonal and implicit, environmental threat as communicated by angry and fearful facial expressions, respectively, was measured using task-based functional MRI.

We hypothesized that greater familial warmth (i.e., FAD affective responsiveness scores) would be associated with decreased amygdala reactivity specifically to angry facial expressions as canonical examples of explicit interpersonal threat, which is more likely to be experienced within family dynamics characterized by higher conflict and less care. Against this primary hypothesis, we further explored the following related questions. Are significant associations driven by familial warmth specifically and not more general family functioning? Moreover, are significant associations independent of early life stress, contemporaneous symptoms of depression and anxiety, and broad familial risk for depression? Lastly, are significant associations moderated by recent stressful life events?

Results

Participant Characteristics

Demographic and behavioral characteristics of our analysis sample are detailed in Table 1. High and low risk groups significantly differed in symptoms of depression [$t(200.4)=2.405$, $p=0.019$] and in early life stress [$t(212.2)=2.228$, $p=0.027$]. However, the high and low risk groups did not differ in FAD general functioning [$t(229)=-1.656$, $p=0.099$] or affective responsiveness [$t(229)=-0.382$, $p=0.703$]. Groups also did not differ in anxiety symptoms [$t(217.3)=1.148$, $p=0.252$] or recent life stress [$t(210.3)=1.234$, $p=0.219$]. There was no significant difference between groups on fMRI task performance as measured by accuracy [$t(225)=-0.230$, $p=0.818$], response time [$t(219)=-0.552$, $p=0.582$], and head displacement [$t(230)=-0.609$, $p=0.543$].

Family Functioning and Amygdala Reactivity

Across all participants, there was robust bilateral amygdala reactivity to both angry and fearful facial expressions (Figure 1). Linear regression analyses using extracted BOLD parameter estimates from clusters exhibiting main effects of expression revealed a significant association between FAD affective responsiveness and amygdala reactivity to angry facial expressions. Contrary to our hypothesis, participants who reported greater affective responsiveness exhibited relatively higher left amygdala reactivity to angry facial expressions [$F(1, 221)=4.305$, $p=0.039$] (Figure 2). This association was independent of broader familial risk for depression, as there was no statistically significant interaction between affective responsiveness and risk group status on reactivity [$F(1, 221)=0.012$, $p=0.912$].

Importantly, planned post hoc analyses revealed there were no significant associations between amygdala reactivity to angry facial expressions and FAD general functioning scores [left: $F(1, 221)=0.611$, $p=0.435$; right: $F(1, 221)=0.025$, $p=0.874$], or between amygdala reactivity to fearful facial expressions and either general functioning [left: $F(1, 221)=0.037$, $p=0.848$; right: $F(1, 221)=0.567$, $p=0.452$] or affective responsiveness [left: $F(1, 221)=0.011$, $p=0.915$; right: $F(1, 221)=0.493$, $p=0.483$]. Additional analyses revealed that the association between affective responsiveness and left amygdala reactivity to angry facial expressions was further independent of early life stress, as the relationship between early adversity and amygdala reactivity has been well documented (14-16, 21-22, 44), as well as contemporaneous symptoms of anxiety and depression [$F(1, 203)=5.741$, $p=0.017$].

Lastly, regression analyses revealed a significant interaction of recent life stress and FAD affective responsiveness [$F(1, 213)=4.581$, $p=0.033$], such that the association between affective responsiveness and amygdala reactivity to angry facial expressions was moderated by the experience of recent life stress (Figure 3). Specifically, the association between lower affective responsiveness and higher left amygdala reactivity to angry facial expressions was significant for participants reporting lower [$r=-0.244$, $p=0.014$] but not higher recent life stress [$r=-0.052$, $p=0.614$].

Discussion

Our findings extend the literature on the impact of early life caregiving extremes on behaviorally-relevant brain function by demonstrating a significant association between normative variability in family functioning and threat-related amygdala reactivity.

Specifically, we find that even subtle differences in the experience of warmth within a family uniquely map onto variability in 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). Contrary to our hypothesis, however, greater familial warmth was paradoxically associated with increased amygdala reactivity to angry facial expressions. This association was robust to the potential influence of participant sex, age, early life stress, contemporaneous symptoms of depression and anxiety, and broad familial risk for depression. Moreover, this association was moderated by the experience of recent stressful life events wherein higher warmth was associated with higher reactivity in participants reporting low but not high recent stress.

Our paradoxical associations are particularly interesting when conceptualizing family functioning as an interpersonal process. For example, in families with relatively low warmth, children may frequently experience interpersonal conflict including anger between family members and thus may be desensitized to interpersonal threat cues, as evidenced by lower amygdala reactivity to angry facial expressions. Alternatively, it is possible that children raised by parents who exhibit greater warmth and emotional responsiveness are sheltered from interpersonal conflict, making them more sensitive to interpersonal threat cues and thus more likely to exhibit amygdala hyper-reactivity to angry facial expressions. Consistent with this speculation, our findings revealed that the negative association between familial warmth and amygdala reactivity to angry expressions was specific to participants who reported having experienced relatively less recent life stress. Thus, adolescents who are raised in warm, supportive family

environments and who experience few external stressors are most likely to exhibit neural hypersensitivity to interpersonal threat perhaps reflecting their lack of exposure to conflict.

Importantly, there is support for our observed paradoxical findings from both basic biobehavioral and clinical developmental research. For example, work by Fanselow & Tighe demonstrates that the length of time between aversive shocks has a greater impact on freezing behavior in rats than does the frequency of shocks. Specifically, unpredictable, infrequent shocks result in stronger freezing behavior than regular, frequent shocks (31). This pattern of potentiated aversive learning, which is mediated by amygdala circuitry, is more generally consistent with the increased effectiveness of spaced versus massed learning (32). Thus, the observed amygdala hyper-reactivity to interpersonal threat cues in adolescents from relatively high warmth families who experience lower recent stress may reflect heightened associative learning following less-frequent, more unpredictable experiences of threat or conflict.

In parallel to this basic research, developmental clinical psychology studies have observed patterns between family functioning and threat-related biobehavioral processes similar to those we observe. For example, Andreotti et al. found that only young adults who reported lower levels of family conflict in early life exhibited increased cortisol reactivity to an acute laboratory stressor that subsequently predicted subliminal attentional bias to threat cues (33). Notably, the amygdala plays a significant role in both processing subliminal threat signals and triggering cortisol release via activation of the hypothalamic-pituitary-adrenal axis (34). More generally, parental overprotection, which may be characterized by either more restrictive and controlling (35) or indulgent

(36) parenting has been associated with later psychosocial dysfunction including depression and anxiety disorders (37). Consistent with these patterns, amygdala hyper-reactivity is a core pathophysiological observation in these disorders (38) and has emerged as a biomarker of future risk (39-42).

To our knowledge, this is one of very few studies investigating the neural correlates of normative variability in caregiving in a non-clinical sample of adolescents. In addition to our current findings, Romund et al. (6) have reported that increased maternal warmth and support are correlated with lower amygdala reactivity to fearful facial expressions amongst a healthy sample of adolescents, although they did not find an association with reactivity to angry facial expressions. The divergence of our associations with that of Romund et al. may reflect differences between broader, non-specific familial dynamics and maternal-specific behaviors, explicit modeling of recent stress, as well as differences in the task-based amygdala reactivity elicited. Of note, unlike our conservative approach using unbiased amygdala reactivity values originating from our main effects of expression contrast in our analyses of a much larger sample (232 vs. 83), Romund et al. did not find significant main effects of angry or fearful facial expressions on amygdala reactivity. Rather, they conducted more liberal correlation analyses identifying specific voxels within the amygdala that exhibited BOLD values correlated with their measures of maternal caregiving.

In another recent study, Thijssen et al. (43) reported that insensitive parenting was associated with a more mature pattern of intrinsic connectivity between the amygdala and medial prefrontal cortex, which together function to integrate and regulate emotional experiences, in healthy children. And in an earlier study, Taylor et al. (44)

reported that lower family stress during childhood was associated with lower correlated activity between the amygdala and regulatory regions of the ventrolateral prefrontal cortex during affective labeling of angry and fearful facial expressions in adulthood. Despite these differences, our findings and those of these prior studies suggest that normative variability in early life caregiving and family functioning is associated with alterations in threat-related brain function similar to those that have been reported for caregiving extremes including trauma, abuse, and neglect.

As exaggerated threat-related amygdala reactivity has been linked to a range of clinical outcomes, particularly following stressful life events (39, 45), next steps will include probing the relationship between family functioning, clinical outcomes following stressors, and amygdala function. Our initial evidence linking normative variability in caregiving with threat-related brain function can now serve as a starting point for pursuing longitudinal data analyses to investigate potential trajectories of amygdala reactivity and family functioning over time. Future research can further explore this question using whole-brain analyses, as well as examining functional connectivity between the amygdala and prefrontal cortex during the explicit regulation of negative emotions to gauge the impact of family functioning on top-down control processes.

Our study, of course, is not without limitations that can be addressed in future research. First, we relied on self-reported, retrospective measures of caregiving using a single instrument. Similarly, we assessed symptoms of anxiety and depression using self-report inventories. Thus, our findings may be subject to reporting bias and are likely more representative of the perception of events rather than of objective events. Second, we did not have an explicit measure of parental overprotection. Thus, our

speculation regarding possible links between warmth and this aspect of family functioning previously linked with sensitization and maladaptive responses to stress outcomes requires explicit testing. Third, we only evaluated amygdala reactivity to threat-related facial expressions. It is possible that normative variability in affective responsiveness may also map onto individual differences in amygdala reactivity to reward-related social signals, namely happy facial expressions, or emotionally neutral facial expressions, and that these associations may reflect additional pathways of risk or resilience to psychopathology. Lastly, TAOS is not a population representative sample but rather selected based on familial risk for depression. Future studies in diverse samples are necessary to evaluate the extent to which our current findings are present more broadly and, consequently, useful for informing risk for psychopathology more generally.

Despite these limitations, our findings suggest that greater familial warmth is associated with a paradoxical increase in amygdala reactivity to explicit signals of interpersonal threat (i.e., angry facial expressions), especially in adolescents reporting lower recent stressful life events. Higher familial warmth may exacerbate neural reactivity to interpersonal threat—particularly when coupled with fewer external stressors—by limiting opportunities for the expression and subsequent self-regulation of negative emotions. While our findings extend a rich literature on the effects of extremes in early life caregiving on emotional brain function to more subtle, normative variability they also indicate that a more careful consideration of putatively protective factors in the expression of risk-related brain function may be necessary in the absence of caregiving extremes.

Materials & Methods

Participants

Study participants were recruited into two groups: “high risk” wherein a first- and second-degree relative has a history of MDD, or “low risk” wherein no first-degree and minimal second-degree relatives (<20%) have such a history. Low risk participants further could not meet criteria for any psychiatric disorder or substance use disorder; however, a diagnosis of an anxiety disorder was permitted in the high risk participants as early-onset anxiety is frequently a precursor to depression (46, 47). Mental health in all participants was assessed through structured clinical interviews with the adolescent and parent separately using the Kiddie Schedule for affective Disorders and Schizophrenia for School-Age Children Present and Lifetime Version (48). All procedures were approved by the Institutional Review Board at the University of Texas Health Sciences Center San Antonio, and participants and their parent provided written informed assent/consent before participation. Additional recruitment details have been described in detail elsewhere (49-51).

Of the 331 participants completing the parent study, Teen Alcohol Outcomes Study, data for our present analyses were available for 232 participants (120 high risk, 112 low risk). Of these 331 participants, data were excluded for 29 due to problems with the scan or raw data, (e.g., ending the scan early, poor coverage of the amygdala, gross anatomical abnormalities, scanner artifacts). Data from another 7 high risk participants were excluded because they had a diagnosis of MDD before the scan, and from another

5 low risk participants because they had an anxiety disorder before the scan. Functional MRI data from the remaining 290 participants underwent pre-processing and, subsequently, another 53 participants were excluded based on quality control criteria for the processed data (see quality control procedures section below for further details). Lastly, data from another 5 participants were excluded for poor accuracy on the fMRI task. The final analysis sample of 232 participants was 50.4% female and approximately 58% Caucasian, 26% Hispanic, 5% African American, and 12% Indian, Asian, Pacific Islander, or multiple races. There was no difference in sex ratio ($\chi^2(1)=0.018$, $p=0.892$) or the distribution of race/ethnicity ($\chi^2(4)=2.413$, $p=0.660$) between the high risk and low risk groups.

Behavioral Measures

Family functioning was assessed using the Family Assessment Device (FAD), a 60-item scale completed by both child- and parent, which yields 7 scales of family functioning. Higher scores on each FAD scale indicate worse levels of family functioning (52, 53). Given the literature on the impact of warmth provided by caregivers, we probed this feature of caregiving on amygdala reactivity specifically using the "affective responsiveness" subscale, which measures familial emotional support, warmth, and affection, with items such as "We are reluctant to show our affection for each other" and "We express tenderness." We addressed the possible unique impact of this caregiving feature on amygdala reactivity by also examining correlations with the "general functioning" subscale, which measures global family functioning with items such as "In times of crisis we can turn to each other for support" and "We don't get along well

together." We utilized child- and not parent-reported scores from these two subscales because we expected that a child's perception of their familial environment would be more impactful than the parent's perception, and we wanted to better approximate scores from the Parental Bonding Instrument, which is a more commonly used inventory of normative caregiving that is child-reported, but unavailable in the present study.

Depression symptoms were assessed using the Mood and Feelings Questionnaire (MFQ), a widely-used measure in children and adolescents (54). We used the child-reported short (13 item) summary score in our analyses. Anxiety symptoms were assessed using the Screen for Child Anxiety Related Disorders (SCARED), a widely-used measure of anxiety symptoms in children and adolescents (55). We use the child-reported SCARED in our analyses.

Early life stress was assessed using the Childhood Trauma Questionnaire (CTQ), a widely-used measure of childhood trauma and early life stress (56). Recent stress was measured using the Stressful Life Events Schedule (SLES), an interview designed to assess for life stressors relevant to children and adolescents (57).

Functional MRI

Participants performed an emotional face matching task that has been shown to consistently elicit robust amygdala activity in numerous studies of adults and adolescents (49-51, 58). Task blocks consisted of matching angry and fearful facial

expressions while control blocks consisted of matching geometric shapes (39, 49-51). Further task details are reported in the supplement.

Analyses were conducted using the general linear model of SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Following preprocessing, linear contrasts employing canonical hemodynamic response functions were used to estimate main effects of expression for each individual. Individual contrast images were then entered in second-level random effects models to determine mean condition-specific regional responses using one-sample t-tests. We extracted parameter estimates from functional clusters within anatomically defined amygdala regions of interest (Automated Anatomical Labeling atlas) at $p<0.05$ family wise error (FWE) corrected across the search volumes for the contrast of angry blocks > control blocks and fearful blocks > control blocks (45). Further quality assurance and extraction details are reported in the supplement.

Statistical Analyses

Mean individual contrast-related BOLD activation values from functional clusters were entered into second-level analyses in SPSS, version 24 (IBM, Armonk, N.Y.). First, we ran linear regressions of the affective responsiveness and general functioning FAD subscales with extracted BOLD values for the contrasts of angry expressions greater than control and fearful expressions greater than control. We then conducted analyses to confirm that the significant association between family functioning and amygdala reactivity was not driven by early life adversity or concurrent depressive and anxiety

symptoms by duplicating regression analyses from step one, this time controlling for CTQ, MFQ and SCARED scores in addition to our other covariates. Lastly, we conducted linear regressions to investigate potential direct effects of recent stress (SLES) and interactions of SLES with FAD. We then conducted post-hoc partial correlation analyses within the high and low stress groups to test for significance of the simple slopes. In all analyses, we controlled for participant age, sex, and risk group.

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Figure Legends

Table 1. Sample Characteristics. Sample characteristics across high-and low risk groups.

Figure 1. Threat-Related Amygdala Reactivity. Mean reactivity to angry (a) and fearful (b) facial expressions within anatomically defined amygdala regions of interest (p<0.05, FWE-corrected). (a) Left: cluster size = 169 voxels, peak voxel = -22, -2, -18; Right: cluster size = 226 voxels, peak voxel = 20, -4, -16; (b) Left: cluster size = 170 voxels, peak voxel = -22, -2, -18; Right: cluster size = 224 voxels, peak voxel = 22, -4, -18. Color bar represents t-scores.

Figure 2. Family Functioning and Amygdala Reactivity. Familial warmth, as index by FAD affective responsiveness scores, is negatively correlated with left amygdala reactivity to interpersonal threat as indexed by angry facial expressions [F(1, 221)=4.305, p=0.039].

Figure 3. Family Functioning, Recent Stress, and Amygdala Reactivity. The negative correlation between familial warmth and amygdala reactivity to interpersonal threat was significant only for participants reporting relatively low levels of recent stressful life events as assessed by the SLES [F(1, 213)=4.581, p=0.033; low stress: r=-0.244, p=0.014; high stress: r=-0.052, p=0.614].

Table 1. Sample Characteristics.

	High Risk (*n*=120)		**Low Risk** (*n*=112)		**Group Comparisons**	
	Mean	**SD**	**Mean**	**SD**	***t***	***p***
Age	13.61	1.01	13.56	0.94	0.416	0.678
FAD Affective Responsiveness	16.69	2.72	16.84	2.96	0.483	0.630
FAD General Functioning	36.79	4.53	37.85	5.11	-0.795	0.427
MFQ	4.06	4.20	2.96	2.60	2.405	0.017*
SCARED	16.64	11.46	15.12	8.51	1.148	0.252
CTQ	34.55	6.85	32.59	6.01	2.228	0.027*
SLES	13.40	10.22	12.20	8.44	1.234	0.219
Mean Accuracy (%)	95.72	17.42	96.23	15.92	-0.237	0.818
Mean Response Time (ms)	1288.61	240.85	1306.66	245.35	-0.522	0.582
Mean Head Displacement (mm)	0.04	0.01	0.04	0.02	-0.609	0.543

Figure 1

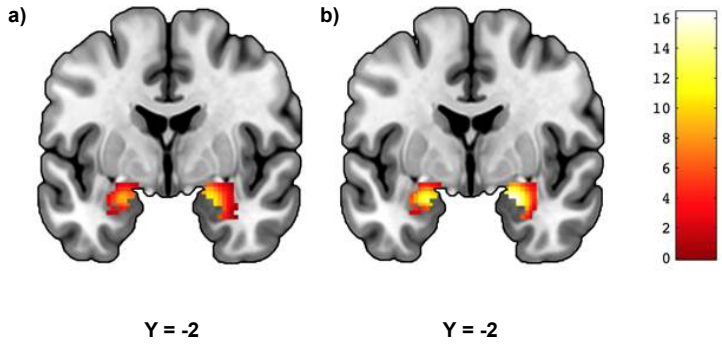

Figure 2

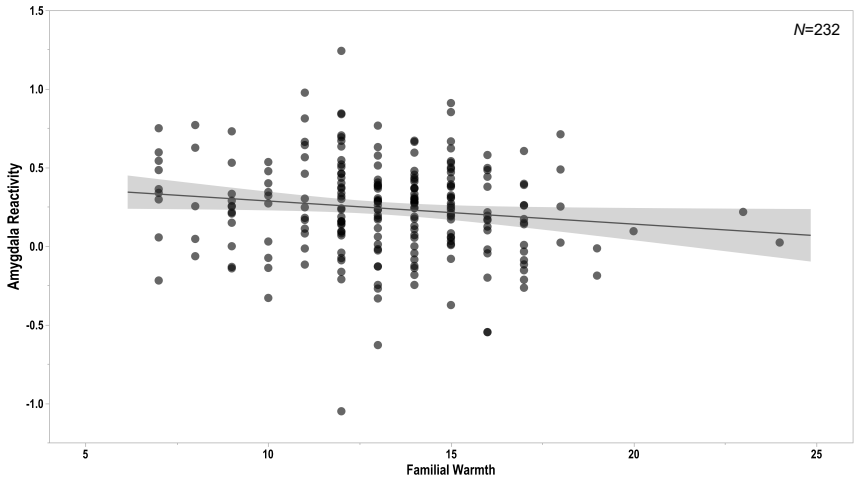
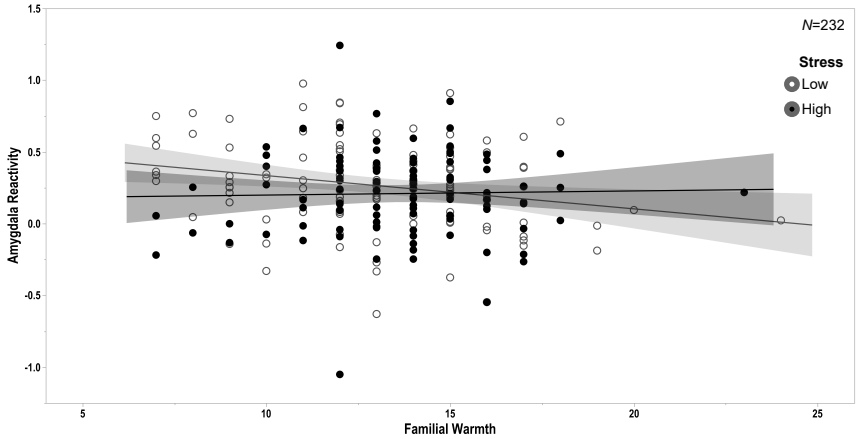
Figure 3