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Chronic Stress May Amplify Gender/Sex Differences in Amygdala Reactivity to Ambiguous Emotional Stimuli

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ABSTRACT

Heightened reactivity to ambiguous emotional stimuli, such as surprized faces, is a transdiagnostic psychopathology risk factor. Women show elevated amygdala activation to ambiguous emotional stimuli relative to men, which may underlie their significantly higher risk for mood disorders. Moreover, there are sex/gender differences in the effects of stress on both emotion processing and emotion regulation, with greater impact of stress on negative emotionality in females. We predicted that chronic stress would be associated with stronger amygdala activation to surprized faces, and these effects would be amplified in girls/women. We tested the interactions of chronic stress and gender/sex on amygdala activity in a sample of 297 healthy participants (59% girls/women, age range 6–75 years) who provided a saliva sample and who viewed emotional face stimuli while undergoing functional neuroimaging. Saliva samples were assayed for two markers of chronic stress: Epstein-Barr Virus (EBV) expression and diversity of *Lactobacilli* species. Among girls/women, higher chronic stress was associated with greater amygdala activation to ambiguous emotional images than lower stress exposure, although this effect was not statistically significant. Counter to predictions, the reverse effect was found among boys/men (i.e., higher stress exposure was associated with *lower* amygdala activation). Results were similar across left and right amygdalae, and across both stress measures. Although our findings are preliminary and should be replicated, they align with findings on gender differences in valence bias, and broadly support the hypothesis that there are gender/sex differences in the effects of chronic stress on neural reactivity to emotional ambiguity, particularly in areas of the brain sensitive to emotion regulation. Possibly, stress does not universally increase negativity, but instead amplifies default emotional biases—which for boys/men, may result in *less* amygdala reactivity.

1 | Introduction

Emotional ambiguity is an ever-present part of life: a friend's blank face might indicate boredom with your story, or breathless anticipation of your every word. While a moderate degree of autonomic and neural reactivity to ambiguous stimuli can help orient attention and facilitate processing, significantly elevated physiologic reactivity to emotionally ambiguous stimuli is associated with greater intolerance of uncertainty (Tanovic et al. 2018) and negative interpretation biases (Collins et al. 2022; H. Kim et al. 2003). This pattern, in turn, contributes to both development and maintenance of mood and anxiety disorders (Würtz and Sanchez-Lopez 2023). Understanding individual differences in reactivity to emotional

stimuli may thus aid in tailoring transdiagnostic clinical interventions.

In the brain, amygdala reactivity is linked to responses to emotional stimuli, including emotionally ambiguous facial expressions (H. Kim et al. 2003; Whalen et al. 2001). In particular, this amygdala reactivity is greater in magnitude (M. J. Kim et al. 2017; Petro et al. 2021) and duration (i.e., more persistent amygdala activation (Petro et al. 2018) when emotional ambiguity is viewed as having a more negative than positive meaning. On the other hand, a more positive interpretation is associated with greater prefrontal activity (e.g., H. Kim et al. 2003; Petro et al. 2021; Petro et al. 2018), a pattern typically associated with emotion regulation (Ochsner et al. 2012; Sun et al. 2023; Wager et al. 2008). Because stress appears to exacerbate negative interpretations of ambiguity, possibly via interference with emotion regulation processes (C. C. Brown et al. 2017; Neta et al. 2017), amygdala reactivity to ambiguous stimuli may be similarly exacerbated in the context of stress. Separately, much research documents that exposure to chronic stress amplifies amygdala response to overt (i.e., unambiguous) negative emotional stimuli, likely through impaired emotion regulation (for review, see Kredlow et al. 2022). In other words, acute and social stress may impede emotion regulation processes and amplify the tendency to default to negative interpretations of emotional ambiguity (C. C. Brown et al. 2017; Raio et al. 2021), leading to greater amygdala response to emotionally ambiguous stimuli (in line with those seen to overtly negative stimuli). In the present study, we compared the effects of chronic stress on both emotionally ambiguous stimuli (surprized faces) and overtly negative stimuli (fear faces).

Despite an ample literature on the effects of gender/sex[1] on stress response (Koenig and Thayer 2016; J. J. Liu et al. 2017), there has been limited research on the interaction of gender/sex and stress on either predicting reactivity to emotional ambiguity or in interfering with emotion regulation processes. Girls/women are generally socialised to show greater emotionality (particularly greater sadness and anxiety) in response to stress, and to attend more to emotional information—particularly social cues such as faces—relative to boys/men (Brody and Hall 2010). Separately, some studies have found women were more likely than men to interpret ambiguous emotional information as negative (Bento de Souza et al. 2014; Clinchard et al. 2024; Gohier et al. 2013), although these studies did not examine stress. Women show greater sensitivity to subtle negative facial expressions than men, but these differences drop out at higher levels of overt emotional display (Hoffmann et al. 2010), suggesting heightened responsiveness to emotional *ambiguity* specifically. However, one study found that acute stress eliminated women's relative advantage in identifying negative facial expressions under conditions of uncertainty (DeDora et al. 2011), highlighting how gender/sex differences in emotional response to ambiguity and emotion regulation may depend on exposure to stress.

Women also report a greater self-reported negativity and show greater physiologic arousal during negative emotions, including elevated sympathetic activity during sadness (Kelly et al. 2008), and elevated amygdala activation as well as depressed vagal tone during negative, but not positive emotions (Min et al. 2023). Other studies have demonstrated sex/gender differences in the neural networks associated with stress reactivity, with men showing greater response to stress in prefrontal cortex regions but women showing greater response in limbic/striatal regions including the amygdala (Goldfarb et al. 2019; Kogler et al. 2015). Taken together, these findings suggest that girls/women may experience stronger effects of stress on the neural response to ambiguous emotional information. In the present study, we conducted a secondary analysis of data collected for another study to examine interactions of gender/sex and putative biomarkers of chronic stress on amygdala activation to ambiguous emotional stimuli in a large sample of healthy participants.

We measured the robustness of effects across two different measures of chronic stress: detection of Epstein-Barr virus (EBV) and diversity of Lactobacilli species. Expression of herpesviruses such as EBV is a well-validated marker of exposure to psychosocial stress that has been used for over 40 years (DeCaro and Helfrecht 2022). Most people are exposed to these viruses at an early age and continue to carry a latent infection into adulthood (Dowd et al. 2013; Panter-Brick et al. 2020; Schmeer et al. 2019). Typically, the immune system can suppress latent EBV into dormancy to the point of non-detection. However, under periods of significant stress, the immune system's ability to suppress the virus is taxed and thus viral load increases to detectable levels (DeCaro and Helfrecht 2022). Salivary measures of EBV shedding are particularly good for capturing chronic stressors such as exposure to discrimination (Ford et al. 2021), with evidence of discriminant validity in children as young as 6 years (Dowd et al. 2013; Schmeer et al. 2019). While EBV indexes exposure to chronic stress equally well across genders/sexes (Panter-Brick et al. 2020), there is one study showing tighter associations between EBV measures and depressive symptoms in adolescent girls versus boys, which authors speculate may arise from boys' greater use of avoidance-related emotion regulation strategies (Ford and Stowe 2017).

Diversity of microbial species in saliva is a relatively newer measure of stress. Much of the recent work links the oral microbiome to a variety of mental and physical health conditions (Kohn et al. 2020) with particular focus on the beneficial effects of species within the Lactobacilli genus (L. Liu and Zhu 2018; R. T. Liu et al. 2019). While there is no consensus on whether the presence or absence of any individual microbial species contributes to affective processes, there is strong evidence that having a range of Lactobacilli species (hereafter referred to as microbial diversity) is associated with resilience to both acute stressors (Keskitalo et al. 2021; Langgartner et al. 2020) and chronic stress (Charalambous et al. 2024). In contrast, low microbial diversity is thought to be a marker of chronic stress, as exposure to stress processes (such as elevated cortisol and inflammation) creates an environment that is inhospitable to many Lactobacilli species (An et al. 2024). Correspondingly, low microbial diversity in saliva is associated with mood pathology including increased risk of depressive disorders (Aleti et al. 2022; Du et al. 2020) and mood disturbances in other disorders such as schizophrenia (Qing et al. 2021) and Post-Traumatic Stress Disorder (Levert-Levitt et al. 2022). Of particular relevance to the present study, a recent study in a sample of depressed volunteers found that increasing

TABLE 1 | Demographics.

	Boys/men (n = 121)		Girls/women (n = 176)		Total sample (n = 297)	
	M	**SD**	***M***	**SD**	***M***	**SD**
Age	22.8	18.1	31.6	22.4	28	21.2
	n	**%**	***n***	**%**	***n***	**%**
Age group						
Child (< 17)	68	56	75	43	143	48
Younger adult (17–30)	43	36	41	23	84	28
Older adult (> 30 years)	10	8	60	34	70	24
Race/ethnicity						
White non-Hispanic/Latino/a	86	71	138	78	224	75
White Hispanic/Latino/a	8	7	10	6	18	6
Asian non-Hispanic Latino/a	6	5	8	5	14	5
Mixed-race non-Hispanic Latino/a	6	5	9	5	15	5
Black non-Hispanic/Latino/a	9	7	4	2	13	4
Mixed race Hispanic/Latino/a	4	3	4	2	8	3
Unknown race/ethnicity	2	2	2	1	4	1
EBV detected	64	53	90	51	154	52
	M	**SD**	***M***	**SD**	***M***	**SD**
Microbial diversity score*	1.06	0.89	0.98	0.92	1.01	0.91

Abbreviations: EBV = Epstein barr virus; M = mean; SD = standard deviation.
*Microbial diversity was calculated by the number of unique Lactobacilli species detected.

microbial diversity through probiotic supplementation was associated with improvements in ability to identify facial emotions, and that these improvements were associated with decreases in depression symptoms (Baião et al. 2022). To our knowledge, there has been no work documenting sex/gender differences in the association between oral microbiome diversity and stress.

In sum, based on the above literatures, we predicted a priori that chronic stress (presence of EBV; low microbial diversity) would be associated with greater amygdala activity in response to ambiguous emotional images (i.e., in line with response to overtly negative emotional images), particularly in girls/women.

2 | Methods

2.1 | Participants

We recruited 297 participants from the Lincoln and Omaha community as part of a larger ongoing project (Harp, Nielsen, et al. 2024; Petro et al. 2021; Petro et al. 2018; Petro, Tottenham, et al. 2021; Pierce et al. 2022; Pierce et al. 2024; Pierce et al. 2024) examining valence bias across the lifespan (age range, 6–80 years; average = 28.00 years, SD = 21.20 years). Briefly, this larger project involves collection of resting-state and task-based functional MRI in a lifespan sample. Participants were all right-handed and none were taking psychotropic medications. Participants self-reported demographics (including gender/sex) and baseline characteristics are presented in Table 1. All participants (and their legal guardian, when applicable) confirmed their understanding of the procedures and provided assent/consent. All procedures were approved by the University of Nebraska-Lincoln Institutional Review Board.

2.2 | Face Viewing Task and Emotional Stimuli

Participants completed a passive face viewing task, including surprized, fearful, and neutral expressions described in prior work (Petro et al. 2018). The face stimuli were selected from the Umeå University Database of Facial Expressions (4 male, 4 female; Samuelsson et al. 2012). As in prior work, blocks contained 32 pseudorandom presentations of faces shown for 200 ms, followed by a 300 ms fixation cross (M. J. Kim and Whalen 2009). Blocks were separated by 14 s fixation. Each of the two functional runs included six blocks of faces: three emotion blocks (surprized or fearful) and three neutral blocks, with block order counterbalanced across participants.

2.3 | Magnetic Resonance Imaging Acquisition and Processing

Data were collected on a 3T Siemens Skrya scanner in the Center for Brain Science at UNL. The structural scan used a T1-weighted MPRAGE sequence (TR = 2.2 s, TE = 3.37 ms, slices = 192 interleaved, 1 mm isotropic voxel size, FOV = 256 mm, flip angle = 7°, total acquisition time = 5:07). Functional scans used a T2-weighted EPI sequence with one of

two protocols: (1) TR = 2.5 s, TE = 30 ms, slices = 42, voxel size = 2.5 × 2.5 × 3.0 mm, matrix = 88 × 88 mm, FOV = 220 mm, flip angle = 80°, total acquisition time = 3:24; (2) (TR = 1.0 s, TE = 30 ms, slices = 51, voxel size = 2.5 mm^3, matrix = 84 × 84 mm, FOV = 210 mm, flip angle = 41°, multiband factor = 3, total acquisition time = 3:42).

2.4 | Preprocessing

Preprocessing was completed with MATLAB (Mathworks, Natick, MA) and AFNI (Cox 1996). We modelled the data as in prior work (Petro et al. 2018). Briefly, we removed the first 10 acquired volumes, aligned the functional to the anatomical image, standardized to Talairach atlas space (TT_N27), applied a smoothing kernel (FWHM 6 mm) and the non-linear warp option to improve the fit for individuals of different ages. Time points with head motion greater than 0.9 mm were censored in the general linear model and the six motion estimates for each TR (x, y, z shift/rotation) from the alignment step were included as regressors of no interest. The task-related regressors were entered for each facial expression (fearful, surprized, neutral).

2.5 | Defining Regions of Interest (ROI)

Given the above-mentioned prior work demonstrating a relationship between stress, emotional reactivity and emotion regulation, we defined two clusters in left (Talairach: $X = 20$, $Y = 2$, $Z = 13$; 17 voxels) and right (Talairach: $X = 32$, $Y = 2$, $Z = 16$; 62 voxels) amygdala as functional regions of interest from prior work on emotion regulation (Petro et al. 2018). Task activation from the current face viewing task was extracted from these amygdala ROIs for each participant using the comparison of surprized versus neutral expressions, and fearful minus neutral expressions. An additional functional ROI, derived from the current participant's activation to all face stimuli versus baseline, was tested as a robustness analysis; as the findings from these parallel analyses overlapped substantially with those reported here, they are presented in Supporting Information S1: Appendix A.

2.6 | Stress Biomarker Collection and Analysis

Participants provided 1 mL of saliva via passive drool into Oragene DNA collection tubes (DNAgenotek LLC, Ontario). Oragene tubes are preloaded with reagents that minimise DNA breakdown and bacterial activity in salivary samples, and can be stored at room temperature for up to 5 years without degradation of the sample's genetic material (Iwasiow et al. 2011; Nunes et al. 2012).

2.7 | Multiplex PCR Genotyping for EBV and Lactobacilli Species in Saliva

Saliva samples were visually evaluated prior to DNA isolation. Nine samples were excluded from analysis due to low sample volume or impurities, as indicated by discolouration suggesting possible blood contamination; data from these participants was excluded from further analysis. DNA was extracted from saliva samples using a Qiagen blood and tissue kit (Redwood, CA) according to manufacturer protocols. DNA concentrations were determined using a nanodrop 1000 spectrophotometer. At least 50 ng of DNA was used for each subsequent PCR analysis. The bacterial multiple primers were as follows: *Lactobacillus. gasseri* primers; forward:5′-AGCGACCGAGAAGAGAGAGA-3′ and reverse: 5′-TGCTATCGCTTCAAGTGCTT-3′, giving a product of 360 base pairs (bp), *Lactobacillus. Iners* primers; forward: 5′-GTCTGCCTTGAAGATCGG-3′ and reverse: 5′-ACAGTTGA-TAGGATCATC-3′, giving a product of 158 bp, *Lactobacillus helveticus* primers; forward: 5′-CTACTTCGCAGGCGTTAACT-3′ and reverse: 5′-GTACTTGATGCTCGCATACC-3′, giving a product of 132 bp, and *Lactobacillus* casei primers; forward: 5′-CCACAATCCTTGGCTGTTCT-3′ and reverse: 5′-GCTTGAGGC GATTGTAATCC-3′, giving a product of 115 bp.

This multiplex PCR assay was performed using Qiagen reagents under the following temperature cycling conditions: 94°C for 10 min pre-denaturation, followed by 40 cycles of: 94°C for 30 s, 60°C for 30 s, 72°C for 30 s, and 72°C for 5 min final extension (E. Kim et al. 2020; Yeruva et al. 2017). EBV DNA was detected in a separate reaction under similar PCR conditions described previously (Julius et al. 2022). For quality control purposes, PCR assays were performed with negative and positive control DNAs for each species. Control DNAs were titrated to determine the lower limits of quantitative detection of each species, which was between 1–10 copies of DNA per reaction.

2.8 | Analytic Plan

We used linear models to examine interactions of gender/sex and chronic stress in predicting amygdala activation to surprized faces (minus activation to neutral faces), with separate models for each hemisphere (R vs. L amygdala) and each marker of chronic stress. As a parallel analysis examining reactivity to overtly (i.e., unambiguous) negative stimuli, we repeated these models with amygdala activation to fearful faces (minus activation to neutral faces). Given prior work documenting substantial effects of age on processing of emotional ambiguity (Petro et al. 2021), all analyses controlled for age. Following recommendations for modelling complex interactions (Aiken et al. 1991), we limited interpretation of effects to the highest order interaction term that showed evidence for significance ($p < 0.10$) and used pairwise contrasts to evaluate direction of significant interactions. All analyses were conducted in R (version 4.3.2).

3 | Results

3.1 | Amygdala Activation to Emotionally Ambiguous (Surprize) Stimuli

The interaction between stress and gender/sex was significant (EBV model: $F(1,292) = 9.71$, $p = 0.002$, $d = 0.258$; Figure 1) or marginally significant (microbial diversity model: $F(1,292) = 3.21$, $p = 0.074$, $d = 0.149$; Figure 2) in predicting left

FIGURE 1 | Gender/sex and EBV shedding interact to predict left amygdala activation to surprized faces. Left amygdala activation to surprize faces, minus activation to neutral faces. Boxes represent interquartile range, wings represent distribution of data, and stars represent significant specific contrasts. The interaction between stress and gender/sex was significant, $F(1,292) = 9.71$, $p = 0.002$.

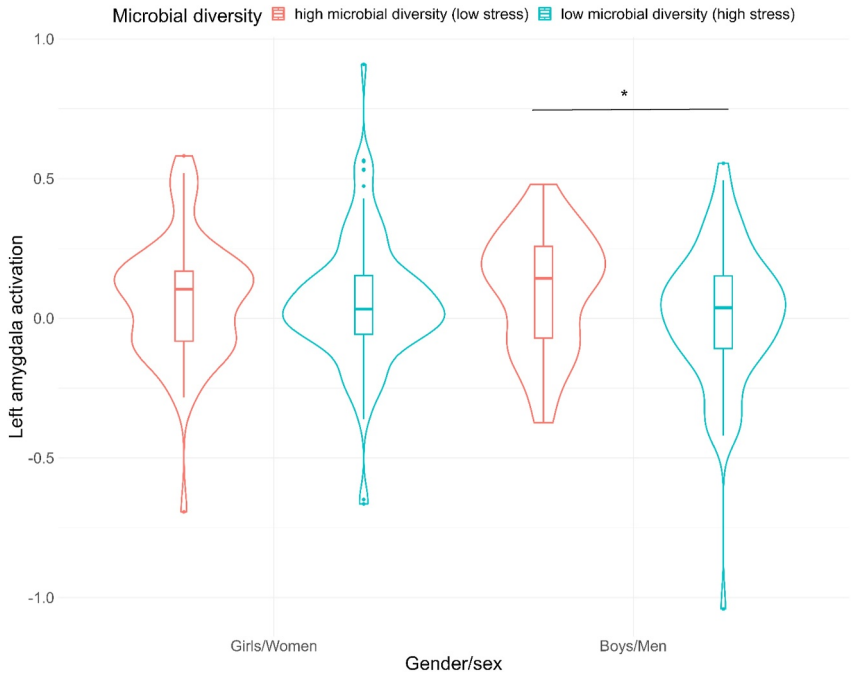

FIGURE 2 | Gender/sex and microbial diversity shedding interact to predict left amygdala activation to surprized faces. Left amygdala activation to surprize faces, minus activation to neutral faces. Boxes represent interquartile range, wings represent distribution of data, and stars represent significant specific contrasts. The interaction between stress and gender/sex was marginally significant, $F(1,292) = 3.21$, $p = 0.074$, $d = 0.149$.

amygdala activation to surprized (vs. neutral) faces. Follow-up pairwise contrasts revealed that among girls/women, participants with higher stress had greater left amygdala activation than those with lower stress, although this effect was not significant (EBV model: $t(292) = -1.58$, $p = 0.115$, $d = 0.131$; microbial diversity model: $t(292) = 0.55$, $p = 0.582$, $d = 0.046$). Among boys/men, participants with higher stress had significantly *lower* left amygdala activation to surprized faces than those with lower stress (EBV model: $t(292) = 2.74$, $p = 0.006$, $d = 0.227$; microbial diversity model: $t(292) = 2.08$, $p = 0.038$, $d = 0.172$).

Findings in the right amygdala were similar to the left. The interaction between stress and gender/sex was significant in

predicting right amygdala activation to surprized faces for both markers of stress (EBV model: $F(1,292) = 5.32$, $p = 0.022$, $d = 0.191$; microbial diversity model: $F(1,292) = 4.16$, $p = 0.042$, $d = 0.169$). As before, pairwise contrasts revealed that among girls/women, higher stress predicted greater right amygdala activation but effects were not significant (EBV model: $t(292) = -1.28$, $p = 0.200$, $d = 0.106$; microbial diversity model: $t(292) = 0.60$, $p = 0.547$, $d = 0.050$). Among boys/men, higher stress was associated with lower right amygdala activation for the EBV measure, although the effect was only marginally significant for EBV ($t(292) = 1.94$, $p = 0.053$, 0.161) and not significant for microbial diversity ($t(292) = -1.41$, $p = 0.158$, 0.117).

3.2 | Amygdala Activation to Unambiguously Negative (Fearful) Stimuli

When considering left amygdala activation to fearful (unambiguously negative) faces, the interaction between stress and sex/gender was significant when considering EBV ($F(1,292) = 6.75$, $p = 0.009$, $d = 0.215$) but not microbial diversity ($F(1,292) = 0.96$, $p = 0.755$, $d = 0.081$). Follow-up pairwise contrasts revealed that among girls/women, participants showing EBV shed (i.e., higher stress) had greater left amygdala activation to fearful faces than those without EBV shedding, although this effect was not significant ($t(292) = -1.50$, $p = 0.134$, $d = 0.124$). As with the effects for surprized faces, however, among boys/men, participants with EBV shed had significantly *lower* left amygdala activation to fearful faces than those without EBV shed ($t(292) = 2.13$, $p = 0.037$, $d = 0.176$; Figure 3).

When considering right amygdala activation, the interaction between stress and sex/gender dropped to either marginal significance (EBV model: $F(1,292) = 3.84$, $p = 0.051$, $d = 0.162$) or non-significance (microbial diversity model: $F(1,292) = 0.44$, $p = 0.505$, $d = 0.055$). As with the above analyses, among boys/men, participants with EBV shed showed weaker right amygdala activation to fearful faces than those without EBV shed, although this difference was not significant ($t(292) = 1.86$, $p = 0.070$, $d = 0.154$; Figure 4).

4 | Discussion

This secondary analysis examined the ways that gender/sex and chronic stress may interact to predict neural processing of ambiguous emotional stimuli. In line with our predictions, among girls/women, biomarkers of chronic stress were associated with (non-significantly) greater amygdala activation to surprized faces. Counter to predictions, however, among boys/men, biomarkers of stress were associated with significantly *lower* amygdala activation to surprized faces. Prior research has suggested that stress may increase the automaticity of emotion processing, which for boys/men, may actually reflect lower physiologic reactivity and less negative valence bias (Bento de Souza et al. 2014; Clinchard et al. 2024; Gohier et al. 2013; Lithari et al. 2010). This, in turn, suggests that chronic stress may act on emotional biases by increasing the *automaticity* of emotion processing of ambiguous emotional stimuli, freeing up cognitive resources to address the stress instead. In partial support of this interpretation, we also found lower amygdala activation to unambiguously negative (fearful) faces among boys/men without EBV shed (i.e., lower stress); however, given these effects did not converge across markers of stress, this interpretation should be considered tentative until replicated independently.

We anticipated greater amygdala activation to surprized faces among individuals experiencing chronic stress (i.e., responses

FIGURE 3 | Gender/sex and EBV shedding interact to predict left amygdala activation to fearful faces. Left amygdala activation to fearful faces, minus activation to neutral faces. Boxes represent interquartile range, and wings represent distribution of data. The interaction between stress and gender/sex was significant, $F(1,292) = 6.75$, $p = 0.009$.

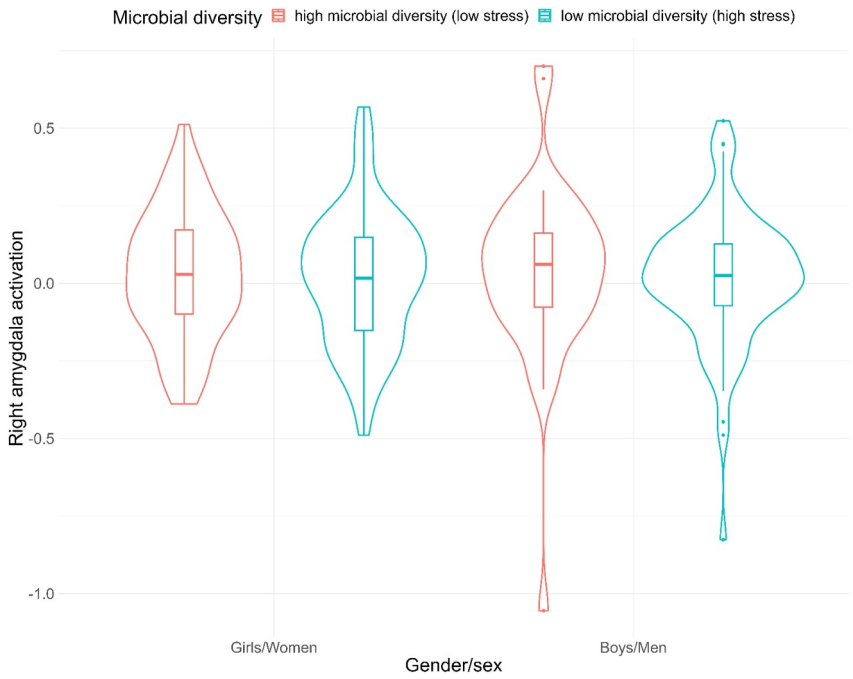

FIGURE 4 | Gender/sex and EBV shedding interact to predict right amygdala activation to fearful faces. Right amygdala activation to fearful faces, minus activation to neutral faces. Boxes represent interquartile range, and wings represent distribution of data. The interaction between stress and gender/sex was marginally significant, $F(1,292) = 3.84$, $p = 0.051$.

more typical when viewing overtly negative emotional stimuli); however, indices of chronic stress were associated with *lower* amygdala activation to both surprize and fear faces in boys/men. One explanation for this pattern is that, although acute stress may increase amygdala activation relative to one's baseline state, and this change can be associated with increases in inflammatory markers (e.g., IL-6; Muscatell et al. 2015), chronic stress may result in a state of blunted reactivity (and thus lower reactivity for stressed than non-stressed persons), at least in boys/men. This is consistent with evidence from patients with treatment-resistant depression, for example, who show blunted amygdala activation during affect labelling relative to control participants (Ferri et al. 2017).

These findings also contribute to the literature on gender/sex differences in emotional valence bias. Negative valence bias—the tendency to interpret ambiguous emotional stimuli as negative—is a transdiagnostic risk factor linked to more severe mood pathology such as depression, anxiety and PTSD symptoms (Clinchard et al. 2024; Harp et al. 2023; Neta and Brock 2021). Negative valence bias is thought to be an automatic or default initial negative response (Petro et al. 2018), which can be overcome through the use of emotion regulation strategies, such as cognitive reappraisal (Harp, Gross, et al. 2024) or mindfulness (Harp et al. 2022). Although valence bias is a relatively stable trait within individuals (Harp et al. 2022; Neta et al. 2009), there is some work suggesting that stress may amplify negative valence bias by prioritising default or automatic emotional responses (C. C. Brown et al. 2017; Neta et al. 2017). Stress may also influence threat perception of emotional stimuli (Ali et al. 2020) and lower cognitive resource availability (Boals and Banks 2012), further exacerbating negativity bias. Supporting this view, the effects of both acute (lab-based) stressors and chronic stress on valence bias appear to be strongest for participants with low cognitive reappraisal skills—that is, individuals for whom reappraisal is most costly (Raio et al. 2021). Because stress tends to promote a more default or automatic response to emotionally ambiguous stimuli (C. C. Brown et al. 2017; Neta et al. 2017), the present findings provide some insight into targets for further investigation of the neural basis of previously reported gender/sex differences in valence bias (Bento de Souza et al. 2014; Clinchard et al. 2024; Gohier et al. 2013).

Speculatively, the present findings suggest that the mechanism underlying interventions for which stress influences mood pathology may differ across gender/sex, with stress reduction leading to less negative reactivity in women but not men. Some work does suggest gender/sex differences in the mechanisms underlying improvement in mood symptoms following mindfulness-based stress reduction programs, with women's improvement stemming from stress reduction but men's improvement more closely tied to reductions in emotion suppression (M. M. Brown et al. 2020; Reangsing et al. 2022). However, further research replicating our findings in a clinical population would be needed to confirm this hypothesis.

Strengths of the present study include patterns of convergence in findings across multiple biomarkers of chronic stress; a sample with a wide age range spanning from young children to older adults—a diversity not often represented in research on the mechanisms underlying mood pathology; and use of a well-validated measure of neural processing of ambiguous emotional information, namely, response to surprised faces. But there were also limitations, including the lack of racial/ethnic diversity in this sample, which may limit participant's exposure to stress (particularly discrimination stress) and minimise the detection of associations between stress and negative emotionality (Deckard et al. 2023).

In sum, we examined the intersection of gender/sex and chronic stress on amygdala activation to ambiguous emotional stimuli in a large lifespan sample, using two unique biomarkers of chronic stress. Results across stress markers converged, with higher stress associated with weaker amygdala activation to ambiguous emotional stimuli in boys/men, but not girls/women. While effects were not fully replicated in models of *unambiguously* negative emotional stimuli, there were similar patterns; tentatively, this suggests that stress may increase automaticity of negative valence bias in boys/men. These preliminary findings warrant replication and extension, to see if the observed effects contribute to the substantial gender/sex differences observed across mood disorders and if findings translate to a clinical population.

Acknowledgements

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Demographics and neuroimaging data supporting this project are available on the National Institute of Mental Health Data Archive at https://nda.nih.gov/edit_collection.html?id=2714. Salivary stress markers data are available at https://osf.io/d2a9w/?view_only=f0091b1bf74446d9bba3663b4640dbc9. These datasets can be linked via a shared participant ID.

Endnotes

[1] While the effects of sex and gender are distinct, the empirical literature on emotion processing has historically conflated the two and rarely measures, let alone reports on, the unique effects of sex versus gender. Moreover, there is some question as to whether such effects can be meaningfully distinguished in neuroimaging research, as a lifetime of gender socialisation can shape neuroanatomy and physiology as readily as gonadal hormones or chromosomes (Eliot et al. 2023). As such, in the present manuscript we refer to 'gender/sex' as a construct that potentially spans embodied gender and socially constructed sex (Fausto-Sterling 2019), and fully acknowledge the flaws inherent in a binary conceptualization of either.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.