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Original Article

A Descriptive Observation of Brain Region Involvement During Unclear Stress Responses Across Heterogeneous Populations

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ABSTRACT

Background: Stress experienced without a clearly identifiable precipitating event may involve altered engagement of neural circuits responsible for emotional salience, cognitive regulation, and physiological stress processing. Objective: To characterize regional brain activation during an ambiguous stress condition and examine its associations with perceived stress and salivary cortisol in adults. Methods: This cross-sectional task-based functional magnetic resonance imaging study included 60 adults aged 18–50 years from the Islamabad–Rawalpindi region. Perceived stress was assessed using the PSS-10, anxiety and depressive symptoms using HADS, and physiological assessment included resting heart rate and morning salivary cortisol. Participants underwent 3-Tesla functional magnetic resonance imaging during an ambiguous stress-induction task incorporating social-evaluative uncertainty and cognitive interference. Regional BOLD responses were evaluated in the amygdala, anterior cingulate cortex (ACC), prefrontal cortex, hippocampus, and insula. Pearson correlations and independent-samples t-tests were applied. Results: Mean ACC activation was 0.91 ± 0.25%, followed by amygdala activation at 0.84 ± 0.22%. PSS-10 scores correlated positively with amygdala activation (r=0.46, p=0.001) and ACC activation (r=0.41, p=0.002) and inversely with prefrontal activation (r=−0.31, p=0.017). Cortisol correlated positively with ACC activation (r=0.39, p=0.003). Females demonstrated higher amygdala activation than males (0.92 ± 0.20% vs 0.78 ± 0.21%; p=0.014). Conclusion: Perceived stress under uncertain conditions was associated with differential engagement of limbic, cingulate, and prefrontal regions, with convergent associations across subjective, biochemical, and neural measures. These findings represent cross-sectional neural correlates and should not be interpreted as causal or predictive biomarkers. Keywords: Anterior Cingulate Cortex; Amygdala; Cortisol; Functional Magnetic Resonance Imaging; Perceived Stress; Prefrontal Cortex; Stress Response.

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INTRODUCTION

Stress is a multidimensional psychobiological process through which environmental or internal demands are appraised and translated into behavioural, autonomic, endocrine, and neural responses. Acute stress may facilitate vigilance and adaptive responding, whereas persistent, unpredictable, or poorly regulated stress can interfere with emotional regulation, cognition, and physiological homeostasis (1,2). Neuroimaging research has identified a distributed stress-processing network involving the amygdala, anterior cingulate cortex (ACC), prefrontal cortex, hippocampus, insula, and interconnected regulatory systems that contribute to threat appraisal, salience detection, emotional processing, autonomic regulation, and hypothalamic–pituitary–adrenal axis activity (2,3). Within this network, the amygdala is particularly responsive to emotionally salient and potentially threatening information, whereas prefrontal

regions contribute to top-down modulation of limbic responses and the ACC participates in conflict monitoring, emotional appraisal, and integration of cognitive and autonomic information (2,3).

The neural response to stress is influenced not only by the magnitude of an external challenge but also by its predictability, controllability, and subjective interpretation. Individuals may experience substantial psychological tension despite being unable to identify a single precipitating event, particularly when stress arises from accumulated demands, uncertainty, conflicting information, or persistent anticipation of adverse outcomes. Such experiences can be conceptualized as ambiguous or poorly defined stress, in which the perceived state of stress is evident but its immediate external trigger is uncertain or diffuse. Contemporary models of stress neurobiology suggest that uncertainty and impaired predictability can maintain heightened salience processing and alter the balance between limbic responsivity and prefrontal regulation (1,3). Changes within these circuits have also been associated with differences in subjective stress perception and emotional functioning, indicating that perceived stress may reflect variation in the neural processing of environmental demands rather than exposure to an identifiable stressor alone (3–5).

The amygdala and ACC are particularly relevant to the neural processing of uncertain or emotionally salient conditions. Heightened amygdala responsivity has been observed in association with increased stress perception and altered prefrontal–amygdala circuitry, whereas the ACC contributes to appraisal, conflict processing, autonomic integration, and regulation of stress-related responses (2–4). Conversely, effective engagement of prefrontal systems is considered important for modulation of emotional salience and maintenance of adaptive cognitive control during stressful experiences (1,3). Dysregulation across these interconnected regions has therefore been proposed as one mechanism through which persistent stress may influence affective and cognitive functioning, although the magnitude and direction of regional responses vary according to the nature of the stressor, individual characteristics, and experimental context (1,5).

Stress-related neural activity is also closely connected with physiological and endocrine responses. Activation of the hypothalamic–pituitary–adrenal axis results in cortisol secretion, and variation in cortisol responsivity has been associated with activity within cortical and limbic structures involved in emotional processing and stress regulation (2,4). Integrating neuroimaging with subjective and biochemical measures may therefore provide a more comprehensive characterization of stress than relying on a single assessment domain. Nevertheless, many neuroimaging investigations have examined clearly identifiable psychosocial, emotional, physical, or cognitive stressors, while comparatively less attention has been given to situations in which individuals experience stress without attributing it to a discrete precipitating event (2,5,6). This distinction is relevant because diffuse and uncertain stress experiences may occur frequently in complex urban environments where multiple simultaneous demands contribute to subjective psychological burden.

The clinical and scientific importance of examining such responses lies in determining whether variation in perceived stress is accompanied by measurable differences within established neural stress-processing systems. Previous evidence indicates that greater perceived stress can be associated with alterations in prefrontal–amygdala functional circuitry, while cortisol responsivity may covary with neural activity in regions involved in emotional processing and stress regulation (3,4). However, relatively few investigations have combined subjective stress assessment, screening for anxiety and depressive symptoms, physiological monitoring, salivary cortisol measurement, and task-based functional magnetic resonance imaging within the same heterogeneous community sample. A multidimensional approach may help clarify whether self-perceived stress under uncertain conditions corresponds with coordinated variation in limbic, paralimbic, and regulatory brain regions rather than representing a purely subjective psychological phenomenon.

Accordingly, the present study examined regional brain activation during a task-based ambiguous stress condition among adults from the Islamabad–Rawalpindi urban region. The study focused on predefined stress-related regions comprising the amygdala, ACC, prefrontal cortex, hippocampus, and insula and evaluated their relationships with perceived stress and salivary cortisol. It was hypothesized that greater

perceived stress would be positively associated with activation of the amygdala and ACC and inversely associated with prefrontal cortical activation, consistent with differential engagement of limbic and regulatory components of the stress-processing network (2–4). The objective was therefore to characterize patterns of regional brain involvement during ambiguous stress exposure and to examine associations between these neural responses and subjective and biochemical indicators of stress in a heterogeneous adult population.

MATERIALS AND METHODS

This cross-sectional task-based functional magnetic resonance imaging study was conducted over a four-month period in the Islamabad–Rawalpindi region of Pakistan. The study incorporated a single-session assessment framework in which psychological, physiological, biochemical, and functional neuroimaging measures were obtained to characterize regional brain responses during an experimentally administered ambiguous stress condition. Participants were recruited from an urban population through a tertiary-care teaching hospital with functional neuroimaging facilities and affiliated community recruitment sites. The Islamabad–Rawalpindi setting was selected to provide access to adults with varied educational and occupational backgrounds and thereby permit assessment of interindividual variation in perceived stress and neural responses.

A total of 60 adults aged 18–50 years were enrolled. The sample size was determined with reference to comparable neuroimaging-based descriptive investigations in which samples of approximately 40–70 participants were considered sufficient to characterize between-participant variation in regional functional activation. Eligible participants were adults of either sex who reported experiencing stress during the preceding month without identifying a single clear precipitating event. For the purposes of participant eligibility, ambiguous stress was therefore operationally defined as a self-reported state of recent psychological stress or tension for which the participant could not attribute the experience to one discrete triggering event. Participants were required to be right-handed to reduce potential variability associated with hemispheric functional organization, to have normal or corrected-to-normal vision, and to be capable of providing informed consent. Individuals were excluded if they had a known neurological disorder, major diagnosed psychiatric disorder including anxiety or depressive disorders, current psychoactive medication use, substance dependence, or a contraindication to magnetic resonance imaging.

Baseline assessment included demographic characteristics and standardized measurement of perceived stress and emotional symptoms. Perceived stress during the preceding month was assessed using the 10-item Perceived Stress Scale (PSS-10), which evaluates the extent to which individuals perceive circumstances in their lives as unpredictable, uncontrollable, or overwhelming and is derived from the established Perceived Stress Scale framework (7). Anxiety and depressive symptoms were assessed using the Hospital Anxiety and Depression Scale (HADS), a self-report instrument developed to identify and quantify symptoms of anxiety and depression in non-psychiatric clinical populations (8). HADS assessment was used to characterize emotional symptoms and to support exclusion of participants with clinically established psychiatric disorders while retaining individuals with subclinical symptom variation relevant to perceived stress.

Physiological assessment was performed before functional neuroimaging and included measurement of resting heart rate and blood pressure using calibrated digital monitoring equipment. Salivary cortisol was used as the biochemical indicator of hypothalamic–pituitary–adrenal axis activity because cortisol is a central endocrine component of the physiological stress response and has demonstrated relationships with neural systems involved in stress regulation (2,4). Saliva samples were collected during the morning using standardized Salivette collection devices and were subsequently analysed by enzyme-linked immunosorbent assay. A single morning measurement was used for the study analyses.

Functional brain responses were examined using task-based blood oxygen level-dependent functional magnetic resonance imaging performed on a 3-Tesla magnetic resonance imaging system. Participants completed an ambiguous stress-induction paradigm incorporating social-evaluative uncertainty and cognitive-interference components interspersed with neutral control conditions. The task was intended to

generate a state in which participants were exposed to evaluative and cognitive demands without a single explicit or clearly identifiable threat cue, thereby permitting assessment of neural activity associated with uncertain or diffuse stress processing. Blood oxygen level-dependent responses during the stress condition were evaluated relative to the control condition, with a priori attention directed toward brain regions implicated in emotional salience, stress appraisal, cognitive regulation, and stress-related physiological processing (1–4).

The predefined regions of interest comprised the amygdala, anterior cingulate cortex, prefrontal cortex, hippocampus, and insula. These regions were selected because convergent neurobiological and functional-imaging evidence implicates limbic, paralimbic, and prefrontal circuitry in stress appraisal, emotional salience, autonomic integration, and regulatory control (1–5). Structural T1-weighted magnetic resonance images were obtained to support anatomical localization of functional activation. Regional blood oxygen level-dependent signal change was quantified for subsequent group-level descriptive and association analyses.

Functional neuroimaging data were processed using Statistical Parametric Mapping version 12 (SPM-12), a software environment based on the statistical parametric mapping framework for analysis of regionally specific effects in functional imaging data (9). Image preprocessing included correction for participant motion and spatial normalization to permit group-level comparison of anatomically corresponding brain regions. Individual-level statistical models were used to generate activation estimates for the stress-related task condition, after which group-level random-effects analyses were performed to characterize regional activation across participants. The resulting activation measures from the predefined regions of interest were used in subsequent analyses examining relationships with subjective stress and salivary cortisol.

Data were summarized using frequencies and percentages for categorical variables and means with standard deviations for continuous variables. Distributional characteristics of continuous variables were evaluated using the Shapiro–Wilk test before parametric analyses. Pearson correlation coefficients were calculated to examine associations between PSS-10 scores, salivary cortisol concentrations, and regional blood oxygen level-dependent signal changes. Independent-samples t tests were used to compare mean regional activation between male and female participants. All statistical tests were two-sided, and a p-value <0.05 was considered statistically significant. Analyses were based on participants with complete psychometric, physiological, biochemical, and neuroimaging measurements.

RESULTS

All 60 enrolled participants completed the psychological, physiological, biochemical, and functional neuroimaging assessments and were included in the final analysis. The mean age was 29.8 ± 7.6 years, with an observed age range of 19–48 years. The sample comprised 34 males (56.7%) and 26 females (43.3%). Graduate-level education was reported by 32 participants (53.3%), while 18 (30.0%) had undergraduate-level and 10 (16.7%) had postgraduate-level education. Twenty-nine participants (48.3%) were employed, 14 (23.3%) were students, 14 (23.3%) simultaneously reported employment and student status, and 3 (5.0%) were unemployed. The mean PSS-10 score was 21.4 ± 5.8. Mean HADS-Anxiety and HADS-Depression scores were 6.8 ± 2.1 and 5.9 ± 1.9, respectively. Mean morning salivary cortisol concentration was 0.42 ± 0.11 µg/dL, and mean resting heart rate was 78.6 ± 8.4 beats/min (Table 1).

Table 1. Baseline Demographic, Psychological, and Physiological Characteristics of Participants (N=60)

Variable	Category	n (%) / Mean ± SD
Age, years	—	29.8 ± 7.6
Sex	Male	34 (56.7)
	Female	26 (43.3)
Education level	Undergraduate	18 (30.0)
	Graduate	32 (53.3)
	Postgraduate	10 (16.7)
Employment status	Employed	29 (48.3)
	Student	14 (23.3)

Variable	Category	n (%) / Mean ± SD
	Employed and student	14 (23.3)
	Unemployed	3 (5.0)
PSS-10 score	—	21.4 ± 5.8
HADS-Anxiety score	—	6.8 ± 2.1
HADS-Depression score	—	5.9 ± 1.9
Salivary cortisol, µg/dL	—	0.42 ± 0.11
Resting heart rate, beats/min	—	78.6 ± 8.4

Abbreviations: HADS, Hospital Anxiety and Depression Scale; PSS-10, 10-item Perceived Stress Scale; SD, standard deviation.

Task-based functional magnetic resonance imaging demonstrated measurable blood oxygen level-dependent signal changes across all five predefined regions of interest. The ACC showed the largest mean percentage signal change at 0.91 ± 0.25%, followed by the amygdala at 0.84 ± 0.22% and the insula at 0.78 ± 0.21%. Mean prefrontal cortical activation was 0.63 ± 0.19%, while the hippocampus showed the lowest mean regional signal change at 0.57 ± 0.18% (Table 2). These values indicate that the largest task-associated responses among the predefined regions occurred within the ACC and amygdala, whereas lower average activation was observed within the prefrontal cortex and hippocampus.

Table 2. Mean Task-Associated BOLD Signal Change Across Predefined Brain Regions (N=60)

Brain region	Mean BOLD signal change, % ± SD
Anterior cingulate cortex	0.91 ± 0.25
Amygdala	0.84 ± 0.22
Insula	0.78 ± 0.21
Prefrontal cortex	0.63 ± 0.19
Hippocampus	0.57 ± 0.18

Abbreviations: BOLD, blood oxygen level-dependent; SD, standard deviation.

Correlation analysis demonstrated that higher perceived stress was associated with greater activation in both the amygdala and ACC. PSS-10 score showed a moderate positive correlation with amygdala activation (r=0.46, 95% CI 0.23 to 0.64; p=0.001) and with ACC activation (r=0.41, 95% CI 0.17 to 0.60; p=0.002). In contrast, PSS-10 score was inversely associated with prefrontal cortical activation (r=−0.31, 95% CI −0.52 to −0.06; p=0.017). Perceived stress was also positively related to salivary cortisol concentration (r=0.34, 95% CI 0.09 to 0.55).

Salivary cortisol demonstrated a positive correlation with ACC activation (r=0.39, 95% CI 0.15 to 0.59; p=0.003). A weaker positive association was observed between cortisol and amygdala activation (r=0.29, 95% CI 0.04 to 0.51), whereas its inverse correlation with prefrontal cortical activation was smaller and did not reach the conventional level of statistical significance (r=−0.18, 95% CI −0.41 to 0.08). Collectively, the correlation pattern indicated that subjective stress and cortisol were more consistently related to limbic and cingulate responses than to prefrontal activation (Table 3).

Table 3. Associations Between Perceived Stress, Salivary Cortisol, and Regional Neural Activation (N=60)

Predictor	Correlate	Pearson r	95% CI for r	p-value
PSS-10	Salivary cortisol	0.34	0.09 to 0.55	<0.01†
PSS-10	Amygdala activation	0.46	0.23 to 0.64	0.001
PSS-10	ACC activation	0.41	0.17 to 0.60	0.002
PSS-10	Prefrontal cortex activation	−0.31	−0.52 to −0.06	0.017
Salivary cortisol	Amygdala activation	0.29	0.04 to 0.51	<0.05†
Salivary cortisol	ACC activation	0.39	0.15 to 0.59	0.003
Salivary cortisol	Prefrontal cortex activation	−0.18	−0.41 to 0.08	>0.05†

Abbreviations: ACC, anterior cingulate cortex; CI, confidence interval; PSS-10, 10-item Perceived Stress Scale. Confidence intervals were derived from the reported correlation coefficients using Fisher's r-to-z transformation. †The source manuscript reported these associations using significance thresholds rather than exact p-values; threshold reporting has therefore been retained rather than replacing it with an assumed exact value.

Sex-stratified analysis showed that females had greater mean amygdala activation than males (0.92 ± 0.20% vs 0.78 ± 0.21%), corresponding to an absolute mean difference of 0.14 percentage points. The difference was statistically significant in the original analysis (p=0.014). Based on the reported group means, standard deviations, and sample sizes, the approximate 95% CI for the female–male mean difference was 0.03 to 0.25 percentage points, with a standardized effect size of approximately Hedges' g=0.67, indicating a moderate magnitude of difference.

Females also showed numerically greater ACC activation than males (0.96 ± 0.26% vs 0.87 ± 0.24%), but the difference was not statistically significant (p=0.121). The mean difference was 0.09 percentage points, with an approximate 95% CI from −0.04 to 0.22 and Hedges' g=0.36. Prefrontal cortical activation was slightly lower among females than males (0.60 ± 0.20% vs 0.65 ± 0.18%), with a mean difference of −0.05 percentage points; this comparison was also non-significant (p=0.180), with an approximate 95% CI from −0.15 to 0.05 and Hedges' g=−0.26. Thus, the clearest sex-related difference was observed for amygdala activation, whereas the ACC and prefrontal cortex estimates remained compatible with little or no group difference (Table 4).

Table 4. Sex-Based Comparison of Regional Neural Activation

Brain region	Male (n=34), Mean ± SD	Female (n=26), Mean ± SD	Female–male mean difference	95% CI	Hedges' g	p-value
Amygdala	0.78 ± 0.21	0.92 ± 0.20	0.14	0.03 to 0.25	0.67	0.014
ACC	0.87 ± 0.24	0.96 ± 0.26	0.09	−0.04 to 0.22	0.36	0.121
Prefrontal cortex	0.65 ± 0.18	0.60 ± 0.20	−0.05	−0.15 to 0.05	−0.26	0.180

Abbreviations: ACC, anterior cingulate cortex; CI, confidence interval; SD, standard deviation. Mean differences are expressed as female minus male. Confidence intervals and Hedges' g were calculated from the reported group sample sizes, means, and standard deviations; because the available summary values are rounded, these derived estimates should be interpreted as approximate. The p-values are those reported in the original manuscript.

Overall, the results demonstrated a coherent pattern across the subjective, biochemical, and functional neuroimaging measures. The strongest average task-associated BOLD responses occurred in the ACC and amygdala. Higher perceived stress was moderately associated with greater activation in both regions, whereas greater perceived stress was associated with lower prefrontal cortical activation. Cortisol showed its strongest reported regional association with the ACC. Although sex-stratified differences were relatively small for the ACC and prefrontal cortex, females showed a statistically significant and moderately sized higher amygdala response compared with males. These findings describe associations within the study sample and do not establish temporal or causal relationships.

DISCUSSION

The present study characterized regional neural responses during an ambiguous stress condition and examined their relationships with subjective and biochemical indicators of stress in a heterogeneous adult sample. The principal findings were greater average task-associated activation in the anterior cingulate cortex (ACC) and amygdala relative to the other predefined regions, moderate positive associations of perceived stress with amygdala and ACC activation, an inverse association between perceived stress and prefrontal cortical activation, and a positive relationship between salivary cortisol and ACC activation. Females also demonstrated greater amygdala activation than males, with an effect of moderate magnitude, whereas sex-related differences in ACC and prefrontal activation were smaller and statistically non-significant. Taken together, these observations suggest that individual differences in perceived stress during uncertain or diffuse stress exposure are accompanied by measurable variation across limbic, cingulate, and prefrontal components of the stress-processing network rather than being confined to subjective psychological experience alone.

The relatively prominent amygdala response observed in this study is compatible with established models in which the amygdala contributes to the detection and appraisal of emotionally salient, uncertain, or potentially threatening information. Stress-related modulation of affective processing involves coordinated activity across limbic and prefrontal systems, and the functional significance of the amygdala

depends on its interactions with cortical regions responsible for contextual evaluation and regulatory control (1,2). In the present sample, perceived stress showed a moderate positive association with amygdala activation (r=0.46), indicating that participants reporting greater subjective stress also tended to demonstrate greater amygdala responsiveness during the task condition. This finding is broadly consistent with neuroimaging evidence linking perceived stress to variation within prefrontal–amygdala circuitry, although previous work also demonstrates that the direction and magnitude of these relationships can vary according to developmental stage, experimental context, and the functional measure examined (3). The current findings should therefore be interpreted as evidence of an association between perceived stress and amygdala responsivity within this sample rather than evidence that increased amygdala activation represents a universal neural signature of ambiguous stress.

The ACC demonstrated the highest average task-associated BOLD signal change and was positively associated with both perceived stress and salivary cortisol. This pattern is biologically plausible because the ACC participates in the integration of cognitive, affective, and autonomic information and forms part of the broader neural circuitry involved in regulation of the hypothalamic–pituitary–adrenal stress response (2). Dedovic et al. described cortical and limbic contributions to cortisol regulation during psychological stress, emphasizing that neuroendocrine responses emerge from distributed neural systems rather than from a single stress-specific region (2). The positive association between cortisol and ACC activation observed in the present analysis (r=0.39) is compatible with this broader brain–endocrine relationship. Nevertheless, cortisol was measured only once in the morning, and the study did not quantify task-evoked cortisol reactivity. Consequently, the observed association should not be interpreted as evidence that ACC activation directly produced the measured cortisol concentration or that the experimental task generated the endocrine response.

The inverse relationship between perceived stress and prefrontal cortical activation represents another potentially important finding. Higher PSS-10 scores were associated with lower prefrontal activation (r=−0.31), whereas perceived stress was positively associated with activation of the amygdala and ACC. Prefrontal regions contribute to cognitive appraisal, flexible behavioural regulation, and modulation of affective responses under stress (1). Previous functional neuroimaging research has further demonstrated relationships between perceived stress and prefrontal–amygdala circuitry, supporting the broader concept that subjective stress is associated with variation in regulatory neural networks (3). However, regional activation alone cannot establish the direction of communication between the prefrontal cortex and limbic structures. The present study did not perform functional-connectivity, effective-connectivity, or dynamic causal modelling analyses; therefore, the lower prefrontal activation observed among participants with higher perceived stress should not be described as direct evidence of impaired “top-down control.” Rather, it indicates differential prefrontal engagement associated with perceived stress that warrants investigation using connectivity-based approaches.

The biochemical findings provide additional support for a multidimensional interpretation of stress responses. Perceived stress was positively related to salivary cortisol, while cortisol showed its strongest reported regional association with ACC activation and a weaker positive relationship with amygdala activation. Neuroendocrine and functional brain responses to stress are closely interdependent but can vary considerably across individuals because of previous stress exposure, psychological characteristics, environmental context, and other moderating factors (2,4). Quidé et al. similarly demonstrated that relationships between cortisol reactivity and emotional brain function can be moderated by individual characteristics and prior life experience, underscoring the importance of avoiding a simple one-to-one interpretation of cortisol and regional brain activation (4). The current findings are therefore more appropriately viewed as convergent associations across subjective, endocrine, and neural domains rather than evidence of a single linear biological pathway.

The greater amygdala activation observed among females compared with males also warrants cautious interpretation. Females demonstrated mean amygdala activation of 0.92 ± 0.20% compared with 0.78 ± 0.21% among males, corresponding to an absolute difference of 0.14 percentage points and an approximate standardized effect of Hedges' g=0.67. In contrast, sex-related differences in ACC and

prefrontal cortical activation did not reach statistical significance. These findings indicate that the sex-related pattern in this dataset was region-specific rather than a generalized difference in neural stress responsivity. Biological sex, previous stress exposure, affective characteristics, social context, and other individual factors can influence neural and neuroendocrine responses to stress (2,4). However, the present study did not measure gonadal hormones, menstrual-cycle phase, reproductive status, gender-related psychosocial exposures, or other mechanisms capable of explaining the observed difference. The greater amygdala response among females should therefore be reported as an empirical subgroup finding rather than attributed to hormonal or sex-specific biological mechanisms that were not measured.

The findings also need to be considered in relation to the operationalization of ambiguous stress. The participants were selected on the basis of recent perceived stress without a single clearly identifiable precipitating event, and the task incorporated social-evaluative uncertainty and cognitive interference. This framework attempts to capture a dimension of stress characterized by uncertainty and diffuse appraisal rather than a discrete external threat. Contemporary research increasingly recognizes that stress perception depends on appraisal and the interaction between environmental demands and regulatory processes (1,3,6). Nevertheless, “ambiguous stress” is not established by the present study as a distinct diagnostic or neurobiological entity. The construct overlaps conceptually with uncertainty, unpredictability, perceived uncontrollability, chronic stress perception, and psychosocial stress. Consequently, subsequent research should determine whether the neural pattern observed here is specifically associated with ambiguity or instead reflects more general stress-related processes.

Several methodological features strengthen the study. Psychological assessment, physiological monitoring, salivary cortisol measurement, and task-based fMRI were incorporated within the same participants, enabling stress to be examined across multiple measurement domains. The analysis also focused on predefined brain regions with established relevance to stress and affective processing rather than relying exclusively on unrestricted post hoc interpretation. Complete data were available for all 60 enrolled participants, and the analysis demonstrated broadly coherent relationships across perceived stress, cortisol, and regional neural activation. Moreover, presenting correlation confidence intervals and effect-size estimates for sex comparisons improves interpretation beyond reliance on p-values alone.

These strengths should be balanced against important limitations. First, the cross-sectional analytical framework prevents determination of temporal sequence or causality. The findings cannot establish whether altered regional activation contributes to greater perceived stress, whether sustained stress modifies neural responsiveness, or whether both arise from other individual characteristics. Second, the sample of 60 participants was modest for neuroimaging subgroup analyses, particularly the comparison of 34 males and 26 females, and precision around some estimates was consequently limited. Third, the reported analyses were primarily bivariate and did not adjust simultaneously for potential confounders such as age, anxiety symptoms, depressive symptoms, educational characteristics, employment status, or other psychosocial factors. Fourth, several relationships were evaluated across multiple regions and stress measures without a reported multiplicity-adjustment procedure, increasing the possibility of chance-positive findings and making replication particularly important.

Additional limitations relate to measurement and neuroimaging interpretation. A single morning salivary cortisol measurement cannot characterize diurnal cortisol secretion or task-related cortisol reactivity, and repeated samples would provide a stronger representation of hypothalamic–pituitary–adrenal dynamics (2,4). Regional BOLD activation was examined without functional-connectivity analysis, preventing conclusions regarding communication or directional regulation among the amygdala, ACC, and prefrontal cortex. The experimental task also cannot reproduce the complexity and duration of diffuse stress encountered in everyday environments. Furthermore, although HADS scores were collected to characterize anxiety and depressive symptoms, these variables were not incorporated into multivariable models capable of determining whether observed stress–neural associations were independent of subclinical emotional symptoms. The heterogeneous urban sample increases variation in participant characteristics but does not, by itself, establish generalizability to other geographical, occupational, socioeconomic, or clinical populations.

Future investigations should therefore evaluate these associations prospectively in larger and more extensively characterized samples. Longitudinal designs would help determine whether neural responses during uncertain stress predict subsequent changes in perceived stress or emotional symptoms rather than merely covarying with them. Repeated cortisol measurements before and after stress exposure could distinguish basal endocrine status from experimentally evoked reactivity, while autonomic indices could provide complementary measures of physiological stress regulation. Functional- and effective-connectivity analyses would be particularly valuable for examining interactions among prefrontal, cingulate, and limbic regions instead of inferring regulatory relationships from isolated regional activation. Multivariable models should also assess whether neural associations remain after accounting for age, sex, anxiety and depressive symptoms, and relevant psychosocial characteristics. Such approaches would clarify whether the patterns identified in this study represent reproducible correlates of stress under uncertainty and whether they have meaningful prospective or clinical relevance.

Overall, the present findings support an association between perceived stress and differential engagement of established stress-related neural circuitry during an ambiguous stress condition. Greater perceived stress was associated with greater amygdala and ACC activation and lower prefrontal cortical activation, while salivary cortisol was positively related to ACC activation. These convergent findings are compatible with distributed models of stress processing involving interactions among affective, regulatory, and neuroendocrine systems (1–4). However, the cross-sectional design, modest sample, unadjusted analyses, single cortisol assessment, and absence of connectivity measures preclude interpretation of these findings as causal mechanisms, diagnostic signatures, or early biomarkers. Their principal contribution is therefore the descriptive identification of neural, subjective, and biochemical associations that can be tested more rigorously in prospective and mechanistically focused studies.

CONCLUSION

Ambiguous stress exposure was associated with differential activation across established stress-related brain regions in this adult sample, with the largest average task-associated responses observed in the ACC and amygdala. Higher perceived stress was positively associated with amygdala and ACC activation and inversely associated with prefrontal cortical activation, while salivary cortisol showed a positive association with ACC activation. Females demonstrated greater amygdala activation than males, whereas sex-related differences in ACC and prefrontal activation were not statistically significant. These findings indicate that subjective stress under uncertain conditions is accompanied by measurable variation across neural and biochemical stress-related measures; however, because the study was cross-sectional and primarily based on unadjusted associations, the observed patterns should be regarded as neural correlates rather than causal mechanisms, predictive biomarkers, or indicators of future psychopathology.

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