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# The relationships between expressed emotion, cortisol, and EEG alpha asymmetry

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#### ABSTRACT

Families can express high criticism, hostility and emotional over-involvement towards a person with or at risk of mental health problems. Perceiving such high expressed emotion (EE) can be a major psychological stressor for individuals, especially those at risk of mental health problems. To reveal the biological mechanisms underlying the effect of EE on health, this study investigated physiological response (salivary cortisol, frontal alpha asymmetry (FAA)) to verbal criticism and their relationship to anxiety and perceived EE. Using a repeated-measures design, healthy participants attended three testing sessions on non-consecutive days. On each day, participants listened to one of three types of auditory stimuli, namely criticism, neutral or praise, and Electroencephalography (EEG) and salivary cortisol were measured. Results showed a reduction in cortisol following criticism but there was no significant change in FAA. Post-criticism cortisol concentration negatively correlated with perceived EE after controlling for baseline mood. Our findings suggest that salivary cortisol change responds to criticism in non-clinical populations might be largely driven by individual differences in the perception of criticism (e.g., arousal and relevance). Criticisms expressed by audio comments may not be explicitly perceived as an acute emotional stressor, and thus, physiological change responds to criticisms could be minimum.

# 1. Introduction

Expressed emotion (EE) is a measure of the level of criticism and hostility, and/or emotional over-involvement that a caregiver/relative expresses about a family member who has a diagnosis of or is at risk for mental disorder [11,37]. Research shows that individuals who encounter high EE tend to exhibit a greater vulnerability for a mental disorder and are more likely to relapse to an already diagnosed disorder [23,27]. This might be related to emotional overarousal through exposure to high degrees of negative affects relevant to the person with mental disorder. It should be noted that individual differences in trait negative emotions (e.g., depression, anxiety, stress) may modulate the intensity and relevance of perceived criticism, its impact on emotional overinvolvement and emotion regulation [32,34]. Emotion regulation in-

cludes monitoring and evaluation of emotional reactions, and encompasses both positive and negative affect [42]. For example, compared to those with little or no depression, those high in depression rated voices higher in both authoritative power and EE [14]. High depression and perceived irritability from a close relative modulated the relationship between positive schizotypy and relevance of criticism [34]. It is argued that low mood increases a person's maladaptive cognitive beliefs about social threat, leading to a greater probability of perceiving the threat of criticism greater and uncontrollable [29], while anxiety results in a greater vulnerability to social stressors and a lower tolerance towards negative emotion from others [18]. Also, low positive mood and greater perceived intrusiveness modulated the relationship between schizotypal disorganisation and relevance of praise [34].

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Fig. 1. Steps involved in the testing session.

Table 1
Descriptive statistics for subject appraisal of auditory comments and mood.

	Arousing	Relevance	Pre-mood		Post-mood	
	Mean (SD)	Mean (SD)	Negative	Positive	Negative	Positive
			Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Praise	6.86	6.76	10.85	13.52	10.90	14.48
	(2.36)	(1.53)	(2.26)	(4.66)	(2.27)	(7.86)
Neutral	3.12	2.32	24.33	22.40	12.87	28.87
	(2.14)	(1.94)	(8.90)	(6.92)	(3.41)	(10.60)
Criticism	5.17	4.32	12.88	30.21	15.71	29.25
	(2.53)	(2.43)	(3.58)	(8.8)	(6.54)	(9.52)
	Depression	1	Anxiety		Stress	
Mean	4.38		4.42(2.75)		6.50(3.88)	
(SD)	(3.97)					

Such observations are predominantly based upon cross-sectional studies, and only a few studies have identified biological mechanisms underpinning these associations. One physiological mechanism may be via excessive activation of the hypothalamic-pituitary-adrenal (HPA) axis function caused by ongoing stress. The HPA axis is a hormonal response system to stress and controls multiple stress hormones such as glucocorticoids that serves to help the organism adapt to change in demand and thereby maintains stability and health [22]. Thus, stronger HPA axis activity when facing major challenges are often implicated higher chance of survival compared to those that mount a weaker response [3]. However, exposure to glucocorticoids can be costly, leading to dysfunction within the HPA axis. Glucocorticoid receptors begin breaking down which in turn can lead to hypercortisolaemia which increases levels of cortisol within the body (Young et al., 2004). An increase in stress hormones due to the lack of HPA axis integrity in the body is known to be a cause for a variety of depressive disorders and cognitive deficits [3,48].

Family interactional behaviours may fit into this equation and serve as a stressor posing ongoing challenge for a biologically vulnerable individual. For instance, heartrate was marginally higher in individuals at high risk for psychosis during EE-type criticism than in individuals will low risk for psychosis [47]. Furthermore, the elevated heartrate was sustained after the criticism, suggesting that these individuals at high risk for psychosis struggle to recover from the pain of criticism. However, the association between subjective response to EE, cortisol responsivity and underpinning brain mechanisms remains unclear.

Little research has addressed the physiological consequence of family interaction; yet, negative family environment [12] and marital criticisms during conflict discussion [37] have been associated with elevated cortisol. Evidence suggests that salivary cortisol response reflects the body's cortisol reaction to a stressful event over time with a rise starting after the event and reaching a peak about 20–30 minutes later, and slowly decaying afterwards until it is close to zero after approximately 90 minutes [24]. Heightened HPA reactivity has been found when couples discuss about recent conflict [37]. Exposing children with Attention Deficit/Hyperactivity Disorder (ADHD) to parental criticism while completing a social stress task increases cortisol level, while exposure to positive comments during the social stress task lowers their cortisol response [13]. In contrast, children without ADHD showed reduced cortisol response to the social stress task during both parental criticism and parental praise [13].

In line with this, the link between neurophysiological activity and perceived EE has also been reported. Research reveals that emotional processing of a stressful event could be reflected in spontaneous Electroencephalography (EEG) activity, with left hemisphere involvement in processing of positive emotions and right hemisphere for processing of negative emotions [1]. The differences between right versus left frontal alpha band power, a so called alpha asymmetry reflect state and trait variations in emotional response [2,15]. As cortical activity is inversely correlated to EEG power, negative values of FAA indicate stronger activity in the right hemisphere and positive values a stronger relative left frontal activation [2,15]. Research shows that left frontal cortex is more active compared to the right under acute stress [9]. Stress-induced atypical asymmetry is considered a mediator of early life stress and the development of psychiatric disorders [28]. Stress research suggests that aversive stimuli trigger a range of stress-associated autonomic responses through endocrine, immune and nervous systems [40], and cortisol regulation could enhance individual coping behaviour to a stressor by modulating levels of neurotransmitters, such as serotonergic and cholinergic, that are involved in response inhibition and active coping [43]. An increase of right, relative to left, frontal neural activity can be induced by acute administration of cortisol (35 mg) [43]. Thus, if family criticism serves as a stressor as expected, cortisol and neurophysiological change should be found. It has been reported that high perceived EE is associated with lower frontal theta power and lower occipital alpha power during criticism and praise in healthy participants with high positive schizotypy [34].

Taken together, the importance of EE in health research and its influential effect on the outcome of an individual's mental health, including depression and aggression in the non-clinical population [35], are well established. However, the psychophysiological mechanisms underlying this link need to be clearly delineated. As such, it is important to investigate the neurobiological changes associated with the response to emotional comments, as it would reveal the mechanisms modulating the interplay between environmental risk factors and vulnerability for mental disorder. This research aims to investigate the role of salivary cortisol level and frontal alpha asymmetry (FAA) in perceived EE as a function of self-reported current mood and general psychological distress, namely depression, anxiety and stress, over the past week. To date, there have been studies on cortisol response to other social stressors, like the Trier Social Stress test, but none on criticism. To our best knowledge, this was the first study to look at the cortisol response to criticism and praise.

It was hypothesised that 1) criticism will induce an increase in cortisol and a shift towards greater right frontal cortical activity; 2) psychophysiological response to criticisms (cortisol, FAA) will be associated with subjective ratings of criticism (arousal and relevance), depression, stress, anxiety and current mood; and 3) the associations between subjective ratings of criticisms and psychophysiological response to criticisms (cortisol, FAA), will be modulated by current mood prior to listening to criticism.

### 2. Methods

### 2.1. Participants

Twenty-six participants (female=14; age mean=26.46 years, SD=6.1) were selected based on the following inclusion criteria, i)  $\geq$  18 years of age; ii) spending  $\geq$ 10 hours/week in face-to-face or phone con-

Table 2
Mean and standard deviation (SD) for cortisol and FAA

			Cortisol		FAA				
			Mean (SD)			Mean (SD)			
	Time	Time	Time	Time	Time	Pre-	Post-	Pre-	Post-
	1	2	3	4	5	EO	EO	EC	EC
Praise	7.12	4.73	4.11	3.47	3.17	0.05	0.06	0.20	0.23
	(6.95)	(3.37)	(2.91)	(2.07)	(1.83)	(0.80)	(0.62)	(0.62)	(0.44)
Neutral	5.96 (4.68)	4.78 (2.51)	3.89 (1.65)	4.00 (3.35)	3.79 (3.03)	-0.75 (3.12)	1.42 (7.47)	0.14 (0.61)	-0.75 (3.12)
Criticism	6.27	4.39	3.78	3.22	2.85	-0.10	0.07	0.19	0.25
	(5.08)	(2.89)	(2.41)	(1.71)	(1.78)	(4.96)	(0.58)	(0.60)	(0.58)

Note: FAA: frontal alpha asymmetry; Pre-EO: Eyes open prior to audio comments; Post-EO: eyes open after audio comments; Pre-EC: Eyes closed prior to audio comments; Post-EC: eyes closed after audio comments.

**Table 3**Spearman's correlations of cortisol following criticism, perceived EE, psychological distress, mood and Post FAA,

'	Cortisol	FAA EC	FAA EO	Arousal-C	Relevance-C	
	<i>r</i> ( <i>p</i> )	r(p)	<i>r</i> ( <i>p</i> )	<i>r</i> ( <i>p</i> )	r(p)	
Arousal-C	0.15 (0.51)	-0.19(0.40)	-0.13 (0.57)			
Relevance-C	-0.09 (0.68)	-0.20 (0.39)	-0.30 (0.17)			
Depression	0.22 (0.30)	-0.22(0.29)	-0.17 (0.42)	0.28 (0.19)	0.50 (0.02)	
Anxiety	0.42 (0.04)	-0.43 (0.04)	0.06 (0.77)	-0.01 (0.96)	0.19(0.40)	
Stress	0.22 (0.29)	-0.13(0.56)	-0.19 (0.38)	0.12(0.58)	0.44 (0.04)	
Post NM	-0.22(0.31)	-0.01(0.96)	0.26 (0.21)	0.46 (0.03)	0.46 (0.02)	
Post PM	0.08 (0.71)	0.16 (0.46)	0.13(0.54)	-0.06 (0.78)	-0.17(0.44)	
Post FAA EC	-0.43 (0.04)					
Post FAA EO	-0.21(0.35)					

Note: Arousal-C: Arousal of criticism; Relevance-C: Relevance of criticism; Post NM: Negative mood following criticism; Post PM: mood following criticism; Post FAA EC: Frontal alpha asymmetry following criticism during Eye closed; Post FAA EO: Frontal alpha asymmetry following criticism during Eye open; Bold indicates significant results.

tact with a close relative, (iii) English was the predominant language in communication. The latter criterion allowed participants to provide informed consent and to perform the experimental task, i.e., listening to and rating positive, neutral, and critical comments in English. The exclusion criteria allowed the potential confounding effects of physical and mental illness on psychophysiological measures to be eliminated. These exclusion criteria included having taken antibiotics or had major surgery in the last 3 months, a historical or current diagnosis of major medical illnesses, mental disorders, or neurological disorders, regular intake of psychotropic or anti-inflammatory drugs. No contraception was reported among female participants. Dropout from a given session resulted in n=25 for the praise condition, n=21 for the neutral comment condition and n=23 for the criticism condition.

#### 2.2. Procedure

Ethics approval was obtained by the institute's Ethics Committee (Ref: 19/231), and written consent was given by participants prior to data collection. Participants attended testing sessions in the morning on three separate days, with a gap of at least one day between sessions). On each day, participants were asked to listen to one type of auditory comments, e.g., criticism, praise or neutral comments, resulting in 40 comments heard per day in a random order (counterbalanced). Participants listened to the comments through headphones and rated the arousal (emotionally demanding) and relevance of the comments on an 11-point Likert scales after each comment. Besides cortisol and EEG recordings, participants rated their current mood on the Positive and Negative Affect Scale (PANAS) [46] prior to and after the auditory comments. Demographic questions and the Depression, Anxiety, and Stress

Scale (DASS-21) [26] were administered to the participants while EEG was set up on the first day of testing. Each session (Fig. 1) took approximately 2 hours.

#### 2.3. Listening to criticism and praise

This audio task included three blocks and each block contains either 40 comments in praising (positive), 40 comments in neutral, or 40 comments in criticism (negative). Participants only listened to one type of comments on a testing day in a random order. Comments were delivered by either a male or female voice. The positive and negative comments reflected remarks typically made by a close relative. For example, "you are good at organising things and paying attention to detail" (praise); "you are too emotional. You shouldn't let things that people say, even things said in a jokey manner, upset you" (criticism). Neutral comments were typically centred around factual statements and irrelevant to the individual, such as "The Emu is the largest native bird in Australia, with long neck and legs". Details of comments and validation methods have been previously published [34,45].

# 2.4. EEG recording and preprocessing

In line with the testing schedule of cortisol, EEG data were recorded on three non-consecutive days, to reduce any carry over effects on emotion of comments from previous conditions (Fig. 1). In each session, EEG was continuously recorded while subjects were sitting relaxed with eyes closed (EC, 2 min) and eyes open (EO, 2 min) in a sound-attenuated room prior to and following the audio task. EEG recording was performed with a SynAmps amplifier and a 64-channel QuickCap, with electrodes configured in the international 10-20 system. The sampling rate was 1,000 Hz. The impedance of the electrodes was kept below 5  $\rm k\Omega$  and the signal was acquired using a common vertex (Cz) reference

EEG data were preprocessed in MATLAB 2019a (The Mathworks, Inc) using custom written scripts and the EEGLAB plugin. Data were downsampled offline to 256Hz and referenced to linked mastoids. A high-pass finite impulse (FIR) filter at 0.01Hz and a low-pass FIR filter at 50Hz was applied offline. Line noise was removed using the Clean-Line function before data was manually inspected for the removal of bad channels. Removed channels were interpolated before an independents components analysis (ICA; runica) decomposition was performed. Independent components were inspected, and muscular and ocular artifacts were removed from the data based on their activity spectra and scalp topographies. Average spectral power was calculated for alpha (8–12Hz) using spectopo function from EEGLAB [16]. The frontal (F3/F4) EEG asymmetry was calculated as F4-F3.

# 2.5. Salivary cortisol

Participants arrived at the lab between 08:00h and 10:00h. Five saliva samples were collected from the participants at each session, with the first sample taken following 10 min of seated rest. The second sample was taken prior to the participant performing the audio comment task (approximately 30 minutes after the first salivary sample collection); the third sample was taken immediately after listening to the auditory comments; the fourth sample was taken 25 minutes after listening to the auditory comments, and the fifth sample was taken 40 minutes after listening to the auditory comments. Saliva samples were collected using 7ml capacity bijou tubes with a screw top, over a two-minute period. When insufficient volume was obtained, the collection period was increased to 3 minutes. During saliva sample collection, participants were asked to be seated, leaning forwards with their heads tilted downwards, which would allow the saliva to fall into the tube with minimal orofacial movement.

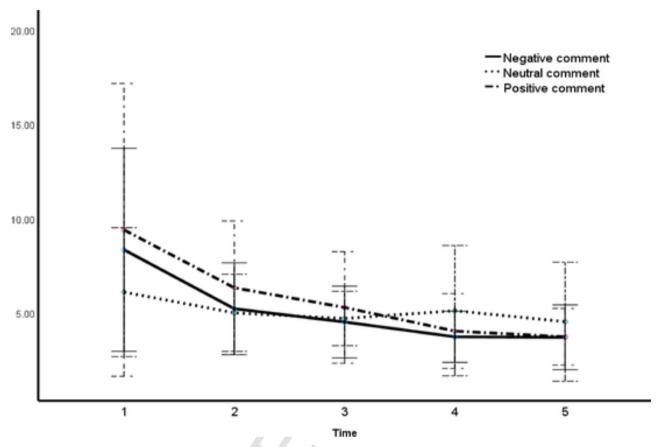


Fig. 2. Cortisol change across type of comments.

Cortisol samples were placed in a  $-20^{\circ}$ C freezer within the psychophysiology lab, and then transported to the Roche Diagnostics Laboratory in a chilly bin with ice packs. Samples were stored at  $-80^{\circ}$ C until batch analysis using a Roche Diagnostics Modular E170 automated instrument was performed. Quantitative results were determined via an instrument-specific full point calibration curve. The lower detection limit of cortisol levels was 1.5 nmol/l which are reliably measured as being different from 0 nmol/l.

# 2.6. Psychometric scales

# 2.6.1. Depression, anxiety and stress

The *Depression, Anxiety, and Stress Scale (DASS-21)* assesses the signs and severity of depression, stress, and anxiety with each subscale having 7 items [26]. The DASS-21 and its subscales have been validated with good convergent and discriminant validity, and reliability [21].

Mood

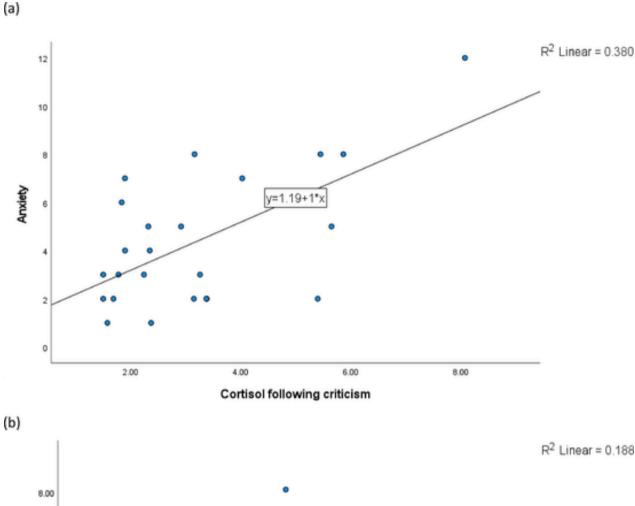
Current mood was measured by the Positive and Negative Affect Scale (PANAS) [46]. There were 20 items in this scale, with 10 descriptors for positive and 10 descriptors for negative mood. Participants were required to rate their current mood on a five-point Likert scale, with 1 being "very slightly or not at all", 2 being "a little", 3 being "moderately", 4 being "quite a bit", and 5 being "extremely" [46]. Participants rated how they felt at that moment. The internal reliability, convergent correlations and discriminant correlations of this scale have been found to be excellent [46].

# 2.6.2. Statistical analyses

A priori power analyses were conducted using G\*Power [19]. For the ANOVA repeated measure test within effect (1 group \* cortisol measure under three conditions), a sample size of 20 would have 80% power to detect an effect size of f=0.30 with a two tailed  $\alpha$  of 0.05.

To simplify our analyses and minimise the effect of small sample size and multiple comparison errors, the cortisol levels from the first and second samples were averaged and the cortisol levels from the third, fourth and fifth samples were averaged, reflecting cortisol prior to, and following audio comments. Consistent with our previous research in EE [45], perceived EE was indicated by median arousal and relevance of criticism and praise. Preliminary analysis using paired ttest respectively showed that there were no significant differences in either cortisol or FAA prior to listening to audio comments between test sessions. Further, independent t-test showed that there was no effect of gender in these measures. Thus, all data were analyzed within subjects with no between-subjects comparisons for gender. A 3 (type of comments: positive, neutral, negative) x 2 (time: before, after) repeatedmeasures ANOVA was used to examine mean differences in cortisol and EEG FAA between comments over time, respectively. The degrees of freedom were adjusted with a Greenhouse-Geisser correction where necessary. Significant main effects were followed-up with post hoc pairwise comparisons which were adjusted for multiple comparisons using a Sidak correction.

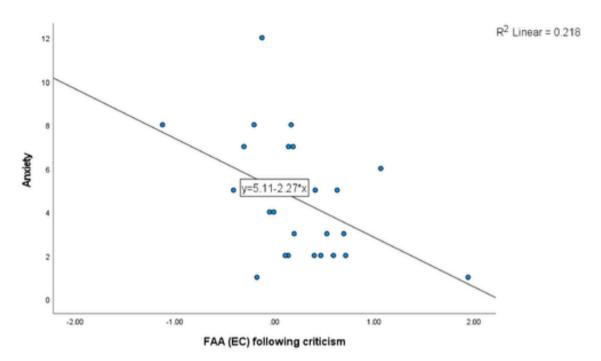
Spearman's Rho correlations were used to explore whether cortisol after listening to criticism was associated with alpha asymmetry following audio comments, perceived EE (median of arousing and relevance in response to critical and positive comments), and negative emotions (depression, anxiety, and stress). A significant change in mood following criticism observed in the present study and previous research indicating the impact of mood on cortisol [7] and FAA [30] meant that non-parametric partial correlation coefficients were calculated between rating of criticism (arousal and relevance) and cortisol response to criticism and post-FAA respectively. These partial correlations were performed while controlling for the effect of positive and negative mood prior to the test. Based on the results of these correlational analyses, a multiple linear regression analysis was performed to determine the pre-



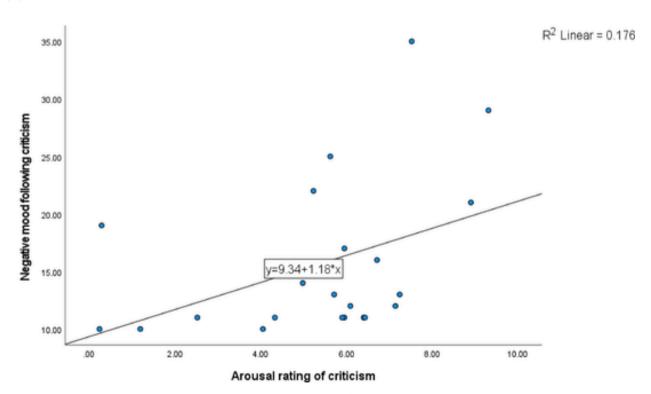
R<sup>2</sup> Linear = 0.186

Fig. 3. Scatterplot for significant relationships observed between (a) Cortisol level following criticism and anxiety, (b) frontal alpha asymmetry following criticism and anxiety, (c) frontal alpha asymmetry following criticism and cortisol following criticism, (d) Arousal rating of criticism and negative mood following criticism, (e) relevance of criticism and negative mood following criticism, (f) relevance of criticism and depression, (g) relevance of criticism and stress.

(c)

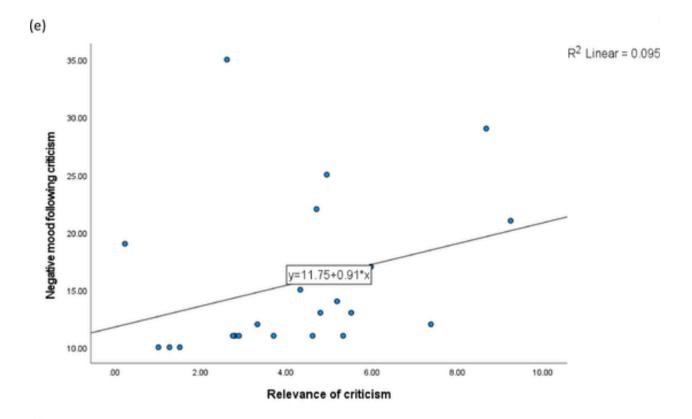


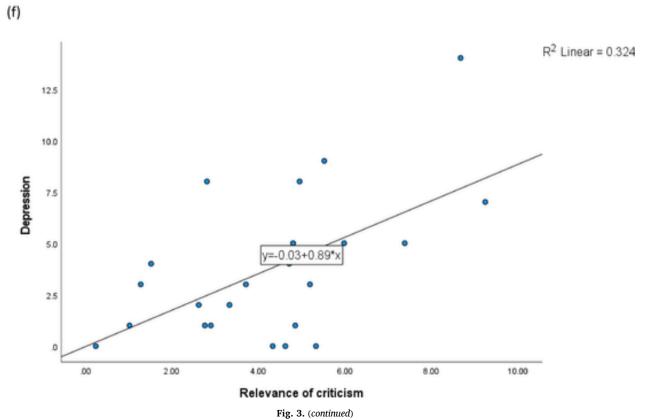
(d)



 $\textbf{Fig. 3.} \ (continued)$ 

diction of anxiety by cortisol and FAA response to criticism. All statistical analyses were conducted using IBM SPSS Statistics (version 27). This study was not preregistered but the data and analytic code are available on request from the authors.





(g)

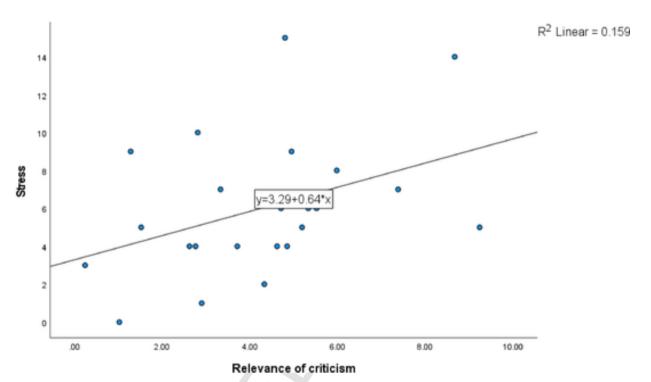


Fig. 3. (continued)

#### 3. Results

# 3.1. Psychological distress, rating of criticism and praise and mood change

Table 1 shows descriptive statistics for depression, anxiety, stress, subjective ratings of EE and mood. Participants' scores on depression, anxiety and stress were within the recommended normal range [26]. There was a significant effect of type of comment on arousal,  $F_{2,38} = 28.05, \, p < 0.001$  and relevance,  $F_{2,38} = 35.95, \, p < 0.001$ . Greater arousal and relevance were reported in response to both criticism and praise, compared to neutral comments. Praise was also perceived as more arousing and relevant, relative to criticism.

Furthermore, negative mood increased following criticism, t= 2.36, p=0.03, while positive mood decreased following neutral comments, t=5.06, p<0.001; however, there was no significant change in mood following praise.

# 3.2. Cortisol change in response to auditory comments

There was an effect of time,  $F_{1,19}=10.03$ , p=0.005, wherein cortisol level decreased following criticism, mean difference (MD) = -2.15, p=0.003, and praise, MD =-2.43, p=0.005. However, neither type of comment,  $F_{1,19}=0.35$ , p=0.71, nor the interaction between time and type of comments,  $F_{1,19}=1.32$ , p=0.28, were significant (Fig. 1 and Table 2).

### 3.3. FAA change in response to auditory comments

There was no significant effect of time, type of comments, and interaction of time  $\times$  type of comments in FFA (p>0.05) (Table 2).

3.4. Correlations between cortisol following criticism, perceived EE (arousal and relevance of criticisms), FAA following criticism, emotional status, and mood

Table 3 presents the correlations between cortisol following criticism, FAA following criticism, perceived EE, depression, anxiety, stress and mood. Cortisol level following criticism was positively correlated with anxiety, r(23) = 0.42, p = 0.04 (Fig. 1a), but negatively correlated with FAA during EC, r(23) = -0.43, p = 0.04 (Fig. 1b). FAA during EC was negatively correlated with anxiety, r(24) = -0.43, p = 0.04 (Fig. 1c). Furthermore, negative mood following criticism was associated with subjective arousal from criticism, r(23) = 0.46, p = 0.03, and relevance of criticism, r(23) = 0.46, p = 0.02. Depression, r(23) = 0.50, p= 0.02 and Stress, r(23) = 0.44, p = 0.04, were also correlated with the relevance of criticism (Fig. 1 d-g). The level of cortisol did not correlate with subjective ratings of the arousal and relevance of criticism. However, after controlling for the effect of mood before listening to the auditory criticism, partial correlation analyses showed that cortisol level after listening to criticism was negatively associated with arousal, r = -0.56, p = 0.02, while its association with relevance of criticism was marginal, r = -0.46, p = 0.06. FAA during EC following criticism did not correlate with subjective ratings of the arousal and relevance of criti-

### 3.5. Prediction of anxiety by cortisol and FAA response to criticism

Logistic regression analyses showed that cortisol level after listening to criticism was the sole significant predictor of anxiety and it explained a significant amount of the variance in the level of anxiety,  $F_2 = 7.71$ , p = 0.003,  $R^2 = 0.44$ ,  $R^2_{\text{Adjusted}} = 0.38$ . However, the FAA response to criticism was not a significant predictor.

#### 4. Discussion

EE reflecting a critical, hostile and/or over-involved family environment is considered a prevalent stressor of symptoms of a mental disorder as well as relapse of an already diagnosed disorder (Hooley & Gotlib, 2000; [27]). According to the diathesis-stress model, EE may be conceptualised as a stressor. Those who come from family environments with high EE have a greater vulnerability for developing a mental disorder (Hooley & Gotlib, 2000; [27]). However, it is equally likely that informal care workers are also caregivers and their criticisms arise because they are observing the early signs of relapse [49]. To reveal the psychophysiological mechanisms underlying the link between vulnerability of mental distress and EE, we examined the association between cortisol levels and neurophysiology following criticism and their association with self-report measures of appraisals of criticism and praise, emotional status of depression, anxiety and stress, and mood in healthy participants in a lab setting. The findings partially supported our hypothesis that there would be an increase in cortisol level and FAA induced by criticism. Surprisingly, cortisol was reduced across the conditions, regardless of the type of comments being administered, and there were no significant differences in cortisol change between the types of comments. FAA remained relatively stable following criticisms. Furthermore, our second hypothesis was that cortisol level and FAA induced by criticism would be associated with subjective ratings of criticism, depression, stress, anxiety and current mood. Cortisol level measured following criticism was also positively correlated with anxiety, but negatively correlated with FAA during EC. Our third hypothesis that cortisol level would be associated with self-rating of arousal during criticism once the effect of mood prior to listening to the comments was controlled for was also supported.

Cortisol is considered a biomarker for various chronic illnesses, showing positive corelations with psychological and physical stresses (Noushad et al., 2021). Alteration in circulating cortisol levels enhance catabolic processes of energy supply so that the body has better adaptation to the changing environment (Lee et al., 2015). When facing stressful situations, elevated cortisol could buffer and reduce the intensity of negative emotional responses, helping individuals to cope with the emotional demands. These changes are referred to as 'allostasis' and can be adaptive or maladaptive depending on its degree or contextual relevance (Ganzel et al., 2010). A significantly higher cortisol level in individuals who experience stress and anxiety through generalised anxiety disorder (Lenze et al., 2011), and healthy university students (Xu et al., 2019) has also been reported. Accordingly, our findings highlight the relationship between higher cortisol levels and the increased likelihood of anxiety in healthy individuals.

However, in contrast to previous research examining momentary emotions and cortisol which shows elevated cortisol associated with increased negativity [10], we did not find short-term increases in cortisol in response to criticisms, despite increased negative mood. Unexpectedly, cortisol levels were decreased in response to either criticism or praise, and cortisol changes over a short period of approximately an hour were not differentiated between criticisms and praise. Healthy individuals could downregulate their physiological arousal upon subjective appraisal of increased arousal from critical through the negative feedback loop in the HPA axis [33]. Indeed, cortisol was not directly correlated with negative ratings, i.e., arousal and relevance of criticisms, unless mood was controlled for. The relationship between cortisol and affect appears complicated in daily life, and direction of their associations could be modulated by subject affective state (Hoyt et al, 2016). In line with this notion, previous research comparing children with attention deficit/hyperactivity disorder (ADHD) with healthy counterparts showed a decrease in cortisol response over time without marked differences between positive and negative comment conditions while completing a cognitive task. Cortisol response in those with ADHD was decreased in a positive condition but increased following

negative emotion provocation [13]. Taken together, our findings suggest that salivary cortisol change to criticism in non-clinical populations might be different from that of a clinical population. For healthy participants, criticisms expressed by audio comments could modulate a person's mood but may not be explicitly perceived as an acute emotional stressor. As a result, a person rather responds to criticisms on a physiological level. In other words, audio stimulation using general comments is expected to be less neuroendocrine challenging than validated stress procedures (e.g., socially evaluated cold-pressor test, Trier social stress test), and thus, would expect neuroendocrine reactivity to be less pronounced. Change in frontal asymmetries tend to be more observable during emotional challenge compared to asymmetries during rest [9]. This might also be the reason for the absence of FAA change associated with EE. Unaffected FAA by stress has also reported in previous studies [8,36], suggesting the general stability of FAA across conditions. It is possible that there is an important involvement of prefrontal cortex in stress-related behavioural and somatic responses, but FAA is more likely to be a trait-related neural marker rather than state-related.

The present findings need to be interpreted with caution due to some limitations of the study. First, small sample size and dropouts compromises the study power and generalizability of the present findings. Furthermore, the impact of standard auditory comments on study participants might be limited due to a lack of personal connections. Research suggests that cortisol may not be responsive to all types of stressors. For example, passive listening to comments. It lacks the integral aspects critical to stress induction, namely uncontrollability and social judgement [17], especially if these comments were not personally connected to the participants. It would be ideal to have these comments delivered by their family member in future research, although other laboratory elicitation of physiological arousal to standard criticism has been evidenced [47]. Furthermore, the present research was conducted in a soundproof and ambiently lit psychophysiological lab, which would not reflect conditions in real life. Thus, the extent to generalise current findings across time and situations might be compromised. It should be acknowledged that there are various techniques such as area under the curve, linear change, baseline-to-peak change that can be used for analysis biological samples, i.e., cortisol sample and lack of control on individual baseline levels of cortisol may over or underestimate the relative change in cortisol following specific experimental tasks. Future research would need to take this into consideration and adapt different approaches for data analysis. Furthermore, future research needs to control for the potential confounds of associations between a fast, immediate neural marker, i.e., FAA and a slow, changing neuroendocrine HPA marker cortisol which has been shown to peak anywhere between 20-30 minutes after stressor onset.

In conclusion, listening to criticism may not always lead to undesirable effect of increasing physiological stress in a healthy population; effective coping strategies could curtail such undesirable sustained effects of criticism. Interpretation of the positive correlation between EE from a carer and risk for onset and relapse of mental illness proposed by previous clinical research need to take the vulnerable persons' current mood and mental health status into consideration as possible buffers of an adverse response to high EE.

#### **Uncited References**

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# **Uncited floats**

Figs. 2 and 3

# Data availability

Data will be made available on request.

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