

The effect of a naturalistic stressor on frontal EEG asymmetry, stress, and health

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Abstract

The aim of the current study was to investigate the effect of a naturalistic stressor, examination stress, on frontal EEG asymmetry, psychological stress, hormonal stress, and negative health. Forty-nine subjects were tested during periods of low and high examination stress. During the high examination stress period, subjects reported higher levels of stress on the Spielberger State Anxiety Inventory and Cohen's Perceived Stress Scale. However, no change in cortisol was detected across the two sessions. Furthermore, a shift from relatively greater left frontal activity during the low examination session to relatively greater right frontal activity during the high examination session was also found. Moreover, the increasing right frontal activity asymmetry associated with the high exam session compared to the low exam session correlated with increasing reports of negative health. No evidence was found for the prediction that cortisol mediated either the relationship between examination stressor and right frontal asymmetry or between right frontal asymmetry and negative health. In conclusion, while the findings from this study are compelling, the mechanism mediating increases in psychological stress, relatively greater right frontal activity, and increases in negative health from naturally occurring stressors is in need of further investigation.

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The major tenet of the valence models of hemispheric specialization of emotion (see Davidson et al., 1979 and Tucker, 1981 for a review of foundational studies) states that the left hemisphere is more involved in the processing of positive emotions and approach-related behaviors, whereas the right hemisphere is more involved in the processing of negative emotions and withdrawal behaviors. The model also suggests that the frontal cortex is particularly critical in emotional processing. There is an abundance of evidence supporting this model from prefrontal EEG alpha asymmetry studies (e.g., Davidson et al., 1979; see Davidson, 2003; Coan and Allen, 2004 for recent reviews of the literature). Specifically, positive moods or reactions have been found to predict relatively greater left prefrontal activity (LFA), whereas negative moods or reactions have been found to predict relatively greater right prefrontal activity (RFA).

However, there is some question regarding whether the region of the prefrontal cortex most involved in emotion is also most relevant to EEG asymmetry findings, especially those related to stress and anxiety. More specifically, Davidson (2004) suggests that EEG mostly records activity at the level of the dorsolateral prefrontal cortex. However, a common finding in anxiety and fear studies is higher metabolic activity in the orbitofrontal prefrontal cortex and ventromedial prefrontal cortex rather than the dorsolateral prefrontal cortex (Davidson, 2002; see Murphy et al., 2003 for a review of these issues). Nevertheless, Davidson (2002) also argues that the strong interconnections among the dorsolateral, orbitofrontal and ventromedial prefrontal cortices, allow for an indirect measure of the level of activity in a variety of prefrontal cortical subregions.

Notwithstanding the need for further elucidation of the specific prefrontal regions involved, numerous findings have confirmed a differential role for the two prefrontal cortices in emotional processing. For instance, in a series of studies investigating shifts in prefrontal activity in infants, smiling faces (Davidson and Fox, 1982), sugar on the tongue (Fox and

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Davidson, 1986), and being approached by the mother (Fox and Davidson, 1987) were related to relatively greater LFA. Furthermore, several studies have found that relatively greater RFA is associated with negatively valenced reactions to emotional stimuli (Davidson and Fox, 1982; Fox and Davidson, 1986, 1988; Hagemann et al., 1998; Tomarken et al., 1990; Wheeler et al., 1993). While relatively greater RFA has been demonstrated with a number of distinct negative mood states or behaviors, including depression, fear and withdrawal (see Coan and Allen, 2004), one negative emotion or mood state that has received less attention is stress. Nevertheless, stress has also been empirically related to relatively greater RFA, especially in rodents (e.g., Sullivan, 2004).

Recent studies have also suggested a differential role for the two cerebral hemispheres in immunocompetence and suppression (e.g., Barneoud et al., 1987; Neveu, 1988, 1993; Quaranta et al., 2004). More specifically, while activity of the left hemisphere appears to be related to increased immunocompetence, activity of the right hemisphere appears to be related to increased immunosuppression. Furthermore, similar to the emotional valence studies, there is evidence that the prefrontal cortex is particularly critical in these hemispheric effects (see Sullivan, 2004 for a review of this issue). While few studies have investigated the more complex associations among prefrontal asymmetry, stress, and health, of those that have, there is evidence to suggest that increased activity in the right hemisphere is related to both increased stress and decreased immune functioning (e.g., Davidson et al., 1999; Kang et al., 1991).

With regard to a mechanism by which stress, health and relatively greater RFA may interact, there is emerging evidence that individuals with relatively greater RFA may also have higher cortisol levels (see Kalin et al., 1998 in rhesus monkeys; Schmidt et al., 1999; Tops et al., 2005 in humans). There is also evidence from hemispheric brain damage and hemispheric presentation studies to suggest that the right hemisphere is more involved in the release of cortisol than is the left hemisphere (see Sullivan, 2004; Wittling, 1995 for reviews). However, few studies have reported a direct linear correlation between the two measures (Buss et al., 2003; Rilling et al., 2001). Again, the complex associations among higher cortisol levels, relatively greater RFA, and greater health problems remain unclear. It is intended that studies like the present one will help to better elucidate these issues.

The goal of the present study was to use an examination stressor to elevate cortisol and psychological stress levels and to test the effect of these elevations on frontal asymmetry and health. There is good motivation for using an examination stressor to elevate cortisol levels and psychological stress in studies investigating these complex issues. For example, there is evidence to suggest that the stress of examinations elicits increased activity in the hypothalamic–pituitary–adrenal (HPA) axis and the release of cortisol (Frankenhaeuser et al., 1978; Lovallo et al., 1986; Lacey et al., 2000; Lucini et al., 2002; Malarkey et al., 1995). However, there is some inconsistency in the literature, with other evidence

suggesting either no change in cortisol or even decreased release of cortisol in the face of examinations (see Glaser et al., 1994; Vedhara et al., 2000). One explanation for these discrepancies across studies is that elevations in cortisol in response to examination stress are simply less consistent and less robust than are those in response to the laboratory stressors. Indeed, one recent review (Weekes and Lewis, 2006) found that changes in cortisol levels in response to examination stress ranged from a 58% decline in cortisol (Vedhara et al., 2000) to a 95% increase in cortisol (Rohrmann et al., 2003). The average change was a 21% increase. In laboratory stress studies (especially using Trier social stress test or TSST), the average change in cortisol is typically in the range of a 70–80% increase (e.g., Kudielka et al., 2004).

While these findings might suggest that examination stress protocols would be a less effective means for investigating the relationship between stress-related changes in prefrontal asymmetry than would laboratory-based protocols, we suggest that such environmental stressors may better mimic the level of stress encountered in everyday life (Weekes et al., 2006), and therefore make for a more externally valid measure.

In the present study, each subject was tested during both a high and a low examination stress period. Equal numbers of males and females served as participants in this study, as our previous studies have shown sex differences in a number of the factors being measured (Weekes et al., 2005, 2006). Stress was measured through both psychological inventories and hormonal assays for cortisol. Health will be measured through a negative symptom inventory.

Finally, prefrontal asymmetry was measured via EEG alpha asymmetry. As is typical within this literature, asymmetry in brain activity will be inferred from the measurement of alpha band EEG. This inference has been empirically supported by studies demonstrating an inverse relationship between alpha and cerebral perfusion using $H_2^{15}O$ with PET (Cook et al., 1998) and BOLD with fMRI (Laufs et al., 2003).

However, it remains unclear which EEG leads are most relevant for measuring emotion related changes in frontal asymmetry. For instance, there is some question regarding whether midline frontal (F3/4) or lateral frontal (F7/8) leads are more sensitive to mood states or individual differences in trait characteristics (see Tops et al., 2005; Rilling et al., 2001 for discussions of this issue). While midline frontal effects are more consistently reported (see Coan and Allen, 2004 for a review of this issue), when lateral effects are included, they are often found as well (Reid et al., 1998; Tops et al., 2005). For this reason, both midline frontal and lateral frontal leads were included in the analyses for this study. Along with this more traditional approach, a full scalp quadrant analysis was also performed in order to better demarcate the extent of any shifts in asymmetry.

It was predicted that the occurrence of examination stress would result in relatively greater RFA, and that greater psychological stress, cortisol levels and negative health would be associated with relatively greater RFA.

1. Methods

1.1. Participants

Of the 66 participants in a larger cortisol and examination stress study (see Weekes et al., 2006), 49 participants (25 males and 24 females) were included in the present EEG analysis. Of the 17 participants who were excluded from EEG analysis, 7 did not have sufficient artifact-free EEG data to analyze during their low examination stress session, and 10 did not have sufficient EEG data during their high examination stress session. Incomplete data also lowered the number of participants in analyses utilizing stress and health surveys and cortisol analyses and will be reflected by lower degrees of freedom in the subsequent analyses. More specifically, incomplete data resulted in exclusion of one participant from the Spielberger state anxiety inventory analyses, two participants from the Spielberger trait anxiety inventory, three participants from the perceived stress scale analyses, four participants from the health symptoms inventory analyses and one participant from the cortisol analyses. Participants were undergraduate students between the ages of 18–22 years at the time of testing. Exclusion criteria included: (i) smokers, (ii) left-handers, (iii) non-native English speakers, (iv) those with vision that was not corrected to normal, (v) antihistamine, glucocorticoid or asthma medication users, (vi) those with exposure to general anesthesia in the last year, (vii) those with a personal or first degree family diagnosis of a DSM-IV, axis I disorder (a list of these disorders was given at time of initial inquiry), and (viii) those with endocrine abnormalities. These exclusionary criteria were self-affirmed by the prospective participants.

1.2. Materials

1.2.1. Spielberger State Trait Anxiety Inventory (STAI; Spielberger, 1983)

1.2.1.1. The State Inventory. This 20-item scale is the subscale of the Spielberger State Trait Anxiety Inventory that assesses anxiety at the time of testing. The Spielberger State Anxiety Inventory questions how participants “feel right now” regarding items such as “I wish I could be as happy as others seem to be” and “I have disturbing thoughts” rated on a 4-point scale in which 1 signifies “almost never” and 4 signifies “almost always.”

1.2.1.2. The Trait Inventory. This 20-item scale is the subscale of the Spielberger State Trait Anxiety Inventory that assesses chronic anxiety. The Spielberger Trait Anxiety Inventory questions how participants “feel generally” regarding items such as “I wish I could be as happy as others seem to be” and “I have disturbing thoughts” rated on a 4-point scale in which 1 signifies “almost never” and 4 signifies “almost always.”

1.2.2. Perceived Stress Scale (Cohen et al., 1983)

This 14-item inventory assesses the frequency of feelings of anxiety regarding certain potentially stressful events. The 5-point scale includes items such as “In the last month, how often have you been angered because of things that happened that were outside of your control?” and “In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?” Responses range from 0 indicating “never” to 4 indicating “very much so.”

1.2.3. Health symptoms inventory

This 21-item inventory assesses the frequency and severity of certain physical symptoms shown in previous studies to be affected by psychological stress. Symptoms include extreme fatigue, allergic reaction, sleep problems, stomach ache, nausea/vomiting, diarrhea, muscle aches and pains, headache or migraine, and weight change (gain or loss of 5 lb or more). These items were rated on a 4-point scale from 0 indicating “not at all” to 3 indicating “severe.” Seven of the items were specific signs and symptoms associated with an upper respiratory tract infection (URTI), including respiratory congestion, runny nose, coughing, sore throat, sneezing, blocked nose, and fever or chills. A shorter version of this same scale was used in our earlier work (Weekes et al., 2005).

1.3. Procedures

Subjects were recruited through mass e-mails to undergraduates at The Claremont Colleges, and through flyers posted around campus. Prospective participants were asked to go to a website set up by the experimenters and complete an online informed consent and survey. After completing the online informed consent and exclusionary criteria survey, subjects who met the criteria were invited to attend recruitment meetings, one before the low examination session and the other before the high examination session. Subjects were asked in advance of their “high examination” meeting to bring documentation of their examination schedule for that academic term.

All subjects came into the laboratory individually, once during the low examination stress period, and once during the high examination stress period. The low examination stress sessions occurred during a week when subjects had no exams and no significant assignments due. The high examination stress sessions occurred during a week when students had three or more exams or significant assignments due. In order to avoid the sharp decline in cortisol observed during the hours following morning awakening (see Kirschbaum and Hellhammer, 1994 for a review of this issue), all subjects were tested in the afternoon or early evening hours. A typical session started at either 3:30 p.m. or 5:30 p.m. For any one individual, there was no more than a 1-h discrepancy between the commencement of the low stress and high stress testing sessions. The two sessions occurred approximately 3 months apart ($M = 2.75$, $S.D. = .96$), and each session was approximately 2 h in length ($M = 1.95$, $S.D. = .85$).

At the beginning of the session, subjects completed a packet of inventories and provided one salivary sample. Then, subjects participated in electrophysiological testing, which included a memory ERP task, with a 30-min distracter period, and the baseline EEG asymmetry task. With regard to the EEG asymmetry recording, subjects participated in eight randomly assigned ‘eyes-open’ and ‘eyes-closed’ 1-min resting EEG blocks. After electrophysiological testing, subjects provided a second salivary sample. The approximate time that elapsed between salivary samples was 1 h and 45 min.

Order of sessions was counterbalanced across subjects, such that approximately half of the subjects had their low examination stress session first (Group A) and the other half had their high examination stress session first (Group B). The study protocol was approved by the Pomona College Institutional Review Board For Human Subject Protection.

1.3.1. Cortisol analysis

Salivettes were stored at -29°C within 1 h of sampling until analysis (storage at room temperature for less than 48 h does not affect the biological measures). Samples were assayed for salivary cortisol in duplicate using a highly sensitive enzyme immunoassay (Salimetrics, PA).

The cortisol assay used 25 μl of saliva (for singlet determinations), has a lower limit of sensitivity of .003 $\mu\text{g/dl}$, a range of sensitivity from .003 to 1.2 $\mu\text{g/dl}$, and average intra- and inter-assay coefficients of variation 4.13% and 8.89%, respectively. Method accuracy (determined by spike recovery) and linearity (determined by serial dilution) are 105% and 95%. Values from matched serum and saliva samples show a strong linear relationship, $r(17) = .94$, $p < .0001$.

Salivary analyses for each participant were always run in series with the same reagents and standard curve. Individuals responsible for analysis were blind to the condition of each subject and to the study design.

1.3.2. EEG recording

EEG was recorded with an Electrical Geodesics Inc. 128-channel Geodesic Netstation System. The EGI Sensor Net used Ag/AgCl sintered electrodes connected to an AC-coupled high input impedance (200 M Ω) Netstation 200 amplifier. Analog voltages (amplified by a factor of 1000 and using a bandpass of .01–100 Hz) were digitized with a 16-bit A/D converter at 250 Hz. Electrodes were adjusted to impedances below 50 k Ω , which preserves the signal integrity (<.1% error) for a system of this design (Ferree et al., 2001). Recording electrodes were referenced to vertex.

1.3.3. EEG analysis

Off-line, EEG was filtered (1–30 Hz), re-referenced to the average reference, and ocular artifacts were removed using a PCA-based procedure (Ille

et al., 2002). EEG analysis followed the protocol set forth in Tomarken et al. (1992). Briefly, for each block of 1-min 'eyes-open' or 'eyes-closed data', alpha power density for frequencies from 8 to 13 Hz was calculated for artifact-free segments of 2.05 s in duration using an overlapping Hamming window. This ensures that each time point contributes equally to the mean spectrum. For a 1-min block of data to remain in the analysis, at least twenty 2.05 s segments had to be free of artifacts. Subjects were excluded from further analysis if they did not have at least six of the eight blocks with a sufficient number of artifact-free segments across both the low exam and high exam sessions. This resulted in the exclusion of 17 subjects. The remaining data included an average of 403 artifact-free segments during the low exam session and 392 artifact-free segments during the high exam session. Alpha power densities were transformed using a natural logarithm transformation to reduce skew and an average weight for the number of artifact-free segments was calculated across the eight conditions. Asymmetry scores represented the difference between log alpha density in the right hemisphere electrodes of interest and log alpha density in the left hemisphere electrodes of interest or $\ln(R/L)$ alpha power (see Allen et al., 2004).

2. Results

2.1. Asymmetry and stress across examination stress sessions

The extent to which psychological and hormonal stress measures changed with the examination stress protocol was investigated through a series of paired sample *t*-tests. The psychological stress measures included the state subscale of the Spielberger State Trait Anxiety Inventory (STAI-S), the trait subscale of the Spielberger State Trait Inventory (STAI-T) and Cohen's Perceived Stress Scale (PSS). The hormonal stress measure was cortisol. Significant session differences were observed for STAI-S, $t(47) = 3.19$; $p < .005$, and PSS, $t(45) = 4.40$, $p < .001$. In both cases, higher scores were observed during the high examination stress session than during the low examination stress session. Neither STAI-T, $t(46) = 1.33$, ns, nor cortisol, $t(47) = 1.14$, ns, showed significant session differences.

In order to investigate the effect of examination stress on alpha asymmetry, the weighted averaged natural log alpha power values for electrodes within four left and four right scalp quadrants were calculated (i.e., anterior superior, anterior inferior, posterior superior, and posterior inferior quadrants; see Curran, 1999 for specific electrode assignments). The left and right anterior superior quadrants contained the commonly analyzed dorsal F3/F4 sites, respectively, and the left and right anterior inferior quadrants contained the commonly analyzed lateral F7/F8 sites, respectively. Means and standard deviations

for the left and right quadrants during low and high exam sessions are presented in Table 1. Scalp distribution of the *q*-values comparing the low and high exam sessions are represented in Fig. 1 (see Storey, 2002). A $2 \times 2 \times 2 \times 2$ ANOVA was conducted with session (low examination stress/high examination stress), hemisphere (right/left), AP (anterior/posterior), and SI (superior/inferior) as the within-subject factors, sex (male, female) as the between-subject factor, and weighted averaged natural log alpha power as the dependent variable. Since our primary interest was the effect of examination stress on alpha asymmetry, we will restrict reporting of the results of the five-way repeated measures ANOVA to effects involving the low exam stress and high exam stress sessions.

There was a main effect of session, $F(1,47) = 8.79$, $p = .005$, such that alpha power was lower (i.e., activity increased) during the high exam session than during the low exam session. There was also a trend toward a session by anterior/posterior interaction, $F(1,48) = 3.82$, $p = .06$, such that there was a greater percentage decline in alpha (i.e., increase in activity) in the anterior scalp region than in the posterior scalp region between the two sessions. There was also a trend toward a significant four-way interaction between session, hemisphere, anterior/posterior and superior/inferior, $F(1,47) = 3.48$, $p = .07$. No other interactions involving session approached significance. Considering the *a priori* prediction of a relatively less right frontal alpha (i.e., relatively greater RFA) during the high exam stress condition, the best way to characterize the four-way interaction is to compare asymmetry scores between the low exam and high exam stress conditions for each of the four quadrants. A significant session difference was observed for only the anterior superior quadrant, $t(48) = 2.00$, $p = .05$, such that there was relatively less right alpha power (i.e., relatively greater RFA) during the high examination stress session than during the low examination session.

To further characterize the change in anterior superior alpha asymmetry across the two sessions, separate means comparisons were performed for the two hemispheres. The shift to relatively greater RFA was the result of both a trend toward a decrease in left anterior superior alpha power (i.e., greater activity), $t(48) = 1.94$; $p = .06$, and a significant decrease in right anterior superior alpha power (i.e., greater activity), $t(48) = 3.43$; $p < .005$, during the high examination stress session. Consequently, these analyses show that there was a shift from relatively greater right frontal alpha (i.e., relatively

Table 1
Means (*M*) and standard deviations (*S.D.*) of weighted average natural log alpha power EEG values by right and left quadrants during low and high exam stress conditions

Quadrant	Baseline				Examination			
	Left hemisphere		Right hemisphere		Left hemisphere		Right hemisphere	
	<i>M</i>	<i>S.D.</i>	<i>M</i>	<i>S.D.</i>	<i>M</i>	<i>S.D.</i>	<i>M</i>	<i>S.D.</i>
Anterior superior	0.2151	0.1174	0.2183	0.1102	0.1982	0.1008	0.1913	0.1085
Anterior inferior	0.2108	0.1022	0.2191	0.1012	0.1912	0.0941	0.1994	0.0932
Posterior superior	0.3114	0.1206	0.3118	0.1275	0.2786	0.1121	0.2856	0.1127
Posterior inferior	0.2865	0.1144	0.2756	0.1291	0.263	0.1096	0.252	0.1120

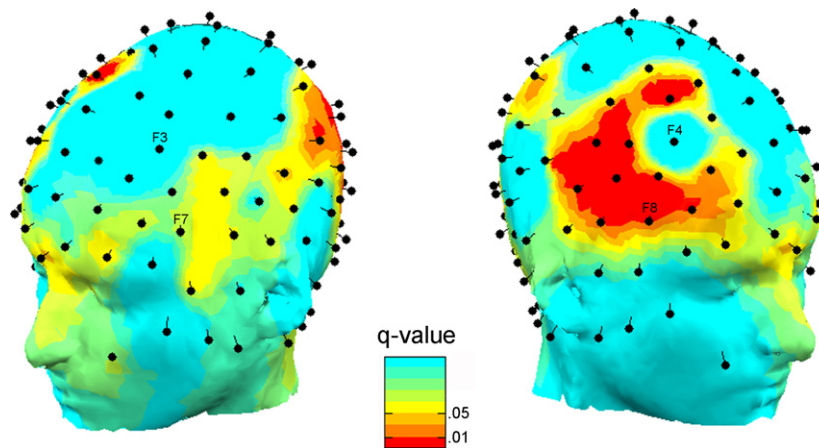


Fig. 1. Scalp distribution of q -values (see Storey, 2002) reflecting differences between low and high exam stress values of the weighted average natural log alpha power EEG. Lower q -values indicate relatively greater EEG activity during the high exam session relative to the low exam session. Figure was created with the aid of EEGLAB.

greater LFA) during low exam stress to relatively greater left frontal alpha (i.e., relatively greater RFA) during high exam stress in the anterior superior quadrant. This shift in asymmetry during high exam stress was due primarily to a greater decrease in alpha power (i.e., greater RFA) in the right anterior superior quadrant than in the left anterior superior quadrant. When group/order of sessions (Group A and Group B) was added as between-subject factors, no additional significant effects were observed.

In order to compare our results with those reported in the literature (where often fewer electrode sites are used), we also ran t -tests including session changes for F3/4 (located within the anterior superior quadrant) asymmetry and session changes for F7/8 (located within the anterior inferior quadrant) asymmetry, the electrode sites most frequently analyzed in the literature. A difference was found for F7/8 asymmetry, $t(48) = 1.91$, $p = .06$, such that significantly less alpha was observed both at F7, $t(48) = 2.12$, $p < .05$, and at F8, $t(48) = 3.76$, $p < .001$, during the high examination stress session than during the low examination stress session. No significant session differences were observed for F3/4 asymmetry, $t(48) = 1.06$, ns. More specifically, there were no session differences either at F3, $t(48) = 1.39$, ns, or at F4, $t(48) = .64$, ns. When t -test analyses were separated by group/order of sessions (Group A and Group B), no additional significant effects were observed.

Correlations were run between the change in stress measures and the change in asymmetry measures (see Table 2). No significant correlations were observed. These findings suggest that while psychological measures of stress and right prefrontal asymmetry increased with examination stress, individual differences in the shift in one variable did not predict individual differences in the shift in the other. Furthermore, changes in prefrontal asymmetry were not related to changes in cortisol.

2.2. Asymmetry and health

The extent to which the negative health symptoms measure fluctuated with examination stress was investigated through a paired sample t -test. There was no significant difference in

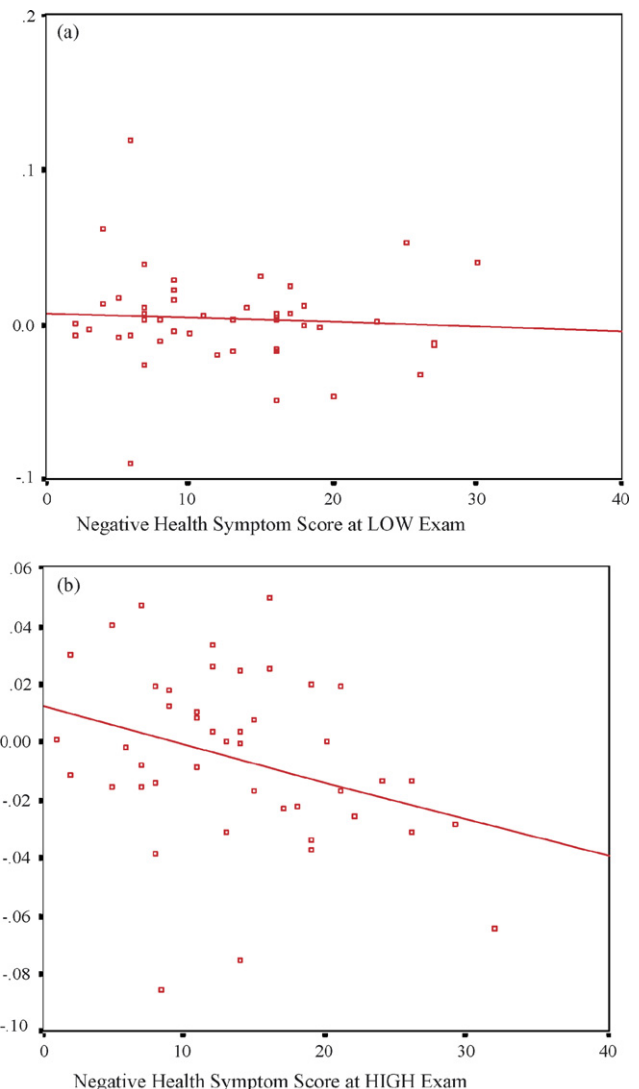


Fig. 2. Scatterplots of the relationship between EEG alpha asymmetry in the anterior superior leads and negative health inventory score during (a) the low examination stress session ($r = -.07$) and (b) the high examination stress session ($r = -.32$). During the high examination stress session relatively less right alpha (i.e., relatively greater right activity) was associated with increasing reports of negative health symptoms.

Table 2

Pearson correlation values of the differences between low and high examination stress values for psychological and hormonal measures of stress, negative health values, and weighted average natural log alpha power EEG asymmetry values

	Anterior superior	Anterior inferior	Posterior superior	Posterior inferior
STAI-S	.04 (<i>N</i> = 48)	-.14 (<i>N</i> = 48)	.04 (<i>N</i> = 48)	.05 (<i>N</i> = 48)
STAI-T	.08 (<i>N</i> = 47)	-.08 (<i>N</i> = 47)	-.04 (<i>N</i> = 47)	.20 (<i>N</i> = 47)
PSS	-.02 (<i>N</i> = 46)	-.03 (<i>N</i> = 46)	-.04 (<i>N</i> = 46)	-.15 (<i>N</i> = 46)
Cortisol	.11 (<i>N</i> = 48)	-.12 (<i>N</i> = 48)	-.17 (<i>N</i> = 48)	-.10 (<i>N</i> = 48)
Negative health	-.037* (<i>N</i> = 45)	.19 (<i>N</i> = 45)	.22 (<i>N</i> = 45)	.16 (<i>N</i> = 45)

Note: The negative correlation between the negative health inventory and the anterior superior EEG alpha asymmetry reflects an increase in the report of negative health symptoms associated with a relative increase in right anterior superior EEG activity.

* $p < .05$ (two-tailed test).

negative symptoms across the two sessions ($t(46) = -1.51$; $p = .14$), though the pattern was such that greater negative health symptoms were reported during the high examination stress session than during the low examination stress session.

Correlations were run between the change in health measures and the change in alpha asymmetry in the four EEG quadrants (see Table 2). A significant correlation was observed between the change in anterior superior alpha asymmetry and change in health ($r = -.37$; $p < .02$), such that as subjects showed relatively less right alpha across the two sessions (i.e., relatively greater RFA), they also showed greater increases in negative health symptoms. However, there were three data points that were outside of the 95% confidence interval. When this correlation was rerun, excluding these data, the correlation diminished to $-.28$, $p < .08$. In order to further investigate the nature of this relationship, we ran correlations between negative health and frontal asymmetry for the low and high examination stress sessions separately. These analyses revealed that the association between alpha asymmetry and negative health was largely driven by the correlation between these two variables during the high examination session (see Fig. 2). This is demonstrated by a low correlation between anterior superior alpha asymmetry and negative health during the baseline session ($r = -.07$, ns) and a significant correlation between these variables during the examination stress session ($r = -.32$, $p < .05$). No other significant effects were observed between EEG asymmetry and the stress and health measures.

Finally, correlations were run among negative health, the psychological stress measures, and cortisol (see Table 3). There

was a significant correlation between the change in STAI-S and the change in STAI-T ($r = .52$, $p < .001$), and between the change in STAI-S and the change in PSS ($r = .38$, $p < .05$), reflecting the fact that these three psychological measures of stress each measure subjective impressions of stress that vary primarily in time frame. There was also a trend for a relationship between STAI-S and negative health ($r = .27$, $p < .1$) such that increases in state stress were associated with increases in reports of negative health.

In order to better understand the relationship among stress, frontal asymmetry and negative health, a regression was run with STAI-S and anterior superior alpha asymmetry serving as the independent variables and negative health as the dependent variable. The overall model was significant ($R^2 = .25$, $p < .01$). Both STAI-S (Beta = .29, $t(43) = 2.07$, $p < .05$) and anterior superior asymmetry (Beta = $-.41$, $t(43) = -1.93$, $p < .01$) were significant predictors of negative health. One possibility is that stress mediates the relationship between frontal asymmetry and negative health. However, since STAI-S was not correlated with frontal asymmetry, and since such a correlation is a necessary condition for such a model (see Baron and Kenny, 1986; Coan and Allen, 2004), the present findings argue against a mediational model. Alternatively, psychological stress could moderate the relationship between frontal asymmetry and negative health. However, our findings also argue against a moderator model because the interaction term of psychological stress and frontal asymmetry was not a significant predictor of negative health, Beta = $-.056$, $t(41) = -.26$, ns. Instead, our data suggest that psychological stress and frontal asymmetry independently influence negative health.

Table 3

Pearson correlations for differences between low and high examination stress values for the psychological and hormonal measures of stress and negative health

	STAI-S	STAI-T	PSS	Cortisol	Negative health
STAI-S	1.00 (<i>N</i> = 47)	.52* (<i>N</i> = 46)	.38* (<i>N</i> = 46)	-.02 (<i>N</i> = 46)	.27** (<i>N</i> = 44)
STAI-T	.52* (<i>N</i> = 46)	1.00 (<i>N</i> = 46)	.11 (<i>N</i> = 45)	.07 (<i>N</i> = 45)	.17 (<i>N</i> = 43)
PSS	.38* (<i>N</i> = 46)	.11 (<i>N</i> = 45)	1.00 (<i>N</i> = 46)	-.15 (<i>N</i> = 46)	.15 (<i>N</i> = 44)
Cortisol	.11 (<i>N</i> = 48)	-.07 (<i>N</i> = 45)	.02 (<i>N</i> = 46)	1.00 (<i>N</i> = 48)	-.04 (<i>N</i> = 45)
Negative health	.27** (<i>N</i> = 44)	.17 (<i>N</i> = 43)	.15 (<i>N</i> = 44)	-.04 (<i>N</i> = 45)	1.00 (<i>N</i> = 45)

Note: The positive correlations between state anxiety (STAI-S) and trait anxiety (STAI-T) and between state anxiety (STAI-S) and perceived (PSS) reflect the fact that all three are psychological measures of stress. The positive correlation between STAI-S and negative health suggests that as reports of stress increased across session, so did reports of negative health.

* $p < .05$ (two-tailed test).

** $p < .1$ (two-tailed test).

3. Discussion

Two major results were observed in the present study. First, as predicted, subjects demonstrated relatively greater right RFA during the high examination stress session than during the low examination stress session. Second, there was a relationship between this shift and an increase in reports of negative health symptoms.

The finding of relatively greater RFA during the high examination stress session (relative to the low examinations stress session) is consistent with an abundance of previous literature (see Coan and Allen, 2004, for a review). Specifically, numerous studies have demonstrated a shift toward greater right frontal activity during exposure to negatively valenced stimuli or conditions that might normally trigger a withdrawal response (e.g., Davidson et al., 1990). Along with confirming the results of previous studies, the present results also extend these findings to include the effects of a naturalistic stressor on prefrontal asymmetry—to our knowledge, the first study to do so.

Although exposure to exam stress was associated with a shift in prefrontal asymmetry, the relationship underlying this association remains obscure. Specifically, no significant correlations were observed between the change in frontal asymmetry and the change in any of the stress measures (either psychological or hormonal). Future studies are clearly needed in order to further investigate the underpinnings of these relationships.

Whereas previous investigations of stress with human and non-human animals have consistently found associations between relatively greater RFA and increases in cortisol (Buss et al., 2003; Kalin et al., 1998; Rilling et al., 2001; Schmidt et al., 1999; Tops et al., 2005), we did not. The present findings, therefore, have an interesting implication for understanding the relationship between stress and frontal asymmetry. Our findings suggest that shifts in frontal asymmetry in response to a stressor need not depend on shifts in cortisol.

Few studies have investigated the relationship between prefrontal asymmetry, immunity, and health. Of the few that have, relatively greater RFA tends to predict immunosuppression (Davidson et al., 1999; Kang et al., 1991). However, the connection between immunosuppression and the report of health symptoms (as was measured in the present study) remains unclear. For instance, due to the complexity of the immune system, significant changes in immune functioning are not necessarily met with obvious changes to health symptoms or health outcome. Furthermore, an increased report of negative health symptoms may simply accompany increased anxiety and the impact such anxiety has on focusing attention to negative events or experiences. Nevertheless, the finding of greater changes in reports of negative health symptoms in those subjects who also showed a shift toward relatively greater RFA is consistent with the immunity findings of previous studies. There is evidence to suggest that prefrontal asymmetry is predictive of immunocompetence independent of stress level (e.g., Barneoud et al., 1987; Davidson et al., 1999; Kang et al., 1991; Neveu, 1988, 1993; Quaranta et al., 2004). While

immunocompetence was not directly tested in the present study, our data are consistent with this view. While examination stress resulted in higher levels of psychological stress, we did not find a correlation between psychological stress and RFA. This is inconsistent with psychological stress mediating the relationship between RFA and negative health. Additionally, we did not find that the interaction of psychological stress and RFA predicted negative health. This finding is inconsistent with a moderator model. Instead, our data suggest that RFA and stress independently predict negative health. This is consistent with Davidson et al. (1999) and Kang et al. (1991). Furthermore, our study also indicates that the relationship between RFA and health is not necessarily cortisol-dependent, which is also consistent with Kang et al. (1991).

The specific contributions of the left and right frontal areas to relatively greater RFA associated with negative emotions have not always been clearly delineated in the literature. This is due to the common use of an asymmetry score as opposed to absolute alpha values. While there are good methodological reasons for using asymmetry scores, ultimately we need to understand each hemisphere's contribution to the asymmetry (see Allen et al., 2004; Coan and Allen, 2004). In the current study, the shift toward relatively greater RFA during the examination stress session was due primarily to an increase in RFA. This finding is consistent with other studies of negative emotions (see Coan and Allen, 2004), and indicates a special role of the right frontal area in negative emotions (see Davidson et al., 2000; Heilman et al., 2003).

Our analyses divided the scalp into eight regions involving the left/right; anterior/posterior; and inferior/superior dimensions. We analyzed the data utilizing regional scalp areas and compared them with the more traditional analyses based on fewer electrodes, most frequently involving mid frontal electrodes (e.g., F3/4) and more laterally placed electrodes (e.g., F7/8). Interestingly, these two analyses led to different conclusions. We found a shift in activity associated with examination stress in the right anterior superior region, which includes electrode F4, but did not find a shift in the right anterior inferior region, which includes F8. However, when we restricted the analysis to only investigating shifts in activity with electrodes F3/4 and F7/8, we only found a shift in activity with F7/8.

Inspection of Fig. 1 shows that the greatest region of activity shift is in the right dorsolateral frontal region, and not the orbitofrontal region. F3/4 and F7/8 are electrodes that are located at the fringes of this active region with F7/8 lying at the inferior extent and F3/4 just superior to the anterior portion of this region. This would explain the apparent contradictory results between the regional and individual electrode analyses. Given the placement of these electrodes on the outer border of the region of the activity shift, analyses focusing on only these electrodes may be more susceptible to environmental factors (e.g., intensity of the stressor) and technical factors (e.g., variability in electrode placement) affecting the location of asymmetry activations. Our findings argue for a comprehensive sampling of frontal sites in EEG asymmetry studies.

While EEG asymmetry studies have proven a fruitful field of exploration into neural mechanisms of emotional regulation, there are significant weaknesses to the usage of this methodology for localizing the neural generators of this activity. fMRI studies will be particularly useful in determining the specific role of orbitofrontal, ventromedial and dorsolateral prefrontal subregions in hemispheric emotional regulation (Davidson, 2004).

In conclusion, we have demonstrated that a naturalistic stressor, in this case examination stress, was associated with a change in frontal activity asymmetry caused primarily by an increase in right dorsolateral frontal activity. This change in frontal asymmetry was also associated with an increase in the report of negative health symptoms. Although the mechanisms of these changes are unclear, they do not appear to be dependent on changes in cortisol. Therefore, stressors experienced in everyday life can result in increases in the perception of stress, a shift in relatively greater right frontal activity, and increased negative health symptoms without requiring an increase in cortisol to accompany them. Therefore, the mechanisms mediating these relationships need further investigation.

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