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Brain Activation during Acute Mental Stress and Outcomes in Patients with Coronary Artery Disease

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Abstract

Psychological stress is a risk factor for angina and major adverse cardiovascular events (MACE) in individuals with coronary artery disease (CAD). Certain brain regions that control both emotional states and cardiac physiology may be involved in this relationship. The inferior frontal lobe and the rostromedial prefrontal cortex (rmPFC) are two important brain regions that processes stress and regulates immune and autonomic functions. However, it is unclear if activation of these regions with stress correlate with angina or MACE in individuals with CAD. Individuals with stable CAD underwent acute mental stress testing using a series of standardized speech/arithmetic stressors in conjunction with high resolution positron emission tomography imaging of the brain. Blood flow to the inferior frontal lobe and rmPFC were evaluated as a ratio compared to whole brain flow for each scan. Interleukin-6 (IL-6) levels 90 minutes post-stress, and high-frequency heart rate variability (HF-HRV) during stress were also assessed. We analyzed 148 individuals with CAD (mean age (SD) 62 (8) years; 69% male, and 35.8% African American). For every doubling in the inferior frontal lobe activation, angina frequency was increased by 13.7 units at baseline ($\hat{\beta}$ 13.7, 95% CI 6.3, 21.7, p=0.008) and 11.6 units during follow up ($\hat{\beta}$ 11.6, 95% CI 4.1, 19.2, p=0.01) in a model adjusted for baseline demographics. . After adjustment for baseline demographics, risk factors, and baseline levels of IL-6 and HF-HRV, higher rmPFC stress reactivity was independently associated with higher IL-6 and lower HF-HRV with stress. During a median follow-up of 3 years, 34 subjects (21.3%) experienced a MACE. Each 1SD increase in rmPFC activation with mental stress was associated with a 21% increase risk of MACE (HR 1.21, 95% CI 1.08-1.37). Stress-induced IL-6 and HF-HRV explained

15.5% and 32.5% of the relationship between rmPFC reactivity and MACE. In conclusion, Inferior frontal lobe activation with mental stress is independently associated with angina at baseline and during follow-up. Also, greater rmPFC stress reactivity is associated with incident MACE and is at least partly mediated by immune and autonomic responses to mental stress.

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Table of Contents

Introduction

Despite significant advances in the prevention and treatment of coronary artery disease (CAD), this condition remains the leading cause of mortality and disability worldwide (1). Angina pectoris is the hallmark symptom of coronary artery disease (CAD) and an important determinant of adverse outcomes, quality of life and healthcare costs in CAD (2, 3). Psychological stress has emerged as a risk factor and prognostic factor for CAD (4). Higher levels of stress have been associated with an increased risk of incident CAD events, such as acute myocardial infarction and CAD mortality (5-7). In most studies, stress has been assessed subjectively as self-report. However, it is possible to gain a more firm understanding of this relationship by directly studying the brain, where the psychological stress response begins (8). To date, there has been no investigation on whether the brain's stress response is associated with angina pectoris or if it predicts future cardiovascular risk. If found, this may lead to important insights into cardiovascular disease risk assessment and prevention.

In the present study, we aimed to investigate the relationship between activation of two brain regions with mental stress and angina and future cardiovascular events in individuals with known CAD, as well as potential mechanisms. We hypothesized that greater activation in the inferior frontal lobe during mental stress will be associated with angina. We also hypothesized that higher rmPFC activation during mental stress would predict future adverse cardiovascular disease outcomes, and that this association would be mediated through autonomic dysfunction and inflammatory responses.

Background

Recent evidence suggests psychological factors are stronger determinants of angina than the burden of CAD (9-13). In addition, the self-reported frequency of angina is related to the degree of myocardial perfusion defects induced by acute mental stress, but not by exercise stress testing (10). However, the central neurological mechanisms for the association between reported symptoms of angina and mental stress are unknown. Given the poor correlation between angina symptoms and extent of CAD, it is important to explore an alternative paradigm for angina as a neurocardiac phenomenon that originates in the brain, which is more in line with other pain syndromes (14).

The inferior frontal lobe is an area of the brain known to be involved in emotional regulation and stress (15, 16). In a meta-analysis of brain imaging studies in humans, this region was consistently activated with psychological stressors (17). We have recently shown that CAD patients with mental stress-induced myocardial ischemia exhibit increased inferior frontal lobe perfusion in response to stress (18). Since the inferior frontal lobe is a key brain region involved in stress reactivity, study of its activation with an acute mental stress challenge may help to unmask functional abnormalities.

The rostromedial prefrontal cortex (rmPFC) region is a key regulator in the default mode network that is central to emotional and cognitive processing (19). In previous work, we have found greater stress activation of the rmPFC in CAD participants exposed to early traumatic events, as well as those who exhibit high stress reactivity with peripheral vasoconstriction (20, 21). As opposed to other regulatory areas of the medial prefrontal cortex, this region is specifically activated during social stressors that include

embarrassment and social rejection, and is one of the most significantly activated regions within the frontal lobe during cognitive stress challenge (22). Its activation may in particular be an important mechanism linking social status and stress with adverse CAD outcomes (23). Given its prominent role in the default mode network (24), as well as connections with the thalamus and other limbic structures, this region likely has many important pleiotropic effects on the cardiovascular system, but further research is warranted (25).

Previous evidence has suggested rmPFC activation with stress associates with inflammation and autonomic activation (26, 27). Changes in vagal tone for example have been related to changes in rmPFC blood flow during working-memory tasks (26). Abnormal activity of rmPFC can also influence immune function as measured by interleukin-6 (IL-6), a marker of systemic inflammation (28). It may also be an important predictor of long-term outcomes as the result of pathological stress reactivity (29). As a next step, a more rigorous evaluation of rmPFC activity with mental stress, inflammation, and cardiovascular disease outcomes is needed to help understand its potential role in disease pathogenesis.

Methods:

We hypothesized that greater activation in the inferior frontal lobe during mental stress will be associated with angina. We also hypothesized that higher rmPFC activation during mental stress would be associated with future adverse cardiovascular disease outcomes, and that this association would be mediated through autonomic dysfunction and inflammatory responses.

Study Design

This is a prospective study where individuals with CAD were prospectively enrolled into the Mental Stress Ischemia Prognosis Study.

Characteristics of the study population

Between June 2011 and August 2014, individuals with confirmed stable CAD were recruited from Emory-affiliated hospitals and clinics as part of the Mental Stress Ischemia Mechanisms and Prognosis Study (MIPS) as described elsewhere (30). CAD was defined based on a positive nuclear stress test, previous cardiac catheterization showing atherosclerosis, history of prior myocardial infarction, or a history of percutaneous coronary intervention or coronary artery bypass grafting. Participants were excluded if they: had a recent episode of unstable angina, decompensated heart failure within one week, were pregnant, had a systolic blood pressure greater than 180 mm Hg or diastolic blood pressure greater than 110 mm Hg on the day of the test, had a history of alcohol or substance abuse or severe psychiatric disorders including schizophrenia, psychotic depression, or bipolar disorder in the past year, or had chronic steroid use

above 1.5 milligram per day. Data regarding the sociodemographic characteristics, medical history, and medication history of all participants were collected using standard questionnaires, and were verified using medical records. All patients provided written informed consent, and the study was approved by the Emory University Institutional Review Board (Figure 1).

Measurements

Sociodemographic factors (age, sex, race, education, and current smoking), medical history (hypertension, hyperlipidemia, diabetes, obesity, previous myocardial infarction, previous revascularization), and list of medication taken were assessed using standardized questionnaires. Depressive symptoms were assessed using the Beck Depression Inventory (BDI) scale (31), and post-traumatic stress disorder symptoms were assessed using the post-traumatic stress disorder symptom checklist (32).

Mental Stress Testing

All participants underwent mental stress testing using a standardized protocol, as previously described (18, 30, 33). Briefly, following 30 minutes of resting in a quiet room, subjects were asked to perform two control tasks (counting aloud and recalling a neutral event), followed by two mentally stressful conditions including a mental arithmetic task and a public speaking task. During the mental arithmetic task, participants were asked to perform a series of increasingly complicated mathematical calculations under time pressure, including addition, subtraction, multiplication, and division, whereas they received negative feedback on their performance from a staff member performing the test who was wearing a white coat (34). The difficulty of mental math was increased

until participants incorrectly answered three successive problems. During the public speaking task, participants were given two stressful situations, one involving a long-term house guest who had overstayed her welcome and the other an uncomfortable situation in which an elderly sister was unfairly hit while driving in a parking lot. They were then asked to prepare and present (2 min) for each situation and were told the speech would be evaluated for content. Blood pressure and heart rate were recorded at one-minute intervals during the control and stress tasks using an automated oscillometric device.

Angina Assessment

Angina symptoms were assessed at baseline and also at 2 years follow-up using the Seattle Angina Questionnaire's (SAQ) angina-frequency subscale, which measures frequency of angina and use of nitroglycerin for chest pain over the previous 4 weeks (35). Scores for angina frequency are rescaled into on a 100-point scale, with 100 representing no chest pain and 0 representing angina occurring ≥ 4 times/day. The minimal clinically-significant change in SAQ scale is a change of 10 points (35). The SAQ has been validated in different populations and correlates with electronic daily diary entries for angina and nitroglycerin use (35, 36). Participants were divided into categories of angina frequency based on SAQ scores, defined as absent (score 100), monthly (score 61 to 99), weekly (score 31 to 60), and daily (score 0 to 30). Since only 3 participants (1.9%) reported daily angina, those with daily or weekly angina were combined into a single category for analysis.

Brain imaging during mental stress

HR-PET brain imaging was conducted using a High Resolution Research Tomograph (Siemens, Inc., Knoxville, TN), with a spatial resolution of 2 mm (37). A total of eight

brain scans were performed for each individual, with two scans during each of the 2 control conditions (counting aloud and recalling a neutral event) and 2 scans for each of the stress conditions (arithmetic and public speaking). Subjects were injected with 20 mCi of O-15 water 10 seconds after the beginning of each task to assess relative cerebral blood flow. The HR-PET has a sensitivity of 1,700,000 counts per second (cps)/(μCi/ml) and a spatial resolution of 2.4mm in the transaxial plane. This provides higher sensitivity than conventional PET cameras.

High Frequency- Heart Rate Variability Assessment

Each participant wore a Holter monitor during the entire testing period that included the control and stress conditions. Holter ECG monitor recordings were obtained with a SEER Light recorder by Marquette (GE) and stored digitally at 128 Hz. Each recording was manually processed and edited for accurate identification of QRS complexes by a trained research assistant. After editing each file, HF-HRV was measured using GE MARS 8.0.2 software (38). High Frequency- Heart Rate Variability (HF HRV) during the control and stress tasks were measured using one-minute time windows, while each task was ongoing. HRV values were log transformed (ln ms^2) as previously described, given the positive skew (39). Given that on average, the duration of mental stress testing was less than 3 minutes for each participant, other HRV bands including low frequency and very-low frequency HRV data were not obtained as these bands required longer recording times (40).

Inflammatory biomarkers measurement

We assessed inflammatory biomarkers at rest, and 90-min after mental stress testing on a separate day (30). Blood samples were also collected at 1 and 2 years follow up for all participants and analyzed for baseline IL-6 levels. Venous blood was collected into ice-cooled citrate tubes and centrifuged immediately at 4 ºC, thereafter, the plasma was snap-frozen at -70 ºC until further processing. High sensitivity assays were employed using the MesoScale system (Meso Scale Diagnostics Rockville, Maryland) using the SECTOR Imager 2400 to quantitate interleukin 6 (IL-6), and high sensitivity C-reactive protein (hsCRP) according to the protocols supplied by the manufacturer. Lower limits of detection for our studies were 0.06 pg/mL and 1.33×10^{-6} mg/L for IL-6 and hsCRP, respectively. The inter-assay coefficient of variations for midpoint standards were 5.78% for IL-6, and 3.06% for hsCRP. The intra-assay coefficients of variations were 3.29% for IL-6, and 2.33% for hsCRP.

Cardiovascular events

Major adverse cardiovascular events (MACE) were collected through follow-up clinic visits at 1 and 2 years, phone calls at 3 years, medical records review, and querying the Social Security Death Index. During the phone calls at 3 years follow up, participants or their family members were inquired about interim hospital admissions and deaths. All diagnoses were verified by an independent adjudication committee with two or more cardiologists using original source documents, which were retrieved in nearly all cases. Any differences were discussed until a consensus was achieved. For individuals who died during follow up, interviews with the next of kin were conducted and copies of death certificates obtained. The adjudication committee was unaware of the results of the brain scans or any other study variables. All outcomes were independently adjudicated by 2

experienced cardiologists. The main outcome of the study was a combined end point of MACE including cardiovascular death, myocardial infarction, unstable angina with revascularization and heart failure hospitalization.

HR-PET Data Analysis

Analysis of HR-PET images, including realigning, normalizing, and smoothing, was completed following established protocols (41) (42, 43) using statistical parametric mapping (SPM12; www.fil.ion.ucl.ac.uk/spm/software/spm12/). HR-PET imaging analysis was completed by creating individual contrast maps to determine increased regional blood flow (Blood FlowNet = Blood FlowMental Stress Task – Blood Flow Control Task). Next, a custom mask limiting brain activity to the inferior frontal lobe, derived from the inferior half of the superior medial frontal gyrus from the Automatic Anatomical Labeling atlas, was applied to the contrast image. We also applied a similar method to identify the magnitude of bilateral rmPFC activity during mental stress by creating a custom mask limiting brain activity to the bilateral rmPFC regions. Individual subject responses (delta blood-flow values from individual contrast map) were extracted from the masked contrast image and averaged across non-zero voxels.

High inferior frontal lobe and rmPFC activation were defined as having a value equal or greater than the median for the sample; the others were classified as having low activation.

Statistical analysis

Baseline characteristics of the population were compared according to increasing levels of angina frequency and severity using the analysis of variance for continuous, normally distributed variables or the chi-square test for categorical variables. Similarly, baseline characteristics of the population were compared between participants with high versus low rmPFC activation.

For our main analysis, we constructed multivariable linear regression models to examine the association between inferior frontal lobe activation (outcome variable) and presence of angina. Models were adjusted for sociodemographic and lifestyle characteristics (age, sex, race, education, and current smoking). We used the base-2 logarithm of the brain activation values to examine the effect of doubling of the brain activation on angina frequency as each unit difference in the log- transformed brain activation represents a doubling of the score. We did not find any association between angina and any of the comorbidities or other medications and therefore these factors were not included in the multivariable regression models to avoid overfitting. We tested for multicollinearity using the Variance Inflation Factor and confirmed that none of the covariates entered in our model met criteria for collinearity. Spearman rank correlation was used to examine the association between rmPFC activation and resting and post-mental stress inflammatory markers, as well as HF-HRV during mental stress testing.

Multivariable competing-risks Cox proportional hazard regression models were constructed to assess the association between mental stress–induced rmPFC activation and adverse events, adjusting for the same variables as above. Cox proportional hazards assumptions were assessed and verified both visually by Kaplan-Meier Curves. All covariates also conformed with the proportionality of hazard assumption and were not

violated by the use of models including time–covariate interactions (all $P > 0.05$ for interaction). Kaplan–Meier curves and the log-rank test were used to explore the association between rmPFC activation and MACE. To describe the association between rmPFC activity and cardiovascular events, stress-induced rmPFC activity was examined as a continuous variable, expressed as standard deviation (SD) increments. Adjusted Hazard ratios (HRs) along with the corresponding 95% confidence intervals (CI) were calculated after controlling for age, sex, race and heart rate-pressure product during mental stress to make statistical inference.

We performed a mediation analysis with bootstrapping (1,000 bootstrap samples and a 95 percent confidence interval) to assess the direct effect of rmPFC activation on MACE, as well as the indirect effect of rmPFC activation via mediators. We explored two mediators (IL-6 and HRV) on the effect of rmPFC activation on MACE. These mediators were found to be mutually independent when conditioned on rmPFC activation and confounders.

Results

Baseline Demographics

A total of 148 individuals with CAD were enrolled with mean (SD) age 62 (8) years; 69% were male, and 36% were African American. Participants reporting higher angina frequency were younger, more often African-American, and higher rates of mental stress-induced ischemia (Table 1). Symptoms of depression, post-traumatic stress disorder, and anxiety as well as use of antidepressants and nitrates, were also more common in participants reporting higher angina frequency (Table 1). Also, participants reporting higher angina frequency were found to have higher levels of distress (SUDS score) immediately following mental stress. Similarly, the DASI score which is reflective of functional capacity of the participants was worse among those reporting higher angina frequency (Table 1). There were no other significant differences in the angina frequency subgroups in demographics, medical history, or severity of CAD. Use of beta-blockers and calcium channel blockers were highest in the group reporting daily or weekly angina but these differences were not statistically significant. No associations were found between the severity of angina symptoms with the presence of ischemia during conventional stress testing (P=0.21).

As shown in Table 2, participants with high rmPFC activation had higher rates of hypertension and higher systolic blood pressures at rest compared to those with low activation (Table 2). There were no other significant demographic or clinical differences between those with low versus high rmPFC activation (Table 2). Also, none of the stress response vital signs to mental stress including heart rate, systolic and diastolic blood

pressure, and heart rate-pressure product were different between the group with high versus low rmPFC activation (Table 2).

Relationship between Angina and Inferior Frontal Lobe activation

As shown in Figure 2A, compared to participants without angina, both groups reporting monthly angina symptoms or weekly/daily symptoms were found to have higher inferior frontal lobe activation to mental stress at both baseline and after 2 years of follow-up ($P<0.001$). Those reporting angina during mental stress testing with cardiac perfusion imaging also had higher inferior frontal lobe activation (1.43 ± 0.42) compared to individuals who did not have active chest pain (1.19 ± 0.14) during mental stress testing ($P=0.03$). These individuals also had fewer years of education and higher BDI-II and PCL scores (Table 3). In addition, those who reported angina during mental stress-testing, were found to have higher distress levels measured by SUDS evaluated immediately after mental stress with both cardiac perfusion imaging and brain PET imaging compared to those who did not have active chest pain with mental stress testing (Table 1). Similarly, the group experiencing angina during stress testing with cardiac perfusion imaging had higher increases in SUDS score during mental stress testing compared to those who did not experience chest pain with mental stress testing (Table 3).

A total of 112 individuals completed the SAQ at 2 years of follow up. The characteristics of these individuals were not significantly different from the baseline cohort (Table 4). Twenty eight individuals (24.1%) reported an increase in the frequency of angina episodes during follow up (Figure 2B). These individuals were found to have a higher mean inferior frontal lobe activation with mental stress at baseline compared to those

who reported a decrease in chest pain frequency (1.82 ± 0.15 vs 0.92 ± 0.28, P=0.01, Figure 2B). There were no significant differences in the baseline demographics and clinical characteristics of the group who reported an increase in angina frequency compared to those with no change or improvement in chest pain symptoms (Table 5).

After adjusting for sociodemographic and lifestyle characteristics, SAQ scores were negatively associated with inferior frontal lobe activation (P=0.01). For every doubling in the inferior frontal lobe activation, angina frequency was increased by 13.71 units, after adjustment for the factors above ($\hat{\beta}$ 13.7, 95% CI 6.3, 21.7, p=0.008). Also, as shown in Figure 3, there was a positive linear association between changes in SAQ during 2 years of follow up and mental stress-induced inferior frontal lobe activation at baseline. After adjusting for the above factors, every doubling in inferior frontal lobe activation was associated with 11.6 units increase in angina frequency score during follow-up ($\hat{\beta}$ 11.6, 95% CI 4.1, 19.2, p=0.01). These changes in SAQ scores are clinically meaningful given that the minimal clinically-significant change in SAQ scores is a change of 10 units.

Relationship between rmPFC activation and HF-HRV

Mental stress testing resulted in a significant decrease in the HF-HRV, indicating vagal withdrawal (P<0.001). Participants with high rmPFC activation had lower HF-HRV during mental stress, but not at rest, compared to the group with low rmPFC activation (Table 2). A linear relationship was found between HF-HRV during mental stress and rmPFC activation (P values for non-linear test >0.05). In a model adjusted for baseline demographics (age, sex, and race) and heart rate-pressure product during mental stress,

rmPFC activation was independently associated with lower HF-HRV during stress (B= -0.10, 95% CI -0.14, -0.02, P=0.008), (Figure 4A).

Relationship between rmPFC activation and inflammation

Mental stress testing resulted in significant increases in the levels of IL-6 (23.1%, P<0.001), but not CRP levels (P= 0.23). While no differences were observed between participants with high vs low rmPFC activation with respect to baseline IL-6, baseline and post-stress CRP levels, participants with high rmPFC activation had 60.4% higher IL-6 levels 90 minutes after mental stress compared to those with low rmPFC activity (Table 1, P=0.02). As shown in Figure 4B, higher rmPFC activation was associated with an increase in post-mental stress IL-6 levels. This association remained significant after adjusting for baseline demographics (age, sex, and race) and heart rate-pressure product during mental stress (B= 0.16, 95% CI 0.07-0.33, P=0.006).

Compared to baseline IL-6 levels, the median levels of IL-6 were not different at 1 year (1.29, IQR 0.93-1.89, P=0.62) and 2 year (1.27, IQR 0.92-2.15, P= 0.83) follow up (Figure 5A). However, those with high rmPFC activation with mental stress at baseline were found to have significantly higher IL-6 levels both at 1 (1.52, IQR 1.01-3.01, P= 0.03) and 2 years (1.64, IQR 1.08-3.23, P= 0.01) of follow up (Figure 5B). As shown in Figure 5A, IL-6 levels were not significantly different for participants with low rmPFC activation at 1 or 2 years follow up compared to baseline levels.

Relationship between rmPFC activation and cardiovascular outcomes

During a median follow-up of 3 years (interquartile range 2.5, 3.6), 34 participants (21.3%) experienced a MACE which included 2 cardiovascular deaths, 1 myocardial

infarction, 5 hospitalizations for congestive heart failure, and 26 cases of unstable angina requiring urgent revascularization.

Figure 6 shows unadjusted Kaplan-Meier curves for MACE according to rmPFC activity. The difference between high vs low rmPFC activation was statistically significant (P=0.01). In a Cox regression analysis adjusting for baseline demographics (age, sex, and race) and heart rate-pressure product during mental stress, each 1SD increase in rmPFC activation with mental stress was associated with a 21% increase risk of MACE (HR 1.21, 95% CI 1.08-1.37).

Mediation analysis

We investigated whether the effect of rmPFC on MACE is at least partially mediated through inflammation and vagal withdrawal using the approach of Lange et al.(44, 45) After adjusting for baseline demographics (age, sex, and race) and heart rate-pressure product during mental stress , both post-stress IL-6 levels and HF-HRV during mental stress separately mediated the relationship between rmPFC activity and cardiovascular outcomes (Figure 7). The proportion mediated through post-stress IL-6 levels and HF-HRV during mental stress was 15.5% and 32.5%, respectively (Figure 7).

Discussion

To our knowledge, the present study is the first to show that among individuals with CAD, greater mental stress-induced activation in the inferior frontal lobe region is positively associated with self-reported angina severity. We found a dose-response relationship between stress-induced inferior frontal lobe activation and angina frequency. There was also a significant positive association between inferior frontal lobe activation during stress and the magnitude of change in angina frequency after 2 years of follow-up, suggesting that brain-related changes predict worsened future angina as well. Higher rmPFC activation with stress was independently associated with worse outcomes compared to lower rmPFC activation with stress. In addition, we found that potential contributors to the worse outcomes in those with higher rmPFC included autonomic dysfunction and inflammation, as summarized in Figure 8. These findings provide objective, neurobiological evidence linking psychological stress and chest pain and adverse cardiac outcomes.

Our findings that focus on brain-related changes with stress complement previous work that has focused on subjective stress measures as key determinants of angina. A key advantage to our findings is that our measures are objective and imaging-based. This may, to a certain extent, provide the underlying neurophysiological basis for previous studies examining angina and depression, post-traumatic stress disorder symptoms, anxiety, and perceived stress (9, 46, 47). Our study builds upon previous research that has implicated the inferior frontal lobe in processing physical and psychological pain, as well as the overall fear of having pain (17, 48-52). Overall, previous research on the relationship of this region with pain and neurobiological mechanisms warrant further research on interventions that

can modify these pathways, such as neurofeedback, deep brain stimulation, and transcranial magnetic brain stimulation (53-55).

The rmPFC is one of the most highly evolved centers of the brain that is responsible for an individual's self-perception (56). This has broad relevance to the stress response, especially with stress challenges that involves social pressure and negative feedback, which was the case in this study design. It is also a critical brain region in emotional regulation and social conditioning, and interacts closely with stress-related subcortical regions, including the amygdala (57). By performing a stress challenge, we were able to ascertain the participants' underlying vulnerabilities to stress as it relates to social interaction and self-perception. Few studies like this have been performed including a previous study by Tawakol *et al*., in which resting amygdalar activity (involved in the fear response) was associated with increased risk of cardiovascular events (58). These data suggest that high rmPFC activity may identify patients who have unusually large amygdala responses to mental stress, and require commensurate amounts of top-down inhibition from higher cortical regions including the rmPFC region.

Our findings are supported by previously described neurocardiac relationships involving the autonomic nervous system. Mental stress increases the output of the sympathetic nervous system with concomitant parasympathetic (vagal) nervous system withdrawal (59). HF-HRV is regarded as a non-invasive, real-time assessment of vagal activity. The rmPFC has been consistently found to be a relevant brain region involved in the control of HF-HRV in two separate meta-analyses (60). Our results are in concordance with these studies demonstrating a significant link between rmPFC reactivity to mental stress and lower HF-HRV indicative of vagal withdrawal.

The systemic inflammatory cascade may also be activated by mental stress (61, 62). A previous meta-analysis showed that IL-6 was the most robust and consistent inflammatory marker that increases with mental stress (62). We and others have also shown that individuals with CAD have higher IL-6 responses to mental stress compared to healthy populations (63, 64). The largest increase in IL-6 appears to be at 90 minutes after mental stress which supports our plasma collection time point at 90 minutes. However, little is known about brain regions that may be involved in the association between stress and systemic inflammation in both health and disease. Recently, it was shown that higher IL-6 levels correlate with higher resting state connectivity of the medial prefrontal cortex and adjacent areas (28). Our results further expand the previous literature by identifying the rmPFC as a brain region linked to higher inflammation with stress.

In our study, adjustment for systemic inflammation and vagal withdrawal in the model accounted for 48% of the total observed association between rmPFC activity and cardiovascular events, findings that are in agreement with a previous report of an association between arterial inflammation, amygdalar activity and cardiovascular events (58). While the exact mechanisms by which mental stress-induced changes in inflammatory markers and autonomic regulation predispose to future events were not explored in our study, we can hypothesize that repeated episodes of everyday life stressors could eventually lead to MACE through the mechanisms described. This hypothesis is strengthened by the finding that those with high rmPFC activation at enrollment had higher IL-6 levels during follow up. Therefore, strategies that mitigate

inflammation and autonomic inflexibility can potentially help reduce the deleterious effects of stress on cardiovascular health in those with CAD.

The major strengths of our study was the ability to measure the cerebral perfusion during stress and not just the resting brain activity. Also, additional measurements of inflammatory markers and autonomic reactivity with mental stress testing enabled us to explore the mechanistic links between brain activation during stress and cardiovascular risk. Our study also had a number of limitations. First, all participants had stable CAD, and therefore our findings may not be generalizable to individuals without CAD. Second, we used standardized mental stress testing in the laboratory setting and thus could not determine whether the rmPFC response recorded reflects everyday life stress. Third, angina symptoms were assess retrospectively using a questionnaire, rather than using a prospective diary documentation of angina symptoms. However, this method is clinically relevant since it is similar to the way health care professionals collect information about patients' symptoms during clinical visits. The SAQ scale is also a well validated tool for assessment of presence and severity of angina, and because we used the questionnaire before mental stress testing, it is unlikely that the responses were biased by the testing itself. Fourth, inflammatory and autonomic responses to mental stress were assessed simultaneously during stressful tasks and therefore it is unclear if these dysregulations persist over time and thus contribute to future adverse events. However, we have also shown that baseline levels of IL-6 increased over time among those with high rmPFC activation with stress which supports the notion of a proinflammtory status for those with high rmPFC stress reactivity. Fifth, the number of events were relatively low with 76% of the events consisting of unstable angina requiring urgent

revascularization. Future studies with longer follow-up are required to confirm whether higher rmPFC activation with stress is associated with higher risk of hard cardiovascular events including death and myocardial infarction.

In summary, we found a positive relationship between inferior frontal lobe activity with stress and reported angina. These findings suggest that altered brain reactivity to stress could possibly represent a key mechanism in chest pain perception in individuals with CAD. We also found that increased stress-induced rmPFC activation is a marker of cardiovascular vulnerability and adverse outcomes in patients with CAD, whose mechanism is at least in part due to changes in autonomic and inflammatory function. Our findings indicate that such stress-induced brain activity contributes to prognosis, independently of established clinical risk indicators. These findings suggest that stress-induced rmPFC activation may represent a new method of risk stratification that can personalize and improve risk discrimination. This highlights the need for future studies that therapeutically target the autonomic and inflammatory neurocardiac pathways, or directly the rmPFC for cardiovascular disease risk reduction.

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Table 1. Baseline Characteristics of the Participants Reporting Angina Frequency

Characteristic	Angina frequency			*p*-value
	Absent N= 94	**Monthly** N=35	**Daily or weekly** N=19	
Demographics				
Age, Mean (SD)	64 (7)	59 (8)	57 (8)	**<0.001**
Men, N (%)	70 (74.5)	21 (60.0)	11 (57.9)	0.093
African American, N (%)	23 (24.5)	17 (48.0)	13 (68.4)	**0.001**
Years of school, Mean (SD)	15 (3)	13 (4)	13 (2)	**0.011**
Current smoker, N (%)	11 (11.7)	8 (22.9)	4 (21.1)	0.28
Medical factors				
Hypertension, N (%)	68 (72.3)	29 (82.9)	16 (84.2)	0.49
Hyperlipidemia, N (%)	78 (83.0)	25 (71.4)	18 (94.7)	0.082
Diabetes, N (%)	25 (26.6)	18 (51.4)	7 (36.8)	0.074
Obesity, N (%)	77 (81.9)	27 (77.1)	15 (78.9)	0.49
Previous MI, N (%)	35 (37.2)	12 (34.3)	6 (31.6)	0.71
Previous revascularization, N (%)	47 (50)	16 (45.7)	9 (47.4)	0.81
Gensini score, Mean (SD)	39.3 (54.6)	32.1 (44.1)	29.0 (32.3)	0.42
Stress-induced ischemia, N (%)				
Conventional (exercise/pharmacologic)	34 (34.0)	18 (51.4)	7 (36.8)	0.21
Mental	19 (20.2)	12 (34.3)	7 (36.8)	**0.02**
Summed difference score, Mean (SD)	2.6 (4.6)	2.9 (3.6)	2.1 (2.8)	0.81
Presence of scar, N (%)	17 (18.1)	4 (11.4)	2 (10.6)	0.33
Ejection Fraction, Mean (SD)	68.4 (15.3)	63.1 (14.7)	72.3 (12.5)	0.12
End Systolic volume, Mean (SD)	44.3 (56.1)	60.0 (58.2)	34.2 (31.3)	0.19
End Diastolic volume, Mean (SD)	118.8 (65.4)	134.7 (75.6)	108 (42.0)	0.31
Psychological factors				
BDI-II score, Mean (SD)	7.77 (6.8)	19.31 (12.4)	21.95 (13.5)	**<0.001**
PCL score, Mean (SD)	26.27 (10.6)	36.37 (13.8)	44.65 (17.8)	**<0.001**
STAI Anxiety-Trait score, Mean (SD)	32.7 (11.0)	42.2 (11.3)	44.8 (12.2)	**<0.001**
Mental stress with brain PET scan				
Baseline SUDS score, Mean (SD)	15.2 (18.0)	17.4 (20.5)	16.6 (18.9)	0.78
Post-mental stress SUDS score, Mean (SD)	15.7 (11.0)	20.9 (7.9)	26.2 (17.2)	**0.009**
Change in SUDS score with stress, Mean (SD)	0.5 (3.4)	2.1 (7.5)	9.9 (10.1)	**0.01**
Mental stress with myocardial perfusion				
Baseline SUDS score, Mean (SD)	10.3 (15.2)	16.6 (27.4)	17.2 (22.2)	**0.29**
Post-mental stress SUDS score, Mean (SD)	17.3 (18.1)	29.7 (33.6)	39.7 (34.0)	**0.007**
Change in SUDS score with stress, Mean (SD)	6.0 (2.1)	13.0 (6.7)	22.5 (5.8)	**0.002**
DASI score, Mean (SD)	43.9 (12.1)	31.2 (17.7)	22.3 (11.5)	**<0.001**
Medications, N (%)				

Antidepressants	29 (30.9)	10 (29.4)	12 (63.2)	**0.043**
ACE inhibitors	44 (46.8)	14 (41.2)	8 (42.1)	0.67
Aspirin	83 (88.3)	30 (88.2)	16 (84.2)	0.88
Beta blocker	70 (74.5)	23 (67.6)	15 (78.9)	0.38
Statin	86 (91.5)	29 (85.3)	18 (94.7)	0.25
Calcium channel blockers	18 (19.1)	8 (23.5)	5 (26.3)	0.72
Nitrates	15 (16.0)	10 (29.4)	8 (42.1)	**0.02**
Increase in angina frequency during follow-up, N (%)	16 (21.3)	11 (40.7)	1 (9.1)	**0.03**

SD: standard deviation, BDI-II : Beck Depression Inventory scale, PCL: Post-traumatic stress disorder symptom checklist, STAI: State- Trait Anxiety Inventory, ACE: angiotensin converting enzyme

Table 2. Baseline Characteristics of the Participants divided by High vs Low rmPFC Activity in Response to Mental Stress

	Low rmPFC activation N= 74	High rmPFC activation N=74	p-value
Demographics			
Age, Mean (SD)	62 (7)	61 (9)	0.67
Men, N (%)	52 (70.3)	50 (67.6)	0.72
African American, N (%)	26 (35.1)	27 (36.5)	0.86
Years of school, Mean (SD)	15 (3)	14 (3)	0.35
Current smoker, N (%)	12 (16.2)	11 (14.9)	0.59
Medical factors			
Hypertension, N (%)	51 (68.9)	62 (83.8)	0.03
Hyperlipidemia, N (%)	61 (82.4)	60 (81.1)	0.83
Diabetes, N (%)	22 (29.2)	28 (37.8)	0.29
Obesity, N (%)	59 (79.7)	60 (81.1)	0.83
Previous MI, N (%)	25 (33.8)	28 (37.8)	0.60
Previous revascularization, N (%)	32 (43.2)	40 (54.1)	0.25
Depression, N (%)	14 (18.9)	15 (20.3)	0.83
Psychological factors			
BDI-II score, Mean (SD)	11.7 (11.1)	13.1 (11.9)	0.46
PCL score, Mean (SD)	30.3 (14.2)	30.9 (13.9)	0.77
Stress test parameters			
Heart rate, mean (SD)			
At rest	64 (9)	63 (10)	0.49
During stress test	79 (12)	76 (11)	0.12
Systolic blood pressure, mean (SD)			
At rest	131 (16)	138 (17)	**0.01**
During stress test	165 (24)	171 (23)	0.12
Diastolic blood pressure, mean (SD)			
At rest	78 (10)	79 (9)	0.49
During stress test	97 (13)	98 (14)	0.45
Rate pressure product, mean (SD)			
At rest	7,878 (1585)	8,173 (1,641)	0.23
During stress test	12,397 (3,000)	12,926 (2,872)	0.98
Inflammatory markers			
Baseline hsCRP, mg/dl Median (IQR)	1.21 (0.66-4.37)	1.99 (0.99-4.43)	0.24
Post-mental stress hsCRP, mg/dl Median (IQR)	1.16 (0.64-3.96)	1.74 (0.83-3.74)	0.19
Baseline IL-6, mg/dl Median (IQR)	1.26 (0.91-2.00)	1.38 (0.87-2.29)	0.32
Post-mental stress IL-6, mg/dl Median (IQR)	1.52 (0.98-2.23)	2.06 (1.46-2.89)	**0.002**

Heart rate Variability (ms^2)			
Baseline, Mean (SD)	14.1 (8.4)	14.2 (8.5)	0.88
During-mental stress (SD)	13.3 (7.6)	10.2 (5.5)	**0.009**
Medications, N (%)			
ACE inhibitors	32 (42)	39 (53.4)	0.32
Aspirin	68 (91.9)	61 (83.6)	0.19
Beta blocker	53 (71.6)	55 (75.3)	0.60
Statin	55 (75.3)	61 (83.6)	0.28

SD: standard deviation, BDI-II: Beck Depression Inventory scale, PCL: Post-traumatic stress disorder symptom checklist, ACE: angiotensin converting enzyme

Table 3. Baseline characteristics of the participants comparing those with angina during mental stress and those without angina during mental stress

Characteristic	Angina during mental stress (N=11)	No Angina during mental stress (N=137)	P-value
Demographics			
Age, Mean (SD)	58 (8)	62 (8)	0.12
Men, N (%)	7 (63.6)	95 (69.9)	0.66
African American, N (%)	5 (45.5)	48 (35.3)	0.50
Years of school, Mean (SD)	13 (3)	15 (3)	**0.003**
Current smoker, N (%)	2 (18.2)	21 (15.4)	0.55
Medical factors			
Hypertension, N (%)	8 (72.7)	104 (76.5)	0.77
Hyperlipidemia, N (%)	8 (72.7)	112 (82.4)	0.42
Diabetes, N (%)	3 (27.3)	46 (33.8)	0.65
Obesity, N (%)	10 (90.9)	108 (79.8)	0.35
Previous MI, N (%)	3 (27.3)	49 (36.0)	0.60
Previous revascularization, N (%)	4 (36.4)	67 (49.3)	0.41
Gensini score, Mean (SD)	34.6 (50.8)	39.7 (51.1)	0.17
Stress-induced ischemia, N (%)	4 (36.4)	31 (22.8)	0.30
Number of atherosclerotic coronary vessels, Median (IQR)	1 (0-2)	1 (0-2)	0.41
Summed difference score, Mean (SD)	2.0 (4.6)	2.3 (3.8)	0.31
Psychological factors			
BDI-II score, Mean (SD)	20.9 (14.5)	11.6 (11.1)	**0.01**
PCL score, Mean (SD)	46.5 (18.2)	29.1 (12.7)	**<0.001**
Mental stress with PET scan			
Baseline SUDS score, Mean (SD)	15.2 (18.3)	18.4 (22.4)	0.22
Post-mental stress SUDS score, Mean (SD)	28.9 (22.3)	21.1 (18.5)	**0.03**
Change in SUDS score with stress, Mean (SD)	11.6 (14.8)	1.91 (15.7)	**0.01**
Mental stress with myocardial perfusion			
Baseline SUDS score, Mean (SD)	17.3 (23.3)	16.1 (27.4)	0.32
Post-mental stress SUDS score, Mean (SD)	46.6 (30.2)	21.5 (25.3)	**0.009**
Change in SUDS score with stress, Mean (SD)	27.3 (26.9)	4.8 (18.2)	**0.01**
DASI score, Mean (SD)	33.8 (18.3)	38.5 (15.5)	0.35
Medications			
Antidepressants	6 (54.5)	44 (32.6)	0.14
ACE inhibitors	4 (36.4)	61 (45.2)	0.57
Aspirin	8 (72.7)	120 (88.9)	0.11

Beta blocker	6 (54.5)	101 (74.8)	0.14
Statin	9 (81.8)	123 (91.1)	0.31

Table 4. Baseline Characteristics of the Participants Reporting Angina Frequency

Characteristic	Baseline cohort (N=148)	Follow up cohort (N=112)	P-value
Demographics			
Age, Mean (SD)	62 (8)	62 (8)	0.65
Men, N (%)	102 (68.9)	78 (69.6)	0.96
African American, N (%)	53 (35.8)	37 (33.0)	0.70
Years of school, Mean (SD)	15 (3)	15 (3)	0.39
Current smoker, N (%)	23 (15.5)	12 (10.7)	0.54
Medical factors			
Hypertension, N (%)	113 (76.4)	82 (73.3)	0.51
Hyperlipidemia, N (%)	121 (81.8)	94 (83.9)	0.68
Diabetes, N (%)	50 (33.8)	35 (31.3)	0.73
Obesity, N (%)	119 (80.4)	91 (81.3)	0.66
Previous MI, N (%)	53 (35.8)	43 (38.4)	0.60
Previous revascularization, N (%)	72 (48.6)	54 (48.2)	0.84
Gensini score, Mean (SD)	36.7 (50.8)	39.9 (52.1)	0.59
Stress-induced ischemia, N (%)	57 (38.5)	38 (33.9)	0.50
Summed difference score, Mean (SD)	2.7 (4.2)	2.3 (3.9)	0.47
Psychological factors			
BDI-II score, Mean (SD)	12.4 (11.5)	11.5 (11.3)	0.37
PCL score, Mean (SD)	30.6 (14.0)	30.1 (14.5)	0.50
DASI score, Mean (SD)	38.1 (15.7)	39.4 (15.35)	0.40
Medications			
Antidepressants	51 (3.7)	38 (33.9)	0.76
ACE inhibitors	66 (44.6)	50 (44.6)	0.85
Aspirin	129 (87.2)	102 (91.1)	0.22
Beta blocker	108 (73.0)	84 (75.0)	0.83
Statin	133 (89.9)	104 (92.9)	0.52

SD: standard deviation, BDI-II : Beck Depression Inventory scale, PCL: Post-traumatic stress disorder symptom checklist, ACE: angiotensin converting enzyme

Table 5. Baseline characteristics of the participants comparing those with an increase in angina frequency during follow up and those without an increase in angina frequency

Characteristic	Increase in Angina during follow-up (N=28)	No Increase in Angina during follow-up (N=137)	P-value
Demographics			
Age, Mean (SD)	62 (9)	63 (8)	0.64
Men, N (%)	17 (60.7)	61 (72.6)	0.23
African American, N (%)	10 (35.7)	27 (32.1)	0.72
Years of school, Mean (SD)	15 (3)	15 (3)	0..71
Current smoker, N (%)	3 (10.7)	9 (10.7)	0.62
Medical factors			
Hypertension, N (%)	23 (82.1)	62 (73.8)	0.37
Hyperlipidemia, N (%)	21 (75.0)	72 (85.7)	0.19
Diabetes, N (%)	6 (21.4)	32 (38.1)	0.10
Obesity, N (%)	20 (71.4)	69 (82.1)	0.22
Previous MI, N (%)	13 (46.4)	33 (39.3)	0.50
Previous revascularization, N (%)	14 (50.0)	41 (48.8)	0.91
Gensini score, Mean (SD)	37.5 (51.2)	38.9 (50.1)	0.38
Stress-induced ischemia, N (%)	7 (25.0)	22 (26.2)	0.90
Number of atherosclerotic coronary vessels, Median (IQR)	1 (0-2)	1 (0-2)	0.38
Summed difference score, Mean (SD)	2.1 (5.1)	2.3 (3.3)	0.22
Psychological factors			
BDI-II score, Mean (SD)	13.7 (13)	10.9 (10.3)	0.24
PCL score, Mean (SD)	31.8 (14.1)	29.3 (13.8)	0.41
DASI score, Mean (SD)	36.1 (15.2)	39.9 (15.4)	0.26
Medications			
Antidepressants	7 (25.0)	30 (35.7)	0.29
ACE inhibitors	14 (50.0)	40 (47.6)	0.82
Aspirin	26 (92.9)	74 (88.1)	0.48
Beta blocker	21 (75.0)	63 (75.0)	1.0
Statin	24 (85.7)	79 (94.0)	0.16

Figure 1. The Consolidated Standards of Reporting Trials (CONSORT)-type flow chart of study cohort.

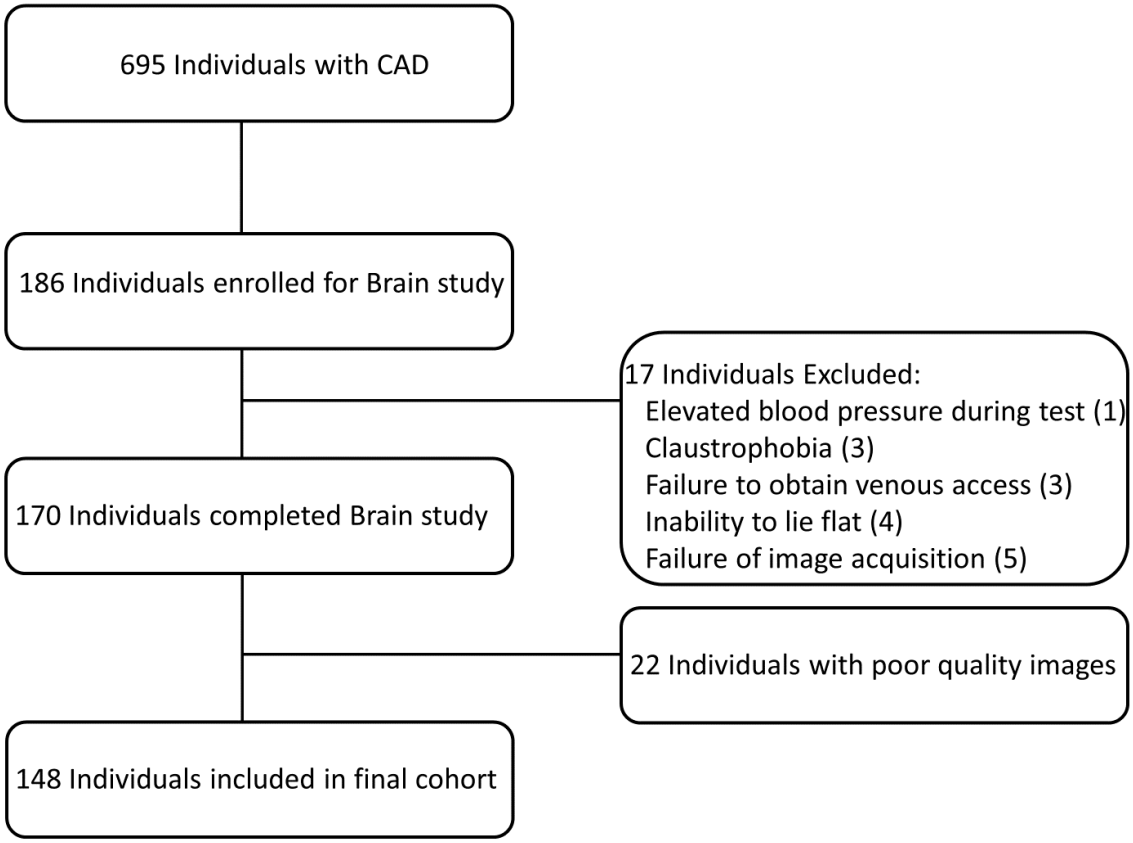
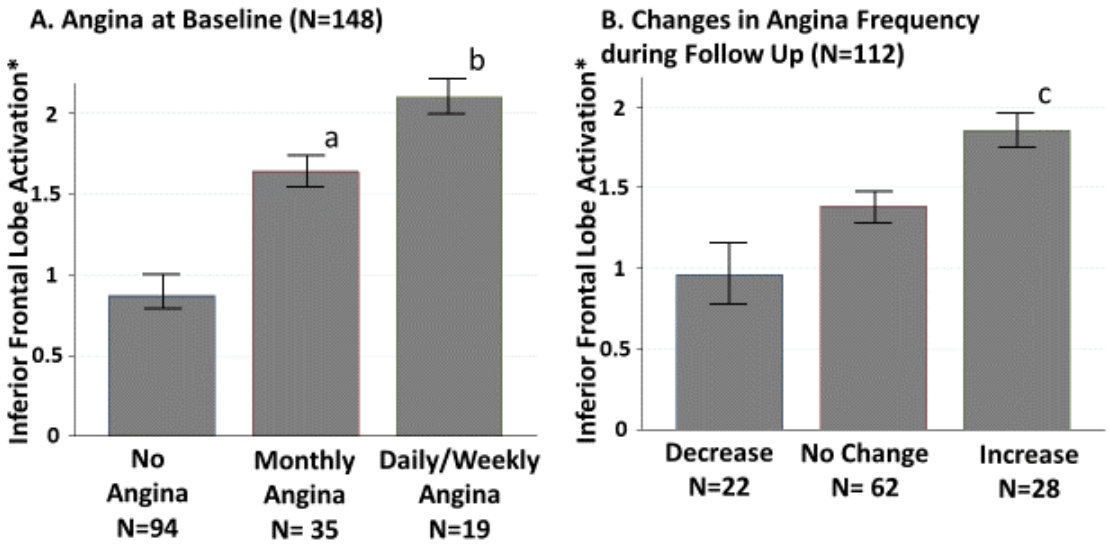
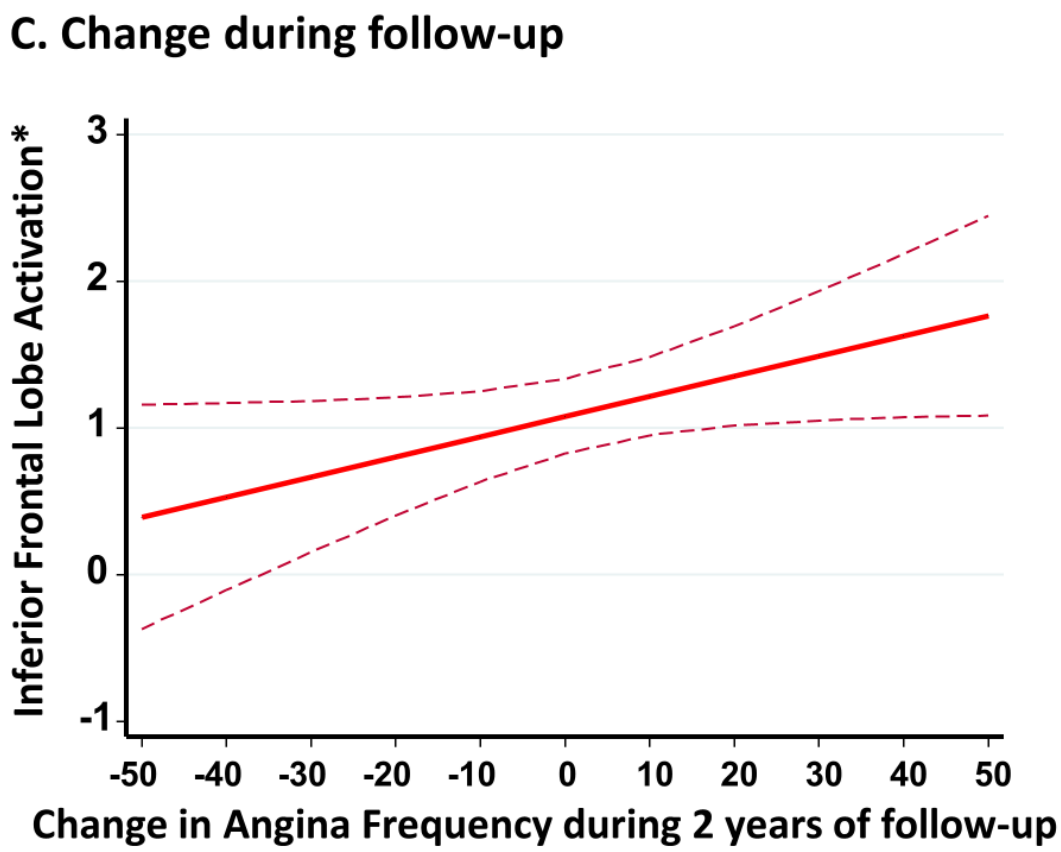


Figure 2. Mental Stress-Induced Inferior Frontal Lobe activation according to angina frequency levels at baseline (A) and changes during 2 years of follow-up (B)



* net difference ml^{-1*} min $^{-1*}100mg^{-1}$
a indicating P-value= 0.03 comparing those with monthly angina to the group with no angina
b indicating P-value- 0.009 comparing those with daily/weekly angina to the group with no angina
c indicating P-value- 0.01 comparing those with daily/weekly angina to the group with no angina

Figure 3. Association between inferior frontal lobe brain activation at baseline and changes in angina frequency during 2 years of follow up. Fitted line adjusted for age, sex and race. Dashed lines representing 95% CI.



***** net difference ml^{-1*} $min^{-1*}100mg^{-1}$

Figure 4. Association between rmPFC activation with HF-HRV during stress (A), and IL-6 levels post mental stress (B). The red dashed lines indicate 95% confidence interval.

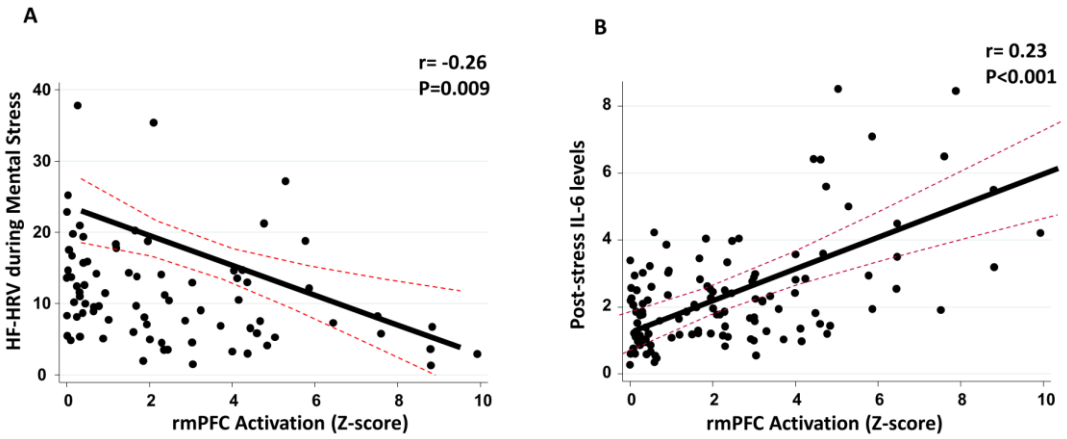
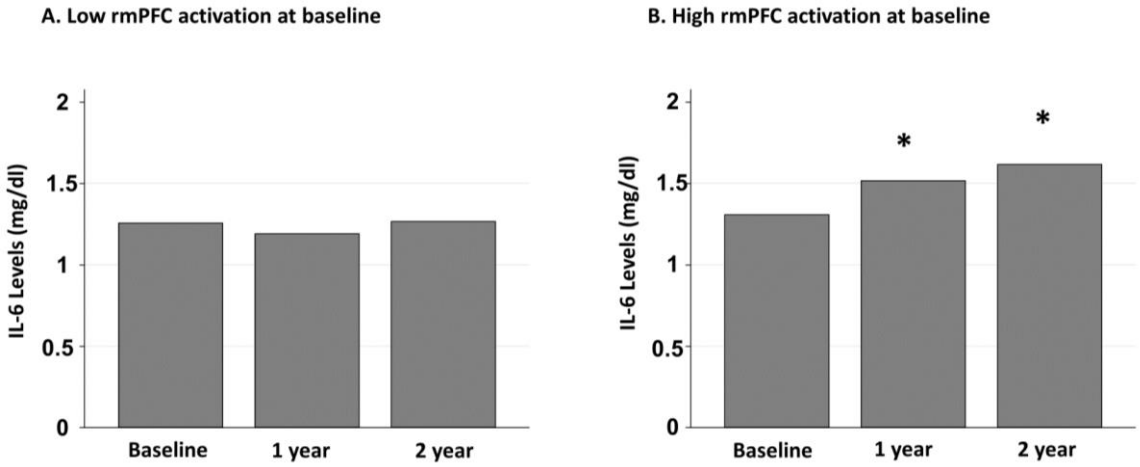
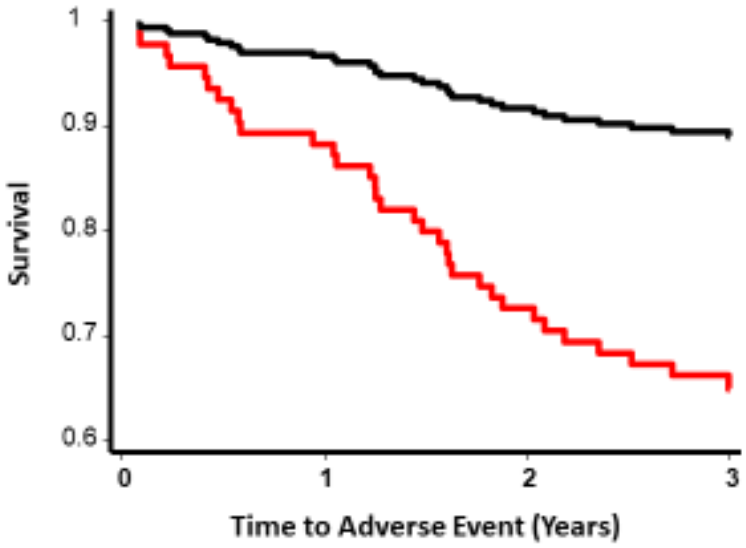


Figure 5. Relationship between IL-6 levels at baseline and follow-up stratified by status of rmPFC activation during mental stress



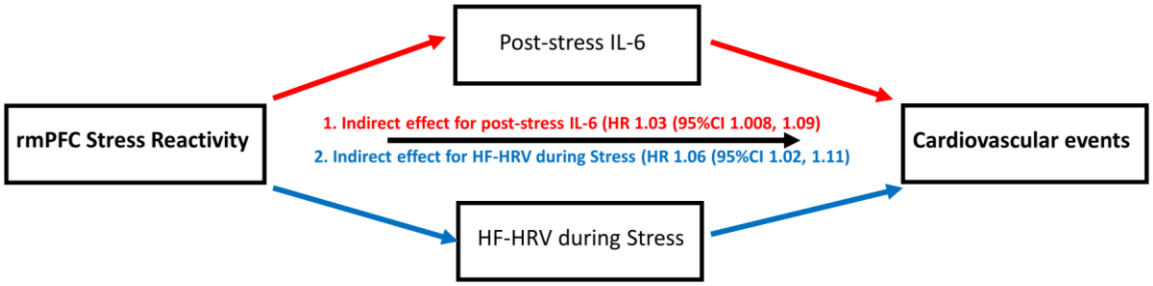
***** indicates a P value of < 0.05

Figure 6. Kaplan–Meier curve for association between rmPFC activation and the composite event of cardiovascular death, myocardial infarction, unstable angina with revascularization and heart failure hospitalization.



Number at risk				
Low rmPFC activation	74	70	68	66
High rmPFC activation	74	67	54	49

Figure 7. Mediation analysis for hypothesized pathways linking rmPFC stress reactivity to cardiovascular events



All models adjusted for baseline demographics (age, sex, and race) and heart rate-pressure product during mental stress.

1: Indirect effects of post-stress IL-6 levels on the association between rmPFC activation and cardiovascular events. Post mental-stress IL-6 levels accounted for 15.5% of the association ($\ln(HR_{\text{indirect effect}}) / \ln(HR_{\text{total effect}})] \times 100$)

2: Indirect effects of HF-HRV during stress on the association between rmPFC activation and cardiovascular events. HF-HRV during stress accounted for 32.5% of the association ($\ln(HR_{\text{indirect effect}}) / \ln(HR_{\text{total effect}})] \times 100$)

Figure 8. A model of psychological stress leading to cardiovascular events. At least two biological pathways, including vagal withdrawal and systemic inflammation mediate the relationship between rmPFC stress reactivity and cardiovascular events.