Circulation

ORIGINAL RESEARCH ARTICLE

Higher Activation of the Rostromedial Prefrontal Cortex During Mental Stress Predicts Major Cardiovascular Disease Events in Individuals With Coronary Artery Disease

BACKGROUND: Psychological stress is a risk factor for major adverse cardiovascular events (MACE) in individuals with coronary artery disease. Certain brain regions that control both emotional states and cardiac physiology may be involved in this relationship. The rostromedial prefrontal cortex (rmPFC) is an important brain region that processes stress and regulates immune and autonomic functions. Changes in rmPFC activity with emotional stress (reactivity) may be informative of future risk for MACE.

METHODS: Participants with stable coronary artery disease underwent acute mental stress testing using a series of standardized speech/arithmetic stressors and simultaneous brain imaging with high-resolution positron emission tomography brain imaging. We defined high rmPFC activation as a difference between stress and control scans greater than the median value for the entire cohort. Interleukin-6 levels 90 minutes after stress, and high-frequency heart rate variability during stress were also assessed. We defined MACE as a composite of cardiovascular death, myocardial infarction, unstable angina with revascularization, and heart failure hospitalization.

RESULTS: We studied 148 subjects (69% male) with mean±SD age of 62±8 years. After adjustment for baseline demographics, risk factors, and baseline levels of interleukin-6 and high-frequency heart rate variability, higher rmPFC stress reactivity was independently associated with higher interleukin-6 and lower high-frequency heart rate variability with stress. During a median follow-up of 3 years, 34 subjects (21.3%) experienced a MACE. Each increase of 1 SD in rmPFC activation with mental stress was associated with a 21% increase risk of MACE (hazard ratio, 1.21 [95% CI, 1.08–1.37]). Stress-induced interleukin-6 and high-frequency heart rate variability explained 15.5% and 32.5% of the relationship between rmPFC reactivity and MACE, respectively. Addition of rmPFC reactivity to conventional risk factors improved risk reclassification for MACE prediction, and C-statistic improved from 0.71 to 0.76 (P=0.03).

CONCLUSIONS: Greater rmPFC stress reactivity is associated with incident MACE. Immune and autonomic responses to mental stress may play a contributory role.

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Clinical Perspective

What Is New?

- Higher rostromedial prefrontal cortex activity with mental stress is independently associated with higher risk of major adverse cardiovascular events.
- Immune and autonomic responses to mental stress contribute to the increased risk of adverse events among those with higher rostromedial prefrontal cortex stress reactivity.

What Are the Clinical Implications?

- Stress-induced rostromedial prefrontal cortex activation contributes to the prognosis of individuals with coronary artery disease independent of established clinical risk indicators.
- Stress-induced rostromedial prefrontal cortex activation may represent a new method of risk stratification of individuals with coronary artery disease.

Despite significant advances in the prevention and treatment of coronary artery disease (CAD), this condition remains the leading cause of mortality and disability worldwide. Psychological stress has emerged as a risk factor and prognostic factor for CAD.[1] Higher levels of stress have been associated with an increased risk of incident CAD events, such as acute myocardial infarction and CAD mortality.[2–4] In most studies, stress has been assessed subjectively as self-report. However, it is possible to gain a firmer understanding of this relationship by directly studying the brain, where the psychological stress response begins.[5] To date, there has been limited investigation on whether the stress response of the brain predicts future cardiovascular risk. If found, this may lead to important insights into cardiovascular disease risk assessment and prevention.

The rostromedial prefrontal cortex (rmPFC) region is a key regulator in the default mode network that is central to emotional and cognitive processing.[6] In previous work, we found greater stress activation of the rmPFC in CAD participants exposed to early traumatic events, and those who exhibit high stress reactivity with peripheral vasoconstriction, as well.[7,8] 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 in the frontal lobe during cognitive stress challenge.[9] Its activation may in particular be an important mechanism linking social status and stress with adverse CAD outcomes.[10] Given its prominent role in the default mode network[11] and 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.[12]

Previous evidence has suggested that rmPFC activation with stress associates with inflammation and autonomic activation.[13,14] Changes in vagal tone, for example, have been related to changes in rmPFC blood flow during working-memory tasks.[13] Abnormal activity of rmPFC can also influence immune function as measured by interleukin-6 (IL-6), a marker of systemic inflammation.[15] It may also be an important predictor of long-term outcomes as the result of pathological stress reactivity.[16] 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.

To test the role of rmPFC activity with mental stress and cardiovascular disease outcomes, we investigated the activity of the rmPFC in participants with CAD undergoing high resolution-positron emission tomography (HR-PET) brain imaging before and during a mental stress challenge and changes in the autonomic and inflammatory pathways. We 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 triggering and inflammatory response.

METHODS

Study Population

The data that support the findings of our study are available from the corresponding author on reasonable request. A total of 695 individuals with confirmed CAD were enrolled into the Mental Stress Ischemia Prognosis Study (US National Institutes of Health grants P01 HL101398 and R01 HL109413) at Emory University Hospital, Grady Memorial Hospital, and the Atlanta VA Medical Center between September 2010 and September 2014.[17] A random sample of 186 patients was included for a brain imaging substudy. Of this sample, 170 completed the brain imaging, and 148 had usable data on rmPFC activity. CAD was defined as an abnormal coronary angiogram demonstrating evidence of atherosclerosis with at least luminal irregularities, a history of myocardial infarction, coronary artery bypass grafting, or percutaneous coronary intervention, or a positive nuclear stress test. Individuals were excluded if they had unstable angina, myocardial infarction, or decompensated heart failure in the past week or if they had any severe comorbidities expected to interfere with the study measure or decrease their life expectancy in the next 5 years. Other exclusion criteria included pregnancy, high blood pressure (systolic ≥180 mm Hg or diastolic ≥100 mm Hg), history of a severe mental disorder (including schizophrenia, psychosis, bipolar disorder, substance dependence within 1 year), and history of a neurological disorder (ie, Parkinson disease, dementia, or stroke). Antianginal medications were withheld for 12 hours (calcium channel blockers, nitrates) or 24 hours (β-adrenergic antagonists) before the study visits. Individuals were also excluded if withholding medication was considered harmful

to them. The brain study was performed only in a subset of all participants, and they were oversampled for depression and mental stress–induced myocardial ischemia.[18] All participants provided written informed consent, and the study was approved by the Emory University Institutional Review Board.

Study Measures

Sociodemographic factors (age, sex, race, education, and current smoking), medical history (hypertension, hyperlipidemia, diabetes mellitus, obesity, previous myocardial infarction, previous revascularization), and list of medications taken were assessed by using standardized questionnaires. Depressive symptoms were assessed using the Beck Depression Inventory-II scale,[19] and posttraumatic stress disorder symptoms were assessed using the Posttraumatic Stress Disorder Symptom Checklist.[20]

Mental Stress Testing

All participants underwent mental stress testing using a standardized protocol, as previously described.[17,18,21] In brief, after 30 minutes of resting in a quiet room, subjects were asked to perform 2 control tasks (counting aloud and recalling a neutral event), followed by 2 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.[22] The difficulty of mental mathematics was increased until participants incorrectly answered 3 successive problems. During the public speaking task, participants were given 2 stressful situations: 1 involving a long-term house guest who had overstayed her welcome and 1 involving 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 minutes) for each situation and were told the speech would be evaluated for content. Blood pressure and heart rate were recorded at 1-minute intervals during the control and stress tasks by using an automated oscillometric device.

Brain Imaging During Mental Stress

HR-PET brain imaging was conducted using a High Resolution Research Tomograph (Siemens, Inc), with a spatial resolution of 2 mm.[23] A total of 8 brain scans were performed for each individual, with 2 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 oxygen-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/(μCi/mL) and a spatial resolution of 2.4 mm 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, high-frequency heart rate variability (HF-HRV) was measured using GE MARS 8.0.2 software.[24] HF-HRV during the control and stress tasks were measured using 1-minute time windows, while each task was ongoing. HF-HRV values were log transformed (ln ms^2) as previously described, given the positive skew.[25] Given that, on average, the duration of mental stress testing was <3 minutes for each participant, other HRV bands including low-frequency and very-low-frequency HRV data were not obtained because these bands required longer recording times.[26]

Inflammatory Biomarkers Measurement

We assessed inflammatory biomarkers at rest and 90 minutes after mental stress testing on a separate day.[17] 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°C; thereafter, the plasma was snap-frozen at –70°C until further processing. High-sensitivity assays were performed using the MesoScale system (Meso Scale Diagnostics) using the SECTOR Imager 2400 to quantitate IL-6 and high-sensitivity C-reactive protein 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 high-sensitivity C-reactive protein, respectively. The interassay coefficient of variations for midpoint standards was 5.78% for IL-6 and 3.06% for high-sensitivity C-reactive protein. The intraassay coefficient of variations was 3.29% for IL-6 and 2.33% for high-sensitivity C-reactive protein.

Cardiovascular Events

Major adverse cardiovascular events (MACEs) 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 ≥2 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[27–29] using statistical parametric mapping (SPM12; https://

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 (Figure 1; http://itksnap.org) limiting brain activity to the bilateral rmPFC, derived from the inferior half of the superior medial frontal gyrus from the Automatic Anatomic Labeling atlas, was applied to the contrast image. We also applied a similar method to identify the magnitude of amygdala activity during mental stress by creating a custom mask limiting brain activity to the bilateral amygdala regions. Individual subject responses (delta blood-flow values from individual contrast map) were extracted from the masked contrast image and averaged across nonzero voxels.

High rmPFC activation was defined as having a value equal or greater than the median for the sample; the others were classified as having low rmPFC activation. Baseline characteristics of the population were compared between participants with high versus low rmPFC activation.

We calculated the Dice coefficient using a built-in MATLAB function to quantify the amount of overlap between 2 stress tasks (mental arithmetic task and a public speaking task). This index was calculated as the ratio of twice the intersection of 2 volumes divided by the sum of the 2 volumes, where A and B are values of the 2 volumes to be compared (DS-Coeffs(A, B) = 2(A ∩ B)/(A + B)). Values range between 0 and 1 with a value of 1 indicating total concordance between 3 volumes. The data showed a Dice coefficient of 0.85 indicating an excellent overlap between the mental arithmetic and public speaking tasks.

Statistical Analysis

Demographic variables were compared using χ^2 tests or Fisher exact tests for categorical variables and 2-sample *t* tests for continuous variables. The distribution of continuous variables was examined for normality as a requirement for parametric testing; if not normal, the Wilcoxon rank-sum test was used.

Spearman rank correlation was used to examine the association between rmPFC activation and resting and post–mental stress inflammatory markers, and HF-HRV during mental stress testing, as well. Linear cubic spline models were derived to assess the relationship between inflammatory markers, HF-HRV, and rmPFC activation. All models were adjusted for a priori chosen covariates, including baseline demographics (age, sex, and race) and heart rate-pressure product during mental stress.

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 described earlier. Cox proportional hazards assumptions were assessed and verified both visually by Kaplan-Meier curves and by using the linear correlation test based on Schoenfeld partial residuals of the model, as well. 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 SD increments. Adjusted hazard ratios (HRs) along with the corresponding 95% CI were calculated after controlling for age, sex, race, and heart rate-pressure product during mental stress to make statistical inference. The incremental value of stress-induced activation of rmPFC to predict risk of MACE was tested by its addition to a model including baseline demographics and heart rate-pressure product during mental stress. The Harrell C-statistic (area under the curve), category-free net reclassification improvement, and the integrated discrimination improvement were calculated as measures of risk discrimination.[30–33]

We performed mediation analysis using the counterfactual approach for causal mediation, as proposed by Lange and Hansen.[34] A marginal structural modeling approach was applied to assess the direct effect of rmPFC activation on MACE, and the indirect effect of rmPFC activation via mediators, as well. We explored 2 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. For each mediator pathway, unbiased estimates were obtained for direct and indirect effects from weighted Cox proportional hazards models with a duplicated data set with 2 replications of the exposure. In the first replication, the exposure E* was set to the observed value of rmPFC activation. In the second replication, E* was set to the counterfactual value of rmPFC activation. Weights were determined by the following formula: Weight = P(M|E*, C) / P(M|E, C), derived from multivariable linear regression model of IL-6 or HRV (M), on rmPFC activation (E and E*) and covariates (age, sex, race, and heart rate-pressure product) (C), as previously described.[35] Standard errors and 95% CIs were determined by 10 000 bootstrap simulation.[34] The

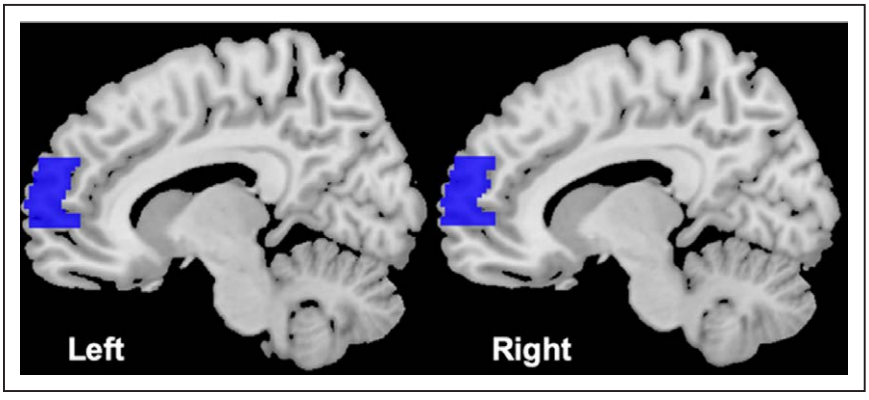

Figure 1. Custom mask limiting brain activity to the bilateral rostromedial prefrontal cortex, derived from the Automatic Anatomic Labeling atlas.
Blue areas (online) indicate the measured rostromedial prefrontal cortex area.

percentage mediated on the log HR scale was calculated as [ln($HR_{indirect\ effect}$) / ln($HR_{total\ effect}$)]×100. Analyses using weighted Cox proportional hazards models were performed in R version 3.1.2. All other statistical analyses was done using Stata 14.0 (StataCorp LP). Two-sided *P* values <0.05 were used for significance testing.

RESULTS

A total of 148 CAD participants were included in the final analysis. The mean age of the cohort was 62±8 years and 102 (68.8%) participants were men. As shown in Table 1, participants with high rmPFC activation had higher rates of hypertension and higher systolic blood pressures at rest than those with low activation (Table 1).There were no other significant demographic or clinical differences between those with low versus high rmPFC activation (Table 1). 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 1).

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, than the group with low rmPFC activation (Table 1). A linear relationship was found between HF-HRV during mental stress and rmPFC activation (*P* values for nonlinear 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 to –0.02]; *P*=0.008) (Figure 2A).

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 C-reactive protein levels (*P*=0.23). Although no differences were observed between participants with high versus low rmPFC activation with respect to baseline IL-6, baseline, and post–stress C-reactive protein levels, participants with high rmPFC activation had 60.4% higher IL-6 levels 90 minutes after mental stress than those with low rmPFC activity (Table 1; *P*=0.02). As shown in Figure 2B, 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).

In comparison with baseline IL-6 levels, the median levels of IL-6 were not different at 1 year (1.29 [interquartile range {IQR}, 0.93–1.89]; *P*=0.62) and 2 years (1.27 [IQR, 0.92–2.15]; *P*=0.83) follow-up (Figure 3A). However, those with high rmPFC activation with mental stress at baseline were found to have significantly higher IL-6 levels both at 1 year (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 3B). As shown in Figure 3A, IL-6 levels were not significantly different for participants with low rmPFC activation at 1- or 2-year follow-up in comparison with baseline levels.

Relationship Between rmPFC Activation and Amygdala Activation

Mental stress resulted in amygdala activation in 104 and deactivation in 44 individuals (Figure 4). All of the individuals with amygdala deactivations were in the group with low rmPFC activation. After adjustment for baseline demographics and medical and psychological factors, every SD increase in amygdala activity was associated with 10% increase in rmPFC activation (B, 0.10 [95% CI, 0.08–0.13]; *P*=0.002).

Relationship Between rmPFC Activation and Cardiovascular Outcomes

During a median follow-up of 3 years (IQR, 2.5–3.6), 34 participants (21.3%) experienced a MACE that included 2 cardiovascular deaths, 1 myocardial infarction, 5 hospitalizations for congestive heart failure, and 26 cases of unstable angina requiring urgent revascularization.

Figure 5 shows unadjusted Kaplan-Meier curves for MACE according to rmPFC activity. The difference between high versus 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 1 SD 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]). Amygdala activation with mental stress was not associated with MACE (HR, 1.03 [95% CI, 0.95–1.14]).

We tested the incremental value of adding the rmPFC activation to a model with baseline demographics and heart rate-pressure product during mental stress in predicting incident MACE. With the addition of rmPFC brain activation, the C-statistic (*P*=0.03), the category-free net reclassification index metric (*P*<0.001), and the integrative discrimination improvement (*P*=0.005) significantly improved for prediction of incident MACE in comparison with the risk factor model (Table 2).

Table 1. Baseline Characteristics of the Participants Divided by High Versus Low rmPFC Activity in Response to Mental Stress

Characteristics	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
Black, 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, n (%)			
Hypertension	51 (68.9)	62 (83.8)	0.03
Hyperlipidemia	61 (82.4)	60 (81.1)	0.83
Diabetes mellitus	22 (29.2)	28 (37.8)	0.29
Obesity	59 (79.7)	60 (81.1)	0.83
Previous myocardial infarction	25 (33.8)	28 (37.8)	0.60
Previous revascularization	32 (43.2)	40 (54.1)	0.25
Depression	14 (18.9)	15 (20.3)	0.83
Psychological factors, mean (SD)			
BDI-II score	11.7 (11.1)	13.1 (11.9)	0.46
PCL score	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	7878 (1585)	8173 (1,641)	0.23
During stress test	12 397 (3,000)	12 926 (2,872)	0.98
Inflammatory markers, median (IQR)			
Baseline hsCRP, mg/dL	1.21 (0.66–4.37)	1.99 (0.99–4.43)	0.24
Post–mental stress hsCRP, mg/dL	1.16 (0.64–3.96)	1.74 (0.83–3.74)	0.19
Baseline IL-6, pg/dL	1.26 (0.91–2.00)	1.38 (0.87–2.29)	0.32
Post–mental stress IL-6, pg/dL	1.52 (0.98–2.23)	2.06 (1.46–2.89)	0.002*
Heart rate variability, ms^2, mean (SD)			
Baseline	14.1 (8.4)	14.2 (8.5)	0.88
During mental stress	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
β-Blocker	53 (71.6)	55 (75.3)	0.60
Statin	55 (75.3)	61 (83.6)	0.28

ACE indicates angiotensin-converting enzyme; BDI-II, Beck Depression Inventory-II scale; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; IQR, interquartile range; PCL, Posttraumatic Stress Disorder Symptom Checklist; and rmPFC, rostromedial prefrontal cortex.
**P* value of <0.05.

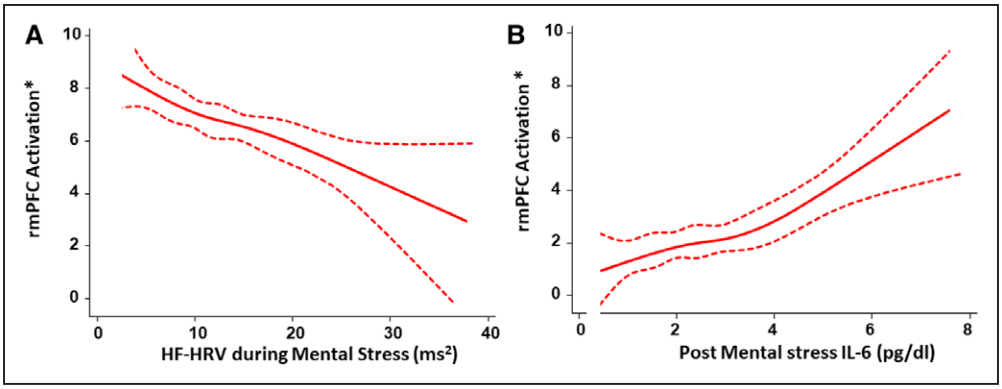

Figure 2. Association between rostromedial prefrontal cortex activation with high-frequency heart rate variability during stress (A), and interleukin-6 levels after mental stress (B).

A linear relationship was found between rostromedial prefrontal cortex activation and both high-frequency heart rate variability during mental stress and post–mental stress interleukin-6. The red dashed lines (online) indicate 95% CI. All models were adjusted for baseline demographics (age, sex, and race) and heart rate-pressure product during mental stress.*Net difference $mL^{-1}\cdot min^{-1}\cdot 100\ mg^{-1}$. HF-HRV indicates high-frequency heart rate variability; IL-6, interleukin-6; and rmPFC, rostromedial prefrontal cortex.

Mediation Analysis

We investigated whether the effect of rmPFC on MACE is at least partially mediated through inflammation and vagal withdrawal by using the approach of Lange and colleagues.[34,36] 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 6). The proportion mediated through post–stress IL-6 levels and HF-HRV during mental stress was 15.5% and 32.5%, respectively (Figure 6).

DISCUSSION

Herein, we demonstrate that in individuals with stable CAD, brain reactivity to mental stress in the rmPFC region predicts future risk of MACE, independently of other clinical risk determinants. Higher rmPFC activation with stress was independently associated with worse outcomes in comparison with lower rmPFC activation with stress, and when added to a standard clinical risk model, greatly improved risk classification and discrimination. 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 7. These findings provide objective, neurobiological evidence linking psychological stress and adverse cardiac outcomes.

The rmPFC is one of the most highly evolved centers of the brain that is responsible for an individual's self-perception.[37] 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.[38] By performing a stress challenge, we were able to ascertain the participants'

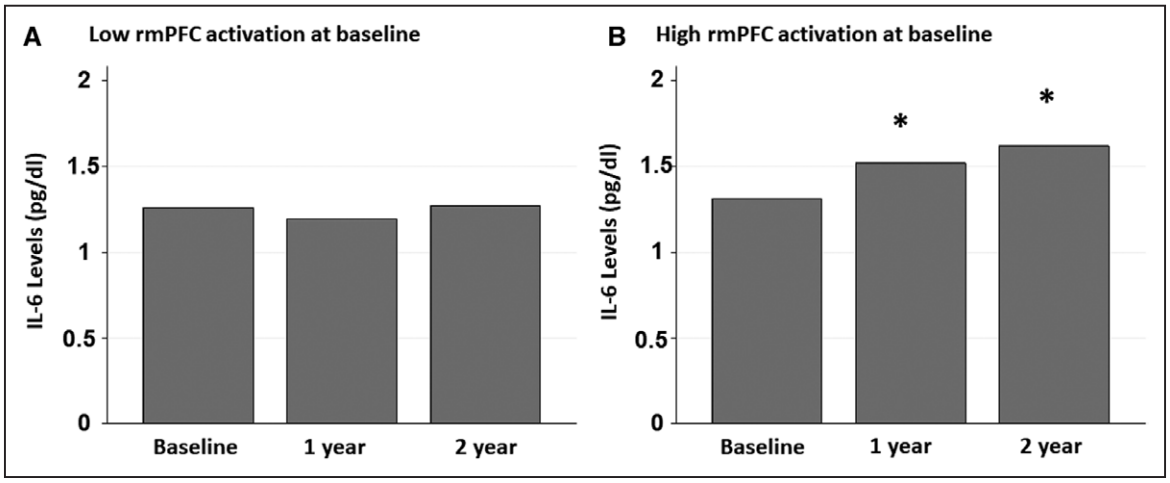

Figure 3. Relationship between interleukin-6 levels at baseline and follow-up stratified by status of rostromedial prefrontal cortex activation during mental stress.

*P<0.05. IL-6 indicates interleukin-6; and rmPFC, rostromedial prefrontal cortex.

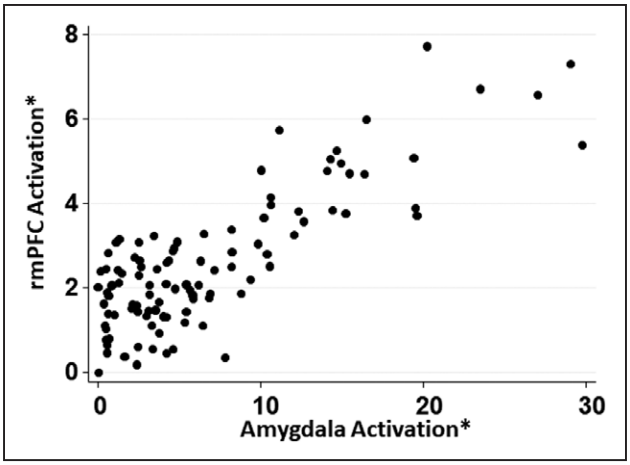

Figure 4. Association between rostromedial prefrontal cortex and amygdala activation during mental stress.
*Net difference $mL^{-1} \cdot min^{-1} \cdot 100\ mg^{-1}$. rmPFC indicates rostromedial prefrontal cortex.

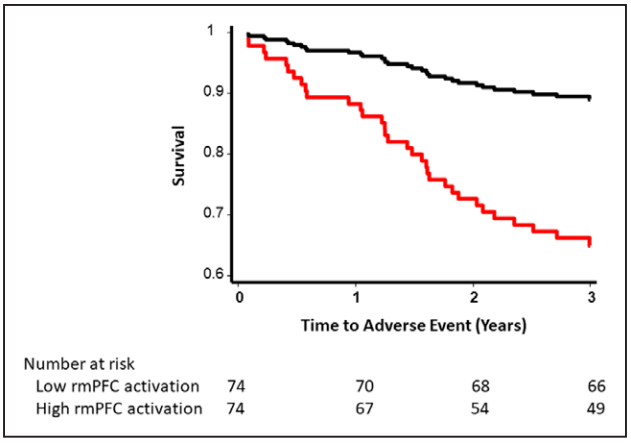

Figure 5. Kaplan-Meier curve for association between rostromedial prefrontal cortex activation and the composite event of cardiovascular death, myocardial infarction, unstable angina with revascularization, and heart failure hospitalization.
rmPFC indicates rostromedial prefrontal cortex.

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[39] in which resting amygdalar activity (involved in the fear response) was associated with increased risk of cardiovascular events. Most areas of the prefrontal cortex inhibit the activity of the amygdala.[40] However, it has been shown that in certain individuals with stress-related disorders, the prefrontal cortex has an excitatory effect on the amygdala.[41] In our study, we found that individuals with particularly high amygdala activation had the highest levels of rmPFC activation. Although we did not find amygdala activation with mental stress to be associated with risk of future cardiovascular events, our results showed clear relationships between amygdala activity and rmPFC activity during mental stress. 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.[42] HF-HRV is regarded as a noninvasive, 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 2 separate meta-analyses.[43] 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.[44,45] A previous meta-analysis showed that IL-6 was the most robust and consistent inflammatory marker that increases with mental stress.[45] We and others have also shown that individuals with CAD have higher IL-6 responses to mental stress than healthy populations.[46,47] 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.[15] 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

Table 2. Risk Discrimination Testing for the Prediction of Major Adverse Cardiovascular Events Including Rostromedial Prefrontal Cortex Activation in Combination With a Clinical Model

Clinical Model	C-Statistic (95% CI)	ΔC-Statistic (95% CI)	Continuous Net Reclassification Index (95% CI)	Integrated Discrimination Index (95% CI)
Base model	0.71 (0.61–0.80)	–		
Base model + rostromedial prefrontal cortex activation	0.76 (0.67–0.85)	0.05 (0.01–0.12)	0.46 (0.18–0.82)	0.04 (0.01–0.09)

The base model includes baseline demographics (age, sex, and race), and medical history (hypertension, hyperlipidemia, diabetes mellitus, obesity, previous myocardial infarction, and previous revascularization).

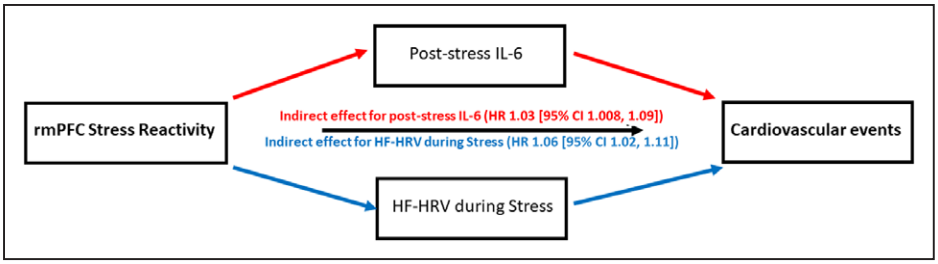

Figure 6. Mediation analysis for hypothesized pathways linking rostromedial prefrontal cortex stress reactivity to cardiovascular events.
All models adjusted for baseline demographics (age, sex, and race) and heart rate-pressure product during mental stress. The red line (online) shows the indirect effects 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($HR_{indirect\ effect}$) / ln($HR_{total\ effect}$)]×100). The blue line (online) shows the 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[($HR_{indirect\ effect}$) / ln($HR_{total\ effect}$)]×100). HF-HRV indicates high-frequency heart rate variability; HR, hazard ratio; IL-6, interleukin-6; and rmPFC, rostromedial prefrontal cortex.

agreement with a previous report of an association between arterial inflammation, amygdalar activity, and cardiovascular events.[39] Although 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.

Our group and others have shown that acute psychological stress can induce mental stress–induced myocardial ischemia, which is associated with a doubling of recurrent events and mortality.[48,49] In our previous work, however, mental stress–induced myocardial ischemia was not found to significantly associate with rmPFC activation or inflammation. These findings, therefore, complement this work by providing an alternative pathway from mental stress–induced myocardial ischemia

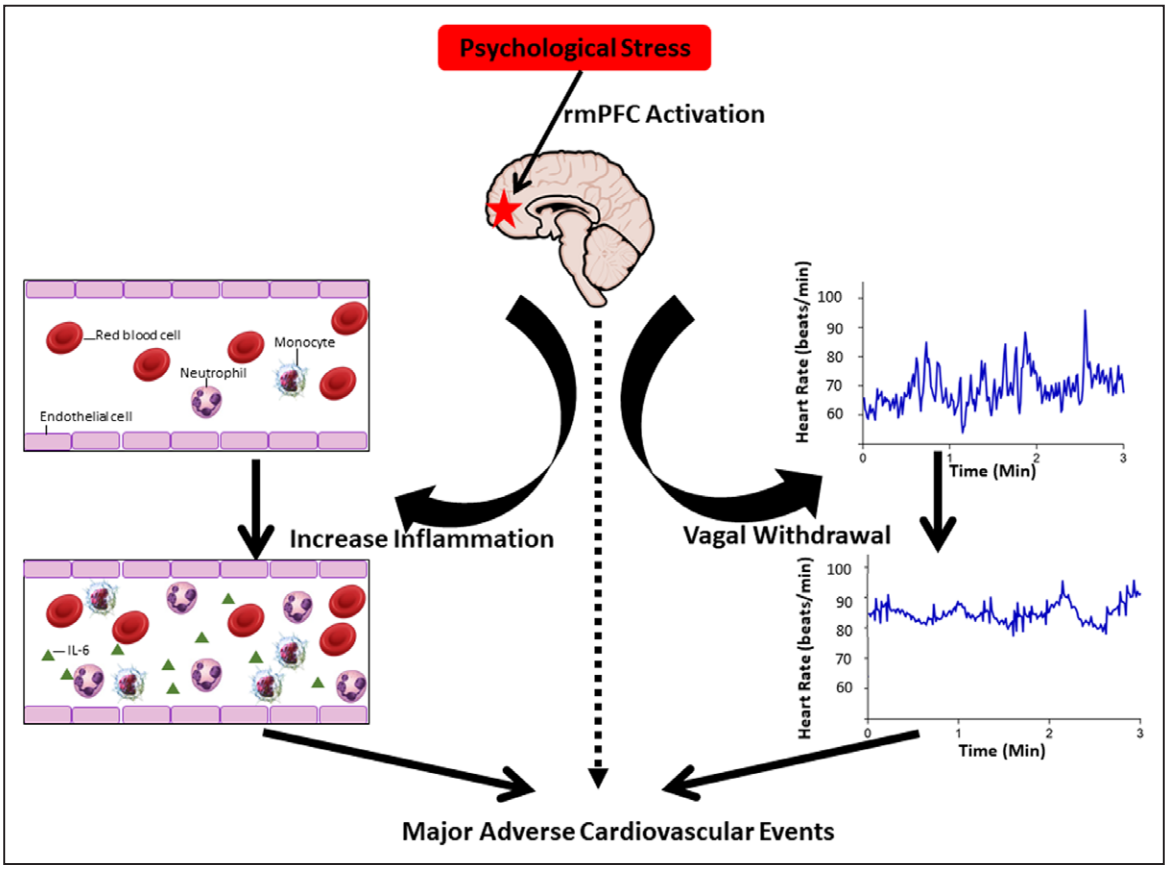

Figure 7. A model of psychological stress leading to cardiovascular events.
At least 2 biological pathways, including vagal withdrawal and systemic inflammation, mediate the relationship between rmPFC stress reactivity and cardiovascular events. IL-6 indicates interleukin-6; and rmPFC, rostromedial prefrontal cortex.

involving inflammation and autonomic dysfunction, rather than vascular mechanisms (as seen with mental stress–induced myocardial ischemia).[18,46]

The major strength 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, inflammatory and autonomic responses to mental stress were assessed simultaneously during stressful tasks; 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 proinflammatory status for those with high rmPFC stress reactivity. Fourth, the number of events was 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.

Conclusions

In summary, we 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, attributable 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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Disclosures

None.

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