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Title: Multivariate brain activity while viewing and reappraising negative affective stimuli and the multiyear progression of preclinical atherosclerosis

Authors:

Peter J. Gianaros[1]
Thomas E. Kraynak[1]
Caitlin M. DuPont[1]
Javier Rasero[2]
James J. Gross[3]
Kateri McRae[4]
Aidan G.C. Wright[1]
Timothy D. Verstynen[2]
Emma Barinas-Mitchell[5]

Author affiliations:

[1]Department of Psychology, University of Pittsburgh, Pittsburgh, PA
[2]Department of Psychology, Carnegie Mellon University, PA
[3]Department of Psychology, Stanford University, Stanford, CA
[4]Department of Psychology, University of Denver, CO
[5]Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA

Study Background and Rationale: Negative affect may confer risk for atherosclerotic cardiovascular disease (CVD; Kraynak, Marsland, & Gianaros, 2018; Suls, 2018). Acute experiences of negative affect, for example, may increase the likelihood of ischemic events, arrhythmias, and sudden cardiac death among vulnerable individuals (Jiang, 2015; Kamarck & Jennings, 1991; Lampert, 2016; Steptoe & Brydon, 2009). A propensity to experience negative affect also appears to confer risk for endpoints of CVD (Kubzansky, Davidson, & Rozanski, 2005; Rozanski, Blumenthal, & Kaplan, 1999) and for an accelerated progression of preclinical atherosclerosis (Stewart, Janicki, Muldoon, Sutton-Tyrrell, & Kamarck, 2007).

The CVD risk conferred by negative affect may be partly attributable to its maladaptive or insufficient regulation (DeSteno, Gross, & Kubzansky, 2013; Kubzansky, Park, Peterson, Vokonas, & Sparrow, 2011); however, the deliberate regulation of negative affect *per se* is rarely examined in the context of CVD risk. One strategy to regulate negative affect and possibly reduce CVD risk is cognitive reappraisal (Appleton & Kubzansky, 2014; Gianaros & Jennings, 2018). By cognitive reappraisal, individuals intentionally change the meaning they ascribe to affectively evocative situations and contexts (Gross, 2014). In clinical contexts, training in reappraisal has been used as a component of adjunctive cognitive behavioral programs designed to decrease CVD risk in patient populations (Cohen, Edmondson, & Kronish, 2015). In epidemiological studies of otherwise healthy adults, the self-reported frequency of using cognitive reappraisal to regulate affect in daily life has been positively associated with cardioprotective health behaviors, as well as negatively with lipid and inflammatory biomarkers of CVD risk (Appleton, Buka, Loucks, Gilman, & Kubzansky, 2013; Appleton, Loucks, Buka, & Kubzansky, 2014; Ellis, Prather, Grenen, & Ferrer, 2019). Across psychophysiological studies, meta-analytic evidence suggests that engaging in reappraisal decreases acute cardiovascular - namely, heart rate – reactions to unpleasant affective stimuli (Zaehringer, Jennen-Steinmetz, Schmahl, Ende, & Paret, 2020). Recent neuroimaging findings indicate further that individuals who exhibit greater activity in the dorsal anterior cingulate cortex (dACC) while attempting to reduce negative affect by cognitive reappraisal also exhibit greater systemic inflammation and a greater severity of preclinical atherosclerosis (Gianaros et al., 2014). Collectively, clinical efforts and findings from epidemiological, psychophysiological, and neuroimaging studies appear compatible with the possibility that individual differences in negative affect regulation by cognitive reappraisal may relate to biological and behavioral factors that influence risk for CVD.

In the latter regard, it is notable that reducing negative affect by cognitive reappraisal not only engages the dACC (Buhle et al., 2014), but also a distributed ensemble of brain systems whose activity patterns relate to (i) processing affective stimuli (Lindquist, Wager, Kober, Bliss-Moreau, & Barrett, 2012) and (ii) controlling and representing autonomic, neuroendocrine, cardiovascular, and immune parameters of peripheral physiology that play a role in the etiology of CVD (Kraynak et al., 2018). This ensemble encompasses so-called visceral control areas of the insula, orbital and medial prefrontal cortex, as well as the amygdala and other subcortical cell groups (Gianaros & Jennings, 2018; Öngür & Price, 2000). Moreover, distributed (multivariate) activity patterns across this ensemble and other brain systems that are elicited by negative affective stimuli (unpleasant affective scenes) associate with baseline (cross-sectional) levels of preclinical atherosclerosis – as reflected by carotid artery intima-media thickness (CA-IMT; Gianaros et al., 2020).

Baseline levels of CA-IMT predict the incident development of atherosclerotic plaques (Tschiderer, Klingenschmid, Seekircher, & Willeit, 2020), and they are interpreted to reflect the cumulative effects of CVD risk factors that have accrued over the lifespan; by comparison, the rate-of-change in CA-IMT over time (e.g., years) is thought to reflect the influence of more recent and proximal etiological determinants of the progression of preclinical atherosclerosis (Chambless et al., 2002). Various metrics reflecting the rate-of-change in CA-IMT are modifiable by behavioral and pharmacological interventions, and they predict clinical CVD endpoints in epidemiological studies (Baldassarre et al., 2013; Willeit et al., 2020). At present, however, an open question about the neurobiology of affect, affect regulation, and CVD risk is whether distributed neural activity patterns – especially beyond the dACC – that are evoked by negative affective stimuli and by cognitive reappraisal predict the prospective and multi-year rate-of-change in

CA-IMT. If so, then such findings would extend prior cross-sectional findings by providing initial evidence as to whether the neural correlates of negative affect and the cognitive regulation of negative affect by reappraisal at a given point in time are able to forecast the future progression of a precursor to clinical CVD endpoints; namely, preclinical atherosclerosis.

Accordingly, the present study assessed brain activity by functional magnetic resonance imaging (fMRI) among a community sample of midlife adults while they viewed and cognitively reappraised complex and unpleasant affective scenes. At the time of initial testing and then a median of 2.8 years later (range = 1.6 to 5.0 years), ultrasonography was used to assess CA-IMT. Whole-brain, multivariate, and penalized regression analyses with dimensionality reduction and cross-validation (Kohoutova et al., 2020), will be used to test whether fMRI activity patterns elicited by viewing and reappraising unpleasant affective scenes predict the annualized rate-of-change in CA-IMT metrics over the follow-up interval. Ancillary analyses will explore the psychometric properties of fMRI activity patterns elicited by viewing and reappraising affective scenes, as well as the potential influence of conventional CVD risk factors and self-reports of negative affect and reappraisal success on any observed predictive associations between fMRI activity and CA-IMT progression. Pre-registration information and study variables will be publicly available online (https://osf.io/hk6qx/).

Study information:

Question title: Does multivariate brain activity while viewing or reappraising negative affective stimuli predict the multiyear progression of preclinical atherosclerosis?

Research Questions:

1. Research Question: Do individual differences in brain activity during the viewing of unpleasant affective scenes predict the multi-year change in carotid artery intima media thickness?

1a. Hypothesis: A multivariate and whole-brain pattern evoked by viewing unpleasant affective scenes will predict the annualized rate of progression of (a) far-wall common carotid artery intima-media thickness and (b) the maximum intima-media thickness in the carotid segment exhibiting the fastest rate of progression for a given individual.

2. Research Question: Do individual differences in brain activity during the cognitive reappraisal of unpleasant affective scenes predict the multi-year change in carotid artery intima media thickness?

2a. Hypothesis: A multivariate and whole-brain pattern evoked by cognitive reappraisal will predict the annualized rate of progression of (a) far-wall common carotid artery intima-media thickness and (b) the maximum intima-media thickness in the carotid segment exhibiting the fastest rate of progression for a given individual.

3. Ancillary Research Question: As predicated on findings from Research Questions 1 – 2, do conventional measures cardiovascular disease risk modify any observed associations between multivariate brain patterns and the progression of carotid artery intima media thickness?

3a. Hypothesis: Individuals with higher Framingham risk scores and higher composite cardiometabolic risk scores at the time of initial testing (baseline) will show a faster annualized progression of carotid artery intima media thickness, but these scores will not statistically moderate predicted vs. observed intima-media thickness associations deriving from Questions 1 - 2.

4. Ancillary Research Question: As predicated on findings from Research Questions 1 – 2, do self-reports of negative affect evoked by viewing or reappraising unpleasant affective scenes (a) correlate with the progression of carotid artery intima media thickness, and if so, (b) modify the predictive associations between multivariate brain patterns and intima-media thickness progression variables?

a. Hypothesis: Self-reports of negative affect will not relate to the progression of carotid artery intima media thickness. Nor will self-reports of negative affect modify the associations between multivariate brain patterns and the progression of carotid artery intima media thickness.

5. Ancillary Research Question: Do brain activity patterns evoked by viewing and reappraising unpleasant affective scenes exhibit within-session reliability during fMRI (i.e., internal consistency)?

a. Hypothesis: The split-half internal consistency of activity patterns evoked by viewing and reappraising unpleasant affective scenes will exhibit variable internal consistency across the brain. If observed as per Research Questions 1-2, whole-brain multivariate brain patterns that predict carotid artery intima media thickness progression will exhibit greater internal consistency than univariate whole-brain activity patterns.

6. Ancillary Research Question: If predictive brain patterns are identified as per Research Questions 1-2, do these predictive patterns generalize to predict the annualized rate of progression in the mean and mean maximum carotid artery intima media thickness from all carotid artery segments (common carotid artery, carotid bulb, and internal carotid artery)?

a. Hypothesis: If observed, predictive patterns identified per Research Questions 1-2 may not generalize, such that predicted vs. observed annualized rate of progression values may not be significantly correlated owing to variation across carotid artery segments in the change in intima-media thickness over time.

7. Ancillary Research Question: Does excluding those individuals who did not engage in reappraisal during the task paradigm alter the associations between predicted vs. observed annualized carotid artery intima media thickness values?

a. Hypothesis: The strength of associations between predicted vs. observed annualized carotid artery intima media thickness values may improve after excluding those individuals who do not engage in reappraisal during the task paradigm.

DATA DESCRIPTION

Name or brief description of data set(s): The current project uses data from the Pittsburgh Imaging Project (PIP), which was funded by the National Institutes of Health (NHLBI R01-089850) under the award title: "Neurobiological Pathways Linking Stress and Emotion to Atherosclerosis." The Principal Investigator of NHLBI R01-089850 was Peter J. Gianaros; co-author, Emma Barina-Mitchell was a co-investigator; consultants were co-authors, James Gross and Kateri McRae.

Is this data open or publicly available? After completion of the analyses described here and prior to manuscript submission, data described in this project will be publicly accessible via: https://osf.io/hk6qx/

Data Source: Data were collected by the laboratories of study authors, PJG and EB-M, as well as the MR Research Center at the University of Pittsburgh.

Sampling and data collection procedures:

Participants

Participants were midlife and community-dwelling adults from the Pittsburgh Imaging Project (PIP), a longitudinal study of biological and behavioral risk factors for preclinical atherosclerosis and CVD risk. The entire PIP cohort consists of 331 individuals (aged 30 – 51 years; 166 women and 165 men; 230

identifying as white; 80 identifying as Black or African American; 16 identifying as Asian or Asian American; and 5 identifying as multiracial). Details regarding recruitment methods, study design, and dates of data collection have been published (Gianaros et al., 2020; Gianaros et al., 2017). *This will be the first analysis and report from PIP on fMRI measures of cognitive reappraisal, as well as the longitudinal progression of preclinical atherosclerosis.*

Potential volunteers for PIP were initially screened by phone and then again by an in-person medical history interview prior to testing to exclude those individuals who endorsed: a history of cardiovascular or cerebrovascular disease (including treatment for or diagnoses of hypertension, stroke, myocardial infarction, congestive heart failure, and arrhythmias); history of any chronic medical or neurological disorder (including, Type 1 and Type 2 diabetes, emphysema, rheumatologic conditions, seizure disorders, and chronic hepatitis); prior neurosurgery; current treatment for or self-reported psychiatric conditions; consuming alcohol equaling or exceeding five servings on three or more occasions per week; regular use over-the-counter or prescribed medications having autonomic, cardiovascular, or neuroendocrine effects (e.g., beta- blockers, decongestants, corticosteroid inhalers); regular use of psychotropic medications; history of metal exposure or presence of metallic implants deemed unsafe for MRI; color-blindness; self-reported claustrophobia; and, for women, pregnancy (as verified by urine test).

Participants were compensated $175.00 US for completing baseline study visits, and $87.50 US for the follow-up study visit. Informed consent was obtained from all study participants, and study approval was granted by The University of Pittsburgh Human Research Protection Office (Protocol number: 07110287).

At the time of initial testing, PIP participants attended multiple study visits. These visits entailed (a) informed consent; (b) medical and demographic interviews; (c) anthropometric assessments of height, weight, and body composition; (d) seated assessments of blood pressure; (e) completion of questionnaires to assess health behaviors and psychosocial characteristics; (f) carotid artery ultrasonography; (g) fasting phlebotomy; and (h) a magnetic resonance imaging (MRI) protocol. Prior to a single initial study visit involving fasting phlebotomy, a light meal, and then MRI, participants were instructed to fast for 8 hours prior to testing. This visit was scheduled to occur between 7:00AM and 11:00AM for all participants.

In September of 2011, an fMRI task involving viewing and reappraising affective images from the International Affective Picture System (Lang, Bradley, & Cuthbert, 2008) was added to the MRI visit of the PIP study protocol. A total of 176 PIP participants completed this fMRI task. For these participants, a median of 55 days separated the MRI and ultrasonography visits at initial testing (range = 2 – 175 days). Of the latter 176 participants, 146 returned for a follow-up carotid artery ultrasound assessment that also included fasting phlebotomy and the re-assessment of medical status and other measures previously assessed at the time of initial testing (83% retention rate, median follow-up interval between the 2 carotid ultrasonography visits = 2.78 years, SD = 0.35 years). The entire span of data collection encompassed 09/2011 through 07/2017.

Demographics and Health Behaviors

At the time of initial testing, demographic information was collected from participants to assess age, sex, race and ethnicity, income, and education (years of schooling). At that time and again at follow-up, participants reported on their smoking status and frequency of alcohol consumption over the past week.

Conventional Cardiovascular Risk Factors

At the time of initial testing and again at follow-up, participants underwent assessments of seated resting systolic and diastolic blood pressure, waist circumference and body mass index, as well as fasting glucose and lipid levels. As detailed previously for the PIP sample (Gianaros et al., 2017), seated resting blood pressures (BPs) were obtained using an oscillometric method following guidelines of the American Heart Association. A total of 3 BPs were taken 2 min apart after an acclimation period, with the average of the last 2 of the 3 BPs being used to compute resting systolic (SBP) and diastolic (DBP) blood pressures. Participants' waist circumference was measured at the level of the umbilicus to the nearest 1/2 centimeter at end expiration. Height was measured by a vertical-mounted stadiometer (with shoes off), and weight was measured by a digital scale. At the time of initial testing and at follow-up, a research nurse performed phlebotomy. Concentrations of total cholesterol and triglycerides were measured by a CHOL and triglyceride GPO reagent, respectively, using an enzymatic, timed-endpoint method on the SYNCHRON LX System (Beckman Coulter, Inc., Brea, California). The concentration of high-density lipoprotein (HDL) cholesterol was measured with a HDLD reagent on the SYNCHRON LX System, which uses an enzymatic, time-endpoint method to uniquely facilitate a detergent that solubilizes only the HDL lipoprotein particles (Beckman Coulter, Inc., Brea, California). Low-density lipoprotein (LDL) cholesterol concentrations were estimated by the Friedewald formula (Friedewald, Levy, & Fredrickson, 1972). Along with other demographic and anthropometric variables, these measures are used to derive Framingham risk scores (D'Agostino et al., 2008) and composite measures of cardiovascular risk (see below).

Medical Conditions and Medication Use

At the time of initial testing and again at follow-up, participants underwent a medical history interview. By this interview, we determined that over the follow-up interval: (a) one participant reported a new diagnosis of and treatment for hypertension; (b) one participant began using lipid-lowering medication; (c) two participants began using medications for glucose control; (d) three participants began using psychotropic medications; (e) two participants began using sleep medication; and (f) one participant began using weight loss medication. Out of the 89 women in the analytic sample with follow-up assessments: 3 underwent hysterectomy prior to initial testing; 1 was postmenopausal prior to initial testing; 5 were premenopausal at initial testing, and then peri-menopausal at follow-up; 1 was peri-menopausal, both at the time of initial testing and at follow-up; and none reported using hormone therapy at initial testing or at follow-up. Given that less than 6% of the sample was found to change in clinical or medication status and less than 6% of women underwent a change in menopausal status over the follow-up interval, there is insufficient statistical power to determine the effects of such changes on primary study outcome variables (CA-IMT metrics).

Carotid Artery Ultrasound

Participants underwent carotid artery ultrasonography. The protocol was performed by a registered vascular technologist in the laboratory of co-author E. B-M. During ultrasonography, the participant lies supine with the head tilted at 45°. Using an Acuson Antares scanner (Acuson-Siemens, Malvern, PA), the technologist performed scout views of the left and right carotid arteries in both the transverse and longitudinal planes. A region-of-interest encompassing the artery walls was identified for more focused B-Mode imaging of 3 carotid areas: (1) the near and far walls of the distal common carotid artery (1 cm proximal to the carotid bulb); (2) the far wall of the carotid bulb (defined as the point where the near and far walls of the common carotid are no longer parallel and extending to the flow divider); and (3) the first cm of the internal carotid (defined distally from the edge of the flow divider). For the 3 carotid areas (common, bulb, and internal), an optimal image was digitized for later scoring with automated edge

detection software (Artery Measurement System; Goteborg University, Gothenburg, Sweden). The software is used to draw two lines: one along the lumen-intima interface and one along the media-adventitia interface. The distances between the line-identified interfaces are measured in 1 cm segments, generating one measurement (in mm) for each pixel in each segment (approximately 140 measurements total). For the carotid 3 areas, the average, standard deviation, minimum, and maximum measurement values are recorded.

Annualized progression of mean common carotid artery intima media thickness (primary outcome variable) and the segment exhibiting the fastest progression of maximum intima media thickness (secondary outcome variable). Carotid artery intima-media thickness is a surrogate marker of generalized preclinical atherosclerosis that predicts future (incident) clinical cardiovascular and cerebrovascular events, as well as incident plaque development (Tschiderer et al., 2020; Willeit et al., 2020). Carotid artery segments exhibit differential progression rates over time, and rates of progression across arterial segments do not equally predict cardiovascular outcomes (Baldassarre et al., 2013; Mackinnon et al., 2004). The majority of evidence regarding CVD risk and the progression of intima-media thickness has relied on measurement of the far walls of the common carotid artery (Willeit et al., 2020). In view of cumulative evidence, to minimize the influence of progression heterogeneity across segments, to account for variable length-of-follow-up intervals, and to limit multiple statistical testing, the primary outcome variable (dependent measure) for analysis is the annualized progression rate of mean common carotid artery intima media thickness, as digitized from the far wall. Secondary analyses use a variable reflecting the segment of the carotid arteries exhibiting the fastest rate of progression in mean maximum carotid artery thickness for each participant, as per the computational methods of prior work (Baldassarre et al., 2013). This choice of a secondary outcome measure is predicated on findings indicating that the carotid segment exhibiting the fastest progression rate of maximum intima media thickness may outperform other metrics in the prediction of future cardiovascular events (Baldassarre et al., 2013). In ancillary tests of model generalizability, the mean and maximum carotid artery intima-media thickness averaged over all carotid segments (common, bulb, internal) will be modeled as alternate outcome variables.

fMRI Reappraisal Task

Participants completed a reappraisal task originally described by Ochsner and colleagues (Ochsner, Bunge, Gross, & Gabrieli, 2002) that was administered as an event-related fMRI paradigm as detailed previously (Gianaros et al., 2014). In brief, participants were trained in cognitive reappraisal and allowed time for practice prior to brain imaging, and they were instructed that they would see unpleasant and neutral images after a cue that provided one of two instructions: “Look” and “Decrease”. All images were drawn from the International Affective Picture System (IAPS; http://csea.phhp.ufl.edu/media.html). When subjects were cued by the “Look” instruction, they were asked to think and feel naturally. When cued by the “Decrease” instruction, they were asked to change the way they thought about the image to feel less negative (i.e., reappraise). Subjects were asked to not look away, but rather to focus on each image and actively try to change their feelings. After each image, they used a 1-5 Likert-type scale to rate how negative they felt at the end of viewing (1 = not at all; 5 = strongly negative). Thus, the task consisted of 3 conditions: ‘Look Neutral’, ‘Look Negative’ and ‘Regulate Negative’, with 15 images per condition. No more than 2 of the same cues were presented consecutively, and no more than 4 unpleasant images were presented consecutively. Each condition was initiated by a cue (“Look”, “Decrease”) for 2-sec, followed by an image for 7-sec, and the rating scale for 4-sec. Lastly, a rest screen was shown for a variable 1-3 sec. IAPS image IDs used as stimuli were as follows:

Unpleasant IAPS image IDs used in the "Look" condition: 2053, 2703, 3051, 3102, 3120, 3350, 3500, 3550, 6831, 9040, 9050, 9252, 9400, 9414, 9921

Unpleasant IAPS image IDs used in the "Regulate" condition: 3030, 3100, 3110, 3170, 3230, 3530, 6212, 9250, 9410, 9420, 9910, 2683, 6520, 6838, 9254

Neutral IAPS image IDs used in the "Look" condition: 2026, 2036, 2102, 2272, 2308, 2377, 2390, 2393, 2411, 2487, 2595, 7130, 7550, 8312, 9210

Likert-type ratings of negative affect after each trial are used to derive 2 variables for Research Question #4: Self-reported affective reactivity ('Look Negative' - 'Look Neutral' ratings) and reappraisal success ('Regulate Negative' - 'Look Negative' ratings).

Task instructions and a post-task experimental questionnaire used to record participant responses about affect regulation strategies and reappraisal success are available online (https://osf.io/hk6qx/).

MRI Data Acquisition and Preprocessing

Imaging was conducted using a 3-Tesla Trio TIM scanner (Siemens, Erlangen, Germany). Prior to functional imaging, a T1-weighted magnetization prepared rapid gradient echo (MPRAGE) structural image was obtained by these parameters: repetition time = 2100 msec; inversion time = 1100 msec; echo time = 3.31 msec; flip angle = 8º. There were 192 sagittal slices (1 mm thick, no spaces between slices) having a matrix size = 256 x 208 pixels (field-of-view [FOV] = 256 x 208 mm). Functional BOLD image acquisition parameters for the IAPS task were: matrix size = 64 × 64 pixels (FOV = 205 × 205 mm), TR = 2000 ms, TE = 28 ms, and FA = 90°. Thirty-nine slices per volume were collected along an inferior-to-superior encoding direction. Each volume was 3 mm in thickness, with no gap (338 task volumes in total). A 6-sec countdown preceded task onset. The 3 volumes of this countdown were not modeled, nor were the 3 volumes collected after the offset of the final rest period (344 functional run volumes in total).

fMRI data for the IAPS tasks have been preprocessed with statistical parametric mapping software (SPM12; http://www.fil.ion.ucl.ac.uk/spm). For spatial preprocessing, T1-weighted MPRAGE images were classified into 6 tissue types. Biased-corrected and deformation field maps were then computed. Functional images were realigned to the first image of the series by 6-parameter rigid-body transformation, using the re-slice step to match the first image on a voxel-by-voxel basis. Before realignment, slice-timing correction was applied to account for acquisition time variation. Realigned images were co-registered to each participant's skull-stripped and biased-corrected MPRAGE image. Co-registered images were normalized to Montreal Neurological Institute (MNI) space. Normalized images were smoothed by a 6mm full-width-at-half-maximum (FWHM) Gaussian kernel.

In within-individual fMRI analyses, univariate general linear models (GLMs) were estimated to compute contrast maps that will be used for prediction analysis described below in the analysis plan. Task events were modeled by rectangular waveforms convolved with the default hemodynamic response function in SPM12. These regressors modeled events of the trial (i.e. cue, picture, rating period, rest). In each GLM, the six realignment parameters from pre-processing were included as nuisance regressors, and low-frequency artifacts were removed by a high-pass filter (128 s). Error variance was estimated and then weighted by restricted maximum likelihood estimation, as implemented in the RobustWLS toolbox, v4.0. Linear contrasts were computed as 'Look negative vs. Look neutral' and 'Regulate negative vs. Look negative' comparisons. Main effects will be determined from individual GLMs at the group (between-individual) level with whole-brain correction for multiple testing using a voxel-wise and false discovery

rate threshold of 0.05 for display.

KNOWLEDGE OF DATA

Prior work based on the dataset: JJG, KM, ACGW are unfamiliar with variables and data from the PIP dataset. PJG, CMD, TEK, TDV, JR, and E B-M have previously worked with and/or published PIP data; however, none of the authors have analyzed or conducted statistical tests regarding fMRI reappraisal data and the longitudinal change in carotid artery IMT, as described in the research questions of this pre-registration. Variables to be used for analysis include participant demographic, health behavior, and anthropometric information; self-reports; fMRI measures; ultrasound measures of carotid artery IMT; and CVD risk factors, which will be made publicly available online and accessible via this project link (https://osf.io/hk6qx/) after analyses are completed and prior to the time of manuscript submission.

Prior Research Activity:

Prior Knowledge of the Current Dataset: PJG, TEK, TDV, and JR have knowledge of the pre-processed fMRI data in the PIP dataset. JJG, KM, ACGW, and EB-M have either no or limited knowledge of the current PIP dataset. All study authors are familiar with descriptive summaries regarding non-fMRI data from the PIP cohort (i.e., those provided in this document above). Please see PJG's laboratory web site for a complete list of publications from PIP (http://www.bnl.pitt.edu/papers.html).

Moment of preregistration: All research questions and variable transformations or calculations were pre-registered prior to statistically analyzing the data to test hypotheses.

Analyses

Prior to addressing the first and subsequent questions via the analysis plans described below, fMRI data will be reviewed for spatial (brain) coverage and image quality. fMRI task performance and experimenter notes will be reviewed. Poor fMRI image quality, incomplete imaging data, experimental error, equipment malfunction during participant testing, and lack of participant task comprehension are exclusion criteria for some (e.g., fMRI) analyses. Also prior to implementing statistical models, group-level effects for the fMRI task contrasts will be determined, as per the whole-brain analyses described above. Lastly, data manipulation (e.g., standardization, transformation to correct detected skew, leverage point and outlier detection, etc.) procedures will be accordingly conducted prior to or as a part of the analysis pipelines to be implemented.

1. Research Question: Do individual differences in brain activity during the viewing of unpleasant affective scenes predict the multi-year change in carotid artery intima media thickness?

1a. Hypothesis: A multivariate and whole-brain pattern evoked by viewing unpleasant affective scenes will predict the annualized rate of progression of (a) far-wall common carotid artery intima-media thickness and (b) the maximum intima-media thickness in the carotid segment exhibiting the fastest rate of progression for a given individual.

The independent variable for this analysis will be the whole-brain fMRI contrast map corresponding to the 'Look negative vs. Look negative' comparison. The primary dependent variable will be the annualized progression rate of common carotid artery intima media thickness from the far wall, expressed as the mm change per year. In secondary analyses, the dependent measure will be the annualized progression rate of the segment showing the fastest progression rate in the mean of the maximum intima media thickness for a given person, expressed as mm change per year as per methods previously detailed (Baldassarre et al., 2013).

To establish generalizability in all statistical models testing predictive associations, nested and k-fold cross-validation will be implemented to (a) optimize regression estimators in an 'inner loop' and then (b) determine predictive generalizability in an 'outer loop' using on leave-one-out cross-validation. Machine learning analyses with regularization (penalization) and feature selection will be executed to test study

hypotheses in a LASSO-PCR framework (Kohoutova et al., 2020). In LASSO-PCR, principal components dimensionality reduction of the predictor space will be followed by optimizing the penalty parameter, λ, in an inner loop applying 5-fold cross-validation. In this way, participants will be divided into training (80%) and testing (20%) samples by stratifying over each outcome variable distribution. Within each cross-validation fold, LASSO will be conducted on training samples using a sequence of 1000 λ values, and the performance of each λ evaluated by calculating the mean squared error (MSE) between predicted and observed outcome variables in respective testing samples. After identifying the optimal λ across all testing samples, the entire LASSO procedure will be repeated using optimal λs on the entire sample, producing a predictive model. To test the generalizability of the LASSO models and to generate estimates of predicted outcomes for each participant, we will repeat the above process in an outer cross-validation loop using the leave-one-out method. The final predictive performance of a given LASSO model will be summarized by concatenating across the outer loops. Here, the similarity between predicted and observed values will be summarized by Pearson correlation coefficients and corresponding 95% bootstrapped confidence intervals (CI) and p-values. The discrepancy between predicted and observed values will be calculated using mean absolute error (MAE). Following guidelines for predictive modeling, variance in observed values explained by predicted values (R2) will be calculated by the sums-of-squares formulation (Poldrack, Huckins, & Varoquaux, 2020). Individual features that reliably contribute to any observed predictive associations will be determined by bootstrap resampling (5000 resamples) at $p < .05$, with correction for the false discovery rate.

2. Research Question: Do individual differences in brain activity during the cognitive reappraisal of unpleasant affective scenes predict the multi-year change in carotid artery intima media thickness?

2a. Hypothesis: A multivariate and whole-brain pattern evoked by cognitive reappraisal will predict the annualized rate of progression of (a) far-wall common carotid artery intima-media thickness and (b) the maximum intima-media thickness in the carotid segment exhibiting the fastest rate of progression for a given individual.

Analyses of the primary and secondary dependent CA-IMT measures will match those detailed above for Research Question 1; however, the multivariate predictor (independent) variable will be the 'Regulate negative vs. Look neutral' contrast map.

3. Ancillary Research Question: As predicated on findings from Research Questions 1 – 2, do conventional measures cardiovascular disease risk modify any observed associations between multivariate brain patterns and the progression of carotid artery intima media thickness?

3a. Hypothesis: Individuals with higher Framingham risk scores and higher composite cardiometabolic risk scores at the time of initial testing (baseline) will show a faster annualized progression of carotid artery intima media thickness, but these scores will not statistically moderate predicted vs. observed intima-media thickness associations deriving from Questions 1 - 2.

For these analyses, a regression/correlation analysis will first test whether Framingham risk scores correlate with primary and secondary variables reflecting the annualized progression of carotid artery intima media thickness. If correlations are observed (and contingent on observing predictive associations per Research Questions 1 -2), Framingham risk scores will be used as effect modifiers by creating an interaction term in multiple regression tests of the association between predicted and observed annualized progression values. As an additional sensitivity test of Ancillary Research Question #3 that does not include the variables age and sex (as in Framingham scores), a composite measure of cardiometabolic risk at baseline will be used in place of Framingham scores by z-scoring and averaging components of the metabolic syndrome; namely blood pressure, waist circumference, body mass index, lipid levels, and glucose. Baseline levels of this composite measure will be used in place of Framingham scores as an effect moderator in interaction tests of predicted vs. observed carotid artery intima media thickness values using an approach described previously (Gianaros et al., 2020).

4. Ancillary Research Question: As predicated on findings from Research Questions 1 – 2, do self-reports of negative affect evoked by viewing or reappraising unpleasant affective scenes (a) correlate with the progression of carotid artery intima media thickness, and if so, (b) modify the predictive associations between multivariate brain patterns and intima-media thickness progression variables?

a. Hypothesis: Self-reports of negative affect will not relate to the progression of carotid artery intima media thickness. Nor will self-reports of negative affect modify the associations between multivariate brain patterns and the progression of carotid artery intima media thickness.

Analyses will match those per Ancillary Question 3 above using correlation and multiple regression analyses. Here, changes in negative affect for each participant will be derived as mean difference scores between 'Look negative vs. Look neutral' trials (reflecting affective reactivity) and 'Regulate negative vs. Look negative' trials (reflecting reappraisal success).

5. Ancillary Research Question: Do brain activity patterns evoked by viewing and reappraising unpleasant affective scenes exhibit within-session reliability during fMRI (i.e., internal consistency)?

a. Hypothesis: The split-half internal consistency of activity patterns evoked by viewing and reappraising unpleasant affective scenes will exhibit variable internal consistency across the brain. If observed as per Research Questions 1-2, whole-brain multivariate brain patterns that predict carotid artery intima media thickness progression will exhibit greater internal consistency than univariate whole-brain activity patterns.

Split-half internal consistency for univariate and multivariate fMRI activity maps will be determined using methods previously detailed (Gianaros et al., 2020). Specifically, GLM analyses will be used to generate contrast images for the approximate first and second halves the fMRI task (i.e., the first 23 and last 22 trials). Internal consistency analyses will then be conducted by applying the Spearman-Brown (SB) correction method on a voxel-wise basis. As predicated on findings from Research Questions 1 – 2, we will compute the internal consistency of the final predictive weight maps generated by LASSO-PCR. Here, we will compute the dot-product of each whole-brain weight map with all of the pairs of contrast maps generated by the split-half procedure described above.

6. Ancillary Research Question: If predictive brain patterns are identified as per Research Questions 1-2, do these predictive patterns generalize to predict the annualized rate of progression in the mean and mean maximum carotid artery intima media thickness from all carotid artery segments (common carotid artery, carotid bulb, and internal carotid artery)?

a. Hypothesis: If observed, predictive patterns identified per Research Questions 1-2 may not generalize, such that predicted vs. observed annualized rate of progression values may not be significantly correlated owing to variation across carotid artery segments in the change in intima-media thickness over time.

For these analyses, multiple regression and correlation will be used to examine the associations between *predicted* annualized carotid artery intima media thickness progression values generated by the LASSO-PCR models of Research Questions 1-2, but observed values will be the variables: (1) mean and (2) mean maximum carotid artery intima media thickness, as quantified from the near and far walls of all carotid artery segments (common carotid artery, carotid bulb, and internal carotid artery).

7. Ancillary Research Question: Does excluding those individuals who did not engage in reappraisal during the task paradigm alter the associations between predicted vs. observed annualized carotid artery intima media thickness values?

a. Hypothesis: The strength of associations between predicted vs. observed annualized carotid artery intima media thickness values may improve after excluding those individuals who do not engage in reappraisal during the task paradigm.

To address Research Question 7, two of the authors (PJG and CD) will independently code free-response descriptions of the emotion regulation strategies participants used during the fMRI task as reflecting reappraisal or not (binary variable: 0 = no, 1 = yes). Inter-rater agreement will be computed by the two-way random effects ICC method to determine absolute inter-rater agreement (Koo & Li, 2016). If necessary, discrepancies between coders will be adjudicated conversation, and verified as needed by a third independent rater (co-author, KM) prior computing final scores used for sensitivity tests below.

At the end of the MRI protocol, participants were asked to describe the strategies they used to decrease their emotional responses to the unpleasant IAPS images in the 'decrease' condition. Specifically, they were asked: "What sorts of things did you tell yourself to try to help you feel differently in response to the negative pictures?" The response format was open ended. Coding of participant responses will follow the methods detailed previously by co-author K. McRae (McRae, Ciesielski, & Gross, 2012). Specifically, responses will be coded as reflecting reappraisal if falling into any of following categories: (1) explicitly positive, (2) a change in current circumstances, (3) a reality challenge, (3) a change of future consequences, (4) ascribing agency to a person capable of changing the circumstances depicted in the image, (5) distancing, (6) problem-solving, (7) acceptance, and (8) non-specific reappraisal. Failures to engage in reappraisal (e.g., avoided looking at or thinking about the image, using expressive suppression as the primary strategy, or using a strategy that is not cognitive) will result in a score of 0. In Ancillary sensitivity tests of Research Questions 1 and 2, those participants having a score of 0 will be removed from analyses.

Contributions: P.J. Gianaros conceived the study and hypotheses, obtained research funding from NIH, planned the project, and oversaw data collection. E. Barinas-Mitchell oversaw the carotid artery ultrasound protocol, as well as scoring and quality control procedures to generate intima-media thickness data. P.J. Gianaros, T.E. Kraynak, C.M. DuPont, J. Rasero, J.J. Gross, K.M. McRae, A.G.C. Wright, T.D. Verstynen, and E. Barinas-Mitchell collaborated on and finalized the pre-registration and data- analysis plan. T.E. Kraynak and J. Rasero will prepare and implement machine learning models of MRI data with P.J. Gianaros and T.D. Verstynen. T.E. Kraynak, J. Rasero, and C.M. DuPont will prepare and implement regression and correlation analyses of self-report, behavioral, MRI, and biological data. P.J. Gianaros, T.E. Kraynak, C.M. DuPont, J. Rasero, J.J. Gross, K.M. McRae, A.G.C. Wright, T.D. Verstynen, and E. Barinas-Mitchell will discuss results, contribute to manuscript preparation and revisions, and approve the manuscript for submission and resubmission.

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