Brain mediators of cardiovascular responses to social threat, Part II: Prefrontal-subcortical pathways and relationship with anxiety

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Running Head: PFC-SUBCORTICAL PATHWAYS IN SOCIAL THREAT

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Abstract

Social evaluative threat (SET) is a potent stressor in humans that is linked to autonomic and endocrine responses, depression, heart disease, and other health problems. Neuroimaging has recently begun to elucidate the brain correlates of SET, but as yet little is known about the mediating cortical-brainstem pathways in humans and how these pathways might differ from stress pathways in animal models. We applied multi-level path analysis to localize brain mediators of SET effects on heart rate (HR) and self-reported anxiety. For HR, SET induced opposite effects in two distinct sub-regions of the medial prefrontal cortex (MPFC), replicating previous findings (T. D. Wager et al., submitted), as well as in the periaqueductal gray (PAG), a critical brainstem coordinator of peripheral autonomic responses. SET activated the rostral dorsal anterior cingulate cortex (rdACC) and PAG but deactivated ventromedial PFC (vmPFC). Both cortical regions were independent mediators of SET effects on HR. Additional analyses provided support for a functional path linking vmPFC, PAG, and HR, and another path linking rdACC, thalamus, and HR. PAG responses were linked with HR changes across time, whereas cortical regions showed stronger connectivity with HR during threat (and pre-stress baseline, in the case of vmPFC). Self-reported anxiety showed a partially overlapping, but weaker, pattern of mediators, including the vmPFC, dorsomedial PFC, and lateral frontal cortex. However, the multi-level path model also provided evidence for substantial individual differences that were largely unexplained. Taken together, these data suggest pathways for the translation of social threats into both physiological and experiential responses, and provide targets for future research on the generation and regulation of emotion.

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Introduction

One of the most remarkable features of the mammalian nervous system is its ability to mount coordinated behavioral and physiological responses to environmental demands. Indeed, when environmental cues signal a potential threat to an organism's well-being, the brain produces a coordinated set of behavioral, autonomic, and metabolic changes that promote an adaptive response. While advantageous in the short term, threat responses that persist over time can have deleterious effects on the brain and body. Chronic threat has been shown to increase the risk of heart disease (Bosma, 1998; Jain, Joska, Lee, Burg, & Lampert, 2001; Rozanski et al., 1988; Sheps, 2002), cause hippocampal deterioration (Smith, Makino, Kvetnansky, & Post, 1995; Stein-Behrens, Mattson, Chang, Yeh, & Sapolsky, 1994; Watanabe, Gould, & McEwen, 1992) and impairments in declarative memory (McEwen & Sapolsky, 1995), promote pro-inflammatory immune responses (Kiecolt-Glaser & Glaser, 2002), and contribute to cognitive and physical aging (Mcewen, 2007), among other adverse effects. Both the threat state and its negative connotations for health are captured in early concepts of "stress" (Selye, 1956) and the more recent concept of "allostatic load" (Mcewen, 2007)—the notion that the brain actively maintains homeostasis and that persistent threat places sustained and diverse demands on brain, autonomic, endocrine, and end-organ systems that have deleterious effects on the brain and body, leading to a variety of health problems (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002). Remarkably, inter-correlated autonomic, endocrine, and immune changes are produced by even acute psychological stressors (Cacioppo, 1994; Cohen et al., 2000; Kiecolt-Glaser, Cacioppo, Malarkey, & Glaser, 1992; Sgoutas-Emch et al., 1994).

Much progress has been made in understanding the neural substrates of threat and stress in animals, but much less is known about stress responses in the human brain. Animal models of threat have incorporated physical threats, including shock, exposure to sensory stimuli related to predators or threatening contexts (e.g., for rats, cat fur and bright lights, respectively; (Davis, 1998)) , social aggression (Sheridan, Stark, Avitsur, & Padgett, 2000), or restraint (Conrad, Magarinos, Ledoux, & McEwen, 1999). Although studies of fear learning have identified homologous behavioral and neural responses to physical threats – like shock – in rodents and primates, including humans (e.g. (Buchel,

Morris, Dolan, & Friston, 1998; Phelps, Delgado, Nearing, & LeDoux, 2004)), the "threats" in modern human life are usually more abstract, and primarily concern the maintenance of our self-esteem, social status, and long-term prospects for mating and longevity. Ecological studies have demonstrated adverse effects of social status-related threats on a number of health-related outcomes, including cardiac health, both in humans and non-human primates (Bosma, 1998; Sapolsky, 2004).

In human laboratory studies social status-related threats have been studied in the context of *social evaluative threat* (SET)—the condition of being judged unfavorably by other individuals in a public setting. SET has been shown to be the most potent human laboratory elicitor of a canonical feature of stress in animal models: the hypothalamic-pituitary-adrenal (HPA) axis response (Dickerson & Kemeny, 2004; Kirschbaum, Pirke, & Hellhammer, 1993). This fits the general finding that HPA-axis responses to stress have been linked specifically to threats to the "social self" (Dickerson, Gruenewald, & Kemeny, 2004) and the specific findings that experimentally induced SET may induce myocardial ischemia (Rozanski et al., 1988) and impairments in left ventricular function (Jain et al., 1998) in patients with coronary artery disease. These effects are clinically relevant; for example, psychological stress-induced ischemia has been shown to predict mortality related to cardiac events over a 5-year followup (Sheps et al., 2002). Given the relevance of SET for cardiovascular disease and other forms of mental and physical health, it is vital to understand the brain pathways that link cortical responses to acute SET with changes in physiological activity, particularly those related to cardiac function.

Whereas a number of human neuroimaging studies have studied responses to and the anticipation of physical threats (like shock), to date only a handful have studied the neural bases of SET or related threats to intellectual competence (H. Critchley, 2003; Dedovic et al., 2005; Eisenberger, Taylor, Gable, Hilmert, & Lieberman, 2007; P. J. Gianaros, F. M. Van Der Veen, & J. R. Jennings, 2004; Kern et al., 2008; Wang et al., 2005). In general, two kinds of findings have been observed. The first and most consistent is activity in midline cortical regions, including the dorsal anterior cingulate cortex (dACC) and medial prefrontal cortex (mPFC). Perceived stress during SET has been associated with increased cortisol and HR reactivity, which is associated with increased dACC activity (Eisenberger et al., 2007) and decreased vmPFC and mOFC

activity (Pruessner et al., 2008). In general, this fits with work implicating these brain systems in visceromotor control and emotion (Price, 1999). The second finding is decreased activity during SET in subcortical regions, including the amygdala and hippocampus (Dedovic et al., 2005; Pruessner et al., 2008). In general, the pattern of cortical activity fits with that found during fear conditioning—conditioned cues elicit dACC increases and vmPFC decreases (Phelps et al., 2004; Schiller, Levy, Niv, LeDoux, & Phelps, 2008)—but diverges from it in the amygdala, whose activity is positively associated with conditioned fear cues. A possible explanation is that fMRI responses in human amygala are driven by salient sensory cues that predict affective value (Paton, Belova, Morrison, & Salzman, 2006; K. L. Phan, Wager, Taylor, & Liberzon, 2004) (Amaral, 2003; Kim et al., 2004; Whalen et al., 1998), and thus plays a key role in conditioned, but not unconditioned, aversive responses (Davis & Lee, 1998; Walker & Davis, 1997; Wallace & Rosen, 2001b).

In a companion paper (T. D. Wager et al., submitted), we found evidence for each of the effects described above. We used fMRI combined with a novel multi-level path analysis approach to identify brain mediators of the effects of a public speech preparation task (social evaluative threat, SET) on heart rate (HR). The results showed evidence for two dissociable, inversely coupled sub-regions of MPFC that independently mediated HR responses. SET induced activity *increases* in a more dorsal pregenual cingulate region (pgACC) and activity *decreases* in a right ventromedial/medial orbital (vmPFC/mOFC) region. Changes in both regions were significant mediators of HR increases across time. That is, the magnitude of pgACC increases and vmPFC decreases predicted the magnitude of HR increases across time. This work provides some of the first evidence for functional connectivity between brain and heart across time (H. D. Critchley, Melmed, Featherstone, Mathias, & Dolan, 2002), which is important for understanding the brain dynamics that give rise to stress-evoked autonomic activity.

The conclusions we can draw from previous work on brain responses to stress are limited in two ways, however. First, they don't make strong contact with a large animal literature that implicates lower subcortical and brainstem regions in the generation of threat responses (Bandler, Keay, Floyd, & Price, 2000; Barbas, Saha, Rempel-Clower, & Ghashghaei, 2003; Devinsky, Morrell, & Vogt, 1995; Saper, 2002). This research has

focused on two major output systems that regulate peripheral physiological responses: the hypothalamus, which regulates the HPA axis and the endocrine system, and the periaqueductal gray (PAG), which regulates autonomic activity—including cardiac responses—largely through nuclei in the medulla, including the dorsal vagal motor nucleus, parabrachial complex, and rostral ventral medulla. Whereas many animal studies focus on links between the brainstem and the periphery, most human studies have focused on the cortex and subcortical telencephalon (basal forebrain). SET influences on functional connections between the cortex, subcortex, and brainstem have not been examined with human imaging. The amygdala and hippocampus offer points of convergence, but their role in human SET, and their relationship to autonomic and endocrine output, remain unclear. Recently, neuroimaging studies have implicated the human PAG in control of pain (Bingel, Lorenz, Schoell, Weiller, & Buchel, 2006; Derbyshire et al., 2002; Fairhurst, Wiech, Dunckley, & Tracey, 2007; Mohr et al., 2008; Valet et al., 2004; T. D. Wager, Rilling et al., 2004; T.D. Wager, Scott, & Zubieta, 2007; Zambreanu, Wise, Brooks, Iannetti, & Tracey, 2005), imminent threat (Mobbs et al., 2007), and negative emotional experience (Kober et al., 2008; T. Wager et al., 2008), suggesting that threat-related activity in the PAG can be detected using fMRI.

Second, extant data have not examined whether the autonomic and experiential correlates of SET are mediated by similar or distinct systems. Emotions include changes in physiology and experience, and it is clear that responses to SET may include changes in both components of emotional responding. As noted above, changes in autonomic responses have been associated with the dorsal cingulate and mPFC as well as putative visceromotor regions in rdACC/pgACC and vmPFC. Changes in emotion experience also have been associated with activity in medial prefrontal and cingulate regions as well as regions of lateral prefrontal cortex (Kober et al., 2008; T. Wager et al., 2008). It is not yet clear, however, which systems mediate each component of responses to SET.

The multilevel path modeling approach

The current study was designed to address these two issues by identifying cortical-subcortical and cortical-brainstem pathways that mediate the effects of SET on changes in HR and self-reported experience. We used multi-level mediation effect parametric mapping (M-MEPM)—an extension of standard path analysis techniques

(Baron & Kenny, 1986; Hyman, 1955; MacCorquodale & Meehl, 1948) suitable for analyzing path models and mediation effects within-persons—to characterize the dynamic relationships between SET, brain, and physiological and experiential measures of response across time.

Rather than making inferences only on single effects (e.g., brain regions that respond to SET), the analysis provides a way to identify brain regions based on a conjunction (intersection) of three different effects, as diagrammed in Figure 1. For example, an activation-based hypothesis might predict only that SET should activate some regions and deactivate others. By contrast, a mediation hypothesis also would make this prediction, but only as part of a larger set of interconnected predictions about activation pathways. For example, if a SET-brain region-HR pathway exists with a specific brain region as a mediator, three significant statistical relationships should be observed: 1) SET should activate the brain region (Path *a* in Figure 1B); 2) Activity in the brain region should predict HR controlling for SET (Path *b*); and 3) The SET-HR relationship should be significantly reduced when controlling for activity in the brain region, which we refer to as the mediation test or the *a*b* effect.

To test these effects, the M-MEPM analysis extends traditional concepts in path modeling in two ways. First, path models were originally formulated for a single level of analysis—i.e., relationships across time within a single participant. However, we were interested in making inferences about variations in SET, brain, and HR across time (within-person) and their generalizability to a population, which requires a multi-level analysis. This allows us to take advantage of the many repeated observations across time in fMRI, and investigate the dynamic relationships between SET, brain, and HR. Effects (Path *a*, Path *b*, *a*b*) are estimated on within-person changes across time, and their statistical significance is tested across persons, treating participant as a random effect. The multi-level path modeling framework was recently formulated by (Kenny, Korchmaros, & Bolger, 2003). Second, path models and related structural equation models traditionally test relationships among variables (e.g., brain regions) specified *a priori*. However, while the general location of mediating pathways can be specified, exactly which voxels are mediators of SET effects are unknown. Most human neuroimaging studies analyze voxel-by-voxel maps of brain activity, because even if one

has a relatively precise anatomical hypothesis (e.g., the PAG) it is often difficult to specify exactly which voxels in that area should show the effect. The M-MEPM software facilitates performing mediation analysis voxel-by-voxel in a region of interest (ROI) or across the brain, allowing researchers to locate multiple brain regions that satisfy the statistical criteria for mediation.

We focused on the generation of HR responses as an outcome measure because a) they are robust (see Figure 1A)—HR increases are elicited by SET in virtually all individuals; b) they can be measured on a roughly second-by-second basis, providing the ability to analyze effective connectivity among key brain regions and HR across time; and c) cardiac dysfunction is among the most prominent health-related effects of SET (see (T. D. Wager et al., submitted). We focused on self-reported anxiety as an outcome measure because a) it increases robustly with SET (see Figure 1 and (Dickerson et al., 2004); b) it provides a direct correlate of negative emotional experience, which c) has been shown to predict mental and physical health outcomes (Brosschot & van der Doef, 2006; Gross & Munoz, 1995; Moskowitz, 2003; Scheier & Carver, 1992; Tugade & Fredrickson, 2004).

With this in mind, we sought to test several hypotheses about mediators of SET effects on HR based on previous literature. First, we expected a bi-valent response to SET in two separate sub-regions of the medial prefrontal cortex/anterior cingulate. We expected *activation* in pgACC/rdACC to mediate increases in HR, and we expected *deactivation* in vmPFC/mOFC to mediate increases in HR. Second, we expected *activation* in the PAG to mediate increases in HR. And third, we expected PAG to mediate the relationship between cortical activity in one or both sub-regions and HR.

These hypotheses are based on findings from our companion paper (T. D. Wager et al., submitted) and on findings from diverse anatomical and neurophysiological studies. The hypothesis of opposite effects in MPFC sub-regions is based on: a) studies identifying separate cortical sub-regions in the rat, with a dorsal region (prelimbic) that, broadly speaking, generally promotes fear learning and a ventral region (infralimbic) involved in extinguishing it (M. R. Milad & Quirk, 2002; M. R. Milad, Vidal-Gonzalez, & Quirk, 2004; Vertes, 2004; Vidal-Gonzalez, Vidal-Gonzalez, Rauch, & Quirk, 2006); b) opposite results in meta-analyses of valenced emotional states, with more dorsal

regions associated with negative emotional experience and medial orbital regions associated with positive experience (T. Wager et al., 2008); c) opposite responses in human fear learning studies, with increases to conditioned cues in rdACC and decreases in vmPFC /mOFC (M. Milad et al., 2007; Phelps et al., 2004; Schiller et al., 2008); d) SET-induced increases in rdACC and other dorsal regions, and decreases in vmPFC/mOFC (H. D. Critchley et al., 2003; Eisenberger et al., 2007; P. Gianaros, F. M. Van Der Veen, & J. R. Jennings, 2004; Gianaros et al., 2005); and e) associations between vmPFC/mOFC and positive hedonic experience and predicted reward value (Daw, O'Doherty, Dayan, Seymour, & Dolan, 2006; Gottfried, O'Doherty, & Dolan, 2003; Knutson & Cooper, 2005; O'Doherty, Critchley, Deichmann, & Dolan, 2003; O'Doherty, Winston et al., 2003). Based on these diverse sources of evidence, we hypothesized that SET would increase activity in 'visceromotor' areas in the MPFC that appear to be localized to the vicinity of the pgACC, and withdrawal of processes related to positive affective experience and increased vagal tone in the mOFC.

The hypothesis of PAG mediation is based on animal studies linking distinct sub-regions of the MPFC to physiological and behavioral "modes" of responses to threat (Bandler et al., 2000) and recent evidence, reviewed above, that suggests that it is a prominent but under-appreciated feature of threat-related responses in human imaging studies. Alternatively, however, other brainstem pathways that are more difficult to detect with fMRI, such as the parabrachial complex, may be key mediators of cognitive threat responses. Finding that SET acts in humans via prefrontal-PAG pathways would advance our understanding of human threat responses and provide a basis for establishing functional pathway-based correlates of fear and anxiety and markers of treatment outcomes in psychopathology.

Methods

Participants

Eighteen healthy, right-handed, native English speakers from Columbia University (mean age 21 years, 9 males) participated in this experiment. Participants were screened to exclude those with a prior history of neurological or psychiatric illness, current or prior psychoactive medication, or factors that would make participants

unsuitable to participate in a typical fMRI experiment. Participants were asked to abstain from tobacco and caffeine use 24 hours before taking place of the experiment, and to get a regular night of sleep the night before. All participants gave written informed consent, and the Columbia University institutional review board approved the experiment.

Procedure and fMRI task design

Before participants entered the scanner, they were informed that during scanning they would be given two 2-minute periods to mentally prepare two different speeches. These speeches, they were told, should be 7 minutes long when presented to two different audiences after scanning is complete. One speech was to be given before a panel of professors and experts in the law and business, and the second was to be scored by a computer analysis program, Latent Semantic computer Analysis (LSA), that they were told could grade college-level essays. Participants were told that they would learn the topics of presentation once they were inside the scanner and that for control purposes, there was a small chance they would not have to give a speech.

Once in the scanner, all instructions were provided via computer presentation during anatomical scans, baseline and tasks. A digital projector displayed stimuli on a back-projection screen placed in the scanner room. All tasks and instructions were programmed using E-prime software (PST Inc.). Finger photoplethysmography (for HR), respiration, and skin conductance were monitored continuously during scanning. Pilot testing showed that no detectable artifacts were introduced by the recording equipment.

A schematic of the design is shown in Figure 1A. After an initial anatomical scan, baseline physiological and brain data were acquired for 120 seconds. Then, for 15 seconds the first speech topic was presented on the screen and participants then had 2 minutes to mentally prepare it. After 2 minutes, participants viewed on-screen instructions with the topic of the second speech. Again, they were given two minutes to prepare it mentally. After this, every participant was told that they were randomly selected to not give a speech, and asked to relax for the remaining 2 minutes. This period was used to measure recovery. The two speech topics were, “the effects of interest rates on stock prices,” and “the relationship between tariffs and free trade.” These topics were selected based on a pilot study evaluating a separate group of participants’ anticipated

anxiety ratings to a number of possible topics (data not shown). Assignment of both topics and audiences to the first or second speech preparation period were counterbalanced across participants.

Every 20s during baseline, speech preparation, and recovery, participants were visually cued to provided a current subjective anxiety rating on an 8-pt Likert scale. Ratings of zero to eight corresponded to ratings of "no anxiety" through "extremely anxious." Participants made ratings by pressing one of 8 buttons on MR-compatible button units held in both hands, and were trained on the use of the buttons prior to scanning. These ratings were interpolated to the TR (2 sec) using linear interpolation for time series analysis.

Data acquisition and analysis

Heart rate was collected continuously with a sampling rate of 100 Hz during fMRI acquisition using photoplethysmography on the left index finger. Successive R-wave peaks were identified using a custom algorithm identifying deviations from a moving average baseline (TDW) implemented in Matlab (Matworks Inc, Natick, MA, USA). The automatic beat detection algorithm was manually reviewed and corrected by a coder blind to condition. HR time series were reconstructed from the R-R intervals and interpolated to the TR. Skin conductance data were collected, but not analyzed in this report.

MR images were collected on a 1.5 GE Signa Twin Speed Excite HD scanner (GE Medical Systems). Structural images were acquired using high-resolution T1 spoiled gradient recall images (SPGR) for anatomical localization and warping to a standard space. Functional images were acquired with a T2*-sensitive EPI BOLD pulse-sequence (TR = 2000 ms, TE = 40 ms, flip angle = 60°), sensitive to blood-oxygen-level-dependent (BOLD) magnetic susceptibility. For each participant we obtained 266 brain volumes during the scanning run (24 ascending odd/even interleaved slices, 3.4375 × 3.475 × 4.5 mm).

Functional images were subjected to standard preprocessing. First, slice timing acquisition correction and realignment of the functional images to correct for head movement were performed using FSL (FMRIB centre, University of Oxford). Remaining

preprocessing steps were performed using the Statistical Parametric Mapping analysis package (SPM2, Wellcome Department of Cognitive Neurology, London, UK). The realigned images from each participant were coregistered to the anatomical space of the structural image. Structural images were normalized to the Montreal Neurological institute (MNI) template space (avg152t1.img). Finally, the normalized functional images were smoothed with an 8-mm (FWHM) Gaussian smoothing kernel to facilitate inter-subject registration in group analysis.

Following this pre-processing, we used linear regression to remove several sources of nuisance variance from each voxel of each participant's time series data. One set of regressors modeled activity during rating probes and instruction periods, convolved with the canonical SPM hemodynamic response function, and their first and second derivatives. In addition, we identified voxels in the major arteries, which were apparent on the SPGR images, as shown in Figure 2. The first three principal components from the vascular time series (generally from the internal carotid, middle cerebral, and anterior cerebral arteries) were also included as nuisance regressors, along with global signal values and a linear drift regressor. Finally, we included the 6 standard head-movement related estimates from realignment (x, y, and z translation, roll, pitch, and yaw), as well as their derivatives, squares, and squared derivatives (24 movement-related regressors total). This procedure helped to minimize the chances that results would be related to artifactual sources of variance or vascular blood flow effects.

Statistical analysis: Multi-level path analysis. Statistical analysis was performed using the M-MEPM toolbox. Mathematical details of the multi-level model can be found in the companion study (Wager et al., 2009, this volume; submitted). Briefly, the mediation analysis can be conceptualized as a series of analyses testing different components of the mediation hypothesis in each voxel within brain regions of interest. The multi-level path model evaluated at each voxel tests the following effects in the context of a single, multi-equation model: (1) Brain responses to the SET challenge, as evidenced by increases in the [Speech Prep – Baseline] contrast within-participants (Path *a* in Figure 1B). (2) Correlations between brain and HR changes across time, within-participants, controlling for SET (Path *b* in Figure 1B). (3) A mediation test for whether the brain voxel explains a significant amount of the SET-HR covariance. This is

accomplished by testing the product of path coefficients *a*b* using a bootstrap test. (4) After identifying mediating regions, we tested whether activity in each pathway was moderated by SET—that is, whether the brain-HR relationship was significantly stronger during threat than baseline. Whereas a mediation effect can identify functional pathways, a moderation effect can test whether brain-heart effects hold across all task conditions or are specific to the SET period.

Though model used was identical to that in the companion paper (T. D. Wager et al., submitted), there is one difference in the inferential procedure used in the current paper. In this paper, a bootstrap test was used to test the significance of *a*, *b*, and *a*b* effects, which provides a more sensitive test of mediation than the approximate test based on normality assumptions (Shrout & Bolger, 2002). 4000 bootstrap samples were used at each voxel. We also note that the combination of the bootstrap test and the integration of individual differences variables is not yet implemented in the MEPM toolbox, so HR was not entered as an individual differences predictor at the second level. Future versions will allow both bootstrapping and individual differences covariates.

For each whole-brain M-MEPM analysis, we controlled the false positive rate using False Discovery Rate control at $q < .05$ (Genovese, Lazar, & Nichols, 2002), across images related to all effects of in the path model (*a*, *b*, and *a*b* effects). The interpretation is thus that the expected false positive rate is 5% of 'significant' voxels across the whole analysis.

Moderation analysis: variations in brain-HR connectivity across task states

This analysis is conceptually similar to testing whether the brain-HR connectivity was stronger during Speech Preparation than during Baseline and Recovery periods. Though simpler analyses on individual brain-HR correlations during different task phases yielded the same results in all ROIs (data not shown), the multi-level model is statistically preferable because it includes weights based on within-subjects variance, and we describe it below.

A [Speech Preparation – Baseline] x brain activity interaction regressor was created in the following way. First, brain activity was averaged over voxels in the region of interest separately for each participant, yielding a set of 18 region-average time series. Second, a matrix of nuisance covariates (Q) was removed from the brain activity, in order

to prevent differences across task periods to drive the interaction results. Q included indicator (dummy-coded) vectors coding for each of the task periods relative to the initial baseline (including Speech Preparation, Relief instructions, and Recovery periods). Additional movement-related and vascular covariates were removed prior to analysis as well. Third, the brain time series for each participant was multiplied with the contrast-coded [Speech Preparation – Baseline] time series for each participant to create series of 18 interaction regressors for each participant. Fourth, and finally, a 60 s tukey window was applied to each interaction regressor, to ensure that transitions between task periods did not drive the interaction results.

Once these interactions were created, they were entered into a multi-level mixed effects GLM. The HR time series for the 18 participants (expressed as change from the individual's baseline HR) was the outcome variable to be predicted.

Limitations on causal inference

Before discussing the results of these analyses, it is important to consider which functional connections can reliably be given a causal interpretation and which cannot. In the present study, SET was experimentally manipulated in an off-on-off design, un-confounding it with processes like fatigue that change monotonically over time. SET-induced brain activity can thus be reasonably interpreted as caused by SET. Brain relationships with HR could plausibly be either causes of HR change, effects of HR as perceived by the brain, or both; however, the locations of active regions in "visceromotor" cortex and known brainstem modulators of HR favor brain-to-heart causality. For these reasons, although the path models we use are directional in form, we do not interpret the directionality of brain-brain connections.

Results

Physiological and subjective effects of SET

Compared with pre- and post-stress baselines, speech preparation induced reliable increases in HR (6.22 beats per minute, BPM, $t = 7.68$, $p < .0001$) and subjective anxiety (1.39 units on a 5-point scale, $t = 3.83$, $p = 0.002$), as shown in Figure 1A. HR increases were positively correlated with Spielberger Trait Anxiety scores (STAI, $r = 0.64$, $p <$

.05), but anxiety increases were not (r = 0.25, n.s.) The first speech preparation period induced larger increases in HR than the second period (3.56 BPM, t = 3.34, p = .005), though HR increases were significant for the second preparation period alone (3.80 BPM, t = 4.88, p = .0003). Anxiety ratings did not vary between the two speeches (-.046 points, n.s.). After the second preparation period and instructions indicating that no speech would be delivered, participants HR returned to baseline. There was no difference between initial-baseline and post-stress baseline values (-1.31 BPM, n.s.). Anxiety ratings showed some evidence for a drop during the post-stress baseline below their initial values (-0.70 points, t = -2.00, p = .066). Within participants, HR changes were positively associated with anxiety changes over time (b = 2.61 BPM/unit anxiety, t = 3.11, p = .007), indicating correlated time courses likely induced by the speech preparation. Individual differences in HR reactivity and anxiety reactivity to the task, however, were uncorrelated (r = 0.07, n.s.).

Physiological analyses reported above controlled for gender, STAI, target audience (Panel vs. Computer scoring), and speech topic ("interest rates" vs. "tariffs"; see Methods), leaving 13 error degrees of freedom for all analyses. None of these variables[1] had significant effects on HR, anxiety reports, or activity in key brain regions (all p's > .1); thus, we do not report on them in detail here.

fMRI results

Our analysis proceeded in the following stages:

(1) First, we searched for brain mediators of the relationship between SET and HR within participants (across time), as diagrammed in Figure 1B, using the M-MEPM toolbox. (2) Second, we further characterized key brain regions from this analysis by testing whether brain-HR correlations varied across task states (Baseline, Speech Prep, and Recovery). That is, we tested whether task state moderated the brain-HR relationship. (3) We performed the MEPM analysis on the PAG, a key brainstem region of interest, to localize

[1] The one exception was that the group assigned to prepare the first speech for computer analysis scoring showed larger HR responses during preparation of speeches for both Computer and Panel audiences, but this effect is not likely to bear on the within-participant analyses we report here. HR and anxiety responses were not significantly different for speeches delivered to Computer and Panel audiences, controlling for task order.

PAG voxels that mediated the SET-HR relationship. (4) We performed a series of path analyses to test for hypothesized relationships between SET, key frontal and brainstem regions, and HR. These analyses differed from the MEPM analysis in that they considered multiple brain regions in the same path models, and could thus test for independent effects of multiple regions. This set of analyses also included a post hoc search for additional brainstem mediators of cortical activity not mediated by the PAG. (5) We performed an MEPM analysis to search for brain mediators of the SET-reported anxiety relationship within participants. In the following, we present results from each of these analyses.

1. Mediators of SET effects on HR

Our first analysis searched for mediators of the SET-HR relationship, including regions identifying SET effects on fMRI activity (Path *a*), fMRI correlates of HR across time (Path *b*), and the *a*b* mediation effect. We performed a whole-brain search at $q < 0.05$ (False Discovery Rate [FDR] corrected, corresponding to $P < 0.0012$)
) with 3 contiguous voxels in each effect. Regions that showed evidence for all three effects are of primary interest, as they show the strongest evidence for mediation. In particular, we sought to test whether the pattern found in our previous study (Wager et al., submitted) of more dorsal MPFC (pgACC and rdACC) increases and mOFC/VMPFC decreases would mediate the Speech Preparation-HR relationship.

The brain regions that responded to the [Speech Preparation – Baseline] comparison (Path a) are shown in the left panel of Figure 3, and a complete listing with statistics is reported in Table 1. As in the companion study, a number of regions responded to the SET challenge, including increases in rdACC and decreases in vmpFC/mOFC, but also including activity in the lateral PFC, insula, medial temporal lobes, occipital cortex, medial cerebellum, and other regions likely to be related to various task demands (such as visual stimulation during instruction presentation, etc.).

Brain correlates of HR controlling for SET are shown in the right panels of Figure 2 and listed in Table 2. These included the rdACC and a large area of the dorsal anterior and posterior cingulate cortices, as well as the PAG, mediodorsal thalamus, caudate,

lateral frontal cortex, and medial cerebellum. Negative associations with HR were found in the vmPFC/mOFC.

Rather than discussing these regions in detail, we focus on those with consistent relationships with both SET and HR, as well as evidence for a significant mediation effect. Only two areas of the brain showed evidence for the conjunction of both SET (Path *a*) and HR (Path *b*) effects and the *a*b* mediation effect. As shown in Figure 3C, areas with at least 3 voxels at $P < .001$ in each of the three tests included the rdACC and vmPFC/mOFC. Individual participant slope plots are shown in Figure 3D for these two regions, and statistical details are presented in Table 3. Both areas showed positive mediation effects, which is consistent with the directions of the *a* and *b* paths reported above. In the rdACC, SET caused *increased* brain activity, which was associated with increased HR. In the vmPFC, SET caused *decreases* in brain activity, and greater *decreases* were associated with increased HR. Thus, because both Path *a* and *b* links were negative in the vmPFC, the *a*b* product that reflects the mediation test was positive.

Figure 4A shows the time courses of activity in the rdACC (red) and vmPFC (blue). The rdACC shows evidence for positive responses during both speech preparation periods, with a break during the instruction period for the second speech preparation. The vmPFC shows prominent decreases that are driven primarily by the first speech preparation period. HR was markedly higher during the first speech period and habituated to some degree, though reported anxiety did not (Figure 1).

2. Moderation analysis: changes in brain-HR correlations across time

Figure 4B shows the results of moderation analyses that tested the strength of brain-HR correlations across different phases of the task (pre-preparation baseline, preparation, and post-preparation recovery). Statistical details are presented in Table 4. Our hypotheses were directional, so one-tailed tests were performed. We expected stronger positive connectivity in rdACC, and stronger negative connectivity in vmPFC, during speech preparation.

Correlations between the rdACC and heart rate (Figure 3B, left panel) varied as a function of task phase, with the strongest correlations during speech preparation. This result indicates that the rdACC-HR pathway was significantly engaged by SET. By contrast, correlations between the vmPFC and heart rate (right panel) showed a non-

significant trend across task periods, but appeared to be strongest before and during preparation. Overall, the results suggest that the balance of (negative) vmPFC contributions and (positive) rdACC contributions to HR changes across time, with vmPFC effects more pronounced earlier and rdACC effects dominating later.

3. PAG region of interest analysis and inter-region connectivity

To test for predicted mediation in the PAG, we conducted an ROI analysis specifically on a pre-defined area surrounding the PAG at a reduced statistical threshold ($P < .05$ in each of the three critical tests). Figure 4A shows the extent and location of the PAG ROI (left panels). We found evidence for significant relationships in PAG voxels in all 3 effects. As shown in the right panels of Figure 4A, PAG increased to SET (Path *a*), predicted HR controlling for SET (Path *b*), and showed evidence for mediation (*a*b*). Statistics for the region showing the overlap of all three effects are presented in Table 3. Notably, the PAG-HR connectivity did not vary substantially across task periods (Table 4, Figure 4).

4. Path models of cortical-brainstem-HR connectivity

We focused our brain effective connectivity hypotheses on three kinds of pathways concerning cortical-subcortical connectivity hypothesized a priori[2]:

1. Whether VMPFC and rdACC were independent mediators of SET effects on HR
2. Whether PAG is a mediator of the relationship between rdACC and/or vmPFC and HR
3. Whether cortical regions mediated SET effects on PAG

To test hypothesis 1, we constructed a path model with SET as the initial variable, both vmPFC and rdACC as mediators, and HR as the outcome. The results showed that each was an independent mediator. vmPFC showed negative path coefficients, indicating

[2] We believe that structural models assessing 'direct' connectivity among each of these key regions would depend heavily on the assumptions of the linear model. Because there would be many variables in such a model, and inferences about direct connectivity depends on assuming an absence of nonlinear effects and higher-order interactions, the results of such a model are less likely to be stable across studies. Thus, we restricted ourselves to a small set of *a priori* tests.

decreases mediated increases in HR (Z_a = -3.72, Z_b = -3.52, Z_{ab} =3.70, all P < .001), whereas rdACC paths were positive (Z_a = -3.48, Z_b = -3.62, Z_{ab} =3.42, all P < .001). Thus, although rdACC and vmPFC were significantly negatively coupled (b = -.05, t(17) = -2.28, p < .05), they independently mediated some of the SET-HR covariance.

To test hypothesis 2, we constructed a path model with each of rdACC and vmPFC as initial variables, PAG as a mediator, and HR as an outcome, controlling for SET. PAG mediated the vmPFC-HR connection (Z_a = -3.59, Z_b = 2.48, Z_{ab} =-2.38, all P < .02). However, rdACC did not, primarily because it was not associated with PAG (Z_a = 0.96, n.s., Z_b = 2.58, P < .01, Z_{ab} =0.32, n.s.).

To test hypothesis 3, we constructed a path model with SET as the initial variable, each of rdACC and vmPFC as mediators, and PAG as an outcome. vmPFC showed strong connectivity with both SET and PAG, and a trend towards significant mediation (Z_a = -3.41, P<.001, Z_b = -3.54, P < .001, Z_{ab} =1.79, P = .07). It was also complete mediator of SET effects on PAG (direct SET-PAG $Z_{c'}$ = 0.37, n.s.). rdACC, by contrast, did not mediate SET effects on PAG. Though it showed a strong response to SET, as in other models, it was not connected with PAG (Z_a = 3.49, P<.001, Z_b = 0.84, n.s., Z_{ab} =1.04, n.s.).

Thus, a consistent picture from these three models is that the vmPFC is most strongly connected to PAG and has an inhibitory effect, whereas rdACC is not strongly connected with PAG. These relationships are summarized graphically in Figure 6.

This result begs the question of which brainstem regions might mediate rdACC relationships with HR, as brainstem nuclei are known to be proximal mediators from animal studies. We performed an additional *post hoc* MEPM analysis within a mask including the brainstem and thalamus. In this analysis, rdACC was the initial variable, HR was the outcome, and we searched for brain mediators of the rdACC-HR relationship. The FDR-corrected threshold across the search space and contrasts was P < .0075. Only two regions showed significant effects in all three path coefficients (Path *a*, rdACC-brain; Path *b*, brain-HR; and the *a*b* mediation test). These regions were in the left and right ventral thalamus. For the left thalamus, MNI coordinates were [x, y, z] = [-14, -17, 4], 3031 mm^3 (57 contiguous voxels). For the right thalamus, [x, y, z] = [17, -17, 4], 638 mm^3 (12 voxels). These regions are shown in Figure 6. The thalamic regions

were strongly coupled with both rdACC and PAG controlling for other key regions ($t(17) = 6.34$, and $t(17) = 11.16$, respectively, both $P < .001$). It did not show significant effective connectivity with vmPFC, however ($t(17) = -1.66$, n.s.).

5. Brain mediators of subjective anxiety

As Figure 1 shows, the temporal profiles of HR and anxiety responses were correlated, but they also differed substantially. Therefore, to test whether the same or different brain areas would be associated with anxiety reports, we conducted an additional M-MEPM analysis to examine the brain areas that mediated subjective anxiety reports across time.

The results from this analysis were not as strong as for the HR analysis: no regions were significant with FDR correction. No results were significant in all three effects (Path a, SET-brain, Path b, brain-anxiety, a*b) at $P < .001$ or even at $P < .005$, indicating less strong and straightforward relationships linking SET, brain activity, and reported anxiety (as compared with HR).

To provide an exploratory analysis of mediators of anxiety, we focused on the *a*b* effect alone, which can, in a multi-level context, reveal evidence for functional pathways that vary across individual as well as those that are consistent in the group. The results are listed in Table 5, and shown in Figure 7. A number of regions showed evidence for significant a*b effects at $P < .001$, including effects in dorsolateral prefrontal cortex and right IFG just anterior to Broca's area, vmPFC/mOFC, temporal poles, caudate head, and the DMPFC superior to rdACC and anterior to pre-SMA. Notably, they overlapped only partially with predictors of HR, most notably in the vmPFC.

Though these results are candidate mediators of reported anxiety responses to social threat, the interpretation of these mediation effects requires some further explanation. The group-average *a* and *b* paths were significant for very few of these regions (see Table 5), indicating a lack of consistent relationships with SET and anxiety across participants. A significant *a*b* effect without significant *a* and *b* effects is only possible in a multi-level mediation context (Kenny et al., 2003), and it indicates that there may be coherent relationships that vary in direction across individuals. For example, consider that some individuals may show brain increases to the SET challenge, and others

may show decreases. If activity predicts increased anxiety (positive Path *b*) for the participants who show increases (positive Path *a*), and stronger decreases predict anxiety (negative Path *b*) for the participants who show decreases (negative Path *a*), then the net result will be consistent mediation, but in different directions (positive vs. negative) for different individuals. This consistency is reflected in the covariance of *a* and *b* paths, which were significantly positive for most regions showing significant mediation effects (r values ranged from .27 to .73; see Table 5). Under what circumstances might this occur? One is if the brain regions have a variable role in shaping anxiety reports—i.e., if they are involved in an appraisal process that increases anxiety for some participants but decreases it for others. We return to this issue in the discussion.

To help interpret the functional role of brain activity in these regions in shaping anxiety, we examined correlations between path coefficients and the strength of overall reported anxiety reactions during speech preparation (as compared with baseline and recovery periods). Few regions showed correlations with anxiety reactivity, but those that did provide some clues. In the IFG, for example, high reported anxiety was associated with positive IFG-anxiety correlations across time. Low reported anxiety was associated with negative IFG-anxiety correlations. The vmPFC showed the opposite pattern, suggesting that as in the HR prediction analyses, decreases in vmPFC are coupled with high anxiety in "reactors." In high reactors, vmPFC decreases were associated with anxiogenic responses.

Discussion

Threats to social status and negative evaluation by other individuals are among the most potent laboratory and real-life stressors in contemporary society. They are particularly relevant for health in modern industrialized countries, in which acute physical threat is relatively rare, but many individuals experience stresses related to social well-being and status on a regular basis. Work in non-human animals has elucidated several kinds of deleterious physiological effects of threat and stress, including social status threats in particular (Blascovich, Mendes, Tomaka, Salomon, & Seery, 2003; Cohen et al., 2000; Kemeny, 2003; Mcewen, 2007; Thayer & Sternberg, 2006), and

important work has been devoted to the brain systems that generate and mediate threat (H. Critchley, 2003; Eisenberger et al., 2007; Gianaros et al., 2007; Kern et al., 2008).

Human neuroimaging research can provide important information on the brain systems that generate (and perceive) physiological responses to social threat. First, while social threats in humans are likely to share many common features with primate social stresses (Cohen et al., 1997; Williams, Vita, Manuck, Selwyn, & Kaplan, 1991), human social status threats are likely to involve both psychological processes and brain systems that are not directly comparable with primate models. In particular, threat appraisals are likely to arise from a complex analysis of inter-personal relationships that go beyond primate social cognition. They are also likely to involve processes that go beyond processing of the presence of any particular simple sensory cue, as in fear conditioning. In this respect, human imaging work can help provide points of convergence with non-human animal models on the brain correlates of social threat.

Second, neuroimaging research on brain-physiological relationships can help to integrate human and non-human research that has focused largely on different levels of the neuraxis. Much animal work has been devoted to the study of brainstem and diencephalic (e.g., hypothalamic) mechanisms that generate physiological responses (Bandler et al., 2000; Korte, Koolhaas, Wingfield, & McEwen, 2005), and specific roles for brainstem nuclei such as the periaqueductral gray (PAG), parabrachial complex, solitary nucleus, and dorsal vagal motor nucleus have been well described (Bandler & Shipley, 1994; Behbehani, 1995; Janig & Habler, 2000; Keay & Bandler, 2001; Saper, 2002). By contrast, human research has focused largely on the cortex, basal ganglia, and amygdala. We focused on the PAG because it is both heavily implicated in central control of lower brainstem autonomic effectors under threat (Bandler et al., 2000; Price, 2005; Verberne & Owens, 1998), and its activity is likely to be more detectable in fMRI than lower brainstem nuclei. PAG appears to be reliably activated in human imaging studies of pain and threat (Bingel et al., 2006; Derbyshire et al., 2002; Fairhurst et al., 2007; Mobbs et al., 2007; Mohr et al., 2008; Valet et al., 2004; T. D. Wager, Rilling et al., 2004; T.D. Wager et al., 2007); in a meta-analysis of 163 studies, we found that PAG was consistently activated in studies involving negative emotional experiences in particular (T. Wager et al., 2008), and was co-activated across studies with the MPFC

(Kober et al., 2008), as might be predicted from the prominent role of OFC- and MPFC-PAG relationships in threat responses.

HR responses to speech preparation as a probe for studying social threat

In this paper and its companion (T. D. Wager et al., submitted), we studied social evaluative threat (SET)—a particular kind of social status threat related to negative evaluation. SET is a cognitively mediated process that depends on social relationships, and so is a good model for the generation of threat responses that pertain to stresses faced by much of society. It is also a particularly potent elicitor of physiological responses. Laboratory SET manipulations trigger reliable patterns of autonomic, neuroendocrine, and immune reactivity (Al'Absi et al., 1997; Benschop et al., 1998; Brosschot et al., 1992; Cohen et al., 2000; Dickerson & Kemeny, 2004; Glaser & Kiecolt-Glaser, 2005; Rozanski et al., 1988; Sgoutas-Emch et al., 1994; Tomaka, Blascovich, Kibler, & Ernst, 1997; Uchino, Cacioppo, Malarkey, & Glaser, 1995). It may be no accident that social processes are particularly interconnected with autonomic output, as some theories have posited that the brain systems that underlie the human capacity for social and emotional interactions developed partly from mechanisms for autonomic control (Porges, 2003). SET is thus likely to engage the human PAG, brainstem autonomic effectors, and neuroendocrine control centers such as the hypothalamus. In this study, we did not observe strong enough evidence for activity in the hypothalamus to meet our statistical criteria, but that does not imply that it and other regions are not involved. Advances in image acquisition and analysis techniques may elucidate the roles of other structures.

We focused on measuring brain correlates of autonomic reactivity as measured by heart rate (HR) responses to a social threat challenge, the silent preparation of a public speech to be given before a panel of expert evaluators. Though it does not provide specific information about sympathetic vs. parasympathetic effects, HR is a reliable integrated index of autonomic responses to this particular type of challenge that is correlated with acute cortisol and cellular immune effects (reviewed above). Unlike cortisol, however, HR can be measured robustly in the MRI environment on a second-by-second basis. Thus, the combination of a SET manipulation with HR time series measurements as a physiological outcome is a good model for studying the cortical and

subcortical brain networks associated with peripheral physiological changes. Our main findings can be summarized briefly as follows.

A dual-process model of HR control: Mediation by reciprocal MPFC subregions

A major cortical correlate of SET, and predictor of HR changes, was the medial frontal cortex. In particular, a bi-valent response that included activity increases in rdACC and decreases in vmPFC/mOFC was linked with increased HR during speech preparation. This pattern replicates findings in the companion study (T. D. Wager et al., submitted) and suggests that different regions of the MPFC have qualitatively different (and perhaps opposite) roles in generating and regulating autonomic responses to SET. In both studies, activity in the more dorsal mid-rostral/pregenual cingulate region (rdACC) and vmPFC/mOFC were negatively correlated, but each was also an independent predictor of HR increases during SET. Such partial independence is in line with theories of sympathetic/parasympathetic interactions, which posit reciprocally interactive but independent systems for cardiac chronotropic control (Berntson, Quigley, & Lozano, 2007; Thayer & Brosschot, 2005). The basic idea is that SET elicits increased sympathetic output and decreased parasympathetic output to the heart, which may be preferentially associated with rdACC and vmPFC, respectively (H. D. Critchley et al., 2003; P. Gianaros et al., 2004; Lane et al., in press).

We believe that the more dorsal of the two MPFC regions we have identified across studies is isomorphic with either the anterior portion of the anterior mid-cingulate cortex or the pre-genual cingulate cortex (pgACC) as identified by Vogt (Palomero-Gallagher, Vogt, Schleicher, Mayberg, & Zilles, 2008; Vogt, 2005). The former area overlaps closely with what we have termed the rdACC. The findings in the companion paper (T. D. Wager et al., submitted) were somewhat more ventral, centered on the pgACC, but this may be due more to differences in the image acquisition techniques than differences in the localization of brain activity. Anatomical localization in the ventral part of the MPFC is non-trivial; it is well-known that magnetic field inhomogeneity caused by air sinuses causes MR signal loss and spatial distortion in the vmPFC in particular (Du, Dalwani, Wylie, Claus, & Tregellas, 2007; Glover & Law, 2001). For this reason, we considered it important to replicate the SET effects we report here in two separate

samples, using two different kinds of pulse sequences. In the companion paper, we used spiral in-out imaging (Glover & Law, 2001), which shows less spatial distortion but more spatial blurring than the EPI sequence used in the present paper. It is possible that the results in the present study, and other previous studies that have used predominantly EPI, appear more dorsal than the actual location of neural activity due to EPI distortion, and thus pgACC is a better estimate of the positive cortical generator of HR. However, high-resolution imaging studies with low image distortion, and converging evidence from other imaging modalities, are needed to be more certain.

The complexities of localization nothwithstanding, these results can help to localize the principal correlates of cardiovascular responses within the MPFC. Precise localization is required if relationships with animal models are to be established and if the regions are to be developed as prospective measures or diagnostic criteria. We note that correlations between dorsal MPFC and cardiovascular reactivity (HR or blood pressure) in several recent studies (Critchley et al. 2004; Critchley, 2005 (P. Gianaros et al., 2004; Gianaros et al., 2008) have been localized primarily to the pre-SMA (Brodmann's Area 6), an area broadly associated with behavioral 'energization' (Stuss & Alexander, 2007) that accompanies diverse forms of working memory and attentional demand (van Snellenberg & Wager, in press; T. D. Wager, Reading, & Jonides, 2004; T. D. Wager & Smith, 2003). Pre-SMA cardiac correlations are not specific to evaluate threat, as pre-SMA correlates with cardiac responses to exercise (H. D. Critchley, Corfield, Chandler, Mathias, & Dolan, 2000; H. D. Critchley, Tang, Glaser, Butterworth, & Dolan, 2005; Wong, Masse, Kimmerly, Menon, & Shoemaker, 2007). Thus, it is the more ventral aspect of the cingulate—the pre-genual cingulate (pgACC, Areas 25/32) and ventro-medial prefrontal cortex/medial orbitofrontal cortex (VMPFC/mOFC, Areas 13, 14, and 11m), in which the mediators in the present study are located, that may be most critical in SET. Unlike pre-SMA, these areas are homologues to the "visceromotor" cortex in monkeys (Devinsky et al., 1995; Öngür, Ferry, & Price, 2003).

It is also the more ventral MPFC regions that are most closely linked in animal models to the PAG, though dorsomedial connections exist as well (Bandler et al., 2000). Specifically, Dorsal MPFC anterior to pre-SMA (Areas 9 and 24b) project to the lateral PAG and dorsal hypothalamus; VMPFC (Areas 25, 32, and medial 10) projects to the

dorsolateral PAG and medial hypothalamus; and medial and lateral OFC project to the ventrolateral PAG and lateral hypothalamus. The pre-SMA (Area 6) does not project to PAG. It is worth noting here that meta-analyses of human neuroimaging studies have shown co-activation between the PAG and the pgACC (Area 24b/32) and dorsal MPFC (Area 9), but in a region clearly anterior to the pre-SMA (Kober et al., 2008)). Thus, in sum, pre-SMA activity is more likely to be associated with behavioral energization of the skeletomotor system, whereas more rostral and ventral MPFC sub-regions are likely drivers of psychosocially mediated visceromotor responses.

Subcortical mediators: Striatum and PAG but not amygdala

In this study, we did not attempt a systematic characterization of all subcortical regions of interest that may partially mediate physiological responses to social threat. We focused specifically on the PAG due to its role in coordinating autonomic and behavioral responses. However, two points on subcortical "limbic" mediators are worth noting. First, de-activation in the right putamen was the only subcortical mediator of HR increases in both studies that passed our stringent correction for multiple comparisons. This activity was located in the mid-dorsal region in the companion study, but in the ventral striatum proper in this study.

Secondly, notably absent in both studies was the amygdala, which was de-activated (not activated) during speech preparation in both studies. These decreases were not strongly enough so to meet the threshold for whole-brain corrected significance in either study, but are apparent in region of interest analyses in both studies. This is notable because the amygdala has been the focus of studies investigating affective responses across a wide variety of paradigms, and it has been linked to human and animal fear conditioning (Phelps et al., 2004), anxiety disorders (Etkin & Wager, 2007; Nitschke et al., 2009), and negative emotional experience reported to photographs (K. N. Ochsner & Gross, 2008; K. Phan, 2004; T. D. Wager, Hughes, Davidson, Lindquist, & Ochsner, 2008). However, meta-analyses have found that the stimuli that most reliably elicit amygdala activation in studies of human emotion is viewing faces of other individuals showing fearful expressions (T. Wager et al., 2008). While the amygdala is clearly involved in simple cued fear conditioning (LeDoux, 2000), it does not appear to be

involved in more prolonged anxiety-like context-driven fear states or responses to unconditioned fear cues such as predator odors (Davis, 1998; Wallace & Rosen, 2001a). This and other evidence has led to the idea that a major role for the amgydala is in the representation of the salience of or behavioral 'arousal' to sensory cues that signal ambiguity or potential threat (Ewbank, Barnard, Croucher, Ramponi, & Calder, 2009; K. L. Phan et al., 2003; Whalen et al., 1998) (Amaral, 2003; Anderson et al., 2003; Anderson & Phelps, 2002; T. Wager et al., 2008), rather than mediating the 'core experience' of fear or anxiety. In this context, our results are notable in that they support a distinction between brain systems that respond to social threat, which elicits relatively strong subjective anxiety, and those that mediate the learning and expression of conditioned 'fear responses.'

In addition to replicating the pattern of cortical and subcortical results in the companion study (T. D. Wager et al., submitted), we extended the results in several ways. The most important was that we specifically focused on the PAG, and found evidence that it, too, was a mediator of the relationship between SET and HR increases. Subsequent confirmatory path models suggested that the MPFC-HR connection in the more ventral vmPFC/mOFC region in particular was mediated by the PAG. The rdACC-HR connection was not mediated by PAG, but was mediated by thalamic activity that was itself connected with PAG. These findings confirm the role of the PAG in human socially generated threat, and provide some preliminary steps towards building a model of cortical-brainstem-autonomic pathways in humans. Based on these findings, it appears that such a model is likely to involve at least two different and opposed systems in the MPFC, a third contribution from the basal ganglia (putamen), and separable mediators at the brainstem/diencephalic level. These results help to establish the locations and functional roles of cortical-brainstem pathways mediating social evaluative threat in humans, and pave the way for examining both more fine-grained patterns of individual differences in response to threat (Blascovich et al., 2003; Tugade & Fredrickson, 2004) and psychosocial interventions and manipulations that affect threat responses (al'Absi, Bongard, & Lovallo, 2000; Eisenberger et al., 2007; Fredrickson, Mancuso, Branigan, & Tugade, 2000).

Overlapping, but distinct, mediators of anxiety reports

This pattern of results diverged substantially from the brain mediators of reported anxiety. Anxiety reports measured across time were positively correlated with HR responses across time (both increased substantially during speech preparation and returned to or below baseline levels afterwards). Candidate brain mediators of anxiety included the vmPFC, in approximately the same location as the region mediating HR responses. However, other mediators of anxiety were divergent from the mediators of HR. One mediator of anxiety was the most dorsal part of the dorsomedial prefrontal cortex (dmPFC). This region has been linked to mentalizing about others and their knowledge and intentions (Mitchell, Macrae, & Banaji, 2004; K. Ochsner & Gross, 2005; Rilling, Dagenais, Goldsmith, Glenn, & Pagnoni, 2008), anxiety-generating cognitive appraisals (Kalisch, Wiech, Critchley, & Dolan, 2006), and negative emotional experience more generally in meta-analyses (T. Wager et al., 2008). Other mediators of anxiety included the left IFG, dorsolateral PFC, and temporal poles. Lateral PFC activity has been linked to both the generation and regulation of negative emotional appaisals (Bishop, Duncan, Brett, & Lawrence, 2004; T. D. Wager et al., 2008), which might suggest a variable role in mediating anxiety responses depending on the contents (anxiogenic or anxiolytic) of task or goal representations maintained in the PFC.

Indeed, in our study, the mediation effect in these regions showed evidence for variability across individuals. Only those who reported strong increases in anxiety, for example, showed the pathway evident in the mediation of HR: a pathway linking SET, vmPFC decreases, and increased anxiety. In addition, only anxiety 'reactors' showed evidence for a positive SET-IFG-anxiety pathway. While it is difficult to interpret these effects post hoc with certainty, there is a precedent for believing that anxiety reports do not mean the same thing for all participants. "Repressors" experience anxiety but do not report it (Weinberger, Schwartz, & Davidson, 1979). One explanation for the variable mediation results in vmPFC, for example, is that some individuals are "repressors" for whom vmPFC deactivation does not predict anxiety because they do not accurately report the anxiety they feel. This explanation is consistent with the negative anxiety reactivity-Path b correlation shown in Figure 7.

Strengthening of cortical, but not PAG, connectivity with HR during SET

An additional extension in this paper was to test whether brain-HR correlations in the vmPFC, rdACC, and PAG were stronger during (moderated by) speech preparation itself than during pre-stress baseline or post-stress recovery. We found evidence that speech prepration strengthened connectivity in both cortical areas, but that PAG was coupled with HR during all task states. The implication is that PAG signal is more closely coupled with HR irrespective of cognitive processes, whereas the coupling between cortical regions and HR is driven by conceptual processing during SET and/or anticipatory anxiety (in the case of the vmPFC).

Whereas mediation implies that SET produces changes in brain activity, which in turn drives HR, moderation is a test of the SET *x* brain interaction on HR. A form of such a moderation test is implemented in popular SPM software as a "psychophysiological interaction" analysis, and is commonly interpreted as evidence for a task-specific functional pathway. However, there are several possible interpretations. The most straightforward one is that the regions are mediators, and that with low SET, restricted range keeps them from correlating with HR as strongly as they otherwise would—thus, the relationship is stronger during threat. Nonlinearity in the SET-brain and brain-HR relationships could also create an interaction. Alternatively, a second brain region activated by SET could be a common cause of both brain and HR increases. Finally, the functional role of the region could be changing during SET, by virtue of its participation in another functional network, or overlapping signals related to other brain processes either added or removed during SET. While these alternatives are impossible to disentangle without converging evidence, we note that stronger brain-HR connectivity during SET is consistent with these regions' role as mediators.

Conclusions

In sum, this paper and its companion report several findings that may assist the integration of human and non-human approaches to studying brain-body communication and its effects on health and health-related physiological processes. First, they demonstrate that a single-epoch social threat challenge can be meaningfully studied using BOLD fMRI. Second, they demonstrate that the multi-level path modeling approach can be used to both constrain inferences on how brain responses to SET are interpreted and to

establish relationships between experimental manipulations, brain activity, and peripheral physiology. Third, they establish a bi-valent pattern of cortical and subcortical changes that mediate HR increases during SET, including activity increases in the pgACC/rdACC and PAG, and de-activation in the vmPFC/mOFC and putamen. The papers also establish the localization of vmPFC-PAG-HR and rdACC-thalamus-HR that are likely locations for cortical-brainstem pathways that translate mental appraisals into adaptive physiological responses. These responses are likely to result in allostatic load on the body (Mcewen, 2007), and the heart in particular (Jiang et al., 1996; Rozanski et al., 1988).

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Table 1.

Path a effects (responses to SET)

Anatomical Group	Name	Coordinates x	y	z	Cluster size voxel	Vol. (mm3)	Path a (SET) Z	P
Positive (activations)								
Medial frontal	rdACC/Pre-SMA	-3	21	50	65	3456	3.58	0.0003
	vFP	-3	65	-18	39	2074	3.58	0.0003
	FP	-14	72	14	4	213	3.38	0.0007
Lateral frontal	L MFG	-34	28	32	9	479	3.48	0.0005
	L MFG	-45	14	50	24	1276	3.58	0.0003
Insula	R AINS	28	28	4	13	691	3.58	0.0003
Medial temporal	R TP	31	28	-40	10	532	3.58	0.0003
	R Hipp	34	-21	-18	83	4413	3.58	0.0003
	L Hipp	-31	-28	-14	24	1276	3.52	0.0004
Basal ganglia	R Cau	17	10	18	128	6806	3.58	0.0003
	L Cau	-17	-17	18	194	10316	3.58	0.0003
Parietal cortex	R Par. Operc.	34	-34	22	4	213	3.33	0.0009
	IPL	-21	-89	45	10	532	3.52	0.0004
Cerebellum	L CB	-48	-62	-36	25	1329	3.58	0.0003
	CB/Occ	3	-72	-4	1727	91831	3.58	0.0003
	R CB	52	-76	-36	5	266	3.56	0.0004
Negative (deactivations)								
Medial frontal	VMPFC/mOFC	0	31	-18	104	5530	-3.58	0.0003
	pACC	-7	3	40	6	319	-3.40	0.0007
Lateral frontal	R SFS	28	14	54	18	957	-3.58	0.0003
	R VLPFC	45	48	-9	67	3563	-3.58	0.0003
Insula/basal ganglia	R vPutamen/vIns	38	-3	-14	19	1010	-3.57	0.0004
Posterior temporal	R ITC	69	-24	-14	7	372	-3.43	0.0006
	R ITC	58	-58	-9	8	425	-3.43	0.0006
	L STG	-58	0	0	8	425	-3.45	0.0006
Parietal cortex	L Operc/IPL	-58	-31	32	176	9359	-3.58	0.0003
	R SPL	52	-41	50	144	7657	-3.58	0.0003
Posterior cingulate	PCC	0	-21	27	13	691	-3.56	0.0004
Cerebellum	R CB	48	-45	-32	13	691	-3.36	0.0008
Sensorimotor	SMC	-7	-45	76	6	319	-3.52	0.0004

Note**.** Brain activation and deactivation to social evaluative threat (SET). Z and P values represent maximum and minimum values over the contiguous suprathreshold region, respectively. Abbreviations:AINS, anterior insula; Cau, caudate; CB, cerebellum; FP, frontal pole; middle frontal gyrus; Hipp, hippocampus; IPL, inferior parietal lobule; ITC, inferior temporal cortex; MFG, middle frontal gyrus; mOFC, medial orbitofrontal cortex; pACC, posterior anterior cingulate; Par. Operc., parietal operculum; PCC, posterior cingulate; rdACC, rostral dorsal anterior cingulate; R, right; L, left; SFS, superior frontal sulcus; SMA, supplementary motor cortex; SMC, sensorimotor cortex; SPL, superior parietal lobule; STG,

superior temporal gyrus; TP, temporal pole; vFP, ventral frontal pole; v, ventral; ; vIns, ventral insula; VLPFC, ventrolateral prefrontal cortex.

Table 2.

Path b effects (connectivity with HR)

		Coordinates			Cluster size		Path a (SET)	
Anatomical Group	**Name**	**x**	**y**	**z**	**voxel**	**Vol. (mm3)**	**Z**	**P**
Positive (activity predicts increased HR)								
Medial frontal	rdACC, PCC	-3	3	40	2112	112303	3.58	0.0003
Lateral frontal	R IFJ	62	3	22	25	1329	3.57	0.0004
	L IFJ/PMC	-52	-3	27	24	1276	3.58	0.0003
	R PMC	41	-3	36	12	638	3.54	0.0004
	L IFJ	-45	10	40	22	1170	3.40	0.0007
	R lPFC	48	10	45	25	1329	3.51	0.0005
Brainstem	dPAG, MD Tha	7	-14	0	309	16431	3.58	0.0003
Medial temporal	R TP	21	21	-40	8	425	3.58	0.0003
Basal ganglia	L Cau	-17	7	4	74	3935	3.58	0.0003
Posterior temporal	R ITC	62	-52	-18	11	585	3.21	0.0013
	R MTG	72	-24	-9	5	266	3.30	0.001
	L MTG	-69	-45	0	27	1436	3.58	0.0003
Parietal cortex	L IPL	-31	-76	32	25	1329	3.58	0.0003
	L IPS	-38	-58	40	24	1276	3.46	0.0005
	R IPS	38	-38	36	8	425	3.37	0.0008
	R Par/Occ	45	-76	18	54	2871	3.58	0.0003
Cerebellum	L CB	-28	-48	-32	22	1170	3.58	0.0003
	R CB	24	-45	-27	3	160	3.36	0.0008
	CB vermis	0	-52	-22	17	904	3.57	0.0004
Negative (activity predicts decreased HR)								
Medial frontal	VMPFC/mOFC	-3	38	-18	287	15261	-3.58	0.0003
Medial temporal	L TP	-38	-7	-45	9	479	-3.55	0.0004
	R Hipp	31	-14	-18	21	1117	-3.58	0.0003
	L ITC	-41	10	-27	38	2021	-3.58	0.0003
Brainstem	Pons	7	-10	-32	11	585	-3.58	0.0003
Cerebellum	R aCB	17	-34	-22	16	851	-3.58	0.0003

Note. Brain connectivity with heart rate (HR), controlling for the social evaluative threat regressor. Z and P values represent maximum and minimum values over the contiguous suprathreshold region, respectively. Abbreviations: aCB, anterior cerebellum; dPAG, dorsal periaqueductal gray; IFJ, inferior frontal junction; MD Thal, mediodorsal thalamus; lPFC, lateral prefrontal cortex; MTG, middle temporal gyrus; rdACC, rostral dorsal anterior cingulate; R, right; L, left; PMC, premotor cortex. Other abbreviations are as in Table 1.

Table 3.

		1st-level: within-subjects				**Path a (SET)**		**Path b (HR)**		**Mediation (a*b)**		**Sig. Voxels**	
Group	**Name**	**x**	**y**	**z**	**Vol. (mm3)**	**max Z**	**P**	**max Z**	**P**	**max Z**	**P**	**P < .001**	**P < .005**
Medial frontal cortex													
	VMPFC/mOFC	-10	38	-14	1010	-3.39	0.0007	-3.44	0.0006	3.58	0.0003	4	19
	rdACC/MCC	-7	24	40	1170	3.03	0.0024	3.53	0.0004	3.58	0.0003	6	22
Periaqueductal gray (PAG) region of interest												**P < .05**	**P < .10**
	PAG	-3	-28	-14	319	1.54	0.12	2.70	0.0069	2.45	0.01	2	6

Note. Brain mediators of social evaluative threat (SET) effects on heart rate (HR), including regions showing significant effects in all three tests of interest (Paths *a, b*, and *a*b*). Statistics are reported only for voxel with the peak effect size for the a*b effect. Significant voxels counts the number of voxels at each threshold (two tailed) in the mediation *(a*b*) effect. Abbreviations are as in Table 1.

Table 4.

Moderation analyses: Brain-HR correlation as a function of task period

	Average time series correlation			Mixed-effects model [Speech Prep - Baseline]				[Baseline - Recovery]			
Region	**Baseline**	**Speech Prep**	**Recovery**	**difference**	**STE**	**t**	**P**	**difference**	**STE**	**t**	**P**
vmPFC/mOFC	-0.1	-0.12	0	-0.05	0.03	-1.54	0.072	-0.09	0.09	-1.5	0.08
dACC	0.05	0.16	0.11	0.08	0.03	2.93	0.005	-0.06	0.07	-0.8	0.22
PAG	0.06	0.06	0.07	0.02	0.02	1.36	0.096	-0.01	-0	-0.2	0.42

Note. Brain-heart rate (HR) association as a function of task period. Variations in association strength constitute a moderation by task state. STE, standard error. Other abbreviations are as in Table 1.

Table 5.

Mediators (a*b) of subjective anxiety profiles

		1st-level: within-subjects				**Path a (SET)**		**Path b (HR)**		**Mediation (a*b)**			**r with anxiety reactivity**		
Group	**Name**	**x**	**y**	**z**	**Vol. (mm3)**	**max Z**	**P**	**max Z**	**P**	**max Z**	**P**	**Cov(a,b)**	**Path a**	**Path b**	**a*b**
Medial frontal															
	DMPFC	14	48	45	3829	0.36	0.72	-1.68	0.09	3.58	0.0003	0.66	-0.22	-0.05	0.16
	R DMPFC	17	48	22	1276	0.17	0.86	-0.71	0.47	3.57	0.0004	0.46	-0.04	0.29	0.08
	L pgACC/VMPFC	-3	41	-14	6966	0.31	0.76	-0.02	0.98	3.58	0.0003	0.54	0.13	-0.36	0.45
	R aVMPFC	14	55	-14	266	-0.76	0.44	-0.40	0.69	3.56	0.0004	0.64	-0.16	-0.52*	0.16
Lateral frontal															
	L DLPFC	-52	14	32	425	0.51	0.61	0.07	0.94	3.55	0.0004	0.64	0.12	0.01	0.37
	L DLPFC	-34	38	40	3988	0.07	0.94	-0.42	0.67	3.58	0.0003	0.48	-0.07	0.39	0.18
	L IFG	-62	24	9	425	2.21	0.03	1.13	0.26	3.58	0.0003	0.50	0.30	0.76*	0.21
Medial temporal															
	R TP	41	7	-40	4041	-1.25	0.21	-2.49	0.01	3.58	0.0003	0.61	-0.14	-0.29	0.21
	L TP	-38	14	-45	1595	-1.33	0.18	-0.60	0.55	3.56	0.0004	0.34	-0.18	-0.47*	0.49*
	R uncus	17	3	-40	213	-0.90	0.37	-1.37	0.17	3.58	0.0003	0.46	-0.13	0.10	0.06
	R PHCP	17	-14	-32	904	-1.02	0.31	-2.59	0.0096	3.57	0.0004	0.28	0.27	-0.26	0.09
Lateral temporal															
	R aSTS	55	10	-27	532	-0.17	0.87	-0.57	0.57	3.56	0.0004	0.73	-0.10	-0.16	0.36
Parietal															
	L IPL	-55	-48	58	266	-1.09	0.28	0.15	0.88	3.50	0.0005	0.57	0.09	-0.21	-0.53*
Occipital															
	L OCC	-17	-82	0	1542	3.49	0.0005	2.23	0.03	3.58	0.0003	0.54	-0.21	-0.09	-0.23

Note. Mediators of subjective anxiety changes across time, as reflected in significant *a*b* effects. The Cov(a,b) column lists the estimated covariance between a and b effects. Large values suggest the presence of coherent pathways that may vary in sign across individuals. The rightmost columns list Pearson's *r* values for the correlation between path coefficients and 'anxiety reactivity,' or overall increases in anxiety for [Speech Preparation – Baseline]. *, $p < .05$. DLPFC, dorsolateral prefrontal cortex; DMPFC, dorsomedial prefrontal cortex; IFG, inferior frontal gyrus; PHCP, parahippocampal cortex; OCC, occipital cortex. Other abbreviations are as in Table 1.

Figure Captions

Figure 1. Social evaluative threat (SET)-related outcomes and mediation model. A) Group-averaged (N = 18) heart-rate changes (red) and self-reported anxiety changes (blue) across time. Anxiety ratings were interpolated from ratings made every 20 sec. The colored bars at the bottom show the phases of the experiment, with non-threat phases in gray, threat phases in pink, and instruction phases in yellow. Shaded regions indicate standard errors of the mean. B) Mediation path diagram showing the mediation effect search strategy. The initial variable (left) was experimentally induced SET across time. The outcome variable (right) was fluctuations in heart rate across time. The mediation effect parametric mapping analysis strategy involved searching for brain voxels in which fMRI time courses mediated the SET-heart rate relationship. A voxel was considered a significant mediator if it showed a significant a path across subjects, indicating brain responses to SET; a significant b path, indicating brain-heart rate correlation, controlling for SET; and a significant mediation effect, defined as the product of path coefficients a and b. Path c reflects cardiovascular responses to the SET challenge. All path analyses controlled for activity related to vascular responses in large vessels, visual and motor activity, and head movement (see Figure 6).

Figure 2. Covariates removed rom each participant's brain, HR, and anxiety time series data prior to multi-level path analysis. The top left panels show a structural T1-weighted image for one representative participant. Major arteries identified using a custom segmentation procedure are shown in red in the panels below, and on a left medial surface representation at right. The bottom panel shows an example of covariates for one participant, with time on the x-axis. These included the first three principal component scores from the participants' vascular voxels and their derivatives, visual stimulation during instructions periods and rating periods and their derivatives, global signal and linear drift, and covariates related to head movement. ACA, anterior cerebral artery; ICA, internal carotid artery; MCA, middle cerebral artery.

Figure 3. Results of the mediation effect parametric mapping search. A) Path a results. Saggital slice showing regions whose activity increased (yellow/orange) or decreased (blue) in response

to the social evaluative threat (SET) challenge. Significant regions of 3 or more contiguous voxels at $q < .05$ False Discovery Rate corrected, and voxels contiguous with these regions at $p < .005$, are shown. rdACC: rostral dorsal anterior cingulate cortex; vmPFC, ventromedial prefrontal cortex. B) Path b results. Sagittal slices showing significant positive (yellow/orange) or negative (blue) correlates of heart rate changes over time, controlling for the time course of the SET manipulation. C) Significant mediators, showing the conjunction of significance in the a path, the b path, and the mediation test on the a*b product. D) Examples of individual subjects' partial regression slopes (blue lines) for the brain-heart relationship (b path) for each significant mediating region. The span of each line along the x-axis reflects the 95% confidence interval of fMRI activity for that participant. The thickness and darkness of the line reflects the weight assigned to that participant based on the within-subject variability, with darker and thicker lines indicating lower within-subject error and more weight in the group analysis. The black line shows the group average, and the shaded gray area shows the 95% confidence interval for the regression slopes. Variability in the intercept values across participants has been removed for display purposes.

Figure 4. Time course of mediating regions and moderation of brain-heart connectivity by task phase. A) Group average time course of fMRI activity in the rostral dorsal cingulate (rdACC, red) and ventromedial prefrontal cortex (vmPFC, blue). Each region is shown on a saggital slice to the right of the plot. Task periods are shown at the bottom of the plot, as in Figure 1. The rdACC time course shows significant elevation during both speeches. The vmPFC time course shows evidence for increases before the beginning of speech preparation and a large negative deflection during the first speech preparation period. Shaded areas show standard errors of the mean, and thicker orange and green lines indicate periods in which the group average differed significantly from zero. All covariates were removed prior to averaging (see Figure 6). To facilitate visualization of low-frequency changes related to task phases, the time series were smoothed with a 60 sec Gaussian full-width half-max moving average. B) Moderation of brain-heart connectivity by the task phase. The plots show the average brain-heart correlation for each task phase, after conversion to Fisher's Z values to normalize the distribution across participants. Correlations between the rdACC and heart rate (left panel) varied as a function of task phase, with the strongest correlations during speech preparation. Correlations between the vmPFC and

heart rate (right panel) did not differ significantly across task periods, but appeared to be strongest before and during preparation. Error bars show standard errors of the mean.

Figure 5. Mediation of the social evaluative threat (SET) effect on heart rate (HR) by the periaqueductal gray (PAG). A) the PAG region of interest (ROI) is shown in green. A) Left: Voxels showing significant responses to SET (a path) at a threshold of $p < .025$ (yellow) and $p < .05$ (orange; one-tailed) within the PAG ROI. Center: Voxels showing a significant relationship with HR, controlling for SET (b path). Right: Voxels showing a significant mediation effect (a*b product). B) The time course of activity in the PAG, which shows the largest peaks during the first 30 sec and the last 30 sec of the first speech. Details of the plot are as in Figure 3A. C) The average PAG brain activity – HR correlation during each task phase. Details are as in Figure 3B. The PAG shows a consistent positive relationship with HR across task phases.

Figure 6. Summary of cortical-brainstem and cortical-heart rate connections. Green lines show positive links, and blue lines show negative links. The causal directionality of effects, except for SET effects, cannot be determined from the data alone (especially as the vast majority brain pathways include bidirectional projections) and so are not marked with arrows. Experimentally induced social evaluative threat (SET) induced increases in rostral dorsal cingulate cortex (rdACC) and periaqueductal gray (PAG), both shown in yellow, and decreases in ventromedial prefrontal cortex (vmPFC). All three areas were significant partial mediators of the SET effect on heart rate (HR). The rdACC influence on HR was partly mediated by a negative coupling with the vmPFC, but a strong direct positive influence on HR remained that was partially mediated by the thalamus, but not the PAG. The vmPFC was negatively coupled with the PAG, which was a strong but partial mediator of the vmPFC-HR relationship. These results are broadly consistent with recent meta-analytic results that show preference for positive valence in the vmPFC and negative valence in the dorsomedial PFC (dmPFC; inset panel, left) , and co-activation across studies between the rdACC and dmPFC cortical regions and and the PAG and medial thalamus (inset panel, right). Overall, the results suggest that multiple medial frontal regions differentially contribute to the generation of stress-induced heart-rate increases.

Figure 7. Mediators of reported anxiety. These regions showed evidence for an a*b mediation effect, but most did not show evidence for average Path a and Path b effects (See Table 5). This implies that functional pathways linking social threat, brain actiivity, and anxiety reports in these regions were variable in sign and/or strength across individuals. Top right: A positive correlation between anxiety reactivity (x-axis) and right inferior frontal gyrus activity (y-axis) suggests that those who report that the task is more anxiogenic show links between social threat, inferior frontal activity, and anxiety. Bottom right: A negative correlation between between anxiety reactivity and anterior ventromedial prefrontal activity suggests that those who report that the task is more anxiogenic show links between social threat, VMPFC *de*activation, and anxiety. Yellow: $P < .001$; Orange: $P < .005$; Pink: $P < .01$.

Figure 1. Task and ben results.

Figure 1.

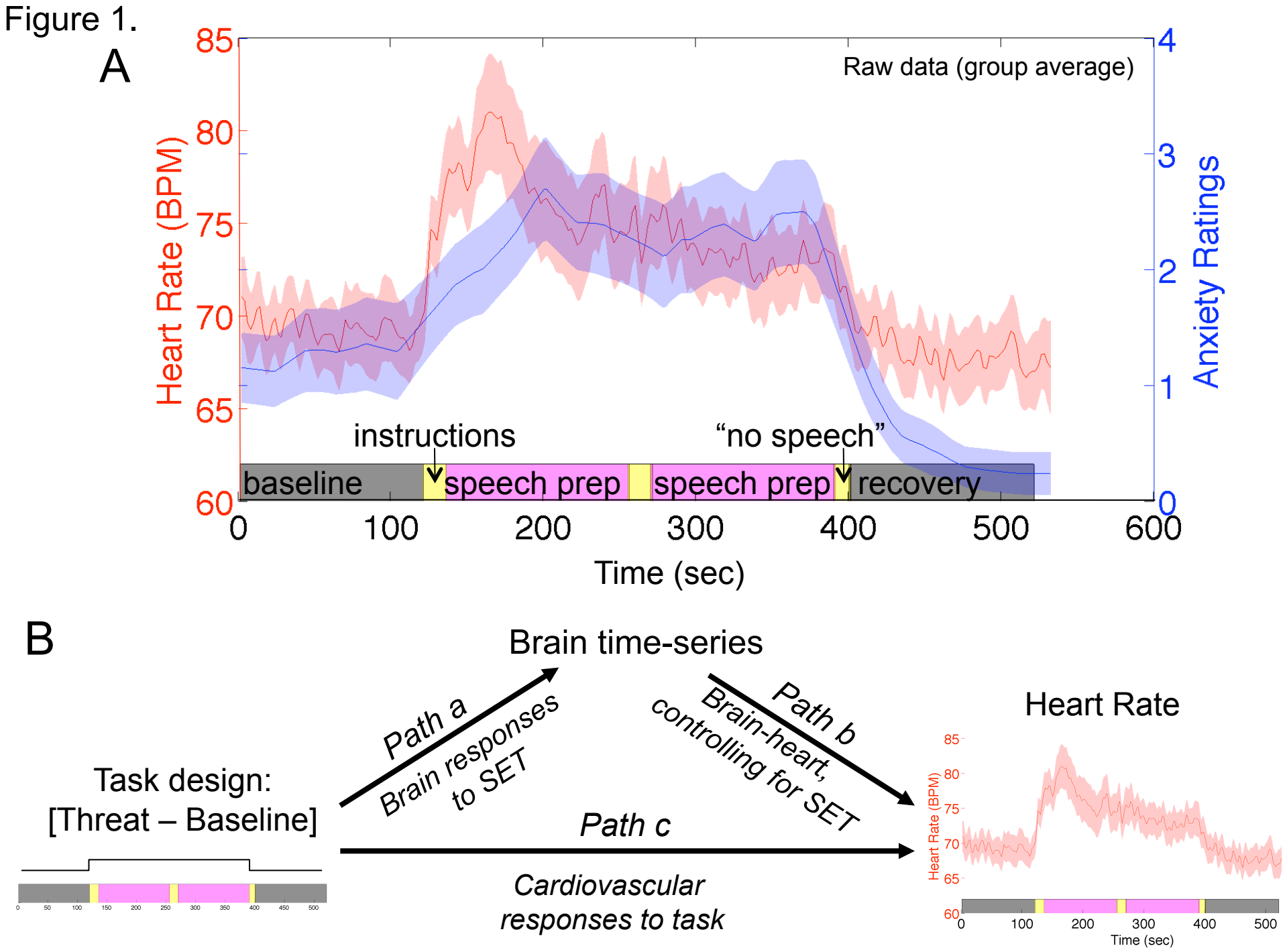
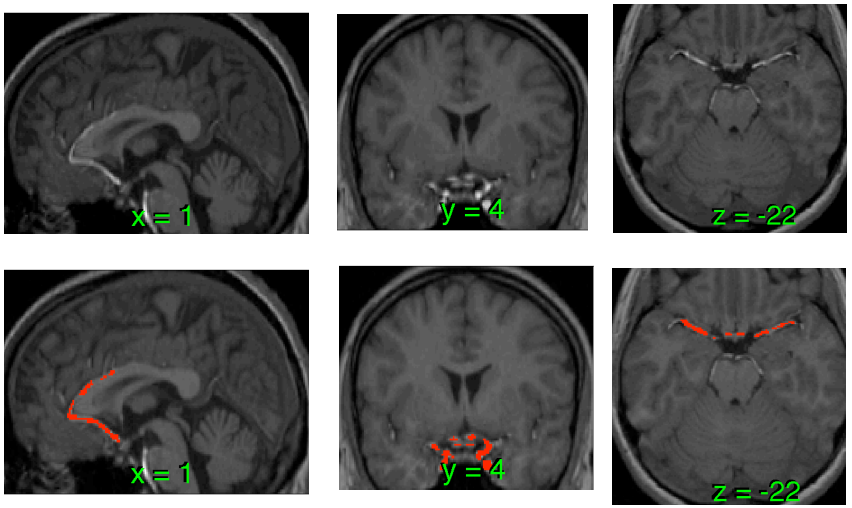
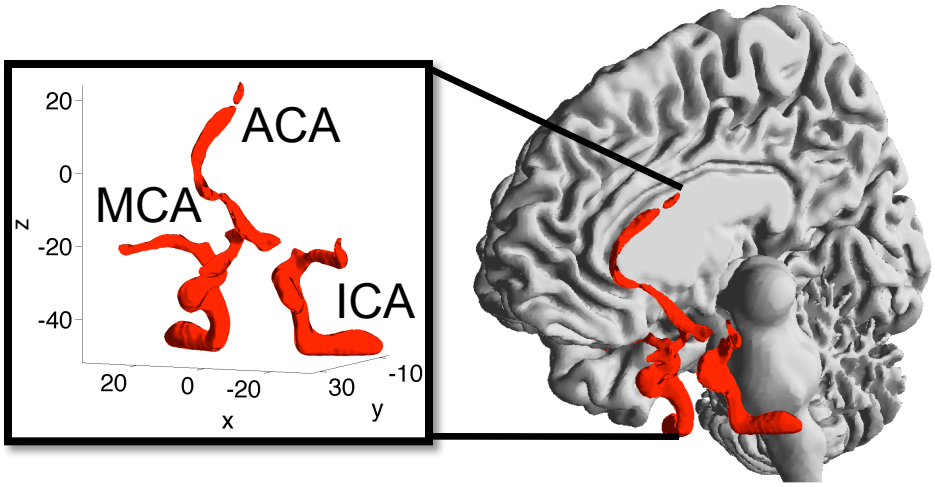
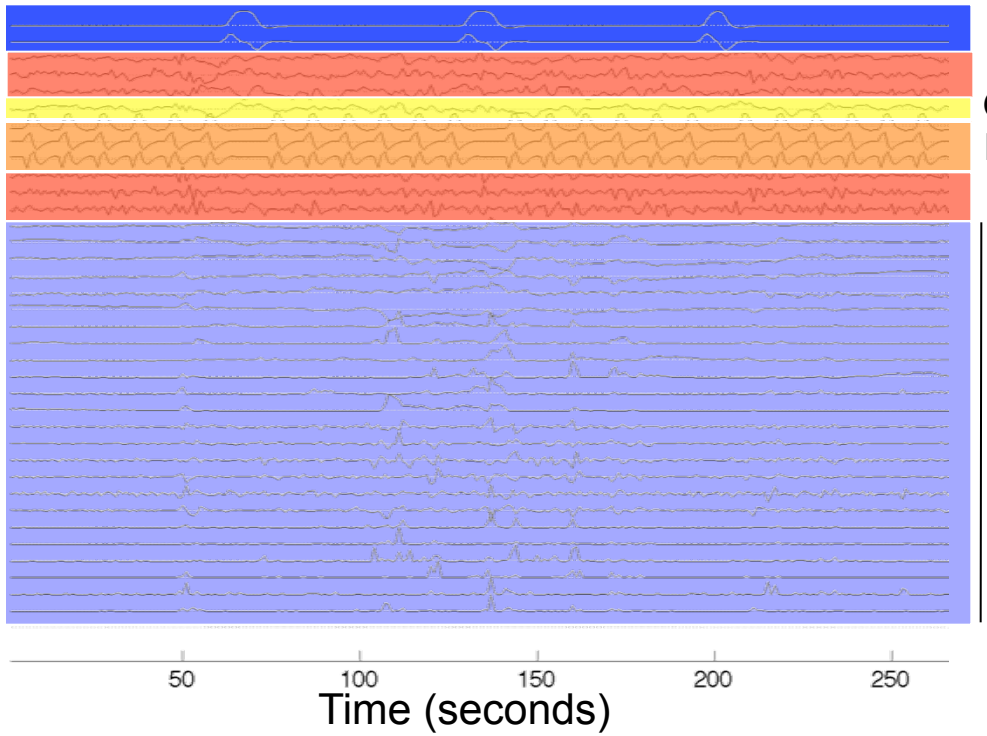


Figure 2.







Visual stimulation
Vascular activity
Global activity
Rating probes
Vascular (derivatives)

Head movement-related
Movement (x6)
Movement^2 (x6)
Derivatives (x6)
Derivatives^2 (x6)

Figure 1

Figure 3.

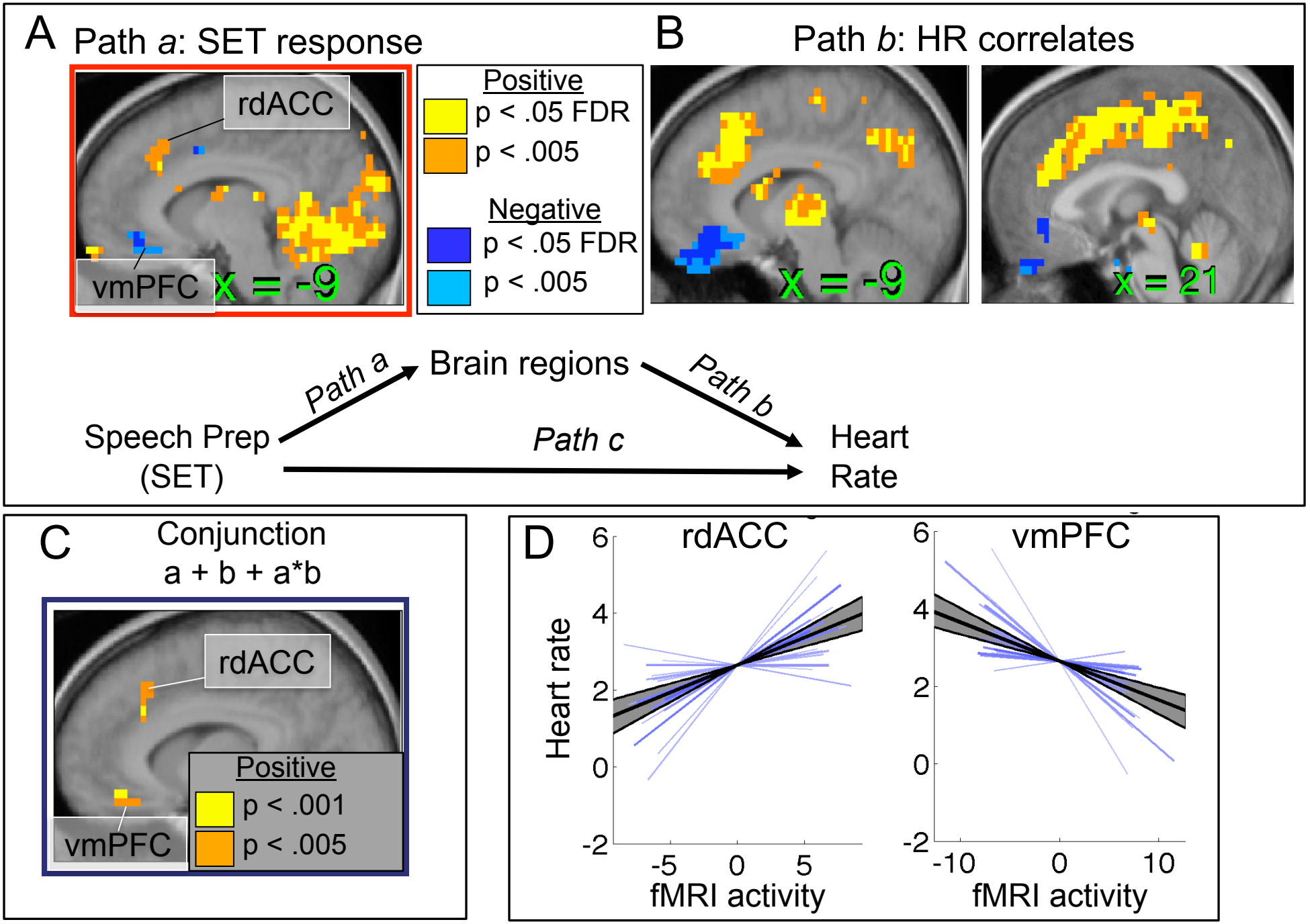


Figure 4.

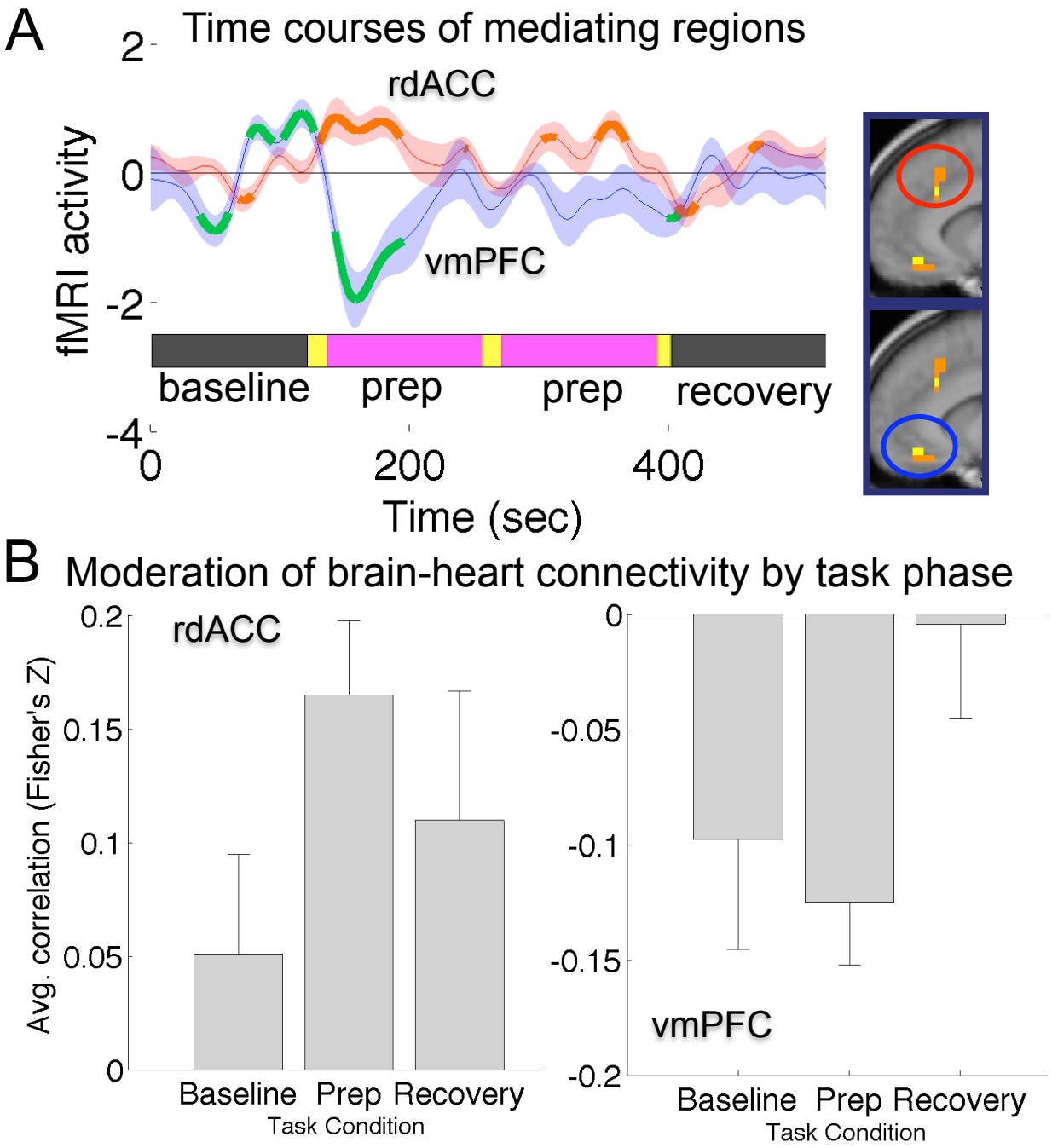

A
Time courses of mediating regions
rdACC
vmPFC
fMRI activity
2
0
-2
-4
baseline
prep
prep
recovery
0
200
400
Time (sec)
B
Moderation of brain-heart connectivity by task phase
rdACC
vmPFC
Avg. correlation (Fisher's Z)
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
Baseline
Prep
Recovery
Task Condition

Figure 5. Mediation by brainstem periaqueductal gray (PAG)

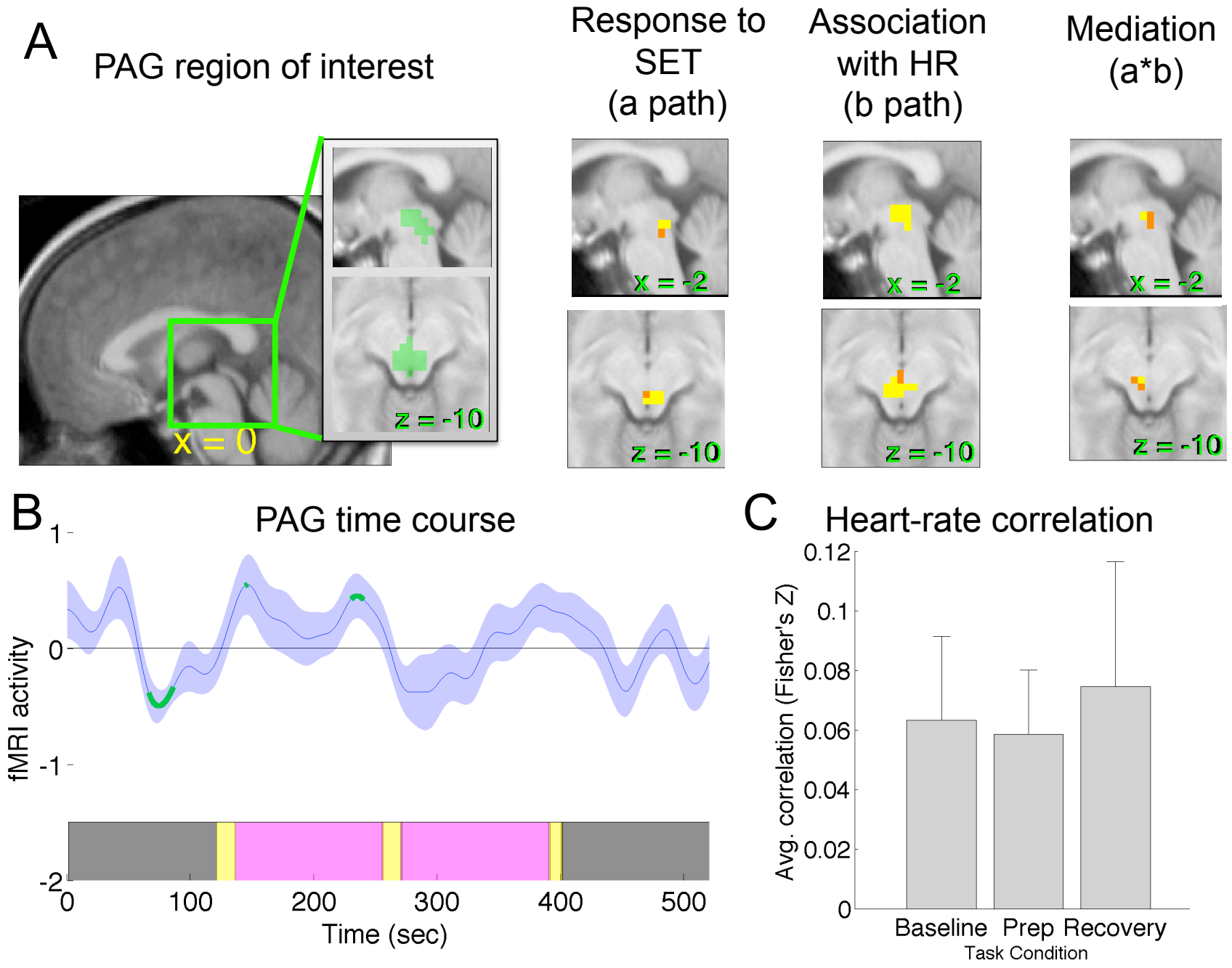


Figure 6. Key regions and pathways

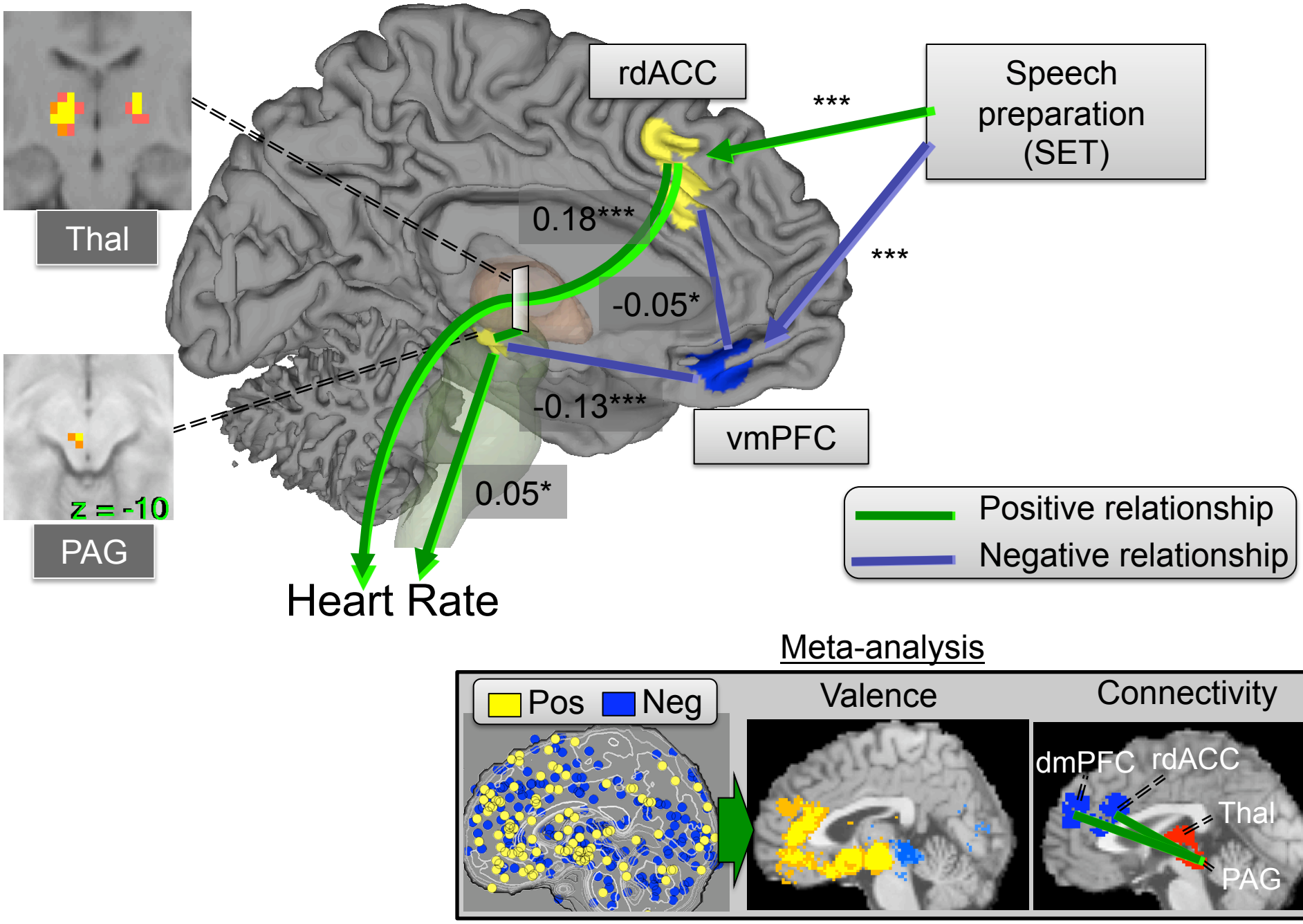


Figure 7. Mediators of reported anxiety (a*b effect)