Heightened Resting Neural Activity Predicts Exaggerated Stressor-Evoked Blood Pressure Reactivity

Peter J. Gianaros, Lei K. Sheu, Allison M. Remo, Israel C. Christie, Hugo D. Critchley, Jiongjiong Wang

Abstract—Individuals who express relatively large-magnitude or “exaggerated” blood pressure (BP) reactions to behavioral stressors are presumably at increased risk for cardiovascular disease. As shown by recent neuroimaging studies, individuals who express exaggerated stressor-evoked BP reactivity also express heightened neural activity in corticolimbic brain areas that centrally regulate the cardiovascular system. These studies, however, have exclusively examined BP reactivity and concomitant neural activity during stressor exposure. To test this hypothesis, perfusion MRI was used to quantify resting regional cerebral blood flow (an indirect metabolic measure of neural activity) in men (n=19) and women (n=20) aged 20 to 37 years who subsequently performed cognitive stressor tasks to evoke BP reactivity. Individuals who expressed larger task-induced rises in systolic and diastolic BP also expressed higher resting regional cerebral blood flow in 4 functionally related corticolimbic areas: the dorsal and perigenual anterior cingulate, medial prefrontal, and insular cortices. Specifically, resting regional cerebral blood flow in these areas accounted, respectively, for 40% and 31% of the variance in systolic (P<0.001) and diastolic (P=0.008) BP reactivity, after accounting for total resting cerebral blood flow, resting BP, task performance, and task-related ratings of unpleasantness, arousal, and perceived psychological control. Heightened resting corticolimbic activity may represent a neurobiological correlate of an individual’s predisposition for exaggerated stressor-evoked BP reactivity and possibly related cardiovascular risk. (Hypertension. 2009;53:819-825.)

Key Words: anterior cingulate cortex ■ blood pressure reactivity ■ individual differences ■ insula ■ medial prefrontal cortex ■ stress

Individuals who express relatively large-magnitude or exaggerated blood pressure (BP) reactions to behavioral stressors are at moderately increased risk for hypertension, stroke, ventricular hypertrophy, preclinical atherosclerosis, and myocardial infarction. Although it is uncertain whether exaggerated stressor-evoked BP reactions are involved causally in the pathophysiology of these precursors and end points of cardiovascular disease, there is psychometric and genetic evidence that an individual’s tendency to express exaggerated stressor-evoked BP reactivity is a reliable and partly heritable dispositional cardiovascular stress response attribute. In parallel, there is human neuroimaging evidence that stressor-evoked BP reactions are associated with patterns of neural activity in corticolimbic brain areas that centrally regulate the cardiovascular system. More precisely, stressor-evoked BP reactions have been associated reliably with activity in the anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC), and insula. Together, these corticolimbic areas can regulate stressor-evoked BP reactions by modulating autonomic and neurohormonal outflow to the myocardium and vasculature via functional neural connections with one another and with subcortical cell groups (eg, amygdala, pontine, and medullary nuclei) that are more proximally involved in cardiovascular control. In addition, individuals who express exaggerated stressor-evoked BP reactivity have been found to also express heightened stressor-evoked activity in the ACC, mPFC, and insula, broadly suggesting that these particular corticolimbic areas may represent components of a neural circuitry that is functionally involved in the dispositional expression of individual differences in cardiovascular stress reactivity.

To date, however, neuroimaging studies of stressor-evoked BP reactivity have exclusively examined concomitant changes in BP and corticolimbic activity during stressor exposure. Critically, if individual differences in BP reactivity originate in part from a centrally regulated and dispositional cardiovascular stress response tendency, then relatively heightened resting (prestressor) corticolimbic activity may directly pre-
dict the subsequent expression of comparatively larger-magnitude (exaggerated) stressor-evoked BP reactions across individuals. To test this hypothesis, perfusion MRI was used to quantify resting regional cerebral blood flow (CBF) [rCBF]; an indirect metabolic measure of neural activity) in young adults who subsequently performed cognitive stressor tasks designed to evoke BP reactivity. First tested was whether relatively heightened resting rCBF in corticolimbic areas would predict (prospectively) comparatively exaggerated BP reactions to the stressor tasks across individuals. Next tested was whether any prospective associations between resting rCBF and BP reactivity would persist after statistically accounting for plausible confounders, namely, total resting CBF, resting BP, task-related performance, and subjective ratings of task-related unpleasantness, arousal, and perceived psychological control. Last tested was whether the corticolimbic areas in which resting rCBF predicted BP reactivity across individuals would exhibit intercorrelations in their time-varying oscillations in the blood-oxygen level–dependent (BOLD) signal, putative indicators of coherent variation in metabolically linked neural activity. Such intercorrelations would provide evidence for so-called resting-state functional connectivity between specific corticolimbic areas that may partly constitute a neural circuitry for the dispositional expression of individual differences in stressor-evoked BP reactivity.

Methods

Participants

Participants were 20 men (mean age ± SD: 24.8 ± 5.1 years) and 20 women (mean age ± SD: 23.8 ± 3.8 years). All were right-handed, and none had any of the following: (1) a history of cardiovascular disease (including hypertension, stroke, myocardial infarction, congestive heart failure, and atrial and ventricular arrhythmias); (2) previous cardiovascular surgery (including coronary bypass, carotid artery, or peripheral vascular surgery); (3) a history of cancer, a chronic kidney or liver condition, type I or II diabetes mellitus, or any pulmonary or respiratory disease; (4) any current or previous self-reported psychiatric diagnosis of a substance abuse or mood disorder; (5) previous cerebrovascular trauma involving loss of consciousness; (6) previous neurosurgery or history of neurological conditions; (7) pregnancy (verified by urine test in females); (8) color blindness; (9) claustrophobia; (10) metallic implants; or (11) a self-reported history of using any psychotropic, lipid-lowering, or cardiovascular medications.

The participants’ average seated resting BP was 117/66 mm Hg (±10.9/6 SD), as determined by the mean of the last 2 of 3 BP readings obtained with an oscillometric device (Critikon Dinamap 8100, Johnson & Johnson) and taken 2 minutes apart after a 20-minute acclimation period before MRI testing. Data from 1 man were excluded because of excessive neuroimaging artifacts attributed to head movements. Thus, results herein are for the remaining 39 participants. Participants gave informed consent to study protocols, approved by the University of Pittsburgh Institutional Review Board. Supplemental information about participant characteristics and screening methods are provided online in Table S1 (please see http://hyper.ahajournals.org).

Study Protocols

Participants abstained from eating, exercising, and consuming caffeinated and tobacco products for 3 hours and drinking alcoholic beverages for 12 hours before testing. At testing, participants underwent a screening interview followed by protocols to assess anthropometric measures, demographic information, and seated BP. Participants then underwent an MRI protocol. For this protocol, participants were fitted with a BP cuff matched to arm size, inserted into the MRI scanner, and asked to rest for ~20 minutes; 12 to 15 minutes later, participants completed 2 stressor tasks while in the scanner.

Neuroimaging Data Acquisition

Neuroimaging data were acquired on a 3T Trio TIM whole-body scanner (Siemens), equipped with a 12-channel, phased-array head coil. Resting perfusion images were acquired with a pulsed arterial spin-labeling sequence. For this sequence, interleaved perfusion images with and without arterial spin labeling were obtained over a 5-minute, 28-second period using gradient-echo echo-planar imaging. The pulsed arterial spin-labeling sequence used a flow-sensitive alternating inversion recovery method, specifically applying a saturation pulse 700 ms after an inversion pulse. To reduce transit artifact, a 1000-ms delay separated the end of the labeling pulse and the time of image acquisition. Resting perfusion image acquisition parameters were: field of view: 240 × 240 mm; matrix size: 64 × 64 mm; repetition time: 4 seconds; echo time: 18 ms; and flip angle: 90°. Twenty-one sections (5 mm thick, 1 mm gap) were acquired sequentially in an inferior-to-superior direction, yielding 80 total perfusion images (40 labeled and 40 unlabeled; 2 initial discarded images allowing for magnetic equilibration). Resting BOLD images used to compute functional connectivity measures were acquired over a 5-minute, 6-second period with a gradient-echo echo-planar imaging sequence using the following parameters: field of view: 205 × 205 mm; matrix size: 64 × 64 mm; repetition time: 2 seconds; echo time: 28 ms; and flip angle: 90°. Thirty-nine sections (3-mm thick, no gap) were obtained sequentially in an inferior-to-superior direction, yielding 150 BOLD images (3 initial discarded images allowing for magnetic equilibration). For spatial coregistration of resting perfusion and BOLD images, T1-weighted 3D magnetization-prepared rapid gradient echo neuroanatomical images were acquired over 7 minutes, 17 seconds by these parameters: field of view: 256 × 256 mm; matrix size: 256 × 256 mm; repetition time: 2100 ms; inversion time: 1100 ms; echo time: 3.29 ms; and flip angle: 8° (192 sections, 1-mm thick, no gap).

Stressor Tasks

After the resting period, participants completed 2 counterbalanced stressor tasks designed to evoke BP reactivity: a modified Stroop color-word interference task and a modified multisource interference task. The tasks were separated by a 10- to 12-minute recovery period, during which subjective ratings of the first task were obtained (see below). Each task lasted 9 minutes, 20 seconds and was composed of trials defining 2 alternating conditions, a less demanding congruent condition and a more demanding incongruent condition. The congruent and incongruent conditions lasted 52 to 60 seconds and were preceded by a 10- to 17-second period during which participants fixated on a cross-hair. Briefly, the congruent and incongruent conditions of both tasks were matched on motor response requirements and visual stimulus characteristics. In addition, the incongruent condition of each task was performance titrated, such that task accuracy was adaptively maintained at ~50% within and between individuals. In this way, task engagement and performance were experimentally approximated across participants. Supplemental task details and trial illustrations and provided online in Figure S1.

Task Accuracy and Subjective Ratings

Task performance (accuracy) was computed as the percentage of trials correctly completed. Posthoc, we verified that mean accuracy during the incongruent condition of each task was titrated across participants to 55.4% (±6.8% SD), as compared with 89.8% (±3.9% SD) during the congruent condition, t138 = 34.8 (P < 0.001). In conjunction with this performance titration, mean response times to trials delivered during the incongruent condition compared with the congruent condition of each task were slowed by 465.8 ms (±152.7 ms SD; t138 = 19.1; P < 0.001).

To assess ratings of valence (1: very unhappy; 9: very happy), arousal (1: very calm; 9: very aroused), and perceived control (1: very little control; 9: very much control), participants completed a modified self-assessment manikin scale after the resting (prestress-
BP Measurement

In the MRI scanner, participant BP was measured during the resting (prestressor) and stressor task periods from the brachial artery of the nondominant (left) arm, which was not used for task responding. BP measurements were taken with an oscillometric device (Multigas 9500, MedRad, Inc), set to inflate every 2.5 minutes during the resting period and once during each condition of the Stroop task and multisource interference task. To compute resting BP, the final 3 measurements were averaged. To compute task-related BP, measurements from the demanding incongruent condition of the Stroop task and multisource interference task were averaged. The incongruent condition (minus) resting BP difference score was used to compute BP reactivity following previous work.7,18 In this sample, men and women did not differ in resting or reactivity measures of systolic BP (SBP) or diastolic BP (DBP; t values: z1.6; P=0.11). In addition, across individuals, SBP and DBP changes from the resting period to the incongruent conditions of the Stroop task and multisource interference task were correlated (ΔSBP r=0.70, P<0.001; ΔDBP r=0.73, P<0.001), indicating that the tasks evoked reliable individual differences in BP reactivity. Following previous guidelines,10 task-averaged SBP and DBP reactivity scores were used for subsequent analyses.

Preprocessing of Neuroimaging Data

Resting perfusion images were preprocessed with computational routines implemented in Statistical Parametric Mapping software (SPM2, Wellcome Trust Centre for Neuroimaging). For preprocessing, perfusion images were realigned to the first image of the series, coregistered to each participant’s magnetization-prepared rapid gradient echo image, spatially normalized to the International Consortium for Brain Mapping 152 template (Montreal Neurological Institute), and resliced to an isotropic voxel size of 3 mm3. Images were then smoothed with a 12-mm full-width at half-maximum isotropic Gaussian kernel, after which the 40 labeled and 40 unlabeled perfusion images were submitted to pairwise subtraction. Subtraction images were converted to an absolute CBF image series using a validated algorithm.28 This perfusion series was then averaged, generating for each individual a single resting voxel-wise rCBF image and a total CBF value, both in units of milliliters per 100 grams per minute.

Resting BOLD images were preprocessed using Statistical Parametric Mapping software (SPM5; Wellcome Trust Centre for Neuroimaging). As for perfusion images, BOLD images were realigned to the first image of the series, coregistered to each participant’s magnetization-prepared rapid gradient echo image, normalized to the Montreal Neurological Institute 152 template, and smoothed with a 6-mm full-width at half-maximum isotropic Gaussian kernel. After preprocessing, a BOLD signal time series was extracted from 4 empirically determined regions of interest (ROIs) for each individual, according to methods detailed previously.18 Specifically, a time series was extracted from the mean BOLD signal of all of the voxels in a 6-mm sphere surrounding the Montreal Neurological Institute coordinates identified in the voxel-wise regression analyses of resting perfusion (as quantified by rCBF) and stressor-evoked BP reactivity reported below. These regions (shown in Figure 1) included the dorsal ACC (dACC; x, y, z Montreal Neurological Institute coordinates in millimeters: −6, 27, 33), perigenual ACC (pACC; −9, 54, 12), mPFC (mPFC; 24, 51, 18; tα=4.2; P<0.001; k=82), and insula (42, −12, 12; tα=3.2; P=0.001; k=24), where resting rCBF predicted SBP reactivity, after controlling for total resting CBF and resting SBP in a voxel-wise regression model. Plots in E through H illustrate SBP reactivity as a function of resting rCBF in the dACC, pACC, mPFC, and insula. Profiled in A through D are areas of the dACC (x, y, z coordinates for peak voxel in mm3: −6, 27, 33; tα=3.1; P=0.002; voxel cluster size [k] in mm3: 32), pACC (−9, 54, 12; tα=3.1; P=0.002; k=23), mPFC (24, 51, 18; tα=4.2; P<0.001; k=82), and insula (42, −12, 12; tα=3.2; P=0.001; k=24), where resting rCBF predicted SBP reactivity, after controlling for total resting CBF and resting SBP. All r values in E through H are significant at P<0.05, 2-tailed.

Figure 1. Individual differences in stressor-evoked SBP reactivity were predicted by resting (prestressor) rCBF in the dACC, pACC, mPFC, and insula. Profiled in A through D are areas of the dACC (x, y, z coordinates for peak voxel in mm3: −6, 27, 33; tα=3.1; P=0.002; voxel cluster size [k] in mm3: 32), pACC (−9, 54, 12; tα=3.1; P=0.002; k=23), mPFC (24, 51, 18; tα=4.2; P<0.001; k=82), and insula (42, −12, 12; tα=3.2; P=0.001; k=24), where resting rCBF predicted SBP reactivity, after controlling for total resting CBF and resting SBP. All r values in E through H are significant at P<0.05, 2-tailed.

values >3 SD of the series mean were replaced by averaging 2 surrounding values. Each outlier-corrected time series was then band-pass filtered from 0.01 to 0.10 Hz to remove nonneural sources of noise using a linear-phase finite impulse response Hamming filter of length 51 (102 seconds, based on the 2-second BOLD signal acquisition repetition time). Each filtered time series was then

Figure 1. Individual differences in stressor-evoked SBP reactivity were predicted by resting (prestressor) rCBF in the dACC, pACC, mPFC, and insula. Profiled in A through D are areas of the dACC (x, y, z coordinates for peak voxel in mm3: −6, 27, 33; tα=3.1; P=0.002; voxel cluster size [k] in mm3: 32), pACC (−9, 54, 12; tα=3.1; P=0.002; k=23), mPFC (24, 51, 18; tα=4.2; P<0.001; k=82), and insula (42, −12, 12; tα=3.2; P=0.001; k=24), where resting rCBF predicted SBP reactivity, after controlling for total resting CBF and resting SBP. All r values in E through H are significant at P<0.05, 2-tailed.

If the predicted SBP reactivity was then

Figure 1. Individual differences in stressor-evoked SBP reactivity were predicted by resting (prestressor) rCBF in the dACC, pACC, mPFC, and insula. Profiled in A through D are areas of the dACC (x, y, z coordinates for peak voxel in mm3: −6, 27, 33; tα=3.1; P=0.002; voxel cluster size [k] in mm3: 32), pACC (−9, 54, 12; tα=3.1; P=0.002; k=23), mPFC (24, 51, 18; tα=4.2; P<0.001; k=82), and insula (42, −12, 12; tα=3.2; P=0.001; k=24), where resting rCBF predicted SBP reactivity, after controlling for total resting CBF and resting SBP. All r values in E through H are significant at P<0.05, 2-tailed.
evaluated by the reactivity explained by the set of extracted rCBF values was the task-averaged SBP (model 1) or DBP (model 2) reactivity. The dependent variable for the models (model 2), task-averaged accuracy, and task-averaged ratings of valence, arousal, and control. Specifically, in step 1 of a 2-step hierarchical regression analysis, total CBF, resting SBP, task accuracy, and task ratings accounted for 14% of the variance in DBP reactivity (F6,32.8 = 0.88; R2 adjusted = 0.02; P = 0.52). In another 2-step regression analysis, total CBF, resting DBP, task accuracy, and task ratings accounted for 18% of the variance in DBP reactivity (F6,32.1 = 0.03; P = 0.33). In step 2 of these regression analyses, resting rCBF values extracted from the dACC, pACC, mPFC, and insula accounted collectively for remaining variance in both SBP (F4,28.6 = 6.1; ΔR2 = 0.40; P = 0.001) and DBP (F4,28.4 = 4.2; ΔR2 = 0.31; P = 0.008) reactivity.

In addition, exploratory regression analyses including the same 4 variables as above demonstrated that resting rCBF values extracted from the dACC, pACC, mPFC, and insula accounted individually for unique variance in both SBP and DBP reactivity (Table S2). Across these exploratory analyses, however, only resting rCBF extracted from the insula did not account for unique variance in DBP reactivity after family wise error rate correction for multiple (posthoc) statistical testing (P family wise error = 0.08). By contrast, resting rCBF from the insula did account for unique variance in SBP reactivity; and, resting rCBF in all of the remaining areas accounted for unique variance in both SBP and DBP reactivity after family wise error correction (Table S2).

Here, we recognize that, during the resting period, participants may have been apprehensive about the forthcoming

Results

Task Ratings and BP Reactivity

The stressor tasks elicited moderate levels of subjective distress sufficient to evoke individual differences in BP reactivity. Specifically, using 9-point rating scales, participants reported that they felt less happy, more aroused, and less in control, while performing the tasks, as compared with the resting period (t values for all task versus resting comparisons: ≥ 3.7; P ≤ 0.001; Figure S2). In addition, both SBP and DBP increased on average while participants performed the tasks, as compared with the resting period (t values for all of the task versus resting comparisons: ≥ 3.0; P ≤ 0.006; Figure S4).

Resting rCBF and BP Reactivity

In a voxel-wise, multiple regression analysis, larger-magnitude, stressor-evoked SBP reactions were predicted across individuals by relatively higher levels of resting rCBF in the dorsal and perigenual areas of the left ACC (dACC and pACC), the dorsal area of the right mPFC (mPFC), and the posterior area of the right insula (Figure 1). Subsequent hierarchical multiple regression analyses further demonstrated that resting rCBF values extracted from the dACC, pACC, mPFC, and insula continued to predict both stressor-evoked SBP and DBP reactivity after accounting for total resting CBF, resting BP, task accuracy, and task ratings of valence, arousal, and control. Specifically, in step 1 of a 2-step hierarchical regression analysis, total CBF, resting SBP, task accuracy, and task ratings accounted for 14% of the variance in SBP reactivity (F6,32.8 = 0.88; R2 adjusted = 0.02; P = 0.52). In another 2-step regression analysis, total CBF, resting DBP, task accuracy, and task ratings accounted for 18% of the variance in DBP reactivity (F6,32.1 = 0.03; P = 0.33). In step 2 of these regression analyses, resting rCBF values extracted from the dACC, pACC, mPFC, and insula accounted collectively for remaining variance in both SBP (F4,28.6 = 6.1; ΔR2 = 0.40; P = 0.001) and DBP (F4,28.4 = 4.2; ΔR2 = 0.31; P = 0.008) reactivity.

In addition, exploratory regression analyses including the same 4 variables as above demonstrated that resting rCBF values extracted from the dACC, pACC, mPFC, and insula accounted individually for unique variance in both SBP and DBP reactivity (Table S2). Across these exploratory analyses, however, only resting rCBF extracted from the insula did not account for unique variance in DBP reactivity after family wise error rate correction for multiple (posthoc) statistical testing (P family wise error = 0.08). By contrast, resting rCBF from the insula did account for unique variance in SBP reactivity; and, resting rCBF in all of the remaining areas accounted for unique variance in both SBP and DBP reactivity after family wise error correction (Table S2).

Here, we recognize that, during the resting period, participants may have been apprehensive about the forthcoming

Figure 2. Brain areas in which resting rCBF predicted stressor-evoked SBP and DBP reactivity exhibited cross-correlations in their time-varying oscillations in the blood-oxygen level-dependent signal, an indirect measure of neural activity. Aggregated cross-correlations (with 99% bootstrapped CIs in parentheses) are shown for cortical surface projections of the (1) dACC, (2) pACC, (3) mPFC, and (4) insula.

This method permitted a test of whether the averaged z values differed from 0 across individuals, indicating so-called functional connectivity between the ROIs. To aid interpretability, the averaged z values and 99% CIs were transformed back to r values for illustration in Figure 2.
stressor tasks, leading to anticipatory unpleasantness or arousal and possibly increased BP reactivity or resting rCBF. This is unlikely, however, because resting ratings of valence and arousal did not correlate significantly with SBP or DBP reactivity or with resting rCBF in the dACC, pACC, mPFC, or insula ($r$ values ranged from $-0.14$ to $0.25$; $P=0.12$). In addition, resting SBP and DBP did not correlate significantly with resting rCBF in the dACC, pACC, mPFC, or insula ($r$ values: $-0.21$ to $0.18$; $P=0.20$).

**Functional Connectivity Between Areas Where Resting rCBF Predicted BP Reactivity**

Across individuals, the dACC, pACC, mPFC, and insula exhibited moderate and directionally positive cross-correlations in their time-varying BOLD signal fluctuations, indicating resting “functional connectivity” between these areas. Figure 2 shows the aggregate cross-correlation coefficients across these areas, along with their 99% bootstrapped CIs, generated by 5000 Monte Carlo simulations.

**Discussion**

The central finding of this study was that relatively heightenened resting (prestressor) rCBF in the dACC, pACC, mPFC, and insula uniquely predicted comparatively larger-magnitude (exaggerated) BP reactions to subsequently completed cognitive stressor tasks across individuals (Figure 1). In addition, these corticolimbic areas exhibited resting inter-correlations in their time-varying BOLD signal oscillations, indicating that they exhibited so-called functional connectivity$^{24}$ with one another (Figure 2). As such, the present study provides further evidence that the dACC, pACC, mPFC, and insula could represent components of a neural circuitry that is functionally involved in the dispositional expression of individual differences in stressor-evoked BP reactivity.

An individual’s tendency to express exaggerated stressor-evoked BP reactivity has long been viewed as a dispositional attribute that may be linked to the pathophysiology of cardiovascular disease.$^{30}$ Heritability studies$^{11,12}$ further support the converging view that individual differences in stressor-evoked BP reactivity arise in part from genetic factors, which may involve the familial transmission of allelic polymorphisms that modify myocardial and vascular sensitivity to centrally regulated patterns of peripheral effector autonomic and neurohormonal outflow.$^{31-33}$ A largely unsupported view, however, is that individual differences in stressor-evoked BP reactivity are solely attributable to interindividual variation in self-reported levels of distress or negative emotionality, because subjective ratings of stress, emotionality, and arousal are weakly (and rarely significantly) associated with BP reactivity in laboratory studies.$^{10,34}$ In agreement, individual differences in stressor-evoked BP reactivity were not significantly correlated with task-related unpleasantness, arousal, or perceived control ratings in this study, nor were the prospective associations between resting corticolimbic activity and stressor-evoked BP reactivity accounted for by such ratings. Finally, self-reported ratings of dispositional negative emotionality (trait anxiety and hostility) and recent levels of perceived life stress were also not significantly correlated with stressor-evoked BP reactivity in this sample (Table S1). Hence, one interpretation of our findings in synthesis with previous work is that a tendency to express exaggerated stressor-evoked BP reactivity could arise in part from interacting central and peripheral neurobiological factors that are subject to genetic modification and relatively independent of subjective (consciously reportable) states of stress, arousal, or emotionality. In this regard, the present findings and those from previous neuroimaging studies may specifically suggest that neurobiological measures, including rCBF and functional connectivity measures, may have advantages over subjective rating measures in understanding the origins of individual differences in stressor-evoked BP reactivity.

To elaborate, it is noteworthy that acute stressors have been demonstrated in previous neuroimaging studies to evoke patterns of neural activity in the dACC, pACC, mPFC, and insula that covary reliably with concomitant changes in BP.$^{14,16-18}$ There is cumulative human and nonhuman animal evidence that these corticolimbic areas are anatomically networked and that they are instrumental for regulating autonomic, neurohormonal, and cardiovascular reactions to stressors, presumably in support of adaptive behavioral action (eg, the canonical “fight-or-flight” response).$^{13,19,20}$ In previous studies, however, measures of corticolimbic activity and BP reactivity were examined concomitantly and exclusively during stressor exposure. As a result, it was unknown whether resting (prestressor) patterns of corticolimbic activity would prospectively predict individual differences in subsequently evoked BP reactivity. Thus, extending previous work, the present findings indicate that corticolimbic activity relates to individual differences in stressor-evoked BP reactivity, even when this activity is measured before stressor exposure. In extension, the cross-correlational (connectivity) findings illustrated in Figure 2 suggest that activity in the dACC, pACC, mPFC, and insula is likely to be functionally integrated within a broader neural circuitry. This notion is consistent with invasive human and nonhuman animal studies that have demonstrated functional and anatomic connections between these corticolimbic areas, which are important for autonomic, neurohormonal, and cardiovascular regulation.$^{19,21,35-40}$ In addition, it is notable that 2 recent neuroimaging studies$^{18,41}$ have specifically demonstrated that exaggerated stressor-evoked BP reactivity and increased carotid intima-media thickness, an indicator of preclinical atherosclerosis linked to stressor-evoked BP reactivity,$^8$ are both associated with a dysregulated pattern of functional connectivity between the pACC and one of its subcortical projection sites involved in cardiovascular regulation: the amygdala. In view of these recent studies and the present study, we are currently testing whether particular patterns of resting state connectivity (eg, time-lagged, directional, or multivariate) exhibited among the pACC, the other corticolimbic areas identified here, and their subcortical targets specifically predict individual differences in stressor-evoked BP reactivity or covary with associated indicators of cardiovascular risk.

Although novel, we appreciate both inferential limitations and unresolved questions raised by the present study. First, by testing younger men and women in good cardiovascular health, extrapolations to older and more heterogeneous samples are precluded. Second, by measuring resting perfusion...
on just one occasion that involved later stress testing, conjectures that relatively heightened resting corticolimbic activity may predispose toward exaggerated stressor-evoked BP reactivity remain tenuous, particularly until individual differences in resting corticolimbic activity (eg, perfusion and functional connectivity) are shown to be reproducible over multiple occasions that do and do not involve later stress testing. Third, by using a study design that did not include assessments of family history of hypertension or cardiovascular disease or other familial assessments permitting inferences regarding genetic influences on our findings, predictions about whether resting corticolimbic activity predicts BP reactivity in part via heritable and risk-related factors are largely premature. Finally, by not measuring preclinical cardiovascular disease indicators or cardiovascular risk factors that have been associated with stressor-evoked BP reactivity, assumptions that our findings are relevant to clinical risk remain untested.

**Perspectives**

To end, individuals expressing relatively exaggerated stressor-evoked BP reactivity also expressed heightened resting rCBF in the cingulate, medial prefrontal, and insular cortices, corticolimbic brain areas that also exhibited functional connectivity with each other. Collectively, these findings could be interpreted from a neurobiological perspective, which views resting measures of rCBF and functional connectivity as corresponding in part with the level of metabolic “preparedness” of functionally related brain areas to respond to future cognitive, emotional, or otherwise behaviorally salient stimuli. In particular, resting rCBF in the cingulate, medial prefrontal, and insular cortices (along with the degree of their functional connectivity) may partially correspond with their “preparedness” to respond to behaviorally salient stressor stimuli and communicate with subcortical cell groups that are involved in regulating BP. These notions could be tested empirically by determining whether and how measures of resting corticolimbic activity and connectivity relate to patterns of neural activity elicited directly by behaviorally salient stressors that evoke BP reactivity. Arguably, testing such notions may aid in further explicated the neurobiological factors and neural circuitries that predispose some individuals toward exaggerated stressor-evoked BP reactivity and perhaps related cardiovascular risk.

**Acknowledgments**

We thank Qi Shi for his technical assistance and Dr Jeanne McCaffery for her constructive comments on a draft of this article.

**Sources of Funding**

This work was supported by National Institutes of Health grants KO1-MH070616 and RO1-HL089850, the Pittsburgh Mind-Body Center (HL076852/076858), and a Commonwealth Universal Research Enhancement Grant from the Pennsylvania Department of Health.

**Disclosures**

None.

**References**


Heightened Resting Neural Activity Predicts Exaggerated Stressor-Evoked Blood Pressure Reactivity

Peter J. Gianaros, Lei K. Sheu, Allison M. Remo, Israel C. Christie, Hugo D. Critchley and Jiongjiong Wang

Hypertension. 2009;53:819-825; originally published online March 9, 2009; doi: 10.1161/HYPERTENSIONAHA.108.126227

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/53/5/819

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2009/03/09/HYPERTENSIONAHA.108.126227.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
Online Supplement

*Heightened resting neural activity predicts exaggerated stressor-evoked blood pressure reactivity*

Peter J. Gianaros¹, Lei K. Sheu¹, Allison M. Remo¹, Israel C. Christie¹, Hugo D. Crtichley², and Jiongjiong Wang³

¹Department of Psychiatry, University of Pittsburgh, 3811 O'Hara Street, Pittsburgh, PA 15213.
²Department of Psychiatry, Brighton and Sussex Medical School, University of Sussex Campus, Falmer, Brighton BN1 3AR, UK.
³Center for Functional Neuroimaging, University of Pennsylvania, 3W Gates, 3400 Spruce Street, Philadelphia, PA 19104.
Table S1. Summary of participant characteristics and screening procedures.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean or (%)</th>
<th>SD or Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Body mass index (kg/m^2)</td>
<td>23.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Number of school years completed</td>
<td>16.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Seated Resting SBP (mmHg)</td>
<td>117.1</td>
<td>9.7</td>
</tr>
<tr>
<td>Seated Resting DBP (mmHg)</td>
<td>65.8</td>
<td>8.8</td>
</tr>
<tr>
<td>Smoking Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>10.3%</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>12.8%</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>76.9%</td>
<td></td>
</tr>
<tr>
<td>Measures of negative emotionality and life stress</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spielberger State-Trait Anxiety Inventory (STAI-T)</td>
<td>32.7</td>
<td>7.0</td>
</tr>
<tr>
<td>Cook-Medley Hostility Scale (total score)</td>
<td>15.6</td>
<td>6.4</td>
</tr>
<tr>
<td>Perceived Stress Scale</td>
<td>12.5</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Note. Participants (n=39) were right-handed, as verified by a self-report inventory. Thirty-six (92%) reported being of Caucasian descent; the remaining 3 participants reporting being of mixed African-American or Asian descent. Screening to exclude those with any self-reported history of a cardiovascular or psychiatric disorder was determined initially by phone and verified in-person by interview prior to testing (interview conducted by AMR, third author). Screening for current suspected psychiatric syndromes, including somatization disorder, major depression, a depressive syndrome, panic or other anxiety disorders, and alcohol dependence, was achieved by the Patient Health Questionnaire (PHQ)\textsuperscript{2}, an instrument validated in outpatient\textsuperscript{3} and community samples\textsuperscript{5} for sensitivity and specificity against the Diagnostic and Statistical Manual of Mental Disorders IV\textsuperscript{6}. Prior to testing, participants also completed inventories to assess dispositional anxiety and hostility, as well as recent levels of life stress experienced in the past month. These inventories included the trait version of the Spielberger State-Trait Anxiety Inventory (STAI-T)\textsuperscript{7}, the Cook-Medley Hostility Scale\textsuperscript{8, 9}, and the Perceived Stress Scale (10 item version)\textsuperscript{10}. Scores on these inventories did not correlate significantly with resting SBP or DBP or with stressor-evoked SBP or DBP reactivity (r’s ranged from -0.04 to 0.22, all \(P \geq 0.17\)). Finally, participants reported consuming a median of 2.1 alcoholic beverages per week (range = 0-11.7). In this sample, neither alcohol consumption nor smoking status [see table above for coding] correlated with resting SBP or DBP or with stressor-evoked SBP or DBP reactivity (Spearman rho’s range: -0.04 to 0.22, all \(P \geq 0.17\)).

References for Table S1


Table S2. Summary of individual 2-step hierarchical regression models predicting systolic (SBP) and diastolic (DBP) blood pressure reactivity by regional cerebral blood flow (rCBF) to the dorsal anterior cingulate cortex (dACC), perigenual anterior cingulate cortex (pACC), medial prefrontal cortex (mPFC), and insula after family-wise error rate (FWE) correction and step-1 control for total resting CBF, resting BP*, task accuracy, and task ratings of valence, arousal, and control.

<table>
<thead>
<tr>
<th>Variables on step</th>
<th>SBP Reactivity Models</th>
<th></th>
<th>DBP Reactivity Models</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Step 1</td>
<td>Step 2</td>
<td></td>
<td>Step 1</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>$P$</td>
<td>$\Delta R^2$</td>
<td>$P_{\text{uncorrected}}$</td>
</tr>
<tr>
<td>Resting CBF, Resting BP, Accuracy, Valence, Arousal, Control</td>
<td>0.1422</td>
<td>0.5179</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dACC rCBF</td>
<td></td>
<td></td>
<td>0.2283</td>
<td>0.0021</td>
</tr>
<tr>
<td>pACC rCBF</td>
<td></td>
<td></td>
<td>0.2182</td>
<td>0.0028</td>
</tr>
<tr>
<td>mPFC rCBF</td>
<td></td>
<td></td>
<td>0.2756</td>
<td>0.0006</td>
</tr>
<tr>
<td>Insula rCBF</td>
<td></td>
<td></td>
<td>0.1732</td>
<td>0.0087</td>
</tr>
</tbody>
</table>

Note. Eight individual 2-step hierarchical regression models were executed, using rCBF from the dACC, pACC, mPFC, and insula as step-2 predictors of SBP reactivity (4 models) and DBP reactivity (4 models). To control for multiple statistical testing across the 8 models, the Type-1 family-wise error rate was maintained by adjusting the $P$-values for the step-2 $\Delta R^2$ with Holm’s sequential implementation of Bonferroni correction$^1, 2$. For models predicting SBP reactivity, resting SBP was included as a step-1 covariate; for models predicting DBP reactivity, resting DBP reactivity was included as a step-1 covariate.

References for Table S2

Figure S1. At right are sample trials from the congruent (panel A) and incongruent (panel B) conditions of the Stroop color-word task and the congruent (panel C) and incongruent (panel D) conditions of the multi-source interference task (MSIT). In both tasks, participants completed 4 blocks of trials defining a congruent condition, which were alternated with 4 blocks of trials defining an incongruent condition. In the Stroop task, participants identified the color of a target word presented in the center of a visual display on each trial by selecting 1 of 4 identifier words presented at bottom. Participants made their selection by pressing 1 of 4 buttons on a response glove, where each button corresponded to the location of the identifier word on the display (e.g., thumb button 1 = identifier word to the participant’s far left; ring finger button 4 = identifier word to the far right). For all trials in the congruent Stroop condition: (1) the target word appeared in a color that was congruent with the target word, and (2) all identifiers appeared in the same color as the target. To illustrate: In panel A, the target word ‘red’ appears in the color red, as do the identifiers. The correct response would be to select the word ‘red’ at bottom by pressing button 1 on the response glove. For all trials in the incongruent Stroop condition: (1) the target word appeared in a color incongruent with the target word, and (2) all identifiers appeared in colors incongruent with the colors that the identifier words named. To illustrate: in panel B, the target word ‘blue’ is shown in the color red, and all identifier words are shown in colors that are different from the colors that the identifier words name. Here, the correct response again would be to select the word ‘red’ at bottom by pressing button 1 on the glove.

In the MSIT, participants identified the number that was different from 2 other numbers in a visual display by pressing 1 of 3 buttons on the response glove. In this task, the buttons on the glove corresponded to a specific number in the display (thumb finger button 1 = number 1, index finger button 2 = number 2, middle finger button 3 = number 3). For all trials in the congruent condition, the target number in the display appeared in a location that was compatible with its location (position) on the glove. To illustrate: in panel C, the target number 1 is shown to the far left, requiring the participant to press the thumb button 1 on the response glove. For all trials in the incongruent condition, the target number appeared in a position that was incompatible with its spatial position on the response glove. To illustrate: in panel C, the number 3 appears in the middle of the display (second position in the row), requiring the participant to suppress the tendency to press the index finger button 2 and instead press the middle finger button 3 on the glove.

During the incongruent condition of both tasks, each participant’s accuracy at target word (Stroop) and number (MSIT) identification was titrated to and maintained at ~50% by adjusting the inter-trial intervals (ITI). Specifically, more accurate performance within a given incongruent condition prompted shorter ITIs and a shorter time in which to respond; conversely, less accurate performance lengthened the ITIs. To control for motor response differences between the incongruent and congruent conditions in both tasks, the number of trials presented in the congruent condition was yoked to the number of trials completed in the incongruent condition. To implement this yoking procedure, (1) a block of incongruent trials was administered first, and (2) congruent condition trials were administered at an ITI that was determined by the participant’s mean ITI of the preceding incongruent block. Consequently, the number of trials completed during the congruent condition was matched to the number completed in the incongruent condition in both tasks. Participants received task instructions and practiced both tasks on a computer before MRI scanning, but performance was not titrated during practice. Also, participants were not informed that performance would be titrated in the incongruent condition during the MRI protocol. During task performance in the MRI protocol, blood-oxygen level-dependent imaging data, which were outside the focus and space limitations of the present report, were collected.
Figure S2. Subjective reports of valence (panel A), arousal (panel B), and perceived psychological control (panel C) were obtained during the MRI protocol using a 9-point rating scale, termed the Self-Assessment Manikin (SAM). For valence ratings summarized in panel A, participants viewed a horizontal row of figures (shown along the ordinate), with an arrow beneath each figure. Participants used a response glove to move the arrow beneath the version of the figure that corresponded to how ‘happy’ or ‘unhappy’ they felt (1) after the 20 min resting (pre-stressor) period, (2) after the Stroop task, (3) after the multi-source interference task (MSIT), and (4) after a 10 min recovery period following the last completed task. Valence ratings ranged from 1 (very unhappy; SAM is frowning) to 9 (very happy; SAM is smiling). For arousal ratings summarized in panel B, participants viewed a horizontal row of SAM figures (shown along the ordinate), with an arrow beneath each figure. Participants used the response glove to move the arrow beneath the version of the figure that corresponded to how ‘aroused’ they felt after (1) the resting period, (2) the Stroop task, (3) the MSIT, and (4) the recovery period. Arousal ratings ranged from 1 (very calm; SAM is relaxed) to 9 (very aroused; SAM is excited). For perceived psychological control ratings summarized in panel C, participants viewed a horizontal row of SAM figures (shown along the ordinate), with an arrow beneath each figure. As for the other ratings, participants used the response glove to move the arrow beneath the version of the figure that corresponded to how much ‘control’ they felt after (1) the resting baseline, (2) the Stroop task, (3) the MSIT, and (4) the recovery period. Control ratings ranged from 1 (very little control; SAM is small) to 9 (very much in control; SAM is large). For all ratings, participants rated how they felt during the period preceding the ratings assessment period. In panel D, task accuracy (computed as the percentage of trials correctly completed) is illustrated for the incongruent condition (IC) and congruent (C) condition of each task.

**P≤0.001**
Figure S3. Illustration of routines for blood-oxygen level-dependent (BOLD) signal extraction, signal filtering, pair-wise cross-correlation analyses, and confidence-interval estimation. Panel A shows the 4 regions of interest (ROIs) for which a mean BOLD signal time-series was extracted for each individual. These ROIs included the dorsal anterior cingulate cortex (dACC), perigenual anterior cingulate cortex (pACC), medial prefrontal cortex (mPFC), and insula. Panel B shows the raw (uncorrected) BOLD signal time-series for each ROI for a single participant selected at random. For each participant, each time-series was corrected and filtered according to the procedures described in the Methods section. To illustrate: Panel C shows the corrected and filtered time series from Panel B. For each individual, pair-wise cross-correlation coefficients (r-values) were computed across the 4 ROIs using the corrected and filtered time-series. After this cross-correlation step, the computed cross-correlation coefficients were transformed to Fisher z-values to approximate distributional normality, where $z = 0.5(1+r)/(1-r)$. These z-values were averaged across participants for each pair-wise cross-correlation analysis for the 4 ROIs, generating 6 participant-averaged values. These values are taken to reflect the average ‘functional connectivity’ between each pair of ROIs. To aid interpretability, z-values were back-transformed to r-values. To assess the reliability of these r-values across participants, we used a bootstrap sampling procedure to generate the frequency distributions of the pair-wise cross-correlation coefficients shown in Panels D-I. From these distributions, we determined the 99% confidence intervals of each average cross-correlation coefficient by determining those coefficients at the 1% and 99% quintiles. The 99% CIs are illustrated by a green horizontal line in the frequency distributions of Panels D-I.
Figure S4. Average systolic blood pressure (SBP) and diastolic blood pressure (DBP) are shown in panels A and B, respectively, for the resting (pre-stressor) period, the incongruent condition of the Stroop task, the incongruent condition of the multi-source interference task (MSIT), and a final recovery period that followed the completion of the tasks.

* $P \leq 0.005$

** $P \leq 0.001$