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Sex Differences in the Neural Correlates of Autonomic Arousal: A Pilot PET Study

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Abstract

Electrophysiology, behavioral, and neuroimaging studies have revealed sex-related differences in autonomic cardiac control, as reflected in measurements of heart rate variability (HRV). Imaging studies indicate that the neurobiological correlates of autonomic nervous system (ANS) function can be investigated by measuring indices of HRV during the performance of mildly strenuous motor tasks or mildly stressful cognitive tasks. In this preliminary study, fifteen male and seven female healthy subjects underwent H₂¹⁵O-positron emission tomography (PET) and electrocardiograph (ECG) recording while performing a handgrip motor task and an n-back task. Indices of HRV were calculated and correlated with regional cerebral blood flow (rCBF). We hypothesized that sex differences would be evident in brain regions known to participate in autonomic regulation: the anterior insula, the anterior cingulate cortex, the orbitofrontal cortex, and the amygdala. Our study found that associations between rCBF and parasympathetic indices differed significantly between female and male subjects in the amygdala. Females showed a positive correlation between rCBF and parasympathetic indices while males exhibited negative correlations. This differential correlation of amygdala rCBF and parasympathetic activity between males and females may reflect differences in parasympathetic/sympathetic balance between sexes, consistent with known sexual dimorphism in the amygdala and closely related structures such as the hypothalamus. These preliminary imaging results are consistent with earlier reports of significant correlation between brain activity and HRV, and extend these findings by demonstrating prominent sex differences in the neural control of the ANS. While the generalizability of our results was limited by the small size of the study samples, the relatively robust effect size of the differences found between groups encourages further work in larger samples to characterize sex differences in the neural correlates of autonomic arousal.

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Keywords

autonomic; HRV; PET; sex; amygdala

1. Introduction

Numerous studies in humans suggest differences in autonomic cardiac control between the sexes. For instance, under arterial occlusion, females are more likely to exhibit bradycardia and a lowering of blood pressure, an essentially cardioprotective response, than males (Airaksinen et al., 1998). Under ischemic challenge, males exhibit a pressor response, while females exhibit either a decrease or no change in arterial pressure (Maixner and Humphrey, 1993). Females also exhibit blunted increases in mean arterial pressure and muscle sympathetic nerve activity during a head-up tilt postural challenge as compared to men (Shoemaker et al., 2001). In males, an acute lower body negative pressure (LBNP) challenge, which acutely lowers blood pressure in the upper body, activates baroreceptors, induces catecholamine release, and activates pressor responses, whereas females show an attenuated physiological response (Kimmerly et al., 2007). Taken together, these reports and others indicate greater reactivity of the parasympathetic system in females, and greater reactivity of the sympathetic nervous system in males (reviewed in (Dart et al., 2002)).

Interestingly, the sex differences in autonomic response to LBNP cited above were associated with differences in hemodynamic response measured with functional magnetic resonance imaging (fMRI). Men showed larger increases than women in blood oxygen level dependent (BOLD) contrast during the LBNP challenge in the right insula and dorsal anterior cingulate, and also showed larger decreases than women in BOLD contrast during the challenge in the right amygdala, left insula, ventral anterior cingulate, and ventral medial prefrontal cortex. A recent functional MRI study by Wong, et al. found that during the performance of a handgrip task, women exhibited less activation in the insular cortex and dorsal cingulate than men. In the same study, heart rate (HR) and mean arterial pressure responses during the handgrip task were measured in a separate session and also found to be blunted in the women as compared to the men (Wong et al., 2007). The functional anatomical basis for sex-differences in autonomic regulation is rarely investigated, however, as most previous neuroimaging studies either limited their subject samples to one sex (Critchley et al., 2000; Lane et al., 2001), or did not directly assess sex effects in samples which combined males and females (Critchley et al., 2003; Matthews et al., 2004; Neumann et al., 2006).

Neuroimaging studies not examining sex implicate a specific network of regions involved in autonomic control, although distinct regions may be more involved in specific behavioral domains. Hemodynamic activity in the anterior cingulate cortex (ACC) and insula correlates with autonomic responses in a variety of behaviors (reviewed in (Critchley, 2005)). The ACC appears to be directly involved in autonomic control, although whether it generates or simply reflects autonomic state has not been established. It has also been suggested that the insula stores a visceral representation of the current autonomic state, particularly in response to emotional stimuli (Critchley, 2005). Similarly, the orbitofrontal cortex (OFC) appears to support a representation of autonomic state during motivational tasks (reviewed in (Nagai et al., 2004)). Neurophysiological activity in the amygdala strongly correlates with autonomic responses during highly emotional situations, such as fear conditioning (reviewed in (Critchley et al., 2005)). Additionally, multiple lines of research in experimental animals implicate the amygdala in sympathetic activation occurring in response to acute stress (reviewed in (Salome et al., 2007)). Furthermore, both animal and human lesion studies have shown that subjects with amygdalar lesions exhibit blunted autonomic responses to stress

(reviewed in (Davis and Whalen, 2001)). Finally, retrograde labeling studies in experimental animals, implicate the amygdala, along with cingulate gyrus, insular cortex, and orbitofrontal cortex, as regions involved in both parasympathetic and sympathetic cardiac control (Ter Horst et al., 1996; Westerhaus and Loewy, 2001).

There is substantial evidence from the literature indicating that mildly effortful or stressful tasks also result in autonomic arousal, or activation of the ANS, and activation of brain areas known to be involved in autonomic control in humans. For example, activity in the amygdala has been associated with autonomic function during performance of a handgrip task, (Napadow et al., 2008), while activity in the ACC, OFC, and insula has been associated with cardiovascular arousal during both a handgrip task and an n-back cognitive task (Critchley et al., 2003). Given these results, we hypothesized that differences in autonomic responses between sexes would be related to underlying differences in activation in the ACC, insula, OFC, and amygdala.

Of these regions, the amygdala is a known site of sexual dimorphism (for example, (Cooke and Woolley, 2005; Simerly, 2002; Stefanova and Ovtcharoff, 2000)). Studies in rats have shown concentrations of estrogen receptors in the amygdala (Milner et al., 2008), while neuroimaging studies in humans have demonstrated alterations of amygdalar connectivity in response to testosterone (van Wingen et al.). The hypothesis that sexual dimorphism in the amygdala may influence autonomic control is also supported by Labus, et al. who found sex-related differences in amygdalar effective connectivity in response to aversive rectal distension in patients with irritable bowel syndrome (Labus et al., 2008). Projections to the central nucleus of the amygdala (ACe) are modulated by both vasopressin (involved in anxiety, stress, and aggression) and oxytocin (involved in bonding and trust behaviors) (Debiec, 2005; Raggenbass, 2008). Vasopressin has been hypothesized to play a role in mediating the “fight or flight” response pattern seen particularly in men in response to stress, while oxytocin has been hypothesized to play a role in mediating the “tend and befriend” reaction seen more often in women in response to stress. It thus may be hypothesized that alterations in amygdalar function may underlie differential responses to stress between sexes (Taylor et al., 2000).

In the current pilot study, we investigated autonomic function in men and women by measuring indices of heart rate variability (HRV). A spectral decomposition of an ECG time series can be divided into frequency bands. The high frequency (HF) component, which is mediated almost entirely by parasympathetic activity transmitted via the vagus nerve (Akselrod et al., 1985; Pomeranz et al., 1985) is calculated as the total spectral power between 0.15 and 0.5 Hz. The peak frequency within this band, fHF, is proportional to the respiratory frequency (Thayer et al., 2002; Yildiz and Ider, 2006). The low frequency component (LF), which partially reflects sympathetic tone (Malliani and Pagani, 1991; Malliani et al., 1994), but is dominated by parasympathetic tone (Thayer et al., In Press) and respiration (Yildiz and Ider, 2006), is calculated as the total power between 0.01 to 0.15 Hz. The ratio LF/HF is often used as a measure of the relative balance of sympathetic and parasympathetic tone (Malliani et al., 1990), although it is an imperfect measure since LF and HF are often highly correlated. The time domain parameter RMSSD is also used as a measure of parasympathetic tone, and is calculated as the root mean squared difference of successive R-R intervals as a measure of beat-to-beat variability (Bigger et al., 1993, 1996). Thus, indices of HRV can be used to examine both parasympathetic and, albeit imperfectly, sympathetic tone, and these measures can be correlated with imaging data to explore relationships between brain activity and autonomic control.

To test the hypothesis that the regions described above as part of our *a priori* hypothesis play a role in sex differences in ANS function, we measured indices of heart rate variability

(HRV) to assess autonomic arousal, while also measuring regional cerebral blood flow (rCBF) using [^{15}O]H $_2$ O-positron emission tomography (PET) in 15 male and 7 female healthy subjects. We obtained these measures both at baseline and during the performance of motor and cognitive tasks to examine the differential autonomic and neurophysiological responses to stress. Although the small sample sizes restrict the weight of our conclusions, this is the first study to examine central gender differences in autonomic control in response to mild stress using neuroimaging.

2. Materials and Methods

2.1 Subjects

Participants included twenty-two right-handed (one female self-identified as having “mixed handedness”), medically healthy individuals (N=22), of which fifteen were male (N=15), and seven were premenopausal females (N=7). Of the male subjects, 11 (73%) were Caucasian, with the remaining being African American (N=3, 20%) or Hispanic (N=1, 7%). Of the female subjects, 4 (57%) were Caucasian, with the remaining being African American, Hispanic, or Asian (N=1, 14.3%, in each of the three groups). Subjects were recruited through IRB approved print advertisements. Although data was not collected on all subjects, men and women for which data was collected did not differ on IQ (N=13) or socio-economic status (N=14).

Physiologic data on the subjects appears in Table 1, including body mass index, blood pressure, and performance on a walk-run task (see below). Subjects underwent full history and physical examinations, a non-structured psychiatric evaluation by a psychiatrist, a structured interview with a clinician (structured interview for DSM-IV; SCID), laboratory testing (complete blood count, electrolytes, liver and thyroid function tests), ECG, and neuromorphological MRI. Exclusion criteria included having cardiovascular disease, history of cardiac arrhythmias, anemia, electrolyte disturbances, ECG abnormalities, exposure to medications (except nicotine) likely to affect CBF or cardiovascular function in the two weeks prior to scanning, history of a major psychiatric disorder, meeting DSM-IV criteria for drug or alcohol abuse within 1 year, or lifetime history of alcohol or drug dependence. Pregnant or lactating women, women with irregular menstrual cycles, and post- or perimenopausal women also were excluded (based on subject self-report). Of the female participants, four were studied in the follicular phase of the menstrual cycle, two in the luteal phase, and one during menses (menstrual phase was assessed using a home ovulation test and self-report for data of most recent menses). Two males and two females were current smokers, and one male and one female were past smokers. A subset of subjects (8 males and 5 females) participated in a walk/run task to assess general fitness levels. Subjects were given 12 minutes in which they were asked to give maximum effort in walking or running to cover as much distance as possible. Results are given (in meters) in Table 1. The study was approved by the NIMH Institutional Review Board and all subjects gave written, informed consent.

2.2 Study Design and Tasks

Neurophysiological measures were obtained using the PET-rCBF technique, and electrophysiological measures were obtained simultaneously using ECG. While undergoing PET and ECG, subjects either rested with eyes fixed on a cross-hair, or performed a working memory or motor task. These particular tasks were chosen because both have been shown to cause autonomic arousal, and have previously been used in neuroimaging studies of cardiac control (Critchley et al., 2003; Wong et al., 2007). For both tasks, subjects underwent training trials before initiation of the scanning session. Each task was performed in a blocked design, where each block was one of the three tasks at one of the difficulty or

intensity levels. Each block lasted 2.5 minutes, and was followed by a 7.5 minute rest period prior to the next block (to allow for decay of the infused ^{15}O radioactivity).

In the baseline fixation task, subjects were asked to fix their eyes on a cross-hair presented on a video monitor; the 2.5 minute baseline fixation task block was presented twice. In the motor task, (adapted from Cramer et al. (Cramer et al., 2002)), subjects were asked to squeeze a handgrip dynamometer (Interface, Inc. Scottsdale, AZ) with their dominant hand to match an intensity target, according to task instructions and performance feedback displayed on the same video monitor. Each handgrip block consisted of ten epochs lasting 15 seconds during which the subjects squeezed for 9 seconds, and then relaxed for 6 seconds. Targets were set at three intensity levels (10, 30, and 50% of each subject's maximal effort), each presented twice, for a total of six blocks (only one strength level was targeted within an individual block). The order of blocks for each intensity level was counterbalanced across subjects.

The cognitive task consisted of a working memory n-back task (1-back, 2-back, and 3-back), after Critchley et al. (Critchley et al., 2003). Within each task block, pseudo-random sequences of consonant letters were presented for 500msec, followed by a 2.5 second inter-trial interval during which subjects viewed a fixation cross-hair. The target stimulus consisted of a letter identical to the letter presented one, two, or three trials before, for the 1-back, 2-back, and 3-back conditions, respectively. Subjects were instructed to respond to target stimuli with their right hand using a button box. Each block consisted of 50 stimuli, 33 percent of which were target stimuli. Each difficulty level was presented twice for a total of six blocks. The order of blocks for each difficulty level was counterbalanced across subjects.

2.3 Behavioral Assessments

During the handgrip task, performance was calculated by measuring the area under the grip intensity vs. time curve. The parameter "handgrip accuracy" was calculated as the deviation of the ratio of actual grip strength and target grip strength from one; thus, "perfect" performance would give a handgrip accuracy of zero (since maximal grip strength was calibrated to each subject individually, this a measure of fine motor control, rather than strength). During the n-back task, "n-back accuracy" was calculated as the sum of the correct responses minus the total number of incorrect responses, divided by the total number of trials.

2.4 Autonomic measurements

The ECG data were acquired throughout scanning at 500 Hz using a 1902 amplifier and Power1401 data acquisition interface (Cambridge Electronic Devices, Cambridge, UK). Spike2 software (Science Products GmbH, Hofheim, Germany) was used to detect time points for individual R-spikes, which were marked as digital events. From R spikes acquired during the final 120 seconds of each behavioral block (which corresponded to the period of actual CBF measurement), we extracted a time series of inter-beat intervals (IBIs). HRV indices, including LF, HF, fHF, and RMSSD, were computed according to published guidelines (Electrophysiology, 1996). From these, we calculated natural logs of total low and high frequency power (lnHF and lnLF) and the ratio LF/HF

2.5 PET Image Acquisition

Subject preparation for PET scanning included intravenous cannulation and head stabilization using a thermoplastic mask. Scans were acquired using a GE Advance (35 contiguous slices with 4.25mm plane separation, 3D resolution=6 to 7mm FWHM, 3D acquisition mode). Transmission scans (about 8 min) were acquired using rotating rods

of $^{68}\text{Ge}/^{68}\text{Ga}$ for attenuation correction. Emission images then were acquired, following H_2^{15}O administration during each of 14 behavioral blocks. Slow infusions of H_2^{15}O were initiated 30 seconds before scanning and continued for 120 seconds, with a total of 10 mCi delivered to the subject during each scan. The behavioral block and the infusion were initiated simultaneously, and the rCBF scanning window occurred between the 30 sec and 150 sec time points within the 150 second behavioral block.

2.6 Statistical Analysis

Demographic variables and performance on the walk run task were analyzed using independent samples t-tests. For the electrocardiographic analysis, autonomic variables were analyzed using a repeated-measures, mixed-model (PROC MIXED) in SAS (SAS Institute, Cary NC), with difficulty level (n-back) or intensity level (handgrip) and sex as fixed-effect factors, and age and fHF (proportional to respiratory rate) included as covariates. Subject was included in the model as a random effect. An unstructured covariance matrix was used, and group was incorporated into the covariance matrix when modeling autonomic variables in which variances differed significantly across sex. This directly models non-sphericity, thus eliminating the need to perform an additional Greenhouse-Geisser correction on the results. Degrees of freedom were calculated using the Kenward-Roger method, a method designed to produce accurate inferences on small samples (Kenward and Roger, 1997), and shown to be robust to violations of normality (Kowalchuk et al., 2004). To assess the relationship between performance and autonomic activity, the mixed model was repeated with the accuracy measure entered as a covariate. Each task (rest, n-back, and handgrip) was assessed separately in order to maximize power. This is justified by the literature (presented in the discussion section) finding autonomic differences in a variety of mildly stressful or autonomically arousing tasks, as well as at baseline.

Image analysis was performed using SPM2 (Wellcome Department of Imaging Neuroscience, University College London, London, UK) within Matlab 6 (Mathworks, Natick, MA, USA). PET images were realigned, and then co-registered to structural T_1 MRI scans. All MRI scans were acquired at 3 Tesla except one (1.5T), using MP-RAGE or IR-fSPGR pulse sequences; all images were acquired with 0.86mm in-plane resolution and a 1.2mm slice thickness. The accuracy of co-registration to the PET images was indistinguishable between these two MRI scan types. The MRI scans were spatially normalized to a template brain, and the resulting transformation then was applied to the co-registered PET images. A separate statistical model was constructed for each each autonomic index. Within each model, we included six factors, one for each task within each sex group, while the autonomic variable and difficulty level (n-back) or intensity level (handgrip) were modeled as covariates. The covariates for task difficulty level (n-back) or intensity level (handgrip) and autonomic variable were allowed to interact by condition, so that the correlation between rCBF and autonomic variable was estimable for each condition and sex separately. Age and fHF were included as potential confounding variables. Within the framework of the SPM2 software, all regressors are considered to be fixed effects. Each repetition was entered into the model as a single observation. Regressors were also included to model subject effects. Subject grand means were scaled to 50 within group, and global normalization was performed using an AnCova by subject (within group).

Contrasts for positive and negative correlations between rCBF and each autonomic index during the performance of each task were calculated for the males and females separately. Additional contrasts were performed to show areas where the correlation between rCBF and the autonomic index differed significantly between males and females. Regression coefficients, which are the extracted beta weights from the SPM2 general linear model, are reported. For the purpose of comparison with mixed-sex studies in the literature, contrasts

including effects in the combined sample of males and females also were constructed *post hoc*. Results of these analyses appear in the supplementary materials.

To reduce the likelihood of Type-I error, the search area included in the primary analysis was limited to regions of interest (ROI) defined *a priori* on the basis of previous literature. The ROI selected were the left and right dorsal and anterior cingulate cortices, anterior insula, amygdala, and OFC. ROI's were defined by hand-tracing of the region on the MRI template used for spatial normalization. Hand-traced ROI were used instead of atlas-based ROI in order to target particular sub-regions of the structures of interest. The anterior insula ROI encompassed the agranular insula, which includes the sulcal portion of lateral OFC (LOFC, Brodmann Area (BA) 47s, see (Ongur et al., 2003)). Small volume corrections (Worsley et al., 1996) were applied to adjust the results of the SPM analyses for the effects of multiple testing. As an additional control against type I error, we only report results for voxels significant at $p_{\text{corrected}} < 0.00625$ (calculated as 0.05 divided by 8, the number of ROI defined *a priori*).

To reduce the likelihood of Type-II error, areas located outside the predefined ROI were explored in whole brain analyses conducted *post hoc*. For each contrast, t-images were displayed with a threshold of $p_{\text{uncorrected}} < 0.05$. Gaussian random field theory was used to identify clusters which remained significant at $p_{\text{corrected}} < 0.05$ after applying the cluster test to correct for multiple comparisons (Poline et al., 1997). Within each significant cluster, the coordinates of the voxel with the highest t-value within the cluster were reported. Family-wise error corrected p-values also were reported for voxels for which the voxel t-value corresponded to $p_{\text{corrected}} < 0.05$. A conjunction analysis of the handgrip and n-back contrasts was performed to assess the significance across both tasks.

Results for male and female subjects together appear in the supplementary materials to permit comparison with the results of previous studies. Additionally, post-hoc within group whole-brain analyses also are included within the supplementary materials. To address our primary aim, only analyses examining *a priori* regions or contrasting males and females directly are presented in the main body of the manuscript.

Finally, to ensure that any differences noted between groups in the correlation between rCBF and autonomic activity were not due to nonspecific global differences in the absolute rCBF between male and female subjects, we averaged across task difficulty (n-back) or intensity level (handgrip) and used a two-sample t-test in SPM to assess group differences. Subject grand means were scaled to 50 within group, and global normalization was performed using proportional scaling.

3. Results

3.1 Behavioral Results

Demographic data for subjects appear in Table 1; no significant difference was found between males and females in BMI, age, or diastolic blood pressure. Male subjects had a significantly higher systolic blood pressure ($t_{20}=4.11$, $p=0.0005$), and traversed a significantly greater distance on the walk-run task (equal variances not assumed, $t_{10.8}=2.29$, $p=0.044$). On the handgrip task, males performed more accurately than females ($F_{1,61}=10.23$, $p=0.0022$). On the n-back task, accuracy did not differ significantly between groups ($p>0.1$).

3.2 Baseline Fixation Task Results

In the mixed model analysis for the fixation condition, females showed greater HR than males ($F_{1,18}=13.83$, $p=0.0016$). No other baseline differences were evident between groups

(See Figure 1). During the fixation condition (Table 2), males showed inverse correlations between HR and rCBF in the right lateral OFC (LOFC) and at several loci in the vicinity of the left and right amygdala. Males also showed inverse correlations in the left OFC between lnLF and rCBF. Also under fixation females showed positive correlations between RMSSD and rCBF in the right amygdala. The relationship between lnHF and rCBF differed significantly between males and females in the right amygdala (See Table 3, Figures 2a and 3a).

3.3 Handgrip Task Results

During the handgrip task, HR increased by an average of 5.5, 12.1, and 20.7 beats per minute (BPM) in females, and by an average of 4.0, 9.7, and 17.5 BPM in males, for each of the 3 intensity levels, respectively, as compared to fixation. In the mixed model analysis for the handgrip task, mean HR was significantly greater in females than males ($F_{1,18.9} = 19.06$, $p=0.0003$), while mean lnHF, lnLF, and RMSSD were significantly greater in males than females ($F_{1,18.9}=6.35$, $p=0.0209$; $F_{1,18.7}=18.03$, $p=0.0004$; $F_{1,17.7}=8.29$, $p=0.0101$, respectively). When handgrip accuracy was included as a covariate, accuracy showed a trend towards being a significant predictor of lnHF and RMSSD ($F_{1,22.4}=3.49$, $p=0.0750$; $F_{1,14.3}=3.52$, $p=0.0813$; respectively), and was a significant predictor of LF/HF ($F_{1,18.4}=4.84$, $p=0.0407$). During the handgrip task, HR correlated inversely with rCBF in the left anterior insula in females, and in the right LOFC and bilateral peri-amygdaloid cortex in males (Table 2). None of the *a priori* regions exhibited a significant difference in the association between autonomic index and rCBF between male and female subjects.

3.4 N-back Task Results

During the n-back task, HR increased by an average of 6.0, 9.3, and 10.7 BPM in females, and by an average of 4.6, 7.3, and 7.3 BPM for males, for each of the 3 difficulty levels, respectively, as compared to fixation. In the mixed model analysis for the n-back task, mean HR was greater in females ($F_{1,19.6}=23.40$, $p=0.0001$), while mean RMSSD was significantly greater in males ($F_{1,17.6} = 9.68$, $p=0.0062$). No autonomic variable showed significant dependence on n-back accuracy, although accuracy showed a trend towards being a significant predictor of lnHF and RMSSD ($F_{1,29.6}=3.85$, $p=0.0583$ and $F_{1,16.1}=4.96$, $p=0.0573$, respectively). During performance of the n-back task (Table 2), HR correlated inversely with rCBF in the dorsal cingulate and ACC in females, and in the left OFC in males. Under the n-back condition, females showed positive correlations between RMSSD and rCBF in the left anterior insula and right amygdala. The correlations between RMSSD and rCBF differed significantly between males and females in the right amygdala and the left anterior insula (Table 3; Figures 2b, 3b). Finally, in a conjunction analysis between the n-back and handgrip task data, rCBF in the right LOFC correlated inversely with HR under both tasks in males.

3.5 Post hoc whole brain analysis

The regions where females and males exhibited significantly different relationships between rCBF and autonomic measures appear in table 4. Notably, in the bilateral superior STG, females and males exhibited differences in the relationship between rCBF and HR during fixation, with females showing a positive association and males showing a weaker negative association. This differential relationship also was found for the relationship between rCBF and RMSSD during the n-back task in the left STG. There were also significant differences in the association between rCBF and both HR and RMSSD between males and females in the PCC during the handgrip task, again with females exhibiting a positive relationship and males exhibiting a weaker inverse relationship. The relationship between rCBF and the autonomic variables differed significantly between males and females in multiple regions of

the PFC. In the handgrip condition, females showed a positive relationship between rCBF and lnHF, while males showed a negative relationship in the dorsolateral PFC.

The globally normalized rCBF differed between groups in the cerebellum during all conditions tested (Table 5).

4. Discussion

In this pilot investigation, we demonstrated that males and females exhibit prominent differences in the relationship between indices of autonomic arousal and rCBF in the amygdala and the insula, as well as other regions involved in autonomic function. We also found differences between males and females in electrocardiographic indices reflective of vagal tone during task performance.

A growing body of literature suggests that females have greater vagally mediated cardiac modulation at rest than men. Some (Kuo et al., 1999) but not all (Sevre et al., 2001) studies report that females have greater HF whereas males consistently show higher LF (Antelmi et al., 2004; Kuo et al., 1999; Sevre et al., 2001) and LF/HF (Kuo et al., 1999; Stein et al., 1997) parameters than females. In our study, at rest, only HR differed significantly between males and females, most likely due to low power to detect a small difference. The difference in HR between female and male subjects at rest may be accounted for by the difference in fitness levels between the two groups, since greater fitness is associated with lowered HR, and increased vagally mediated HRV. We would not, however, expect differences in physical fitness to account for the imaging differences between males and female subjects, where females showed greater positive correlations with blood flow and parasympathetic indices, opposite the direction of change expected on the basis of physical fitness alone.

In both sexes, performing either the n-back or the handgrip task was associated with increased HR and LF/HF but lowered lnHF, lnLF, and RMSSD relative to the fixation condition, suggesting a shift toward reduced parasympathetic modulation and greater sympathetic relative to parasympathetic modulation of HR (main effect of task was significant in a sex by task ANOVA, data not shown). Other researchers reported similar changes in autonomic indices during performance of a Stroop task and a simple motor task (Garde et al., 2002), as well as during controlled breathing, reading, and mental arithmetic (Bernardi et al., 2000). During task performance, we found that males exhibited lower HR but higher lnHF, lnLF, and RMSSD as compared to females. This suggests that parasympathetic activity decreased to a greater degree during task performance in female subjects than in male subjects. Although we did not find a significant sex-by-task interaction, our results are consistent with Sato and Miyake (Sato and Miyake, 2004), who found that LF increased and HF decreased more in females than males during performance of a stressful mirror tracing task and mental arithmetic, but in contrast to Wong et al. (Wong et al., 2007), who found that males showed a greater degree of vagal withdrawal during a handgrip task as compared to females (although that study did not example spectral power indices of the HR). Our findings also suggest that the parasympathetic withdrawal seen in all subjects is more prominent in the female subjects. Taken in total, our data, as well as data from other studies, are consistent with the conclusion that cardiac control is characterized by greater parasympathetic modulation in females (Airaksinen et al., 1998; Dart et al., 2002; Evans et al., 2001; Snieder et al., 2007).

Our findings in the amygdala also emphasize the importance of the parasympathetic system in cardiac control in women. During the fixation and n-back conditions, the relationship between rCBF in the right amygdala and lnHF and RMSSD, respectively, differed significantly between females and males. We show the location and extent of the amygdalar

clusters in Figure 2, and plot the data from the peak voxels in Figure 3. In both cases, females showed positive associations between amygdala activity and vagal measures, while males exhibited negative or no associations, although only the positive correlation between rCBF and RMSSD during the n-back task in females was independently significant. A prior study of men and women also revealed positive correlations between HF and rCBF during a working memory task, although no subgroup analyses were performed to determine if the correlation was driven by the female subjects (Gianaros et al., 2004).

The differences observed in the neural correlates of autonomic function between males and females are consistent with evidence that several brain structures, including the amygdala, are sexually dimorphic. Synaptic organization of the medial amygdala in mice shows prominent sex effects, with prepubertal males exhibiting 80% more excitatory synapses than females (Cooke and Woolley, 2005). Sex differences involving amygdala response to emotionally valenced sensory stimuli have often been reported in human brain mapping studies (Cahill, 2003).

There is significant evidence that gonadal hormones play a role in sexual dimorphism of autonomic control. In rats, estrogen has been shown to enhance vagal tone and suppress sympathetic activity (Dart et al., 2002). Both estrogen and testosterone are known to modulate the synthesis of catecholamines, the primary transmitter of the sympathetic nervous system (Dart et al., 2002). Furthermore, there is evidence that autonomic indices vary significantly across the menstrual cycle. Parasympathetic activity is more prominent in the luteal phase, suggesting a key role for estrogen in the regulation of parasympathetic activity (Sato and Miyake, 2004). In a study by Goldstein, et al., autonomic arousal correlated positively with neurophysiological activity in the amygdala, ventromedial nucleus of the hypothalamus, and OFC during the midcycle but not the early follicular phase, suggesting that estrogen may inhibit arousal in women via amygdalar, hypothalamic, and OFC control (Goldstein et al., 2005).

The behavioral conditions under which we found differences between men and women in the correlation of amygdala rCBF with HRV indices were each associated with mild stress. Challenging cognitive tasks are known to result in increases in HR and anxiety ratings (e.g., (Simpson et al., 2001)). Similarly, the 30% and 50% strength levels required on the hand-grip task were perceived as mildly stressful by our subjects. Finally, although the fixation condition has been used widely as a low performance level baseline task in functional neuroimaging studies, Heinz et al. argued that this task generally is perceived as stressful by research subjects (Heinz et al., 2007), an effect which would be additive to the stress of being confined in the relatively small space of the scanner gantry with the head restrained. Nevertheless, the analyses we performed were not designed to establish the specificity of each task in modulating the differential correlation of rCBF and parasympathetic activity between males and females. Thus sex differences in the correlations between brain activity and autonomic indices were evident in each of our experimental conditions, and the extent to which these relationships are modulated by the individual tasks must be established in future studies.

Although levels of stress-related steroids and peptides were not assessed in this preliminary study, the behavioral and neurophysiological differences we observed in response to mild stressors conceivably relate to the sex-differences found in these biochemical systems in other paradigms. Taylor et al. proposed that female responses to stress more likely follow a pattern of “tend-and-befriend”, while male responses more likely follow a “fight-or-flight” pattern related to sympathetic arousal (Taylor et al., 2000). Ennis and colleagues further hypothesized that down-regulation of the hypothalamic-pituitary-adrenal (HPA) axis supports the “tend and befriend” response, based upon findings that cortisol levels decrease

in females, but increase in males, under mild stress (Ennis et al., 2001). In rodents endogenous opioids and corticotrophin releasing hormone are expressed in overlapping regions of the central nucleus of the amygdala (Marchant et al., 2007), while distinct populations of neurons in this nucleus respond to vasopressin and oxytocin (Huber et al., 2005). Thus the sex differences between amygdalar rCBF and autonomic tone we observed in may be particularly informed by further studies that additionally assess biochemical components of the stress response.

In addition to the amygdala, our imaging results confirm and extend previous reports regarding other brain regions where neurophysiological activation correlated with electrocardiographic indices of HRV. Although most previous investigations assessed the functional neuroanatomical correlates of ANS tone at rest, several pioneered its study during the performance of physical and cognitive tasks, and our results are consistent with these reports. Critchley, et al. studied male subjects performing physical and mental stress tasks with PET (Critchley et al., 2000). They studied a combined sample of males and females performing cognitive and motor tasks using fMRI, and our study shares many of the same findings. Although the Critchley fMRI study reported positive correlations between hemodynamic response and both HF and sympathetic tone (LF orthogonalized to HF) not replicated by our study, we did not use an orthogonalized LF, and additionally covaried by respiratory rate. Another fMRI study examining sex differences found that relative to males, females exhibited a lower HR and mean arterial pressure while performing a handgrip task. In this study, HR was not measured concomitantly with the neurophysiology; ECG recordings were obtained in a separate trial of the task performed outside the scanner. During task performance in the scanner, women showed a deactivation in the dorsal anterior cingulate while men showed activation, and women exhibited reduced activation in the right insula as compared to men (Wong et al., 2007). Although our results do not confirm the results from the Wong et al. study, this discrepancy may reflect differences in study design.

This study was unique in its use of neuroimaging to investigate rCBF and autonomic differences as simultaneously indexed by HRV between men and women as they performed cognitive and motor tasks. The chief limitation of this study is the small size of the female sample, which reduced our sensitivity to detect correlations within this sex group, as well as differences in the autonomic correlations between groups. Our SPM analysis is limited from the standpoint that variances were not calculated separately for intra- and inter-subject effects. Given these limitations, our conclusions should be considered preliminary. Another limitation of the study is that the females were not studied in the same phase of their menstrual cycle. Correlations between ANS function and serial estrogen levels in the blood may also prove informative to elucidate the biological basis of sex differences in autonomic tone. Our design, however, may emphasize sex differences independent of menstrual phase. Finally, it could be argued that we should have performed this study using functional MRI; however, we were concerned that BOLD signal susceptibility artifacts would potentially reduce sensitivity to differences in hemodynamic activity in the ventral ACC, OFC, and amygdala. Moreover, the temporal resolution of the PET-[O-15]water technique was well suited to our experimental design since the assessment of HRV required integration of the beat-to-beat variability over one to two minutes. Finally, the spatial resolution of this method was sufficient to assess blood flow changes in our pre-selected ROIs, in a manner that avoided potential mis-localization artifacts caused by the draining vein effect on the fMRI-BOLD signal.

Elucidating the functional anatomical correlates of ANS control may inform future research on the biobehavioral response to stress. As disease course and general quality of life are profoundly affected by stress, additional studies such as these in larger samples with improved methodology may reveal neurobiological targets for interventions designed to

encourage more adaptive physiologic responses to stress. Similarly, understanding the neuroanatomical correlates of autonomic regulation may elucidate sex-based differences in a variety of pathological conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations/Glossary

ACC	anterior cingulate cortex
ACe	central nucleus of the amygdala
ANS	autonomic nervous system
BLA	basolateral amygdala
BNST	bed nucleus of the stria terminalis
BOLD	blood oxygen level dependent (fMRI contrast mechanism)
ECG	electrocardiograph
fMRI	functional magnetic resonance imaging
HR	Heart rate
HRV	heart rate variability
IBI	inter-beat interval
LBNP	Lower body negative pressure (autonomic challenge)
lnHF	Natural log of high frequency power of the heart rate spectrum. An indicator of parasympathetic activation
lnLF	Natural log of low frequency power of the heart rate spectrum. Partially dependent on sympathetic tone, but strongly dependent on parasympathetic activity as well
LF/HF	Ratio of low to high frequency power, generally used as an index of the balance of sympathetic to parasympathetic tone
OFC	orbitofrontal cortex
PCC	posterior cingulate cortex
PET	positron emission tomography
PFC	prefrontal cortex
rCBF	regional cerebral blood flow
RMSSD	Root mean square standard deviation of successive R-R intervals of the heart rate time series. An index of parasympathetic tone
ROI	region of interest
STG	superior temporal cortex

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Figure 1.
Autonomic variables (mean + SEM) for each task by sex.

Figure 2.

Coronal section from the SPM analysis showing voxels in the right amygdala where a) the correlations between lnHF and rCBF (during fixation) and b) the correlations between RMSSD and rCBF (during the n-back task) differed significantly between males and females. The cross-hairs locate the voxel containing the peak t-values as indicated in Table 3. The coronal section in a) is situated 36 mm posterior to the anterior commissure (i.e. $y = -3$), the coronal section in b) is situated 6mm posterior to the anterior commissure. The color bar indicates the magnitude of voxel t-values. The statistical parametric map is thresholded at $p < 0.005$ uncorrected, with extent threshold of a) 344 voxels and b) 190 voxels to highlight the relevant cluster.

Figure 3.

Plots showing the relationships between a) lnHF and rCBF during fixation and b) between RMSSD and rCBF during n-back in the points in the right amygdala indicated in Table 3. Each data point represents one repetition of the task at a single difficulty (n-back) or intensity (handgrip) level for each subject; thus each subject has two fixation data points and six data points for each task. Regional CBF values were adjusted in SPM for all effects except the autonomic variable, and values for both rCBF and the autonomic variable have been mean centered. Note that the apparent bimodal distribution of RMSSD in males is a reflection of the decrease in that parameter during task performance. Line shown is a simple linear regression for illustrative purposes; slope is not necessarily equal to regression coefficient in Table 3.

Table 1

Demographic information for subject groups

	Age (yrs)	BMI	BP-Systolic (mm-Hg)	BP-Diastolic (mm-Hg)	Walk-Run Distance (m) [‡]
Males	30±7.7	27.4±4.50	128±13.6 ^{**}	71±6.1	1806 ± 453.6 [*]
Females	34±10.3	23.4±5.91	104±11.6 ^{**}	66±7.8	1369 ± 233.0 [*]

* Group difference significant at p<0.05

** Group difference significant at p<0.001

[‡] Eight males and 5 females participated in the walk/run test

Table II

Areas within the regions of interest defined *a priori* where the correlations between autonomic indices and rCBF remained significant after applying small volume corrections for multiple comparisons in the male and female samples.

task	sex	autonomic Index	ROI for SVC	region	direction of correlation	voxel Z	Stereotaxic Coordinate (x,y,z) ^d
Fixation	Males	HR	LA	Left Amygdala	-	3.95 ^b	-22, 1, -17
		HR	LA	Left Amygdala/Hipp junction	-	3.68 ^a	-20, -9, -16
		HR	RA	Right Amygdala	-	3.77 ^a	14, -5, -15
		HR	RA	Right Amygdala/Hipp junction	-	3.74 ^a	12, -9, -18
		HR	RA	Right Amygdala	-	3.58 ^a	16, -1, -15
		HR	RAI	Lateral Orbitofrontal Cortex (BA47) ^c	-	3.83 ^a	44, 19, -6
Handgrip	Females	InLF	LOC	Medial Orbital Cortex (BA 10o)	-	4.05 ^a	-8 44 -22
		RMSSD	RA	Right Amygdala	+	3.63 ^a	22, -5, -13
	Males	HR	LA	Periamygdaloid C	-	4.39 ^b	-10, 1, -19
		HR	RA	Periamygdaloid C	-	4.09 ^b	16, 1, -19
		HR	RAI	Lateral Orbitofrontal Cortex (BA47) ^c	-	4.49 ^b	44, 21, -9
	Females	HR	LAI	Anterior Insula	-	4.34 ^b	-34, 16, 1
N-Back	Males	HR	RAI	Lateral Orbitofrontal Cortex (BA47) ^c	-	4.01 ^a	42, 19, -11
	Females	HR	LCC	Motor Area	-	4.37 ^a	-6, -2, 39
		HR	RCC	Dorsal Anterior Cingulate	-	4.10 ^a	8, 9, 27
		RMSSD	LAI	Left Anterior Insula	+	3.74 ^a	-38, 21, 3
	Females	RMSSD	RA	Right Amygdala	+	3.72 ^a	18, -6, -13
		HR	RAI	Lateral Orbitofrontal Cortex (BA47) ^c	-	4.01 ^a	42, 19, -11

^a Indicates that the voxel is significant in "small volume correction" (SVC) at $p \leq 0.05$ (family-wise error)

^b Indicates that the voxel is significant in SVC at $p \leq 0.001$ (family-wise error)

^c The lateral OFC region corresponded to the intrasulcal portion of BA47 which was situated within the insular ROI because of its close proximity to the insular cortex (Ongur et al. 2003).

^d Stereotaxic coordinates correspond to the spatial array of Talairach and Tournoux (1988) in which values indicate the distance in mm from the origin (anterior commissure), with positive x indicating right, positive y indicating anterior, and positive z indicating dorsal to a plane containing both the anterior and posterior commissures.

Abbreviations: PFC = prefrontal cortex, Gy=gyrus, BA=Brodmann area; BA designations are from (Ongur and Price 2000)

Regions where females exhibited significantly larger associations between rCBF and cardiac autonomic indices than males in the regions of interest defined *a priori*. Regression coefficients from the general linear model are shown, with the larger regression coefficient in bold font.

Table III

Task	Autonomic Index	ROI for SVC	Region	Regression coefficient female subjects	Regression coefficient male subjects	Stereotaxic Coordinate (x,y,z) ^b
Fixation	LnHF	RA	Right Amygala	2.8134	-0.6182	22, -3, -13
N-Back	RMSSD	LAI	Left Anterior Insula	0.1164	-0.0064	-36, 20, 5
	RMSSD	RA	Right Amygdala	0.106	-0.0075	18, -6, -13

^a Indicates that the voxel level p-value was significant after applying correction for multiple testing using the Small Volume Correction (SVC) option within SPM2 at p≤0.00625 (family-wise error)

^b Stereotaxic coordinates and abbreviations interpreted as in Table 2.

Table IV

Regions where males and females exhibit significantly different associations between rCBF and cardiac autonomic indices. The region name corresponds to the location of the voxel with the maximum Z-value within each cluster, although some voxels are significant only by voxel-wise test. Regression coefficients from the general linear model are given, with the larger regression coefficient in bold font.

Task	Autonomic Index	Region	regression coefficient Females	regression coefficient Males	Voxel Z	Stereotaxic Coordinate (x,y,z) ^d
Fixation	HR	Superior Temporal Gy	0.6573	-0.2856	5.11 ^{b,c}	57 0 0
	HR	Superior Temporal Gy	0.6606	-0.1629	4.62 ^{b,c}	-55 6 -5
	HR	Frontal Polar C, BA (10p)	-0.4806	0.2659	4.05 ^a	26 48 -7
	HR	Posterior Thalamus	-0.5028	0.164	3.65 ^a	18 -16 1
	InLF	Inferior Parietal C	-2.1487	1.5851	3.70 ^a	-44 -33 46
	RMSSD	Temporal Polar C	0.1242	-0.0204	4.95 ^b	-42 -14 -13
handgrip	RMSSD	Inferior Parietal C	0.1427	0.0075	4.03 ^a	57 -34 26
	HR	Posterior Cingulate C	0.2429	-0.2284	4.54 ^c	-4 -73 7
	HR	Precentral/Postcentral Gyrus	0.2593	-0.1404	3.88 ^a	14 -34 64
	InHF	Dorsolateral PFC	0.9226	-1.2421	4.01 ^a	22 18 52
	RMSSD	Posterior Cingulate C	0.1429	-0.0081	4.41 ^a	-12 -19 38
	InHF	Posterior Thalamus	1.4957	-0.7098	3.83 ^a	20 -23 14
n-back	InLF	Lingual Gy	-1.7487	0.9138	3.82 ^a	12 -82 -6
	RMSSD	Superior Temporal Gy	0.1009	-0.0269	4.88 ^{b,c}	-57 -41 6
	RMSSD	Inferior Parietal C	0.1268	-0.0014	4.55 ^c	57 -32 25

^a significant at p≤0.05 after applying corrections for multiple comparisons using the cluster test

^b significant at p≤0.05 (family-wise error) after voxel-wise correction for multiple comparisons

^c significant at p≤0.001 after applying corrections for multiple comparisons using the cluster test

^d Stereotaxic coordinates and abbreviations interpreted as in Table 2

Table V

Regions where normalized rCBF differed significantly between male and female subjects during each task condition. The region name corresponds to the location of the voxel with the maximum Z-value within each cluster.

Task	Contrast	Region	clust p	Voxel Z	Talairach coordinate ^d
Fixation	Female>Male	Cerebellum, Pyramis	0.005	3.92 ^a	22 -81 -32
Handgrip	Female>Male	Cerebellum, Declive	0.001	4.09 ^b	41 -75 -22
N-Back	Female>Male	Cerebellum, Declive	0.048	4.03 ^a	37 -58 -17

^a significant at p≤0.05 after applying corrections for multiple comparisons using the cluster test

^b significant at p≤0.001 after applying corrections for multiple comparisons using the cluster test

^d Stereotaxic coordinates and abbreviations interpreted as in Table 2