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The effects of acute stress on human prefrontal working memory systems

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ABSTRACT

We examined the relationship between acute stress and prefrontal-cortex (PFC) based working memory (WM) systems using behavioral (Experiment 1) and functional magnetic resonance imaging (fMRI; Experiment 2) paradigms. Subjects performed a delayed-response item-recognition task, with alternating blocks of high and low WM demand trials. During scanning, participants performed this task under three stress conditions: cold stress (induced by cold-water hand-immersion), a room temperature water control (induced by tepid-water hand-immersion), and no-water control (no hand-immersion). Performance was affected by WM demand, but not stress. Cold stress elicited greater salivary cortisol readings in behavioral subjects, and greater PFC signal change in fMRI subjects, than control conditions. These results suggest that, under stress, increases in PFC activity may be necessary to mediate cognitive processes that maintain behavioral organization.

1. Introduction

Working memory (WM) may be defined as the retention and/or manipulation of to-be-remembered information over brief time intervals. It is believed to underlie many higher cognitive processes [6,59] including reasoning [51], planning [18] and problem solving [13,50]. Research indicates that the prefrontal cortex (PFC) is a vital neural substrate for WM functions [10,27]. Neuroimaging studies with humans have consistently demonstrated increased PFC activation during delayed-response tasks that require temporary storage of information [11,55]. In particular, event-related fMRI studies indicate that dorsolateral PFC mediates WM processes at high WM demands [55,57]. These results are consistent with primate WM studies showing sustained firing of PFC neurons during delay periods of WM tasks [19] and significant decreases in performance on delayed-response tasks following PFC lesions [17,26]. Primates also show performance decrements with stress induced PFC catecholaminergic changes [5].

Stress-regulation exerts influences on cognition and behavior. The presence of an acute environmental stressor can modify cognitive functions in humans, including WM systems [1,14,23,37,38,44,48]. Furthermore, WM processes may be particularly susceptible to the effects of acute stress under high memory loads [7,46] and during the resistance of interference from competing sources of information, especially for older adults [67]. Given the fundamental nature of relationships between WM and higher cognitive processes, delineating the underlying mechanisms of stress-related performance changes is critical, not only to a complete understanding of WM systems in particular, but to understanding the nature of stress–cognition relations generally.

Studies that have examined the effects of acute stress on WM have produced mixed results. Negative effects of acute stress on WM task performance have been observed in some studies [32,35,46,48,61]. Other studies, however, have not shown such effects [12,42,58]. Empirical discrepancies have been difficult to reconcile because, across studies, a variety of stress manipulations and WM measures have been used. Some stress manipulations may be more susceptible to individual reactivity differences than others [2,68]. Some performance measures may also be more susceptible to individual reactivity differences than others. For instance, some studies suggest that gender mediates stress–WM performance relationships [35,68]. Procedural differences between experiments may also lead to differences in

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results across studies. These include temporal relationships between stress-administration and cognitive assessment, cortisol collection methods, endogenous collection or exogenous cortisol administration, measured behavioral parameters (i.e., reaction time; RT and accuracy), and within- vs. between-subject stress manipulations.

Human and animal research suggests anatomic and neurochemical relationships between sub-cortical structures that respond to stress and affect PFC [21]. Rodent medial PFC is one target of the stress-related neurochemical response [8,15,16] via connections with amygdalar basolateral complex [41]. Additionally, lesions within these amygdalo–PFC pathways have been shown to attenuate catecholamine release within PFC [3,5]. Stress-related catecholaminergic changes may affect PFC-based WM processes in primates [5]. In one study, for instance, monkeys performed a spatial delayed-response task with varying delay intervals [3]. On some occasions, WM performance followed sustained exposure to loud noise (100–110 db wide-band frequency). Noise-related performance decrements were greater with longer delay intervals. Performance decrements were attributed to a "hyperdopaminergic" stress response in PFC because the behavioral stress response was mediated by administration of dopamine-receptor antagonists. In humans, excitation of the hypothalamic–pituitary–adrenal (HPA) axis leads to corticosteroid (e.g., cortisol) hypersecretion due to stress exposure. These hormones exert global effects on the brain and body and also affect mental states [20,22,45]. Results from multiple studies converge to indicate that increases in glucocorticoid levels exert a profound influence over PFC structure and functioning, in both animals and non-human primates. For example, corticosterone (the central cortisol analogue in rodents) has been associated with a reorganization of PFC dendritic fibers in rats [8]. Additionally, injections of hydrocortisone (a synthetic form of cortisol) have been linked to impairment of medial PFC-based behavioral inhibitory capabilities in non-human primates [39]. By impairing PFC function, excessive levels of cortisol also appear to disinhibit HPA activation thus increasing sympathetic nervous system activity.

These studies are consistent with the notion that WM systems are especially susceptible to the deleterious effects of acute stress. They illustrate a plausible mechanism through which stress could affect PFC-dependent WM processes, through PFC–amygdala interactions. To observe this mechanism in humans, we had subjects perform a delayed-response WM task during behavioral performance and fMRI scanning. In behavioral (Experiment 1) and fMRI (Experiment 2) studies we periodically immersed subjects' hands in ice-cold water (4 °C) to induce acute stress. For Experiment 1, we hypothesized that there would be a significant difference in salivary cortisol levels during cold stress compared to control conditions. Specifically, we hypothesized that salivary cortisol levels would be higher when subjects' hands were immersed in cold water than when they were immersed in room temperature water. For Experiment 2, we predicted that PFC activity would be most affected by the application of cold stress, relative to non-cold stress conditions. It is our hypothesis that the cold press experience results in increased cortisol levels, and that these higher cortisol levels disrupt typical prefrontal functioning. Additionally we predicted that this increase, if present, may be mediated by amygdalar activity. Because behavioral results from studies of acute stress have been mixed [35,42,46,49,58], we were less certain about predictions regarding behavioral performance. By convolving the presence or absence of acute cold-pressor stress with high and low WM demand we sought to clarify the manner in which these factors interact with PFC activity, amygdala activity and WM performance. The current study sheds new light on the nature of the interaction of PFC areas underlying WM processes and the amygdala, and how these neural regions interact to regulate the effects of acute stress in order to maintain organized and goal-directed behavior.

2. Experiment 1

2.1. Method

2.1.1. Participants

Eighteen healthy young volunteers (mean age=20.4; 6 men) were recruited from the undergraduate and medical campus of Rutgers University — Newark and UMDNJ. Participants were excluded if they had any medical (including type I or type II diabetes, hypertension, cardiac condition, significant weight loss or major surgery within the last 6 months), psychiatric (including depression, anxiety or substance abuse), or neurological (including epilepsy and migraine syndrome) conditions. Participants were also excluded if they were pregnant or taking oral contraceptives, currently on psychotropic medication, presently menstruating, smoked more than ten cigarettes per day or if they consumed more than fifteen drinks of alcohol per week. Participants were prohibited from consuming a large meal 60 min prior to the experiment, dairy products at least 30 min prior to the experiment and smoking or consuming alcohol 24 h prior to the study. All participants provided informed consent.

2.1.2. Procedure

In order to control for circadian fluctuations in cortisol levels, all sessions took place between 11 am and 1 pm. This choice of an earlier time in the day was made to enable subjects to easily follow the dietary guidelines necessary for accurate salivary cortisol collection (i.e. no eating or drinking for about 1 to 1.5 h prior to sample collection). Participants were greeted by an experimenter upon arrival to the laboratory and told that they would be involved in a study of memory. They were told that they would be taking several computerized cognitive tasks in addition to having their hands immersed in water intermittently. After signing IRB-approved consent forms, participants were escorted to an isolated experiment room, which maintained pleasantly neutral lighting so that subjects could acclimate to the lab environment. They were asked to settle in for about 20 min, provided with neutral reading material, and told that the experimenter would return shortly with instructions.

Following this acclimation period, the experimenter returned with a bucket of either chilled water or room temperature water for the hand-immersion procedure (see Section 2.1.2.2 below for description) immediately prior to the cognitive task. After the completion of the first run of the WM task (see Section 2.1.2.1), the participant was told to relax, continue reading until the experimenter returned with further instructions. After a 20-minute period, the experimenter returned with the bucket of either chilled water or room temperature water. The participant began the second run of the WM task. Salivary cortisol samples were obtained at baseline (immediately after acclimation period and prior to beginning the cognitive task), 20 min after task I and 20 min after task II (see Section 2.1.2.3). These time-delays permitted us to capture the effects of the room temperature and cold-pressor stress on salivary cortisol levels, as it generally takes 15–20 min for task-related changes in unbound cortisol levels to be expressed in the saliva.

2.1.2.1. Working memory assessment. The WM task was a modified version of the Sternberg Item Recognition Task. Subjects were presented sequentially with blocks of 1 letter (low WM demand condition) or 6 letter (high WM demand condition) trials. There were 5 trials per block. Subjects received 2 runs of the Sternberg Item Recognition Task, each consisting of a total of 60 trials with an ITI of 1 s. On each trial, either 1 or 6 letters appeared on the computer screen for 4 s. At the end of this period, the letters disappeared and a 5 s delay period ensued (fixation). Then a probe letter appeared for 3.5 s and the subject indicated whether the letter was part of the previously presented string of letters or not.

2.1.2.2. Cold-pressor task (CPT). A bucket of ice water chilled to a temperature of 4 °C was used. Temperature was assessed intermittently

by a laboratory grade thermometer. Subjects were instructed to immerse their left hand, with the assistance of the experimenter, up to the wrist, for a period of 2 min.

2.1.2.3. Salivary cortisol sampling. Baseline measures of cortisol were taken prior to water exposure of either type: room temperature or cold. Subjects were asked to lightly chew on cotton like material (salivette) for a brief period of time. Upon completion of this procedure, the subject withdrew the salivette and the experimenter immediately placed it in its individual centrifuge tube. At the end of each day, samples were packaged and sent to Salimetrics Laboratory (State College, PA) for duplicate biochemical assay analysis.

2.1.2.4. Data analysis. Data were analyzed with repeated-measures analysis of variance (ANOVA), student's paired *t*-tests, correlations and Friedman ranking procedures. Effect sizes were calculated for student's paired *t*-tests using Cohen's [9] criteria. The alpha level used to determine statistical significance was $p<.05$, two-tailed.

2.2. Results

2.2.1. Behavioral results

Paired *t*-tests were performed on reaction time (RT) medians (for accurate trials only) in all conditions. In addition, we calculated the effect sizes (*d*) for each comparison. Within each stress condition subjects performed significantly slower on high WM demand as compared to low WM demand trials (see Fig. 1). This effect was observed within the cold stress $t(17)=-3.009$, $p<.01$, $d=.70$, and room temperature control $t(17)=-3.732$, $p<.01$, $d=.88$, conditions individually. No significant main effect of the two stress conditions were observed in RT $F(1,17)=.020$, $p>.05$ or accuracy $F(1,17)=2.058$, $p>.05$, data. No significant interaction between the factors of stress and WM demand was observed for RT data $F(1,17)=1.211$, $p>.05$. Interestingly, a trend towards significance was observed in the interaction between stress and WM demand in the accuracy data $F(1,17)=4.013$, $p<.10$). Table 1 presents the means (±SEM) of performance on the WM tasks for Reaction Time and Accuracy during cold stress and room temperature control conditions.

Previous research on the effect of acute stress on WM performance has produced inconsistent results. Recent empirical findings have highlighted gender as a mediator to stress–WM performance relationships [35,68]. Based on these findings, a repeated-measures ANOVA was performed in order to explore the role of gender on WM performance. Results indicated no significant interaction or main effects of accuracy or reaction time as a function of gender (all *p* values>.10)

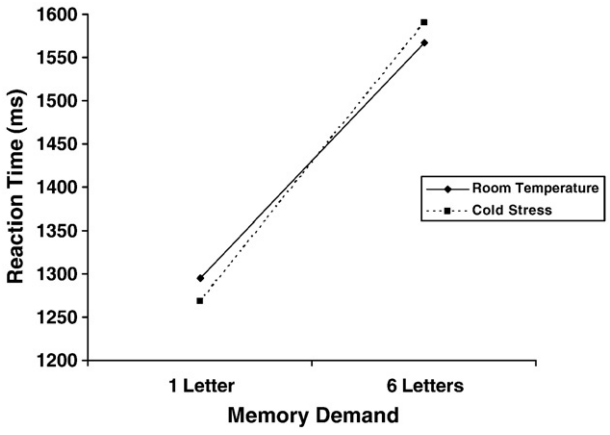

Fig. 1. Reaction time (RT) in milliseconds on the WM task between the stress and room temperature control for Experiment 1. There was no significant difference between groups however within each stress condition, RT was significantly slower on high WM demand compared to low WM demand trials.

Table 1
Mean (±SEM) performance on working memory tasks for reaction time and accuracy during cold stress and room temperature control conditions

Control	Cold stress	Room temperature
Reaction time		
Low memory load (ms)	1310 (73.6)	1267 (71.5)
High memory load (ms)	1522 (69.4)	1552 (80.2)
Accuracy		
Low memory load (% correct)	.86 (.05)	.92 (.04)
High memory load (% correct)	.86 (.03)	.86 (.03)

2.2.2. Salivary cortisol results

Each subject's baseline salivary cortisol levels were subtracted from those obtained during the room temperature control and cold stress portions of the experiment. When these adjusted cortisol readings were compared using a paired *t*-test, we observed that cortisol levels in the cold stress condition were significantly higher than those obtained during the room temperature control condition, $t(17)=-3.728$, $p<.01$, $d=.85$ (see Fig. 2).

Preliminary examination of our results suggested that gender could control significant proportions of variability. Gender differences in stress responsiveness have been observed [35,68]. To examine the relationship between gender and cortisol stress reactivity, a 2 (Gender)×3 (Stress Condition) repeated-measures ANOVA was conducted. On average, men had higher cortisol levels (.386±.09 nmol/l) than woman (.171±.02 nmol/l). Repeated-measures ANOVA indicated a significant main effect of Gender, $F(1,16)=8.148$, $p<.05$ in the absence of a Stress $F(2,16)=1.729$, $p>.10$ or Stress×Gender interaction $F(2,16)=1.093$, $p>.10$. A power analysis was conducted using SPSS (Version 15 for Windows). Effect size was calculated based on a partial squared curvilinear correlation, which removes the effects of individual differences by eliminating variability between subjects [66]. Observed power was calculated at .76 with an obtained effect size of partial $\eta^2=.34$; power was sufficient to detect a gender effect if it was present in the data.

3. Experiment 2

3.1. Method

3.1.1. Participants

Twelve right-handed healthy volunteers (mean age 22.67, 6 men) were recruited from the undergraduate campus of Rutgers University — Newark. Only subjects determined to be free of psychotropic medications, substance abuse or addiction, medical, neurological or psychiatric illness via a screening interview were allowed permitted to participate.

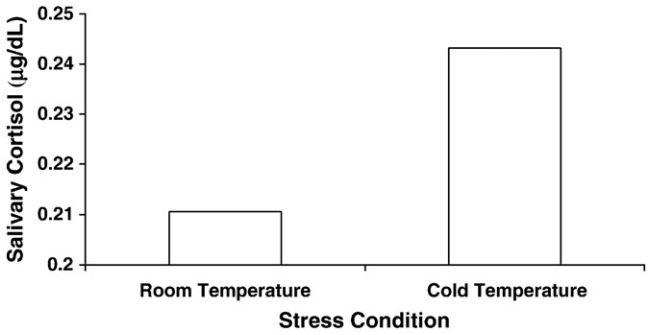

Fig. 2. Salivary cortisol levels for room temperature control and cold stress conditions for Experiment 1. Salivary cortisol levels were significantly higher in the cold stress condition compared to the room temperature control.

Subjects were told that they would be performing several computerized cognitive tasks during fMRI scanning. In addition they were informed of the water hand-immersion procedure. Subjects were also given the option to withdraw at any time during the experiment without penalty.

3.1.2. Procedure

3.1.2.1. Working memory assessment. In order to control for circadian fluctuations in cortisol levels, all Experiment 2 sessions took place between 2 pm and 5 pm in the afternoon. Because cortisol samples were not being collected, however, we shifted experimental sessions to the afternoon as compared to Experiment 1 to assure that all subjects could be scanned at the same time of day. Participants were situated in the scanner in a supine position while they viewed a backlit projection screen from within the magnet bore, through a mirror mounted on the head coil. On each trial, subjects were first presented with either 1 letter or 6 letters for 2.5 s. A shorter encoding time was used in Experiment 2 as compared to Experiment 1 in order to reduce subject's total scanning time, thereby reducing their discomfort and minimizing head motion while maximizing subject's focus and interest in the task. Following a 5 s delay (during which subjects viewed a blank screen), subjects were presented with a probe letter for 3.5 s. During this time, subjects indicated whether or not the letter was part of the previously presented string of letters by responding with an index finger button press if the probe letter was contained in the memory set or a middle finger button press if the probe letter was not contained in the memory set. Each trial was followed by a 1-second inter-trial-interval (ITI). All subjects completed 3 scanning runs, all consisting of 12 min. A scanning run consisted of 60 trials, in which WM demand varied systematically using a block design (alternating every 5 trials between high and low WM demand). Three different stress conditions were administered to each subject in 3 consecutive scanning runs (see Fig. 3). The subjects' left arm extended out of the magnet bore with the left hand resting on a towel. The orders of the three conditions were counterbalanced between subjects. Salivary cortisol measurements were not collected in Experiment 2 to obviate increases in head motion that might interfere with fMRI data acquisition.

3.1.2.2. Cold stress. A container of ice water chilled to a temperature of 4 °C was prepared for the cold condition prior to the start of the scan. Temperature was assessed intermittently using a laboratory grade thermometer. During the cold scan, subjects' left hands were immersed up to the wrist for a period of 30 s by an experimenter who received a signal from the control room. After the hand-immersion procedure, the subjects' hands were removed for a period of 2 min 30 s. This pattern was repeated 3 more times over the course of the 12-minute cold stress run, resulting in 4 total stress applications. Data acquired from this scan represented a cold stress condition.

3.1.2.3. Room temperature control. To dissociate possible cold effects from hand-immersion effects, we implemented the following control condition. A container of water (25 °C) was prepared for the room temperature condition prior to the start of the scan. Temperature was assessed intermittently using a thermometer. During the room temperature scan, subjects' left hands were immersed up to the wrist, for a period of 30 s. After the hand-immersion procedure, subjects' hands were removed for a period of 2 min 30 s. This pattern was repeated 3 more times over the course of the 12-minute room temperature control run, resulting in 4 total stress applications. Data acquired during this scan represented a room temperature control condition.

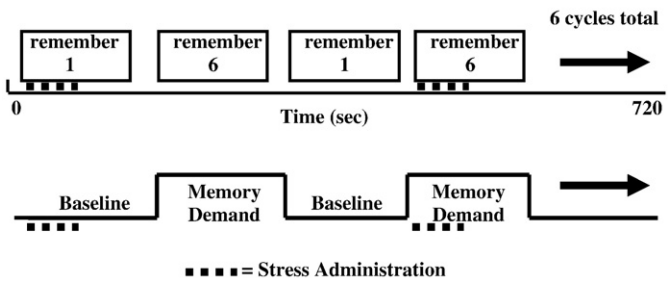

Fig. 3. Representation of the design of the behavioral task, with stress applications where applicable (no applications in the no-water control condition).

3.1.2.4. No-water control. To permit observation of any possible hand-immersion effects, we implemented the following control condition. During the no-water control condition, the subjects' hand was free from any hand-immersion while they performed the delayed-response task. Data acquired during this scan comprised a no-water control condition.

3.1.3. MRI technique

Imaging was performed on a 3T Siemens Allegra scanner equipped with a fast gradient system for echoplanar imaging. A standard radiofrequency head coil with foam padding was used to restrict participants' head motion while minimizing discomfort. High-resolution axial T1-weighted images were obtained from all subjects. A gradient echo, echoplanar sequence (TR=2000 ms, TE=50 ms) was used to acquire data sensitive to the blood-oxygen-level-dependent (BOLD) signal. Anatomical imaging resolution was .8594 mm × .8594 mm in-plane and a slice thickness of 4 mm (32 axial slices were acquired). Resolution of acquired BOLD functional data was 3.4375 mm × 3.4375 mm (32 axial slices acquired). Eighteen seconds of gradient and radio-frequency pulses preceded the actual data acquisition to allow tissue to reach steady-state magnetization.

3.1.4. MRI data analysis

The methods we used for data analysis have been presented in a number of papers using similar experimental designs [54,70]. A blocked fMRI analysis was used in this study. BOLD signal changes during each stress and WM demand condition were modeled with a reference function that represented the blocked design, smoothed with a time-shifted BOLD impulse response function. Reference functions were convolved with hemodynamic reference functions specific to each individual, modeled using central sulcus BOLD activity measured during a preliminary finger tapping task at the beginning of the experimental scan.

Data processing was conducted on Linux workstations. Subsequent to the reconstruction of the fMRI image, data were sync interpolated in time in order to account for phase-shifts related to slice-wise data acquisition. Motion compensation involved both the removal of spatially coherent signal changes using a partial correlation method, and the application of a six-parameter, rigid-body, least squares alignment routine. Data were analyzed using a modified general linear model for serially correlated error terms [69]. All data processing and analysis was conducted using VoxBo statistical software.

To observe stress-related and WM demand-related effects in PFC, two PFC regions of interest were specified, averaging across the left and right hemispheres. These regions of interest were defined in each participant individually, using anatomical landmarks [60]. Masks for each region of interest (ROI) were drawn individually by one experimenter (AJP). One ROI was the dorsolateral prefrontal cortex (DLPFC). It included the middle and superior frontal gyri corresponding to Brodmann's Areas (BA) 9 and 46. The second region of interest was ventrolateral prefrontal cortex (VLPFC). It included inferior frontal gyrus corresponding to BAs 44, 45, and 47. To observe stress-related and WM demand-related effects in amygdala, we outlined this structure bilaterally on the basis of landmarks described in Pruessner et al. [52] for each subject. The spatial extent of activation in these regions was determined by calculating the number of

suprathreshold voxels (specifically voxels whose *t*-value exceeded 1.96 uncorrected). The magnitude of activation in these regions was determined by calculating average percent-signal change from baseline. These measures were assessed for all stress and WM demand conditions.

3.2. Results

3.2.1. Behavioral results

Paired *t*-tests were performed on reaction time (RT) medians for trials in which subjects responded correctly. In addition, we calculated the effect sizes (*d*) for each comparison [9]. The results of these tests indicated that subjects performed significantly slower on high WM demand (6-letter; $M=1088.04$ ms) trials as compared to low demand (1-letter; $M=884.29$ ms) when collapsed across stress condition, $t(11)=-5.184$, $p<.01$, $d=.93$. This effect was observed within the cold stress $t(11)=-4.103$, $p<.01$, $d=1.02$), room temperature control $t(11)=-4.472$, $p<.01$, $d=.86$, and no-water control $t(11)=-5.306$, $p<.01$, $d=.86$, conditions individually as well. No significant main effect of the three different stress conditions was observed in RT or accuracy, (both p values $>.10$). Furthermore, no significant interaction was observed between the factors of stress and WM demand for RT or accuracy (both p values $>.10$).

3.2.2. Prefrontal cortex

We tested for the presence of monotonic stress-related activation changes using two measures. The first was spatial extent, as measured by numbers of suprathreshold voxels. Numbers of voxels showing significant increases in magnitude between low WM and high WM demand trials were Friedman ranked and a distribution-free test of ordered alternatives (Page Test) comparing the three stress conditions was performed [24]. These tests indicated a monotonic effect in VLPFC, $L=2.14$, $p=.02$, also present as a trend in DLPFC, $L=1.43$, $p=.08$ (see Fig. 4). The second measure was activation magnitude as measured by percent-signal change. Analyses similar to those above were performed on those voxels in which a significant increase in average percent-signal change was observed, using low WM demand trials as a baseline. Results indicated similar monotonic effects between stress conditions in both regions. In DLPFC percent-signal change was greatest with cold stress, lower with room temperature control, and lowest with no-water control, $L=1.84$, $p=.03$. There was a similar effect within VLPFC, $L=1.63$, $p=.05$ (see Fig. 5). No significant gender effect was observed in PFC response to cold stress.

3.2.3. Amygdala

We hypothesized that the effects we observed in PFC may be mediated by amygdala activity. To test this hypothesis, we correlated activity as measured by *t*-tests comparing low WM and high WM demand trials in the PFC ROIs with activity in amygdala in each subject. The results indicated strong inverse relationships between amygdala and PFC ROIs in the cold stress condition alone. Amygdala activity decreased as VLPFC activity increased; these regions were correlated negatively ($r=-.76$, $p<.004$). Trends toward a similar correlation were observed between amygdala and DLPFC activity ($r=-.53$, $p<.08$). No such effects were observed in the other two stress conditions (all p values $>.10$). In order to better understand the differential relationships between amygdala and dorsal and ventral prefrontal regions, we tested the idea that the stress-related amygdalar activity we reported above may be mediated by subjects' gender. Relationships between neural activity and stress have been shown to be mediated by gender in previous studies [67]. A 2 (Gender)×3 (Stress Condition) repeated-measures ANOVA on amygdala data indicated that in the cold stress condition males showed significantly greater increases in amygdalar activity than females, $F(2,20)=3.564$, $p<.05$.

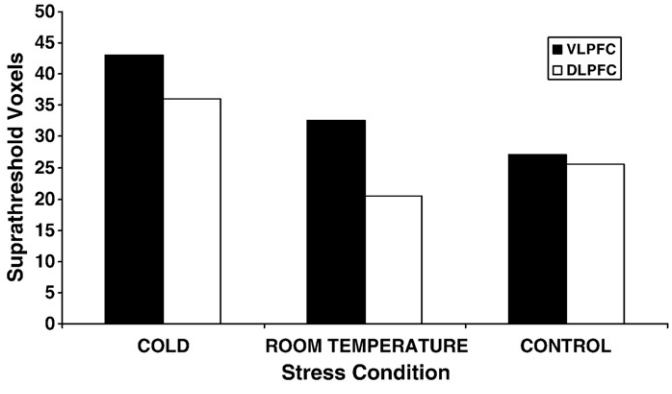

Fig. 4. Median spatial extent of PFC activation in each PFC ROI in each condition.

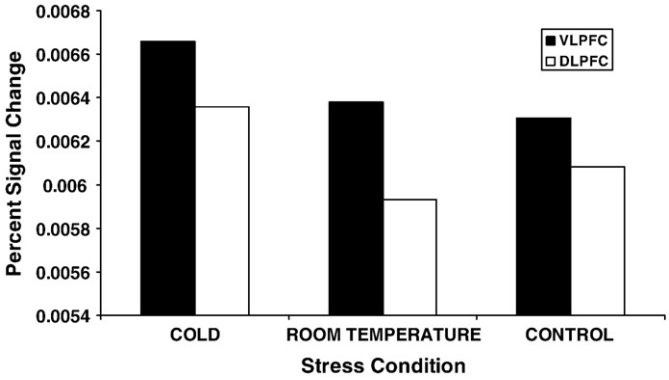

Fig. 5. Average percent-signal change in each PFC ROI in each condition.

4. Discussion

Experiment 1 was conducted to determine the effects of stress, as measured by salivary cortisol levels, on WM performance. Subjects performed a WM task with high and low WM demand trials, while their hands were immersed in either room temperature or cold water. We observed minimal stress-related performance differences. There were significant performance differences, however, as a function of WM demand. Subjects performed significantly slower on high WM demand as compared to low WM demand trials. These results are consistent with previous findings [46]. Subjects experienced stress when their hands were immersed in cold water; salivary cortisol was significantly higher during cold stress than during room temperature control. Consistent with previous findings [35,68] we observed gender differences in stress reactivity; mean salivary cortisol levels were higher in men compared to woman.

In Experiment 2 we utilized a block-design fMRI technique to examine the effects of acute stress on PFC-based WM systems. During scanning, subjects performed a delayed-response task with alternating low and high levels of WM demand (1 vs. 6 letters) under no-water control, room temperature control, and cold stress conditions. There were differential effects of stress on BOLD activity in PFC and differential effects of WM demand on performance. There was a greater spatial extent of activity in the cold stress condition than in the other stress conditions in both PFC ROIs in high WM demand activation as compared to low WM demand. We observed that fMRI activation differed significantly more from baseline in the cold stress condition than in room temperature control and no-water control conditions. In DLPFC we observed that with cold stress, percent-signal change was the largest, followed by room temperature control, and then no-water control.

Acute stress exposure may elicit a cascade of responses that mediate WM systems at physiological and cognitive systems levels. Excessive PFC glucocorticoid and catecholamines may lead to a "hyperdopaminergic" response [5]. Specifically, high PFC dopamine levels couple with protein kinase A (PKA) via G proteins and/or protein Kinase C (PKC) via calcyon

[4]. This coupling may serve to synergistically impair intracellular PFC signaling mechanisms. These neurochemical changes in PFC signaling and neurotransmission during periods of stress may compromise PFC functions, thus affecting cognition and behavior [3]. Neurochemical modulation of hemodynamic coupling could also affect stress-related PFC signal change. For instance, relationships between dopamine (a known PFC-based WM neurotransmitter) and vasoconstrictive responses in the cortical vasculature have been observed [25,34].

Amygdalo–prefrontal interactions are known to mediate stress responses in PFC [53]. For instance, amygdalar lesions have been shown to block modulatory glucocorticoid effects on cognitive function [53]. Amygdala projections pervasively innervate PFC [40]. The strong inverse relationship between amygdala and PFC activity observed in Experiment 2 supports these conclusions. It is plausible that amygdala activity may moderate PFC-based WM functions while under acute stress. Also of interest is research indicating that amygdala activity is inversely related to ventral prefrontal activity during the regulation of negative affect [62]. Based on the results of Experiment 2, a plausible hypothesis may be that participants are engaging these areas in order to maintain organized behavior under stress and that – once engaged – ventral prefrontal areas reciprocally inhibit stress-related amygdala activity in the furtherance of that goal. In addition, the gender by stress interaction we observed in amygdala activity suggests a differential role for these neural systems when males are exposed to stress as compared to females. Further research examining amygdalo–PFC interactions, the role that neurotransmitters may play in mediating neural and vascular responses to stress, as well as the differential impact of gender will be necessary to understand the complex interplay of these systems. The findings from Kavushansky and Richter-Levin [30], of a positive relationship between entorhinal cortex and amygdala activity, suggest that additional research will be required to understand the relationship between findings from animal models and those from human models. Technical development of such translational methods is currently underway (e.g., [29]).

The present study has a number of limitations. First, we were not able to collect salivary cortisol samples in Experiment 2. This was because motion introduces significant artifact in fMRI signal. While we are equipped to deal with naturally occurring sources of motion including eye-blinks and respiration, excessive head motion would be created from the sampling of salivary cortisol while subjects were in the scanner because the subject is required to chew on a cotton salivette for no less than 1 min per sample. However, past research that has clarified the temporal nature of cortisol dynamics after exposure to cold stress can clarify its role in the current study. Cold induced stress is sustained over time. McRae and colleagues [43], for instance, observed immediate and sustained cortisol increases after exposure to cold stress. These results are consistent with other findings investigating cortisol kinetics in response to CPT (i.e., [64]) and easily fall within the time window of stress exposure in the current experiment. Future studies are warranted to address the relationship between cortisol activity, prefrontal cortex and amygdala functioning in humans during the performance of cognitive operations in the presence of acute stress.

A second limitation of the present study was that we did not collect data on subjective measures of stress in these experiments. Other research, however, suggests that cold stress does not correlate with anxious mood [46]. Furthermore, participants have rated cold stress procedures as only moderately painful, with longer hand-immersion intervals (3 min) and lower water temperatures (2–3 °C) than were utilized in our experiment [47]. Reduced tactile sensitivity with prolonged cold stress may attenuate pain responses. However, in a study by Patil et al. [48] participants were exposed to alternating cold- and luke-warm water hand-immersions while performing a series of cognitive tasks. The results of physiological indices of stress suggested that participants did not habituate or demonstrate increased sensitization as a result of the repeated water immersions. Similarly, Oshima et al. [47] found that repeated hand-immersions in cold water produced a significant increase in cortisol responsivity and prolonged the response curve. Nonetheless our data support the hypothesis that PFC plays a central role in maintaining organized behavior during acute stress.

In the present study we used a single stressor, the cold-pressor stress task (CPT), administered concurrently with subjects' performance of a relatively simple delayed-response WM task with known sensitivity to cognitive demand (the Sternberg item-recognition task; 59). The CPT has been shown to induce a reliable stress response. In one study the CPT resulted in significant changes in cardiovascular functioning compared to baseline across multiple measures of cardiovascular functioning. Reliability measures for internal consistency in that study ranged from .74 to .92 with relatively small standard error of measurement values [31]. To assess the effects of cold stress on WM-related PFC regions, we also had subjects perform this task during fMRI scanning. The current study contributes to the understanding of individual differences in stress reactivity by presenting evidence of gender differences in neural activity related to acute stress and concurrent WM operations. Although no behavioral differences between genders were observed, our results further support the hypothesis that gender is a significant source of variability when measuring stress reactivity. This variability is present not only at the hormonal level, but can be quantified at the neural level as well. Additional research clarifying the neural correlates of gender differences in stress reactivity may be of great use in addressing the nature of individual differences in stress reactivity.

Our results indicated that an acute stressor does influence PFC-based WM processes. We observed distinct patterns of BOLD activity between stress conditions in the presence of relatively homogeneous behavioral performance across stress conditions. There were significant BOLD differences between stress conditions. One possibility suggested by this pattern of results is that stress-related PFC activation increases reflect operations in the service of maintaining organized behavior during stress. Behavioral research highlights the role of PFC in resistance to interference or distraction via executive processes fundamental to WM capacity [28]. Such processes may permit individuals to maintain performance under sub-maximal stress conditions. The cold-water induced stress in our study did not reach sufficient levels to incur performance deficits [68]. Further research will be required to precisely determine what cognitive mechanisms are reflected by stress-related PFC activation increases, and how those processes change when stress-levels induce performance decrements.

Our observations support the existence of system-level interactions between physiological and cognitive responses to acute stress. Individual differences may play a role at both of these levels. Individual subjects vary in physiological reactions to acute stress, and the same is true of individuals' WM capabilities. It is possible that some differences are related to PFC-based executive-attentional capabilities [28,54,56,63]. For example, Vogel et al. [63] have observed that human subjects vary in their efficiency at inhibiting irrelevant information during WM encoding. Some individuals appear to encode only task-relevant stimulus properties whereas others encode stimuli less discriminately. It may be that physiological acute stress responses disrupt such PFC-based inhibitory processes, requiring increased PFC activity to maintain the interference resistance capabilities that are necessary for high-quality behavioral performance.

Acute stress is known to affect performance in demanding workplace environments. Physiological stress measures are elevated during performance in such environments [33,65] and may adversely affect vital aspects of performance such as drug-dose calculations[36]. Responses to acute stress may have evolutionarily adaptive value. Nonetheless, higher-order cognition may be compromised in the service of instinctual responses designed to deal with such stress.

Our results, showing amygdala-mediated PFC increases during cold-pressor stress, suggest a central role for this region in maintaining organized and complex human behavior in stressful environments.

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