

Part A

Methods

Participants

Information on participant demographics and symptom severity (Clinician-Administered Posttraumatic Stress Disorder [PTSD] Scale [1], Impact of Events Scale-Revised [2], Toronto Alexithymia Scale [3], Beck Depression Inventory [4]) are shown in Table 1. Three (19%) of the PTSD subjects (N=16) had a history of major depressive episodes. The healthy combat-exposed and noncombat comparison subjects had no history of PTSD symptoms or major depressive episodes. Four (25%) of the PTSD patients had a history of alcohol dependence (in remission); one (7%) of the healthy combat-exposed comparison subjects (N=15) and two (13%) of the healthy noncombat comparison subjects (N=15) had a lifetime history of alcohol abuse or dependence (in remission). All subjects were free of psychotropic medications for at least 4 weeks prior to the study, had no current axis I disorder (6 months for substance abuse), and had no history of chronic medical or neurological illness.

Table 1

Group Characteristics

	Healthy Comparison Subjects N=15		Combat Comparison Subjects N=15		PTSD Patients N=16	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	49.4	7.9	56.5	4.9	53.8	4.2
Clinician-Administered PTSD Scale total	2.8	3.4	6.3	7.3	61.3 ^a	19.9
Impact of Events Scale-Revised total	36.2	15.5	35.9	14.7	68.4 ^a	20.8

Toronto Alexithymia Scale	58.0	8.0	52.5	7.9	71.9 ^a	15.5
Beck Depression Inventory	4.6	4.3	5.2	4.9	21.3 ^a	9.9

^aSignificant between-group differences ($p < 0.001$) from one-way analysis of variance (ANOVAs).

Emotional Challenges

Participants were exposed to emotional stimuli at varying intensity levels using two presentation modes—emotional pictures and autobiographic scripts—as previously described (5, 6). Two sets of ***emotional*** (aversive and neutral) gray-scaled pictures from the International Affective Picture System (7) and a third set of blank images (luminance matched fixation cross) were presented in blocks of 10 for 3 seconds each (30 seconds/block). ***Autobiographical script-driven imagery*** was administered per Pitman et al. (8). Two kinds of 40-second autobiographical “scripts” were constructed from subject interviews, describing either neutral or aversive (combat traumas for combat veterans, highly stressful for healthy comparison subjects) events. These scripts were recorded and then played to subjects during positron emission tomography (PET) scanning.

Stimulus Presentation

Participants received a total of 10 emotional challenges over the 2-hour experiment (two presentations each of neutral scripts, traumatic/stressful scripts, neutral International Affective Picture System pictures, aversive International Affective Picture System pictures, and “blank” pictures (fixation cross). Presentation was counter-balanced by stimulus type and valence. Script valence was counter-balanced on an AABB schedule to help control “carry-over” effects when traumatic scripts were presented first, and pictures were counter-balanced on ABCABC schedule. Participants were instructed to focus on the images and scripts and to then

maintain the evoked states and rate emotional states (1–10 scale) after each scan using a modified Positive Affectivity-Negative Affectivity Scale.

Neuroendocrine Measures

Blood samples (5ml in EDTA containing tube) were obtained from an in-dwelling intravenous catheter (inserted 60 minutes earlier) at the start of the experiment (~2:00 p.m.) at 5 minutes after each emotional challenge and 2 minutes prior to the next scan. Two samples were also obtained at morning baseline (8:00 a.m.). Blood was placed on ice and spun within 1 hour. Adrenocorticotrophic hormone was assayed using Nichols Institute (San Juan Capistrano, Calif.) Adrenocorticotrophic hormone radioimmunoassay, specific for intact adrenocorticotrophic hormone; detection limit 0.5 pg/ml, intra-assay variability 7%. Cortisol was assayed using DPC (Los Angeles, Calif.), RIA, detection limit 0.2 mg/dl, intra-assay variability <5%.

Neuroendocrine data showed rightward skew and were natural log transformed to reduce skew.

Positron Emission Tomography Procedures

[¹⁵O] H₂O PET scans were performed on Siemens ECAT EXACT and HR+ scanners (both CTI, Knoxville, TN), which yield 47 slices simultaneously for whole-brain coverage at an axial field of view (FOV) of approximately 15 cm and a resolution of 4.5 mm full width half-maximum. Participants were positioned using the orbito-meatal line as a reference and a forehead restraint to reduce intrascan movement. For each scan, 10–15 mCi of [¹⁵O] H₂O was given as an intravenous bolus. Data were collected in three-dimensional acquisition mode as a single 60-second frame beginning 2 seconds after the radioactivity arrived in the brain. Scans were separated by 12 minutes. Both ECAT scanners have similar technical specifications, and steps were taken to minimize differences between them as reported previously (5), including use of

within-subject subtractions, an equal number of subjects from each group studied in each scanner, and data transformation using an empirically derived algorithm to equate sensitivity thresholds. Validation analyses performed with “scanner type” as a categorical variable found no changes in activation foci.

Statistical Analyses

The analyses of primary interest in this paper are analyses of covariance, regressing regional cerebral blood flow (rCBF) with hormone levels to examine: 1) links between brain activation patterns and subsequent adrenocorticotrophic hormone response and 2) the impact of circulating cortisol levels on rCBF responses. These are described in detail below in Imaging Analyses.

We also conducted separate analyses of neuroendocrine data alone to assess whether 1) the experimental manipulations produced significant changes in subjective negative emotional responses, 2) the experimental manipulations produced significant changes in adrenocorticotrophic hormone, and 3) that there was also meaningful variation in circulating cortisol during the 2-hour experiment. Because the obtained plasma adrenocorticotrophic hormone and cortisol levels showed a positive skew (skewness = 1, range 1.35–4.1 using the “Frequencies” module in SPSS statistical software), adrenocorticotrophic hormone and cortisol data were natural log transformed to reduce skew in the distribution.

To examine (1) *subjective emotional responses*, we first examined effects of aversive pictures vs. neutral pictures and traumatic/stressful scripts using repeated measures-ANOVA to test main effects of condition, and then tested group differences using Tukey post hoc tests.

To examine (2) *plasma adrenocorticotrophic hormone responses to emotional challenges*, we first compared “morning baseline” values between the groups using one-way

ANOVA. To analyze acute adrenocorticotrophic hormone reactivity to individual stimuli (conditions), we first conducted an omnibus repeated measures ANOVA of 5-minute postscan plasma adrenocorticotrophic hormone levels to test overall effects of condition (six levels, baseline, fixation cross, neutral International Affective Picture System, aversive International Affective Picture System, neutral scripts, and traumatic/stressful scripts), group (PTSD, healthy combat, and healthy non-combat comparison subjects), and any interaction effects. A finding of overall significance in the conservative repeated measures ANOVA was followed by further investigations of plasma adrenocorticotrophic hormone reactivity within each condition and each group by comparisons of 5-minute postscan adrenocorticotrophic hormone levels with morning baseline levels, and also with 2-minute prescan adrenocorticotrophic hormone levels (i.e., acute baseline) to examine acute reactivity to individual conditions, using paired t tests.

To examine (3) *plasma cortisol responses to emotional challenges*, we again first examined morning baseline plasma cortisol levels between groups using a one-way ANOVA. To examine potential changes in cortisol levels in response to our stimuli, we conducted an omnibus repeated measures ANOVA of cortisol levels (10-minute postscan levels) over the 5 conditions during the 2-hour experiment (five levels, fixation cross, neutral International Affective Picture System, aversive International Affective Picture System, neutral scripts, and traumatic/stressful scripts condition), and group (PTSD, healthy combat, and healthy non-combat comparison subjects). Since our interest was in changes in cortisol levels during the emotional stimuli presented in the experiment, we did not include morning baseline cortisol in this analysis, as the expected diurnal variation in cortisol levels would lead to false positive findings. We also examined the cortisol levels during the 2-hour experiment as a time series in a separate repeated measures ANOVA analyzing main effects (and linear tendencies) of time, group, and group-by-

time interactions. Since the timing of our emotional stimuli presentation (12-minute interstimulus interval) was not designed to optimally detect cortisol responses to individual conditions (peak cortisol responses usually occur at 15-30 minutes following emotional challenge), we also compared overall plasma cortisol during the two separate 48-minute periods when subjects either 1) viewed four sets of emotional International Affective Picture System pictures or 2) listened to four emotional autobiographical scripts using a one-way ANOVA.

Imaging Analyses

PET images were realigned to correct for head movement, anatomically standardized to MNI coordinates, and smoothed with a 12 mm full width half-maximum filter using SPM '99 (Wellcome Department of Cognitive Neurology, London). To identify regions of brain activity associated with HPA axis activation, we performed voxel-wise analyses of covariance of [¹⁵O] PET activity using 5-minute postscan plasma adrenocorticotrophic hormone concentrations as a regressor on all 10 emotional challenge scans (“multisubject, covariates-only” module in SPM99, ANCOVA by subject global normalization). To examine adrenocorticotrophic hormone responses specifically to autobiographical scripts, we separately examined covariations with the rCBF during the scripts scans. To identify regions of brain activity associated with prestimulus levels of circulating cortisol, analyses of covariance were performed using 2-minute prestimulus plasma cortisol concentrations. Based on prior work in humans and rodents, we hypothesized covariations in paralimbic cortex (orbitofrontal cortex, rostral ACC, insular cortex and parahippocampal gyrus), subcortical limbic structures of the medial temporal lobes (amygdala and hippocampus), and/or mPFC. We used cluster/extent threshold of five contiguous voxels, and small volume correction using a $p < 0.005$ threshold with a false discovery rate correction, for activations within *a priori* regions: [right insula/amygdala, rectangular boxes 1 and 2: $x = 30$ mm

(SD=20) or -30 mm (SD=20), y=-10 mm (SD=45), z=-5 mm (SD=25); left insula/amygdala, rectangular box 3: x=-30 (SD=20) mm, y=-10 mm (SD=45), z=-5 mm (SD=25); mPFC/ACC]. Foci of covariation outside our *a priori* small volume correction areas were thresholded at $p < .001$ ($z > 3.19$, uncorrected).

References

1

Blake DD, Weathers FW, Nagy LM, Kaloupek DG, Gusman FD, Charney DS, Keane TM: The Development of a Clinician-Administered PTSD Scale. *J Trauma Stress* 1995; 8:75-90

2

Horowitz M, Wilner N, Alvarez W: Impact of Event Scale: a measure of subjective stress. *Psychosom Med* 1979; 41:209-218

3

Taylor GJ, Bagby RM, Ryan DP, Parker JD, Doody KF, Keefe P: Criterion validity of the Toronto Alexithymia Scale. *Psychosom Med* 1988; 50:500-509

4

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J: An inventory for measuring depression. *Arch Gen Psychiatry* 1961; 4:561-571

5

Liberzon I, Zubieta JK, Fig LM, Phan KL, Koeppe RA, Taylor SF: mu-opioid receptors and limbic responses to aversive emotional stimuli. *Proc Natl Acad Sci U S A* 2002; 99:7084-7089

6

Britton JC, Phan KL, Taylor SF, Fig LM, Liberzon I: Corticolimbic blood flow in posttraumatic stress disorder during script-driven imagery. *Biol Psychiatry* 2005; 57:832–840

7

Lang PJ BM, Cuthbert BN: International Affective Picture System (IAPS): Technical Manual and Affective Ratings. Gainesville, Fla., University of Florida: NIMH Center for the Study of Emotion and Attention, 1997

8

Pitman RK, Orr SP, Forgue DF, de Jong JB, Claiborn JM: Psychophysiologic assessment of posttraumatic stress disorder imagery in Vietnam combat veterans. *Arch Gen Psychiatry* 1987; 44:970–975

Part B

Results

Subjective Ratings

Participants in all three groups reported greater negative subjective emotional response on the PANAS scale for aversive vs. neutral pictures ($F=231.4$, $df=1, 43$, $p<0.001$), and for traumatic/ stressful vs. neutral scripts ($F=121.1$, $df=1, 41$, $p<0.001$) in repeated measures ANOVA. PTSD subjects reported greater negative emotional response to trauma scripts than combat comparison subjects ($F=5.9$, $df=2, 41$, $p<0.01$), Tukey post hoc $p<0.001$).

Neuroendocrine Findings

Adrenocorticotrophic Hormone

Groups did not differ in baseline adrenocorticotrophic hormone ($F=0.94$, $df=2, 3$, $p=0.39$); however, an overall repeated measures ANOVA of 5-minute postchallenge adrenocorticotrophic hormone levels during the PET scanning experiment showed a significant effect of condition ($F=2.48$, $df=5, 27$, $p=0.04$). Examination of the data suggested that this condition effect was entirely due to increased adrenocorticotrophic hormone responses to scripts in both PTSD patients and combat comparison subjects. Follow-up paired t-tests confirmed that PTSD subjects showed significantly elevated adrenocorticotrophic hormone levels 5 minutes after the first trauma script, compared to both morning adrenocorticotrophic hormone levels ($t=1.93$, $df=14$, $p=0.03$) and immediate baseline (2 minutes before the script) ($t=2.42$, $df=14$, $p=0.03$). These effects were at trend level for combat comparison subjects ($p=0.06$), but the variance was inflated due to a single subject with a very high adrenocorticotrophic hormone response (102 pg/ml). After exclusion of this outlier, combat comparison subjects also showed significant 5-minute adrenocorticotrophic hormone response to first trauma script, compared with morning ($t=2.76$, $df=9$, $p=0.01$) and immediate baseline ($t=4.09$, $df=9$, $p<0.01$). Healthy comparison subjects showed no acute adrenocorticotrophic hormone responses. As expected, adrenocorticotrophic hormone elevations following the second trauma scripts were less prominent and more variable (likely due to both habituation and negative feedback) and did not reach statistical significance. Adrenocorticotrophic hormone responses to trauma scripts in PTSD subjects were strongly and positively correlated with the severity of PTSD symptoms as measured by the Impact of Event Scale Revised (Pearson correlation $r=0.69$, $p=0.005$), supporting the clinical meaningfulness of adrenocorticotrophic hormone response data.

Cortisol

Groups did not differ in baseline cortisol ($F=0.94$, $df=2, 36$, $p=0.39$ [Table 1]) and cortisol levels did not decrease over the course of the 2-hour experiment; (RM-ANOVA - no effect for time or group x time interaction). The timing of sample collection was not designed to test for the direct effects of emotional challenges on plasma cortisol responses; however, cortisol in plasma corresponded to a degree with the adrenocorticotrophic hormone responses. Significant differences were found between the conditions ($F=4.39$, $df=4, 29$, $p<0.01$), with higher cortisol during scripts than during pictures presentation ($F=5.16$, $df=1, 32$, $p=0.03$), and as with adrenocorticotrophic hormone, this effect was due to the combat-exposed groups. Separate ANOVAs revealed higher cortisol levels during the scripts than the pictures (10.3 vs. 8.2 g/dl; $F=8.19$, $df=1, 11$, $p=0.01$) in PTSD patients, and combat comparison subjects (9.7 vs. 8.1 g/dl; $F=7.55$, $df=1, 10$, $p=0.02$), and no difference in healthy comparison subjects (8.9 vs. 8.4 g/dl; $F=0.12$, $df=1, 11$, $p=0.77$).

References

1

Liberzon I, Zubieta JK, Fig LM, Phan KL, Koeppe RA, Taylor SF: mu-Opioid receptors and limbic responses to aversive emotional stimuli. *Proc Natl Acad Sci U S A* 2002; 99:7084–7089

2

Britton JC, Phan KL, Taylor SF, Fig LM, Liberzon I: Corticolimbic blood flow in posttraumatic stress disorder during script-driven imagery. *Biol Psychiatry* 2005; 57:832–840

3

Lang PJ BM, Cuthbert BN.: International Affective Picture System (IAPS): Technical Manual and Affective Ratings. Gainesville, Fla, University of Florida: NIMH Center for the Study of Emotion and Attention, 1997

4

Pitman RK, Orr SP, Forgue DF, de Jong JB, Claiborn JM: Psychophysiologic assessment of posttraumatic stress disorder imagery in Vietnam combat veterans. Arch Gen Psychiatry 1987; 44:970-975