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Neuroendocrine and Psychophysiologic Responses in PTSD:

A Symptom Provocation Study

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Biological research on post-traumatic stress disorder (PTSD) has focused on autonomic, sympatho-adrenal, and hypothalamo-pituitary-adrenal (HPA) axis systems. Interactions among these response modalities have not been well studied and may be illuminating. We examined subjective, autonomic, adrenergic, and HPA axis responses in a trauma-cue paradigm and explored the hypothesis that the ability of linked stress-response systems to mount integrated responses to environmental threat would produce strong correlations across systems. Seventeen veterans with PTSD, 11 veteran controls without PTSD, and 14 nonveteran controls were exposed to white noise and combat sounds on separate days. Subjective distress, heart rate, skin conductance, plasma catecholamines, ACTH, and cortisol, at baseline and in response to the auditory stimuli, were analyzed for group differences and for patterns of interrelationships. PTSD patients exhibited higher skin conductance, heart rate, plasma cortisol, and catecholamines at baseline, and exaggerated responses to combat sounds in skin conductance, heart rate, plasma epinephrine, and norepinephrine, but not ACTH. The control groups did not differ on any measure. In canonical correlation analyses, no significant correlations were found between response systems. Thus, PTSD patients showed heightened responsivity to trauma-related cues in some, but not all, response modalities. The data did not support the integrated, multisystem stress response in PTSD that had been hypothesized. Individual response differences or differing pathophysiological processes may determine which neurobiological system is affected in any given patient. ***[Neuropsychopharmacology 21: 40–50, 1999]*** *© 1999 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

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Biological research on post-traumatic stress disorder (PTSD) has grown rapidly, focusing on psychophysiological and neuroendocrine study of autonomic, sympathoadrenal (SA), and hypothalamo-pituitary-adrenal (HPA) axis systems. Both baseline (or "tonic") abnormalities and abnormal responsivity ("phasic abnormalities") of these stress response systems have been reported. Response to traumatogenic stimuli, both externally presented and internally generated (mental imagery), is probably the most discriminating approach for examination of biological abnormalities in PTSD (Friedman 1991; Shalev et al. 1993). However, there are inconsistencies in available data, and much remains to be learned about the responsivity of these systems and their interrelationships.

Among psychophysiological measures, enhanced heart rate (HR) responses have been found consistently

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reported (Blanchard et al. 1982; McFall et al. 1990; Pallmeyer et al. 1986; Pitman et al. 1990); whereas, enhanced skin conductance (SC) and electromyogram (EMG) responses have been mainly reported during internally generated mental imagery (Pitman and Orr 1993; Pitman et al. 1987). This response heterogeneity is not surprising, because the SC response reflects primarily sympathetic tone, the HR response is governed by both sympathetic and parasympathetic activity, and facial EMG response reflects primarily stimulus appraisal, or valence, but not arousal (Bradley et al. 1996). However, cue intensity may be a relevant variable, and further work is needed to clarify the ability of intense standardized cues to elicit enhanced appraisal and autonomic arousal responses in PTSD patients.

Neuroendocrine studies have examined catecholamine and HPA axis activity in PTSD patients. Catecholamine studies have produced variable results. Tonic secretion has been elevated in some studies (Kosten et al. 1987); whereas, baseline measures were found normal in others (Murburg et al. 1994b). Blanchard (Blanchard et al. 1991a) reported enhanced plasma norepinephrine (NE) responses to traumatic stimuli; whereas, others (McFall et al. 1990; Murburg et al. 1994a) found enhanced epinephrine (Epi), but not norepinephrine, reponses. Although technical aspects of sample collection (arterialized vs. nonarterialized) and norepinephrine metabolism (Murburg 1994) could account for some discrepancies, stress studies in normals report good correspondence between epinephrine and norepinephrine responses (Richter et al. 1996), which is yet to be demonstrated in PTSD. HPA axis findings have been more consistent, with evidence of lower 24-hour cortisol secretion (Yehuda et al. 1990), hypersensitive glucocorticoid negative feedback (Yehuda et al. 1993b), and higher numbers of lymphocyte glucocorticoid receptors (Yehuda et al. 1993a). However, others have reported elevated 24-hour cortisol secretion (Pitman and Orr 1990). Only two HPA axis stimulation studies have been done in PTSD, producing evidence of reduced ACTH response to corticotrophin releasing factor (CRF) in the presence of elevated baseline cortisol (Smith et al. 1989) , and an enhanced ACTH response to metyrapone (Yehuda et al. 1996a). No PTSD study to date has examined immediate ACTH responses to traumatic stimuli. Examination of HPA axis responses in a trauma-cue paradigm may be illuminating, especially if combined with further exploration of Epi and NE responses.

Although various "stress system" abnormalities in PTSD have been independently reported, neuroanatomically and neurophysiologically these systems interact. The presence of adrenergic innervation of the adrenal medulla, adrenergic receptors in the hypothalamus, and glucocorticoid and CRF receptors in the locus coeruleus (LC) (Harfstrand et al. 1986; Reul and de Kloet 1985), and the role of CRF as a neurotransmitter in the LC and amygdala (Aston-Jones et al. 1994; Valentino et al. 1983; Valentino et al. 1992) suggest that both central and peripheral catecholamine systems, and the HPA axis may be linked in a way that could explain the abnormalities in all these systems in PTSD. It remains unclear, however, whether different neurobiological abnormalities in PTSD occur in the same patients, and in the same paradigm. If so, it might suggest the presence of a common pathophysiological mechanism, such as CRF hypersecretion. We might, then, predict that during periods of increased CRF activity (anxiety, fear, stress), there will be a strong correlation between HPA axis responses, plasma catecholamine secretion, and indices of sympathetic tone (HR, SC). Alternatively, abnormalities in different systems may reflect heightened sensitivity of various stress response measures to specific paradigms or individual differences in dominant response system. PTSD might represent a syndrome, or "final common pathway," which can result from a number of different neurobiological abnormalities. These considerations underscore the need to simultaneously investigate multiple stress systems in the same PTSD patients.

In this study, we examined subjective, autonomic, adrenergic, and HPA axis responses of PTSD patients and controls in an auditory, trauma-cue paradigm. We predicted a strong correlation across systems in PTSD patients, especially in the activation condition.

METHODS

Subjects

Seventeen Vietnam veterans with PTSD, 11 Vietnam veterans without PTSD (combat controls), and 14 nonveterans (noncombat controls), participated in the study. All were males recruited through advertisements. PTSD was diagnosed by DSM-III-R criteria using a clinician-administered structured interview (SCID) (Spitzer 1990). Subjects were excluded if they had any history of psychosis, dementia, or substance abuse within the previous 6 months. All subjects were free of psychotropic medication for at least 4 weeks (6 weeks in the case of fluoxetine). Control subjects had no current Axis I conditions and no history of PTSD. Groups did not differ in age or ethnicity. Combat controls did not differ from noncombat controls on symptom measures; whereas, PTSD subjects were highly symptomatic (Table 1). After a thorough description of the study to the subjects, written informed consent was obtained.

Procedures

Subjects were studied in two sessions in randomized order, on separate days 48 hours apart, at about 9:00

Table 1. Subject Characteristics and Baseline Measures

	PTSD (n = 17)	Normal Controls (n = 14)	Combat Controls (n = 11)
Mean age (SD)	46.4 (3.2)	45.4 (6.1)	51.2 (1.7)
Ethnicity			
White	16	11	9
Black	1	2	2
Hispanic	0	1	0
Marital status			
Divorced/single	7	6	2
Married	10	8	9
Comorbid diagnoses			
MDD[a]	9	0	0
Other anxiety dis.	3	0	1
Substance ab./dep.[a]	10	5	1
PTSD symptoms			
Impact of events	49 (7)	7 (10)	11 (4)
Mississippi	134 (14)	63 (4)	67 (4)
MMPI - PTSD	37 (12)	9 (1)	7 (2)
DES[b]	29 (19)	6 (2)	12 (5)
SUDS[c]	54 (31)	26 (5)	19 (7)
Baseline measures[d]			
Heart rate (bpm)	81 (12)	70 (10)	72 (6)
Skin conductance (μmho)	14.2 (9.6)	4.7 (2.3)	8.6 (5.1)
Norepinephrine (pg/ml)	362 (151)	311 (143)	211 (83)
Epinephrine (pg/ml)	76.4 (40)	74.2 (109.6)	26.1 (14)
ACTH (pg/ml)	15.1 (9.9)	19.8 (15.3)	16.9 (6.5)
Cortisol (μg/dl)	12.1 (3.9)	9.3 (2.9)	7.9 (2.4)

[a] In remission.
[b] Dissociative experiences scale.
[c] Subjective units of distress scale (at baseline).
[d] Standard deviation in parenthesis. Note that baseline measures include both prewhite noise and precombat sounds measures.

AM. One session consisted of exposure to nonspecific arousing stimuli (white noise) and the other of exposure to trauma-related stimuli (combat sounds). After securing intravenous access and electrophysiologic "hook-up," subjects were allowed a 60-min adaptation period. Measures of heart rate and skin conductance were recorded during this adaptation period and throughout the session. The environment was controlled by dimming lights, minimizing interaction, instructing subjects to keep eyes closed during stimulation, and using headphones to present stimuli and block ambient noise.

A 3-min audiotape of combat sounds (e.g., helicopter sounds, explosions, small arms fire) was played at gradually increasing volume (up to 75 dB) (Blanchard et al. 1986) on one testing day. A tape of white noise with the same frequency spectrum was played at the identical, ramped intensity on the other day. Order was counterbalanced (on day 1, half the subjects within each group received combat sounds and half received white noise). Subjects closed their eyes to facilitate imagery and were instructed to remain seated and continue to imagine whatever came to mind following the termination of auditory stimuli, for approximately 5 min.

Electrophysiological data were recorded using a I-330 Interface System (J&J Enterprises, Poulsbo, WA). Skin conductance was recorded using Ag/AgCl electrodes attached to the second phalanx of the first and third fingers of the nondominant hand and connected to a T-601 Electrodermograph Module of the I-330 System, which utilized a constant voltage procedure (0.166 VDC), with a range of 0 to 50 micromhos. Heart rate was recorded with a photoplethysmograph attached to the second phalanx of the second finger of the nondominant hand. It was connected to a P-401 Plethysmograph Module that converted interpeak interval data to beats per minute, with a range of 40 to 200 bpm. Surface frontalis muscle EMG was measured using three electrodes (ground in the middle position) centered on the forehead and connected to a M-501 module. Heart rate, skin conductance, and rectified EMG were sampled at 20 Hz. An IBM-compatible microcomputer running USE software (J&J Enterprises) controlled data acquisition. Mean electrophysiological scores were computed for a baseline period prior to onset of audiotaped sounds (duration = 120 s) and another period immediately following termination of the audiotaped sounds (duration = 30 s).

Plasma samples were drawn via syringe from the IV catheter (inserted 60 min earlier) immediately before and after playing the audiotape. Time interval between

the samples was 4.5 min. Samples for ACTH were mixed with EDTA, placed on ice, and spun within 1 hour. ACTH was assayed in unextracted plasma using Allegro HS ACTH IRMA, a two-site assay specific for intact ACTH, with detection limit of 1 pg/ml and with intra-assay variability of 3%. Briefly, the sample and [^{125}I] labeled monoclonal antibody are added to a tube. A bead with a second antibody attached to it is then added, and the tube is incubated for 20 h at room temperature. After incubation, the beads are washed, and the tubes are counted in a gamma counter. Cortisol was assayed using the Diagnostic Products Corporation (DPC) Coat-a Count cortisol kits, an RIA methodology with a detection limit of 0.2 mg/dl and with intra-assay variability less than 5%. Briefly, plasma sample and [^{125}I] cortisol are added to an antibody-coated tube, and, after incubation at 37°C in a water bath, tubes are decanted and counted in a gamma counter. A calibration curve is then used to calculate the unknown samples. Catecholamine samples were drawn into tubes containing EDTA and reducing agent. Levels were analyzed by a commercial laboratory using conventional high-performance liquid chromatography (HPLC) with electrochemical detection. Subjective units of distress or SUDs (Wolpe 1973) were recorded using a 100-mm visual analog scale, before initiation and after termination of the sounds and the psychophysiological monitoring.

Analyses

Analyses were conducted using the SPSS/Windows version 7.5 and SAS version 6.12. Initial analyses examined baseline (prestimulus) variables for effects of order of stimulus presentation and expectancies. All subjects had equivalent expectancies before their first session, because they all knew they could receive either combat sounds or white noise. However, expectancies and, thus, anticipatory effects could differ entering the second session, because one group had already received white noise and could anticipate hearing combat sounds; whereas, the other group had already heard combat sounds; therefore, knew they would receive white noise. To assess stimulus order and expectancy effects on baseline measures, we conducted three-way, repeated measures analyses of variance (ANOVAs) with two between-subjects variables (order: those receiving white noise first vs. those receiving combat sounds first, and diagnosis: PTSD patients, combat controls, no-combat controls) and one repeated measure (stimulus condition: white noise vs. combat sounds). If anticipatory anxiety associated with expectancies had an impact on our dependent measures, this should be reflected in significant order effects or order-by-stimulus condition interactions in these analyses. If order/expectancies affected PTSD patients differently than controls, this would be reflected in interactions involving diagnosis.

Our main analyses of responsivity to auditory stimuli and differential responsivity of PTSD patients, on seven biological variables and subjective distress, involved separate three-way, repeated measures ANOVAs, examining the main effects of diagnosis as a between-subjects variable and time (pre- to poststimulus) and stimulus condition as two repeated measures, within-subjects variables. In this model, main effects reflect responsivity to stimuli across groups and conditions, effects of stimulus type across groups, and effects of diagnosis across time points. Interactions reflect differential responsivity to different stimuli across diagnosis, and differential responsivity of diagnostic groups.

To examine relationships between biological variables, we constructed correlational matrices including all the subjects and partialling out baselines and subjects' diagnoses. We also performed canonical correlation analyses to examine possible coregulation of psychophysiologic (SC, HR, EMG) and neuroendocrine (NE, Epi, ACTH) reactivity. We ran these analyses with controls for baseline values and diagnosis and both with and without controls for degree of subjective distress. We also examined relationships between autonomic (SC, HR, E, NE) and HPA (ACTH) responsivity using a similar procedure. In this case, with ACTH as a single variable factor, the procedure is equivalent to multiple regression.

RESULTS

Order and Expectancy Effects

We did not detect any evidence that stimulus order altered expectancies and made an impact on baseline measures. Subjects who received white noise first did not differ overall, on any baseline biological measure or on the level of subjective distress (SUD), from those receiving combat sounds first. In the three-way ANOVAs, the main effect of order and all interactions were not significant. Pre-stimulus baselines did not differ between stimulus conditions. Baseline levels at the second session were unaffected by the type of stimulus presented at session one. Because of the lack of significant order, stimulus condition, or interaction effects, we ignored order and expectancy effects in subsequent analyses.

On the other hand, PTSD patients, as compared to controls and regardless of order of presentation, had higher baseline subjective distress, skin conductance, NE, and cortisol [significant main effects of diagnosis, $F_{(2,35)} = 4.78$, $p = .015$; $F_{(2,37)} = 3.87$, $p = .03$; $F_{(2,35)} = 4.5$, $p = .018$); $F_{(2,35)} = 4.69$, $p = .016$, respectively]. There were also trend level effects of diagnosis for HR [$F_{(2,30)} = 2.88$, $p = .071$], EMG [$F_{(2,32)} = 2.50$ $p = .098$], and Epi [$F_{(2,31)} = 2.52$ $p = 0.092$]. In all cases, the diagnosis ef-

fects were attributable to baseline elevations in the PTSD patients relative to one or both of the control groups (see Table 1, post hoc Sheffe tests showed $p < .05$ for SUDS, SC, HR, and Cortisol, and $p < .07$ for NE and Epi). The two control groups did not differ significantly on any baseline measures. There was no evidence for an effect of diagnosis on prestimulus ACTH levels [$F_{(2,35)} = .12$, $p = .89$].

Subjective Distress

PTSD patients reported higher levels of distress across all the time points and conditions, as compared to both control groups [main effects of diagnosis $F_{(2,37)} = 10.19$, $p < .001$]. Mean change scores on the SUDS rating (pre- to poststimulus) are presented in Figure 1. Distress levels were higher after stimulation for all groups and both types of stimuli [time effect $F_{(1,37)} = 30.4$, $p < .001$], but the PTSD patients reacted with more distress to stimulation than either control group [diagnosis-by-time interaction $F_{(2,37)} = 4.02$, $p = .026$]. Their elevation in level of distress was largest in the combat sounds condition [see Figure 1, diagnosis-by-stimulus-by-time interaction $F_{(2,37)} = 6.31$, $p = .004$]. There was no evidence for differences between combat and noncombat controls on any distress measure.

Psychophysiologic Response

PTSD patients had elevated skin conductance across all time points relative to both control groups (main effect of diagnosis [$F_{(2,37)} = 4.02$, $p = .026$, data not shown]. Pre- to poststimuli change scores, for each group and type of stimulus, are presented in Figure 2. As can be seen, skin conductance increased from pre- to poststimulus, in all three groups and for both types of stimuli [main effect of time, $F_{(1,37)} = 21.03$, $p < .001$]. The largest changes shown were those of the PTSD patients responding to the combat sounds [diagnosis-by-time-by-stimulus interaction, $F_{(2,37)} = 3.49$, $p = .041$].

Similarly, there was a general elevation in HR in PTSD patients relative to both control groups [main effect of diagnosis, $F_{(2,33)} = 3.94$, $p = .029$, data not shown]. Pre- to poststimuli change scores, for each group and type of stimulus, are presented in Figure 2. Normal controls showed little or no HR change to either stimulus, but the presence of a differential response (acceleration to combat sounds, deceleration to white noise) in patients, and combat controls produced a significant time-by-stimulus interaction [$F_{(1,33)} = 7.87$, $p = .008$]. As with skin conductance, the largest changes shown were those of the PTSD patients responding to the combat sounds stimuli [diagnosis-by-time-by-stimulus condition interaction, $F_{(2,33)} = 3.2$, $p = 005$].

PTSD patients did not differ from control groups in frontalis muscle activity [EMG, main effect of diagnosis,

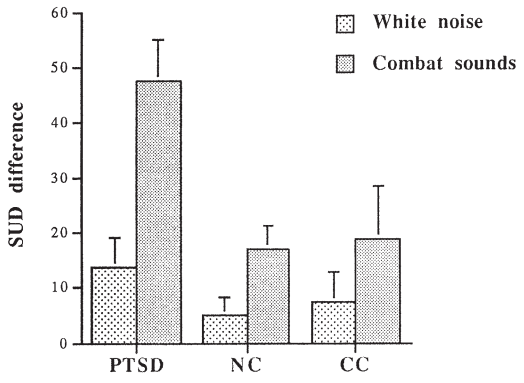

Figure 1. Change in the level of distress, in response to white noise and combat sounds, in PTSD, normal control, and combat control groups. Expressed as difference in subjective units of distress (SUD) from pre- to postexposure.

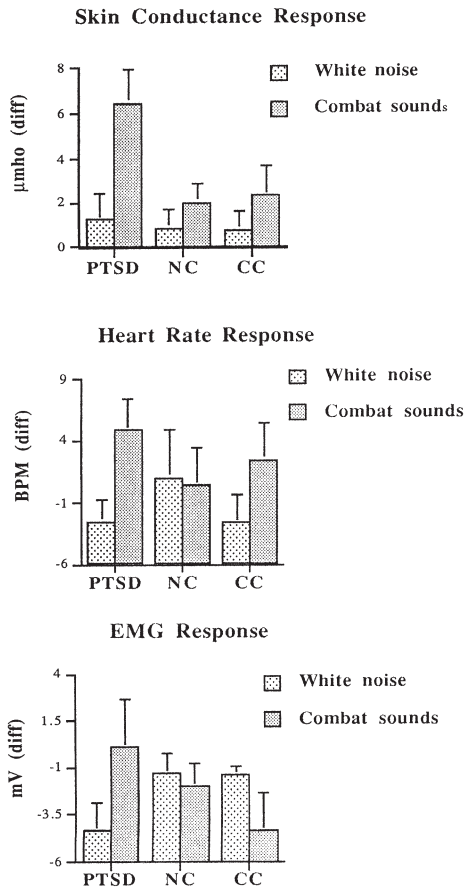

Figure 2. Skin conductance, heart rate, and EMG responses to white noise and combat sounds in PTSD, normal control, and combat control groups. Expressed as difference from pre- to postexposure conditions, in mmhos, bpm (beats per minute), and mV, respectively.

$F_{(2,35)} = 2.49$, $p = .096$]. Pre- to poststimuli change scores, for each group and type of stimulus, are shown in Figure 2. Most subjects showed reduced activity from pre- to poststimulus for both types of stimuli [main effect of time, $F_{(1,35)} = 11.97$, $p < .001$], reflecting possible relaxation of frontalis muscle with termination of acoustic stimuli. The only exception was the PTSD group responding to combat sounds, where no decrease in EMG activity was seen.

Plasma Catecholamines

PTSD patients had elevated norepinephrine levels relative to the two control groups [main effects of diagnosis, $F_{(2,36)} = 8.73$, $p = 001$, post hoc Sheffe, $p < .01$]. Norepinephrine data are presented in Figure 3. Both combat groups had small NE responses to white noise [main effect of time, $F_{(1,36)} = 7.01$, $p = .012$], but there was an NE response to combat sounds in the PTSD group only, [diagnosis-by-stimulus-by-time interaction, $F_{(2,36)} = 5.77$ $p = .007$]. The two control groups did not differ from each other on poststimulus norepinephrine measures.

Epinephrine showed a very similar pattern to the NE results, but the group, time, and diagnosis-by-stimulus-by-time interaction effects reached trend level significance (p values .06 to .076). As with NE, the effects seen were primarily attributable to an Epi response to combat sounds that was specific to the PTSD group (Figure 3).

Cortisol and ACTH

PTSD patients had elevated cortisol levels relative to both control groups at all measurement points; but there were no consistent cortisol changes in the time frame of this experiment, (see Figure 4). The only significant effect in the three-way ANOVA of cortisol data was a main effect of group [$F_{(2,38)} = 4.44$, $p = .018$]. To test the impact of depression histories on cortisol levels, PTSD patients with and without a history of MDD (Ma-

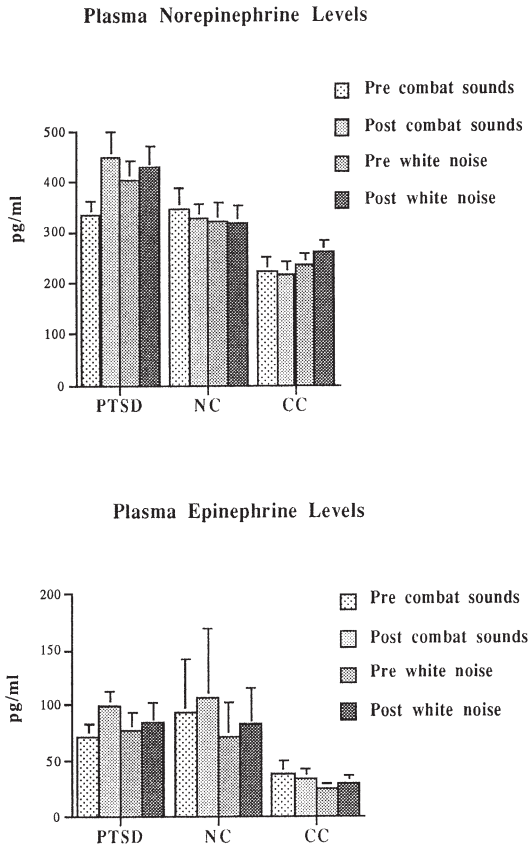

Figure 3. Plasma catecholamine levels (pg/ml), both during baseline (pre-) and after termination (post-) of auditory stimuli (white noise and combat sounds) for PTSD, normal control and combat control groups.

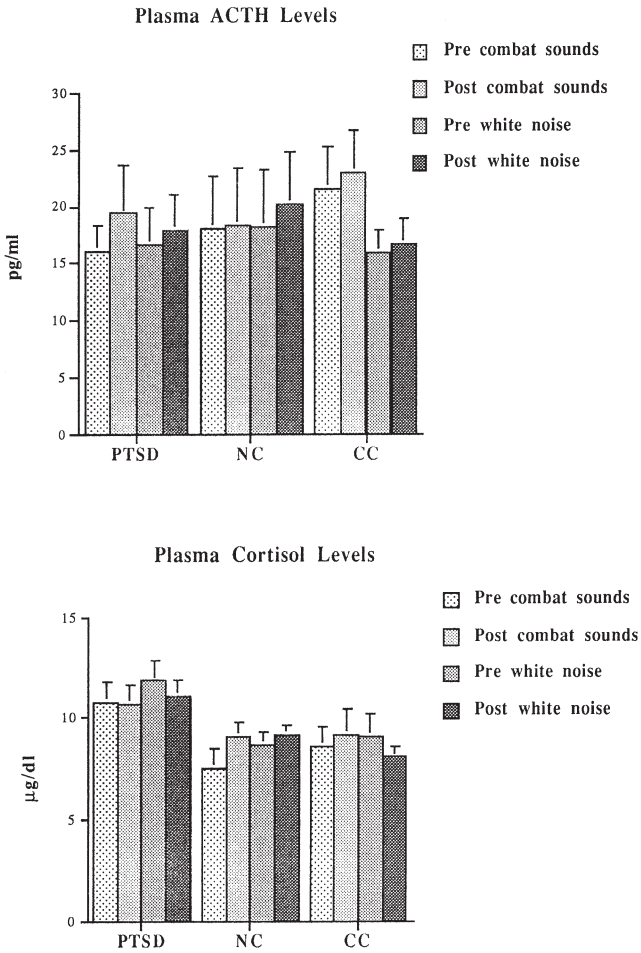

Figure 4. ACTH (pg/ml) and cortisol (µg/dl) levels, both during baseline (pre-) and after termination (post-) of auditory stimuli (white noise and combat sounds), for PTSD, normal control and combat control groups.

jor Depressive Disorder) were compared to each other. No differences were detected (means were almost identical, 11.1 vs. 10.8 μg/dl).

There was a small, but consistent increase in ACTH in response to both white noise and combat sounds across all groups [main effect of time ($F_{(1,38)} = 5.16$, $p = .029$]; but there was no evidence for a diagnosis effect [$F_{(2,38)} = 0.07$, $p = .93$] or for group or condition differences in responsivity (no significant interactions). Adding plasma cortisol as a covariate did not affect the results.

Correlations

Overall, in the canonical correlation analysis, no significant correlations were found between the psychophysiologic and neuroendocrine factors or between the "autonomic" and the HPA factors, when both WN (White Noise) and CS (Combat Sounds) days are considered. When the baseline measures were considered, significant canonical correlation for neuroendocrine and psychophysiologic factors was found on the white noise day only. The first canonical correlation was $r_1^* = .65$, $p = .019$. The canonical correlation was not significant on the combat sounds day ($r_1^* = 0.32$, $p = .92$). No evidence for a relationship between the "HPA" and "autonomic" factors in baseline measures was found on either day. When the responsivity or "post" measures were considered, there were no significant canonicial correlations on either day for psychophysiologic and neuroendocrine factors. There was a significant canonical correlation of "HPA" and "autonomic" factors on the WN day ($r_1^* = 0.61$, $p = .02$); but this correlation was not found for the combat sounds response ($r_1^* = 0.42$, $p = .27$). Repeating these analyses without partialling out subjective distress did not alter the results.

When Pearson partial correlation matrix for single variables within the canonical factors was examined, only epinephrine and norepinephrine measures were significantly correlated, with coefficients ranging from 0.33 on (post WN) to 0.5 (post CS), $p = .08$ and .004, respectively.

DISCUSSION

We examined psychophysiologic and neuroendocrine activity at baseline and in response to trauma-related stimuli, in PTSD patients and control groups. Several baseline abnormalities were found in PTSD patients. The trauma-related stimulus produced a robust activation, eliciting significant subjective and objective responses in all three groups. Using conservative data analyses, we found differential reactivity to combat sounds in PTSD patients. The PTSD patients generally differed from both combat-exposed and noncombat-exposed controls, suggesting that detected differences are associated with the presence of PTSD and not a history of trauma exposure. The two control groups generally did not differ from each other. Their few apparent differences suggest even lower stress system activity in the combat controls (e.g., over-all lower catecholamine levels), perhaps indicating resilience or better coping in these subjects. We also examined coregulation of response modalities using canonical correlation analysis and Pearson partial correlations, and found little relationship between modalities. We discuss baseline, reactivity, and coregulation findings separately below.

Baseline Measures

Compared to controls, PTSD patients at baseline reported higher distress and had elevated skin conductance, norepinephrine, and cortisol, with less pronounced elevations in HR and Epi. Baseline ACTH did not differ across groups. Elevated baseline autonomic activity (HR, BP) has been seen in PTSD patients in some studies (Pallmeyer et al. 1986), but not all (McFall et al. 1992; Orr et al. 1998). The baseline catecholamine elevations are consistent with reports of elevated 24-hour urinary catecholamines in PTSD (Yehuda et al. 1992), but baseline elevations were not seen in another challenge study (McFall et al. 1992) . The elevation of baseline cortisol in our PTSD patients is surprising in light of evidence of reduced cortisol secretion in this population (Yehuda et al. 1990). However, others have noted increased HPA axis activity in PTSD (Pitman and Orr 1990), and in a detailed 24-hour study (Yehuda et al. 1996b), reduced cortisol secretion was present in the afternoon, but not in the morning (when our samples were drawn). Chronobiological analysis of these data suggested an enhanced signal-to-noise ratio (SNR) in HPA function in PTSD, which, in conjunction with enhanced negative feedback findings, was interpreted as evidence of low background activity and increased capacity of the axis to respond to the environment. Our finding of elevated cortisol could reflect environmental responsivity, but the HPA axes of our patients did not seem to be excessively environmentally reactive, either to the anticipation of combat sounds or to the sounds themselves (discussed below). The elevated cortisol in our PTSD patients also does not seem explicable on the basis of past histories of depression, because patients with such histories did not differ from those without such histories.

Our PTSD subjects showed evidence of baseline elevations in subjective distress, autonomic and sympatho-adrenal activation, and plasma cortisol. We cannot state definitively whether these findings reflect tonic abnormalities in PTSD, because our "baseline" was before a psychological challenge. It is possible that our subjects were anxious in anticipation of the proce-

dure. The normal baseline ACTH levels do not support this interpretation, but elevations attributable to anticipatory anxiety that began before our first measurement could still be evident in cortisol levels, with return of ACTH to normal (because of cortisol feedback). However, if heightened anticipatory anxiety caused baseline elevations, we would expect to see some baseline differences, during the second visit, between patients who received white noise first and those who received combat sounds first. Patients receiving white noise first knew that they would be exposed to combat sounds on their second visit, and we would expect the greatest baseline elevations during their second visit. We could find no effects of stimulus order and no interactions between order and diagnosis. Thus, it seems that the baseline elevations in our PTSD patients do not simply reflect heightened anticipatory anxiety and could represent fairly chronic activation. However, the real meaning of chronic or tonic activation in these patients can be questioned, because they are chronically symptomatic and may continuously perceive ambiguous stimuli of everyday life as threats. The cross-system activation we found at baseline in PTSD may be a biological correlate of this psychological activation. CNS circuitry linking the HPA axis and the noradrenergic system (CRF and locus coeruleus?) can drive both, thus producing the increased subjective distress, autonomic and adrenergic activity, and increased cortisol. If so, however, we would expect significant correlations between measures of subjective, adrenergic/autonomic, and HPA axis activity.

Responsivity

As expected, all subjects experienced combat sounds as more distressing then white noise. PTSD patients were more distressed than both control groups, by both combat sounds and white noise, supporting the notion of hyper-responsivity to meaningful and ambiguous cues. In agreement with earlier studies (Blanchard et al. 1991a; Pitman and Orr 1993), skin conduction and heart rate responses to combat sounds were significantly larger in PTSD, supporting the notion of autonomic hyper-reactivity to trauma-related stimuli and demonstrating that excessive SC and HR responses can be elicited by a "generic" auditory activation paradigm as well as by individualized imagery scripts. SC was a more sensitive index of arousal, responding to both traumatic and nonspecific auditory stimulation; whereas, HR was more specifically responsive to combat sounds. EMG measures were sensitive to the change from pre- to poststimulus conditions but did not clearly differentiate between the diagnostic groups. It is possible that the timing of the measures (with the termination of the sounds) precluded us from detecting PTSD-specific abnormalities in muscular activity.

Our PTSD patients showed exaggerated responses of both epinephrine and norepinephrine to combat sounds. Prior studies found similar elevations in one or the other, but not both, of these hormones (Blanchard et al. 1991b; McFall et al. 1990). The differences may relate to stimulus intensity, because simultaneous elevations in both Epi and NE are seen in normals exposed to sufficiently stressful challenges, such as sky diving (Richter et al. 1996). We detected small, but consistent, ACTH responses to auditory stimulation in our total subject group, but ACTH was not differentially responsive to the type of stimulus, and patients did not differ from controls in ACTH responsivity. We did not detect cortisol responses to stimulation, but we did not have poststimulation samples at the appropriate times to detect such responses. The cortisol data cannot, therefore, be interpreted as suggesting a lack of cortisol responsivity in this paradigm. Sampling over a longer time frame is needed.

More extended sampling following the challenge may have increased the probability of detecting group differences in ACTH as well. However, acute activation can produce rapid ACTH responses (Abelson et al. 1994; Kirschbaum et al. 1993; Meyerhoff et al. 1988), and HPA axis activation in humans often begins in anticipatory stages, even before an acute stressor is applied (Kirschbaum et al. 1993; Richter et al. 1996). Subtle differences in HPA axis reactivity may also be more detectable in the afternoon, when the intrinsic activity in the axis is lower. However, the few studies that have examined both morning and afternoon responsivity have not shown time-of-day differences in ACTH response to either neuroendocrine (De Cherney et al. 1985) or psychological challenge (Kirschbaum et al. 1993). Our paradigm was sensitive to ACTH changes in that we did see significant rises from pre- to poststimulation. Thus, there is reason to believe that we would be able to detect HPA axis reactivity in ACTH measures, even in the morning and within the short time frame of our HPA axis monitoring. However, our failure to detect evidence of ACTH hyper-reactivity to trauma-related cues in PTSD must still be qualified by the brief time course over which ACTH was monitored. Although, as noted, our sampling schedule would be unlikely to detect acute cortisol reactivity, the over-all elevations in cortisol that were detected are consistent with other evidence for altered HPA axis function in PTSD. The precise nature of the HPA axis alterations in PTSD still needs further clarification, particularly the hypothesis that PTSD patients may have heightened acute, HPA axis responsivity to environmental challenge. The elevated cortisol levels seen in our PTSD patients could be attributable to heightened reactivity in anticipation of the expected challenge; but the rest of our data do not strongly support the presence of HPA axis hyper-responsivity to acute challenge. Clearly, further work is needed, utiliz-

ing a variety of challenges at different times of the day and with more extended sample timing.

Our data do demonstrate heightened responsivity to trauma-related cues in PTSD patients, detectable in subjective, autonomic, and adrenergic responses. The high decibel, combat sounds paradigm used, therefore, seems to be robustly activating in multiple channels in PTSD patients. This makes it highly unlikely that a lack of stimulus salience could account for the lack of ACTH hyper-responsivity and supports the potential utility of this paradigm for examining cross-system relationships. By measuring multiple systems, we could explore whether the responsivity of PTSD patients to traumatic cues reflects a single integrated process, or separable emotional, autonomic, catecholaminergic, and HPA axis responses.

Coregulation

Based on the idea of linked stress-response "systems" that can mount integrated responses to environmental threat, perhaps mediated by CRH, we expected significant correlations between stress response components in PTSD patients, particularly during exposure to combat sounds. Canonical correlation analyses, however, did not support this prediction. There were no significant relationships between response systems in the over-all analyses, nor when combat sound exposure data were analyzed separately. Significant correlations between baseline psychophysiologic and neuroendocrine factors were detected on the white noise exposure day, but given the lack of order or expectancy effects that differentiated white noise baselines from combat sounds baselines, it is difficult to attribute this to anything but day-to-day variability. No simple linear correlations between stress-response systems, either at baseline or in response to a robustly activating challenge, can be ascertained; therefore, from our data. This suggests that although this paradigm activated multiple stress response systems in our PTSD patients, they were not activated in an integrated fashion, and different patients may have been responding in different systems. In contrast with previous symptom provocation studies in PTSD, we did find significant correlations between plasma epinephrine and norepinephrine levels both at baseline and in response to stimulation in all the subjects. When Epi and NE responses were studied in other paradigms, epinephrine was found to respond consistently to all types of stress—mental arithmetic, Stroop Test, public speaking, psychiatric examination, parachute jumping and traumatic films (Dimsdale et al. 1987; Kaji et al. 1989; Kemmer et al. 1986; Richter et al. 1996); whereas, NE responses were detected in fewer specific situations—public speaking, interview, and parachute jumping (Dimsdale et al. 1987; Kemmer et al. 1986; Richter et al. 1996). In these studies, consistent with our findings, when a NE response was present, a significant correlation existed between Epi and NE responses. At times, the NE response appeared later then the Epi response (sometimes with the termination of stress), and it was proposed that changes in muscular sympathetic tone are responsible for this effect (Akerstedt et al. 1983). In agreement with our findings, when both HPA and catecholamine responses were studied, no evidence for a relationship between HPA axis and catecholamine responses was found, suggesting independent regulating mechanisms for these systems (Malarkey et al. 1995).

Limitations

One important limitation of this study is the fact that biological sampling included a single poststress measure. The timing of measurements was shaped by the expectable time frame for responses in each modality and by practical limitations that truncated the duration of biological data collection. Because of the lack of sequential sampling, we did not capture possible cortisol responses, rendering our conclusions about HPA axis responsivity somewhat tentative. Additionally, "responses" to the stressor were sampled after cessation of the auditory stimulus. Although subjects were instructed to continue imagining whatever came to mind for 5 minutes after the stimulus stopped, and this instruction can sustain or increase arousal in PTSD patients (Pitman et al. 1987), we cannot definitively determine whether differential responsivity of patients reflects stress responsivity per se, or differences in recovery from stress. Furthermore, because the time course of response in various response systems might differ, it is conceivable that at the time of sampling, we captured a stress response in one system while another system was already recovering. This could contribute to the failure to find relationships across systems. Finally, we utilized a standardized, auditory-cue stressor rather than personalized scripts. Although we obtained robust, multimodal responses, it is conceivable that personalized scripts may have elicited even stronger or more integrated stress responses. Clearly, follow-up work is needed, using varying stressor paradigms and with more frequent and more prolonged sample acquisition. Our findings do support the recommendation that future work should monitor multiple "stress" channels in each subject. We cannot assume that finding HPA axis abnormalities in one study and catecholamine abnormalities in another indicates that both exist within the same PTSD patients.

CONCLUSION

The current study provides evidence for baseline abnormalities in PTSD patients and replicates and expands

other major psychophysiologic and plasma catecholamine hyper-responsivity findings. Our data suggest that skin conductance may be a useful and sensitive index of arousal in a robust auditory activation paradigm, and that in this paradigm, NE and Epi responses of PTSD patients are exaggerated and correlated. Our ACTH findings argue against similar hyper-responsitivity of the HPA axis; and the cortisol data, taken together with prior work, support the presence of a complex alteration of HPA axis activity in PTSD patients.

We did not find evidence to support the proposed coregulation hypothesis, although it is possible that nonlinear and more complicated relationships exist between stress-response systems. Our data also do not support "paradigm specificity" of neurobiological changes, because catecholamine, psychophysiological, and HPA axis responses were all demonstrated in this single paradigm. However, the lack of correlation between these changes suggests that individual differences may influence which neurobiological system is affected in each specific case, and that different subjects within the same diagnostic category may have different response profiles. A recent pharmacological challenge study also suggested the presence of neurobiologically distinct subgroups of PTSD patients (Southwick et al. 1997). This is consistent with our suggestion that PTSD patients are not a neurobiologically homogenous group. Examination of possible linkages between individual differences in stress response profiles and pharmacological sensitivities is needed. Larger-scale studies using multiple, repeated measures and within-subject designs are also needed to search for more complicated coregulation patterns. Follow-up studies might explore potential clinical implications of varying neurobiological stress response profiles. Such work can examine the hypothesis, raised both by our data and those of Southwick et al., that PTSD is, in fact, a syndrome, or "final common pathway," that can result from a number of distinct pathophysiological processes.

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