

Physiologic Responses to Sudden, Loud Tones in Monozygotic Twins Discordant for Combat Exposure

Association With Posttraumatic Stress Disorder

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Background: Larger heart rate responses to sudden, loud (startling) tones represent one of the best-replicated psychophysiologic markers for posttraumatic stress disorder (PTSD). This abnormality may be a pretrauma vulnerability factor, ie, it may have been present prior to the event's occurrence and increased the individual's likelihood of developing PTSD on traumatic exposure. Alternately, it may be an acquired PTSD sign, ie, it may have developed after the traumatic exposure, along with the PTSD. Studying identical twins discordant for traumatic exposure offers an opportunity to resolve these competing origins.

Methods: Subjects included pairs of Vietnam combat veterans and their non-combat-exposed, monozygotic twins. Combat veterans were diagnosed as having current PTSD (n=50) or non-PTSD (ie, never had) (n=53). All subjects listened to a series of 15 sudden,

loud tone presentations while heart rate, skin conductance, and orbicularis oculi electromyogram responses were measured.

Results: Consistent with previous reports, averaged heart rate responses to the tones were larger in Vietnam combat veterans with PTSD. These larger responses were not shared by their non-combat-exposed co-twins, whose responses were similar to those of the non-PTSD combat veterans and their non-combat-exposed co-twins. This result remained significant after adjusting for a number of potentially confounding factors.

Conclusions: The results suggest that larger heart rate responses to sudden, loud tones represent an acquired sign of PTSD rather than a familial vulnerability factor.

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POSTTRAUMATIC STRESS disorder (PTSD) is among the few mental disorders with a formally recognized cause, in this case, a psychologically traumatic environmental event.¹ However, this does not necessarily mean that all abnormalities found in PTSD patients are caused by the etiologic event. An abnormality may be a pretrauma vulnerability factor, ie, it may have been present prior to the event's occurrence and increased the individual's likelihood of developing PTSD following traumatic exposure. Alternately, an abnormality may be an acquired PTSD sign, ie, it may have developed after the traumatic exposure, along with the PTSD.

The co-twin control study of monozygotic twins discordant for traumatic exposure has successfully been used to study the origin of PTSD.² In the present study, it was used in an effort to resolve competing explanations of the origin of physiologic abnormalities that are found in PTSD. Investigating these abnormalities has advanced our understanding of

PTSD because they provide objective measures of the disorder that go beyond self-reported symptoms. They also have the potential for informing PTSD's pathogenesis. If an abnormality is genetic or due to environmental influences shared by twins during their rearing—ie, if the abnormality is a “familial” pretrauma vulnerability factor—then it should also be found in the non-trauma-exposed co-twins of trauma-exposed twins with PTSD. On the other hand, if the abnormality results from an environmental factor unique to exposed twins, eg, a traumatic event, then their co-twins should not share the abnormality.

The above approach is unsuitable for the study of abnormal responses to trauma-related stimuli because such stimuli would not be salient for non-trauma-exposed co-twins. However, a number of physiologic abnormalities that do not involve responses to such stimuli have been observed in PTSD.³ Among these, the best replicated in both combat-related^{4,5} and non-combat-related⁶⁻⁹ PTSD is increased heart rate response (HRR) to a series of

sudden, loud (startling) tone presentations, believed to be indicative of a “defensive” response. Increased skin conductance (SC) and orbicularis oculi electromyographic (EMG) responses to sudden, loud tones, and slower habituation of SC responses, have also been reported in some of these studies. The investigation reported here examined responses to sudden, loud-tone stimuli in monozygotic twins discordant for combat exposure in Vietnam in an attempt to clarify the origin of physiologic abnormalities in PTSD.

METHODS

SUBJECTS

The overall study sample consisted of 130 male monozygotic twin pairs, in which one twin served in combat in Vietnam and his co-twin did not. Zygosity was determined by a questionnaire that has been found to be 97% accurate when validated against blood typing.¹⁰ The only exclusion criterion for participation in this noninvasive study was a serious contraindicating medical (eg, cardiac) or psychiatric (eg, psychosis, dementia) condition.

Most of the twin pairs studied were recruited from the Vietnam Era Twin (VET) Registry, which has been described in detail elsewhere.¹¹ Staff at the VET Registry identified a pool of 624 living monozygotic pairs of Vietnam era veterans discordant for combat exposure in Vietnam with known addresses and/or telephone numbers. They succeeded in obtaining preliminary data over the telephone in both twins in 442 (71%) of these pairs. These data included scores on the Mississippi Scale for Combat-Related PTSD for the combat-exposed twin.¹² The staff initially sent letters to each member of the twin pairs randomly selected from the subpool of 442 pairs, inviting him to participate in the study. The letter contained a brief description of the study and copies of the consent documents the subject would be required to sign prior to participation. The letters were followed by telephone contact to answer any questions the subject candidate might have and to encourage participation. Unwillingness of one twin disqualified the pair. When recruitment of PTSD twin pairs (ie, pairs in which the combat-exposed twin had PTSD) fell behind that of non-PTSD twin pairs, random recruitment was abandoned and efforts were focused on the combat-exposed subject candidates who had the highest Mississippi scale scores or other suggestive evidence of PTSD from past mail and telephone surveys. Of 210 (48% of 442) registry twin pairs invited to come to the Manchester, NH, Veterans Affairs Research Service Psychophysiology Laboratory, 102 (49%) participated. Most subject candidates who did not participate either declined because they were uninterested or too busy or could not be scheduled at a time of mutual convenience.

After the VET Registry resource had apparently been exhausted for suitable PTSD pair candidates, to make up the deficit, letters were sent to the approximately 80 000 Vietnam veterans receiving compensation for PTSD from the Veterans Benefits Administration (Washington, DC). The letter inquired whether the recipient had an identical twin who had not served in combat in Vietnam, and if so, whether he and his brother were interested in participating in a research study. Twenty-seven additional PTSD pairs were recruited in this manner. Of these, 20 (74%) contained a non-combat-exposed co-twin who, like all registry co-twins, had also served in the military but not in combat in Vietnam. On arrival at the laboratory, written informed consent was obtained from each subject after the procedures had been fully explained.

PSYCHOMETRICS

Prior to arrival, the VET Registry had administered to each combat-exposed subject (with his informed consent) (1) an 18-item Combat Severity Scale¹³ previously validated against combat-related medals and employed in VET Registry research, which yielded a measure of the extent of his overall combat experience in Vietnam, and (2) the Mississippi Scale for Combat-Related PTSD,¹² a 35-item instrument quantifying PTSD and related symptoms.

PSYCHODIAGNOSTICS

An experienced, doctoral-level psychologist (N.B.L.) used the Clinician-Administered PTSD Scale (CAPS)¹⁴ to evaluate the PTSD diagnostic status of each combat-exposed twin, with reference to his experience in Vietnam. The reliability of total CAPS score in the hands of this psychologist, as measured against another interviewer in our laboratory, is interrater = 0.99, test-retest = 0.94 (intraclass correlation coefficients).

Each subject completed a Stressful Life Events Checklist (Frank W. Weathers, PhD, and R.K.P., unpublished data, 1996; available on request) designed to quantify the number of lifetime (noncombat) experiences that potentially meet the DSM-IV A.1 (stressor) and A.2 (response) criteria. The psychologist asked the subject to identify the worst of these and then administered the CAPS to ascertain whether the subject met criteria for non-combat-related PTSD. She also administered the Structured Clinical Interview for DSM-IV¹⁵ to determine the presence or absence of non-PTSD Axis I mental disorders.

STIMULI

The experimental techniques, including stimuli, dependent measures, procedure, and data reduction, were all as employed in our previously reported studies of the startle response in PTSD.⁴⁻⁹ Stimuli consisted of fifteen 95-dB (sound pressure level), 1000-Hz, 500-millisecond pure tones with 0 milliseconds of rise and fall times. Stimuli of this intensity and configuration typically elicit a startle response on initial presentation. The tones were generated using Neuro Stim software (Neuro Scan Inc, Herndon, Va), and the dB level was verified using an Abbeon (AB-85) sound level meter (Indianapolis, Ind). The tones were presented binaurally over E-ARTONE (Aearo Co, Santa Barbara, Calif) insert earphones. Intertrial intervals were randomly selected by the computer and ranged from 27 to 52 seconds.

DEPENDENT MEASURES

Dependent physiologic measures included HRR, SC, and left orbicularis oculi (eye blink) EMG response. Heart rate was recorded from standard limb electrocardiogram leads connecting to a Coulbourn (Allentown, Pa) High Gain Bioamplifier; a Coulbourn Tachometer yielded interbeat intervals that were converted to HR. Skin conductance was measured directly by a Coulbourn Isolated Skin Conductance coupler using a constant potential of 0.5 V through 9-mm (sensor diameter) Sensor Medics (Anaheim, Calif) Ag/AgCl electrodes filled with an isotonic paste and placed on the hypothenar surface of the nondominant hand. For EMG, 4-mm (sensor diameter) Sensor Medics Ag/AgCl surface electrodes were filled with electrolyte paste and placed over the left orbicularis oculi muscle. The EMG signal was amplified by a Coulbourn High Gain Bioamplifier and filtered (90-250 Hz); a 300-millisecond time constant was used for integration by a Coulbourn Contour Following Integrator. All physiologic analog signals were digitized at 500 Hz and stored using a Neuro Scan system.

Table 1. Group Demographic and Psychometric Measures for Combat-Exposed Subjects

| Variable | Non-Registry PTSD (n = 27) Mean (SD) | Registry PTSD (n = 23) Mean (SD) | Registry vs Non-Registry PTSD | | All PTSD (n = 50) Mean (SD) | All Registry Non-PTSD (n = 53) Mean (SD) | Registry PTSD vs Non-PTSD | | All PTSD vs Non-PTSD | | Entire Registry Pool (n = 624)* Mean (SD) |
|-------------------------|---|-------------------------------------|-------------------------------|------|--------------------------------|---|---------------------------|------|----------------------|------|--|
| | | | t | P | | | t | P | t | P | |
| | | | | | | | | | | | |
| Age† | 51.3 (3.2) | 48.5 (2.5) | 3.5 | .001 | 50.0 (3.2) | 49.2 (2.5) | 1.1 | .27 | 1.4 | .18 | 48.8 (2.4) |
| Combat severity | 8.1 (2.3) | 7.1 (2.7) | 1.4 | .16 | 7.6 (2.5) | 3.8 (2.7) | 4.9 | .001 | 7.4 | .001 | 4.2 (3.0) |
| Months in Vietnam | 13.0 (6.5) | 11.2 (3.7) | 1.2 | .25 | 12.2 (5.4) | 11.5 (4.2) | 0.3 | .76 | 0.8 | .44 | 12.6 (6.8) |
| Mississippi PTSD scale‡ | 130 (19) | 111 (20) | 3.6 | .001 | 121 (22) | 72 (18) | 8.4 | .001 | 12.6 | .001 | 71.8 (20.7) |
| CAPS | 71.7 (14.4) | 60.9 (18.7) | 2.3 | .03 | 66.7 (17.2) | 6.5 (7.9) | 17.9 | .001 | 23.0 | .001 | ... |

Abbreviations: CAPS, Clinician-Administered PTSD scale; PTSD, posttraumatic stress disorder.

*For Mississippi PTSD scale, n = 512.

†Age as of October 1, 1997.

‡Mississippi Scale for Combat-Related PTSD.

PROCEDURE

The testing took place in a sound-attenuated room connected via wires to the laboratory in which the experimental apparatus was located. Subjects were seated upright in a comfortable armchair, had earphones and recording electrodes attached, and then were instructed as follows: “We will start this procedure with 10 minutes of relaxation and baseline measurement. Following the 10-minute baseline, you will hear a series of tones. This part of the procedure will last about 10 minutes. Please sit quietly because movement can affect the physiologic recordings. Also, please keep your eyes open during the procedure. Do you have any questions?”

DATA REDUCTION

An HRR score was calculated for each trial by subtracting the average HR level for the 1 second immediately preceding tone onset from the maximum level within 1 to 4 seconds after tone onset. An SC response (SCR) score was calculated in the same manner. An EMG response (EMGR) score was calculated by subtracting the average EMG level for the 1 second immediately preceding tone onset from the maximum level within 40 to 200 milliseconds of tone onset. Square root transformations were performed on the HRR, SCR, and EMGR scores to reduce heteroscedasticity. For each physiologic variable, an averaged response score was calculated for the transformed responses to the 15 tones.

In addition, the regression equation $Y=bX+a$ for trials 2 to 15, where Y represents the magnitude of the response and X the log trial number, was calculated, yielding a response slope (a measure of relative habituation). An SCR that was less than 0.05 microsiemens (untransformed) to a tone was considered to be an SC nonresponse trial. An EMGR that was less than 0.10 μ V (untransformed) to a tone was considered to be an EMG nonresponse trial. A subject was considered to reach an SC or EMG nonresponse criterion (a measure of absolute habituation) when there were 2 consecutive nonresponse trials. The possible range for this criterion is 0 (nonresponse on each of the first 2 trials) to 14 (no 2 consecutive trials with nonresponses). (In our previous studies with this technique,^{4,9} habituation has not proven to be a useful dimension in the analysis of HRR data of this nature.)

STATISTICAL ANALYSIS

Due to ambiguous predictions for past-PTSD twin pairs, some of whom may have substantial residual PTSD symptoms and some of whom may not, data from the past-PTSD

twin pairs were excluded from the analyses. Data were analyzed by means of a mixed model that treated PTSD diagnosis (ie, PTSD vs non-PTSD in the combat-exposed twin) as a between-pairs fixed effect, combat exposure as a within-pair fixed effect, and twin pairs as a random effect.¹⁶ The mixed model analysis yields a t statistic (not to be confused with the t test). If a physiologic dependent variable represents a PTSD vulnerability factor, the model predicts a significant diagnosis effect in the absence of a diagnosis \times exposure interaction. If a physiologic dependent variable represents an acquired PTSD sign, the model predicts a significant diagnosis \times exposure interaction. (We also performed parallel analyses to those reported herein using total CAPS score, which can serve as a continuous measure of PTSD. However, because the results obtained were only duplicative of those that treated PTSD as a dichotomous measure, we do not report them herein.)

RESULTS

DESCRIPTIVE DATA

Group mean demographic and psychometric data for the combat-exposed subjects are presented in **Table 1**. At the $P<.05$ level, compared with non-PTSD subjects, PTSD subjects had significantly greater combat severity and greater scores on the Mississippi Scale and CAPS. Compared with registry PTSD subjects, non-registry PTSD subjects had significantly greater age, Mississippi Scale scores, and CAPS scores. For comparison purposes, means for the entire registry recruitment pool of combat-exposed subjects appear in the far right column. Statistical analyses (not shown) indicated that the non-PTSD subjects (all of whom came from the registry) did not differ from the pool on any demographic or psychometric measure at the $P<.05$ level. Compared with the pool, registry PTSD subjects had significantly greater combat severity and Mississippi Scale scores (CAPS scores were not available for the registry pool).

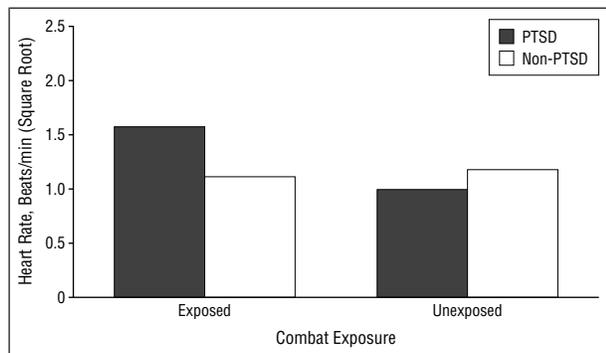
PHYSIOLOGIC GROUP MEANS

Table 2 presents psychophysiologic group means along with the results of the mixed-model analyses. The physiologic variable that clearly yielded statistically signifi-

Table 2. Group Physiologic Measures

| Variable | Twins | PTSD (n = 50) Mean (SD) | Non-PTSD (n = 53) Mean (SD) | PTSD Diagnosis | | Combat Exposure | | Interaction | |
|---|-----------|-------------------------------|-----------------------------------|----------------|------|-----------------|------|-------------|-------|
| | | | | t* | P | t* | P | t* | P |
| Resting levels | | | | | | | | | |
| Heart rate, beats/min | Exposed | 77.5 (15.8) | 73.6 (11.3) | 0.8 | .44 | 1.0 | .33 | 1.7 | .09 |
| | Unexposed | 73.4 (11.1) | 74.4 (10.9) | | | | | | |
| Skin conductance, microsiemens | Exposed | 3.81 (3.30) | 4.80 (3.22) | 0.9 | .36 | 0.7 | .48 | 1.7 | .09 |
| | Unexposed | 4.19 (2.67) | 3.97 (2.68) | | | | | | |
| Orbicularis oculi EMG, μ V | Exposed | 1.85 (1.63) | 1.77 (1.39) | 0.5 | .60 | 1.0 | .31 | 0.1 | .95 |
| | Unexposed | 1.67 (0.72) | 1.60 (0.66) | | | | | | |
| Averaged responses | | | | | | | | | |
| Heart rate, beats/min $\frac{1}{2}$ | Exposed | 1.57 (0.92) | 1.11 (0.51) | 1.5 | .14 | 3.1 | .002 | 4.3 | <.001 |
| | Unexposed | 0.99 (0.54) | 1.17 (0.49) | | | | | | |
| Skin conductance, μ siemens $\frac{1}{2}$ | Exposed | 0.45 (0.32) | 0.60 (0.44) | 1.7 | .09 | 0.2 | .82 | 0.8 | .41 |
| | Unexposed | 0.49 (0.38) | 0.57 (0.39) | | | | | | |
| Orbicularis oculi EMG, μ V $\frac{1}{2}$ | Exposed | 1.04 (0.66) | 1.06 (0.83) | 1.0 | .34 | 1.0 | .33 | 1.7 | .10 |
| | Unexposed | 0.87 (0.58) | 1.09 (0.79) | | | | | | |
| Response slopes | | | | | | | | | |
| Heart rate | Exposed | -0.10 (0.86) | -0.07 (0.57) | 0.1 | .92 | 0.6 | .56 | 0.4 | .67 |
| | Unexposed | -0.01 (0.59) | -0.06 (0.56) | | | | | | |
| Skin conductance | Exposed | -0.18 (0.16) | -0.25 (0.20) | 2.0 | <.05 | 0.3 | .78 | 0.6 | .55 |
| | Unexposed | -0.20 (0.14) | -0.25 (0.20) | | | | | | |
| Orbicularis oculi EMG | Exposed | -0.38 (0.37) | -0.42 (0.36) | 0.4 | .67 | 1.8 | .07 | 0.4 | .67 |
| | Unexposed | -0.31 (0.41) | -0.31 (0.35) | | | | | | |
| Trials to criterion | | | | | | | | | |
| Skin conductance | Exposed | 7.8 (5.9) | 9.6 (5.1) | 1.4 | .15 | 0.7 | .48 | 0.8 | .42 |
| | Unexposed | 8.7 (5.7) | 9.5 (5.2) | | | | | | |
| Orbicularis oculi EMG | Exposed | 10.8 (4.6) | 10.8 (4.8) | 0.5 | .63 | 0.0 | .97 | 0.7 | .50 |
| | Unexposed | 10.4 (5.0) | 11.1 (4.5) | | | | | | |

Abbreviations: EMG, electromyogram; PTSD, posttraumatic stress disorder.
*Mixed model t.



Group mean averaged heart rate response (HRR) to a series of 15 sudden, loud (startling) tone presentations in male Vietnam combat veterans with and without combat-related posttraumatic stress disorder (PTSD), and their non-combat-exposed monozygotic twins. PTSD (solid bars) (n=50) and non-PTSD (open bars) (n=53) refer to the diagnostic status of the combat-exposed twin.

cant results was averaged HRR to the tones (**Figure**). This variable conformed to the profile of an acquired PTSD sign, as indicated by the highly significant diagnosis \times exposure interaction. Specifically, averaged HRR was elevated in the combat-exposed PTSD veterans in comparison with the other 3 groups. (For informational purposes, the HRR means [SDs] of the 26 past PTSD twins were as follows: combat-exposed, 1.44 [0.91] square root beats/min [BPM $\frac{1}{2}$]; non-combat-exposed, 1.12 [0.50] BPM $\frac{1}{2}$.)

ANALYSIS OF VARIABLES POTENTIALLY CONFOUNDING HRRs

We identified the following variables that could potentially confound the interpretation of the diagnosis \times exposure interaction for HRR: (1) source of subjects (VET Registry vs non-VET Registry); (2) age; (3) combat severity (exposed subjects only); (4) noncombat stressful life events over the lifespan; (5) alcohol and (6) caffeine consumed and (7) cigarettes smoked during the 24 hours preceding testing; (8) reported use of 1 or more potentially confounding medications or substances (including antihistamines, sympathomimetics, sympatholytics, parasympathomimetics, parasympatholytics, skeletal muscle relaxants, hypotensive agents, vasodilating agents, pressor agents, β -blockers, antiarrhythmics, calcium channel blockers, narcotics, anticonvulsants, antidepressants, neuroleptics, benzodiazepines, other psychotherapeutic agents, cerebral stimulants, sedatives, and hypnotics) during the month prior to testing, or a "dirty" urine specimen (ie, containing amphetamines, barbiturates, cocaine, opiates, benzodiazepines, methaqualone, propoxyphene, phencyclidine, methadone, or cannabinoids); (9) current affective disorder (bipolar, depressive, cyclothymic, or dysthymic); (10) current non-PTSD anxiety disorder; and (11) current non-combat-related PTSD.

To address these potentially confounding factors, we first examined the association between each of the above factors and the difference in HRR between combat-

exposed and non-combat-exposed twins.¹⁷ For the significance of these screening tests, we employed a conservative cutoff of $P < .20$. These tests ruled out all but the following as potentially confounding factors: source, noncombat stressful life events in the combat-exposed twin, and affective disorder in the combat-exposed twin.

Source

Because there were no non-PTSD pairs who were not recruited from the VET registry, this variable could not be used as a covariate. Instead, we re-performed the mixed model analyses on registry pairs only. Subgroup mean (SD) HRRs (BPM $^{1/2}$) for PTSD pairs ($n=23$) were: combat-exposed, 1.50 (0.85); non-combat-exposed, 1.09 (0.45); for non-PTSD pairs ($n=53$), combat-exposed, 1.11 (0.51); non-combat-exposed, 1.17 (0.49). The diagnosis \times exposure interaction for HRR remained highly significant: $t=3.1$, $P=.003$.

Noncombat Stressful Life Events in the Exposed Twin

Because this variable's interaction with exposure was homogeneous in PTSD and non-PTSD pairs, we were able to use it as a covariate in the mixed model. The results indicated that the adjusted diagnosis \times exposure interaction for HRR remained highly significant: $t=4.2$, $P<.001$.

Affective Disorder in the Exposed Twin

Because only one pair contained an exposed twin with an affective disorder who did not have PTSD, this variable also could not be used as a covariate. Instead, we re-performed the analyses on pairs in which the exposed twin did not have an affective disorder. Subgroup mean (SD) HRRs (BPM $^{1/2}$) for PTSD pairs ($n=27$) were: combat-exposed, 1.34 (0.70); non-combat-exposed, 1.05 (0.50); for non-PTSD pairs ($n=52$): combat-exposed, 1.10 (0.51); non-combat-exposed, 1.15 (0.48). The diagnosis \times exposure interaction for HRR remained significant: $t=2.3$, $P=.03$.

OTHER PHYSIOLOGIC MEASURES

The only noteworthy result among the other physiologic dependent variables was a significant diagnosis main effect, in the absence of a significant diagnosis \times exposure interaction, for SCR slope. This result suggests that more slowly habituating SCRs to sudden, loud tones may represent a pretrauma vulnerability factor for PTSD. A more specific test for a pretrauma vulnerability factor, however, is a t test of the difference between the responses of the ("high-risk") non-combat-exposed co-twins of PTSD twins vs the ("low-risk") non-combat-exposed co-twins of non-PTSD twins. For SCR slope, this yielded $t=1.5$, $P=.15$ (2-tailed). Therefore, this result should only be regarded as suggestive.

COMMENT

Consistent with previous reports,^{4,9} HRRs to a series of sudden, loud tones were larger in Vietnam combat vet-

erans with than without PTSD. The larger HRRs of the PTSD combat veterans were not shared by their non-combat-exposed, identical twin brothers, whose responses were similar to those of the non-PTSD combat veterans and their non-combat-exposed brothers. This result could not be explained by any of a number of potentially confounding factors. According to the present findings, increased HRRs to sudden, loud tones cannot be attributed to either heredity or familial environment shared by a PTSD veteran and his non-combat-exposed, identical twin brother. Rather, it must reflect the contribution of unique environmental difference(s) between these twins.

A limitation of the design employed here is that it cannot identify the specific environmental difference(s) between the combat-exposed and non-combat-exposed twins that is responsible for the abnormality. However, because the most salient, common difference was the presence of combat-related PTSD in the twins with the higher HRRs, and because the effect remained significant after considering the contributions of numerous potentially confounding variables, it is reasonable to attribute the larger HRRs to the presence of combat-related PTSD. The design employed here cannot rule out the possibility that the non-combat-exposed co-twins of combat veterans with PTSD share latent inherited phenotypes predisposing to increased heart rate responses that require traumatic exposure to be activated.¹⁸

The conclusion that increased HRRs to sudden, loud tones are acquired along with PTSD is supported by results of a longitudinal study in Israeli civilians who had experienced acute psychologically traumatic events. Those who developed PTSD had similar HRRs to those who did not develop PTSD 1 week after the event, but larger HRRs 1 and 4 months later. Results of that study and the one reported here converge on the conclusion that larger HRRs to sudden, loud tones represent an acquired sign of PTSD.

Although the neuroanatomy of the HRR to startling stimuli is not as clearly delineated as the muscular response, the amygdala likely plays a crucial role. In rodents, amygdala neurons are preferentially activated by high-intensity acoustic stimuli.¹⁹ Amygdalotomized monkeys are impaired in their HRRs to tones.²⁰ Lesions of the central nucleus of the amygdala have been found to significantly reduce HRRs to startling white noise in rats, a finding that led investigators to conclude that the amygdala mediates responses to nonsignal acoustic stimuli and that acceleratory HRRs can reflect the development of fear during acoustic startle testing.²¹ We have theorized that hyper(re)activity of the amygdala underlies a number of PTSD symptoms.²²⁻²⁴ The results of the present study suggest that amygdala hyper(re)activity is not present prior to the occurrence of the traumatic event, but rather, develops following its occurrence in persons with PTSD. This postulate is worth testing within a twin design that employs more direct measures of amygdala (re)activity.²⁵

There was a nondefinitive trend in the data for slower habituation of SCRs to the tones to conform to the pattern of a familial vulnerability factor for PTSD. However, this may only have reflected the unexpectedly smaller mean averaged SCRs found in the PTSD veterans and their co-twins. The most that can be said of this result is that

it warrants further investigation. For example, this might be done in a non-twin study that measures physiologic responses prior to exposure to a traumatic event in persons at high risk for such exposure in an attempt to predict the PTSD outcome.

None of the other SC or EMG physiologic response measures yielded significant results. However, compared with HRR, these features of the startle response have not yielded a consistent discrimination between PTSD and non-PTSD subjects in previous studies.⁴⁻⁹ Also, the proportion of subjects who were receiving treatment in the present study was lower than in previous studies in which the PTSD subjects were recruited from clinics. Subjects who are not receiving treatment may not show physiologic abnormalities to the same degree. Additionally, more severely ill PTSD twins may either have not participated in the VET Registry, not responded to our solicitation letters, or not been willing to undergo the stress of traveling by air to a distant city to participate. Because the PTSD and non-PTSD combat veterans studied here were not randomly ascertained from the VET Registry population, not to mention from the Vietnam veteran population at large, epidemiologic inferences should be drawn with caution.

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1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*. Washington, DC: American Psychiatric Association; 1994.
2. Goldberg J, True WR, Eisen SA, Henderson WG. A twin study of the effects of the Vietnam War on posttraumatic stress disorder. *JAMA*. 1990;263:1227-1232.
3. Pitman RK, Orr SP, Shalev AY, Metzger LJ, Mellman, TA. Psychophysiological alterations in post-traumatic stress disorder. *Semin Clin Neuropsychiatry*. 1999; 4:234-241.
4. Orr SP, Solomon A, Peri T, Pitman RK, Shalev AY. Physiologic responses to loud tones in Israeli veterans of the 1973 Yom Kippur War. *Biol Psychiatry*. 1997;41: 319-326.
5. Orr SP, Lasko NB, Shalev AY, Pitman RK. Physiologic responses to loud tones in Vietnam veterans with post-traumatic stress disorder. *J Abnorm Psychol*. 1995; 104:75-82.
6. Shalev AY, Orr SP, Peri P, Schreiber S, Pitman RK. Physiologic responses to loud tones in Israeli post-traumatic stress disorder patients. *Arch Gen Psychiatry*. 1992;49:870-875.
7. Shalev AY, Peri T, Brandes D, Freedman S, Orr SP, Pitman RK. Auditory startle responses in trauma survivors with PTSD: a prospective study. *Am J Psychiatry*. 2000;157:255-261.
8. Metzger LJ, Orr SP, Berry NJ, Ahern CE, Lasko NB, Pitman RK. Physiologic reactivity to startling tones in women with PTSD. *J Abnorm Psychol*. 1999;108: 347-352.
9. Shalev AY, Peri T, Orr SP, Bonne O, Pitman RK. Auditory startle responses in help seeking trauma survivors. *Psychiatry Res*. 1997;69:1-7.
10. Eisen S, Neuman R, Goldberg J, Rice J, True W. Determining zygosity in the Vietnam Era Twin Registry: an approach using questionnaires. *Clin Genet*. 1989;35: 423-432.
11. Henderson WG, Eisen S, Goldberg J, True WR, Barnes JE, Vitek ME. The Vietnam Era Twin Registry: a resource for medical research. *Public Health Rep*. 1990; 105:368-373.
12. Keane TM, Caddell JM, Taylor KL. Mississippi Scale for Combat-Related Post-traumatic Stress Disorder: three studies in reliability and validity. *J Consult Clin Psychol*. 1988;56:85-90.
13. Janes GR, Goldberg J. Reliability and validity of a combat exposure index for Vietnam era veterans. *J Clin Psychol*. 1991;47:80-86.
14. Weathers FW, Keane TM, Davidson JR. Clinician-Administered PTSD Scale: a review of the first ten years of research. *Depress Anxiety*. 2001;13:132-156.
15. First MB, Spitzer RL, Gibbon M, Williams JBW. *Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), Clinician Version*. Washington, DC: American Psychiatric Press; 1997.
16. Little RC, Milliken GA, Stroup WW, Wolfinger RD. *SAS System for Mixed Models*. Cary, NC: SAS Institute Inc; 1996.
17. Milliken GA. SAS tutorial: analysis of covariance: models, strategies, and interpretations. In: *Proceedings of the Ninth Annual SAS Users Group International*. Cary, NC: SAS Institute Inc; 1984:990-999.
18. Kendler KS. Twin studies of psychiatric illness: an update. *Arch Gen Psychiatry*. 2001;58:1005-1014.
19. Bordi F, LeDoux J. Sensory tuning beyond the sensory system: an initial analysis of auditory response properties of neurons in the lateral amygdaloid nucleus and overlying areas of the striatum. *J Neurosci*. 1992;12:2493-2503.
20. Bagshaw MH, Benzie S. Multiple measures of the orienting reaction and their dissociation after amygdectomy in monkeys. *Exp Neurol*. 1968;20:175-187.
21. Young BJ, Leaton RN. Amygdala central nucleus lesions attenuate acoustic startle stimulus-evoked heart rate changes in rats. *Behav Neurosci*. 1996;110:228-237.
22. Shalev AY, Rogel-Fuchs Y, Pitman RK. Conditioned fear and psychological trauma. *Biol Psychiatry*. 1992;31:863-865.
23. Pitman RK, Orr SP, Shalev AY. Once bitten, twice shy: beyond the conditioning model of PTSD. *Biol Psychiatry*. 1993;33:145-146.
24. Pitman RK, Shalev AY, Orr SP. Post-traumatic stress disorder: emotion, conditioning, and memory. In: Gazzaniga MS, ed. *The Cognitive Neurosciences*. Cambridge, Mass: MIT Press; 2000:1133-1147.
25. Rauch SL, Whalen PJ, Shin LM, McInerney SC, Macklin ML, Lasko NB, Orr SP, Pitman RK. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. *Biol Psychiatry*. 2000;47: 769-776.