Psychiatry Research: Neuroimaging 283 (2019) 7–15

Contents lists available at ScienceDirect

Psychiatry Research: Neuroimaging

journal homepage: www.elsevier.com/locate/psychresns

Gulf War illness associated with abnormal auditory P1 event-related potential: Evidence of impaired cholinergic processing replicated in a national sample

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ARTICLE INFO

Keywords:
Hyperarousal
ERP
Dopaminergic
Desert storm
Desert shield
Neurotoxic

ABSTRACT

Our team previously reported event-related potential (ERP) and hyperarousal patterns from a study of one construction battalion of the U.S. Naval Reserve who served during the 1991 Persian Gulf War. We sought to replicate these findings in a sample that was more representative of the entire Gulf War-era veteran population, including male and female participants from four branches of the military. We collected ERP data from 40 veterans meeting Haley criteria for Gulf War syndromes 1–3 and from 22 matched Gulf War veteran controls while they performed an auditory oddball task. Reports of hyperarousal from the ill veterans were significantly greater than those from the control veterans, and P1 amplitudes in Syndromes 2 and 3 were significantly higher than P1 amplitudes in Syndrome 1, replicating our previous findings. Many of the contributors to the generation of the P1 potential are also involved in the regulation of arousal and are modulated by cholinergic and dopaminergic systems—two systems whose dysfunction has been implicated in Gulf War illness. These differences among the three syndrome groups where their means were on either side of controls is a replication of our previous ERP study and is consistent with previous imaging studies of this population.

1. Introduction

An array of chronic health problems have been reported by veterans who served in the Persian Gulf during Operation Desert shield/Desert storm at significantly higher rates than have been observed in other military populations (Fukuda et al., 1998; Haley et al., 1997a, 1997b; Kang et al., 2000; Research Advisory Committee [RAC] on Gulf War Veterans' Illnesses, 2008; Steele, 2000; Unwin et al., 1999). Taken together, these health issues are commonly referred to as Gulf War Illness (GWI). Clusters of symptoms within GWI that tend to occur together have been identified among the ill veterans (Cherry et al., 2001; Fukuda et al., 1998; Haley et al., 1997b2001; Iannocchione et al., 2011; Kang et al., 2002). Subsequent studies have found objective differences in brain function between those meeting the definitions for GWI and veterans who were deployed but endorsed none of these symptoms (e.g., Hubbard et al., 2014; Tillman et al., 2010) and between ill veterans from the different syndrome variant groups (e.g., Calley et al., 2010; Haley et al., 2009; Li et al., 2011; Liu et al., 2011; Tillman et al., 2012).

In a previous study conducted in 2008 and 2009 (Tillman et al., 2012), our group identified unique event-related potential (ERP) patterns among Haley syndrome groups 1–3 (Haley et al., 1997b; Iannocchione et al., 2011) in a homogenous sample of male veterans from one construction battalion of the U.S. Naval Reserve (Seabees). GWI Syndrome 1 is associated with impaired cognition, including memory problems, confused thought, distractibility, and fatigue; Syndrome 2 is associated with more debilitating neurocognitive issues—confusion, word-finding and reasoning difficulties, emotional lability—and balance problems such as frequent stumbling and vertigo; and Syndrome 3 is generally associated with somatic complaints such as fatigue, joint and muscle pain, weakness, and numb or tingling extremities as well as neurocognitive complaints. Veterans who were not deployed or were similarly deployed but remained well served as

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https://doi.org/10.1016/j.pscychresns.2018.11.006
Received 5 June 2018; Received in revised form 5 October 2018; Accepted 9 November 2018
Available online 10 November 2018
0925-4927/

controls.

Investigating the highly reported hyperarousal symptom (Thompson et al., 2004), we recorded the electroencephalogram (EEG) of these veterans as they performed a three-condition auditory oddball task. We included the auditory P1 event-related potential (ERP) component given its association with states of stress (Ermutlu et al., 2005) and with conditions marked by anxiety and attentional difficulties (e.g., Berman et al., 2002; Sarno et al., 2006; Uc et al., 2003), including posttraumatic stress disorder (PTSD; Gillette et al., 1997, Skinner et al., 1999), a condition to which much of symptomology reported by ill Gulf War veterans was originally attributed (see Haley, 1997). Only five of the veterans in our previous study (and 12 in the present study) had been diagnosed with PTSD via structured clinical interview, but all three Gulf War illness syndrome groups reported significantly greater hyperarousal symptoms, even when the data from those with a PTSD diagnosis had been removed. Uc et al. (2003) reported group differences in P1 amplitude between Huntington's disease patients and controls but found no linear relationship between P1 amplitude and anxiety measures. Thus, we sought to explore the hyperarousal symptom by examining ERPs that had previously been associated with such symptoms in many conditions, including PTSD. We observed that, relative to the control group, the amplitude of the P1 component among veterans meeting criteria for Syndromes 2 and 3 was significantly higher, especially in response to threatening distractor stimuli, and was significantly lower among veterans meeting criteria for Syndrome 1, especially in response to threatening distractor stimuli. These findings were consistent with reported differences in white matter integrity (e.g., Heaton et al., 2007), basal ganglia and dopaminergic function (e.g., Haley et al., 2000a, 2000b; Meyeroff et al., 2001), and, especially, given the complex role of acetylcholine in the generation and modulation of the P1 potential (Reese et al., 1995), cholinergic system dysfunction (e.g., Haley et al., 2009, 2013; Li et al., 2011; Liu et al., 2011).

We sought to replicate these findings in a randomly selected nested case-control sample from a larger dataset that is nationally representative of the Gulf War veteran population, including male and female participants from four branches of the military. This design feature avoided the selection bias of studying veterans who volunteer while increasing the probability that the findings are true of the entire GW veteran population. This national sample included male and female veterans from four branches of the military who met the case definition of the three Haley syndrome variants of GWI, obtained with standardize factor analysis and validated by structural equation modeling in a clinical sample (Haley et al., 2001) and in a large nationally representative sample (Iannacchione et al., 2011). Our hypotheses were that the same patterns would be observed in this national sample as were observed in the Seabees sample. We expected to find greater hyperarousal in all three syndrome groups than in the control group, higher P1 amplitudes in Syndromes 2 and 3, and lower P1 amplitudes in Syndrome 1 relative to controls.

2. Method

2.1. Participants

Data were collected as part of a multi-study protocol from 97 participants (24 female). All the participants had been active U.S. military service members during the 1991 Persian Gulf War. Since data from a previous study indicated erratic effects of physostigmine on ERP components (unpublished results), only the data from 62 participants (13 female) whose EEG was recorded *before* their physostigmine infusion for a companion study were included in the analysis. Of these 62 veterans, 50 served in the Army, 3 in the Navy, 4 in the Airforce, and 5 in the Marines. Forty of these 62 met the Haley case definition (Haley et al., 1997b, 2001; Iannocchione et al., 2011) subclassified into one of the three syndrome variants of GWI. Chi square analysis indicated an expected distribution of male and female across syndrome groups, $\chi^2 = 3.046$, $p = .38$. Fourteen (five female) met criteria for GWI Syndrome 1 (impaired cognition), 14 (two female) were identified as Syndrome 2 (neurocognitive and balance issues), and 12 (three female) were identified as Syndrome 3 (somatic complaints). The control group comprised the remaining 22 (3 female) veterans who remained well, 12 of whom were deployed to the Persian Gulf theatre and 10 who remained within the U.S. The control group ranged in age from 38 to 66 years ($M = 48.4$, $SD = 7.9$), Syndrome 1 from 40 to 69 years ($M = 49.2$, $SD = 10.4$), Syndrome 2 from 38 to 65 years ($M = 52.0$, $SD = 7.3$), and Syndrome 3 from 40 to 62 years ($M = 49.7$, $SD = 7.8$). Age did not differ significantly among the groups ($F(3, 58) = 0.598$, $p = .619$). The study sample of 62 was carefully drawn from the larger full survey sample ($N = 8,022$) by selecting randomly from strata to ensure reasonably comparable estimates of the full U.S. military population. The success of the strategy is illustrated by Table 1, which shows the similarity of distributions of demographic characteristics and geographic location in the war made from the study sample and full national survey sample. See Iannacchione et al. (2011) for details of the survey design.

Table 1
Comparison of the distributions of demographic characteristics location in the war in the 1991 U.S. military population estimated from the study sample and the full survey sample.

		U.S. military population estimates* made from:			
	Study sample	Study sample ($N = 62$)	Full national survey sample ($N = 8,022$)		$P^{\dagger}$
Characteristics	N	%	N	%	
Age					
< 49	34	54.5	1,934,103	62.0	
≥49	28	45.5	1,183,884	38.0	0.63
Sex					
Male	49	86.2	2,625,108	84.2	
Female	13	13.8	492,879	15.8	0.81
Race					
Caucasian	47	87.1	2,435,404	78.1	
Other	15	12.9	682,583	21.9	0.28
Service branch					
Army	50	48.7	1,439,540	46.2	
Other	12	51.3	1,678,447	53.8	0.87
Force status					
Active duty	40	58.4	1,839,127	59.0	
Guard/ Reserve/ civilian	22	41.6	1,278,860	41.0	0.62
Geographic location in the war					
Frontline (Iraq/Kuwait)	31	11.5	259,433	8.3	
Other in Kuwaiti Theater	22	12.9	367,656	11.8	
Not deployed to Theater	9	75.6	2,490,868	79.9	0.72
Total	62	100.0	3,117,987	100.0	

* Numbers and percentages in the 1991 U.S. military population were estimated by weighting the subjects with sampling weights that correct for unequal probabilities of selection from the strata and selection bias from non-participation.

† *P* values allowing for the complex survey sampling design tested the difference in percentage distributions of the characteristics between the study sample ($N = 62$) and the rest of the full survey sample ($N = 7,960$).

The subjects were hospitalized and monitored at The University of Texas Southwestern Medical Center's Clinical and Translational Research Center from 2008 through 2010, and underwent a week-long multi-modal neuropsychological, neuroimaging, and biomarker study. An audiometric examination was performed on each veteran. An analysis of variance showed neither main effects of syndrome group or of

ear laterality nor an interaction on pure-tone average thresholds, $p > .35$. All subjects gave written informed consent according to a protocol approved by the university's institutional review board.

2.2. Hyperarousal ratings

A hyperarousal rating was derived from a subset of items from the Mississippi Scale for Combat-Related PTSD (Keane et al., 1988), administered to the veterans as part of their psychological evaluation during their week-long participation in this study. Five doctoral-level clinicians agreed on seven items (item nos. 16, 20, 21, 25, 30, 31, and 34) that were most representative of hyperarousal. Reliability analysis for the seven-item subset yielded a Cronbach's alpha coefficient of .878. Mississippi Scale scores were not obtained from two of the participants. Twelve of the 40 ill veterans (five from the Syndrome 1 group, four from Syndrome 2, and three from Syndrome 3) and none of the controls had been diagnosed with PTSD by psychiatrist's or psychologist's clinical interview following a structured interview technique (SCID).

2.3. Task stimuli

The auditory task consisted of 224 trials presented 2 sec apart and made up of four stimuli that were presented using a three-condition oddball paradigm. A 1000-Hz square wave tone served as the frequent nontarget stimulus and represented 54% of the trials. A 250-Hz square wave tone served as the target stimulus, which was presented for 18% of the trials. The sound of a gunshot (14% of the trials) and the sound of a mountain lion roar (14% of trials) served as the threatening distractor stimuli. Each auditory stimulus was 500 m in duration and was sampled at a rate of 22050 Hz with 16-bit amplitude resolution. The root-mean-squared amplitudes of all four sounds were digitally equated.

The stimuli were presented from two speakers positioned approximately 1 m in front of the subject. Each participant was assigned to one of six randomized sequences of the 224 stimulus presentations. Intensity was adjusted for each subject to a level that was reported as audible and comfortable. Each subject sat in a comfortable chair in a sound-proof booth and was told to keep his or her eyes open during the task.

2.4. Procedure

Participants were fitted with an EEG electrode cap prior to the beginning of the task. The task instructions were read aloud as each participant viewed the printed instructions. Experimenters answered participants' questions and ascertained that the participant understood the task before it began. Once the task began, the subject heard a recorded version of the task instructions. Participants heard examples of the target low tone (250-Hz) and frequent high tone (1000-Hz) during these instructions, and were instructed to press the response button under their right middle finger only for the low tone and to press the response button under their right index finger for *all other* sounds during the task. The subject was not informed that any of the task stimuli would represent threatening circumstances. During the task, each stimulus onset and subject's response-button presses generated a time-locked mark recorded on the continuous EEG, from which response accuracy and reaction time data were collected.

2.5. EEG acquisition

EEG activity was recorded using a 128-electrode array mounted within an elastic cap that was placed on the participant's head. Electrodes placed at the superior and inferior orbital margins recorded blinks and vertical eye movements. The reference electrode was located near the vertex and the APZ electrode served as the ground electrode. Impedance for each electrode did not exceed 10 kΩ as measured before the beginning of the task.

The EEG was recorded using a Neuroscan Synamps2 amplifier at a 500-Hz sampling rate. Data from the continuous EEG were high-pass filtered at 0.15 Hz and were re-referenced to the global mean amplitude. Blink artifacts were filtered from the EEG file using a spatial filter process in the Scan 4.4 Edit (Compumedics Neuroscan) software. From each participant's continuous EEG, 224 1400-ms epochs, consisting of 200 m prior to the presentation of each stimulus through 1200 m after the onset of each stimulus, were used to create four averages: an average of the responses to the target tone, an average of responses to the nontarget tone, and the same for the responses to the lion roar and to the gunshot. Each average consisted of epochs that had been baseline-corrected based on the 200-ms prestimulus data.

3. Results

3.1. Hyperarousal score

An analysis of variance (ANOVA) where the hyperarousal sub-score from the Mississippi Scale for Combat-Related PTSD (Keane et al., 1988) was the dependent variable and GWI syndrome group (deployed control, nondeployed control, Syndrome 1, Syndrome 2, Syndrome 3) was the between-subjects factor indicated a significant effect of GWI group on hyperarousal scores, $F(4, 55) = 22.042$, $MS_{error} = 20.726$, $p < .0001$, $\eta^2 = .616$. The means and standard deviations for each group are shown in Table 2. Post hoc analyses showed that the deployed and undeployed control groups' hyperarousal scores were not different from each other ($p = .903$) and were significantly lower than scores from each of the ill veteran groups, $p < .001$. This is the same pattern we observed in the hyperarousal scores from the Seabees battalion sample (Tillman et al., 2012), as shown in Fig. 1.

When data from the 12 veterans who had been diagnosed with PTSD were removed from the analysis, the same pattern was maintained: Each syndrome group's mean hyperarousal score was significantly higher than that of the control group, $p < .003$.

3.2. Behavioral data

Percent correct and reaction time were used as dependent variables in two separate ANOVAs where GWI syndrome group was the between-subjects factor and condition (target tone, nontarget tone, gunshot, lion roar) was the within-subjects factor. Neither accuracy nor reaction time showed an effect of GWI. Among the veterans who remained well and served as controls, there was no difference between those who were deployed and those who were not deployed in reaction time ($p = .424$) or accuracy ($p = .235$); thus, these data were collapsed into one control group. (See Table 2 for means and standard deviations for each group.)

There was an effect of condition on accuracy, $F(3, 174) = 4.582$, $MS_{error} = 0.041$, $p = .0041$, $\eta^2 = .03$. Post hoc comparisons showed that this was due solely to a significantly lower correct response rate to the lion roar stimulus than to the target tone stimulus ($p = .0009$) and to the nontarget tone stimulus ($p = .001$). There was neither a main effect of group ($F(3, 58) = 0.594$, $MS_{error} = 0.172$, $p = .6217$) nor an interaction between group and condition ($F(9, 174) = 0.599$, $MS_{error} = 0.041$, $p = .7967$).

Removing the data from the veterans with a PTSD diagnosis also showed an effect of condition ($F(3, 138) = 3.281$, $MS_{error} = 0.042$, $p = .023$, $\eta^2 = .03$), but no effect of group ($F(3, 46) = 0.735$, $MS_{error} = 0.149$, $p = .735$) and no interaction ($F(9, 138) = 0.741$, $MS_{error} = 0.042$, $p = .671$).

There was also a main effect of condition on reaction time, $F(3, 174) = 11.578$, $MS_{error} = 143102.163$, $p < .0001$, $\eta^2 = .07$. Reaction times for both the gunshot and the lion roar stimuli were significantly longer than the reaction time for the target tone ($p < .0001$). Reaction time to the lion roar was also significantly longer than reaction time to the nontarget tone ($p = .0038$). There was neither a main effect of group ($F(3, 58) = 1.336$, $MS_{error} = 658240.502$, $p = .2714$) nor an

Table 2
Means and standard deviations of measures on deployed and nondeployed controls and syndrome groups 1–3.

Measure		Nondeployed* Controls *N* = 10 *M(SD)*	Deployed* Controls N = 12 *M(SD)*		Syndrome 1 *N* = 14 *M(SD)*	Syndrome 2 *N* = 14 *M(SD)*	Syndrome 3 *N* = 12 *M(SD)*
Hyperarousal score		12.2(4.26)	12.4(3.97)		24.3(4.36)	24.9(4.85)	20.8(5.2)
P1 amplitude in μV							
	Target tone	0.68(.51)	0.97(.95)		1.12(1.26)	1.31(.70)	1.63(1.03)
	Nontarget tone	0.79(.49)	0.94(.76)		0.40(.78)	0.94(.69)	0.54(.94)
	Lion roar	0.85(.70)	1.00(1.07)		0.32(1.06)	1.24(.86)	1.27(.74)
	Gunshot	0.80(.71)	0.91(.70)		0.59(1.14)	1.27(.92)	1.59(.27)
P1 latency in ms							
	Target tone	46.6(8.1)	45.8(12.9)		48.7(11.0)	50.6(9.0)	48.2(11.0)
	Nontarget tone	48.2(7.1)	48.2(11.8)		45.4(10.8)	49.1(9.7)	44.7(7.1)
	Lion roar	48.4(12.3)	49.8(13.4)		49.9(11.3)	51.0(10.3)	49.8(11.6)
	Gunshot	57.0(10.5)	48.8(14.1)		53.4(12.1)	56.0(12.6)	44.5(13.5)
Response accuracy (%)							
	Target tone	94.3(7.4)	87.3(13.6)	84.5(16.7)		76.8(15.0)	81.3(13.7)
	Nontarget tone	87.2(17.7)	90.3(14.5)	81.1(30.2)		78.2(28.5)	85.6(20.6)
	Lion roar	80.9(29.4)	64.2(40.6)	76.534.5		65.4(36.3)	74.7(34.2)
	Gunshot	84.7(16.4)	65.639.9	83.924.5		70.3(30.7)	74.5(33.5)
Reaction time in ms							
	Target tone	689(126)	729(131)	855(438)		895(467)	704(165)
	Nontarget tone	828(324)	754(306)	1036(667)		1080(542)	968(439)
	Lion roar	1007(369)	1188(769)	908(294)		1403(740)	1189(588)
	Gunshot	894(291)	1058(784)	1031(604)		1299(678)	1197(678)

* Deployed and nondeployed controls were found not to be different on any of these measures ($p > .23$), and were thus combined into one control group for the analyses.

interaction between group and condition ($F(9, 174) = 1.2$, $MS_{error} = 143102.163$, $p = .2978$).

Removing the data from the veterans with a PTSD diagnosis did not change the pattern. There was an effect of condition ($F(3, 138) = 5.828$, $MS_{error} = 118098.489$, $p = .001$, $\eta^2 = .04$), but neither an effect of group ($F(3, 46) = 0.835$, $MS_{error} = 587506.369$, $p = .482$) nor an interaction ($F(9, 138) = 1.441$, $MS_{error} = 118098.489$, $p = .176$).

3.3. ERP data

The P1 component was defined as the most positive point in the event-related potential average from the frontal midline electrode 61 in the interval between 30 and 75 m after the onset of the auditory stimulus. The amplitude and latency measures were dependent variables in two separate mixed-effects general linear models, where GWI syndrome group was the between-subjects factor, condition (target tone, nontarget tone, gunshot, lion roar) was the within-subjects factor, and subjects were the random factor. The means and standard deviations for P1 amplitudes and latencies are indicated in Table 2. There was no difference between deployed and nondeployed controls in P1 amplitude ($p = .550$) or latency ($p = .555$); thus their data were collapsed into one control group. A planned comparison based on our previous findings (Tillman et al., 2012) was also computed comparing Syndrome 1 to Syndromes 2 and 3. We also used three orthogonal single-*df* interaction contrasts to test whether the groups differed in their mean change between distractor stimuli (gunshot and lion roar) and task-relevant stimuli (target and nontarget tones): a) comparing controls to Syndromes 1, 2, and 3; b) comparing Syndromes 2 and 3 to Syndrome 1; and c) comparing Syndrome 2 to Syndrome 3. Age did not predict P1

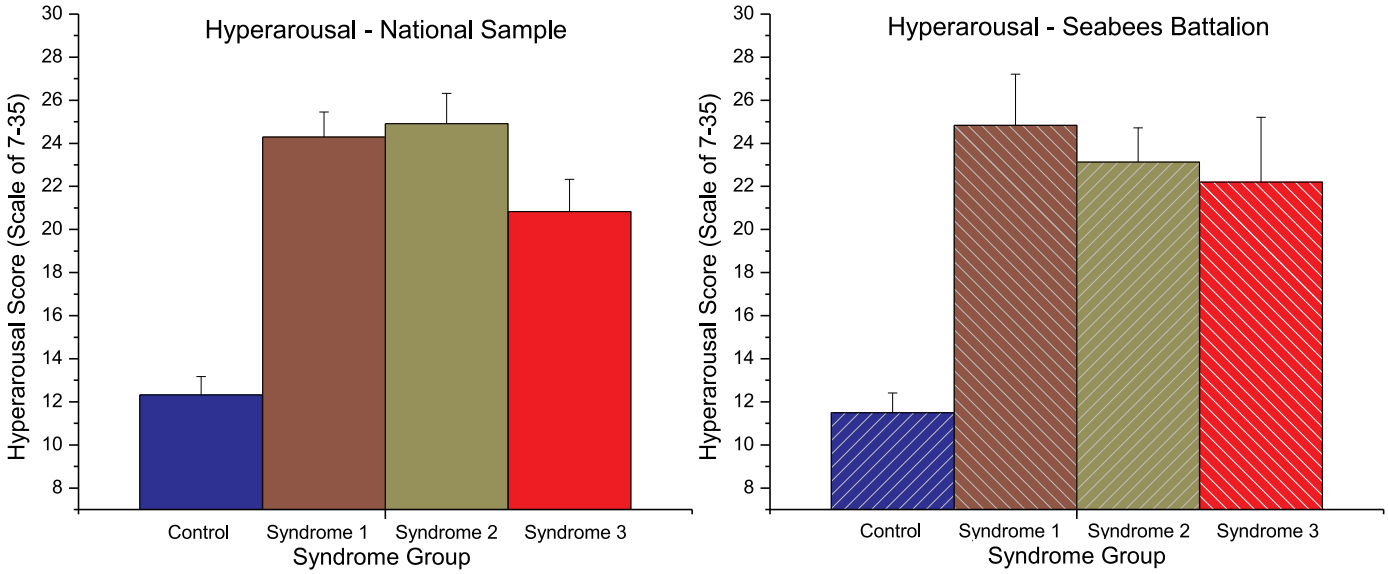

Fig. 1. *Left panel:* Hyperarousal scores of the three syndrome groups were significantly higher than those of the control group in the sample reported here. *Right panel:* The same pattern was observed in the hyperarousal scores of the Seabees battalion (Tillman et al., 2012).

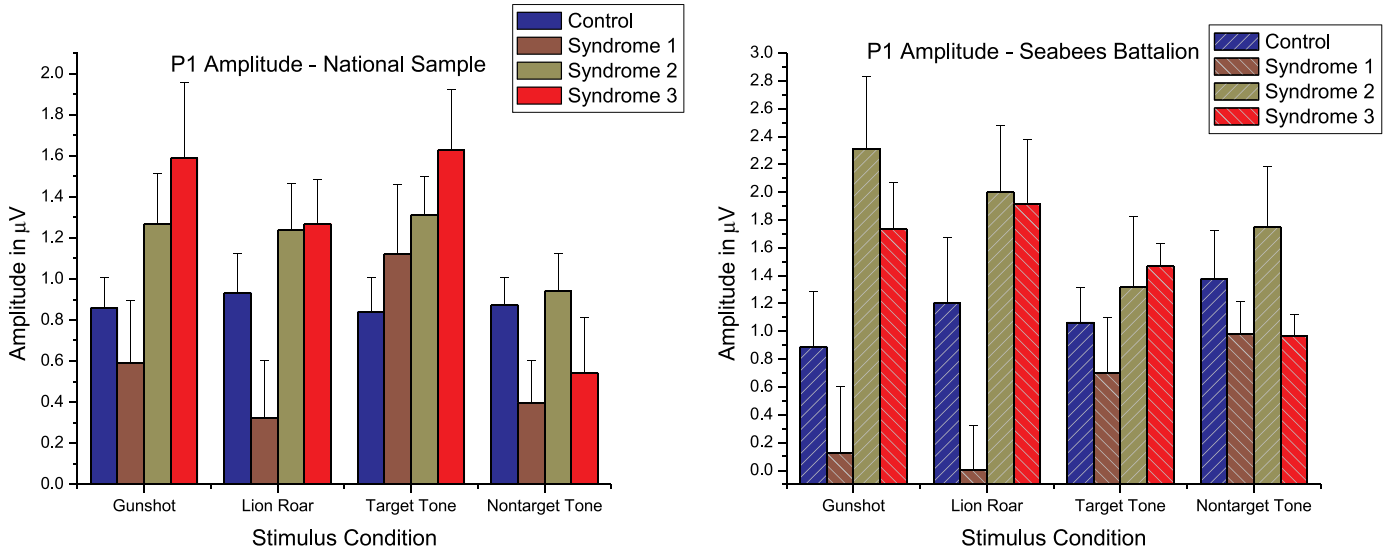

Fig. 2. *Left panel:* Syndrome 1 P1 amplitudes were significantly lower than those of Syndromes 2 and 3. Also, among Syndromes 2 and 3, the average P1 amplitude to distractor stimuli (gunshot, lion roar) is greater than that to task-related tone stimuli (target tone, nontarget tone), whereas the opposite pattern is observed in Syndrome 1. *Right panel:* The pattern of P1 amplitudes replicated those observed in our previous study (Tillman et al., 2012).

amplitude or P1 latency in this sample; thus, it was not used as a covariate in the analyses.

There was a main effect of condition on P1 amplitude, $F(3, 174) = 7.075$, $MS_{error} = 0.433$, $p = .0002$, $\eta^2 = .042$. As shown in Fig. 2, the P1 response to the nontarget tone was significantly smaller than the P1 response to the target tone, $p = .0003$ ($p = .0016$, Bonferroni-corrected). There was a trend toward a significant main effect of GWI syndrome group on P1 amplitude, $F(3, 58) = 2.567$, $MS_{error} = 1.916$, $p = .0631$, $\eta^2 = .117$. The comparison of Syndromes 2 and 3 to Syndrome 1 was significant, $F(1, 58) = 4.869$, $p = .031$. Syndrome 1 amplitudes were lower than those of Syndromes 2 and 3. An interaction between group and condition was also indicated, $F(9, 174) = 2.383$, $MS_{error} = 0.433$, $p = .014$, $\eta^2 = .042$. Only the second interaction contrast, which compared the mean change between distractor stimuli and task-relevant tone stimuli of Syndromes 2 and 3 to that of Syndrome 1, was significant, $p = .0145$. The mean *decrease* in P1 amplitude observed in Syndromes 2 and 3 was significantly different from the mean *increase* in P1 amplitude from distractor to tone stimuli for the Syndrome 1 group; that is, the Syndrome 1 amplitudes to tone stimuli were higher than their amplitudes to distractor stimuli, whereas Syndrome 2 and 3 amplitudes to distractor stimuli were higher than their amplitudes to tone stimuli. This pattern was also observed in the P1 amplitudes from the Seabees battalion sample (Tillman et al., 2012), illustrated in Fig. 2.

Removing the data of the veterans with a PTSD diagnosis did not change the pattern of results. A main effect of condition was indicated, $F(3, 138) = 7.356$, $MS_{error} = 0.280$, $p = .0001$, $\eta^2 = .039$. The amplitude of the response to the target stimulus was higher than that to the nontarget stimulus, $p = .0004$. The Group x Condition interaction was significant, $F(9, 138) = 2.574$, $MS_{error} = 0.280$, $p = .009$, $\eta^2 = .041$; however, there was no main effect of group on P1 amplitudes among the veterans without PTSD, $F(3, 46) = 1.385$, $MS_{error} = 2.160$, $p = .259$.

P1 latency also showed a main effect of condition, $F(3, 174) = 3.213$, $MS_{error} = 75.297$, $p = .024$, $\eta^2 = .023$. The latency of the P1 to the gunshot stimulus was significantly longer than the latency of the P1 to the nontarget stimulus, $p = .002$ ($p = .012$, Bonferroni-corrected; Fig. 3). There was neither an effect of GWI syndrome group on P1 latency ($F(3, 58) = 0.748$, $MS_{error} = 275.714$, $p = .528$) nor an interaction between group and condition ($F(9, 174) = 0.955$, $MS_{error} = 75.297$, $p = .479$).

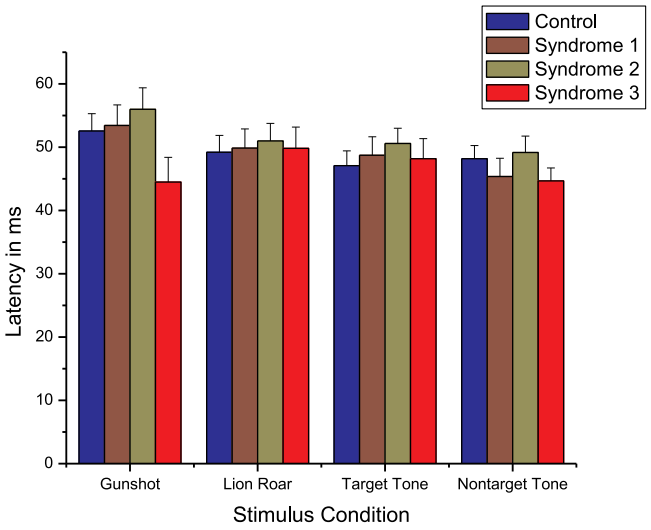

Fig. 3. P1 latencies showed only an effect of condition. P1 latency to the gunshot stimulus was significantly longer than that to the nontarget tone.

Removing the data from the veterans with a PTSD diagnosis showed a similar pattern of results but no significant omnibus effects. There was a trend toward a main effect of condition, $F(3, 138) = 2.489$, $MS_{error} = 74.847$, $p = .063$, due to the latency of the response to the target stimulus being longer than that to the nontarget stimulus ($p = .007$). Neither a main effect of group ($F(3, 46) = 0.591$, $MS_{error} = 270.463$, $p = .624$) nor a Group x Condition interaction ($F(9, 138) = 1.471$, $MS_{error} = 74.847$, $p = .165$) was indicated.

There was a trend toward hyperarousal score reliably predicting P1 amplitude to the nontarget (frequent) stimulus ($\beta = -.242$, $p = .062$), where higher hyperarousal scores predicted lower P1 amplitude in response to the frequent tone. Hyperarousal score bore no other linear relationships with the P1 component ($p > .14$). When the data from the veterans with a PTSD diagnosis were removed, no linear relationship between hyperarousal score and the P1 component reached significance ($p > .11$).

4. Discussion

In this study of a larger and more representative national sample of Gulf War-era veterans we expected to find a pattern of ERP responses to auditory threat that was similar to that exhibited in a previous study (Tillman et al., 2012) of veterans who had served in one construction battalion of the United States Naval Reserve during the 1991 Persian Gulf War. As in the prior study, hyperarousal scores were significantly higher among the ill veterans of all 3 syndrome groups than among the control group veterans. The early auditory P1 component showed the same pattern by syndrome group: Syndromes 2 and 3 exhibited significantly higher and Syndrome 1 significantly lower P1 amplitudes, with the amplitudes of the control group intermediate between them. We also observed the same interaction between group and condition: P1 responses of the Syndrome 1 group were attenuated for threat-related stimuli (gunshot and lion roar) relative to task-related stimuli, whereas those of the Syndromes 2 and 3 groups showed the opposite pattern.

The significant response pattern observed in this sample's P1 amplitudes is the same pattern observed in our previous study (Tillman et al., 2012) and is consistent with dysfunction of the cholinergic system, which has been proposed in many studies of GWI (Haley et al., 2009, 2013; Hubbard et al., 2014; Li et al., 2011; Liu et al., 2011; Tillman et al., 2012; Turner et al., 2016). Cholinergic input from the pedunculopontine nucleus (PPN) of the reticular activating system in the brainstem exerts substantial influence on P1 amplitude (see Reese et al., 1995).

Christova et al. (2017) found that brainstem volumes of veterans with GWI were significantly reduced relative to veterans in the control group. Brainstem abnormalities in this veteran population have also been indicated in studies using magnetic resonance imaging (MRI) voxel-based morphometry (Rayhan et al., 2013) and MR spectroscopy (Haley et al., 2000b). In addition, auditory brainstem response data collected in an investigation of vestibular dysfunction in Gulf War veterans (Roland et al., 2000) strongly indicated brainstem injury related to neurotoxic exposure.

The PPN sends cholinergic projections to muscarinic receptor sites in the thalamus (Reese et al., 1995), including the intralaminar nuclei (Dickerson and Buchwald, 1991). These in turn project to the amygdalae (LeDoux et al., 1991) as part of the rapid nonlemniscal “low road,” described by LeDoux (1998) as a pathway for transmitting auditory information that is low in detail but possibly vital to survival. Calley et al. (2010) compared deployed GW veterans without GWI to those with GWI, especially Syndrome 2, and found different functional MRI BOLD responses in thalamus and caudate. Haley et al. (2009) used SPECT imaging to examine changes in regional cerebral blood flow (rCBF) in eight deep brain structures in response to infusion of the cholinesterase inhibitor physostigmine. Veterans in syndrome groups 1 and 3 showed greater decreases in rCBF than controls in different nuclei of the left thalamus, while the Syndrome 2 group showed paradoxical increases in rCBF in the right posterior hippocampus and amygdala and the caudate heads bilaterally.

Abou-Donia et al. (2001, 2002, 2004) and Abdel-Rahman et al. (2004) have studied in rats the combinatory and synergistic effects of three widely used chemicals suspected of playing a role in Gulf War illnesses (RAC, 2008): permethrin, pyridostigmine bromide (PB), and DEET. Their data have demonstrated that different combinations of and levels of exposure result in differential effects on acetylcholine (ACh) and ACh receptors. They specifically reported effects on the muscarinic ACh receptor M2. Abou-Donia et al. (2001, 2002, 2004) reported increased ligand binding in rats’ cortical M2 receptors, and Abdel-Rahmen et al. (2004) reported decreased M2 ligand binding in midbrain and cerebellum after rats were exposed to both stress and low doses of permethrin, PB, and DEET combinations. M2 receptors are highly involved in the modulation of the auditory P1. M2 receptors modulate PPN input to thalamus via their inhibition not only of cholinergic projections to thalamus but also of GABAergic and glutamatergic projections (Ye et al., 2010). In addition, the nonlemniscal pathway expresses a higher percentage of M2 receptors relative to the lemniscal pathway, which expresses a higher percentage of M1 receptors (Mooney et al., 2004). Nonlemniscal M2 receptors are largely postsynaptic and inhibitory (Mooney et al., 2004); application of muscarine to nonlemniscal cells results in their hyperpolarization, whereas muscarine application to lemniscal cells results in depolarization (Mooney et al., 2004). Mooney et al. argued that such hyperpolarization enabled in nonlemniscal cells a selective sensitivity to synchronized input that can result in burst firing. M2 receptors on the more depolarized neurons of the nonlemniscal pathway suppress synaptic transmission. Thus, cholinergic tone within this nonlemniscal low road (LeDoux, 1998) is important in the regulation of arousal and attention. Dysfunctional M2 receptors in the nonlemniscal pathway could disrupt the sensitivity to temporally correlated input or overly suppress input from auditory stimuli. Many of the intralaminar nuclei efferents are to amygdala (LeDoux et al., 1990), where M2 receptor activity is critical for consolidating memories of emotionally arousing experiences (Power et al., 2003). M2-mediated mechanisms in the basolateral nucleus of amygdala include presynaptic regulation of ACh, glutamate, and GABA release, and postsynaptic effects in dendrites (Muller et al., 2016; Rouse et al., 1997). Amygdalar dysfunction has been indicated in several conditions that are marked by hyperarousal, such as social and general anxiety disorder (Rabany et al., 2017), panic disorder (Brinkmann et al., 2017), and PTSD (Weston, 2014). Thus, if the M2 receptor is especially susceptible to the chemicals to which troops were exposed in theatre in 1991 (Abou-Donia et al., 2001, 2002, 2004; Abdel-Rahman et al., 2004), such M2 receptor irregularities could easily be contributing to the different P1 amplitude patterns and hyperarousal observed in the ill Gulf War veterans.

Most literature reporting modulation of the auditory P1 have been based on studies of the gating mechanism. The P1 shows habituation to rapidly repeated auditory stimuli in healthy subjects (Erwin and Buchwald, 1986). This habituation is expressed as a ratio (S1/S2) of the P1 amplitude in response to an initial click stimulus (S1) to the P1 amplitude to an identical click stimulus (S2) presented, most commonly, 500 m later. This habituation has been shown to be attenuated in individuals diagnosed with many conditions associated with anxiety and attention difficulties such as traumatic brain injury (Arciniegas et al., 2000), PTSD (Skinner et al., 1999), Huntington's disease (Uc et al., 2003), and schizophrenia (Siegel et al., 1984). Persistent but non-habituating P1 observed in decerebrate animals (Reese et al., 1995) strongly suggests that top-down influences are modulating this habituation. Several studies have indicated that cholinergic projections to nicotinic ACh receptors in hippocampus mediate P1 habituation (Adler et al., 1998; Bickford et al., 1993; Luntz-Leybman et al., 1992). Freedman et al. (1995) suggested that the failure of P1 suppression in patients with schizophrenia is due to their paucity of nicotinic ACh receptors on hippocampal neurons, which are thought to activate the inhibitory interneurons responsible for the suppression of response to repeated stimuli. Some of these GABAergic interneurons are modulated by postsynaptic M2 receptors (Rouse et al., 1997**).**

The smaller gating ratio observed in schizophrenia patients is partially due to a reduced P1 amplitude in response to the first stimulus (Johannesen et al., 2005; Turetsky et al., 2012), suggesting that response amplitude and sensory gating are separate mechanisms. Whereas cholinergic receptor subtypes have been implicated in observed P1 gating (Adler et al., 1998; Bickford et al., 1993; Freedman et al., 1995; Leonard et al., 1996; Luntz-Leybman et al., 1992;), P1 amplitude may be modulated by dopaminergic action. Abnormal central dopamine metabolism has been implicated in GWI (Haley et al., 2000a; RAC, 2008). Moxon et al. (2003) argued that both increased and decreased dopaminergic activity in hippocampus perturbs the synchrony of the P1 response. Such asynchrony reduces the signal-to-noise ratio, increasing temporal variability and resulting in reduced P1 amplitudes (Patterson et al., 2000). The role of dopaminergic activity is

further supported by research showing that pharmacological treatment of and genetic differences in dopamine receptor D2 activity modify P1 amplitude but not P1 gating (Adler et al., 1990; Freedman et al., 1983; Knott et al., 2010). Activation of dopamine receptor sites in amygdala are thought to be responsible for releasing the amygdala from the inhibitory input from the medial prefrontal cortex (De la Mora et al., 2010), thus enabling preparation for dealing with real or potential threat. Whereas amygdala D1 receptors principally facilitate retrieval of affective associations of a stimulus, D2 receptors mediate brainstem-based reflex responses and the establishment of adaptive coping responses to threatening stimuli; they are critical in the control of overgeneralization of threat responses (De Bundel et al., 2016). Thus the abnormal dopaminergic metabolism among ill Gulf War veterans may be contributing to their heightened hyperarousal and disparate P1 amplitude patterns.

Our finding of responses in the Syndrome 2 and 3 groups opposite from those in the Syndrome 1 group in this national sample replicates the finding in our prior application of this EEG paradigm in the Seabees battalion and is also consistent with prior functional neuroimaging studies employing the Haley factor case definition. Specifically, the Haley et al. (2009) study performed in 1998 found response to a cholinergic challenge by the three syndrome groups in opposite directions from the control group in SPECT-measured rCBF in deep gray matter, and this finding was replicated with a similar cholinergic challenge protocol in a similar sample 10 years later by studies measuring changes in rCBF with arterial spin labeling MRI (ASL) in deep gray matter (Liu et al., 2011) and in a high resolution ASL study of the hippocampus (Li et al., 2011). This diverse response pattern may reflect differences in environmental exposures of the 3 syndrome groups documented in two epidemiologic studies. In an epidemiologic study of the Seabees battalion, Haley and Kurt (1997) found Syndrome 2 associated with wartime exposure to low-level nerve gas; Syndromes 2 and 3, with excess cholinergic side effects from the pyridostigmine anti-nerve gas medications; but Syndrome 1, with pesticide exposure. Epidemiologic study of a large national sample of Gulf War-era veterans strongly associated Syndromes 2 and 3, but not Syndrome 1, with exposure to low-level nerve gas (Haley and Tuite, 2013; Tuite and Haley, 2013). The continuing replication of important differences among the three syndromes, with responses in opposite directions relative to controls, supports our use of three groups of differing symptomologies, given that the means of such opposite effects among ill veterans might obscure important differences from controls.

Many auditory oddball paradigm studies that have examined the P1 have shown an effect of target condition, wherein the P1 amplitude in the response to the target stimulus is significantly higher than the amplitude in the response to the nontarget stimulus (e.g., Boutros et al., 1995; Boutros and Belger, 1999). Whereas the main effect of condition reported here was due to the P1 response to the target being stronger than the P1 response to the nontarget, this pattern was not observed in the control group. A plausible explanation for this effect being so small as to render it insignificant is based on research examining whether the target tone is higher or lower than the nontarget tone (e.g., Boutros and Belger, 1999) and research examining the effects of age (e.g., Golob and Starr, 2000). Boutros and Belger compared P1 responses in oddball paradigms when the target stimulus (1500 Hz) was higher than the nontarget stimulus (1000 Hz) and when the target stimulus (500 Hz) was lower than the nontarget stimulus (1000 Hz) in 19–45-year-olds. The effect size of condition when the target stimulus was lower was much smaller than this effect size when the target stimulus was higher. In the auditory oddball tasks we used in our previous study and in the study reported here, the target tone frequency (250 Hz) was lower than that of the nontarget tone frequency (1000 Hz), which likely contributed to there being no difference between target and nontarget amplitudes among controls. Also, Golob and Starr reported no effect of condition on P1 amplitudes among older adults with (mean age of 72) or without (mean age of 66.3) an Alzheimer's disease diagnosis; however, the mean amplitude to the nontarget was higher than mean amplitude to the target in both groups. Although the veterans in the current study (mean age of 49.6, ± 7.97) and in our previous study (mean age of 58.8, ± 8.00) are somewhat younger than the participants in the Golob and Starr study, we feel that their age was likely playing a role in the lessening difference between target and nontarget P1 amplitudes.

4.1. Conclusion

Our finding replication in the pattern of the early P1 suggests that the criteria for Haley syndrome group membership are closely aligned with the conditions that contribute to different P1 amplitude patterns. Hyperarousal, which is the symptom that prompted the design of this study, is not specifically assessed by the symptom questionnaire that classifies veterans into the syndrome groups. Hyperarousal was higher in the ill veterans than in the control group, with and without the data from the veterans with a PTSD diagnosis, but the syndrome groups did not differ from one another. Poorly modulated input from PPN to thalamus, attenuated sensitivity to temporally correlated input in non-lemniscal M2 receptors, dysfunctional amygdalar or hippocampal dopaminergic mechanisms, or any combination of these may be contributing to the behavioral outcome of hyperarousal. Since the Seabees comprised a more homogenous group with more similar posts and experiences, the contributors to and the ERP patterns of hyperarousal were more consistent within each syndrome group. However, it appears that items from the symptom reports used to classify a veteran as belonging to one of the syndrome groups *do* identify symptoms that are highly related to the generation of a P1 ERP component.

The present study of a randomly selected nested case-control sample from a larger dataset that is nationally representative of the Gulf War veteran population replicated a pattern of early auditory P1 component differences across various Haley syndrome groups found in a prior study of male veterans from a single naval construction battalion (Tillman et al., 2012): Syndromes 2 and 3 exhibited significantly higher and Syndrome 1 significantly lower P1 amplitudes, especially to threat-related distractor stimuli. This finding of the Syndrome 1 responses and the Syndromes 2 and 3 responses being on opposite sides of the control group responses is consistent not only with our previous study (Tillman et al., 2012) but also with prior neuroimaging studies (Haley et al., 2009; Li et al., 2011; Liu et al., 2011). Data from this study also indicated the same pattern of higher hyperarousal in the syndrome groups relative to controls even after the data from veterans with PTSD were removed. Veterans with PTSD also negligibly contributed to observed ERP P1 group differences. Contributors to the auditory P1 potential are highly relevant in the complex regulation of arousal and are modulated by neurotransmitter and receptor systems—dopaminergic and especially cholinergic—that have been strongly implicated in the etiology of GWI.

Acknowledgements

This study was supported by IDIQ contract VA549-P-0027, awarded and administered by the Department of Veterans Affairs Medical Center, Dallas, TX; U.S. Army Medical Research and Materiel Command grant number DAMD17-01-1-0741; and Grant Number UL1RR024982, titled North and Central Texas Clinical and Translational Science Initiative (Milton Packer, M.D., PI), from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and NIH Roadmap for Medical Research. The content does not necessarily reflect the position or the policy of the Federal government or the sponsoring agencies, and no official endorsement should be inferred. The corresponding author had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Authors contributions

Tillman, Hart, and Kraut designed the experiment. Tillman, Briggs, Haley, and Hart wrote the manuscript with consultation, input, and critical feedback from all the authors. Tillman and Spence performed the analyses.

Conflict of interest

None of the authors have conflicts of interest to report.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.pscychresns.2018.11.006.

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