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Visual event-related potentials as markers of hyperarousal in Gulf War Illness: evidence against a stress-related etiology

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Abstract

An exaggerated response to emotional stimuli is among the many symptoms widely reported by veterans of the 1991 Persian Gulf War. These symptomologies have been attributed to damage and dysfunction associated with deployment-related exposures. We collected event-related potential data from 22 veterans meeting Haley criteria for Gulf War (GW) Syndromes 1-3 and from 8 matched GW veteran controls, who were deployed but not symptomatic, while they performed a visual three-condition oddball task where images authenticated to be associated with the 1991 Persian Gulf War were the distractor stimuli. Hyperarousal reported by ill veterans was significantly greater than that by control veterans, but this was not paralleled by higher amplitude P3a in their ERP responses to GW-related distractor stimuli. Whereas previous studies of PTSD patients have shown higher amplitude P3b responses to target stimuli that are placed amid trauma-related nontarget stimuli, ill veterans in this study showed P3b amplitudes to target stimuli—placed amid GW-related nontarget stimuli—that were significantly lower than those of the control group. Hyperarousal scores reliably predicted P3b, but not P3a, amplitudes. Although many factors may contribute to P3b amplitude differences—most notably depression and poor sleep quality, symptoms that are prevalent in the GW syndrome groups—our findings in context of previous studies on this population are consistent with the contention that dysfunction in cholinergic and dopaminergic neurotransmitter systems, and in white matter and basal ganglia may be contributing to impairments in GW veterans.

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Keywords

ERPs; P3a; P3b; cholinergic; dopaminergic

1. Introduction

1. Veterans who were deployed during the 1991 Persian Gulf War have reported clusters of symptoms that have been attributed to deployment-related exposures (Research Advisory Committee on Gulf War Veterans' Illnesses, 2008). There have been several studies (e.g., Haley et al., 1997a; Fukuda et al., 1998; Doebbeling et al., 2000; Kang et al., 2002; Iannacchione et al., 2011) that have identified through factor analysis most commonly three main clusters of symptoms. One cluster is associated with impaired cognition: distractibility, memory problems, confused thought, and fatigue. A second cluster describes more debilitating neurocognitive problems—reasoning problems, confusion, disorientation, word-finding difficulty, emotional lability—and balance problems, such as vertigo and frequent stumbling. The third cluster of symptoms is associated more with somatic complaints, such as joint and muscle pain, weakness and fatigue, and numb or tingling extremities.
2. Hyperarousal is a symptom that has been widely reported by Gulf War veterans (Thompson et al., 2004). Hyperarousal is also observed among persons with posttraumatic stress disorder, (Morina et al., 2010), traumatic brain injury (Rapoport et al., 2002), anxiety disorders (Ruscio and Borkovec, 2004; Sachs et al., 2004; Erwin et al., 2006; Pillay et al., 2006), and schizophrenia (Nakamura et al., 2003). Hyperarousal among persons with conditions associated with hyperarousal has been assessed using event-related potentials (ERPs) derived from electroencephalographic (EEG) data (Bruder et al. 2002; Dodin and Nandrino, 2003; Karl et al., 2006; Rossignol et al., 2008). Using ERPs, a three-condition oddball paradigm is especially appropriate for studying exaggerated arousal to trauma-related but task-irrelevant stimuli. In a three-condition oddball task, the ERP elicited by response to the target stimuli (~20% of the trials) shows a positive deflection occurring around 300 ms and has been called the target P3 or the P3b. The standard nontarget stimuli are presented ~60% of the time and require a standard response or no response. The nontarget distractor is a novel stimulus that is presented the remaining 20% of the time. The subject is to give the same response to these novel stimuli as to the standard nontarget stimuli. The ERP response to the nontarget distractor is a positive deflection with a peak latency that is earlier than that of the P3b and has been termed the novelty P3 or P3a. The P3a is purported to index an involuntary capture of attention (Friedman et al., 2001) and can be used as an index of hyperarousal.

1.2. Target P3b

A meta-analysis of ERP studies of PTSD (Karl et al., 2006) showed that relative to controls, persons with PTSD exhibited increased target P3b amplitude to neutral stimuli when those stimuli were interspersed among trauma-related stimuli, but showed decreased target P3b amplitude to targets when both the target and the nontarget stimuli were neutral. Kolassa et al. (2005, 2006) has shown that both schematic and photographic images of spiders elicit higher P3b amplitudes in spider-phobics. Lower P3b amplitudes have been observed in patients with traumatic brain injury (Doi et al., 2007) and in patients with schizophrenia (Kogoj et al., 2005). The P3b to target stimuli has also been shown to be sensitive to agents that affect the systems that most likely suffered insult from known toxic exposures during

Desert Storm: the cholinergic system, the basal ganglia and dopaminergic system, and white matter.

The cholinergic system is affected by many of the agents to which veterans deployed to the Persian Gulf were exposed and has been linked to many of the symptoms reported by them. Veterans classified as Haley syndrome groups 2 and 3 (Haley et al., 1999) tended to show exaggerated reactions to the anti-nerve-gas agent pyridostigmine bromide, a reversible cholinesterase inhibitor. Veterans who showed many of the neurological symptoms associated with GW illness were more likely to have an R allele of the PON1 gene, the genotype with slow hydrolysis of the cholinesterase inhibitors soman, sarin, and diazinon, and were more likely to have suffered a severe reaction to the pyridostigmine bromide (Haley et al., 1999). In addition, magnetic resonance spectroscopy (MRS; Haley et al. 2000b) and single photon emission computed tomography (SPECT; Haley et al., 2009; Liu et al., 2011) studies have indicated dysfunctional cholinergic systems in ill Gulf War veterans. The insect repellent *N,N*-diethyl-*m*-toluamide (DEET), the insecticide permethrin, and other cholinesterase-inhibiting organophosphates were widely used to manage the endemic insect problem in the Gulf War theatre (Institute of Medicine, 1995). Studies have shown that, when combined with stress, exposing adult rats to pyridostigmine bromide, DEET, and permethrin can result in disruption of their blood-brain barrier, neuronal death, decreased acetylcholinesterase activity, and decreased acetylcholine receptor binding (Abou-Donia et al. 1996; Abdel-Rahman et al., 2004). Human subjects have exhibited increased P3b amplitudes (Müntz et al., 1988) after the administration of the ACh receptor agonist WEB1881 FU (Nebracetam), whereas administration of the cholinergic antagonist scopolamine resulted in decreased P3b amplitudes and impaired performance on memory tasks (Hammond et al., 1987; Meador et al., 1989).

ACh is also known to play a key role in striatal function (Calabresi et al., 2000; Bonsi et al., 2011) and in modulating dopaminergic activity (Exley and Cragg, 2008), which in turn modulates ACh activity (Deboer et al., 1996; Aosaki et al., 2010). Thus, dysfunction in either system can result in dysfunction in the other. Dysfunction in basal ganglia and the dopaminergic system among Gulf War veterans has been reported (Haley et al., 2000a; Meyerhoff et al., 2001). Magnetic resonance spectroscopy (MRS) studies measuring *N*-acetylaspartate-to-creatine (NAA/Cr) ratio showed evidence of reduced neuronal integrity in basal ganglia in Haley GW Syndromes 1 and 2, and in brainstem in Haley Syndromes 2 and 3. The basal ganglia choline-to-creatine (Cho/Cr) ratio was significantly lower in the Syndrome 1 group (Haley et al., 2000b), and lower NAA/Cr ratio in left basal ganglia was closely associated with higher dopaminergic activity (Haley et al., 2000a).

The basal ganglia have also been shown to contribute to the P3b. Rektor et al. (2003) recorded from electrodes implanted in basal ganglia, primary motor cortex, and lateral and medial supplementary motor cortices during subjects' performance of auditory and visual oddball tasks. Target P3 amplitude was significantly higher in basal ganglia than in cortical areas, indicating contribution by this noncortical area to the generation of the P3b. Systems both with low DA activity, as in Parkinson patients (Galvan and Wichmann, 2008), and with high activity, as in schizophrenia (Howes and Kapur, 2009), show decreased P3b amplitudes (Li et al., 2003; Ergen et al., 2008), thus implicating dopamine in P3b amplitude variance.

1.3. Novelty P3a

Higher novelty P3a amplitudes have been observed in responses to phobia-related images among persons with spider- (Kolassa et al., 2005) and dental-phobias (Schienle et al., 2011), and to emotional faces in female patients diagnosed with mixed anxiety-depression (Rossignol et al., 2008). Similarly, the Karl et al. (2006) meta-analysis of ERP studies of PTSD concluded that P3a amplitudes to trauma-related distractor pictures were significantly

higher in PTSD trauma-exposed groups than in trauma-exposed groups without PTSD. Lower P3a amplitudes to novel stimuli have been observed among schizophrenia patients and their siblings (Turetsky et al., 2000), and in patients with traumatic brain injury (Roche et al., 2004). The P3a response to novel stimuli is generated by several contributing brain areas, including the hippocampus (Knight, 1996) and mediofrontal (Elting et al., 2008), inferior frontal (Baudena et al., 1995), dorsal prefrontal (Knight, 1984), and anterior cingulate cortex (Dien et al., 2003). These areas receive, or are modulated by, dopaminergic input (Allman et al., 2001; Monchi et al. 2004; Cilia et al., 2011, Fusar-Poli et al., 2011) and are implicated in emotional processing and regulation (Ochsner et al., 2004; Badgaiyan et al., 2009; Schienle et al., 2009; Etkin et al., 2011). While dopamine is purported to be a significant neurotransmitter contributor to the generation of the P3a component (Polich, 2007), little has been reported on the role of the cholinergic system in its generation.

To assess the nature of the hyperarousal symptoms of GWI patients, we analyzed ERP data of 30 Gulf War veterans during their performance on a visual three-condition oddball task where pictures of animals were the targets, scenes from the 1991 Persian Gulf War and weapons were the threatening distractors, and nonthreatening pictures of objects, people, and nature were the standard stimuli. We hypothesized that we would find inconsistent effects on P3a amplitudes, given the countervailing factors of a higher P3a being associated with hyperarousal, but a lower amplitude being associated with a diminished contribution to this component by dopaminergic neural systems. We also hypothesized that we would observe reduced P3b amplitudes in ill GW veterans given the documented contributions of both cholinergic and dopaminergic systems to the generation of this potential.

2. Method

2.1. Participants

The participants were 30 GW veterans who had served in the same construction battalion in the United States Naval Reserve deployed during the 1991 Persian Gulf War. Twenty-two of these met the Haley (Haley et al., 1997a; Iannacchione et al., 2011) criteria for one of the syndromes of Gulf War Illness: Seven met criteria for Gulf War Syndrome 1, nine were identified as Syndrome 2, and six were identified as Syndrome 3. Eight age-sex-education-matched veterans without symptoms served as controls. All subjects were male. The ill group ranged in age from 46 to 73 years ($M=57.2$), and the control group, from 51 to 76 years ($M=61.6$). All participants had participated in prior studies of Gulf War illness (Haley et al., 1997a, 1997b, 2000a, 2000b). Subjects were hospitalized and monitored at The University of Texas Southwestern Medical Center's Clinical and Translational Research Center in 2008 and 2009 while they participated in a week-long multi-modal neuropsychological, neuroimaging, and biomarker study. The design of the study as it pertains to the data discussed here is shown in Figure 1. All subjects gave written informed consent according to a protocol approved by the university's institutional review board.

2.2. Hyperarousal ratings

We evaluated hyperarousal using a subset of items from the Mississippi Scale for Combat-Related PTSD (Keane et al., 1988), which was included as part of each veteran's psychological evaluation during his week-long participation in this study. Seven items that were most representative of hyperarousal were chosen by five doctoral-level clinicians. Internal consistency reliability for the seven-item subset was high (Cronbach's $\alpha = .88$). Only 5 of the 22 ill veterans (two from the Syndrome 1 group, two from Syndrome 2, and one from Syndrome 3), and none of the controls, were diagnosed with PTSD by psychiatrist's or psychologist's clinical interview using the Clinician-Administered PTSD

Scale (CAPS; Blake et al., 1995) and the Structured Clinical Interview for DSM-IV (SCID; First et al., 1996).

2.3. Stimuli

The visual stimuli consisted of 160 color photographs, 138 of which were taken from the International Affective Picture Set (IAPS). Sixteen images authenticated to be associated with the 1991 Persian Gulf War and six images of weapons were added to the ten weapon pictures taken from the IAPS, and these 32 images comprised the novel threatening distractor stimuli. Another 32 pictures of animals comprised the target stimuli. The remaining 96 images, which consisted of nature scenes, people, food, and objects, served as the nonthreatening nontarget stimuli. The ratio of target stimuli to threatening distractor stimuli to nontarget nonthreatening stimuli was 20:20:60, respectively.

The stimuli were presented on a color computer video monitor positioned approximately 1 meter in front of the subject. The picture stimuli subtended approximately 18° of visual angle. As shown in Figure 2, each picture stimulus was presented for 1 sec followed by a 1-sec fixation point, producing a 2-sec interstimulus interval. Each subject sat in a comfortable chair in a sound-proof booth and was told to keep his eyes focused on the center of the screen.

2.4. Procedures

After the participants were fitted with the electrode cap and, prior to the beginning of the task, they were shown and read the written instructions and were allowed to have their questions answered. The participants were instructed to press the response button under their right index finger for the picture stimuli that were of animals and to press the response button under their right middle finger for everything else. The response buttons interfaced with Stim² (Compumedics Neuroscan, Charlotte, NC, USA) software, which recorded the accuracy of the responses and their reaction times. A time-locked mark of each stimulus onset and response was recorded on the continuous EEG.

The subject was not informed that any of the stimuli would represent threatening circumstances. At the beginning of each task, the first image repeated the instructions they had learned prior to the beginning of the task.

2.5. EEG Acquisition

Ongoing EEG activity was recorded via a 64-electrode array mounted within an elastic cap that the participant wore during the task. Electrodes placed at the superior and inferior orbital margins monitored 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 was kept below 10 kΩ as measured before the beginning of the task.

The EEG was recorded using a Neuroscan Synamps2 (Compumedics Neuroscan, Charlotte, NC, USA) amplifier at a 1000-Hz sampling rate. The continuous EEG data were high-pass filtered at 0.15 Hz and re-referenced to the global mean amplitude. Blink artifacts were filtered from the continuous EEG file by using a spatial filter process in the Scan 4.4 Edit (Compumedics Neuroscan, Charlotte, NC, USA) software. Data from 200 ms before the onset to 1200 ms after the onset of each stimulus were included in each epoch. From each subject's task data, three conditions—target animals, nontarget threat-related distractors, and nontarget nonthreatening objects—were averaged. Each average consisted of epochs that had been baseline-corrected based on the 200-ms prestimulus data and low-pass filtered at 20 Hz.

2.6. Data Analysis

Each subject's averages were used to generate target, threatening distractor, and nonthreatening nontarget group ERP averages for Syndromes 1–3 and controls. Visual inspection of the ERPs from the responses to nonthreatening nontarget, threatening distractor, and target stimuli revealed a consistent anterior negative deflection around 300 ms, identified as the N300. A posterior positive deflection in the threatening distractor averages around 300 ms was identified as the P3a, and a posterior positive deflection occurring around 550 ms in the target averages was identified as the P3b component.

The N300, shown in Figure 3, is a monopolar component demonstrated to be sensitive to the emotional or arousal level of visual stimuli (Rossignol et al., 2005). Others have interpreted it to be associated with the degree of effort involved in integrating semantic information into a higher level of conceptual representation (McPherson and Holcomb, 1999). To ascertain the effect of the emotional nature of the threatening distractor, target, and nonthreatening nontarget stimuli on this component, the negative most point between 250 and 350 ms at anterior sites was recorded from each individual average. The most negative deflection was represented in electrode site FZ in the group averages and in most individual averages; thus, the most negative amplitude and its corresponding latency at FZ were chosen from each individual's ERP to be the best representative of the N300 component.

Due to the N300, a traditional anterior P3a could not be assessed. Thus the positive-most point occurring between 275 and 370 ms at posterior sites were recorded from each participant's threatening distractor ERP average. The posterior representation of the P3a has been shown to be part of the response to novelty that is less sensitive to habituation (see Friedman et al., 2001, for a review). The occipitoparietal midline electrode POZ showed the highest P3a peak amplitude in the group averages as well as in most individuals' averages (see Figure 4). Thus, peak amplitude and its corresponding latency at POZ were chosen from each individual's ERP to be the best representative of the P3a component.

A positive deflection occurring between 350 and 650 ms in the group ERP averages to target stimuli was identified as the target P3b. This component amplitude was maximal at electrode CPZ in the group averages (shown in Figure 5) and in most individual averages of the ERP response to target stimuli. Thus, the most positive point between 350 and 650 ms at electrode CPZ in each participant's average ERP was considered the best representative of the P3b response to target stimuli.

A hyperarousal score was recorded for each subject based on responses from a 7-item subset of items on from the Mississippi Scale for Combat-Related PTSD (Keane et al., 1988), which was included in the evaluation of each veteran who participated in the week-long study.

A one-way analysis of variance (ANOVA) was used to assess differences in hyperarousal among the four groups (control, Syndrome1, Syndrome2, Syndrome3; Haley et al., 1997a). Two 4×3 ANOVAs, where group was the between-subjects factor and condition (target, nontarget nonthreatening, nontarget threatening distractor) was the within-subjects factor, was computed to assess affects on N300 amplitude and latency. Two one-way ANOVAs where group was the between-subjects factor were computed on P3a amplitude to distractor stimuli and on P3a latency. Two similar ANOVAs were computed using P3b amplitude to target stimuli and P3b latency as dependent variables. Post hoc analyses were used to clarify the omnibus effects. Data regarding alcohol abuse and dependence, from the SCID, and data regarding medication use collected as part of the week-long multimodal study were used as covariates in post hoc analyses. To further our understanding of the relationship between

hyperarousal scores and the amplitudes of P3a and P3b, simple regression analyses were computed.

3. Results

3.1. Hyperarousal scores

An 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 (control, Syndrome1, Syndrome2, Syndrome3; Haley et al., 1997) was the between-subjects factor indicated a significant effect of Gulf War syndrome group on hyperarousal scores, omnibus test, $F(3, 26) = 11.802$, $P < 0.0001$, $\eta^2 = 0.5766$. Post hoc comparison showed that the control group's hyperarousal scores were significantly lower than each of the ill veteran groups, $P < 0.0005$. When the scores from the five veterans diagnosed with PTSD were removed from the analysis, both the omnibus effect ($P = 0.0001$) and the post hoc comparison ($P < 0.0001$) remained significant.

3.2. ERPs

3.2.1. P3a—A one-way ANOVA using Gulf War syndrome group (control, Syndrome1, Syndrome2, Syndrome3; Haley et al., 1997) as the between-subjects factor showed no effect of syndrome group on P3a amplitude, $F(3, 26) = .339$, $P = 0.7973$. See Figure 6. Removing data from the five veterans who had been diagnosed with PTSD from the analysis did not change the lack of effect, $F(3, 21) = .413$, $P = 0.7454$.

3.2.2. P3b—The later P3b to target stimuli was maximal at central parietal site CPZ. The P3b peak amplitude was defined as the most positive point between 350 and 650 ms in each participant's ERP average of responses to the target (animal) stimuli at central parietal midline electrode CPZ. A one-way analysis of variance with Gulf War syndrome group as the between-subjects factor showed a significant effect of syndrome group on P3b amplitude, $F(3, 26) = 5.282$, $P = 0.0056$, $\eta^2 = 0.3787$. Post hoc comparison showed that this was due to the P3b amplitude of the control group being significantly higher than that of the ill groups, $P = .0004$. The P3b component from each group is shown in Figure 7. When data from the five veterans who had been diagnosed with PTSD were removed from the analysis the effect of group was maintained ($F(3, 21) = 5.014$, $P = 0.0089$, $\eta^2 = 0.4174$), as was the significance of the post hoc test comparing the P3b of the control group to that of the ill groups ($P = 0.001$).

Although fewer control subjects than Syndrome group subjects endorsed alcohol abuse or dependence (measured with the SCID), alcohol abuse or dependence showed an expected distribution among all four groups, $\chi^2 = 3.702$, $p = .3$. Adding alcohol abuse or dependence as a covariable in the analysis reduced the effect size of group on the P3b amplitude but did not nullify its significance, $F(3, 22) = 3.683$, $MS_e = 5.474$, $p = .0274$, $\eta^2 = .334$.

Information regarding medication was collected from each of the 30 veterans. Number of medications for each participant was also recorded. These data included use of H2 receptor blockers, proton pump inhibitor, statins, other cholesterol-reducing medication, opiates, anti-convulsant pain medication, SSRIs, benzodiazepine, aspirin, NSAIDs, ACE inhibitors, beta blockers, diuretics, oral hypoglycemic medication, antihistamine, thyroid replacement, alpha blockers, and beta agonists (See Table 1). For SSRI use, Chi-square analysis showed that the distribution across groups was not even ($\chi^2 = 7.829$, $p = .0497$, Cramer's $V = .511$) due to almost half of the veterans in the Syndrome 2 group taking SSRIs. Only one other veteran, in the Syndrome 1 group, was taking an SSRI medication. Using SSRI use as a covariate did not change the significance of or the effect size of the effect of group on P3a or P3b.

amplitudes. An ANOVA using number of medications as the dependent variable showed a trend toward the number of medications taken by veterans in the Syndrome 2 group being significantly higher than that of the other three groups, $F(3, 26) = 2.466$, $MS_e = 13.194$, $p = .0846$, $\eta^2 = .22$. However, when number of medications was added as a covariate, the effect of group on P3b amplitude remained significant, $F(3, 22) = 4.480$, $MS_e = 4.183$, $p = .0134$, $\eta^2 = .323$.

Hyperarousal scores did not reliably predict the amplitudes of the P3a response to threatening stimuli ($\beta = -0.157$, $P = 0.409$), whereas they did reliably predict and show a considerable amount of shared variance with the amplitude of the P3b response to target stimuli, $\beta = -0.506$, $P = 0.004$, $R^2 = 0.628$. This regression maintained its reliability when the data from the five veterans who had been diagnosed with PTSD were removed from the analysis, $\beta[g58] = -0.598$, $P = 0.002$, $R^2 = 0.358$. Higher hyperarousal scores predicted more attenuated P3b amplitudes. A regression analysis using only the hyperarousal scores and P3b amplitudes from the five veterans diagnosed with PTSD revealed a positive slope, where higher hyperarousal scores were associated with higher P3b amplitudes, $\beta = 0.492$, $P = 0.40$.

The latencies of neither P3a nor P3b differed significantly by Gulf War syndrome group, $P > 0.27$.

3.2.3. N300—The image-specific N300 component has been shown to reflect the degree of effort involved in integrating semantic information into a higher level of conceptual representation (McPherson and Holcomb, 1999). As each of the stimuli the participant encountered was a *new* stimulus, this component showed neither a main effect of condition or group nor an interaction, $P > 0.38$.

3.3. Behavioral data

Percent correct and reaction times were used as dependent variables in two separate ANOVAs where GWI syndrome group was the between-subjects factor and condition was the within-subjects factor. There was an effect of condition on reaction times, $F(2, 52) = 4.809$, $P = 0.0121$, $\eta^2 = 0.0161$. Mean reaction time to threatening stimuli was significantly longer than reaction time to nontarget nonthreatening stimuli, $P = 0.0038$. Reaction time to neither threatening distractor stimuli nor nontarget stimuli differed significantly from reaction time to target stimuli. There were no effects of GWI syndrome group and no interaction on reaction time, $P > 0.30$. Percent correct showed no effects of condition or of GWI group, and no interaction, $P > 0.29$.

4. Discussion

This study found that ill Gulf War veterans, whose hyperarousal scores were significantly higher than those of the matched controls, exhibit ERP profiles more indicative of cholinergic, basal ganglia, and dopaminergic dysfunction than of anxiety disorders such as PTSD. That is, the P3a amplitudes to Persian Gulf War photographs were not higher in the groups reporting high hyperarousal, nor were their P3b amplitudes to target stimuli similar to or higher than those of the controls, as would be expected if these participants were similar to patients with PTSD who have been studied using event-related potentials (Karl et al., 2006). Rather, the subjects reporting high hyperarousal exhibited similar P3a amplitudes to threatening nontarget stimuli but reduced P3b amplitudes to target stimuli, profiles that are likely indicative of the cholinergic dysfunction and/or basal ganglia/dopaminergic dysfunction that have previously been reported as having resulted from neurotoxic exposures during their deployment to the Persian Gulf (Haley et al. 1999, 2000a, 2000b; Liu et al., 2011). The results of this study not only show toxin-related physiological differences that correlate with reported symptoms, but they provide an ERP profile that can aid in

distinguishing patients with Gulf War Illness, and can provide an objective marker for future treatment trials.

The contributions of ACh, DA, and basal ganglia to the generation and variance of the P3b response have been previously established. Administration of a cholinergic receptor agonist to human subjects has resulted in increased P3b amplitudes (Müntz et al., 1988), whereas decreased P3b amplitudes were observed in subjects who received a cholinergic antagonist (Hammond et al., 1987; Meador et al., 1989). Parikh et al. (2007) measured ACh release during rats' performance in attention-demanding tasks and found that cholinergic neurotransmission to cortex played important roles in the detection of relevant cues and thereby in the performance of attention-demanding tasks. In addition, administration of the cholinesterase inhibitor donepezil to human subjects was shown to enhance voluntary attention more than involuntary attention (Rokem et al., 2010) in visual spatial cuing tasks. This is consistent with the cholinergic system playing a greater role in the intentional allocation of attentional resources (P3b) than in the involuntary capture of attention (P3a). Adamec et al. (2008) found evidence of the read-through variant of acetylcholinesterase (AChE-R), which had been found to be overexpressed in mice that had been stressed with immobilization (Nijholt et al., 2004), playing a role in the hyperarousal resulting from predator stress in mice. Mice that were treated with EN101, which reduced the transcription of AChE-R, did not show the increased startle amplitude following their unprotected and inescapable exposure to a cat, whereas mice treated with a similar vehicle that had no effect on AChE-R showed the expected long-lasting increase in startle amplitude (Adamec et al., 2008). After receiving an infusion of the anticholinesterase physostigmine, human subjects showed enhanced activity in response to emotional faces in frontal areas in attended and unattended conditions (Bentley et al., 2003). Thus it is plausible that acetylcholine dysfunction evident in the GWI syndromes may be contributing to their lower P3b responses, their attentional difficulties, and their hyperarousal.

ACh is also involved in basal ganglia function (Calabresi et al., 2000; Bonsi et al., 2011) and in modulating dopaminergic activity (Exley and Cragg, 2008). P3b amplitude variation has been attributed to variation in DA activity in persons with Parkinson's disease (Li et al., 2003) and attention-deficit/hyperactive disorder (López et al., 2004). Recordings obtained from electrodes implanted in basal ganglia (Rektor et al., 2003) have revealed a basal ganglia contribution to the generation of the P3b. Dopamine and basal ganglia are also implicated in hyperarousal. De la Mora et al. (2010) suggested that activation of dopamine receptor sites in amygdala are responsible for releasing the amygdala from the inhibitory input from the medial prefrontal cortex (PFC), thus enabling preparation for dealing with real or potential threat. Amygdala D1 receptors principally facilitate retrieval of affective associations of a stimulus, whereas D2 receptors mediate brainstem-based reflex responses and the establishment of adaptive coping responses to threatening stimuli. The subthalamic nucleus of the basal ganglia plays a role in emotional regulation (Volkman et al., 2010; Greenhouse et al., 2011). The volume of the inferior frontal cortex, specifically the ventromedial PFC, covaries with emotional regulation (Welborn et al., 2009); the inferior frontal cortex is the initiator of the fronto-striatal inhibitory loop described by Aron et al. (2007), which includes the subthalamic nucleus. Although we found no effect of GW syndrome group on the response to emotional stimuli (P3a), a dysfunction in this inhibitory circuit could be contributing both to the hyperarousal and to the attentional and concentration problems reported by many GW veterans (Haley et al., 1997a; Ford et al., 2001). The ill veterans of this study having significantly reduced amplitude of the P3b, which is purported to indicate the volitional allocation of attentional resources, is consistent with their reports of attention and concentration difficulties (Haley et al., 1997a). Thus, basal ganglia damage or dysfunction of the dopamine system—secondary to basal ganglia damage, cholinergic system dysregulation, or both—very likely contributed to the

significantly attenuated P3b amplitudes and perhaps the hyperarousal observed among the ill veterans in this study.

Evidence has accrued to suggest that the dopaminergic system may play a principal role also in the generation of the P3a response to novel or distractor stimuli. Reduced P3a amplitudes have been observed among groups diagnosed with conditions marked by atypical dopaminergic systems (Sagvolden et al., 2005; Toda and Abi-Dargham, 2007; Galvan and Wichmann, 2008; Connor et al., 2009), such as schizophrenia (Merrin and Floyd, 1994), restless leg syndrome or Parkinson's disease (Poceta et al., 2006), attention-deficit/hyperactivity disorder (Kemner et al., 1996), and the *met/met* allelic variant of the catechol-O-methyltransferase (COMT) gene (Marco-Pallarés et al., 2010). The roles of the cholinergic system, however, seem to be more peripheral (Aloisi et al., 1997; Giovannini et al., 2001). A study using a Go-NoGo spatial cuing task found that nicotine had no effect on early sensory components but did enhance a frontally distributed positive deflection 300–400 ms post-stimulus in response to invalid cues (Meinke et al., 2006). The authors' interpretation of these findings, similar to that of the Rokem et al. (2010) study, was that the cholinergic system affects voluntary attention more than it affects involuntary attention.

However, the 300–400 ms response component examined in the Meinke et al. (2006) study is consistent with the NoGo P3 in ERP literature examining response inhibition (e.g., Eimer, 1993; Weisbrod et al., 2000; Maguire et al., 2009). We have previously shown that the anterior P3 to NoGo stimuli is attenuated in ill Gulf War veterans (Tillman et al., 2010). The present study, however, found no effect of Gulf War Illness syndrome group on P3a measures. Thus, while the interpretation of the findings from the Rokem et al. (2010) and Meinke et al. (2006) studies regarding voluntary and involuntary attention would also apply to our findings, we must acknowledge the caveats that must be applied to our particular P3a measure.

Polich (2007) evaluated the components that have been identified as P3a, novelty P3, and NoGo P3 and concluded that the three components are most likely variations of the same component. In the present study's visual paradigm, the more widely used frontal P3a could not be measured due to a strong and pervasive frontal N300. The P3a component for this study was measured from the midline occipitoparietal electrode POZ, the posterior aspect of the P3a component. ERP studies using auditory stimuli have indicated that the anterior and posterior aspects of the P3a are modulated to greater or lesser degrees by sleep quality, stimulus repetition and familiarity, attention, stimulus physical characteristic, and task relevance, which is attributed to the observations that the this component has multiple generators (Friedman et al., 2001; Bledowski et al., 2004; Volpe et al., 2007). Salmi et al. (2005) found that in healthy subjects the amplitude of the auditory P3a parietal aspect showed correlations with sleep efficiency, sleep onset latency, and percentage of sleep that were stronger than correlations between those sleep measures and anterior P3a amplitudes. Sleep disturbances are widely reported among GW veterans, and are included among the symptoms that may represent all GWI syndromes (Haley et al., 2001); thus, poor sleep may have contributed to the variance in the P3a amplitude of the ill veterans at POZ to a greater degree than it would have had we been able to assess the P3a at anterior sites.

In auditory tasks, the amplitude of the posterior aspect of the P3a, when compared to the anterior aspect, is less attenuated by the repetition of familiar sounds but is increased more in response to the repetition of unfamiliar stimuli (Cycowicz and Friedman, 1998). Additionally, Friedman et al. (1998) found that the greater habituation-driven attenuation of the anterior aspect with respect to the posterior aspect was present only when attention was engaged. In a subsequent study, Gaeta et al. (2003) determined that the anterior aspect of the P3a component was more sensitive to the contextual salience of the physical characteristics

of auditory stimuli whereas the posterior aspect was more sensitive to task category, clarifying the importance of task-relevance to the variance in the posterior aspect. Thus, the P3a data from this study allow us to conclude only that both the symptomatic and the nonsymptomatic GW veterans were similarly engaged in the task-relevance assessments of the threatening stimuli, or that we may not have adequately measured hyperarousal using these visual stimuli, as such reported responses may be more often triggered by other than GW-related images, or by smells, sounds, or social cues. We were unable to assess the frontal aspect of the P3a, which could have furthered our understanding of observations of fronto-striatal deficits in this cohort (Tillman et al., 2010).

White matter integrity has also been implicated in symptom complaints of Gulf War veterans and is a source of P3b variability. White matter volume reduction has been shown to be significantly correlated with the amount of sarin exposure that Gulf War veterans received (Heaton et al., 2007). In their study using data from both MRI and EEGs recorded during a visual three-condition oddball task, Cardenas et al. (2005) found that, relative to P3a, P3b variance was related more to white matter volume than to gray matter factors, implying that the connections between generators influence the latency variability of the P3b more the generators themselves. The degree of white matter damage in TBI sufferers has been found to be closely associated with hyperarousal (Rapoport et al., 2002). While white matter damage has been suggested in patients with GWI (Heaton et al., 2007) and can affect the P3b response, it typically affects P3b latency (Docree and Robertson, 2011), which is not significantly different in the syndrome groups in this study. Thus, while we cannot discount that white matter pathology secondarily could play a role in the reduced P3b responses reported here, it appears less likely than the dopamine-acetylcholine etiologies.

A reduced P3b has been observed in many ERP studies. Whereas P3b amplitude has been observed to increase as more processing resources are dedicated to a task when memory load increases, P3b amplitude was found to decrease when processing resources were shared by a secondary task (Kramer and Strayer, 1988; Watter et al., 2001). For example, Watter et al. found that the P3b amplitude of subjects when they were required to remember the stimulus that occurred three trials prior (*3-back* condition) was significantly lower than the P3b amplitude during the *1-back* condition; yet, the latency remained consistent across conditions. This may apply to our results as well. The P3b amplitudes of the ill veterans were significantly lower, but P3b latencies were not different from those of controls. It could be argued that, relative to controls, the ill veterans found much of the stimuli in the task more distracting, and required that the processing of the primary task (responding differently to pictures of animals) be reduced by the portion of processing resources that had to be dedicated to inhibiting the distraction from the non-animal pictures. In a similar vein, automatic processing of some aspects of the task could have come more easily to the control group than to the ill veterans (Kramer and Strayer, 1988). A reduced P3b has also been observed in studies of schizophrenia (Devrim-Üçok et al., 2006) and multiple systems atrophy (Kamitani et al., 2002). However, these diagnoses were not present in the subject groups of the current study. A reduced P3b has also been observed among treated alcoholics and those with a genetic predisposition toward alcoholism (Porjesz et al., 1998), but our analysis found no effect of use or abuse of alcohol on the P3b amplitude in these groups.

Other possible contributors to the reduced P3b seen in the ill veterans include depression and sleep quality. Depression has been identified as a major symptom in the clusters identified by both Haley and colleagues (Haley et al., 1997b, 2001) and Fukuda et al. (1998). There was a significant difference between controls and ill veterans in depression as assessed by SCID ($\chi^2=17.632$, $p=.0005$, Cramer's $V=.767$) in that only two of the veterans in Syndromes 1–3 were not identified as depressed and only one of the veterans in the control group was identified as depressed, a disparity that precluded using depression

diagnosis as a factor in the analyses. Studies examining the amplitude and latency of P3 components show conflicting results, which have been attributed to task paradigm and difficulty and to differing ERP profiles for differing types of depression (see Bruder et al., 2012). Both P3 components tend to be attenuated in depressed patients and tend to occur at longer latencies in melancholic and bipolar depression. In addition, hyperarousal and poor sleep quality, which impact ERP amplitudes (Salmi et al., 2005; Trujillo et al., 2009) and are widely reported among GW veterans (Haley et al., 2001; Thompson et al., 2004), are closely associated with depression (Riemann and Voderholzer, 2003). Only the target P3b amplitude in the veterans meeting criteria for Gulf War Illness in the current study was attenuated and neither component's latency was significantly different from that of the controls. Assessing whether depression contributed to the reduction in target P3b amplitude, toxic exposure contributed to the depression and P3b amplitude reduction, or both was beyond the scope of this study. Disentangling the relationships among hyperarousal, depression, sleep quality, toxic exposure, and ERP profiles will require further study.

In summary, ill GW veterans reported hyperarousal rates that were significantly higher than those reported by matched controls. Yet, only five of the ill veterans had been diagnosed with PTSD using a structured clinical interview. Hyperarousal was not paralleled by overly robust responses to Gulf War-related images (P3a), as would be expected in persons with war-induced PTSD. Rather, hyperarousal scores were inversely related to the amplitudes of their responses to target stimuli (P3b). Whereas previous studies of PTSD have indicated higher P3b amplitudes in individuals when the target stimuli is accompanied by trauma-related nontarget stimuli, ill veterans in this study showed P3b amplitudes that were significantly lower than those of the control group. This pattern is consistent with previous findings of dysfunction in white matter and basal ganglia, and in cholinergic and dopaminergic neurotransmitter systems in GW veterans. Each of these plausible neurobiologic disruptions can be linked to neurotoxic effects of exposure to specific agents during GW deployment. Since all but two of the ill veterans and only one of the controls were identified as depressed, we could not ascertain to what degree the pervasive comorbidity of depression in Gulf War Illness is making an independent contribution to the P3b amplitude attenuation or to what degree the neurotransmitter systems dysfunctions are contributing to the reduced P3b, hyperarousal, and/or depression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Gulf War veterans were classified as either controls, Syndrome 1, Syndrome 2, or Syndrome 3 based on Haley et al. (1997, 2000a) criteria, and underwent a week-long multi-modal neuropsychological, neuroimaging, and biomarker study, which included electroencephalography and measurement of hyperarousal using the Mississippi Scale for Combat-Related PTSD (Keane et al., 1988).

Schematic diagram of the three-condition oddball paradigm used. After instructions, stimuli were presented for 1 sec followed by a 1-sec fixation. Participants responded with a button push that was different for target stimuli (animals) than for nonthreatening nontarget stimuli and threatening nontarget distractor stimuli.

Figure 3.

A prevalent anterior N300 was present for all conditions, preventing the measure of an anterior P3a. There were no significant effects of group or condition on the N300 amplitude or latency.

Figure 4.
P3a response to threatening distractor stimuli

P3b for Three Gulf War Illness Groups and Controls (400-700 ms)

Figure 5.
P3b response to target stimuli

Figure 6.

P3a component to distractor threatening stimuli at midline occipitoparietal electrode site POZ for all four Gulf War Illness syndrome groups.

Figure 7. P3b component to target stimuli at midline centroparietal electrode site CPZ for all four Gulf War Illness syndrome groups.

Table 1

Demographic and Medication Data

	Control	Syndrome 1	Syndrome 2	Syndrome 3
<i>N</i>	8	7	9	6
Age (<i>M(SD)</i>)	61.6 (7.58)	53.8 (5.03)	62.6 (7.50)	53.6 (6.85)
Number of Medications (<i>M(SD)</i>)	3.5 (3.74)	3.57 (2.87)	7 (4.03)	2.33 (2.13)
SSRI *	0.0%	14.3%	44.4%	0.0%
H2 Receptor Blocker	37.5%	28.6%	88.9%	33.3%
Proton Pump Inhibitor	37.5%	14.3%	0.0%	0.0%
Statins	25.0%	57.1%	55.5%	0.0%
Other Cholesterol-Reducing Medication	12.5%	14.3%	22.2%	0.0%
Opiates	12.5%	0.0%	33.3%	16.7%
Anti-Convulsant Pain Medication	12.5%	0.0%	11.1%	0.0%
Benzodiazepine	0.0%	14.3%	11.1%	0.0%
Aspirin	12.5%	28.6%	33.3%	0.0%
NSAIDs	12.5%	42.9%	33.3%	16.7%
ACE Inhibitors	12.5%	28.6%	22.2%	16.7%
Beta Blockers	25.0%	14.3%	33.3%	16.7%
Diuretics	12.5%	0.0%	33.3%	0.0%
Oral Hypoglycemic Medication	12.5%	14.3%	44.4%	0.0%
Antihistamines	0.0%	28.3%	11.1%	16.7%
Thyroid Replacement	25.0%	14.3%	11.1%	0.0%
Alpha Blockers	12.5%	14.3%	33.3%	33.3%
Beta Agonists	0.0%	0.0%	22.2%	0.0%
Smoking	0.0%	14.3%	11.1%	50%
Alcohol Abuse or Dependence	12.5%	42.9%	55.6%	50%

* indicates a significant difference among the groups.