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Baseline psychophysiological and cortisol reactivity as a predictor of PTSD treatment outcome in virtual reality exposure therapy

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Abstract

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Baseline cue-dependent physiological reactivity may serve as an objective measure of posttraumatic stress disorder (PTSD) symptoms. Additionally, prior animal model and psychological studies would suggest that subjects with greatest symptoms at baseline may have the greatest violation of expectancy to danger when undergoing exposure based psychotherapy; thus treatment approaches which enhanced the learning under these conditions would be optimal for those with maximal baseline cue-dependent reactivity. However methods to study this hypothesis objectively are lacking. Virtual reality (VR) methodologies have been successfully employed as an enhanced form of imaginal prolonged exposure therapy for the treatment of PTSD.

Our goal was to examine the predictive nature of initial psychophysiological (e.g., startle, skin conductance, heart rate) and stress hormone responses (e.g., cortisol) during presentation of VR-based combat-related stimuli on PTSD treatment outcome. Combat veterans with PTSD underwent 6 weeks of VR exposure therapy combined with either D-cycloserine (DCS), alprazolam (ALP), or placebo (PBO). In the DCS group, startle response to VR scenes prior to initiation of treatment accounted for 76% of the variance in CAPS change scores, $p < 0.001$, in that higher responses predicted greater changes in symptom severity over time. Additionally, baseline cortisol reactivity was inversely associated with treatment response in the ALP group, $p = 0.04$. We propose that baseline cue-activated physiological measures will be sensitive to predicting patients' level of response to exposure therapy, in particular in the presence of enhancement (e.g., DCS).

Keywords

Acoustic startle; Posttraumatic stress disorder; Virtual reality; Psychophysiology; Exposure therapy; Cortisol

1. Introduction

Clinical and empirical evidence strongly supports the use of prolonged exposure therapy as first-line intervention for reducing posttraumatic stress disorder (PTSD) symptom severity and for improving overall mental health (Bradley, Greene, Russ, Dutra, & Westen, 2005; DoD/VA PTSD Working Group, 2010; Institute of Medicine, 2007; Sherman, 1998; Steenkamp & Litz, 2013). According to the principles of Emotional Processing Theory (Foa & Kozak, 1986), prolonged exposure fosters a level of patient engagement that facilitates habituation to trauma-related cues and promotes the extinction of conditioned responses to fearful cues. Virtual reality methodologies have been successfully employed as an enhanced form of imaginal prolonged exposure therapy for the treatment of stressor-, trauma-, and anxiety-related disorders such as PTSD for more than a decade (Difede et al., 2007; Rizzo et al., 2010; Rothbaum, Hodges, Ready, Graap, & Alarcon, 2001). Virtual reality exposure (VRE) therapy is believed to foster engagement and elevate patient arousal (Robison-Andrew et al., 2014) through the inclusion of computer-generated simulations of trauma-related stimuli that span sensory modalities, including the ambient sights, sounds, smells, and tactile stimuli present during a traumatic event. In addition to its clinical impact, VR technology has also been used successfully to elicit robust fear reactions in patients with anxiety disorders (Diemer, Muhlberger, Pauli, & Zwanzger, 2014).

To date, the efficacy of prolonged exposure therapies, including those that are VR-based, has been largely indexed through the use of clinician-administered or self-report measures of patient symptom severity or within-session distress. More recently, psychophysiological measures have been employed as complementary assessment tools for use in traumatized populations presenting with PTSD symptoms (Costanzo et al., 2014; Griffin, Resick, & Galovski, 2012; Rabe, Dorfel, Zollner, Maercker, & Karl, 2006; Rhudy et al., 2010; Robison-Andrew et al., 2014; Rothbaum et al., 2014; Roy et al., 2013). The addition of psychophysiological indices, such as heart rate (HR), skin conductance (SC) and electromyography (EMG) responses to trauma-related cues can provide the potential prediction of treatment outcome, objective assessments of treatment outcome, and evidence of the underlying biological changes that accompany successful PTSD treatment.

Heightened psychophysiological reactivity in response to trauma cues has been observed in PTSD patients for several decades. More specifically, increased HR, SC, and startle EMG responses have repeatedly been found in chronic PTSD (Blanchard, Kolb, Gerardi, Ryan, & Pallmeyer, 1986; Blanchard, Kolb, Pallmeyer, & Gerardi, 1982; Buckley & Kaloupek, 2001; McTeague et al., 2010; Orr, Metzger, & Pitman, 2002), and have been a predictor of PTSD in acutely traumatized populations (Orr et al., 2012; Roy et al., 2013; Shalev et al., 2000). Pre- and post-treatment data suggest that exposure therapy reduces heart rate response to trauma related stimuli (Rabe et al., 2006). Similarly, EMG, HR, and SC in response to loud tones decreases after cognitive behavior therapy in those who show a positive treatment outcome (Griffin et al., 2012).

Exposure therapists have repeatedly shown that increased arousal is associated with treatment response. In fact, it is a core tenet of the theoretical underpinnings for exposure therapy in PTSD (Foa & Kozak, 1986). Many therapeutic resources and clinical supervision efforts are focused on better enabling clinicians to increase the emotional and physiological responses of clients/ patients early in the exposure therapy process (Foa, 2011). Although the theory is widely supported as evidenced by clinically significant effect sizes for exposure therapy in PTSD, investigations of the specific empirical links between psychophysiological indices of patient engagement in exposure therapy and positive treatment outcomes have only recently been initiated (Price et al., 2015). There is evidence from the literature supporting a link between biological reactivity pre-treatment with positive (Foa, Riggs, Massie, & Yarczower, 1995; Rauch et al., 2015) and negative (Yehuda et al., 2009) treatment outcomes.

More recent investigations have explored acoustic startle responses to trauma-related stimuli (termed trauma-potentiated startle) within the presentation of a virtual reality environment (Costanzo et al., 2014; Robison-Andrew et al., 2014; Roy et al., 2013). Robison-Andrew et al. (2014) reported that combat veterans who responded well to prolonged exposure therapy (defined as a >50% reduction in their CAPS score over the course of treatment) showed an initial increase in trauma-potentiated startle followed by a significant decrease in startle responses to trauma-related cues compared to veterans who did not respond to treatment. As in our aforementioned previous work, we examined participants' startle response to cues specifically drawn from their collective traumatic experiences. Whereas the trauma-relevant cues depicted in the VR environment may not represent the exact nature of the index trauma

experienced by the participant, it remains plausible that general combat-related stimuli produce robust arousal and psychophysiological reactivity in traumatized Veteran samples. While these physiological markers show promise as objective assessments of treatment efficacy, their predictive value has not been well studied.

Prolonged exposure therapy represents one of the most effective treatments for PTSD (group, 2010) and can be characterized as a clinical homolog of laboratory fear extinction learning (Rothbaum & Davis, 2003). D-cycloserine (DCS), an N-methyl-D-aspartate (NMDA) glutamate receptor partial agonist (Dravid et al., 2010), has improved the short- and long-term efficacy of exposure therapy when administered as a single dose just prior to treatment for several anxiety disorders (Guastella, Lovibond, Dadds, Mitchell, & Richardson, 2007; Kushner et al., 2007; Otto et al., 2010; Ressler et al., 2004; Rothbaum et al., 2014; Wilhelm et al., 2008). However, DCS has also been shown to be ineffective under specific conditions with some patient populations (e.g., cognitive behavioral therapy for social phobia Hofmann et al., 2013); for review see (Rodrigues et al., 2014). Nevertheless, acute administration of DCS has been shown to be effective at facilitating emotional learning in humans (e.g., behavioral exposure therapy Rothbaum & Davis, 2003), and fear extinction in rodent models of fear- and anxiety-based psychopathologies (e.g., Ledgerwood, Richardson, & Cranney, 2003, 2004, 2005; Richardson, Ledgerwood, & Cranney, 2004; Walker, Ressler, Lu, & Davis, 2002). Benzodiazepines, which increase GABA activity, have been used clinically to treat PTSD (Lund, Bernardy, Vaughan-Sarrazin, Alexander, & Friedman, 2013) but their effect on exposure therapies for PTSD remains largely unknown. There is evidence that the use of benzodiazepines is contraindicated for exposure therapy due to the potential to reduce engagement and arousal resulting in a lack of improvement, and, in turn, increase the likelihood of patient dropout (van Minnen, Arntz, & Keijsers, 2002; van Minnen, Harned, Zoellner, & Mills, 2012).

In addition to psychophysiological responses, alterations in stress hormones have long been a target of investigation in PTSD (Heim et al., 2000). However, findings on baseline cortisol levels have been mixed, and a recent meta-analysis based on a large number of patients and controls concluded that there are no consistent differences between PTSD and controls (Meewisse, Reitsma, De Vries, Gersons, & Olf, 2007). A much more promising approach is measuring cortisol reactivity to a stress challenge. As part of the hypothalamic-pituitary-adrenal axis (HPA)-mediated stress hormone cascade, glucocorticoid receptors (GR) in the hypothalamus regulate cortisol release and higher GR sensitivity has been proposed as a consistent alteration in PTSD (Yehuda, 2009). To our knowledge, few studies have conducted a randomized clinical trial for PTSD with cortisol reactivity as an outcome measure. For example, one study examined treatment effects on cortisol response to a cognitive challenge and found that 12-month open label SSRI treatment reduced reactivity in women with PTSD (Vermetten et al., 2006).

In addition to the above background, it is hypothesized that subjects with the greatest within-session extinction will respond most robustly to D-cycloserine enhancement of exposure therapy (Guastella, Lovibond, Dadds, Mitchell, & Richardson, 2007; Hofmann et al., 2006; Ressler et al., 2004). How might we predict, prior to treatment, which subjects may have the most robust within-session extinction learning? Animal models have begun to suggest that

prediction error may be required to explain neural plasticity in extinction learning (Delamater & Westbrook, 2014). Historically, learning theory suggests that extinction is enhanced by increased attentional salience of the conditional stimulus (Mackintosh, 1965, 1975; Pearce & Hall, 1980). Furthermore, a number of human studies have shown that greater expectancy violation may be among the most important variables involved in exposure therapy (reviewed in Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014). This seems to occur across learning paradigms including auditory attentional processing (Vachon, Hughes, & Jones, 2012), contextual learning (Ferdinand, Mecklinger, & Opitz, 2015), and exposure therapy for social phobia (Price, Mehta, Tone, & Anderson, 2011). Thus, we hypothesized that subjects with the highest baseline levels of cue-dependent biological reactivity would have the greatest likelihood for expectancy violation with VR-based exposure therapy, and thus those with the most robust DCS response.

The purpose of the present study was to examine the predictive nature of psychophysiological and cortisol responses during presentation of combat-related stimuli in a VR environment on PTSD treatment outcome in a population of combat veterans from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF). We examined both immediate treatment outcomes and sustained treatment effects at 6 months in patients receiving VRE therapy in combination with DCS, a benzodiazepine (alprazolam; ALP), or placebo (PBO). The neural processes underlying mammalian fear extinction learning and human emotional learning through exposure therapy appear to use similar molecular and cellular mechanisms (e.g., glutamatergic NMDA receptor systems; (Davis & Myers, 2002; Davis, Ressler, Rothbaum, & Richardson, 2006). Ongoing translational work from our laboratories has shown that psychophysiological reactivity serves as a reliable, objective measure of learned (or extinguished) fear. This work coupled with more recent findings suggest the use of psychophysiological reactivity as an index of emotional engagement. As such, we hypothesized that psychophysiological reactivity to trauma reminders, would be predictive of enhanced extinction and thus better treatment outcomes in the DCS group, but not in the other treatment groups. Our hypothesized effects also consider the potential for benzodiazepines to reduce arousal levels (see van Minnen et al., 2002) and the intentional “under-dosing” of prolonged exposure to minimize floor effects (ie., mask the enhancing effects of DCS). All groups received only 6 sessions of exposure therapy (as opposed to a full regimen of 10–15 sessions, (Foa, Hembree, & Rothbaum, 2007).

2. Materials and methods

The results presented here were collected as part of a large-scale investigation of the treatment efficacy of DCS, ALP, or PBO administered in combination with VRE therapy in a population of combat veterans from OIF/OEF (Rothbaum et al., 2014). The parent study was a double-blind, placebo-controlled study that included a baseline screening assessment, six VRE treatment visits, and follow-up assessments at 3, 6, and 12 months post-treatment. Participants were randomly assigned using a 1:1:1 ratio to one of three conditions: VRE plus 50 mg DCS, VRE plus 0.25 mg ALP, or VRE plus PBO. Study staff members and patients were blind to treatment group.

2.1. Participants

Participants reviewed and signed an informed consent form approved by the Emory University Institutional Review Board and the Atlanta VAMC Research and Development Committee. Participants enrolled in this study met criteria for PTSD per DSM-IV criteria with index trauma(s) related to combat experience in Iraq and/or Afghanistan. Exclusion criteria included the following: lifetime history of psychosis, bipolar disorder, current risk of suicide, current alcohol or drug dependence, pregnancy, and the current use of psychotropic or endocrine treatments that could confound the study data (e.g., benzodiazepines, opioids, glucocorticoids). Veterans with mild traumatic brain injury were enrolled in the study. Due to the inclusion of a benzodiazepine as a psychiatric drug control condition, participants were required to discontinue long-acting benzodiazepines for 1 month and short-acting benzodiazepines for 2 weeks prior to the initial screening visit. Individuals taking psychotropic drugs that were not considered exclusionary were required to be on a stable dose for at least 2 weeks prior to study enrollment and to maintain a stable dose throughout the course of their participation. The current study included 50 participants who had pre-treatment psychophysiological data, completed 6 weeks of treatment and a 6-month follow-up visit. The mean age of the study population was 36.14 (range 23–55 years of age) and the sample was 94% male and 6% female. Per participant self-report, the race/ethnicity distribution was 38% Caucasian/non Hispanic, 52% African American/non Hispanic, 6% Hispanic, 2% Asian, and 2% other. Of the 50 patients who had complete data, 13 were in the DCS group, 20 were in the ALP group, and 17 were in the PBO group. Table 1 shows the demographic data, trauma history and pre-treatment symptom severity for the three groups. There were no differences between the groups in any measures.

2.2. Treatment description

Six sessions of treatment were included in the current study. The first session was a 90-min introductory period during which time the implementation and rationale for the virtual reality treatment regimen was discussed. This initial session was followed by five weekly, 90-min sessions of VRE therapy provided by a doctoral level clinician. Thirty minutes prior to each VRE session, the patient completed questionnaires and took a study pill under supervision of a study staff member. Following the guidelines for traditional, standardized exposure therapy, patients were encouraged to focus on their most distressing traumatic memories during the VRE sessions (Foa et al., 2007). During VRE treatment, the therapist viewed and controlled the VR environment via a computer monitor while the patient experienced the VR environment through an integrated VR system that included auditory, visual, olfactory, and tactile stimulation. The therapist presented stimuli that closely matched what was being described by the patient. Continued exposure to trauma-related stimuli was encouraged until within-session anxiety diminished (approximately 30–45 min). Anxiety ratings were collected every 5 min through administration of a Subjective Units of Discomfort scale (SUDs; 0 = no anxiety, 100 = high anxiety). The VRE session concluded with a 15–20 min period for processing the experience that included the active integration of newly acquired associations (often termed extinction learning in laboratory models; Rothbaum & Davis, 2003).

Treatment was administered according to previous published work (Rothbaum et al., 2014). For VRE treatment sessions, patients viewed the visual virtual imagery through a head-mounted display (eMagine Z800, eMagine Corp., Bellevue, WA) that was coupled to a head tracker and stereo earphones. The virtual Iraq and Afghanistan environments changed in a naturalistic way in response to head and body movement. The participant used a handheld video game style controller to navigate through each environment. Environments included either driving a Humvee on a desert road alone or in a convoy or walking on foot through a Middle Eastern city similar to those experienced in Iraq and Afghanistan. As mentioned previously, trigger stimuli were of four modalities: auditory (e.g., weapons fire, explosions), visual (e.g., smoke, time of day), olfactory (e.g., spices, burning rubber), and tactile (vehicular vibrations, nearby explosions).

2.3. Clinical assessments

All assessments occurred when the participants were free of study medication. The initial study visit was a pre-treatment baseline screening assessment that included administration of the Clinician Administered PTSD Scale (CAPS; Blake et al., 1995) to determine PTSD symptom severity. This assessment was completed by a master's level clinician who focused on the most traumatic combat experience as identified by the patient. The presence of comorbid Axis I disorders was determined using the Mini International Neuropsychiatric Interview (MINI; Sheehan, Lecrubier, & Sheehan, 1998). Complementary self-report measures were also administered including the PTSD Symptom Scale (PSS; Foa, Riggs, Dancu, & Rothbaum, 1993). Inter-rater reliability was measured via videotaping of assessment interviews with ten percent of taped CAPS and MINI interviews randomly selected for evaluation of reliability. This subset of diagnostic interviews was reviewed by a master's-level clinician and the agreement for the primary diagnosis across assessments was 100%. Trauma history was assessed using the Childhood Trauma Questionnaire (CTQ, Bernstein and Fink) and the combat exposure subscale of the Deployment Risk and Resilience Inventory (DRRI).

2.3.1. Psychophysiological assessment—Psychophysiological assessments were completed after the interviews and at pre-, and post-treatment, and at 6 months follow-up visits. Psychophysiological responses were acquired using the Biopac MP150 data acquisition system (Biopac Systems, Inc., Goleta, CA). Heart rate was based on R-peaks detected from electrocardiogram (ECG) recordings from two 5-mm Ag/AgCl electrodes placed on the upper right torso and left wrist. Skin conductance was measured using the galvanic skin response (GSR) module of Biopac, with two 5-mm Ag/AgCl electrodes placed on the middle fingers of the non-dominant hand. Startle responses were measured through EMG activity of the *orbicularis oculi* muscle using two 5-mm Ag/AgCl electrodes. Similar to our previous reports (Norrholm et al., 2011), one electrode was placed 1 cm below the pupil and another placed 1 cm below the lateral canthus of the right eye. EMG startle response was assessed in response to an acoustic white noise burst of 40 ms at 108 dB. ECG, SC and EMG responses were sampled at 1000 Hz, amplified, digitized and filtered using Biopac. The data were exported for further analysis using Mindware software (Mindware Technologies, Gahanna, OH). The EMG signal was acquired with a gain of 5000, and bandpass filtered at 28 and 500 Hz, according to guidelines for human startle measurement

(Blumenthal et al., 2005). Peak startle amplitude within 20–200 ms after acoustic probe onset was used as the startle response measurement.

During assessments, participants viewed the standardized VR clips using an eMagin Z800 head mounted display and audio stimuli were presented binaurally with headphones. Stimulus presentation occurred through the use of SuperLab 4.0 for Windows (Cedrus Corp., San Pedro, CA). Unlike during the VR treatment sessions, participants were not able to navigate in the VR scenes or tailor them to their own specific experiences. This was done in order for all participants to view the same stimuli with the same duration during assessment. The scenes that were used for assessment were those most commonly experienced by OIF and OEF soldiers. The psychophysiology assessment sessions consisted of three 2-min VR scenes (Roy et al., 2010) separated by presentation of a 30-sec blank blue screen (Blank Screen), see Fig. 1A. Two scenes were presented from the perspective of a soldier in either a gun turret (Turret) or Humvee cabin (Cabin) as their convoy encounters improvised explosive devices (IEDs), gunfire, and insurgent ambushes. The third scene (City) was shown from the perspective of a soldier on foot patrol walking through an Iraqi city marketplace who encounters explosions, civilians, and rocket propelled grenades. The blank screen and VR scenes were not counterbalanced, but alternated through the session. Startle probes were presented at times in the VR scenes when ambient noise was absent or minimal. Two startle probes were presented prior to the onset of VR scenes, and during each blank screen. Five startle probes were presented during each VR scenes, during periods of low level background sounds. A total of 25 startle probes was delivered. The time between startle probes was between 9 and 22 s as in our previous work (Norrholm et al., 2006).

2.3.2. Cortisol assays—Saliva for cortisol was collected using Salivette collection system. When possible, sampling occurred between 11:00a.m. and 2:00 PM at each visit. In four cases cortisol was collected between 9:30a.m. and 10:30a.m., and in four cases samples were collected between 3:00p.m. and 4:00p.m.. There were no difference in sampling times between groups, and collection time was not associated with cortisol levels ($p > 0.1$). Samples were collected at three points during each assessment visit. The first sample was collected immediately prior to viewing the Virtual Iraq scenes, immediately after the 15-min VR assessment, and 15 min after the end of the scenes. The samples were immediately transferred to a -80° freezer. Saliva cortisol was measured by a chemiluminescent immunoassay available on the Beckman Access analyzer. Prior to assay, the saliva was thawed and centrifuged. The assay was calibrated against the European GC/MS reference method. All samples were run in duplicate and three levels of quality control were processed in every assay run. The detection limit for the salivary assay was 0.1 nmol/L. The inter-assay and the intra-assay CVs were under 10%.

2.4. Data analysis

Startle response was measured as the peak EMG amplitude 20–200 ms following the onset of the acoustic startle probe. The ECG data were analyzed as average HR in beats per minute. Electrodermal activity analyses captured skin conductance levels. Startle, HR, and SC data were averaged across the three VR scenes and compared to Blank screens with a

repeated measures analysis of variance (RM-ANOVA) with the factor of Stimulus type (VR vs. Blank screen) in order to see if exposure to VR combat scenes increased responding.

In order to test whether psychophysiological reactivity changed with treatment, we compared startle, HR, and SC at 3 time points (pre-, post-treatment and at 6 months) using a RM-ANOVA. In addition, the psychophysiological reactivity to VR scenes at pre-treatment was used as predictors of change in PTSD symptoms at post-treatment and at 6 months follow-up assessments. Change in PTSD symptoms was calculated by subtracting the CAPS scores at post-treatment and 6 months from the pre-treatment scores. Linear regression analyses were used to analyze whether EMG startle, HR, or SC, data during VR scenes predicted change scores within each treatment group (DCS, ALP, PBO).

Cortisol levels were analyzed using RM-ANOVA testing the effects of the VR scenes across time points. Within-subjects variables included VR sample (3 levels: baseline, post-VR, and 15 min post-VR) and Time (3 levels: pre-treatment, post-treatment, and 6 months follow-up). Stepwise linear regression analyses were performed to examine the relationship between cortisol change score and CAPS change after controlling for baseline levels of cortisol and initial CAPS score. Cortisol levels and change scores were Z-score transformed to normalize the distribution of the data. All analyses were performed using SPSS 20.0 for Windows. Alpha level was set at 0.05, and the Greenhouse-Geisser correction was used for repeated measures analyses.

3. Results

3.1. Psychophysiological reactivity

For pre-treatment testing, a RM-ANOVA of VR scenes compared to the neutral blue screen revealed a significant increase in startle response, $F(1,49) = 10.64$, $p = 0.002$ and HR, $F(1,49) = 12.12$, $p = 0.001$, but not SC, $F(1,49) < 1$ during VR. Fig. 2A and B shows increased responses to startle and HR, and Fig. 2C shows SC. However, none of the measures were correlated with CAPS or PSS scores at pre-treatment.

Changes in reactivity across treatment conditions in this dataset have already been published elsewhere (Rothbaum et al., 2014); here we briefly show that these indices changed with treatment. Specifically, startle EMG responses to VR scenes decreased across time points, $F(2,62) = 6.76$, $p = 0.006$, with a significant linear association from pre-treatment to 6 months ($p = 0.007$). In addition there was a significant interaction of condition and time on startle responses, $F(4,62) = 3.88$, $p < 0.05$. Follow-up analyses within each treatment condition found that startle response during the VR scenes were reduced only in the DCS group, $F(2,12) = 6.24$, $p = 0.01$. Likewise, SC responses decreased after treatment, $F(2,70) = 5.13$, $p = 0.01$, with the quadratic pattern of association having higher significance ($p = 0.01$), in that SC responses did not continue to decrease from post-treatment to 6 months follow-up. In contrast, HR responses did not show any significant changes across treatment timepoints. The SC and HR measures did not show interaction effect of condition by time.

Next we examined whether startle, SC, or HR during VR scenes were predictive of the change in CAPS scores from pre to post-treatment using a linear regression within each

treatment group. We examined both immediate CAPS change (pre- to post-treatment) and sustained CAPS change (pre-treatment to 6 month follow-up). In the DCS group, startle and HR showed trends in predicting immediate CAPS change ($p = 0.08$ and $p = 0.06$, respectively), but SC did not. When CAPS change was examined as percent change from pre-treatment baseline, startle response was significantly predictive of change ($F(1,12) = 8.13$, $p < 0.05$), but HR and SC were not. None of the variables predicted change in the ALP and PBO groups.

However, we found a stronger prediction when we examined the change in CAPS scores from pre-treatment to 6 months follow-up. In the DCS group, startle response to VR scenes prior to treatment accounted for 76% of the variance in CAPS change scores, ($F(1,12) = 34.92$, $p < 0.001$), in that higher responses predicted greater changes in symptom severity (i.e. better outcome). The same was true when CAPS was analyzed as percent change from pre-treatment to 6 months follow-up ($F(1,12) = 18.80$, $p = 0.001$). Neither regression was significant for either the ALP or PBO groups (both p 's > 0.1). Fig. 3A shows the CAPS change score at 6 months follow-up and the pre-treatment startle response for DCS and PBO groups. Higher HR and SC during VR scenes also predicted better 6 months change scores ($R^2 = 0.47$, $F(1,12) = 6.46$, $p = 0.03$ and $R^2 = 0.50$, $F(1,12) = 9.82$, $p = 0.01$, respectively; see Fig. 3B and C). The results were partially replicated using percent change in CAPS as the dependent variable (HR $R^2 = 0.37$, $p = 0.06$ and SC $R^2 = 0.38$, $p < 0.05$, respectively). As was the case with startle, the ALP and PBO groups did not show significant effects. The regression analysis in the DCS group remained significant after controlling for initial CAPS scores at pre-treatment (startle, $R^2 = 0.69$, $p < 0.001$; HR, $R^2 = 0.43$, $p = 0.04$; and SC, $R^2 = 0.51$, $p = 0.01$).

3.2. Cortisol levels

Cortisol data were available for 45 individuals at all 3 assessment visits (pre-treatment, post-treatment, and 6 month follow-up). As with the psychophysiological variables, cortisol reactivity to VR scenes changed across treatment time points (RM ANOVA Time \times VR interaction, $F(4,152) = 2.43$, $p = 0.05$). The contrast term for VR samples indicated that the difference between cortisol collected at baseline and 15 min post-VR decreased from pre- to post-treatment ($F(1,38) = 5.23$, $p < 0.05$).

Next we examined whether cortisol levels prior to treatment predicted changes in PTSD symptoms immediately after treatment and at 6 months follow-up. Linear regression analyses of baseline cortisol levels revealed a trend association with treatment response, in that higher baseline cortisol predicted greater changes in CAPS scores immediately post treatment only in the ALP group ($F(1,16) = 4.14$, $p = 0.06$). The same trend was found using percent CAPS change ($F(1,16) = 3.86$, $p = 0.07$). Cortisol reactivity (the difference between cortisol 15 min after VR scenes and baseline cortisol) had an opposite prediction to treatment response, in that higher reactivity was significantly associated with worse outcome. In the ALP group, cortisol reactivity prior to treatment accounted for 27% of the variance in change in CAPS scores ($F(1,16) = 5.56$, $p = 0.03$, see Fig. 4), and in percent CAPS change ($F(1,16) = 6.64$, $p = 0.02$). This association remained significant after controlling for baseline cortisol levels ($R^2 = 36\%$, $p = 0.04$). In the DCS group, there was a

trend for the same prediction, but the sample size was smaller ($F(1,9) = 4.13, p = 0.08$). Neither baseline nor reactive levels of cortisol predicted treatment response in the PBO group. In addition, sustained changes in CAPS scores (pre-treatment to 6 month follow-up), were not predicted by cortisol levels.

4. Discussion

In the present study, startle responses to VR scenes prior to treatment accounted for 76% of the variance in PTSD symptom reduction from baseline to 6-month follow-up (as measured via change in CAPS score) in the group that received DCS just prior to VR exposure therapy. A prior publication reported that the patients receiving DCS displayed markedly greater changes in trauma-potentiated startle responses when assessed pre- and post-VR exposure treatment as compared to groups receiving ALP or PBO (Rothbaum et al., 2014). The present paper focuses on pre-treatment psychophysiological responses, including SC, HR in addition to startle EMG as predictors of clinical outcomes separately for each treatment condition. It has been suggested that increased engagement with exposure therapy can be predictive of better treatment outcome (Robison-Andrew et al., 2014). An objective measure of engagement may be one's psychophysiological response in the presence of trauma-related cues. DCS has shown effectiveness as an adjunct treatment in concert with prolonged exposure (with some notable exceptions and experimental caveats cited in the literature, e.g., Rodrigues et al., 2014). It is important to note that the current study "under-dosed" the exposure therapy, inasmuch as only 5 VR exposure sessions were used. Therefore, we expected minimal reductions in fear in the placebo group as compared to the group that received DCS; effects similar to those observed by Ressler et al. (2004) who compared "under-dosed" VR exposure therapy with and without adjunct DCS. The results of the present study suggest that a higher level of engagement coupled with the cognitive enhancing properties of DCS may lead to more substantial improvements in patient symptom severity.

There is currently a significant void in the literature with respect to objective biomarkers that are predictive of PTSD treatment efficacy. The results of the current study suggest that the inclusion of psychophysiological assessments prior to and over the course of treatment and follow-up may prove to be an invaluable tool for monitoring therapeutic progress. Our findings represent one of the first demonstrations of the predictive value of the acoustic startle response with respect to PTSD treatment outcome. There have been several investigations showing strong associations between acoustic startle-based assessments and PTSD symptom severity (e.g., Jovanovic et al., 2009; Norrholm et al., 2011) as well as studies illustrating the potential predictive nature of acoustic startle response and PTSD vulnerability in the aftermath of a traumatic experience (Pole et al., 2009). We now extend these earlier findings to show the potential clinical utility of assessing baseline and trauma-potentiated startle over the course of treatment.

This study also showed that VR exposure therapy attenuated cortisol response to a trauma-relevant scene. Attenuation of the cortisol response with PTSD treatment was also observed by Vermetten et al. (2006); however, the current study expanded this finding to a placebo-controlled RCT, and demonstrated the effect of treatment after 6 weeks, rather than 12

months as in the previous study. The results suggest that neuroendocrine biomarkers may serve as objective measures of treatment response.

Finally, higher cortisol reactivity prior to starting treatment was predictive of poorer treatment outcome. It is possible that individual variability in HPA axis response increases treatment resistance. Such individual variability may be due to genotype. For example, FKBP5 risk polymorphisms are associated with increased glucocorticoid sensitivity and higher PTSD symptoms (Binder et al., 2008). Alternatively, since the cortisol change score was derived by subtracting sample values collected 15 min after the end of the VR scenes from the pre-VR sample values, it is possible the individual differences are due to slower recovery of the cortisol response and more glucocorticoid resistance. While a limitation of the current study, future studies should include a later sample collection time to examine whether cortisol recovery is predictive of treatment outcome. It is important to note that baseline cortisol levels were associated with treatment response in the opposite direction from cortisol reactivity, i.e. higher baseline was associated with better outcome, further indicating that GR sensitivity may be a unique predictor relative to baseline HPA measures.

In addition, other hormones (such as DHEA) or hormone metabolites (such as allopregnanolone) may be interacting with the cortisol response to contribute to treatment resistance (Rasmusson et al., 2006). Interestingly, treatment drug did not impact the association between cortisol response and treatment outcome, suggesting that the individual neuroendocrine risk factors may not be sensitive to DCS interventions. Thus the DCS effect may be specific to factors that determine how stressful the perception of the traumatic VR scenes is but not the glucocorticoid response to that perception.

Interestingly, cortisol and psychophysiological reactivity to trauma-related stimuli (i.e. VR scenes) differed in their predictive value in three important ways: 1) they had opposite predictions in terms of direction, 2) they predicted different post-treatment timepoints, and 3) they were predictive in different drug treatment groups.

With regard to the first point, higher cortisol reactivity predicted poorer outcome, whereas greater psychophysiological reactivity predicted better outcome. Several studies have found that exposure therapy is more effective when patients are more engaged in the trauma memories (Jaycox, Foa, & Morral, 1998). Increased psychophysiological activity may be indicative of higher engagement with the stimuli and activation of the sympathetic nervous system (SNS). Given increases in the autonomic measures, SCL and HR, SNS activation seems highly likely. In addition, the startle reflex can be modulated by norepinephrine, which is part of the fight-or-flight SNS response to fear eliciting stimuli. On the other hand, cortisol reactivity is part of the HPA axis, and may be related to neuroendocrine coping mechanisms governed by negative feedback signaled by GR. It is possible that heightened GR sensitivity may be related to poor stress hormone regulation during traumatic stimulation rather than higher engagement with the VR environment.

The psychophysiological responses predicted better sustained treatment outcome at the 6 month follow-up visit in the DCS group, while cortisol reactivity data predicted immediate treatment outcome in the ALP group. DCS is known to enhance extinction (Davis et al.,

2006; Vervliet, 2008). Therefore, higher early engagement may facilitate learning during VR exposure therapy with better long-term gains. On the other hand, higher cortisol reactivity to stressors may have overwhelmed the beneficial effects of engagement in VR stimuli, and that the addition of benzodiazepines provided enough “numbing” to allow for better engagement. In any case, these effects appear to be temporary, as they were not associated with lower PTSD symptoms 6 months after treatment. Importantly, the drugs were given ONLY during individual treatment sessions and the assessments were performed free of study drugs; therefore there was no direct contribution to the predictors.

Better understanding of the potential augmentation of cognitive behavioral strategies and the evaluation of biological predictors of treatment outcome will emerge as studies move forward in this area. The current results, taken together with several reports from the literature (for review see Foa, Gillihan, & Bryant, 2013), suggest that increased arousal (often referred to as engagement) during early exposure therapy can foster more positive treatment outcomes. There is some evidence that decreased arousal *over the course of several weeks of treatment* can increase outcome (Telch et al., 2014). Future examination of the time course, psychophysiological index, and time since trauma will enable clinical investigators to focus on the most promising biological markers for exposure based treatment measures (see discussion by Tuerk, 2014).

The current analyses were part of a treatment study and not a psychophysiological study *per se*. There is a rather large body of literature, including sample sizes of varies size and demographics that support a strong association between psychophysiological reactivity and PTSD (e.g., for meta analysis see Pole et al., 2009). There continues to be a compelling rationale to include physiological measures in behavioral treatment outcome studies. Unfortunately, these studies can be costly and time-consuming. However, these measures can be interwoven into clinical studies as in the current study. Further reasoning for doing so rests in the observations that positive treatment outcomes can be assessed by reductions in objectively measured arousal (Lindauer et al., 2008). Lastly, several translational studies using these methods have included sample sizes of in this range and have been appropriately powered to detect significant differences (e.g., Norrholm et al., 2014).

In summary, this study suggests that psychophysiological responses may serve as biomarkers that predict treatment outcome. Specifically, these measures are likely to capture the patients’ level of engagement with the exposure therapy, which in turn predicts better treatment response.

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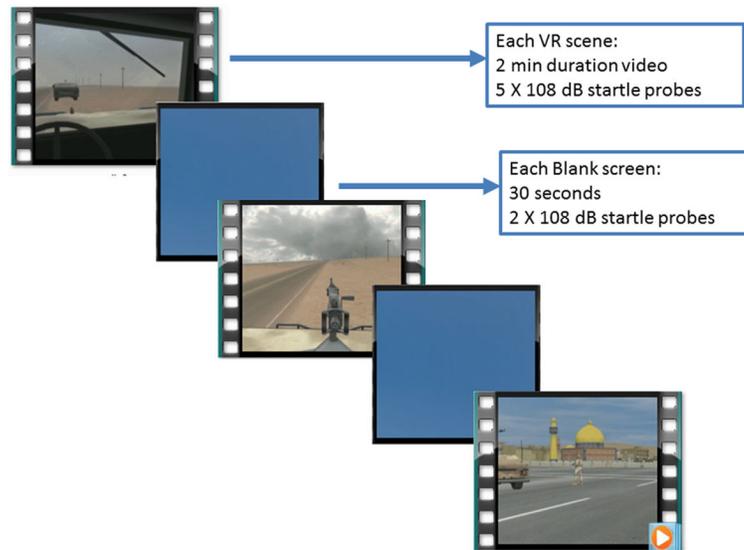


Fig. 1.
Panel A. Schematic illustration of the virtual reality (VR) session presented to assess baseline and trauma-potentiated acoustic startle responses. Three 2-min VR video clips were presented with 30-sec blank blue screen images interleaved between the VR clips. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

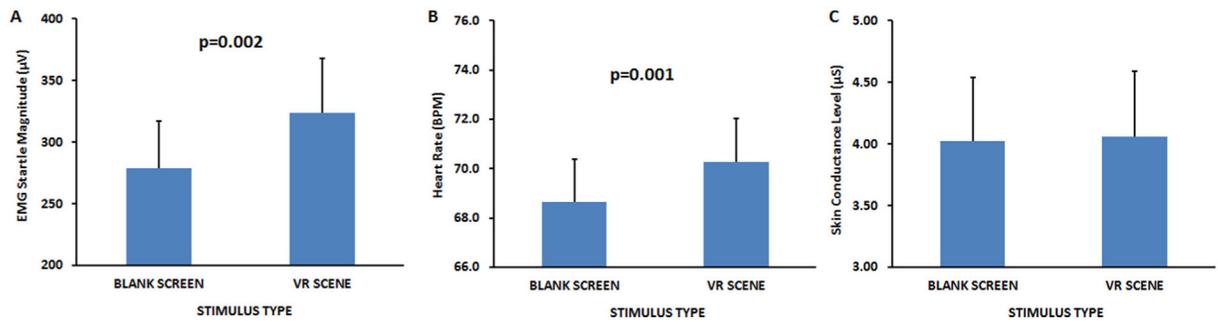


Fig. 2.

Acoustic startle, heart rate, and skin conductance responses (mean \pm SEM) assessed prior to initiation of virtual reality exposure (VRE) therapy in the presence of d-cyloserine (DCS), alprazolam (ALP), or placebo (PBO). Acoustic startle (panel A) and heart rate (panel B), but not skin conductance (panel C), responses were elevated in the presence of the combat-related virtual reality clips as compared to blank blue screen. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

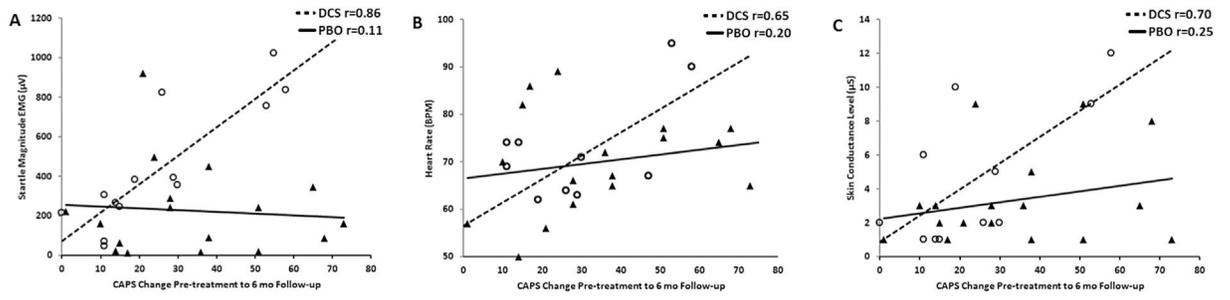


Fig. 3. Correlations between pre-treatment acoustic startle (panel A), heart rate (panel B), and skin conductance (panel C) responses and PTSD symptom change in the groups receiving DCS or PBO. Higher degrees of startle, heart rate, and skin conductance predicted better 6-month change scores in PTSD symptoms as measured by CAPS interview.

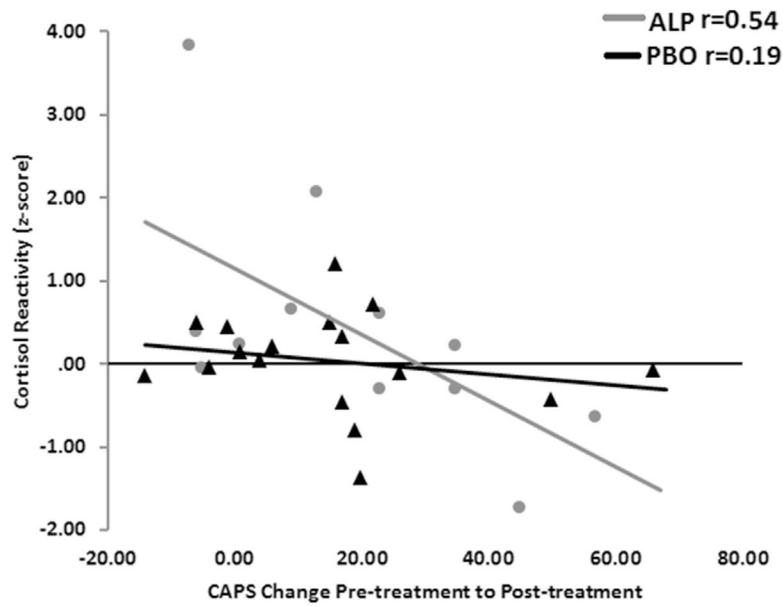


Fig. 4. Correlations between pre-treatment cortisol reactivity to VR scenes and PTSD symptom change in the groups receiving DCS or ALP. In the ALP group, higher cortisol reactivity was associated with worse treatment outcome when comparing PTSD symptoms at the pre- and post-treatment timepoints.

Table 1

Demographic, trauma history and pre-treatment PTSD symptom severity between the three treatment groups, d-cyloserine (DCS), alprazolam (ALP), and placebo (PBO). There were no differences in any variables. Abbreviations: CTQ = Childhood Trauma Questionnaire, DRRI = Deployment Risk and Resilience Inventory, CAPS = Clinician Administered PTSD Scale.

	DCS (n = 13)	ALP (n = 20)	PBO (n = 17)	
Demographics				
Sex (N, % male)	13 (100.0%)	19 (95.0%)	15 (88.2%)	p = 0.39
Age (M, SE)	36.15 (2.49)	37.5 (2.11)	34.73 (2.31)	p = 0.68
Childhood trauma				
CTQ (M, SE)	36.54 (3.50)	36.39 (2.98)	39.20 (3.26)	p = 0.79
Combat trauma				
DRRI (M, SE)	9.36 (0.89)	10.77 (0.75)	9.36 (0.83)	p = 0.36
PTSD symptoms				
CAPS (M, SE)	92.77 (5.56)	90.89 (4.72)	86.73 (5.17)	p = 0.71