Responses to Ambiguous Facial Expressions in Posttraumatic Stress Disorder

An Honors Thesis for the Department of Psychology

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Table of Contents

Abstract

Posttraumatic stress disorder (PTSD) is associated with increased arousal and exaggerated responses to potential threats. Research has used images of different facial expressions as stimuli to stimulate a threat response in participants. This study aims to determine whether individuals with PTSD perceive surprised faces as a potential threat. Surprise is an ambiguous emotion that can represent a positive or negative context. Previous studies have shown that individuals with PTSD show abnormal brain activation to fearful and angry faces compared to control participants, which suggests that PTSD is associated with a heightened threat response to emotional facial expressions. Therefore, we hypothesized that individuals with PTSD would have an exaggerated threat response to surprised facial expressions compared to a trauma-exposed non-PTSD (TENP) control group. Participants viewed surprised facial expressions, neutral facial expressions, and a fixation cross during an fMRI scan and then rated the arousal and valence of the facial expressions. There were no significant group differences in the surprise vs. neutral contrast, but there was significantly greater amygdala activation in the PTSD group compared to the TENP group in both the surprise vs. fixation and the neutral vs. fixation contrasts. Overall, our results showed that PTSD participants displayed increased amygdala activation to surprised faces and may interpret them as a potential threat. Our results also suggest that individuals with PTSD respond more negatively to neutral facial expressions than the TENP group and that neutral facial expressions may be interpreted as a potential threat in PTSD.

Responses to Ambiguous Facial Expressions in Posttraumatic Stress Disorder

Posttraumatic stress disorder (PTSD) is a psychiatric disorder that can result after a person experiences a life-threatening and/or traumatic event such as violent crime, abuse, or combat. Symptoms of PTSD may include intrusive and distressing recollections of the event(s), nightmares, avoiding reminders of the event, hypervigilance, difficulty sleeping, and an exaggerated startle response (American Psychiatric Association, 2013). Researchers have investigated how individuals with PTSD respond to potentially threatening stimuli in order to understand the effects of the disorder on perceptive and emotional processes in the brain. One example of potentially threatening stimuli is images of human faces displaying different emotions. Facial expressions are important social cues that provide information about the environment. Facial expressions like fear or anger are shown in response to a potential danger or negative situation and, therefore, can reflect potential threat.

Individuals with PTSD have shown abnormal responses to fearful faces (Rauch et al., 2000; Shin et al., 2005; Kim et al, 2008; Fonzo et al., 2010; Poljac, Montagne, & de Haan, 2011; Simmons et al, 2011; Shenk, Putnam, & Noll, 2013; Stevens et al., 2013; Almli et al., 2014). Specifically, a previous study found that veterans with PTSD were less able to accurately label fearful faces than trauma exposed non-PTSD participants, suggesting processes in the brain of fearful facial expression recognition, but not emotion recognition in general, are disrupted in PTSD (Poljac, et al., 2011).

Other studies have further investigated these abnormalities using brain imaging techniques. Functional magnetic resonance imaging (fMRI) studies have reported greater activation in the amygdala in PTSD relative to controls when viewing fearful vs happy faces

(Rauch et al., 2000; Shin et al., 2005; Fonzo et al, 2010; Simmons et al., 2011) and fearful vs. neutral faces (Stevens et al., 2013). Other abnormal brain responses to fearful faces include greater insula activation (Fonzo et al., 2010) and reduced medial prefrontal cortex activation to fearful vs. happy faces (Shin et al., 2005), as well as reduced rostral anterior cingulate cortex (rACC) activation to fearful vs. neutral faces (Kim et al., 2008). Other studies have found decreased functional connectivity between the amygdala and medial prefrontal cortex (mPFC) in PTSD participants, suggesting that PTSD involves deficits in top-down modulation of emotional responses to potential threats (Simmons et al., 2011, Stevens et al, 2013, Almli et al, 2014).

Other emotional facial expressions have also been shown to elicit abnormal responses in PTSD participants. Angry face stimuli have been used to represent threat; results show increased insula activation to angry vs. happy faces (Fonzo et al., 2010) and increased amplitude of N170 response to angry faces vs. neutral faces in PTSD participants (Houlihan et al., 2007). Overall, results of studies comparing responses of PTSD patients and healthy individuals to fearful and angry faces suggest that individuals with PTSD have abnormal brain responses to potential threats.

Despite the variety of studies on responses to facial expressions in PTSD, few studies have investigated responses to ambiguous facial expressions. Surprise is one ambiguous emotion that can occur in response to a positive novel stimulus, e.g., guests jumping out from behind a couch at a surprise party, or a negative novel stimulus, e.g., an attacker appearing around a corner. Indeed, Cheal and Rutherford (2013) determined that interpretation of surprised faces is context dependent, meaning a positive or negative context can influence how a surprised face is processed. As previous studies have shown that individuals with PTSD have greater brain activation and more negative behavioral responses to potential threats, assessing responses by PTSD patients to surprised faces could provide insight into how those with PTSD respond to ambiguous stimuli.

Despite this, only three previous studies have assessed responses to surprised faces in individuals with PTSD (Houlihan et al., 2007; Poljac et al, 2011; Shenk et al., 2013). Houlihan et al. (2007) measured responses to viewing fearful faces using EEG. Poljac et al. (2011) and Shenk et al. (2013) measured recognition accuracy and sensitivity to facial expressions by having participants view videos of models making facial expressions and then pause the videos as soon as they recognized the portrayed emotion. None of these studies found significant abnormalities pertaining to surprise in PTSD. However, it is likely that using imaging techniques along with behavioral measures would capture more nuanced reactions to surprised faces. This study aims to assess how individuals with PTSD and trauma-exposed individuals without PTSD respond to viewing surprised faces vs neutral faces using both behavioral and imaging techniques.

We based our study design on Kim, Somerville, Johnstone, Alexander, and Whalen (2003), which used fMRI to assess how healthy individuals respond to surprised faces. That study observed greater activation in the right amygdala and lower activation in the vmPFC to surprised faces compared to neutral faces. Furthermore, they observed significant correlations between activation in the amygdala and vmPFC and valence ratings of surprised faces, such that individuals who rated surprised faces with a negative valence showed increased amygdala activation and reduced vmPFC activation, and vice versa. The current study uses the general design of Kim et al. (2003) to compare the results of individuals with PTSD and trauma-exposed individuals without PTSD.

Based on the results of previous studies and the fact that surprised faces are ambiguous and can represent a potential threat, we predicted that participants with PTSD would respond to surprised faces with ratings of greater arousal and more negative valance than trauma-exposed participants without PTSD and be more likely than TENP participants to mislabel surprised facial expressions as fearful. We predicted that individuals with PTSD would have longer response than the TENP group times to surprised vs. neutral facial expressions during the rating task. Furthermore, we hypothesized based on Kim et al. (2003) that surprised faces would elicit relatively greater activation from PTSD versus TENP participants in the amygdala, and relatively reduced activation in the medial prefrontal cortex. We also predicted that PTSD participants' ratings of valence would correlate negatively with activation in the amygdala and positively with activity in the medial prefrontal cortex to a greater degree than in trauma-exposed participants without PTSD. Stronger correlations in the PTSD group would suggest that increased activation in the brain results in more extreme ratings of valence.

Methods

Participants

We collected data from 28 male participants (mean age=62.3 years, SD=5.1). All participants were exposed to combat in Vietnam. Twelve met *DSM-IV* diagnostic criteria for PTSD, according to a structured clinical interview (the Clinician-Administered PTSD Scale [CAPS]) (Blake et al., 1995). The remaining sixteen participants did not have PTSD and are referred to as trauma-exposed non-PTSD (TENP) participants. Exclusion criteria for the study consisted of complicating medical conditions (e.g. cardiac disorder, seizure disorder) or psychiatric conditions (e.g. psychosis, dementia), as well as contraindications to the MRI environment (e.g. metal implants, pacemakers). Although participants were recruited through the Vietnam Era Twin Registry (VETR) and the University of Washington Twin Registry, none of the participants included in the current report were twins or members of the same family.

Of the PTSD participants, 7 were taking psychotropic medications: selective serotonin reuptake inhibitors (SSRIs) (n=5), other serotonergic antidepressants like serotonin antagonist and reuptake inhibitors (SARIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) (n=3), other antidepressants (n=2), and atypical antipsychotics (n=2). One TENP participant was taking an SSRI (n=1).

Table 1. Demographics

	PTSD (n=12)		**TENP** (n=16)	
	Mean	SD	Mean	SD
Age	61.8	6.7	62.7	3.7
Education (years)	14.0	2.8	15.2	3.5
CAPS*	54.9	26.6	4.8	8.0
BDI	10.0	9.9	4.6	4.0

*****The PTSD group had significantly higher scores than the TENP group, t(12.5)=6.31, p<.001

Materials

Apparatus. A MacBook Pro laptop was used to present stimuli to participants during fMRI scanning, using MacStim 3.2.1 software (WhiteAnt Occasional Publishing, West Melbourne, VIC, Australia).

Stimuli. "Surprised" and "neutral" faces were grayscale images selected from Ekman's (1976) well-validated set of emotional facial expressions. The stimulus set included the faces of 8 different individuals, 4 male and 4 female, and each individual was shown with a surprised expression and a neutral expression (for a total of 16 different images).

Figure 1: Sample surprised and neutral stimuli.

Clinical Assessment. Severity of PTSD symptomology was measured with the Clinician-Administered PTSD Scale (CAPS). The CAPS has been shown to be reliable and valid for use in assessing PTSD symptom severity (Blake et al., 1995). Severity of depressive symptomology was measured with the Beck Depression Inventory (BDI), which has been shown to be reliable and valid (Beck, Steer, & Carbin, 1988).

Procedures

This study was approved by the institutional review boards of the Massachusetts General Hospital and the Puget Sound VA. Written informed consent was obtained from each individual before participation.

Facial Expression Categorization Task. Participants were given a paper and pencil questionnaire in two parts: Free Response and Forced Choice. In the Free Response part, participants were shown 16 grayscale images of facial expressions one at a time and were instructed to write down the emotion they thought the person in the image was experiencing. 8 stimuli which featured “surprised” expressions and 8 of which featured “neutral” expressions. In the Forced Choice part, participants were shown the same faces in the same order, but below each image were the names of seven emotions: Neutral, Sadness, Anger, Surprise, Happiness, Fear, and Disgust. Participants were instructed to circle the emotion that they thought the person in the image was experiencing.

Face Rating Task. After the categorization task, participants rated the emotional valence and arousal of face images presented on the computer screen. Following the procedures of Kim et al. (2003), faces were displayed and rated using two different methods. In the block task, participants viewed a block of stimuli and rated the block as a whole. In the individual task, participants gave a rating for each stimulus. In both tasks, participants were instructed to respond as quickly as possible. However, there was no time limit for response. One participant in the PTSD group did not complete the behavioral portion of the study.

The block rating task began with a focus cross appearing on the screen for 4 seconds. Then grayscale faces (sized 412 x 310 pixels) sequentially appeared on the screen with a presentation rate of 0.5 seconds and an interstimulus interval of 0.2 seconds. The faces were presented in blocks by emotional expression. Each block consisted of 32 images. Within each block, the same 4 faces (2 male, 2 female) experiencing the same emotion were presented 8 times, in a pseudorandom order so that any given image was never presented twice in a row. After all 32 pictures had flashed on the screen, participants were asked to rate their impression of the entire block for both valence and arousal using labeled keys on a keyboard. Scores for valence ranged from -4 to 4 (very negative to very positive). Ratings for arousal ranged from 1 -9 (low arousal to high arousal). This procedure was completed for four total blocks, two consisting of surprised faces and two of neutral faces, starting with neutral and alternating with surprise.

For the individual face rating task, participants were shown 16 sequentially presented images of grayscale faces with alternating surprised and neutral expressions. After the presentation of each face, participants were asked to rate the valence of the image using the same scale as in the block rating task before proceeding to the next image. Each grayscale face (sized 412 x 310 pixels) remained on the screen for 3 seconds. After rating all 16 faces for valence, participants were then asked to rate the same faces in the same order for arousal, again using the same scale from the block rating task.

fMRI Task. We conducted fMRI scanning using a Siemens Trio Tim 3 Tesla MRI with a 12-channel gradient head coil at the Massachusetts General Hospital (MGH)'s Athinoula A. Martinos Center for Medical Imaging (Charlestown, MA). Functional MRI blood-oxygen-level dependent (BOLD) images were acquired using a gradient echo T2-weighted sequence (repetition time=2.5 sec, echo time=.52msec, flip angle=90) in 46 coronal slices (thickness=2.5mm, 20% distance factor, 0.5mm skip). Total scan time was 3 minutes, 42 seconds.

Surprised and neutral faces (sized 7.3 cm x 11.1 cm on the MacBook Pro screen) were presented to participants in the scanner over two runs (208 seconds each) using MacStim 3.2.1 (WhiteAnt Occasional Publishing, West Melbourne, VIC, Australia). Participants passively viewed each face as it was presented. Each run began and ended with a 16-second focus cross presentation. Within each run, participants viewed eight blocks of images with a 16 second focus cross between each pair of blocks. Each block consisted of 32 total images of faces of the same emotion. These images were images of 4 faces, 2 male and 2 female, repeated 8 times in pseudo-random order such that an image was never displayed twice in a row. Faces were presented for 200 ms with an interstimulus interval of 300 ms. The order of presentation of blocks was counterbalanced across subjects and groups.

Data Analysis

Categorization Analyses. To assess the categorization of faces, we first coded answers on the Free Response portion. For Surprise trials, responses were coded as Surprise, Fear, Happiness, or Other. For Neutral trials, responses were coded as Surprise, Fear, Happiness, Neutral, or Other. We then calculated the proportion of faces categorized as each emotion by each participant. The Free Response data for Surprise were analyzed using a 2 (Diagnosis: PTSD or TENP) x 4 (Emotion: Fear, Surprise, Happiness, Other) ANOVA. The Free Response data for Neutral trials were analyzed using a 2 (Diagnosis: PTSD or TENP) x 5 (Emotion: Fear, Surprise, Happiness, Neutral, Other) way ANOVA. The Forced Choice data for Surprise and Neutral trials were each analyzed using a 2 (Diagnosis: PTSD or TENP) x 7 (Emotion: Fear, Surprise, Happiness, Anger, Disgust, Neutral) ANOVAs. Follow-up independent samples t-tests were used to determine which emotions were categorized differently by the two groups.

Face Rating Analyses. We ran separate 2 (Group: PTSD or TENP) x 2 (Emotion: Surprise or Neutral) repeated measures ANOVAs to statistically evaluate participants' ratings of valence and arousal for faces presented during both the blocked and individual rating tasks, as well as the response times (RTs) associated with those ratings. RT data were not analyzed for individual arousal data, as we did not hypothesis any significant results based on the results of Kim et al. (2003).

fMRI Analyses. Data preprocessing and fMRI analyses were completed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/software/spm2/; Copyright © 1994-2012 FIL). Functional images were coregistered to each participant's high-resolution structural MRI image, normalized, and smoothed using a 4-mm Gaussian kernel. The BOLD data were fitted to a linear statistical model by the method of least squares. We tested our hypotheses as contrasts in which the linear model parameters were compared using two-sample t tests, the results of which were converted into z scores. The three contrasts of interest were Surprise versus Neutral, Surprise versus Fixation, and Neutral versus Fixation using the first run of the stimuli. These contrasts were run within each group and between PTSD and TENP participants. In addition, correlations between these contrasts maps and both blocked and individual valence ratings were run.

Several areas of the brain were established as a priori regions of interest (ROIs) based on our hypotheses: the amygdala, medial prefrontal cortex (including anterior cingulate cortex), and insula. Given our a priori hypotheses, we applied a significance threshold of .001, one-tailed and uncorrected (z score ≥3.09) to activations in these regions. For regions where we had no a priori predictions, we applied a more conservative significance threshold of .00002, two-tailed and uncorrected (z score ≥4.27). Data were extracted from spherical ROIs (centered around the peak voxel) with a 4 mm radius.

Correlational Analyses. Activations showing significantly greater activity viewing surprised faces than a fixation cross in the PTSD group were determined using SPM2. Neutral vs. fixation data were not analyzed, as the focus of this study was brain activation to surprised faces based on the results of Kim et al. (2003). Activation data were extracted from these coordinates for all participants. The same procedure was repeated for coordinates showing greater activity viewing surprised faces than a fixation cross in the TENP group. Activation data were then correlated with arousal and valence ratings of surprised and neutral facial expressions, as well as CAPS score. Activation levels were also correlated with depression levels determined by Beck Depression Inventory (BDI) in order to ensure that results were related to PTSD symptomology and not depressive symptomology.

Results

Facial Expression Categorization

Surprise Faces

Free Response. There was a significant main effect of Emotion, $F(3,75)=110.34$, $p<.001$. Inspection of the means revealed that the faces were most likely to be correctly categorized as surprise rather than any other emotion. There was no significant of Group (df=1,29) and there was no significant Emotion x Group interaction, $F(3, 75)=.32$, $p=.81$. There was no significant between-group difference in proportions of surprised faces labeled as fear ($p=.91$) (Figure 2).

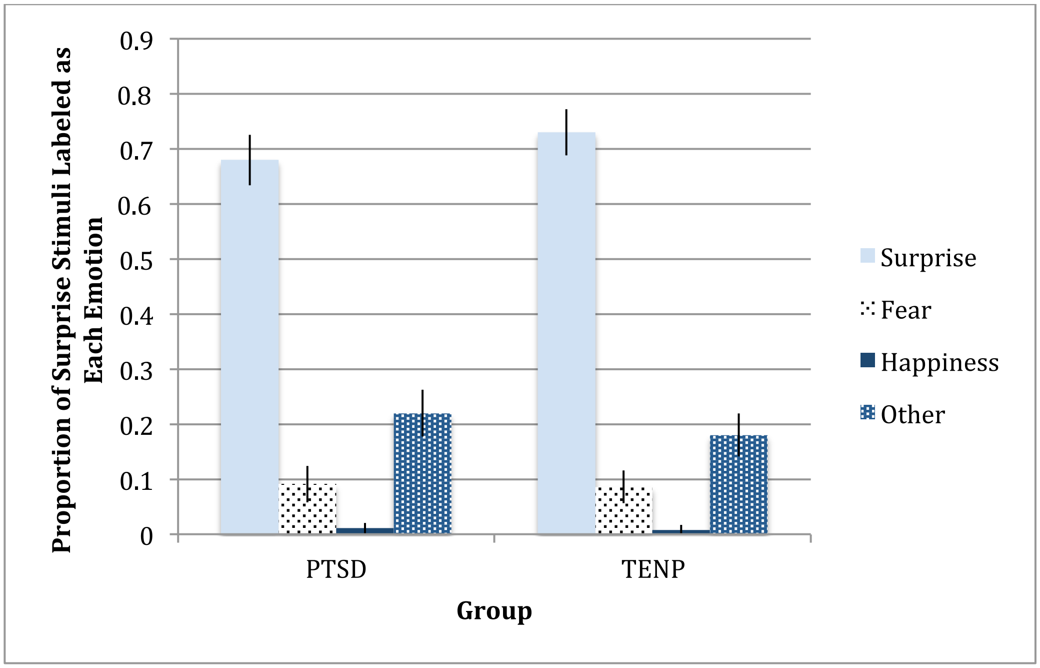

Figure 2: Proportion of surprised facial expressions categorized as surprise, fear, happiness, or other in the Surprise Free Response Categorization task. All error bars represent standard error. There was a significant main effect of emotion, F(3,75)=110.34, p<.001. Stimuli were most likely to be correctly categorized as surprise.

Forced Choice. There was a significant main effect of Emotion, F(6,150)=184.30, p<.001. Inspection of the means revealed that the faces were most likely to be correctly categorized as surprise. There was no significant main effect of Group (df=1,29) and no significant Emotion x Group interaction, F(6,150)=1.20, p=.31 (Figure 3). There were no significant differences between PTSD and TENP participants in proportions of surprised faces labeled fear (p=.45) (Figure 3).

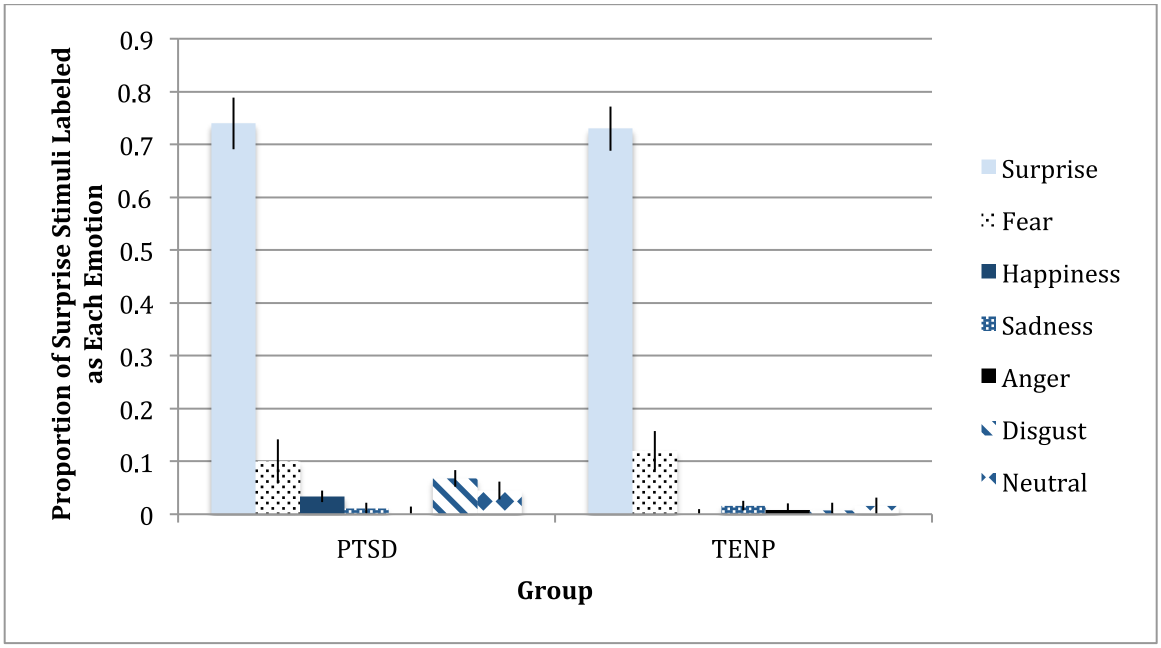

Figure 3: Proportion of surprised facial expressions categorized as surprise, fear, happiness, sadness, anger, disgust, or neutral in the Surprise Forced Choice Categorization task. There was a significant main effect of emotion, F(6,150)=184.30, p<.001. Stimuli were most likely to be correctly categorized as surprise.

Neutral Faces

Free Response. There was a significant main effect of Emotion, F(4,100)=23.79, p<.001. The faces were most likely to be categorized as other. There was no significant main effect of Group (df=1,29), and no significant Emotion x Group interaction, F(4,100)=1.66, p=.16 (Figure 4).

Forced Choice. There was a significant main effect of Emotion, F(6,150)=85.91, p<.001. The faces were most likely to be correctly categorized as neutral. There was no significant main effect of Group, F(1,25)=1.48, p=.24, and no significant Emotion x Group interaction, F(6,150)=.50, p=.81 (Figure 5).

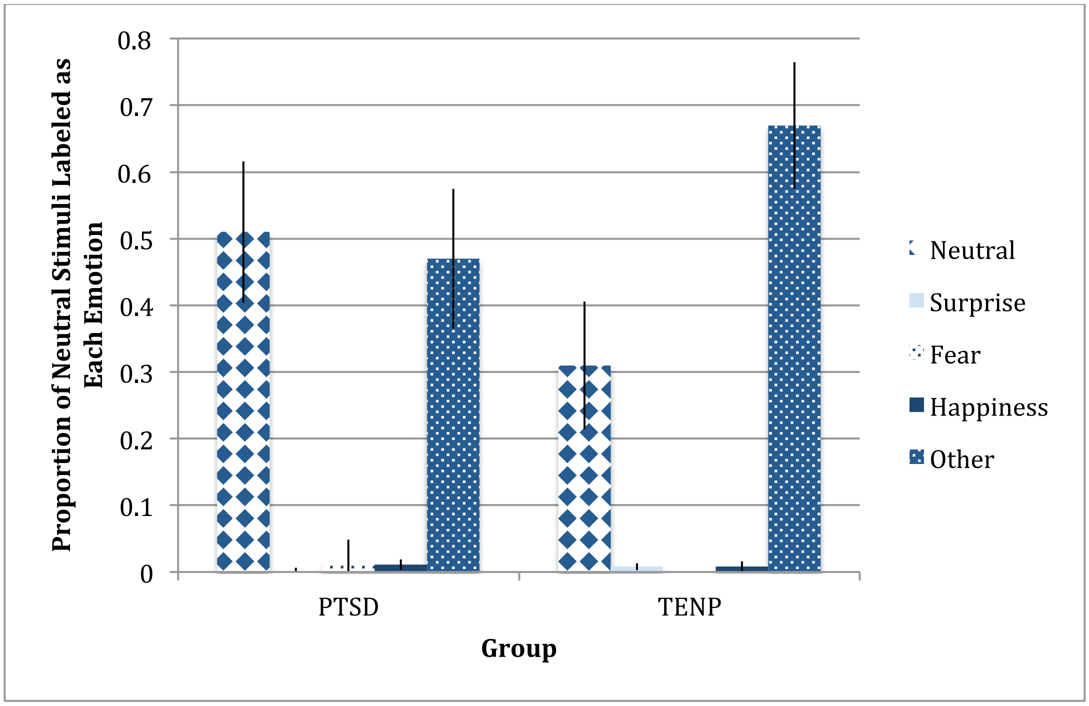

Figure 4: Proportion of neutral facial expressions categorized as neutral, surprise, fear, happiness, or other in the Neutral Free Response Categorization task. There was a significant main effect of emotion, $F(4,100)=23.79$, $p<.001$. Stimuli were most likely to be categorized as other.

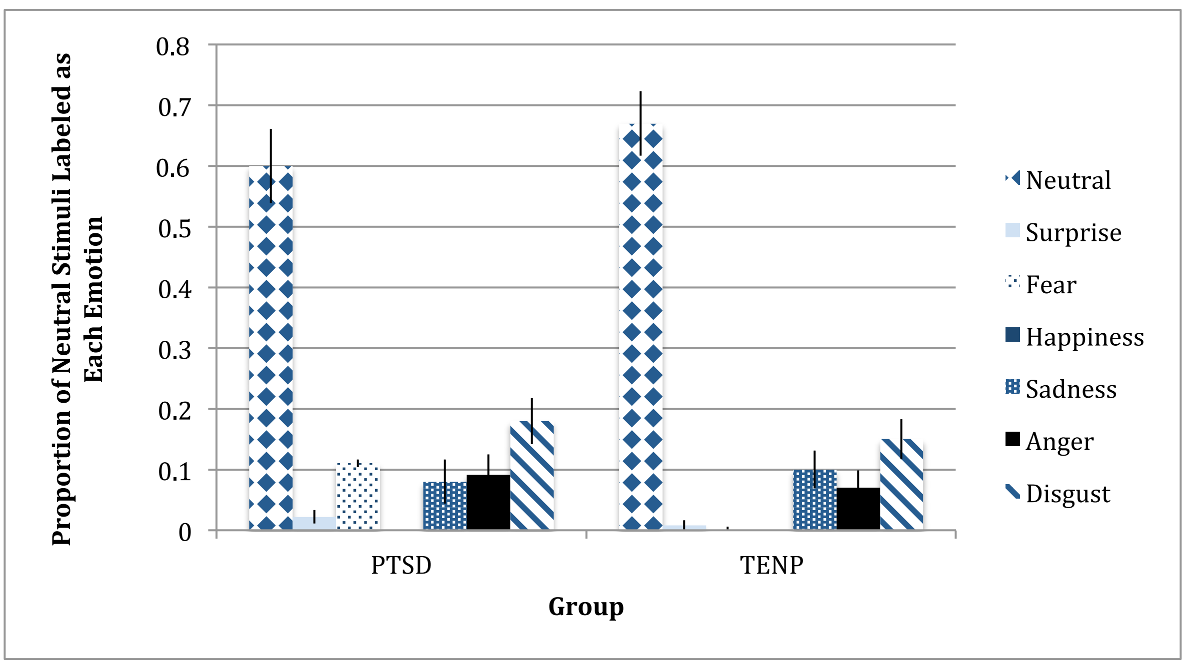

Figure 5: Proportion of neutral facial expressions categorized as neutral, surprise, fear, happiness, sadness, anger, or disgust in the Neutral Forced Choice Categorization task. There was a significant main effect of emotion, $F(6,150)=85.91$, $p<.00$. Stimuli were most likely to be correctly categorized as neutral.

Block Ratings

Arousal. The main effect of Emotion was significant, $F(1,25)=28.19$, $p<.001$. Surprised faces were rated higher on arousal than neutral faces. There was also a significant Emotion by Group interaction, $F(1,25)=5.59$, $p=.026$. Inspection of the means revealed that ratings of surprised faces did not differ significantly between groups, $t(25)= -0.78$, $p=.44$., and ratings of neutral faces did not differ significantly between group, $t(25)=1.01$, $p=.32$. (see Figure 6). The main effect of Group was not significant, $F(1,25)=.001$, $p=.98$.

Valence. There was a trend for a significant main effect of Emotion, $F(1,25)=3.70$, $p=.066$. Surprised faces tended to be rated lower in valence than neutral faces. There was no significant main effect of Group, $F(1,25)=.52$, $p=.48$, or significant Emotion by Group interaction, $F(1,25)=.14$, $p=.70$ (Figure 7).

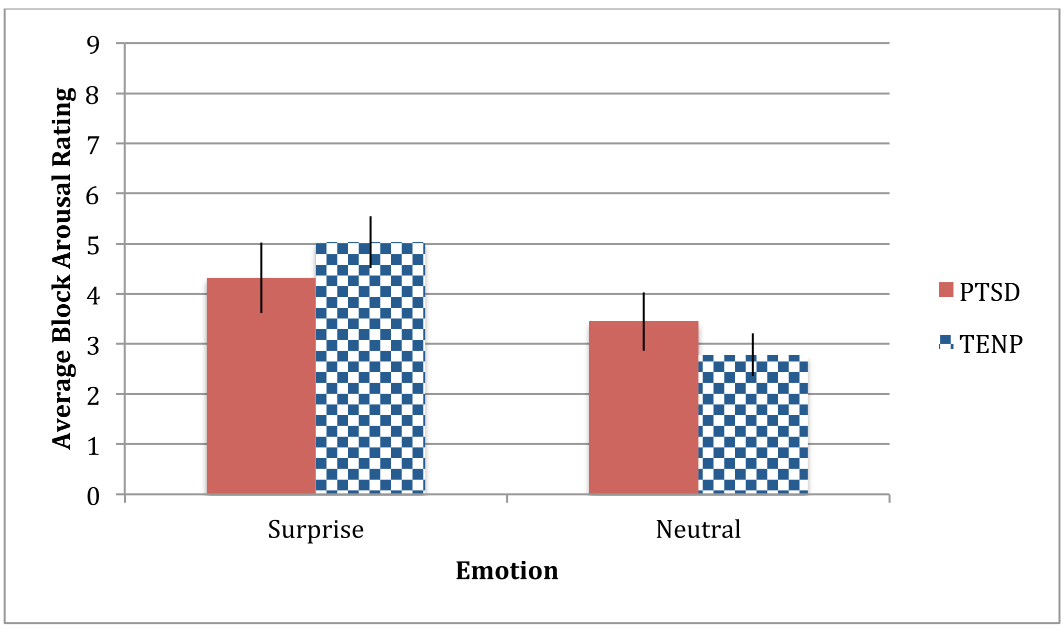

Figure 6: Average block arousal rating of surprised and neutral facial expressions by group. The main effect of Emotion was significant, $F(1,25)=28.19$, $p<.001$. There was a significant Emotion by Group interaction, $F(1,25)=5.59$, $p=.026$.

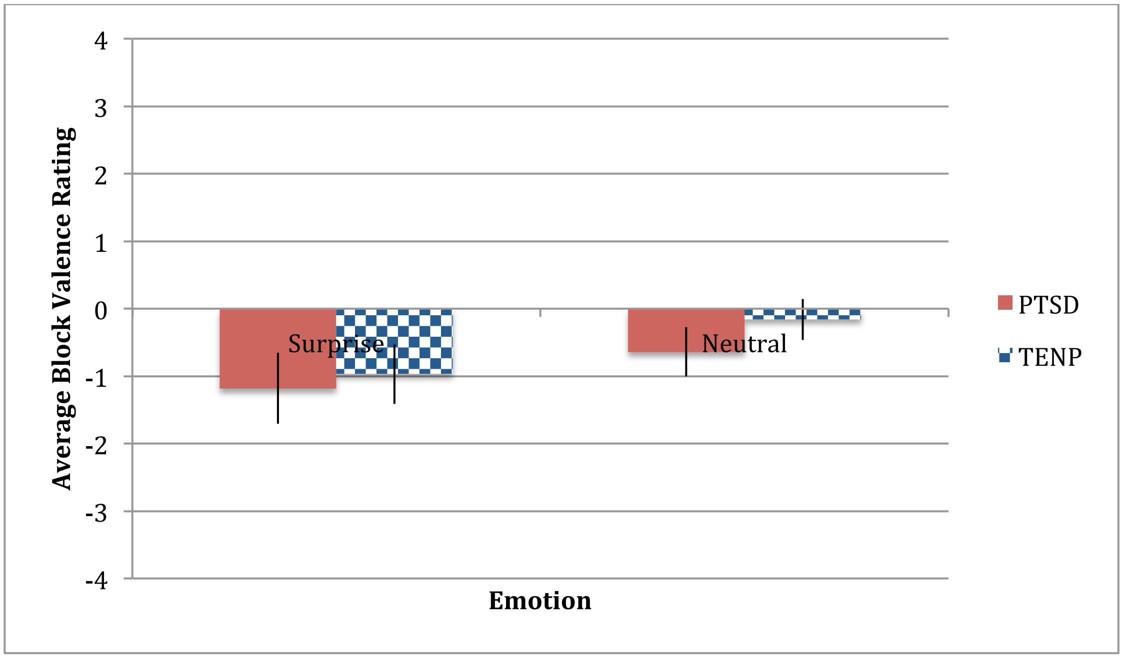

Figure 7: Average block valence rating of surprised and neutral facial expressions by group. There were no significant main effects and no significant interaction, but there was a trend for a significant main effect of Emotion, $F(1,25)=3.70$, $p=.066$.

Response Times

Arousal. We removed one outlier from the analysis due to response times greater than two standard deviations over the mean. There was a trend for a significant main effect of Emotion, $F(1,24)=4.06$, $p=.055$. Response times to neutral faces were greater than response times to surprised faces. There was no significant main effect of Group, $F(1,24)=.11$ $p=.75$, and no significant Emotion by Group interaction, $F(1,28)=.010$, $p=.92$ (Figure 8).

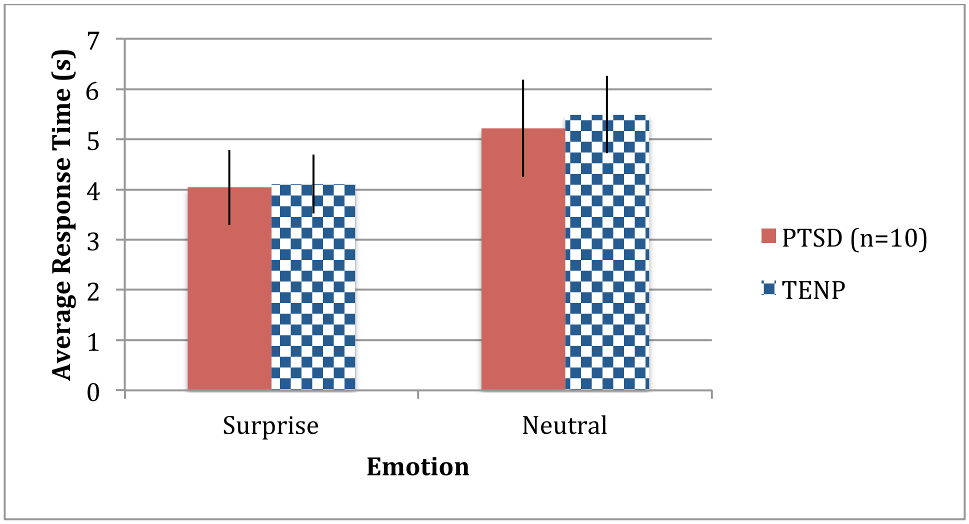

Figure 8: Average response time during block arousal rating task. There was a trend for a significant main effect of Emotion, $F(1,25)=3.70$, $p=.066$.

Valence. No effects were significant (all ps > .14) (Figure 9).

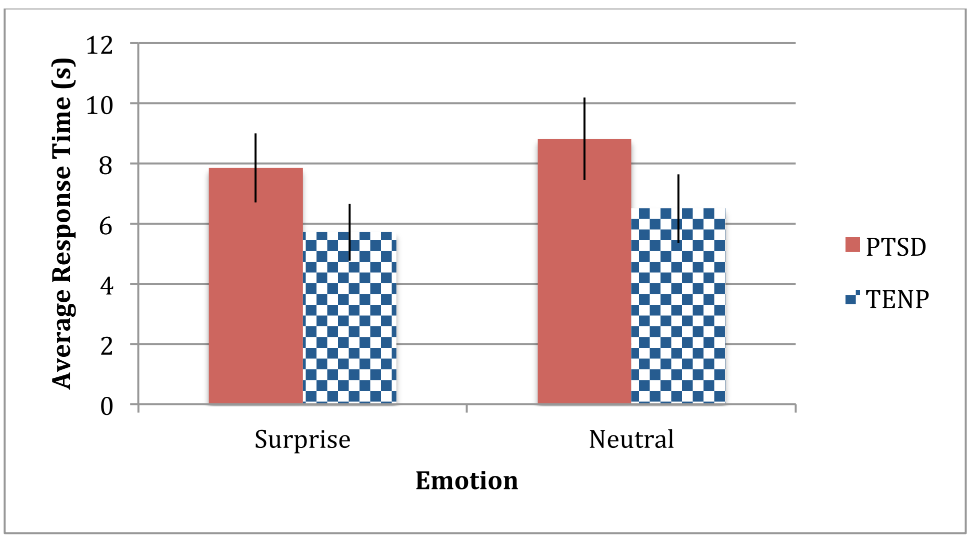

Figure 9: Average response time during block valence rating task. There were no significant effects.

Individual Ratings

Arousal. The main effect of Emotion was significant, $F(1,25)=23.99$, $p<.001$. Across all participants, surprise faces were rated higher on arousal than neutral faces. The Emotion by Group interaction was significant, $F(1,25)=4.83$, $p=.037$. Inspection of the means revealed that ratings of surprised faces did not differ significantly between groups, $t(25)=.073$, $p=.94$, nor did ratings of neutral faces, $t(25)=1.72$, $p=.098$(See Figure 10). The main effect of Group was not significant, F(1,25)=.72, p=.40.

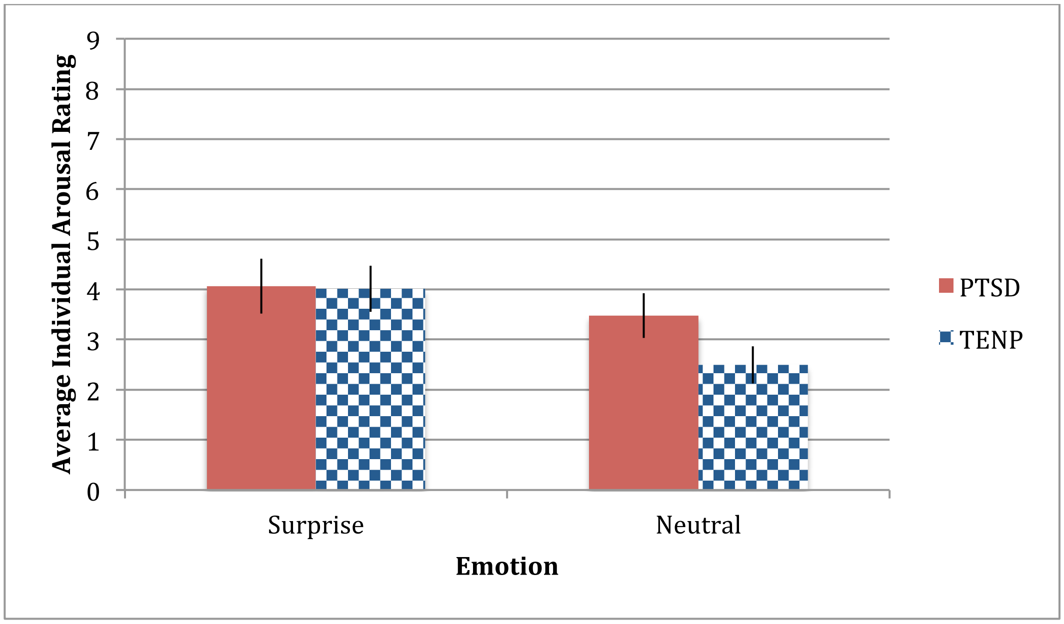

Figure 10: Average individual arousal rating of surprised and neutral facial expressions by group. The main effect of Emotion was significant, F(1,25)=23.99, p<.001. There was a significant Emotion by Group interaction, F(1,25)=4.83, p=.037.

Valence. One participant in the PTSD group was missing 6 individual ratings (2 surprise items and 4 neutral items). The 2x2 repeated measures ANOVA was conducted excluding this participant. There was a significant main effect of Group, F(1,24)=4.80, p=.038. PTSD participants gave higher ratings than TENP participants. There was a trend for Emotion by Group interaction, F(1,24)=3.75, p=.065. Inspection of the means revealed that there was a weak trend for a significant difference in ratings of surprised facial expressions between the groups, *t*(10.83)=1.85, p=.091, where PTSD gave higher ratings. There was no significant difference in ratings of neutral facial expressions between groups, t(24)= 0.49, p=.63 (see Figure 11). There was no main effect of Emotion, F(1,24)=2.78, p=.11.

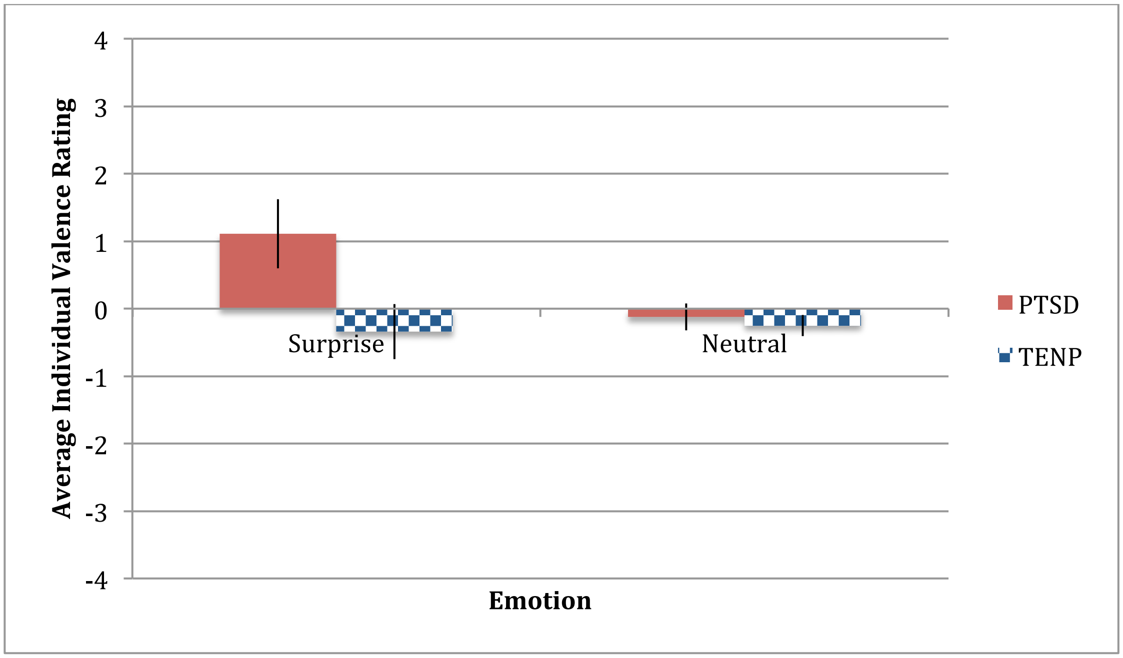

Figure 11: Average individual arousal rating of surprised and neutral facial expressions by group. The main effect of Group was significant, F(1,24)=4.80, p=.038, with the PTSD group giving higher ratings. There was a trend for significant Emotion by Group interaction, F(1,24)=3.75, p=.065.

Response Times

Valence. The main effect of Emotion was significant, F(1,25)=5.29, p=.030. Participants took significantly longer to evaluate the valence of surprised faces than neutral faces. There was a significant main effect of Group, F(1,25)=4.32, p=.048. PTSD participants took significantly longer to rate faces than TENP participants did. There was a trend for a significant Emotion by Group interaction, F(1,25)=4.18, p=.051. Inspection of the means revealed that there was a significant difference in response time to neutral facial expressions between the groups, with the PTSD group having slower response times, $t(25)=2.94$, p=.007. There was no significant difference between the groups in response time to surprised facial expressions, $t(25)=0.75$, p=.46(see Figure 12).

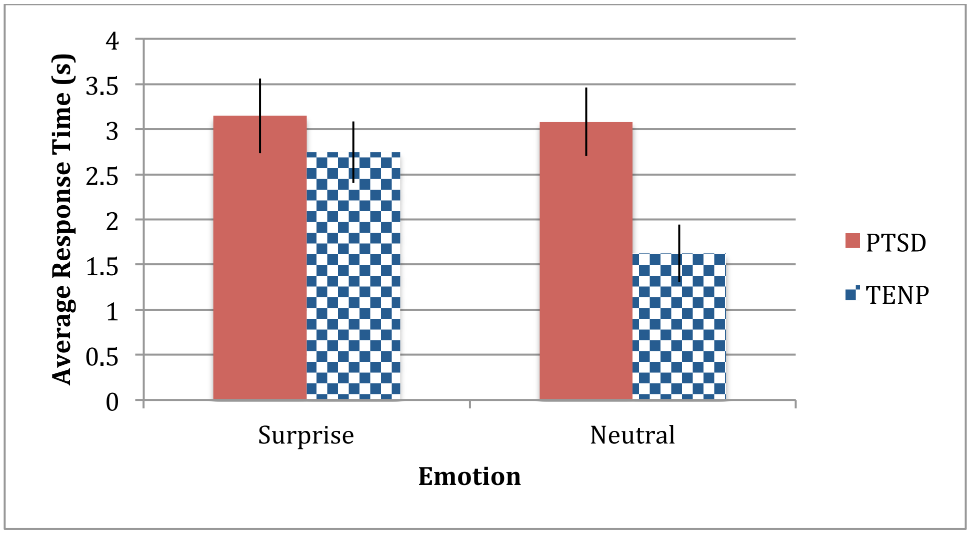

Figure 12: Average response time during block valence rating task. There was a significant main effect of Group, $F(1,25)=4.32$, $p=.048$. There was a trend for a significant Emotion by Group interaction, $F(1,25)=4.18$, $p=.051$.

fMRI Data

Surprise vs Neutral. Within the PTSD group, the right insula showed an increase in BOLD signal when viewing surprised versus neutral faces (Table 2).

In the TENP group, there was an increase in BOLD signal in the dACC when viewing surprised versus neutral faces.

There were no significant differences in levels of BOLD signal increase or decrease between the PTSD and TENP groups in any brain region (Figure 13).

Surprise vs. Fixation. In the PTSD group, the right amygdala, the dACC, and the right insula, as well as the non a priori regions of the left and right parietal operculum, the right fusiform gyrus, and the occipital cortex, showed greater BOLD signal when viewing surprised faces than when viewing the fixation cross. The rACC showed reduced BOLD signal when viewing surprised faces compared to fixation (Table 3).

In the TENP group, the right and left amygdala, and the dACC, as well as the non a priori regions of the lingual gyrus, the right fusiform gyrus, and the cerebellum, showed greater BOLD signal when viewing surprised faces versus fixation. The rACC, and right and left insula showed less activation to surprised faces versus fixation, as did the non a priori regions of the somatosensory cortex and premotor cortex.

PTSD participants had greater BOLD signal increases than TENP participants in the right amygdala (Figure 14), vmPFC, dACC, rACC, and the left and right insula.

Neutral vs. Fixation. In the PTSD group alone, BOLD signal increases occurred in the right amygdala, the rACC, and the right insula, as well as the non a priori regions of the occipital cortex and the right fusiform gyrus (Table 4).

In the TENP group, BOLD signal increases occurred in in the left and right amygdala and the rACC, as well as the non a priori regions the occipital cortex, the right fusiform gyrus, and the lingual gyrus (Table 4). The TENP group showed reduced BOLD signal in the rACC, dACC, and left and right insula, as well as the a priori regions of the superior parietal cortex and the medial temporal gyrus.

PTSD participants had greater BOLD signal increases than TENP participants in the left amygdala (Figure 14), the dACC, the rACC, the left and right insula, as well as the non a priori regions of the auditory cortex, the superior temporal cortex, and the cerebellum (Table 4).

Table 2.

Voxelwise Analysis Results					
				MNI Coordinates	
Comparison	**Region**	**z Score**	**x**	**y**	**z**
	PTSD (n=12)				
Surprise > Neutral	**Right Insula**	3.68	46	-16	16
Neutral > Surprise	**Brainstem**	3.33	10	-18	16
	TENP (n=16)				
Surprise > Neutral	**dACC**	4.13	4	0	38
	dACC	3.75	-12	2	40
	dACC	3.50	6	16	32
	Occipital Cortex	4.27	-32	-90	-2
Neutral > Surprise	None				
	PTSD vs TENP				
PTSD > Control, Surprise v Neutral	None				
PTSD < Control, Surprise v Neutral	None				

Abbreviations: dACC, dorsal anterior cingulate cortex; rACC, rostral anterior cingulate cortex; drACC, dorsal/rostral ACC; dmPFC, dorsal medial prefrontal cortex; MNI, Montreal Neurological Institute; PTSD, posttraumatic stress disorder. For a priori regions, a significance threshold was applied at z≥ 3.09. Significant a priori regions are listed in bold print. For non-a priori regions, the applied significance threshold was z≥ 4.27.

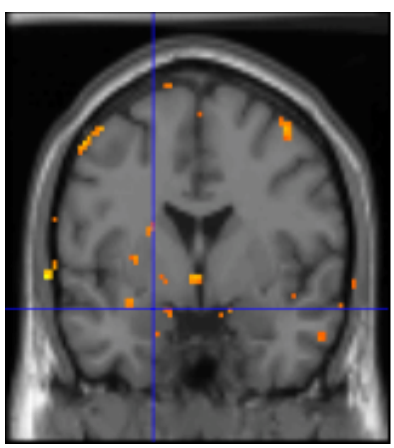

Figure 13: The fMRI image displays that there were no significant differences in activation between the PTSD and TENP groups to surprise vs neutral facial expressions in the amygdala (MNI coordinates: -20.25, -.71, -16.04 [crosshairs]).

Table 3.

Voxelwise Analysis Results					
			MNI Coordinates		
Comparison	**Region**	**z Score**	**x**	**y**	**z**
	PTSD (n=12)				
Surprise > Fixation	**Right Amygdala**	3.40	24	-6	-22
	Right Amygdala	3.80	26	4	-20
	dACC	3.51	10	20	26
	Right Insula	3.25	44	18	-4
	Right Insula	3.50	42	16	6
	Right Insula	3.60	42	-16	16
	Left Parietal Operculum	4.80	-46	12	26
	Right Parietal Operculum	5.00	44	10	22
	Right Fusiform Gyrus	5.33	38	-46	-20
	Occipital Cortex	5.21	-36	-90	-16
	Occipital Cortex	5.08	20	-90	-12
Fixation > Surprise	**rACC**	3.29	2	42	-2
	TENP (n=16)				
Surprise > Fixation	**Right Amygdala**	4.30	22	-4	-12
	Left Amygdala	4.00	-18	-4	-10
	dACC	3.66	-10	4	54
	Brainstem	3.25	-12	-28	-24
	Lingual Gyrus	5.48	20	-88	-14
	Right Fusiform Gyrus	5.34	40	-44	-20
	Cerebellum	5.25	40	-74	-20
Fixation > Surprise	**Hippocampus**	3.25	28	-12	-26
	rACC	3.38	2	46	0
	Right Insula	3.42	46	-12	-2
	Left Insula	3.72	-38	-24	4
	Left Insula	3.37	-40	-12	8
	Left Insula	3.22	-42	8	6
	Somatosensory Cortex	4.72	-15	-56	62
	Premotor Cortex	4.54	18	-16	76
	PTSD vs TENP				
PTSD > Control, Surprise v Fixation	**Right Amygdala**	3.19	24	-8	-24
	vmPFC	3.16	4	44	-20
	dACC	3.33	2	26	24
	dACC	3.38	6	24	22
	rACC	3.41	6	40	14
	Left Insula	4.58	-40	-18	0
	Left Insula	3.53	-40	-12	8
	Right Insula	3.11	46	-12	-4
	Right Insula	3.59	42	-16	16
Control > PTSD, Surprise v Fixation	None				

Abbreviations: dACC, dorsal anterior cingulate cortex; rACC, rostral anterior cingulate cortex; drACC, dorsal/rostral ACC; vmPFC, ventromedial prefrontal cortex; MNI, Montreal Neurological Institute; PTSD, posttraumatic stress disorder. For a priori regions, a significance threshold was applied at z≥ 3.09. Significant a priori regions are listed in bold print. For non-a priori regions, the applied significance threshold was z≥ 4.27.

Table 4

Voxelwise Analysis Results					
			MNI Coordinates		
Comparison	**Region**	**z Score**	**x**	**y**	**z**
	PTSD (n=12)				
Neutral>Fixation	**Right Amygdala**	4.25	22	-8	-12
	rACC	3.33	-4	48	2
	Right Insula	2.78	42	20	4
	Occipital Cortex	5.46	36	-72	-12
	Right Fusiform Gyrus	5.20	44	-64	-18
	Right Fusiform Gyrus	4.92	40	-48	-18
Fixation > Neutral	None				
	TENP (n=16)				
Neutral>Fixation	**Left Amygdala**	3.11	-22	-4	-10
	Right Amygdala	3.33	20	-6	-14
	rACC	3.26	16	40	26
	Occipital Cortex	5.87	44	-76	-14
	Right Fusiform Gyrus	5.55	36	-42	-18
	Lingual Gyrus	5.46	16	-94	-12
Fixation > Neutral	**rACC**	3.52	2	46	2
	rACC	3.20	6	26	-8
	rACC	3.38	6	36	-10
	dACC	3.46	4	24	28
	dACC	3.45	2	14	34
	Left Insula	3.43	-36	-8	8
	Right Insula	3.54	38	4	14
	Superior Parietal Cortex	4.58	20	-50	76
	Superior Parietal Cortex	4.31	14	-56	58
	Medial Temporal Gyrus	4.52	-58	-18	0
	PTSD vs TENP				
PTSD> Control, Neutral v Fixation	**Left Amygdala**	3.62	-18	2	-18
	dACC	3.96	-2	20	32
	dACC	3.32	0	24	24
	rACC	3.62	-2	30	-8
	rACC	3.18	-4	40	4
	rACC	4.10	8	38	16
	vmPFC	3.49	2	46	4
	Left Insula	3.98	-40	-10	8
	Left Insula	3.90	-38	-18	0
	Left Insula	3.53	-34	4	6
	Right Insula	3.49	44	-12	14
	Right Insula	3.36	40	-26	12
	Right insula	4.13	44	2	-10
	Auditory Cortex	5.07	-54	-26	-22
	Auditory Cortex	4.63	-50	-12	4
	Superior Temporal Cortex/Uncus	4.35	-30	12	2
	Cerebellum	4.34	16	-36	-24
Control>PTSD, Neutral v Fixation	None				

Abbreviations: dACC, dorsal anterior cingulate cortex; rACC, rostral anterior cingulate cortex; vmPFC, ventromedial prefrontal cortex; MNI, Montreal Neurological Institute; PTSD, posttraumatic stress disorder. For a priori regions, a significance threshold was applied at z≥ 3.09. Significant a priori regions are listed in bold print. For non-a priori regions, the applied significance threshold was z≥ 4.27.

A **B**

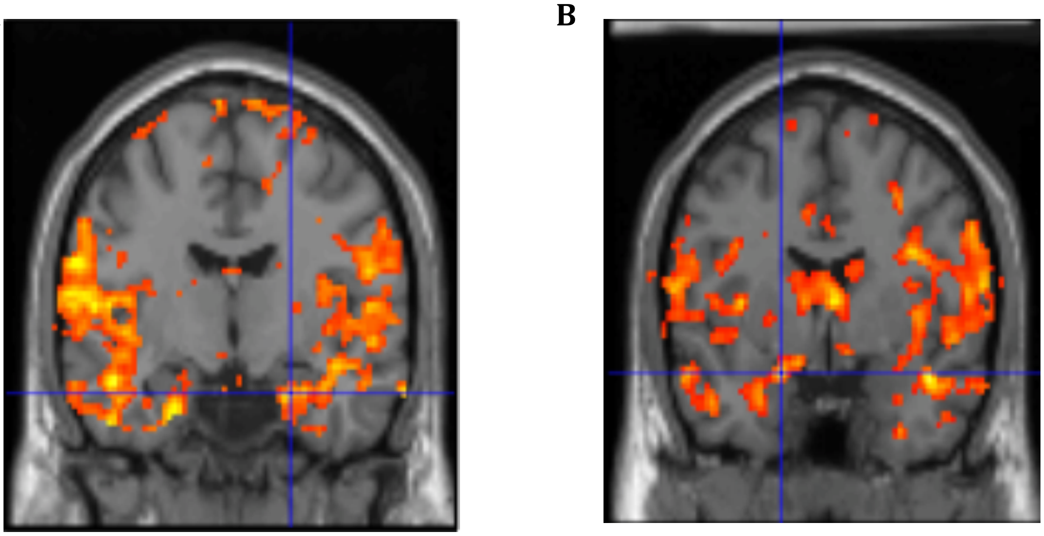

Figure 24: The fMRI image (A) displays activation to surprised facial expressions vs a fixation cross in the right amygdala (MNI coordinates: +24, -8, -24 and z=3.19 [crosshairs]) that were greater in the PTSD group vs the TENP group. The fMRI image (B) displays activation to neutral facial expressions vs a fixation cross in the left amygdala (MNI coordinates: -18 +2, -18 and z=3.62 [crosshairs]) that were greater in the PTSD group vs the TENP group.

Correlational Analyses

Within PTSD Group. There was a significant negative correlation between the activation of the dACC in PTSD participants and their arousal ratings of neutral faces in both the individual task, *r*(9)=-.62, p=.041, and block task, r(9)=-.63, p=.038. There was a trend for a significant negative correlation between dACC activation and arousal ratings of surprised faces during the individual task, *r*(9)=-.58, p=.064.

There was a trend for a significant correlation between right amygdala activity at two coordinates and score on the B subunit of the CAPS, MNI coordinates 24, -6, -22, *r*(10)=.54, p=.067; and MNI coordinates 26, 4, -20, *r*(10)=.56 p=.60.

There were no other significant correlations between brain activity and behavioral measures (all ps > .14).

Within TENP Group. There were no significant correlations between brain activity in the TENP group and behavioral measures (all ps > .097).

Discussion

Overall, our results showed mixed support for our hypotheses. With regard to ratings data, we predicted that individuals with PTSD would rate surprised facial expressions with a more negative valence than TENP individuals would; however, the data did not support this prediction. Instead we found that PTSD participants gave more positive ratings to both surprised and neutral facial expressions than TENP participants, shown by a main effect of group in the individual ratings task. Overall, the ratings results do not support our hypotheses, but indicate that individuals with PTSD interpret the valance of ambiguous facial expressions differently than controls.

Unlike Kim et al. (2003), we also gathered and analyzed arousal ratings. We predicted that PTSD individuals would give higher arousal ratings to surprised vs neutral facial expressions compared to the TENP group. Instead, we found that PTSD participants rated surprised facial expressions in the block arousal task as displaying lower arousal than TENP individuals did. Our results suggest that individuals with PTSD show abnormalities in assigning appropriate arousal to neutral expressions.

We predicted that individuals with PTSD would have greater response times during the rating tasks to surprised vs. neutral faces than TENP participants. This was not supported by our data, though analysis of the response time data from the individual valence rating task showed a trend for a significant Group x Emotion interaction, where PTSD participants had a significantly greater response time to neutral facial expressions than the TENP group, but this pattern was not observed with surprised facial expressions. If PTSD participants found it more difficult to rate faces for valence than TENP participants and, therefore, took longer to respond during the task, as shown by a significant main effect of Group, this may explain the unusual rating patterns that were observed.

Overall, the reason why PTSD individuals rated surprise with a higher valence than TENP participants is unclear. What the results from the behavioral tasks of our study do suggest is that individuals with PTSD show abnormal processing of ambiguous facial expressions, including neutral facial expressions, and that individuals with PTSD took longer to assign ratings to facial expressions.

We hypothesized that the PTSD group would show greater activation in the amygdala and reduced activation in the mPFC than the TENP group in the surprised versus neutral contrast. However, the fMRI results showed no significant between-group differences in this contrast. Instead, significant between-group activations were seen in the surprised vs. fixation contrast. PTSD participants showed greater activation in the right amygdala. This supports our hypothesis that the PTSD is associated with increased amygdala activation to surprised faces. Furthermore, the PTSD group displayed greater activation in the insula, dACC, and rACC. These areas seem to be implicated in processing of surprised faces. However, the data did not support our hypothesis that PTSD would be associated with reduced mPFC activation, as the PTSD group showed greater vmPFC activity to surprised faces than controls.

Though we did not predict any group differences during the neutral versus fixation contrast, these data revealed similar patterns of activation to the surprise versus fixation contrast. The PTSD group showed greater activation in the left amygdala to neutral faces than the TENP group. As with surprise, the PTSD group displayed increased vmPFC activation compared to controls, as well as increased activity in the insula, dACC, and rACC. As we found that both surprised and neutral facial expressions produced increased amygdala activity in participants with PTSD, our data suggest that surprised and neutral facial expressions are processed as a potential threat and that individuals with PTSD display a stronger reaction to this threat than TENP individuals. Overall, the data from neutral vs. fixation trials seems to point to neutral facial expressions being interpreted in similar ways to surprised facial expressions and suggests that PTSD is associated with an increased threat response to ambiguous faces.

We predicted that correlational analyses would reveal significant negative correlations between valence ratings and amygdala activity in the PTSD group. However, our data did not support these predictions. We also predicted that CAPS scores of the PTSD group would positively correlate with amygdala activation. Analyses revealed a trend for a significant positive correlation between right amygdala activity and score on the B subscale of the CAPS in the PTSD group, which measures severity of re-experiencing symptoms. Since more severe re-experiencing symptoms were associated with greater amygdala activity to surprised faces, this further supports abnormal processing of surprised faces in PTSD. Additionally, there were no significant correlations between amygdala activation and BDI scores. This shows that correlations between amygdala activation and PTSD symptom severity are not likely explained by depressive symptomology. There was also a significant negative correlation between dACC activation to surprised facial expressions and arousal ratings for neutral faces in the block and individual task. The ACC has been associated with cognitive control (Kim et al. 2008), so it follows that reduced dACC activation could result in a greater threat response to surprised facial expressions if they were being perceived as a potential threat. These data also provide further similarities between processing of surprised and neutral faces.

Overall, our results show that surprised facial expressions (vs fixation) trigger an increased amygdala response in individuals with PTSD compared to controls. Furthermore, similar activation patterns were seen in response to neutral facial expressions vs fixation. Therefore, our results suggest that surprised and neutral facial expressions are interpreted similarly. These results add to an existing body of literature on responses in PTSD to emotional facial expressions and provide insight into increased reactivity during non – threatening situations of individuals with PTSD. Furthermore, our results imply that neutral facial expressions may not be a valid control for other facial expressions in studies of PTSD. These results have ramifications for understanding how individuals with PTSD perceive potential threats in ambiguous situations, including social situations.

There are several limitations to this study. First, we used stimuli from the Ekman face set. The neutral images in this set may be more negative than intended. This is shown by a trend for a main effect of Emotion where neutral facial expressions were rated as having more negative valence than surprised faces in the individual valence ratings task, despite both emotions being ambiguous. Furthermore, the results of the neutral Free Response categorization task showed that participants were most likely to categorize neutral faces as "other", not as neutral. Responses coded as "other" include anger, sadness, boredom, and seriousness. If the neutral images are not perceived as neutral, they may not be appropriate controls for surprised facial expressions. This could have caused heightened brain responses to neutral faces across subjects. However, even if this is the case, the PTSD group still showed greater amygdala activation to neutral faces than the TENP group. Furthermore, both groups were most likely to categorize neutral faces as neutral in the Forced Choice task and there were no significant differences between groups in how they categorized neutral faces. This shows that despite the potential negative appearance of the facial expressions, participants still interpreted them as neutral faces categorically.

Another limitation is the use of static facial stimuli. We utilized static facial expression stimuli to ensure simplicity of the task. However, using video images of facial expressions may capture a more naturalistic example of facial expressions. Using a paradigm that mimics a more natural context of emotion, rather than viewing a still picture, may more successfully elicit a threat response. Additionally, we were missing data on some of our analyses due to participants not completing all of the tasks. This could have affected our results, as more data was missing from the PTSD group. Lastly, our study population was made up of solely older male combat veterans, which could threaten the external validity of our study. It is unclear whether the effects seen would also be present in different populations affected by PTSD. Nevertheless, our results suggest that further study of the response to ambiguous facial expressions in PTSD is needed.

Future research should investigate neutral facial expressions and how they are perceived in PTSD. There is precedence for abnormal responses to neutral faces in psychiatric disorders, as atypical amygdala activation to neutral faces has been observed in Social Anxiety Disorder (SAD) (Cooney, Atlas, Joormann, Eugène, & Gotlib, 2006). Therefore, researchers should investigate how individuals with PTSD respond to neutral facial expressions instead of only using these stimuli as a control group. Neutral facial expressions are encountered commonly in social situations and, therefore, it is important to understand how individuals with PTSD perceive these expressions. Studies investigating response to neutral facial expressions could potentially use happy facial expressions as control items. There is a strong foundation for the use of happy facial expressions as control stimuli, as they have been used as control stimuli in several studies measuring responses to fearful facial expressions in PTSD (Fonzo et al., 2010; Rauch et al., 2000; Shin et al., 2005; Simmons et al., 2011). This paradigm could be utilized in order to further understand the response to neutral facial expressions. However, happy faces also elicit their own amygdala activations (Felmingham et al., 2014) and therefore are not perfect control stimuli either. Regardless, researchers should take caution in future studies of facial expressions to be aware of increased activation to neutral facial expressions in PTSD.

Future studies should also investigate the origin of increased amygdala activation in PTSD to ambiguous facial expressions. Our participants are all identical twins; future research should measure responses to ambiguous facial expressions in the co-twins of our participants. If greater amygdala activation to ambiguous facial expressions were also seen in the combat unexposed twins of the PTSD group, this would imply that increased amygdala activation to ambiguous facial stimuli is a preexisting familial vulnerability factor for PTSD. If not, this would suggest that increased amygdala activation to ambiguous facial stimuli is an acquired characteristic of PTSD. This paradigm has been used previously to determine the origin of deficits in configural processing (Gilbertson et al., 2007) and exaggerated activation of the dACC during cognitive interference in PTSD (Shin et al., 2011) and could be applied to our study. Overall, our results provide evidence for abnormal processing of both surprised and neutral facial expressions in PTSD, due to an exaggerated threat response in the amygdala.

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