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# Impaired fear inhibition learning predicts the persistence of symptoms of posttraumatic stress disorder (PTSD)

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ABSTRACT

Recent cross-sectional studies have shown that the inability to suppress fear under safe conditions is a key problem in people with posttraumatic stress disorder (PTSD). The current longitudinal study examined whether individual differences in fear inhibition predict the persistence of PTSD symptoms. Approximately 2 months after deployment to Afghanistan, 144 trauma-exposed Dutch soldiers were administered a conditional discrimination task (AX+/BX−). In this paradigm, A, B, and X are neutral stimuli. X combined with A is paired with a shock (AX+ trials); X combined with B is not (BX− trials). Fear inhibition was measured (AB trials). Startle electromyogram responses and shock expectancy ratings were recorded. PTSD symptoms were measured at 2 months and at 9 months after deployment.

Results showed that greater startle responses during AB trials in individuals who discriminated between danger (AX+) and safety (BX−) during conditioning, predicted higher PTSD symptoms at 2 months and 9 months post-deployment. The predictive effect at 9 months remained significant after controlling for critical incidents during previous deployments and PTSD symptoms at 2 months. Responses to AX+ or BX− trials, or discrimination learning (AX+ minus BX−) did not predict PTSD symptoms. It is concluded that impaired fear inhibition learning seems to be involved in the persistence of PTSD symptoms.



## 1. Introduction

Posttraumatic stress disorder (PTSD) is characterized by re-experiencing of the trauma, avoidance of its reminders, and hyperarousal (American Psychiatric Association, 1994). Early after trauma, PTSD symptoms are relatively common (Shalev et al., 1996), but, generally, only about 9% of trauma-exposed individuals develop PTSD (Breslau et al., 1998).

Fear conditioning models may explain why PTSD symptoms persist (Engelhard et al., 2009; Pitman et al., 1993). According to contemporary conditioning models (see Engelhard et al., 2009) the traumatic event (unconditioned stimulus; US) triggers an unconditioned response, characterized by strong arousal and fear. Previously neutral (conditioned) stimuli (CSs), like sights, sounds, and smells present at the time, become associated with the US. As a result of this CS–US pairing, CSs may later activate the representation of the US in absence of the actual US, leading to a conditioned fear response such as re-experiencing and hyperarousal symptoms. Usually, when the CS is no longer followed by the US, acquired fear extinguishes (the individual learns that the CS no longer predicts the US). A breakthrough in the understanding of persistent fear is that extinction involves inhibitory learning (Bouton, 2002; Myers et al., 2006) which results in two acquired meanings of the CS: the originally-learned excitatory meaning (CS–US) and the new inhibitory meaning (CS - no US). In trauma-exposed individuals with persisting PTSD symptoms, no or incomplete inhibitory learning may occur.

It has been proposed that the failure to inhibit the fear response in the presence of safety signals plays a prominent role in PTSD's development and persistence (Davis et al., 2000). Essentially, the inability to suppress fear responses in the presence of safety may be due to (a) the inability to discriminate between danger and safety

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signals and (b) the inability to inhibit the fear response to safety signals. The first notion suggests that during acquisition, people with PTSD may mistake the safety signal for the danger signal. In most conditioning paradigms, these stimuli share many stimulus properties (e.g., both are colored shapes; Lissek et al., 2005). Support for such stimulus-generalization is given in fear-conditioning studies reporting more pronounced psychophysiological responses during safety signals (but not during danger signals) in PTSD-patients than in trauma-exposed controls, including electrodermal responses (Peri et al., 2000) and fear-potentiated startle (Grillon and Morgan, 1999). In the latter study, this lack of differential responding was not attributable to a failure to learn the CS–US contingency on a cognitive level (Grillon and Morgan, 1999).

The second notion has received much less research attention. Thus, it is unclear whether individuals with PTSD are less able to inhibit the fear response in the presence of safety cues, even if they have learned to discriminate between danger and safety cues. Critically testing this requires an experimental paradigm that allows the independent assessment of excitatory and inhibitory associations and transfer of inhibition, e.g., the conditional discrimination paradigm called "AX+/BX−" (Jovanovic et al., 2005), originally developed for animal research (Myers and Davis, 2004). In this paradigm, neutral stimulus X is paired with a US (i.e., airblast to the throat) when X is presented with stimulus A (AX+), and not when X is presented with B (BX−). Thus, after AX+ and BX− trials, A has become excitatory, and B is inhibitory. In subsequent crucial AB trials, reduced fear to A is expected, because B transfers its inhibitory properties to A (Jovanovic et al., 2005). A recent study using this paradigm found that individuals with high PTSD symptom-levels showed (a) no significant difference in discrimination between danger and safety (AX+ vs. BX− trials), and (b) did not show reduced fear potentiated startle to AB trials (Jovanovic et al., 2009a). The second finding may directly follow from the first: if no discrimination learning occurs, no inhibitory learning can take place. Similar results were found in another study that compared trauma-exposed individuals with PTSD to trauma-exposed individuals with no disorder or with major depression (Jovanovic et al., 2010a). Results of both studies indicate that individuals with PTSD show a lack of discrimination between danger and safety cues, and do not show fear inhibition under safe conditions. Although the lack of fear inhibition in PTSD may be secondary to failed discrimination learning, the effect size for impaired inhibition learning in the second study (Jovanovic et al., 2010a) was twice as large as the effect size for impaired discrimination learning. This suggests that at least some individuals with PTSD show impaired transfer of inhibition after successful safety cue learning or, alternatively, that impaired transfer of inhibition is a more robust measure of reduced fear inhibition (cf. Jovanovic et al., 2010a). To elucidate whether deficient fear inhibition learning is implicated in the development of PTSD, analyses should focus on participants who showed successful discrimination between the danger and the safety cue.

An important question is whether impaired discrimination learning and impaired fear inhibition learning predict the development of persistent PTSD symptoms. Since previous studies were cross-sectional (Jovanovic et al., 2009b, 2010a), studies using longitudinal designs in individuals at risk for PTSD symptoms are needed to elucidate whether abnormalities in fear conditioning are vulnerability factors or epiphenomena of disease processes.

The current study examined whether reduced fear inhibition learning predicts the persistence of PTSD symptoms using a longitudinal design in a sample of recently trauma-exposed soldiers deployed to Afghanistan. More specifically, we tested whether the persistence of PTSD is predicted by (a) a failure to discriminate between danger and safety (i.e., smaller differences between fear responses during AX+ trials relative to BX− trials) or by (b) a failure to inhibit the fear response in the presence of safety (i.e., stronger fear responses during AB trials).

## 2. Method

### 2.1. Participants and procedure

Participants were Dutch Royal Army soldiers ($N = 144$) deployed to Afghanistan from November 2009 to March 2010 and participating in a larger project (Lommen et al., 2013). About 2 months post-deployment, every two out of three soldiers participating in the larger project were approached for participation in the current study. Assessments at pre-deployment (baseline characteristics), 2 months post-deployment (conditional inhibition paradigm, PTSD-diagnosis and PTSD-questionnaire) and 9 months post-deployment (PTSD-questionnaire) took place at the military bases in the Netherlands. They were performed by trained clinical psychologists.

Participants gave oral and written informed consent. The study was approved by the Institutional Review Board of the University Hospital Maastricht.

### 2.2. Experimental procedure

The AX+/BX− conditional discrimination paradigm (cf. Jovanovic et al., 2005; Jovanovic et al., 2009a) was presented using the software 'Presentation' (Neurobehavioral Systems Inc, www.neurobs.com). Each session consisted of a startle habituation phase followed by three conditioning blocks and a fear inhibition block without any breaks. Conditioned stimuli (CSs) were a compound of two different shapes presented on a computer screen. AX+ trials consisted of cue 'A' paired with a common cue 'X', BX− trials consisted of cue 'B' paired with cue 'X'. The fear inhibition test stimulus was a compound of the previously conditioned A and B cues and was used to determine transfer of inhibition of B to the fear response to A. Cues A, B, and X were blue, black or purple shapes (star, triangle or square; counterbalanced across CSs) and any given pair of cues involved two different colors and shapes. For each compound stimulus, the cues were presented simultaneously with a plus sign between the shapes to facilitate elemental processing (Jovanovic et al., 2010a, 2010b). The aversive stimulus (US) was a mild electric shock (500 ms, .2–4.0 mA) delivered to two fingers of the non-dominant hand. Before the task it was individually set at a 'highly annoying but not painful' level using a work-up procedure (cf. Orr et al., 2000).

The habituation phase consisted of six startle probes presented alone (noise-alone trials, NA). The conditioning phase consisted of three blocks. Each block included 12 trials: four AX+ trials, four BX− trials and four NA trials, in random order. Each trial included a startle probe. Immediately after the conditioning phase, a block of three AB trials was presented. AX+ trials were always followed by the US (reinforced stimulus), whereas the BX− and AB trials were not (non-reinforced stimulus). In the AX+ trials, shape A and X were presented on the computer screen during 6040 ms. The 40 ms startle probe was presented at the end of the first 5 s, and was followed after 500 ms by the US (duration: 500 ms). The shapes remained on the screen for an additional 250 ms, such that both shapes were visible during the startle probe and the US. During the BX− trials, B and X were presented simultaneously during 5040 ms, and the startle probes were presented at 5 s from the start of the trial. The AB trials were similar to the BX− trials. In all trials, visual analog scales (VASs) for measuring US-expectancy were presented at the bottom of the screen during the first 5 s, after which they disappeared. Inter-trial intervals were of randomized duration (range: 9–22 s).

### 2.3. Fear potentiated startle

Fear-potentiated startle (i.e., relative increase in the magnitude of the acoustic startle reflex elicited in the presence of a CS previously paired with an aversive US; Grillon and Baas, 2003) was assessed to obtain an objective measure of fear responses, tapping directly into the amygdala (Davis et al., 1993).

Acoustic startle probes were 40-ms 95-dB(A) bursts of white noise with an instant rise and fall time, and delivered binaurally through headphones. The eye-blink reflex was measured by recording electromyogram (EMG) activity from the orbicularis oculi muscle below the left eye with two disk electrodes (Ag–AgCl; 4-mm inside diameter). The ground electrode was placed on the forehead. The raw EMG signal, sampled at 1000 Hz, was amplified (10 K) and filtered (13 Hz high-pass; 150 Hz low-pass) by a Coulbourn V75-04 Isolated Bioamplifier with Bandpass Filter (Blumenthal et al., 2005).

Startle amplitudes were computed as the difference between the maximum EMG value within 20–150 ms after stimulus onset and the average EMG value during baseline (−40 to +10 ms around stimulus onset). Response onset latency was set at 21–80 ms (Blumenthal et al., 2005). All amplitudes were standardized into *Z*-scores.

We computed the mean startle amplitude across the final 3 trials for each trial type (AX+ and BX−), subtracted by the mean of the 3 final NA trials in that block. This resulted in mean AX+ and mean BX− startle response scores. The AB startle score was defined as the mean of the 3 AB trials minus the mean of the 3 NA trials in that condition. In addition, differential startle responding at the end of the conditioning phase was defined as the startle response score for the final three AX+ trials minus this score for BX−. Higher scores indicated better differential responding on the startle outcome.

### 2.4. US-expectancy

Participants rated their expectation of the US to follow during each stimulus presentation on a VAS (0 = certain no electric stimulation; 100 = certain electric stimulation; cf. Engelhard et al., 2009). AX+ and BX− expectancy scores were the mean of the final 3 trials in that phase. The AB expectancy score was the mean expectancy score of the 3 AB trials. In addition, differential responding at the end of the conditioning phase was defined as the mean expectancy score of the final 3 AX+ trials minus this score for BX−. Higher scores indicate stronger differential startle responding.

### 2.5. Other measures

Baseline characteristics (gender, age, marital status, education, years in the army) were assessed.

The number of critical incidents during previous deployments was measured using an adapted version of the Potentially Traumatizing Events Scale (PTES; Engelhard et al., 2007a). The original PTES includes 21 items recording war-zone related stressors. The item "patrolling areas with landmines" was omitted and two items were added: "having injured civilians due to own action", "being told that a colleague got killed". For each event, individuals rated its negative impact at the time on a 1 (no impact) to 4 (extremely)-point Likert scale. We calculated the total number of stressors (range 0–22). To assess critical incidents during the latest deployment two items were added to the PTES, based on information provided by the Defense staff concerning mission-related situations. These items were "seeing dead or injured Afghan soldiers/police" and "conflict situation with the Afghan police". We calculated the number of reported incidents (range 0–24).

**Table 1**
Characteristics of the sample ($n = 144$).

| Characteristic | *n* (%) |
|---|---|
| Male gender | 143 (99.3) |
| With partner[1] | 102 (70.8) |
| Education[2] | |
| Elementary school | 5 (3.4) |
| High school | 127 (88.4) |
| College/university | 9 (6.2) |
| Previously deployed | 76 (52.8) |
| PTSD diagnosis according to SCID[3] | 1 (.7) |
| | M (SD) |
| Age | 23.5 (5.0) |
| Years in the army | 4.9 (4.3) |
| Number of critical incidents during previous deployments | 6.4 (7.0) |
| Number of critical incidents during latest deployment | 14.3 (4.5) |
| PTSD symptoms (PSS-SR total score) at 2 months | 3.3 (4.2) |
| PTSD symptoms (PSS-SR total score) at 9 months | 4.1 (5.4) |

*Note* [1]Data of 140 participants available; [2]Data of 141 participants available; [3]Structured Clinical Interview for DSM-IV Axis I Disorders Patient Edition (30,31).

PTSD symptoms during the previous month were measured with the Posttraumatic Symptom Scale–Self Report (PSS-SR; Foa et al., 1993; Engelhard et al., 2007a). The PSS-SR contains 17 items corresponding to the DSM-IV symptoms of PTSD ranging from 0 (not at all) to 3 (almost always) and scores range from 0 to 51. In this study, Cronbach's alpha was $\alpha = .83$ at 2 months and $\alpha = .88$ at 9 months.

The Structured Clinical Interview for DSM-IV Axis I Disorders Patient Edition (SCID-I, Patient Edition; First et al., 1996; Van Groenestein et al., 1999) was used to diagnose PTSD.

### 2.6. Statistical analysis

Repeated measures analysis of variance (ANOVA) was performed with trial type (AX+ vs. BX− vs. AB) as within-subjects factor. Dependent variables were differential startle responses (defined above) and expectancy scores. Significant effects between trial types were analyzed with post-hoc *t*-tests. To separate difficulty in discriminating AX+ and BX− from difficulty in inhibiting responses to A when combined with B, additional analyses included only those participants who displayed differential conditioned responding ("learners"). Learners were participants showing a larger mean startle potentiation to the final three AX+ trials than to the final three BX− trials. In contrast, non-learners showed no difference between startle potentiation to the final three AX+ and BX− trials or startle potentiation to the final three AX+ trials was smaller than to the final three BX− trials. We chose this relatively liberal criterion since requiring a larger difference between AX+ and BX− would exclude too many participants. Moreover, we included only the participants who had US-expectancy higher than 60 to the final three AX+ trials (cf. Lommen et al., 2013) and lower than 40 to the final three BX− trials.

Hierarchical linear regression analyses were used to analyze whether inhibition scores during the conditional discrimination task administered at 2 months post-deployment predicted PSS-SR total score at 9 months post-deployment, employing the compound scores for AX+, BX− and AB responding and differential responding to AX+BX− as defined above. Furthermore, since PSS-SR total score was skewed to the right, it was root-square transformed to normal distribution. Since pre-specified hypotheses were tested, no formal corrections for multiple comparison were carried out (Perneger, 1998). Analyses were carried out in SPSS 12, and two-tailed tests are reported, with $p < .05$.

**Table 2**
Critical incidents experienced during deployment by Dutch soldiers in Afghanistan ($N = 144$).

| Critical incident | Item experienced (%) | Item rated as moderately to extremely negative, % |
|---|---|---|
| 1. Standing guard during patrol | 94.4 | 8.3 |
| 2. Disarming civilians | 69.9 | .7 |
| 3. Fear of being ambushed or attacked | 93.7 | 9.1 |
| 4. Going on patrols or performing other dangerous duties | 96.5 | 3.5 |
| 5. Fear of having unit fired on | 95.8 | 8.4 |
| 6. Locating unexploded land mines | 86.0 | 7.0 |
| 7. Needing to manage civilians in chaotic conditions | 73.6 | 2.8 |
| 8. Fear that you might be taken hostage | 55.6 | 4.2 |
| 9. Witnessing violence | 88.1 | 4.2 |
| 10. Witnessing an explosion | 84.7 | 9 |
| 11. Having to aid in the removal of human remains | 39.6 | 3.5 |
| 12. Having to aid in the removal of unexploded ordnance | 45.1 | 0 |
| 13. Being injured because of an accident | 12.5 | 0 |
| 14. Being shot at | 59.7 | 6.2 |
| 15. Being injured because of an assault/attack | 8.3 | .7 |
| 16. Seeing dead or injured civilians | 79.2 | 4.9 |
| 17. Seeing dead or injured NATO (non-Dutch) soldiers | 28.5 | 4.2 |
| 18. Seeing dead or injured Afghan soldiers/police | 61.1 | 1.4 |
| 19. Seeing dead or injured Dutch soldiers | 20.1 | 6.2 |
| 20. Seeing human remains | 70.8 | 5.6 |
| 22. Experienced sexual harassment during the deployment | 7.6 | .7 |
| 23. Having injured civilians by own action | 31.9 | 1.4 |
| 24. Being informed of a Dutch soldier who got killed | 66.0 | 22.2 |
| 25. Conflict situation with the Afghan police | 66.9 | 23.2 |

## 3. Results

### 3.1. Characteristics of participants

Table 1 shows participants characteristics and Table 2 critical incidents during the latest deployment to Afghanistan. Participants lost to follow-up ($n = 17$) had lower educational levels ($\chi^2(2) = 12.2$, $p < .01$), were younger (mean difference 1.8 years; 95%CI .1–3.5, $p < .05$), spent more years in the army (mean difference 1.5 years; 95%CI .3–2.8, $p < .05$), and reported more incidents during previous deployments (mean difference 5.1; 95%CI .2–9.9, $p < .05$).

Learners ($n = 66$; 45.8%) did not differ on any of the baseline characteristics compared to non-learners ($n =$ ; 54.2%), except that learners had experienced fewer incidents during previous deployments (mean number of incidents: 4.8; SD = 6.0) than non-learners (mean number: 7.7; SD = 7.6; $t(141,50) = 2.56$; $p < .05$).

### 3.2. Fear-potentiated startle

Fig. 1 depicts mean startle potentiation relative to NA in the three trial types. The repeated measurements ANOVA for startle magnitude, with trial type (AX+ vs. BX− vs. AB) as within-subjects factor, showed a main effect for trial type ($F(2,286) = 6.13$, $p < .01$). Post-hoc comparisons revealed that, as expected, startle was robustly potentiated since AX+ startle responses were higher than BX− startle responses (mean difference = .18√μV, 95% CI = .01–.34, $p < .05$). BX− startle responses were lower than AB startle responses (mean difference = −.32√μV, 95% CI = −.51–.13, $p < .01$), but there was no difference between AX+ and AB startle responses (mean difference = .14√μV, 95% CI = −.04-.32, $p = .14$), suggesting that participants in our study did not show transfer of inhibition on the AB trials. Similar results were obtained when only the learners ($n = 66$) were included. However, after including only the participants ($n = 48$) of whom startle potentiation to AX+ than to BX− was more than .5 points higher, we found that startle to AX+ was significantly higher than to AB (mean difference = .34√μV, 95% CI = −.00 to .68, $p = .05$).

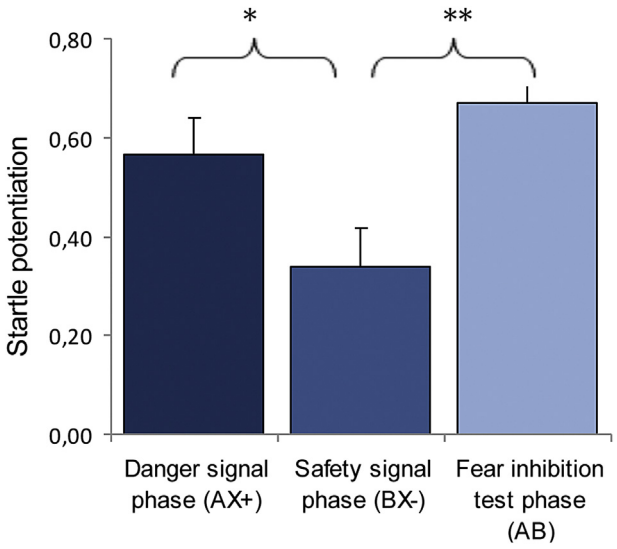


**Fig. 1.** Fear-potentiated startle responses on AX+, BX−, and AB trials. *$p < .05$; **$p < .01$ ($N = 144$; total sample). Scores are raw means.

Hierarchical linear regression analyses including all participants showed that PSS-SR scores at 2 months were not predicted by startle to AX+ ($R^2 = .01$; $\beta = -.08$, $p = .34$), BX− ($R^2 = .00$; $\beta = .02$, $p = .86$), AB ($R^2 = .01$; $\beta = .09$, $p = .27$) or differential responding ($R^2 = .01$; $\beta = -.08$, $p = .35$). In addition, PSS-SR scores at 9 months were not predicted by startle to AX+ ($R^2 = .00$; $\beta = .06$, $p = .47$), BX− ($R^2 = .00$; $\beta = .06$, $p = .48$), AB ($R^2 = .40$; $\beta = .12$, $p = .18$) or differential responding ($R^2 = .40$; $\beta = .00$, $p = .97$).

After including only the learners ($n = 66$), PSS-SR scores at 2 months were significantly predicted by startle to AB ($R^2 = .12$; $\beta = .34$, $p < .01$). Furthermore, PSS-SR scores at 9 months were significantly predicted by startle to AB ($R^2 = .02$; $\beta = .38$, $p < .01$) and remained a significant predictor for PSS-SR scores at 9 months ($R^2 = .40$; $\beta = .26$, $p < .05$) after controlling for critical incidents during previous deployments ($\beta = -.20$; $p = .06$) and PSS-SR scores at 2 months ($\beta = .52$; $p < .001$).

Spearman correlations between 9 months PSS-SR scores and AB startle responses are plotted in Fig. 2.

### 3.3. US-expectancy

Fig. 3 depicts mean US-expectancy scores across the three trial types. Repeated measures ANOVA with trial type as within-subjects factor showed a main effect for trial type ($F(2,143) = 347.66$, $p < .001$). Post-hoc comparisons revealed that US-expectancy to AX+ was higher than to BX− (mean difference = 74.6, 95% CI = 80.7–68.5, $p < .001$) and, as expected, higher than to AB (mean difference = 54.3, 95% CI = 48.5–60.0, $p < .001$). In addition, US-expectancy to AB was higher than to BX− (mean difference = 20.3, 95% CI = 16.2–24.5, $p < .001$). Similar results were obtained when only the learners ($n = 66$) were included.

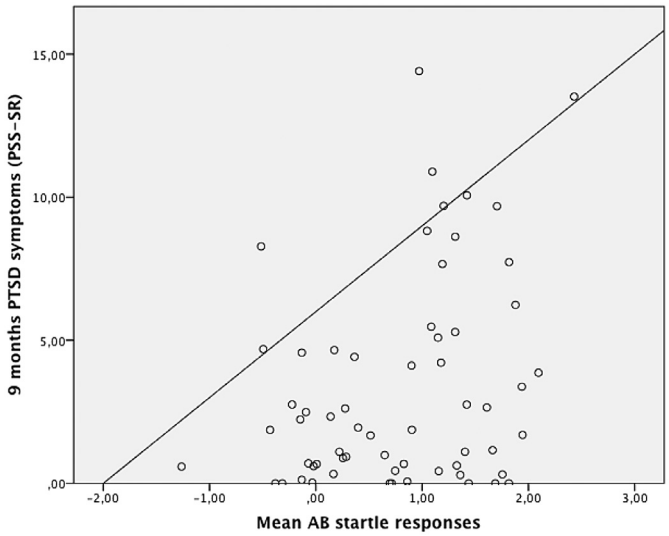


**Fig. 2.** Correlations between AB startle responses and 9 months PTSD symptoms ($n = 66$; learners).

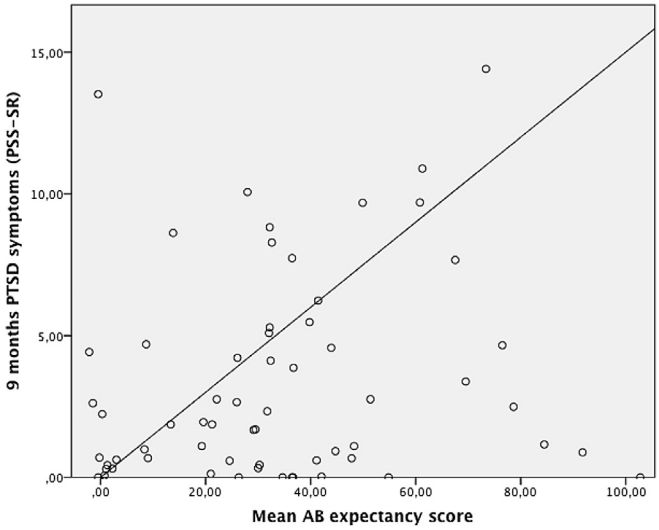


**Fig. 4.** Correlations between mean AB expectancy score and 9 months PTSD symptoms ($n = 66$; learners).

In the total sample ($n = 144$), PSS-SR scores at 2 months were not predicted by US-expectancy to AX+ ($R^2 = .00$; $\beta = .05$, $p = .59$), BX− ($R^2 = .00$; $\beta = .82$, $p = .41$) or differential responding ($R^2 = .40$; $\beta = -.02$, $p = .85$) but they were predicted by AB expectancy ($R^2 = .03$; $\beta = .17$, $p < .05$). Further, PSS-SR scores at 9 months were not predicted by US-expectancy to AX+ ($R^2 = .00$; $\beta = -.06$, $p = .51$), BX− ($R^2 = .01$; $\beta = .10$, $p = .25$) or differential responding ($R^2 = .01$; $\beta = -.09$, $p = .30$), but they were predicted by AB expectancy ($R^2 = .04$; $\beta = .20$, $p < .05$).

In the learners subsample ($n = 66$), PSS-SR scores at 2 months were significantly predicted by AB expectancy ($R^2 = .08$; $\beta = .27$, $p < .05$), but PSS-SR scores at 9 months were not predicted by AB expectancy ($R^2 = .05$; $\beta = .23$; $p = .08$). No predictive effect of AB expectancy ($R^2 = .34$; $\beta = .06$; $p = .60$) was found when controlling for critical incidents during previous deployments ($\beta = -.16$; $p = .16$) and PSS-SR scores at 2 months ($\beta = .57$; $p < .001$).

Fig. 4 shows Spearman correlations between AB expectancy scores and PSS-SR scores at 9 months.

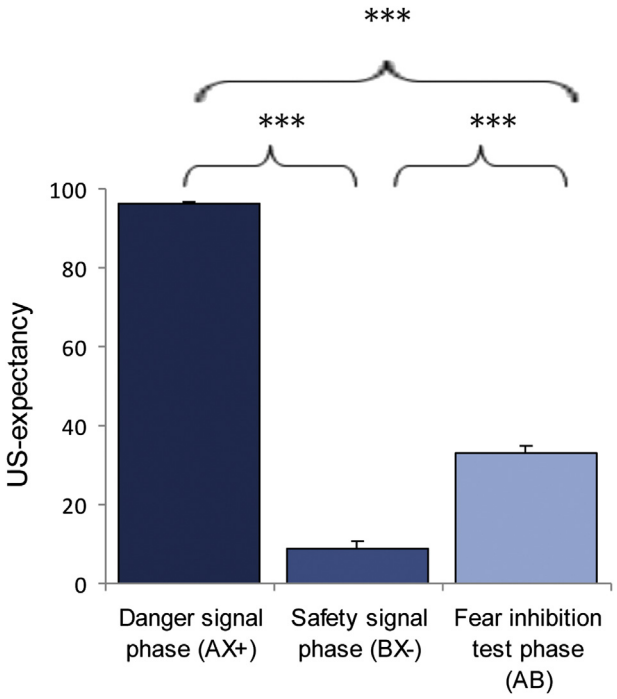


**Fig. 3.** Mean US-expectancy scores on AX+, BX−, and AB trials. ***$p < .001$ ($N = 144$; total sample). Scores are raw means.

## 4. Discussion

This study examined whether the inability to discriminate between danger and safety signals and inhibit the fear response was associated with PTSD symptoms at 2 and at 9 months after deployment to Afghanistan. Results showed that impaired fear inhibition learning, measured with fear-potentiated startle in individuals who discriminated between danger (AX+) and safety (BX−) during conditioning, was associated with PTSD symptoms at 2 and 9 months post-deployment. The predictive effect at 9 months remained significant over and beyond previous critical incidents and concurrent PTSD symptoms. Impaired discrimination learning was not associated with PTSD symptoms at both assessments, nor were responses to the danger or the safety cue. Results for the cognitive outcome, US-expectancy, were slightly different. Impaired fear inhibition learning predicted PTSD symptoms at 2 and 9 months post-deployment, but the predictive effect at 9 months was no longer present when including only the individuals showing discrimination learning during conditioning.

In this study, startle responses to AB trials were not generally smaller compared to AX+ trials, suggesting that not all participants learned to attribute safety to the cue predicting absence of the shock. However, the expected transfer of inhibition effect to the AB trials was found in participants showing clear differential responding on the startle measure. Possibly, fear inhibition learning may be reduced in recently trauma-exposed individuals, even when symptoms are mild. A recent study with the conditional discrimination paradigm in participants with acute stress disorder showed that impairments in safety learning are already evident within the first month after trauma (Jovanovic et al., 2013).

The US-expectancy outcome, however, revealed that fear inhibition occurred at a cognitive level, since US-expectancy scores to AB were lower than to AX+. Obviously, participants can be cognitively aware that no shock will follow when presented a safety signal, while not being able to suppress the amygdala-driven startle response (Davis, 2006; Baas, 2013). This confirms

previous studies showing that startle measurements and US-expectancy scores do not necessarily concur (Soeter and Kindt, 2011), especially when measuring responses to safety signals (Jovanovic et al., 2006).

Another interesting finding is that non-learners, i.e., participants not displaying differential conditioned responding on startle and US-expectancy, had experienced more critical incidents during previous deployments than learners. Although this may indicate a causal relationship between trauma history and deficits in discrimination learning, another possibility is that non-learners reported more past incidents than learners, e.g., due to memory deficits.

In line with conditioning theories, our study underscores the role of impaired inhibition of acquired fear in PTSD's development (Lissek et al., 2005; Mineka and Oehlberg, 2008). This impaired inhibition may be explained by insufficient inhibitory control of the prefrontal cortex over the amygdala (Jovanovic and Norrholm, 2011). When individuals learn that a CS no longer signals a US, the prefrontal cortex areas inhibit the amygdala-driven fear response such that the individual may refrain from a fear response at future CS presentations. This may resolve acute re-experiencing and hyperarousal, precluding development of chronic PTSD symptoms. It should be noted, however, that impaired fear inhibition learning explained only a small proportion of the variance in PTSD severity. This implies that other variables, such as the acute response (Ozer et al., 2003; Shalev and Freedman, 2005) or a lack of social support (Brewin et al., 2000) may be more important risk factors.

It is unclear whether reduced fear inhibition learning also predicts the onset of symptoms, since fear inhibition learning was not assessed before deployment. However, impaired pre-trauma extinction learning (Guthrie and Bryant, 2006; Lommen et al., 2013) and enhanced pre-trauma startle reactivity under low threat (Pole et al., 2009) predict the onset of PTSD symptoms. Since fear inhibition learning is assumed to play a role in fear extinction, fear inhibition learning may also be a pre-trauma vulnerability factor for PTSD symptoms. On the other hand, a study comparing extinction recall between monozygotic twins discordant for combat exposure indicated that only twins with PTSD had an extinction recall deficiency (Milad et al., 2008), suggesting that extinction recall is acquired as a result of PTSD.

A limitation of our study includes the low PTSD incidence. Although the low rates may appear remarkable when compared to PTSD rates previously reported in US army soldiers (see Sundin et al., 2010), they are consistent with other studies of Dutch (Engelhard et al., 2007b), British (Hotopf et al., 2006), and Danish (Berntsen et al., 2012) soldiers deployed to Iraq and/or Afghanistan, and recent methodologically rigorous studies in US soldiers (see McNally, 2012). However, low symptom levels may have limited statistical power and may impede generalizability to populations with higher levels of PTSD symptoms. In addition, our sample consisted mainly of young, male soldiers, which may be another factor limiting generalization of the results.

In sum, this study showed that impaired fear inhibition learning predicts the persistence of PTSD symptoms. Future studies may identify neurobiological and genetic factors implicated in fear inhibition learning. Findings from such studies may contribute to our knowledge about PTSD's etiology.

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## Conflicts of interest

No conflicts of interests are declared.

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