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# Amygdala Hyperactivity in Posttraumatic Stress Disorder: Disentangling Predisposing From Consequential Factors Using a Prospective Longitudinal Design

Lycia D. de Voogd, Mahur M. Hashemi, Wei Zhang, Reinoud Kaldewaij, Saskia B.J. Koch, Vanessa A. van Ast, Floris Klumpers, and Karin Roelofs

## ABSTRACT

**BACKGROUND:** Substantial interindividual differences exist in the vulnerability to develop posttraumatic stress disorder (PTSD) symptoms following trauma exposure. Identification of neurocognitive risk markers for PTSD symptoms could aid early assessment and identification of preventive intervention targets for PTSD, particularly in high-risk professionals. Therefore, large prospective longitudinal studies with pretrauma measurements are essential to disentangle whether previously observed neurobiological alterations in PTSD are a cause or consequence of trauma exposure or PTSD symptoms.

**METHODS:** In police recruits ( $n = 221$ ) without current trauma symptoms but at high risk for trauma exposure, we used functional magnetic resonance imaging to disentangle predictive and acquired neural markers of posttraumatic stress symptoms. Using an experimental paradigm, we investigated anticipatory threat responses and the switch into defensive action.

**RESULTS:** Recruits who showed relatively heightened dorsal amygdala responses and heightened amygdala-precuneus coupling during threat anticipation demonstrated relatively stronger increase in PTSD symptoms after trauma exposure. The experience of traumatic events, independent of PTSD symptoms, was associated with increased lateral amygdala activation in response to an aversive stimulus (i.e., receiving an electrical shock).

**CONCLUSIONS:** This prospective longitudinal study shows a predictive role for dorsal amygdala responsivity during threat anticipation for the development of trauma symptoms, while lateral amygdala responding to aversive events after trauma may reflect a failure to regulate. Our findings not only inform neurobiological theories of PTSD risk and vulnerability but also provide a starting point for prediction and intervention studies.

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Many individuals experience a traumatic event at some point during their life. Of those, approximately 5% to 20% develop posttraumatic stress disorder (PTSD), although these rates increase to ~35% in conflict-affected countries (1–4). Primary responders, such as police, experience particularly high rates of trauma exposure and therefore are at especially high risk for developing PTSD. Despite selection and training, 34% to 62% of police officers develop subsyndromal PTSD in the line of duty, while 7% to 13% develop full-blown PTSD [e.g., (5,6)]. Given the enormous personal and societal costs of PTSD symptoms (7), advancing understanding of individual risk variation would aid in early detection and allow targeted preventive interventions. However, current definitions of PTSD do not capture the mechanistic origins of these individual differences and how they emerge from our neurobiology (8,9).

Early seminal longitudinal neuroimaging studies pointed out that neural circuits crucially involved in threat detection [e.g., (10–16)] and regulation [e.g., (17,18)], such as the amygdala and prefrontal cortex, show structural and functional

alterations related to PTSD symptom development. Together, these studies suggest that PTSD is associated with heightened anticipatory threat arousal and poor regulation of these arousal responses [for reviews, see (19–23)]. However, because the number of well-powered longitudinal studies is still low, it is not clear whether heightened arousal and poor regulation occur because of trauma exposure (or related PTSD symptoms) or alternatively may rather present predisposing risk factors. To dissociate acquired from predictive factors, sufficiently powered prospective longitudinal studies are necessary with assessments before and after trauma exposure occurs.

Initial studies pointed out that the amygdala, a region implicated in threat detection (24,25), shows stronger threat reactivity in patients with PTSD than in control participants [e.g., (21,22,26,27)]. Such exacerbated amygdala threat response is already present immediately after trauma and is predictive of subsequent PTSD symptoms. Namely, previous studies in which individuals were assessed at emergency departments (28–30) demonstrated that hyperamygdala

SEE COMMENTARY ON PAGE 376

responding, or altered connectivity patterns, in response to threat could be predisposing factors. Based on the results of those studies, one cannot rule out the possibility that PTSD-relevant processes are a consequence of trauma exposure, and dissociating acquired from predictive factors requires assessments that occur before and after trauma occurs. Consistent with these observations, prospective neuroimaging studies have provided evidence that hyperamygdala responding to threat or threat anticipation may be a predisposing factor. Increased amygdala responding to threat is already present in individuals who later develop PTSD symptoms before any trauma exposure occurs (10,13,16,31) [but see (15)]. Additionally, salience network connectivity changes during rest were identified as a potential marker for trauma-related symptom development (32). It is important to note that prospective neuroimaging studies are methodologically challenging and therefore are scarce to date (33). They also typically have relatively small sample sizes that may not be sufficiently powered to detect interindividual differences (34) in underlying neurobiology. Moreover, these studies have mostly been performed with specific groups of individuals, such as military personnel, who will experience excessive trauma. In addition, they experience specific life-threatening and combat-related violence. To validate and generalize previous findings, it is crucial to replicate and extend these findings in other populations and with other traumatic events. Finally, previous studies (10,13,16,31) have typically compared average responses in an experimental group to responses in a control group. This approach does not allow for assessment of interindividual differences on a continuum from strongly resilient to full-blown psychopathology (8,9,35,36).

Here, in a large cohort of police recruits ( $n = 221$ ), we prospectively investigated the neural threat circuitry that underlies the early development of PTSD symptoms. Police recruits were tested at the start of their police training before they were sent into field work for their first emergency aid duty (baseline session) and were tested again after this period (follow-up session, ~16 months postbaseline) [see (37) for the protocol paper]. Participants performed a well-established Go/NoGo under threat (GUNT) paradigm (38–40) while undergoing functional magnetic resonance imaging (fMRI). We opted for an active coping paradigm, unlike previous studies that exclusively measured blood oxygen level-dependent (BOLD) response patterns in passive paradigms (e.g., response to faces). Such a paradigm allowed us to study potential alterations in threat processes involved in active threat coping beyond the amygdala, including the periaqueductal gray (PAG), a region related to freezing states and defensive actions (38–40). Measurements of BOLD-fMRI during acute threat of electrical stimulation were taken during threat anticipation and subsequent defensive action. The PTSD Checklist for DSM-5 (PCL-5) was administered at baseline and follow-up to measure the development of PTSD symptoms. Based on previous findings (10,13,16), we predicted that interindividual differences in BOLD-fMRI response patterns in the neural threat circuitry, including the amygdala, during acute threat anticipation would predict later PTSD symptom development. Specifically, we expected a positive correlation between amygdala activation at baseline and subsequent PTSD symptom increase. We also investigated whether (de)

activation patterns that have previously been observed (39) during threat anticipation and switch to defensive actions, including the PAG, would predict later PTSD symptom development. Finally, we investigated acquired changes in these circuits following trauma exposure.

## METHODS AND MATERIALS

### Participants

Participants were recruits from the Dutch Police Academy. A total of 340 participants completed the baseline assessment, and 271 (79.7%) completed the follow-up assessment. See the [Supplement](#) for more details.

Because we aimed to predict the development of trauma-related symptoms, we included participants who experienced their core traumatic event between baseline and follow-up ( $n = 222$ ) (17,32,41,42), as assessed by a clinical interview using the Clinician-Administered PTSD Scale for DSM-5 (43), and who did not have PTSD symptoms above the clinical cutoff at baseline (PCL-5 total score  $>33$ ) (44), which led to the exclusion of 1 participant. In most cases, the core traumatic events occurred in the context of police work (86%) but could also involve personal events unrelated to work (14%). The final sample was  $n = 221$  (60 females, 161 males; 18–45 years [mean = 24, SD = 5]), and the maximum available data were included for each analysis. Three of 221 participants had PTSD symptoms above the formal clinical cutoff at follow-up (PCL-5 total score  $>33$ ) (44). However, taking all proposed prevalence criteria into account, 12 individuals met criteria for PTSD, and 61 individuals met criteria for subthreshold PTSD (see the [Supplement](#) for details). There was missing data for trauma exposure at follow-up ( $n = 8$ ). MRI data were available for 210 participants at baseline, 182 at follow-up, and 179 for both sessions. The project was approved by the Independent Review Board Nijmegen and was conducted in accordance with their guidelines (Registration No. NL48861.072.14).

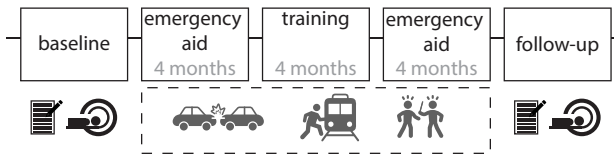
### Procedure

This study was part of a larger prospective study (Netherlands Trial Registry NTR6355; <https://onderzoekmetmensen.nl/en/trial/24254>). The procedure was similar for the baseline and follow-up sessions. During the baseline session, police recruits were at the start of their police academy training and without exposure to emergency aids. During the follow-up session, police recruits had served in police-related emergency aid services for approximately 8 months, during which they had been exposed to traumatic events. See [Figure 1A](#) and the [Supplement](#) for details.

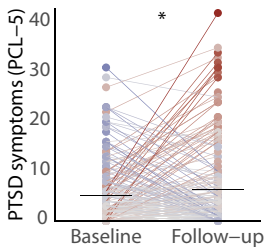
### Questionnaires

As registered in the protocol article (37), the primary outcome measure was change in PTSD symptom severity as assessed by the PCL-5 (43,44). The PCL-5 was completed based on an event that was selected as most disruptive by the recruits from the Life Events Checklist for DSM-5. Participants also completed the Police Life Events Scale (PLES) twice to measure police work-related trauma incidence once before and once during the training period (45). See the [Supplement](#) and [Figure S1](#) for more details.

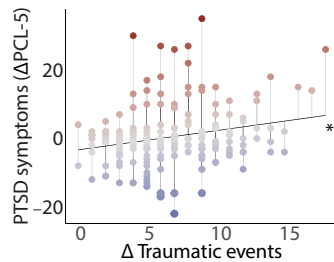
## A Study timeline



## B PTSD symptoms



## C Vulnerability



PTSD symptom change ( $\Delta$ PCL-5)

Vulnerability

**Figure 1.** (A) Timeline of the study. Between baseline and follow-up, police officers gained experience in emergency aid (2 periods) as part of their training, encountering potentially trauma-related events. (B) Post-traumatic stress disorder (PTSD) symptoms on the PTSD Checklist for DSM-5 (PCL-5) for the baseline and follow-up measurement. The colors of the lines indicate individual differences in symptom change. (C) Positive correlation between baseline and follow-up ( $\Delta$ PLES) and PTSD symptom change ( $\Delta$ PCL-5) from baseline to follow-up. The colors indicate the relative change in PTSD symptoms with regards to the traumatic events experienced. Red dots above the regression line indicate more vulnerability, and blue dots below the regression line indicate less vulnerability. \* $p < .05$ . PLES, Police Life Events Scale.

The PCL-5 baseline score, PLES baseline score, and  $\Delta$ PLES score were log-transformed to correct for a skewed distribution before inclusion as covariates.

## The GUNT Paradigm

Participants completed the GUNT paradigm (38–40) during each session (baseline, follow-up). The paradigm involved 4 practice trials (not included in the analyses) and 5 blocks of 28 trials (for a total of 140 trials). Participants were instructed to detect whether a virtual opponent drew a gun or a phone and to only shoot the opponent upon gun draw. They were instructed to refrain from shooting upon phone draw. There was 1 high-threat opponent and 1 low-threat opponent (counterbalanced across participants). If participants refrained from shooting (or were too late in responding) in response to a gun draw, they were punished by being shot by the opponent. If participants shot the opponent with a phone, participants were punished by being shot by a virtual police officer who was standing in the back of the garage. On high-threat trials, being shot was associated with receiving visual feedback and aversive electric shocks. On low-threat trials, being shot was associated only with visual feedback. The duration of the response window was titrated to prevent ceiling effects for performance. See the Supplement for details.

## Peripheral Measurements and Stimulation

We measured heart rate through finger pulse recordings using a pulse oximeter affixed to the ring finger of the left hand. Electrical shocks were delivered via 2 Ag/AgCl electrodes attached to the distal phalanges of the second and third fingers of the right hand using a MAXTENS 2000 (Bio-Protech) device. See the Supplement for details.

## MRI Statistical Analyses

MRI data were preprocessed in standard stereotactic (MNI152) space using SPM12 (<http://www.fil.ion.ucl.ac.uk/spm>; Wellcome Department of Imaging Neuroscience). See the Supplement for details.

For statistical analysis, during the anticipation phase, responses to the high-threat opponent and low-threat opponent were modeled. During the draw phase, responses to the draw were modeled using 6 separate regressors for correctly Go, correctly NoGo, and incorrect trials, for high-threat and low-threat trials separately. There were separate regressors for button presses and electrical shocks. Additionally, nuisance regressors were included in the model. See the Supplement for details.

Single-subject contrast maps from the first-level models of the anticipation phase and the draw phase were entered into second-level one-sample  $t$  tests. There were 3 main contrasts of interest: 1) anticipation high threat versus low threat, 2) draw threat level (high threat, low threat) by response (Go, NoGo) for correct responses only, and 3) responses to electrical shocks (compared with implicit baseline). We used a cluster-forming voxel-level threshold of  $p < .001$  (uncorrected). Alpha was set at .05, whole-brain familywise error (FWE) corrected at the cluster level using Gaussian random field theory-based methods (46).

In addition, we performed small-volume correction (SVC) (at the peak level  $p < .05$ , FWE corrected) on our regions of interest (ROIs) including the amygdala (defined by the Automated Anatomical Labeling [AAL] atlas) and PAG [defined by manual segmentation of a previous study: (47)]. Additionally, although we did not make specific predictions concerning the role of amygdala subregions due to the lack of relevant previous human literature, we opted to report the location of reported amygdala activations relative to known cytoarchitectonic subregions of the amygdala using the SPM anatomy toolbox (48) following previous literature (49). While the anterior prefrontal cortex (aPFC) was previously found to be associated with trauma resilience (17), the current paradigm lacked an explicit emotion regulation component, and therefore the aPFC was not specifically investigated here.

For the prediction analysis,  $\Delta$ PCL-5 (follow-up minus baseline), the log-transformed PCL-5 baseline score, log-transformed PLES baseline score, and log-transformed  $\Delta$ PLES score were included as covariates of interest in the second-level model. It should be noted that in the model, predictor and outcome variables are reversed to allow voxelwise modeling with all appropriate covariates. While counterintuitive, the correlational nature of these analyses renders the temporal order of events (where neural activity preceded the change in symptoms) irrelevant for the outcome of the statistical tests used.

To test the acquired effects of PTSD symptomatology on activation, single-subject contrast maps (baseline vs. follow-up) of the anticipation phase and the draw phase were

entered into second-level one-sample *t* tests.  $\Delta$ PCL-5 (follow-up minus baseline), the log-transformed PCL-5 baseline score, log-transformed PLES baseline score, and log-transformed  $\Delta$ PLES score were included as covariates of interest.

**MRI Data: Functional Connectivity**

As follow-up on the predictive activation findings, we conducted a psychophysiological interaction analysis with the amygdala (defined by the bilateral amygdala AAL mask) as a seed for the high-threat versus low-threat anticipation contrast. See the Supplement for details.

**RESULTS**

**Trauma Exposure and Symptom Development**

Police recruits experienced a greater number of traumatic events between baseline and follow-up than all of the traumatic events they reported having experienced before in their life (baseline: mean = 1.74, SD = 2.24; follow-up: mean = 6.67, SD = 3.42), indicating an increase in trauma load ( $\Delta$ PLES vs. PLES<sub>baseline</sub>:  $F_{1,212} = 440.62$ ;  $p < .001$ ;  $\eta^2 = 0.68$ ; 95% CI, 0.62

to 0.72). Mean PTSD symptom severity showed a small but significant rise following this increase in trauma load (mean = 6.37, SD = 8.47) compared with baseline (mean = 5.14, SD = 6.18;  $F_{1,220} = 4.70$ ;  $p = .031$ ;  $\eta^2 = 0.02$ ; 95% CI, -0.01 to 0.05), and there was substantial variation in PTSD symptom change. Moreover, the number of traumatic events experienced between baseline and follow-up ( $\Delta$ PLES) correlated positively with PTSD symptom increase over the same time period ( $r_{211} = 0.16$ ;  $p = .02$ ; 95% CI, 0.03 to 0.3) (see Figure 1).

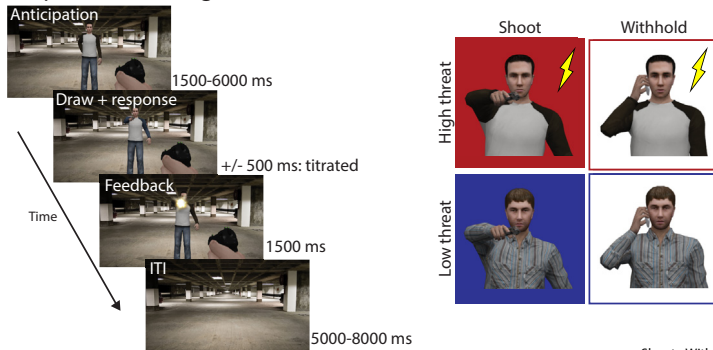
**Threat-Related Performance and Cardiac Changes**

We replicated typical GUNT effects on both behavior and heart rate responses (39). See Figure 2B, C and the Supplement for all statistical analyses.

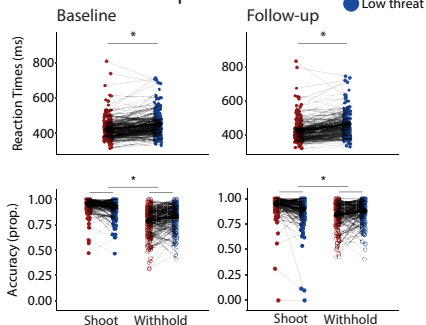
**Amygdala BOLD-fMRI Response and Connectivity Patterns During Threat Anticipation Predict Later Symptom Development**

During anticipation, increased activation in the left amygdala (high-threat compared with low-threat) at baseline ( $x, y, z = -18, -2, -14$ ; peak voxel  $z = 3.42, p = .018, FWE-SVC$

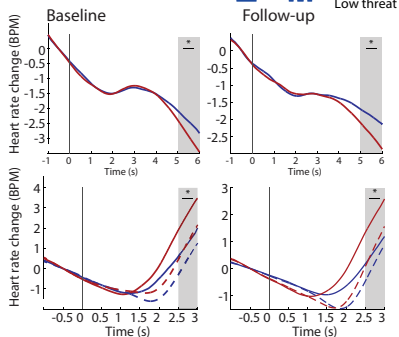
**A Experimental design**



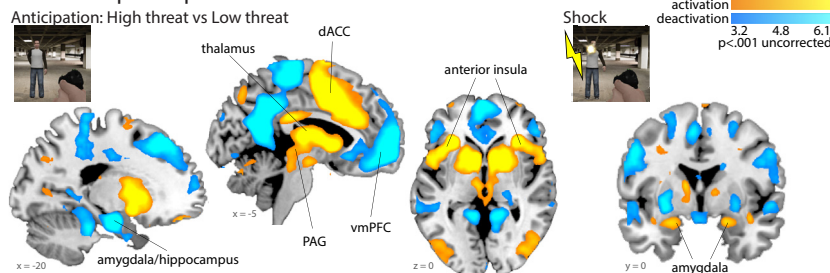
**B Behavioral responses**



**C Heart rate**



**D fMRI response patterns**



**Figure 2.** Experimental paradigm and main effects.

(A) In the Go/NoGo under threat paradigm, participants were presented with an avatar (high threat or low threat) for a variable amount of time (80% of trials 6000–6500 ms, 10% of trials 500–1500 ms, 10% of trials 1500–6000 ms), after which the avatar either drew a gun or a phone. Upon a gun draw, participants were required to shoot (make a Go action) or refrain from shooting (a NoGo action). In the high-threat condition, if participants made an incorrect decision, they received an electric shock to the fingers. (B) On average, participants responded faster on high-threat trials than low-threat trials and made more Go responses under high threat, resulting in higher accuracy on Go trials and lower accuracy on NoGo trials. See the Supplement for statistical analyses. (C) Average cardiac response across participants during the full trial time locked to the cue onset during baseline and follow-up (upper 2 panels). Participants showed threat-related bradycardia (high threat vs. low threat) during anticipation and trial time locked to the draw onset (lower 2 panels). Participants showed a heart rate increase for Go vs. NoGo trials. This increase was stronger during high-threat than low-threat trials. See the Supplement for statistical analyses. (D) Blood oxygen level-dependent-functional magnetic resonance imaging (BOLD-fMRI) response patterns for the contrast high threat vs. low threat during anticipation of the gun draw (left panel) and for the contrast shock vs. implicit baseline (right panel). For visualization purposes, a threshold of uncorrected  $p < .001$  was used. \* $p < .05$ . BPM, beats per minute; dACC, dorsal anterior cingulate cortex; PAG, periaqueductal gray; vmPFC, ventromedial prefrontal cortex.

Amygdala Hyperactivity in PTSD

was associated with a subsequent increase in PTSD symptoms at follow-up ( $\Delta$ PCL-5), while correcting for baseline symptom severity (baseline PCL-5) and trauma exposure history (baseline PLES and  $\Delta$ PLES) (see Figure 3). Increased activation in the left amygdala ( $x, y, z = -18, -2, -14$ ; peak voxel  $z = 3.34, p = .022$ , FWE-SVC) predicting subsequent increase in PTSD symptoms was also found when only the  $\Delta$ PCL-5 variable was included as a covariate, thereby mitigating the chance of confounds related to multicollinearity. Follow-up exploration revealed that the activation was centered in more dorsal areas in the basal forebrain and centromedial amygdala (CMA) ( $p_{\text{excess basal forebrain}} = 1.57$ , CMA = 0.37). No significant association was present within the PAG.

Given that this result was of central interest, we performed additional functional connectivity analyses with the bilateral amygdala as a seed. They revealed that amygdala-precuneus coupling (cluster size = 1088 mm<sup>3</sup>, cluster  $p = .006$ , FWE corrected) was positively associated with this increase in PTSD symptoms at follow-up ( $\Delta$ PCL-5) during threat anticipation (high threat > low threat), while correcting for baseline

symptom severity (baseline PCL-5) and trauma exposure history (baseline PLES and  $\Delta$ PLES).

In response to the draw (threat  $\times$  response interaction), no significant associations with increased PTSD symptoms at follow-up ( $\Delta$ PCL-5) were present across the whole brain or our ROIs (i.e., the amygdala and PAG).

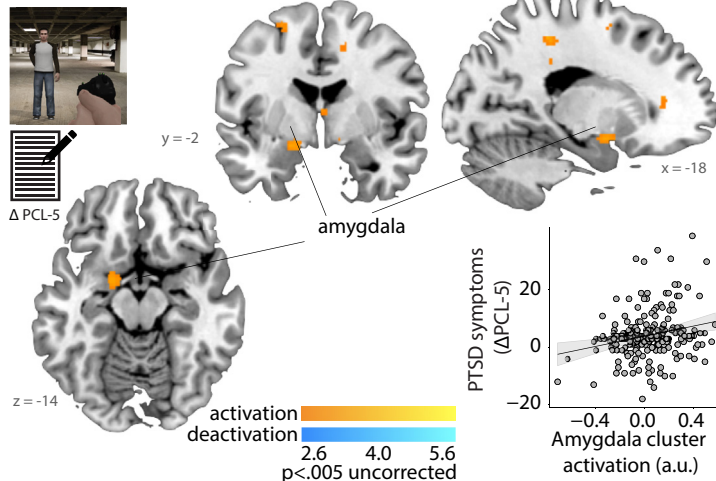
Together, these results indicate that increased threat reactivity of the amygdala and the amygdala-precuneus circuit may serve as a prospective vulnerability marker for the development of PTSD symptoms (see Figure 3A).

**Acquired Changes in BOLD-fMRI Response Patterns Related to Traumatic Events and Symptoms**

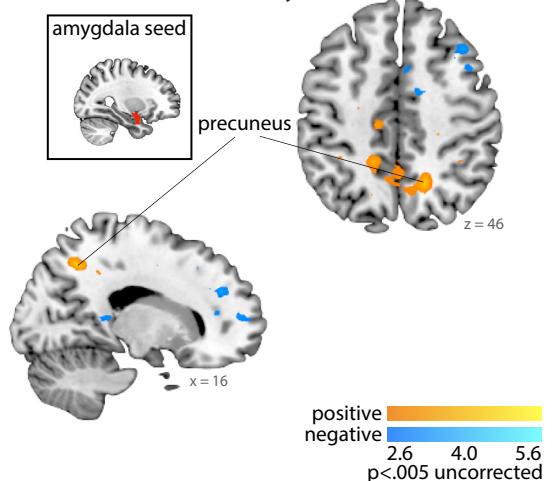
Finally, we assessed which changes in neural activity were associated with trauma exposure and PTSD symptom development. The change in BOLD responses during threat anticipation (high threat vs. low threat) and draw (threat  $\times$  response interaction) from baseline to follow-up were not significantly related to trauma exposure ( $\Delta$ PLES) or the

**A PTSD symptom prediction**

Anticipation: High threat vs Low threat

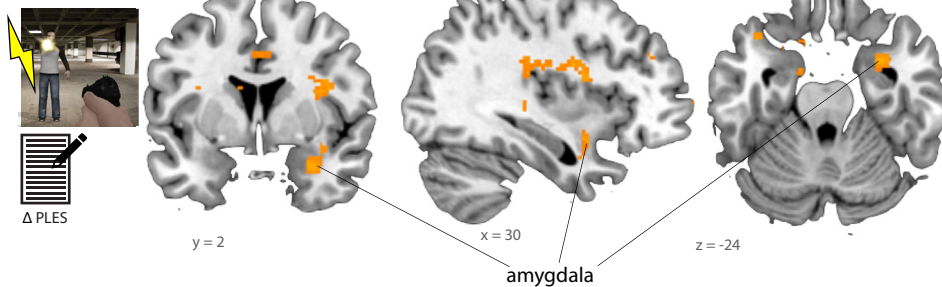


Functional connectivity (PPI)



**B Following trauma exposure**

Shock



**Figure 3.** (A) Relatively high amygdala activation during high-threat (vs. low-threat) anticipation at baseline prospectively predicts later symptom development. (B) Increased amygdala responses to the aversive stimulus following trauma exposure (follow-up session). For visualization purposes only, a threshold of uncorrected  $p < .005$  was used. a.u., arbitrary unit; PCL-5, PTSD Checklist for DSM-5; PLES, Police Life Events Scale; PPI, psychophysiological interaction; PTSD, posttraumatic stress disorder.

increase in PTSD symptoms from baseline to follow-up ( $\Delta$ PCL-5).

Individuals with more trauma exposure ( $\Delta$ PLES) showed a reduction in right amygdala responses to the aversive stimulus (i.e., electrical shock) ( $x, y, z = 36, 2, -24$ ; peak voxel  $z = 3.12, p = .048$ , FWE-SVC) relative to the baseline session. This activation centered in the basolateral amygdala (BLA) ( $p_{\text{excess BLA}} = 1.06$ ). Follow-up analyses revealed no impact of trauma load at baseline, while during the follow-up, session participants with more trauma exposure ( $\Delta$ PLES) showed an increase in amygdala responses ( $x, y, z = 30, 2, -24$ , peak voxel  $z = 3.63, p = .006$ , FWE-SVC). This activation centered in the BLA ( $p_{\text{excess BLA}} = 1.03$ ). No significant association was present within the PAG. These findings further suggest altered amygdala activation patterns in response to aversive stimuli following trauma exposure (independent of symptom changes).

## DISCUSSION

This prospective longitudinal study shows that dorsal amygdala hyperresponsivity during threat anticipation is associated with increased vulnerability for developing PTSD symptoms. In a sample of newly selected, symptom-free police recruits at high risk for trauma exposure, we were able to disentangle predictive and acquired effects of PTSD symptoms during threat anticipation and responsivity. Recruits with stronger pretrauma dorsal amygdala responses during threat anticipation demonstrated a stronger increase in PTSD symptoms after trauma exposure, while controlling for trauma load. In addition, stronger amygdala-precuneus coupling was similarly associated with a stronger increase in PTSD symptoms after trauma. Regarding acquired effects, the experience of traumatic events, independent of PTSD symptoms, was associated with increased lateral amygdala activation in response to the aversive stimulus (i.e., electrical shock). Thus, when disentangling predictive from acquired associations, we found evidence that distinct amygdala subregions may be implicated in the causes and consequences of PTSD symptoms. We thereby extend previous prospective studies by showing these findings in a well-powered sample, using an active (compared with passive) threat paradigm, and providing more specificity regarding the role of amygdala subregions.

Our findings are consistent with theoretical models that postulate that a hyperreactive salience network, including amygdala reactivity and connectivity, is a predictor of PTSD development (19,50). Early studies pointed out that regions crucially involved in threat processing are altered in individuals with PTSD (15,31) [see (51,52) for a meta-analysis]. Similarly, peritrauma studies with participants who were recruited from an emergency department and scanned 1 month posttrauma have found amygdala reactivity to negative emotional stimuli to be correlated with PTSD symptoms months later (30). However, posttrauma studies have indicated altered amygdala reactivity (15) that possibly normalizes over time (53). This finding suggests that amygdala hyperactivity could be a consequence of trauma exposure rather than a predisposing factor.

Our finding of increased dorsal amygdala responsivity to threat predicting PTSD symptoms is consistent with early

prospective neuroimaging studies with considerably smaller sample sizes (all  $n_s < 40$ ) (10,13,16). Our study extends these prospective studies in several ways. First, our sample allows for individual difference analyses. We were able to verify that the amygdala reactivity was correlated with PTSD symptoms dimensionally rather than only increasing in participants with core symptoms at the high extreme of the spectrum. Second, the participants in previous studies were combat paramedics or victims of a terroristic attack. Our findings show that such earlier findings generalize to a broader population that are confronted with more heterogeneous daily adversity (including, e.g., traffic accidents, physical assault, death, and illness) [see (17,32)]. Note that in our sample, a small minority of cases met full-blown PTSD criteria (1%–5% depending on the criteria applied) but covers a range of PTSD symptom levels. Third, we used an active coping paradigm under threat of shock. Previous paradigms involved passive amygdala reactivity to salient or facial stimuli. Our results show that these findings generalize to different contexts and different levels or types of threat. In our paradigm, participants had to make accurate decisions to minimize the risk of receiving an electrical shock. Fourth, in our analyses, we controlled for trauma load and thereby took into account the PTSD symptom increase relative to the individual deviation from the study sample's normative relationship between adversity and symptoms (e.g., the regression residual), similar to previous studies that focused on resiliency [e.g., (54)]. Finally, our results highlight that activation of distinct amygdala subregions may contribute to vulnerability for developing PTSD symptoms while disentangling predictive from acquired consequences.

How do our findings inform theoretical models regarding the role of the amygdala in PTSD vulnerability?

The most consistent functional abnormality in human PTSD studies is a hyperactive amygdala in response to emotional or trauma stimuli (19). Theoretical models on the amygdala have stated that the amygdala is crucial for threat detection and cardiac and behavioral threat responses (24). Therefore, enhanced amygdala reactivity is thought to contribute to hyperarousal symptoms in PTSD (30) and to impairments in top-down emotion regulation (17,51) or extinction (55).

Specifically, we observed enhanced amygdala activations during threat anticipation predicting later PTSD symptoms in the more dorsal part of the amygdala (i.e., CM) extending into the basal forebrain. It should be noted that we also observed general amygdala deactivation during threat anticipation as observed previously (56,57), but the location of that cluster is more ventral and does not overlap with the location of this prediction finding. Within the amygdala, the basal forebrain forms the bridge from the CM to the bed nucleus of the stria terminalis and includes projections to the cortex (58). Due to its dense population of magnocellular and cholinergic neurons, the basal forebrain is seen as the main regulator of cholinergic output and cortical activation. The basal forebrain is associated with the control of vigilance, arousal, and memory processes (59,60). Comparisons between subregions of the amygdala using BOLD-fMRI are inherently difficult because of signal loss and distortion due to magnetic field inhomogeneity increases from dorsal to ventral parts of the amygdala (61,62). However, our results show activations in different subregions at different moments during threat processing and thus rule

out the possibility that signal dropout prevented us from acquiring data from the BLA and CMA.

Enhanced connectivity between the amygdala and precuneus also predicted later PTSD symptoms. The precuneus is implicated in the integration of external and self-referential information and has been associated with motor imagery (indexing motor intentions) and processing of visuospatial aspects during action preparation (63,64). As a central hub of the default mode network, the precuneus has typically not been included in the threat network or in models of PTSD. However, a growing literature supports its role in the context of PTSD risk and resilience [e.g., see (50)], and amygdala-precuneus connectivity has been implicated in stress-related affect processing (32,65). Amygdala-precuneus connectivity during rest is also associated with reported childhood trauma in patients suffering from depression (66). Similar, in a group of adult trauma survivors, amygdala-precuneus connectivity during rest was associated with reported childhood trauma (67). Our finding that such connectivity pattern can even predict later PTSD symptom development calls for more attention to the role of the precuneus in trauma processing.

If enhanced amygdala activations, and amygdala-precuneus connectivity, during threat anticipation provide a neurocognitive risk marker of trauma vulnerability, then it raises the question of whether prevention or training responsiveness in these circuits may increase resiliency [e.g., using imagery-based interventions (68) including fMRI neurofeedback techniques]. Initial neurofeedback training studies have indicated that amygdala feedback during passive viewing of aversive scenes is followed by downregulation of later amygdala responses (69). Moreover, amygdala downregulation training using fMRI neurofeedback in patients with PTSD after exposure to personalized trauma scripts was associated with increase amygdala control (70). Although this was not directly linked to improvements in symptom scores, it may suggest a potential clinical application of neurofeedback in PTSD treatment. Another study (71) found greater posterior cingulate cortex (PCC)–amygdala connectivity in patients with PTSD (compared with control participants) during neurofeedback regulation, while both groups showed greater PCC-precuneus connectivity, providing targets for preventive intervention.

Trauma-induced increase in PTSD symptoms was not related to individual differences in threat-anticipatory amygdala activation at the follow-up measurement (after trauma exposure). However, the degree of trauma exposure, but not PTSD symptom increase, was related to individual differences in amygdala reactivity to the aversive shock stimulus. Participants with more trauma exposure showed enhanced lateral amygdala responses to the electrical shock. This observation is consistent with a recent study that showed posttrauma enhanced BLA activation in response to a trauma-related context in susceptible compared with resilient animals (72). However, previous studies with patients with PTSD have yielded mixed findings, with some studies finding increased amygdala responses (73) and others finding decreased amygdala responses to an electrical shock (74). We found that the number of experienced traumatic events, not PTSD symptoms, correlated with amygdala reactivity to the shock. This may explain differences between earlier studies and provides longitudinal evidence of a dose-response relationship

between trauma and amygdala reactivity to aversive pain stimuli.

## Conclusions

This prospective study demonstrates that enhanced dorsal amygdala activations and increased connectivity with the precuneus during threat anticipation predict later PTSD symptoms. These patterns may provide a neurocognitive risk marker of trauma vulnerability. Additionally, following trauma exposure, enhanced lateral amygdala was related to the number of traumatic events experienced, independent of PTSD symptoms. Therefore, activation of distinct amygdala subregions may contribute to vulnerability for developing PTSD symptoms. Increased knowledge of biomarkers that predict PTSD symptoms may be instrumental in designing future innovative training and prevention programs.

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MMH, WZ, RK, SBJK, VAvA, FK, and KR designed the study. RK, WZ, and MMH carried out the data collection. LDdV and MMH verified the underlying data. LDdV and MMH carried out the statistical analyses and produced figures. LDdV and MMH wrote the first draft of the article, and all authors contributed to editing and commenting on the final version.

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