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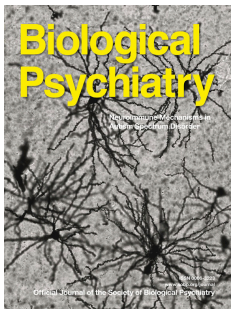

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Amygdala hyperactivity in PTSD: disentangling predisposing from consequential factors in a prospective longitudinal design

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Abstract

Background: Substantial inter-individual differences exist in the vulnerability to develop post-traumatic 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 pre-trauma 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 employed functional magnetic resonance imaging (fMRI) to disentangle predictive and acquired neural markers of post-traumatic stress symptoms. Using an experimental paradigm, we investigated anticipatory threat responses and the switch into defensive action.

Results: Those 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. While the experience of traumatic events, independent of PTSD symptoms, was associated with increased lateral amygdala activation in response to the 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.

Introduction

Many individuals experience a traumatic event at some point in life. Of those, approximately 5-20 % develop post-traumatic stress disorder (PTSD), although these rates increase to ~35% in conflict-affected countries (1–4). Primary responders, such as police, experience particularly high trauma-exposure and are therefore especially at risk for developing PTSD. Despite selection and training, 34-62 % of police officers develop sub-syndromal PTSD in the line of duty, while 7-13 % develop full-blown PTSD (e.g. 5,6). Given the enormous personal and societal costs of PTSD symptoms (7), advance understanding of individual risk variation would be of great benefit, to aid early detection and allow targeted preventive interventions. However, current definitions of PTSD are not capturing 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 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 always clear whether heightened arousal and poor regulation occur because of trauma exposure (or related PTSD symptoms). Or alternatively may rather present a predisposing risk factor. To dissociate acquired from predictive factors, sufficiently powered prospective longitudinal studies are necessary with assessments timed 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 versus controls (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 assessing individuals at emergency departments (28–30) demonstrated that hyper amygdala responding, or altered connectivity patterns, in response to threat could be a predisposing factor. In those studies, one cannot rule out that PTSD-relevant processes are a consequence of trauma exposure and dissociating acquired from predictive factors requires assessments timed before and after trauma occurs. In line with these observations, prospective neuroimaging studies have provided evidence that hyper amygdala responding to threat or threat anticipation may be a predisposing factor. Indeed, increased amygdala responding to threat is already present in individuals that later develop PTSD symptoms, before any trauma exposure occurred (10,13,16,31,but see 32). Additionally, salience network connectivity-changes during rest, were identified as a potential marker for trauma-related symptom development (33). It is important to note that prospective neuroimaging studies are methodologically challenging and are therefore scarce to date (34). They also typically have sample sizes that may not be sufficiently powered to detect inter-individual differences (35) in underlying neurobiology. Moreover, these studies are mostly performed with specific groups of individuals, such as military personnel, that 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 traumatic events. Finally, previous studies (10,13,16,31) typically compare average responses in an experimental group to a control group. This approach does not allow for assessment of inter-individual differences on a continuum from strongly resilient to full-blown psychopathology (8,9,36,37).

Here, in a large cohort of police recruits (N=221), we prospectively investigated the neural threat circuitry underlying the early development of PTSD symptoms. Police recruits were tested at the start of their police training before being sent into field work for their first emergency aid duty (Baseline session) and again tested after this period (Follow-up session, ~16 months following Baseline), (see 38 for the protocol paper). Participants performed a well-established Go/NoGo Under Threat (GUNT) paradigm (39–41) while undergoing functional MRI. We opted for an active coping paradigm, unlike previous studies that exclusively measured 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 (39–41). Measurements of blood-oxygen-level-dependent functional magnetic resonance imaging (BOLD-fMRI) during acute threat of electrical stimulation were taken during threat anticipation and subsequent defensive action. The PTSD Checklist (PCL-5) was administered during Baseline and Follow-up to measure the development of PTSD symptoms. We predicted, based on previous findings (10,13,16), that inter-individual 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 during baseline and subsequent PTSD symptom increase. We further investigate whether (de)activation patterns previously observed (40) 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 follow trauma exposure.

Materials and Methods

Participants

Participants were recruits from the Dutch Police Academy. 340 participants completed the Baseline assessment and 271 (79.7%) completed Follow-up. See ***Supplement*** for more details.

As we aimed to predict development of trauma-related symptoms, we included participants who experienced their core traumatic event between Baseline and Follow-up (N = 222; ,17,33,42,43), as assessed with a clinical interview Clinician-Administered PTSD scale (CAPS-5 44) and who did not have PTSD symptoms above the clinical cut-off at Baseline (PCL-5 total score >33; ,45), which led to the exclusion of one participant. The core traumatic events occurred in most cases in the context of police-work (86%) but could also involve work-unrelated, personal events (14%). The final sample was therefore n=221 (60 females, 161 males; 18–45 years [M = 24, SD = 5]) and for each analysis the maximum available data were included. From the 221 there were 3 individuals with PTSD symptoms above the formal clinical cut-off at Follow-up (PCL-5 total score >33; ,45). However, taking all proposed prevalence criteria into account there are 12 individuals that met criteria for PTSD and 61 individuals met criteria for sub-threshold PTSD (see ***Supplement*** for details). There was missing data for trauma exposure at Follow-up (n=8). MRI data was available for n=210 at Baseline, n=182 at Follow-up, and n=179 for both sessions. The project was approved by the Independent Review Board Nijmegen and was conducted in accordance with these guidelines (IRBN registration number NL48861.072.14).

Procedure

This study was part of a larger prospective study (Netherlands Trial Registry NTR6355). The procedure was similar for the Baseline and Follow-up session. During the Baseline session

police recruits were at the start of their police academy training without exposure to emergency aids. During the Follow-up session, police recruits had served police-related emergency aid services for approximately 8 months in which they had been exposed to traumatic events. See **Figure 1a** and ***Supplement*** for details.

Questionnaires

As registered in the protocol article (38), the primary outcome measure was change in PTSD symptom severity assessed by the PTSD checklist for DSM-5 (PCL-5; 44,45). The PCL-5 was filled in based on an event that was selected as most disruptive by the recruits from The Life Events Checklist for DSM-5 (LEC-5). Participants additionally filled out the Police Life Events Scale (PLES) twice to measure police work-related trauma incidence once before and during the training period (46). See ***Supplement*** (and **Figure S1**) for more details.

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

The Go/Nogo under threat (GUNT) paradigm

Participants completed the GUNT paradigm (39–41) during each session (Baseline, Follow-up). The paradigm involved 4 practice trials (not included in the analyses) and five blocks of 28 trials (total 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 withhold from shooting upon phone draw. There was one High Threat oppenent and one Low Threat opponent (counterbalanced across participants). If participants withheld from shooting (or too late) in response to a gun draw, participant were punished by being shot by the opponent. If participants shot the opponent with a phone, the participant was punished

by being shot by a virtual police-officer standing in the back of the garage. In 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 performance. See ***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 two 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 ***Supplement*** for details.

MRI statistical analyses

MRI data was pre-processed in standard stereotactic (MNI152) space (using SPM12 (http://www.fil.ion.ucl.ac.uk/spm; Welcome Department of Imaging Neuroscience, London, UK). See ***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 modelled using six separate regressors for Correctly Go, Correctly No Go, and Incorrect trials, for High Threat and Low Threat trial separately. There were separate regressors for button presses and electrical shocks. Additionally, nuisance regressors were included in the model. See ***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 vs Low Threat, 2) draw Threat level (High

Threat, Low Threat) by Response (Go, NoGo) for the correct responses only, and 3) responses to electrical shocks (compared to implicit baseline). We used a cluster-forming voxel-level threshold of p<.001 (uncorrected). Alpha was set at .05, whole-brain family-wise error (FWE) corrected at the cluster level using Gaussian Random Field Theory based methods (47).

In addition, we performed small volume correction (at the peak level p<.05 FWE) on our regions of interests (ROI) including the amygdala (defined by the Anatomical Automatic Labelling; AAL) and PAG (defined by manual segmentation of a previous study: (48)). Additionally, although we did not make specific predictions concerning the role of amygdala subregions due 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 (49) following previous literature (50). While the 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, ΔPCL (follow-up minus baseline), log-transformed PCL baseline score, log-transformed PLES baseline score and log-transformed ΔPLES score were included as covariates of interest to the second level model. Please note that in the model predictor and outcome variables are reversed to allow voxel-wise modelling 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 employed.

To test the acquired effects of PTSD symptomatology on activation, single-subject contrast maps (Baseline versus Follow-up) of the anticipation phase and the draw phase were entered into second-level one-sample t tests. ΔPCL (follow-up minus baseline), log-

transformed PCL baseline score, log-transformed PLES baseline score and log-transformed ΔPLES score were included as covariates of interest.

MRI data - functional connectivity

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

Results

Trauma exposure and symptom development

Police recruits experienced a greater number of traumatic events between Baseline and follow-up compared to all traumatic events experienced before in their life [Baseline: M=1.74, SD=2.24; Follow-up: M=6.67, SD=3.42], indicating an increase in trauma load [ΔPLES versus $PLES_{baseline}$; $F(1, 212) = 440.62$, $p < .001$, $\eta^2 = .68$, 95% CI (0.62, 0.72)]. Mean PTSD symptom severity showed a small but significant rise following this increase in trauma load [M=6.37, SD=8.47] compared to Baseline [M=5.14, SD=6.18; $F(1, 220) = 4.70$, $p = .031$, $\eta^2 = .02$, 95% CI (-0.01, 0.05)] and there was substantial variation in PTSD symptom change. Moreover, the number of traumatic events experienced between Baseline and Follow-up (ΔPLES) correlated positively with PTSD symptom increase [r(211)=0.16, p=0.02, 95% CI (0.03, 0.3)]. See **Figure 1**.

Figure 1 – *A) Timeline of the study. Between Baseline and Follow-up, police officers gained experience in emergency aid (two periods) as part of their training, encountering potentially trauma-related events. B) PTSD symptoms on the PCL checklist (PCL-5) for the Baseline and Follow-up measurement. Colors of the lines indicate individual differences in symptom change. C) Positive correlation between number of traumatic events experienced between Baseline and Follow-up (ΔPLES) and PTSD symptom change (ΔPCL-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*

Threat-related performance and cardiac changes

We replicated typical GUNT effects on both behaviour and heart rate responses (40). See **Figure 2b-c** and ***Supplement*** for all statistical analyses.

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 time (80% of trials 6000-6500 ms, 10% of trials 500-1500, 10% of trials 1500-6000) after which the avatar either drew a gun or a phone. Upon gun draw participants were required to shoot (make a go action) or withhold from shooting (no go action). In the High threat condition, if participants made an incorrect decision, they would receive an electrical shock to the fingers. B) On average participants responded faster on High threat trials compared to Low threat trials and made more Go responses under High treat resulting in higher accuracy on Go trials and lower accuracy on No Go trials.* See ***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 two panels). Participants showed threat-related bradycardia (High threat versus Low threat) during anticipation. And trial time-locked to the draw onset (lower two panels). Participants showed heart rate increase for Go versus NoGo trials. This increase was stronger during High threat compared to Low threat.* See ***Supplement*** for statistical analyses*. D) BOLD-fMRI response patterns for the contrast High Threat versus Low threat during anticipation of the gun draw (left panel) and for the contract Shock versus implicit baseline (right panel). For visualization purposes a threshold of p < 0.001 uncorrected was used. * p<.05*

Amygdala BOLD-fMRI response and connectivity patterns during threat anticipation predict later symptom development

Prediction: During anticipation, increased activation in the left amygdala (High Threat compared to Low Threat) at Baseline [xyz = -18, -2, -14, peak voxel z=3.42, p=.018 FWE-SVC] was associated with a subsequent increase in PTSD symptoms at follow-up (ΔPCL), while correcting for baseline symptom severity (baseline PCL) and trauma exposure history (baseline PLES and ΔPLES; see Figure 3). Increased activation in the left amygdala [xyz = -18, -2, -14, peak voxel z=3.34, p=.022 FWE-SVC] predicting subsequent increase in PTSD symptoms was also found when only the ΔPCL variable was included as a covariate, mitigating the chance on confounds related to multicollinearity. Follow-up exploration revealed the activation is centered in more dorsal areas in the basal forebrain and centromedial amygdala [P_{excess} BF = 1.57, centromedial amygdala (CMA) = .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^3, cluster p=.006, FWE-corrected] was positively associated with this increase in PTSD symptoms at Follow-up (ΔPCL) during threat anticipation [High threat > Low threat], while correcting for baseline symptom severity (baseline PCL) and trauma exposure history (baseline PLES and ΔPLES).

In response to the draw (Threat by Response interaction), no significant associations with increased PTSD symptoms at Follow-up (ΔPCL) were present across the whole brain nor our ROIs (i.e. amygdala and PAG).

These results together 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.

After Trauma exposure: 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 by Response interaction) from Baseline to Follow-up were not significantly related to trauma exposure (ΔPLES) nor the increase in PTSD symptoms at Follow-up (ΔPCL).

Individuals with more trauma exposure (ΔPLES) showed a relative reduction in the right amygdala responses to the aversive stimulus (i.e. electrical shock) [xyz = 36, 2, -24, peak voxel z=3.12, p=.048, FWE-SVC] relative to the Baseline session. This activation centered in the basolateral amygdala [P_{excess} basolateral amygdala (BLA) = 1.06]. Follow-up analyses revealed no impact of trauma load at Baseline while during the Follow-up session those with more trauma exposure (ΔPLES) showed an increase in amygdala responses [xyz = 30, 2, -24, peak voxel z=3.63, p=.006, FWE-SVC]. This activation centered in the basolateral amygdala [P_{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).

Figure 3 – *A) Relatively high amygdala activation during High threat (versus 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 p < 0.005 uncorrected was used.*

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 could disentangle predictive and acquired effects of PTSD symptoms during threat anticipation and responsivity. Recruits with stronger pre-trauma dorsal amygdala responses during threat anticipation demonstrated relatively 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 prediction from acquired associations, we found evidence that distinct amygdala subregions may be implicated in the cause and consequence of PTSD symptoms. We hereby extend previous prospective studies by showing these findings in a well-powered sample, an active (compared to passive) threat paradigm, and provide more specificity regarding the role of amygdala subregions.

Our findings are consistent with theoretical models postulating that a hyperreactive salience network, including amygdala reactivity and connectivity, is a predictor for PTSD development (19,51). Early studies pointed out that regions crucially involved in threat processing are altered in individuals with PTSD (15,31,see for a meta-analysis 52,53). Similarly, peri-trauma studies with participants that were recruited from the emergency department and who were scanned 1-month post-trauma, have found amygdala reactivity to negative emotional stimuli to be correlated with PTSD symptoms months later (30). However, post-trauma studies indicated altered amygdala reactivity (15) that possibly normalizes over

time (54). This finding suggests 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 in line with early prospective neuroimaging studies with considerably smaller sample sizes (10,13,16 all n <40). Our study extends these prospective studies in several ways. First, our sample allows for individual difference analyses. We could verify that the amygdala reactivity was correlated with PTSD symptoms dimensionally rather than only increasing in those participants with core symptoms at the high extreme of the spectrum. Second, the population of 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 heterogenous daily adversity (including e.g. traffic accidents, physical assault, death and illness), see (17,33). Note that within 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 thus 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. Forth, in our analyses we controlled for trauma load and thereby take 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 focusing on resiliency (e.g. 55). 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 behavioural threat responses (24). Enhanced amygdala reactivity is therefore thought to contribute to hyperarousal symptoms in PTSD (30) and to impairments in top-down emotion regulation (17,52) or extinction (56).

Specifically, we observed enhanced amygdala activations during threat anticipation predicting later PTSD symptoms in the more dorsal part of the amygdala (centromedial: CM) extending into the basal forebrain. Please note we also observed general amygdala deactivation during threat anticipation as observed previously (57,58), 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 (BNST), and includes projections to the cortex (59). 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 (60,61). Comparison between subregions of the amygdala using BOLD-fMRI is inherently difficult because of signal loss and distortion due to magnetic field inhomogeneity increases from dorsal to ventral parts of the amygdala (62,63). However, our results show activations in different subregions at different moments during threat processing and thus rule out that signal dropout prevented us from acquiring data from the basolateral amygdala (BLA) and centromedial amygdala (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 visuo-spatial aspects during action preparation (64,65). As a central hub of the default mode network, the precuneus is typically not 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 51) and amygdala-precuneus connectivity has been implicated with stress-related affect processing (33,66). Amygdala-precuneus connectivity during rest is also associated with reported childhood trauma in patients suffering from depression (67). Similar, in a group of adult trauma survivors, amygdala-precuneus connectivity during rest was associated with reported childhood trauma (68). 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 provides a neurocognitive risk marker of trauma vulnerability, then it raises the question whether prevention or training responsivity in these circuits may increase resiliency (e.g. using imagery-based interventions (69) including fMRI neurofeedback techniques). Initial neurofeedback training studies have indicated that amygdala feedback during passive viewing of aversive scenes is followed by down-regulation of later amygdala responses (70). Moreover, amygdala downregulation training using fMRI neurofeedback in PTSD patients after exposure to personalized trauma scripts was associated with increase amygdala control (71). 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 (72) found greater PCC-amygdala connectivity in PTSD patients (compared to

controls) 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 in trauma exposure, but not PTSD symptom increase, was related to individual differences in amygdala reactivity to the aversive shock stimulus. Those with more trauma exposure showed enhanced lateral amygdala responses to the electrical shock. This observation is in line with a recent study showing post-trauma enhance BLA activation in response to a trauma-related context in susceptible compared to resilient animals (73). However, previous studies with PTSD patients have shown mixed findings where some found increased amygdala responses (74), while others found decreased amygdala responses to an electrical shock (75). We found that the number of experienced traumatic events, not PTSD symptoms, correlated with amygdala reactivity to the shock. This might explain differences between earlier studies and provides longitudinal evidence of a dose-response relationship between trauma and amygdala reactivity to aversive pain stimuli.

In conclusion, 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. While post trauma, enhanced lateral amygdala was related the number of experienced traumatic events, independent of PTSD symptoms. Therefore, activation of distinct amygdala subregions may contribute to vulnerability for developing PTSD symptoms. Increased knowledge of biomarkers predicting PTSD symptoms may be instrumental in designing future innovative training and prevention programs.

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MMH, WZ, RK, SBJK, VvA, FK, and KR designed the study. RK, WZ, and MMH carried out the data collection. LdV and MMH verified the underlying data. LdV and MMH carried out the statistical analysis and produced figures. LDV and MMH wrote the first draft of the manuscript and all authors contributed to editing and commenting on the final version.

The authors report no biomedical financial interests or potential conflicts of interest.

Supplement Description:

Supplement Methods, Results, Figures S1-S2, Tables S1-S5

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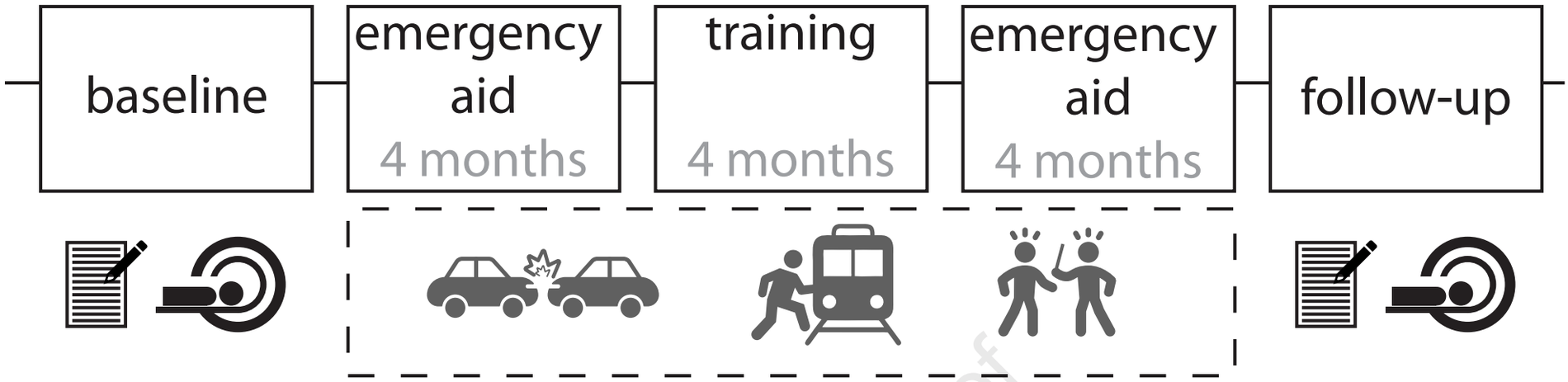
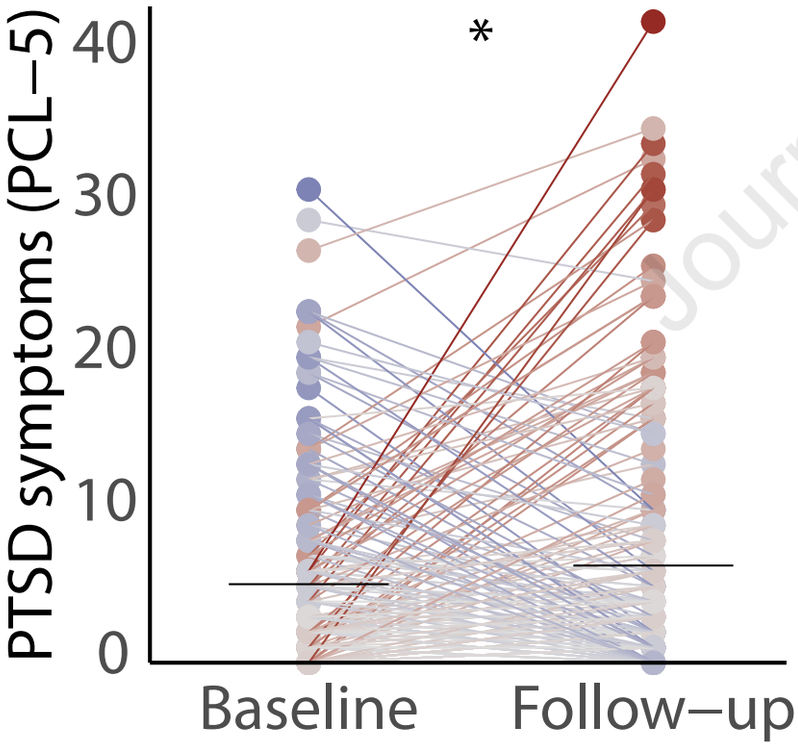
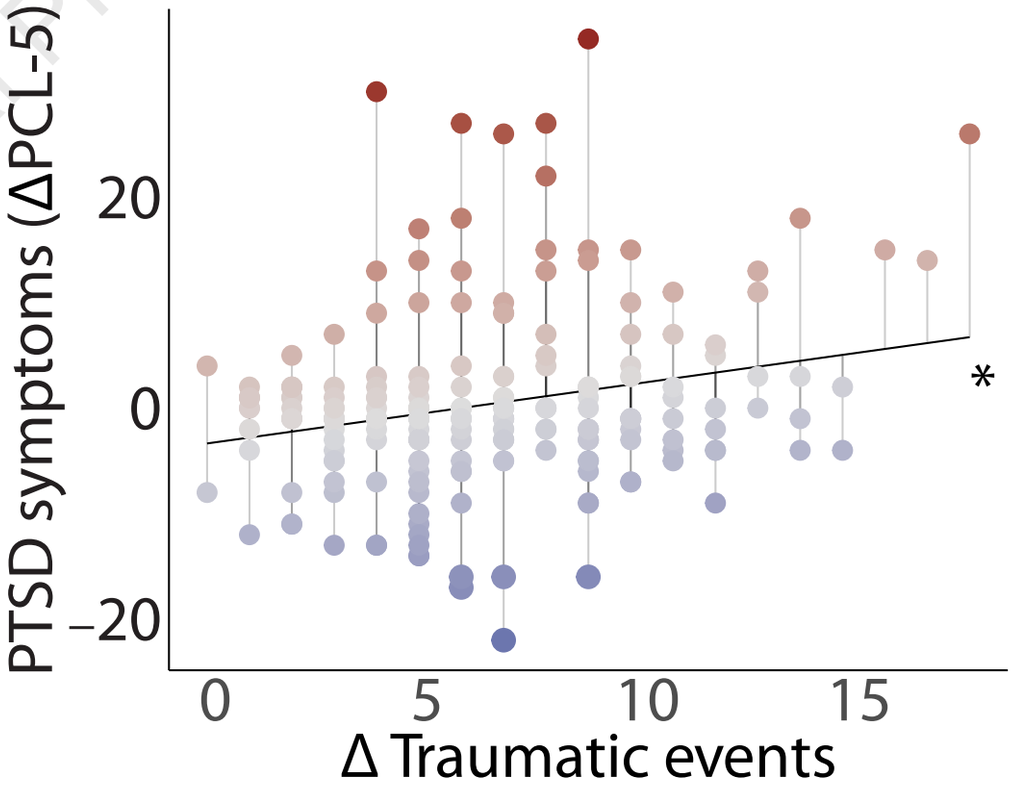
A) Study timeline



B) PTSD symptoms



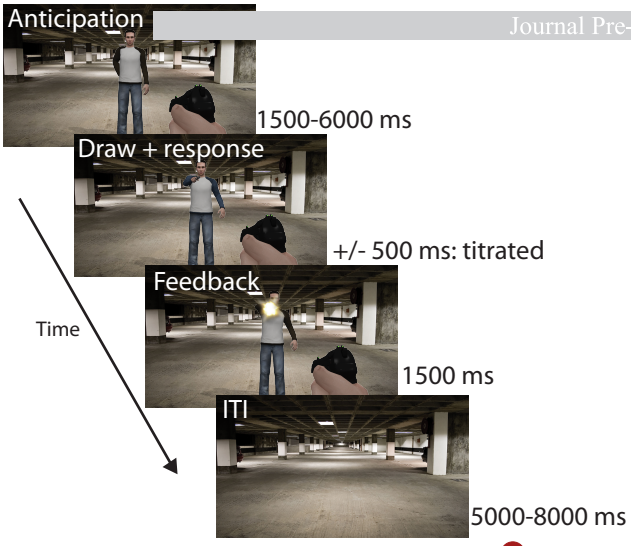
C) Vulnerability

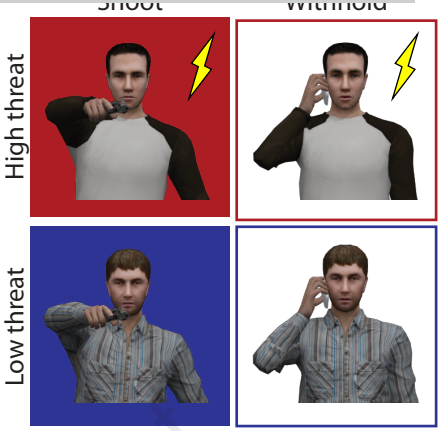


PTSD symptom change (ΔPCL-5)

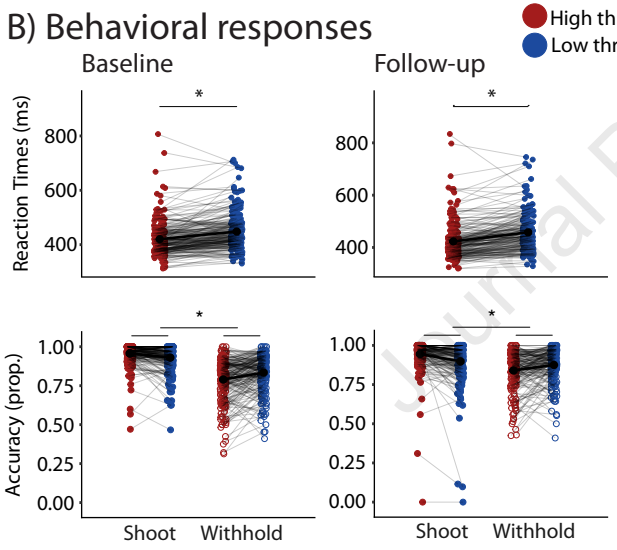
Vulnerability

A) Experimental design

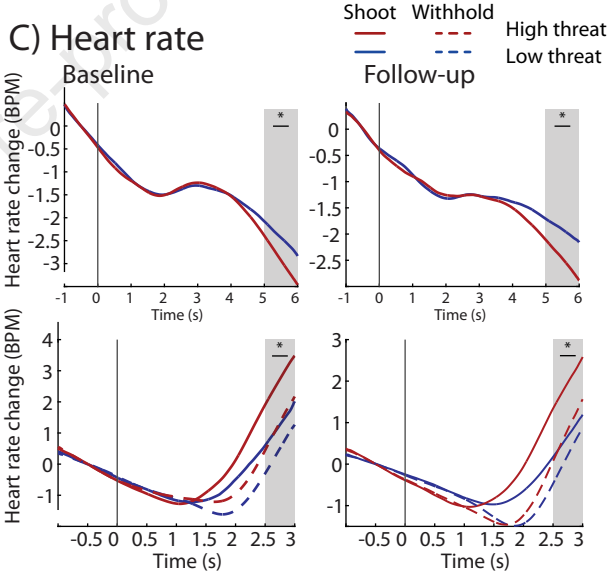




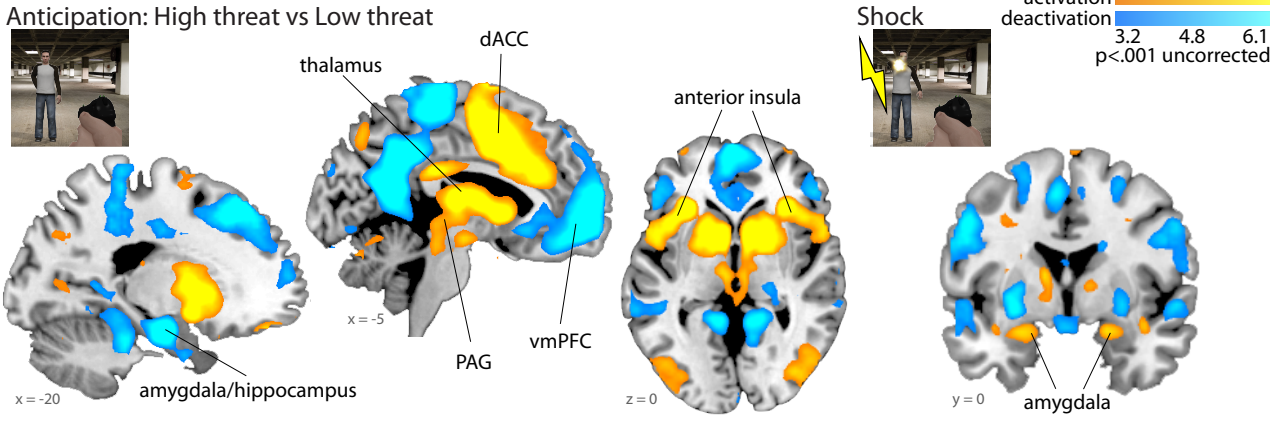
B) Behavioral responses



C) Heart rate



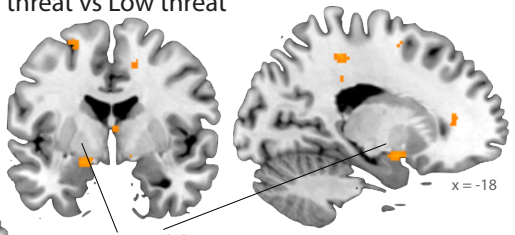
D) fMRI response patterns

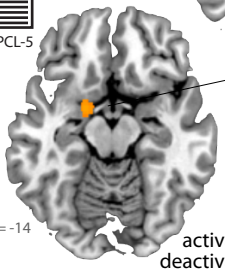


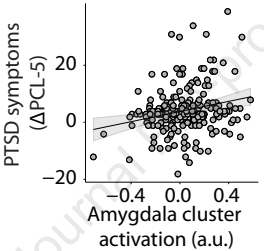
A) PTSD symptom prediction

Anticipation: High threat vs Low threat

Δ PCL-5

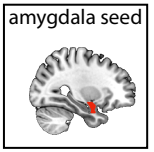


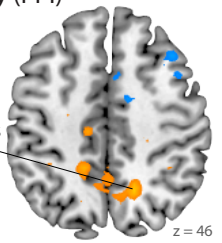


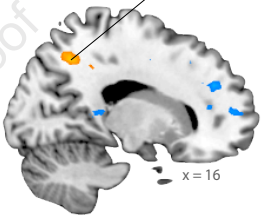


activation
deactivation
2.6 4.0 5.6
p<.005 uncorrected

Functional connectivity (PPI)





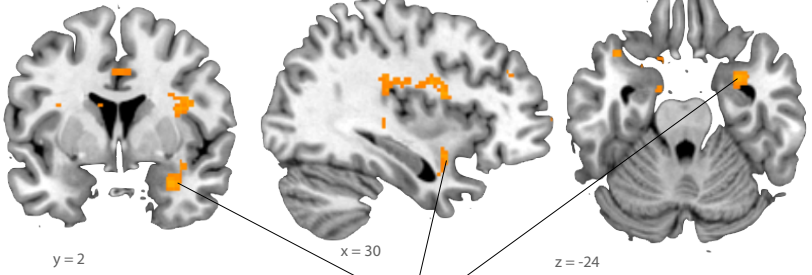


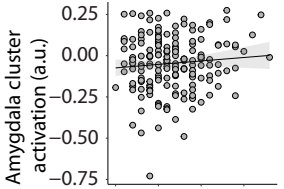
positive
negative
2.6 4.0 5.6
p<.005 uncorrected

B) Following trauma exposure

Shock

Δ PLES





activation
deactivation
2.6 4.0 5.6
p<.005 uncorrected