SUPPLEMENTARY MATERIAL:

GENERALISATION OF FEAR IN PTSD

RELATED TO PROLONGED CHILDHOOD MALTREATMENT:

AN EXPERIMENTAL STUDY

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**1. Supplementary Methods**

1.1 Participants

*1.1.1 Diagnostic and consenting procedures*

The diagnostic interviews in order to assess inclusion - and exclusion criteria were conducted by trained clinical psychologists by using the borderline personality disorder section of the International Personality Disorder Examination (IPDE;Loranger *et al.*, 1999), and the Structured Interview for DSM - IV (SCID - I; Wittchen *et al.*, 1997). We additionally applied the Clinician Administered posttraumatic stress disorder (PTSD) Scale for DSM - 5 (CAPS - 5; Weathers *et al.*, 2013, Mueller-Engelmann *et al.*, submitted) only in case of a PTSD diagnosis to facilitate a comparison of PTSD symptom severity in our publication to existing literature (mean = 39.67, SD = 9.77). PTSD patients were recruited from an ongoing study at the Department for Psychosomatic Medicine and Psychotherapy, CIMH Mannheim. Both, trauma control (TC) and healthy control (HC) groups were recruited through the database at the Department for Psychosomatic Medicine and Psychotherapy, CIMH Mannheim, as well as through newspaper advertisements and the distribution of flyers at public places (e.g. cafés, supermarkets). Approval was obtained from the independent Ethics Committee of the Medical Faculty Mannheim at Heidelberg University. All participants provided written informed consent.

*1.1.2 Inclusion, and exclusion criteria*

The inclusion criterion for trauma exposed individuals (PTSD and TC group) was the exposure to physical and/ or sexual violence prior to the age of 18. Since PTSD individuals took part of a longitudinal treatment study, which evaluated two trauma treatments for individuals with posttraumatic stress disorder related to childhood and adolescent maltreatment (RELEASE - study), they also had to fulfill at least 3 borderline personality disorder criteria, as defined by the IPDE (Loranger *et al.*, 1999).The exclusion criteria for TC, as well as HC individuals contained a lifetime diagnosis of any axis - I or borderline personality disorder, the intake of psychotropic drugs or experiences of psychotherapeutic interventions. Exclusion criteria for the PTSD sample included a lifetime diagnosis of schizophrenia or bipolar - I disorder, current substance dependence, a body mass index <16, or the intake of the following psychotropic drugs: tricyclic antidepressants, neuroleptics, trazedon, benzodiazepines, anxiolytic drugs, as well as beta adrenergic blocking agents. For safety reasons, PTSD individuals who had attempted suicide within the last two months were excluded as well.

1.2 Experimental Procedure

*1.2.2 Fear conditioning and generalisation paradigm*

*Stimuli:* Two circles served as conditional stimuli. For half of the participants within each group, the small circle (5.5 cm) was associated with the aversive outcome (danger cue, CS+) and for the remaining subjects this was reversed, meaning that the big circle (12.5 cm) served as the CS+. Within the generalisation test, eight additional circles were presented. The generalisation stimuli decreased in similarity in regard to the CS+ and increased in their similarity to the safety cue (CS-).In detail, each generalisation stimulus increased 0.875 cm in diameter starting from the smallest circle (5.5 cm) and thus representing a continuum from the smallest to the biggest circle. Prior to analyses, responses to every two generalisation stimuli were averaged resulting in four generalisation classes. This procedure was based on the concern that including each generalisation stimulus as a separate stimulus would lead to a too long experiment, while including only four generalisation stimuli would not allow a gradual enough continuum between CS+ and CS-. Thus, presenting eight generalisation stimuli, while analyzing four generalisation classes seemed to be a good compromise (see Lissek *et al*., 2008). A fixation - cross (size: 1 cm) appeared on the screen during inter - trial intervals (ITI). Electrical stimulation (20 ms) served as a unconditioned stimulus (US) and was controlled by Digitimer DS7A constant current stimulator (Digitimer, Herfordshire, UK) via a bar stimulating electrode with two durable stainless steel disk electrodes of 8 mm diameter each and with 30 mm spacing between, placed on the upper wrist of the non - preferred hand and fixated with a Velcro strap. Electrodes were filled with conductive gel (Signa Gel, Parker). US intensity calibration was set individually starting with 10 mA, while intensity was increased by steps of 20 mA until it felt ‘unpleasant but not painful’. Herein, the following steps have been important: The participant was instructed to provide feedback as soon as the electrical shock felt ‘unpleasant’ for the first time. Next, intensity was further increased until the participant mentioned that the electric shock felt ‘painful’. The intensity subsequently was decreased by 20 mA. The participant was than instructed to rate, whether this intensity felt ‘unpleasant, but not painful’, while it should further feel more unpleasant as compared to the startle probe, which has been presented one time before starting the US intensity calibration.

*Stimulus Sequence:* *S*timulus sequence for the pre - acquisition, acquisition as well as generalisation phase were pseudorandomized across groups and participants. Presentation of the stimulus sequence, electrical stimulation and startle probes were controlled by Presentation (Neurobehavioral System). All stimuli were presented on a monitor screen (17’’, resolution 1024 x 786 x 32 pixel, picture size 456 x 456 pixel).Regarding the *pre - acquisition* and *acquisition* *phase*, a random sequence for six blocks (pre - acquisition) and 12 blocks (acquisition), each containing a CS+/CS- and an ITI, was created. Next, three pseudorandomized sequences (swapping position of a) CS+ and CS- stimuli, b) CS+ and ITI; c) CS- and ITI) were developed. The *generalisation* *phase* comprised a random sequence for four blocks, each containing two presentations of the CS+/CS- and the ITI, as well as one presentation of each generalisation stimulus. Next, 17 pseudorandomized sequences (within each block, stimuli were rotated, meaning that each stimuli is placed on each possible position within a particular block) were created.

*Behavioral Measures:* Online risk ratings were the evaluations of the risk of the occurrence of an electric shock associated with the presented stimulus (10 point - Likert scale ranging from 1 = no risk, to 10 = high risk). Additionally, reaction times of ratings were measured. To indicate their response, subjects had to move a red dot from a starting area to one of ten target areas displayed in an equal distance to the starting area.

*Physiological Measures:* Fear potentiated startle (FPS)was measured as the potentiated eyeblink startle reflex to a loud noise by electromyography (EMG) of the (left) orbicularis oculi muscle. The acoustic startle reflex is a specific measure for fear (Hamm *et al.*, 2005), and is directly connected with and modulated by the amygdala (Davis, 2006). The noise consisted of a burst of white noise (40 ms, 95 dB, bandwith of 20 Hz – 20 kHz) and was presented binaurally via headphones (Sennheiser HD 25-1-II). Two electrodes with a diameter of 13 mm each, filled with electrolyte gel (Synapse, Costumer Kinetics) positioned approximately 1 cm under the pupil and 1 cm below the lateral canthus (Blumenthal *et al*., 2005). The eye - blink EMG activity was measured with an EMG amplifier (Varioport, Becker Meditec, input resistance of 500 MΩ, bandwidth of 19–500 Hz (-3dB), sampling rate 1024Hz). EMG data were pre - processed using in - house software (MatLab 2011b, MathWorks) following a standard procedure: Raw data were filtered (50Hz notch filter, 28-Hz high - pass filter, 4th order Butterworth filter), rectified and smoothed (low - pass filter 50Hz). Startle amplitude was measured as the maximum peak within a time window of 20 - 150 ms following the onset of the startle probe referenced to mean baseline level (50 ms before onset of the startle probe). Outliers were defined (z - transformation overall trials, within - subject; Z > 3) and replaced by the maximum z - score. Trials were visually inspected and excluded from further analyses, when the baseline period was contaminated with noise and movement artifacts (Blumenthal *et al.*, 2005). Missing startle responses were scored as 0 and entered in the calculation of the FPS magnitude. To normalize the data and to reduce the influence of between - subjects variability, startle amplitudes in response to inter - stimulus intervals (NA trials) across all phases of the study were standardized together using within - subject t - score conversion (Lissek *et al.*, 2008).

*1.2.3 Statistical Analyses: Exclusion Criteria*

Overall, two participants within the TC group, as well as three participants within the PTSD, and four of the HC group had to be excluded of all FPS analyses due to a startle signal contaminated by technical artifacts.

Regarding missing values, subjects were excluded in case of more than one out of three trials during pre - acquisition, more than two out of six trials during acquisition and more than two trials out of four during generalisation testing (herein, one trial needed to correspond to each generalisation stimuli of each class) were missing for each dependent variable and phase separately. With respect to online risk ratings and RTs, four subjects had to be excluded within the pre - acquisition analyses (PTSD = 1, TC = 3). Regarding FPS, one subject had to be excluded within the pre - acquisition analyses (TC = 1), and four subjects within the acquisition and generalisation analyses (TC = 1, HC = 3).

 In addition, subjects were excluded, if they were statistical outliers. Boxplot analyses have been conducted for each phase, respectively. Herein, the difference in response between the CS+ and the CS- within each phase represented the variable of interest. Subjects were excluded, if the depended variable exceeded >/<= 3 \* interquartile range for each dependent variable and phase separately. Regarding online risk ratings, one subject had to be excluded during generalisation testing (PTSD = 1). With respect to RT, one subject had to be excluded of all analyses (TC = 1).

 Regarding online risk ratings, subjects were excluded if they did not report a CS+/US and CS-/no US contingency. Declarative memory of a CS+/US and CS-/no US contingency was defined as a greater online risk ratings to the CS+ than CS- during the end of fear acquisition phase (trial 5 and 6). Subjects were thus further excluded from online risk ratings generalisation testing (HC = 1, TC = 1).

**2. Supplementary Results**

2.1 Generalisation Testing

*2.1.1 Online Risk Ratings*

Online risk ratings differed between stimulus types, irrespective of the group (*F5,420* = 253.05, *p* <.001; stimulus type x group: *F10,420* = 1.44, *p* = .162; Table 2C, Figure 2). Trend analyses indicated significant linear and quadratic components (linear: stimulus type: *F1,84 = 563.81*, *p* < .001; stimulus type x group: *F2,84*= 1.38, *p* = .258; quadratic: stimulus type: *F1,84 = 100.76*, *p* < .001; stimulus type x group: *F2,84*= .72, *p* = .491). To further describe this effect, online risk ratings for each generalisation stimulus und CS+ stimuli, respectively were compared with the CS-, according to the procedure of Lissek et al (2010). These comparisons serve to determine at which degree of dissimilarity between a stimulus and the CS- increased risk is reported, i.e., to which degree of perceptual similarity fear generalized. All groups reported higher online risk ratings to the CS+ and the three generalisation classes most similar to the CS+, i.e., class 1, 2, and 3 (*p* < .001), but not for the generalisation class most similar to the CS-, i.e., class 4 (*p* > .1).

*2.1.2 Reaction Times (RT)*

RT differed between groups depending on stimulus type (*F10,430* = 1.85, *p* = .050; Table 2C, Figure 2). Trend analysis revealed that this difference is linked to the linear component of the shape of the relationships between the speed of risk ratings and the increasing similarity from the safety cue across the different generalisation classes to the danger cue (*F2,86* = 4.44, *p* = .015). To further describe this effect, RTs for each GS respectively were compared to both cues representing unambiguous threat information, i.e., CS-, and CS+ to determine to which level of perceptual similarity RT indicated heightened uncertainty during risk evaluation. In HCs increased RTs, compared to the safety cue were found towards the two generalisation classes (class 1 and 2) least similar to the safety cue (*p*’s < .031), while compared to the danger cue, faster RTs were found towards the danger cue least similar class, i.e., class 4 (class 4, *p* = .031). In contrast, trauma - exposed participants, i.e., PTSD patients and TCs, responded slowest only to generalisation class 2 compared to the safety cue (*p*’s < .031). PTSD participants further responded slower to class 2 compared to the danger cue (*p* = .019). Thus, they experienced a higher uncertainty during risk rating towards generalisation stimuli only when GS were less similar to both the danger and the safety cue.

*2.1.3 Fear Potentiated Startle (FPS)*

FPS did differ between stimulus types, irrespective of the group (*F5,370* = 18.86, *p* < .001; *F10,370* = .56, *p* = .844; Table 2C, Figure 2). Trend analyses revealed a significant linear component (stimulus type: *F1,74* = 92.39, *p* < .001; stimulus type x group: *F2,74* = 1.33, *p* = .270). To further describe this effect, FPS in response to each generalisation stimulus, and CS+ was compared with the CS-, respectively (for rational see section 2.1.1). All groups showed increased FPS to the CS+ and the two generalisation classes most similar to the CS+, i.e., class 1 and 2 (*p* < .001), but not for the remaining generalisation classes, i.e., class 3 and 4 (all *p* > .189).

2.2 Perceptual Discrimination Task

*2.2.1 Similarity ratings*

Similarity ratings did not differ between groups (*F2,86* = 1.83, *p* = .166). Similarity ratings differed between comparison stimuli depending on the reference stimuli (*F5,430* = 13.27, *p* < .001): Similarity ratings were higher, when the CS+ was the reference stimulus (*p*’s < .023), except when comparing the CS+ and CS- to each other (*p* = .669). There was no further significant interaction effect (all *p*’s > .238) (STable 1).

*2.2.2 Reaction times associated to similarity ratings*

 RT of similarity ratings did not differ between groups (*F2,86* = .12, *p* = .887). RT of similarity ratings differed between comparison stimuli depending on the reference stimulus (*F5,430* = 4.09, *p* = .001). Participants were faster when rating the similarity of the closest approximation of the CS+, i.e., class 1, when the reference was the CS+ compared to the closest approximation of the CS-, i.e., class 4, when the reference was the CS- (*p* < .001). There was no further interaction effect (all *p*’s > .261) (STable 1).

**STable 1.** **Results of the analyses of variance for similarity ratings (A.), and reaction times (B.) of the perceptual discrimination task.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | F | df | *p* | d |   |
| **A. Similarity Rating** |   |   |   |   |   |
|  |   |   |   |   |   |
| Group | 1.83 | 2/86 | .166 | .003 |   |
|   |   |   |   |   |   |
| Stimulus Type | 810.378 | 5/430 | <.001 | .771 | \* |
|   |   |   |   |   |   |
| Reference | 27.46 | 1/86 | <.001 | .004 | \* |
|   |   |   |   |   |   |
| Group x Stimulus Type | 1.28 | 10/430 | .238 | .003 |   |
|   |   |   |   |   |   |
| Group x Reference  | 0.30 | 2/86 | .739 | <.001 |   |
|   |   |   |   |   |   |
| Reference x Stimulus Type | 13.27 | 5/430 | <.001 | .006 | \* |
|   |   |   |   |   |   |
| Group x Stimulus Type x Reference | 0.47 | 10/430 | .912 | <.001 |   |
|   |   |   |   |   |   |
| **A. Reaction Times** |   |   |   |   |   |
|   |   |   |   |   |   |
| Group | 0.12 | 2/86 | .887 | .001 |   |
|   |   |   |   |   |   |
| Stimulus Type | 15.15 | 5/430 | <.001 | .045 | \* |
|   |   |   |   |   |   |
| Reference | 5.82 | 1/86 | .018 | .003 | \* |
|   |   |   |   |   |   |
| Group x Stimulus Type | 1.24 | 10/430 | .261 | .008 |   |
|   |   |   |   |   |   |
| Group x Reference  | 0.69 | 2/86 | .501 | <.001 |   |
|   |   |   |   |   |   |
| Reference x Stimulus Type | 4.09 | 5/430 | .001 | .009 | \* |
|   |   |   |   |   |   |
| Group x Stimulus Type x Reference | 1.11 | 10/430 | .354 | .005 |   |
|   |   |   |   |   |   |

*Significance threshold \* p <.05*

*Abbreviations: df = degrees of freedom, d = Cohen’s d*

**3. Supplementary References**

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