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*Running head: Conditioned Responses to Trauma Reminders*

## **Conditioned Responses to Trauma Reminders: How Durable are They Over Time and Does Memory Integration Reduce Them?**

Markus Streb<sup>1</sup>, Martin A. Conway<sup>2</sup>, and Tanja Michael<sup>3</sup>

<sup>1</sup>*Department of Psychiatry and Psychotherapy, University Hospital of Freiburg, Germany*

<sup>2</sup>*Department of Psychology, City University London, London, United Kingdom*

<sup>3</sup>*Division of Clinical Psychology and Psychotherapy, Department of Psychology, Saarland University, Saarbrücken, Germany*

Corresponding author:

Professor Tanja Michael, DPhil,  
Department of Clinical Psychology and Psychotherapy,  
Saarland University,  
Campus, Gebäude A 1.3  
66123 Saarbrücken  
Germany

Phone: +49- (0)681 302 71001

Fax: +49- (0)681 302 4437

Email: [t.michael@mx.uni-saarland.de](mailto:t.michael@mx.uni-saarland.de)

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ACCEPTED MANUSCRIPT

## 1. Introduction

Intrusive memories of traumatic events are a hallmark symptom of posttraumatic stress disorder (PTSD; American Psychiatric Association, 2013). These memories consist of vividly experienced thoughts, images, and perceptions that cause immense distress (Michael, 2000; Michael, Ehlers, Halligan, et al., 2005). Intrusive memories are often triggered by stimuli that have been encountered in the context of the traumatic event even though they do not necessarily have a meaningful relationship to the traumatic event (e.g. a pattern of light, a particular sound; Brewin et al., 1996; Foa et al., 1989; Ehlers et al., 2004). According to learning or conditioning models of PTSD, temporal co-occurrence causes neutral stimuli to become associated with the aversive experience of the traumatic event and subsequently have the potential to trigger intrusive reexperiencing of the trauma, including memories, emotions, and physiological arousal (Foa et al., 1989; Keane et al., 1985). Thus, intrusive memories in PTSD are regarded as conditioned reactions (CR) and triggers can be seen as conditioned stimuli (CS) that predict a traumatic event (unconditioned stimulus, UCS; Foa et al., 1989; Keane et al., 1985; Rothbaum & Davis, 2003).

It has been proposed that, in general, this stimulus-driven retrieval is inhibited when episodic memories are integrated into the autobiographical memory system (Conway, 2005; Conway & Pleydell-Pearce, 2000). This system is regarded as a representation of conceptually organized autobiographical knowledge, regulated by a central control process, the working self, which controls the retrieval and encoding of episodic memories (Conway, 2003, 2005). Based on this model, Ehlers and Clark (2000) have suggested that poor memory integration of the traumatic experience in PTSD patients leads to insufficient inhibition of stimulus-driven retrieval of trauma memories.

Accordingly, memory integration should lead to a reduction of conditioned reactions and intrusive memories triggered by trauma-related stimuli. Indeed, clinical efficacy studies show that intervention techniques that focus on trauma memories and include a verbalization of the traumatic experience (e.g. memory integration through imaginal exposure) provide the best therapeutic outcomes (Bisson et al., 2007). It is not clear, however, whether memory integration actually leads to a reduction of associative learning, the memory processes supposedly underlying intrusive memories.

There is growing evidence for the important role associative learning plays in the development and maintenance of PTSD (Duits et al., 2015). Orr et al. (2000) investigated fear conditioning in PTSD patients and trauma-exposed participants without PTSD using a differential fear conditioning paradigm. Neutral visual stimuli were used as CS and either paired with an electrical stimulus as UCS or not. During acquisition, PTSD patients showed larger differential skin conductance (SC), heart rate (HR), and electromyogram responses to the CS+ (stimulus paired with the UCS) versus the CS- (stimulus not paired with the UCS) compared to trauma-survivors without PTSD. When CS+ and CS- were subsequently repeatedly presented without being followed by the UCS (extinction), only PTSD patients continued to show differential SC responses to CS+ versus CS-. Delayed extinction in PTSD patients compared to trauma-exposed or healthy control groups has also been found in larger heart rate responses (Peri, Ben-Shakhar, Orr, & Shalev, 2000), startle responses (Norrholm et al., 2011), and subjective ratings of valence and US-expectancy (Blechert et al., 2007). In a prospective study of soldiers who were tested before and after their deployment, reduced extinction learning was found to be a pre-trauma vulnerability factor for PTSD symptom severity (Lommen et al., 2013). Taken together, these findings indicate that conditioned reactions to trauma reminders play an important role for the development of intrusive reexperiencing. However, so far, fear conditioning for neutral stimuli actually present during the traumatic event could not be investigated directly.

Another limitation of fear conditioning experiments is their relatively poor ecological validity. The UCSs implemented to simulate a traumatic event in the laboratory are electrical stimulation or aversive noises (Duits et al., 2015; Lissek et al., 2005). These stimuli are suitable for investigating conditioned fear reactions like SCR, but allow no inferences about the question whether fear conditioning underlies intrusive trauma memories. Because of these shortcomings, Wegerer et al. (2013) have recently developed the conditioned intrusion paradigm. In this paradigm, neutral sounds are either paired with short aversive film clips (CS+) or presented alone (CS-; for a similar approach see Kunze, Arntz, & Kindt, 2015). Subsequently, the CS+ when presented again while embedded in a neutral background soundscape triggered intrusive memories, and induced anxiety and physiological arousal (as indexed by SC levels) as a conditioned reaction (CR). Furthermore, conditionability of subjective valence ratings and fear reactions in this task was associated with later ambulatory intrusive memories. This paradigm was an important step toward investigating fear conditioning in a more naturalistic laboratory setting, as it was the first study to show that intrusive trauma memories can occur as a CR to a CS+. However, it does not resemble the typical time course of a traumatic event.

A more naturalistic laboratory analogue of traumatic experiences is the trauma film paradigm (for a review see Holmes & Bourne, 2008). In this paradigm healthy participants are exposed to a stressful film (typical duration: 8-12 min), depicting traumatic events, such as actual or threatened death and serious physical injuries. Over the following days, participants keep a diary to document their intrusive memories of the presented film. In a recent meta-analysis of 458 participants the mean number of intrusive memories in the week following a “traumatic” film was 5.53 (SD = 6.52) (Clark et al., 2015). There is a broad consensus that the trauma film paradigm provides a valuable experimental tool for investigating memory processes underlying PTSD with high ecological validity (Holmes & Bourne, 2008).

In order to examine how stable conditioned responses to trauma reminders are over time, and how they are affected by memory integration (Michael & Ehlers, 2007), we combined the conditioned intrusions paradigm from Wegerer et al. (2013) with the standard trauma film paradigm. Specifically, neutral sounds were repeatedly presented during either a “traumatic” film clip (CS+) depicting interpersonal violence or a neutral control film (CS-) depicting neutral social interactions. To test whether trauma-associated sounds (CS+) trigger traumatic memories and increase anxiety as conditioned responses, the memory triggering task, developed by Wegerer et al. (2013), was performed after presentation of a well-established trauma film paradigm (Streb, Mecklinger, Anderson, Lass-Hennemann, & Michael, 2016). To examine how durable these conditioned responses are over time, we implemented the memory triggering task again one day and one week after presenting the film. To study whether fear conditioning plays a role in the effects of memory integration, one day after seeing the film, participants were instructed to imagine and verbalize either the events of the “traumatic” or neutral film, following Ehlers’ (1999) rationale for imaginal exposure. Our design has the advantage of using the well-established standard trauma film paradigm that is known to reliably induce analogue trauma intrusions and allows experimental control of which neutral stimuli are present during the analogue trauma, as in a fear conditioning paradigm. It therefore enables the investigation of associative learning for neutral stimuli present during traumatic events in a relatively natural setting. Additionally, by having participants repeatedly perform the memory triggering task after the “traumatic” film, we are able to assess whether intrusive memories as a conditioned response (CR) to trauma reminders (CS+) remain stable over a longer time span and how they are impacted by early memory integration.

This experimental analogue study had two main aims: (1) Investigating associative learning for intrusive memories and conditioned fear in a traumatic context and (2) examining the effects of memory integration on differential conditioning and subsequent intrusive trauma memories.

Regarding conditioned fear reactions, we expected that neutral sound stimuli repeatedly presented during a “traumatic“ film (CS+), would lead, when presented again in a neutral context, to more intense intrusive memories, more anxiety, and greater physiological arousal (as indexed by enhanced skin conductance levels and heart rates) as compared to neutral stimuli that were originally presented during a neutral film (CS-). This effect was expected to be observed directly after film presentation (t1), on the following day (t2, t3), and one week after film presentation (t4). Furthermore, enhanced conditionability, as assessed by differential conditioned reactions (CS+ minus CS-) directly after film presentation (t1), was expected to predict the intensity of subsequent ambulatory intrusive trauma memories, assessed with an electronic diary over the following seven days. Concerning the effects of memory integration, we expected reduced differential conditioning effects (CS+ minus CS-) on intrusive memories, anxiety, and physiological arousal directly after memory integration (t3) and six days later (t4) for the memory integration group as compared to the control group. Furthermore, we expected the memory integration group to show reduced ambulatory intrusive memories on subsequent days as compared to the control group.

## **2. Materials and Methods**

### *2.1. Participants*

Forty-eight female non-psychology students (mean age: 23.8, range 19-34 years) were recruited on the campus of Saarland University and participated in exchange for 56 Euros. All participants had normal or corrected to normal vision, were native German speakers, reported no history of neurological or psychiatric disorders or past traumatic experience, and gave informed consent. The research was approved by the Department of Psychology Ethics Committee of Saarland University.



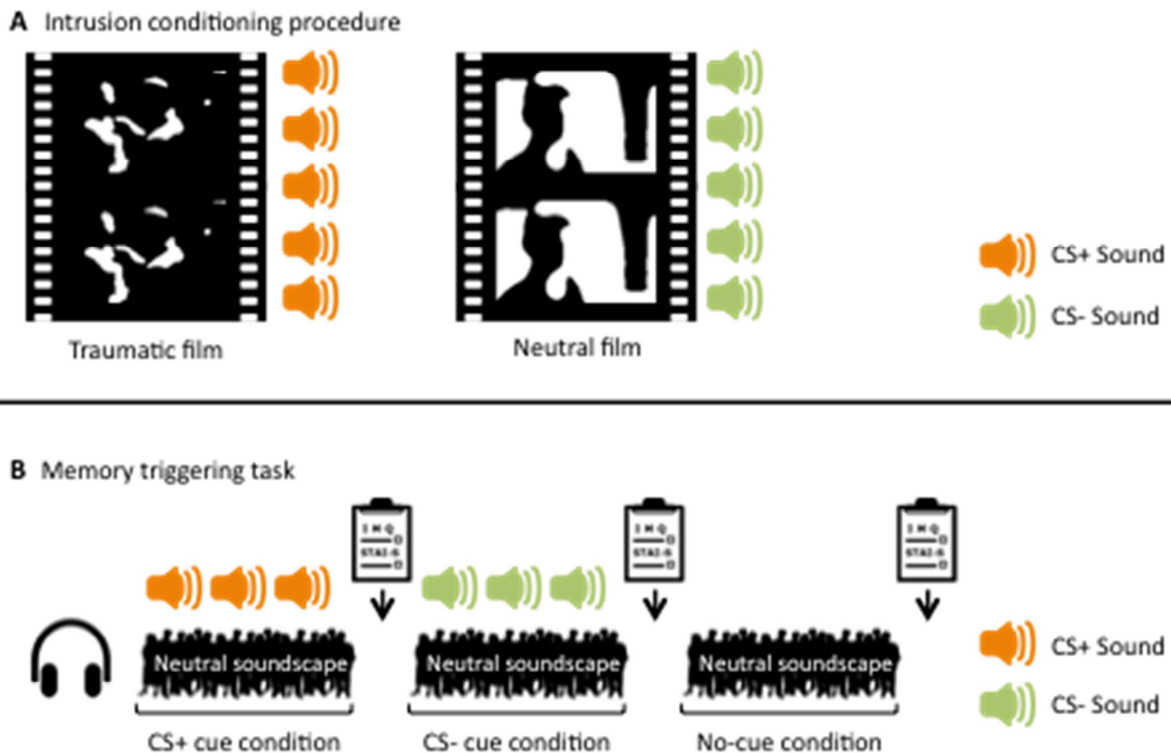
## 2.2. Analogue trauma and intrusion conditioning

All participants saw two film clips (one neutral and one traumatic) in pseudo-randomized order. The neutral film was a compilation of neutral scenes (11 min) from the movie “Three Colors: Blue” directed by Krzysztof Kieslowski (1993). The “traumatic” film consisted of neutral and violent scenes (11 min) from the movie “Irreversible” by Gaspar Noé (2002). During presentation of each film clip one of two neutral sounds with a duration of 5 s (sound A: clock ticking, sound B: sound of a passing train) was presented every minute (11 times) to serve as conditioned stimuli (CS; see Fig. 1). The CS sounds were assigned to CS+ (i.e., sound that was presented during the aversive film clip and serves as danger signal) and CS- (i.e., sound that was presented during the neutral film clip and served as safety signal), pseudo-randomized across participants. After watching each film clip, an adapted version of the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988) was administered, assessing how participants felt while watching the preceding film. Participants were subsequently asked to rate how strongly the preceding film caused physiological arousal on a 5-point scale going from “very slightly or not at all” to “extremely”.

## 2.3. Memory triggering task

The memory triggering task was designed to simulate situations of everyday life in which trauma survivors experience intrusive memories that are triggered by CSs (Wegerer et al., 2013). Following a 1 min physiological baseline measurement, participants were informed that they would then be presented with a background soundscape via headphones while they could let their mind wander freely. The soundscapes were of 3 min duration and featured various people talking with neither content nor language identifiable. In the *CS+ cue condition*, the CS+ sound (clock ticking or train passing) was faded in six times with 5 s duration during sound-scape presentation (see Fig. 1). In the *CS- cue condition*, the CS- sound was faded in six times with 5 s

duration. In the *no-cue condition*, no sound cues were faded in. In both the CS+ and the CS- cue conditions, sound cues were presented subtly but perceptibly at the same points in time (at 15 s, 45 s, 75 s, 105 s, 135 s, 165 s from soundscape onset). The order of cue conditions was counterbalanced across participants (including all six permutations), whereas the order of cue conditions was the same across repetitions for each participant.



**Fig. 1:** Schematic depiction of the conditioned-intrusions paradigm. (A) Intrusion conditioning procedure with “traumatic” and neutral film scenes as unconditioned stimuli (UCS) and neutral sounds as conditioned stimuli (CS+ and CS-). (B) Memory triggering task. Neutral soundscape with faded in CS+ sounds (CS+ cue condition) CS- sounds (CS- cue condition), or no additional sound faded in (no-cue condition); IMQ: Intrusive Memory Questionnaire; STAI-S: STAI state anxiety scale. (modified from Wegerer et al., 2013)

Following each 3 min soundscape presentation, participants filled in the STAI state questionnaire (Laux, Glanzmann, Schaffner, & Spielberger, 1981) and the Intrusive Memory Questionnaire (IMQ; Michael, 2000; Michael & Ehlers, 2007; ). The IMQ was adapted to assess frequency and duration (in seconds) as well as distress (visual analogue scale going from “0 = not at all” to “100 = extremely”) during the preceding soundscape. Intrusions were defined as involuntary memories that could include thoughts, pictures, noises, and emotions. Intrusions

were defined as recurrent, sudden, spontaneous and non-initiated memories of film scenes that might be very vivid and consist of pictures, sounds, thoughts, words or sentences, feelings or combinations of those. Intrusions do not include reflective and conscious thinking or ruminating about the film (translated). Participants were carefully instructed to only report memories that met these criteria. Participants first completed the IMQ with regard to intrusive memories of the “traumatic” film. To give participants the opportunity to report intrusions to the neutral film, thus asserting that only trauma-related memories were included in the assessment of “traumatic” intrusions, the IMQ was subsequently administered again with regard to memories of the neutral film. Only the version assessing trauma-related memories was used for data analysis. To obtain a more reliable score for intrusive trauma memories, we additionally calculated an index of intrusive trauma memories by building a composite score of the IMQ by standardizing ( $z$ -transformation) and summing all single items for the “traumatic” film (For purposes of better illustration the composite scores were transformed into T-scores.) The three soundscapes from the memory triggering task were presented before film presentation (t0) without subsequent questionnaires to habituate participants to the stimuli and to examine potential pre-experimental differences in physiological reactions to the cue conditions. The memory triggering task was assessed at four different measurement points in time (see Fig. 2): (t1) after film presentation, (t2) one day after film presentation before (t2) and after (t3) memory integration (see next section), (t4) at follow-up session (one week after film presentation).

#### 2.4. *Memory integration*

On the day following film presentation, participants were asked to imagine and verbalize one of the two previously seen film clips (neutral or traumatic). Participants were randomly assigned to the two groups. The one group was asked to imagine the “traumatic” film (memory integration group) and the other group (control group) was asked to imagine the neutral film. The instructions were modeled on imaginal exposure (Ehlers, 1999). Participants were asked to close

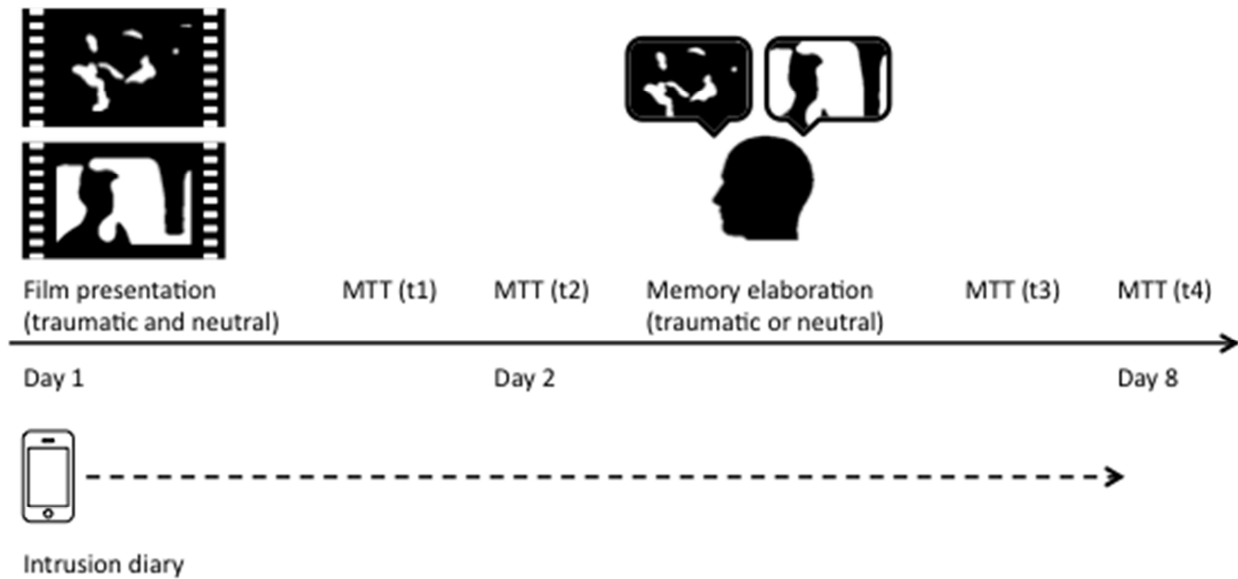
their eyes and imagine the respective film clip as vividly as they could. Participants were encouraged to remember as much detail as possible and recall their original feelings and thoughts. They were asked to remember the action of the film in chronological order.

### *2.5. Assessment of ambulatory intrusive memories*

During the seven days following film presentation, participants documented every intrusive film memory, using an iPod Touch (4th gen., Apple Inc., Cupertino, USA) running Forms VI (Pendragon Software Corporation, Chicago, USA). The frequency of intrusive memories was determined by summing up their frequency for the neutral and the “traumatic” film separately. For each memory, participants stated its duration (in seconds) and rated how distressing it was on a 10-point scale going from “not at all” to “extremely”. These ratings were averaged for the neutral and the “traumatic” film separately (Pfaltz et al., 2013; Streb et al., 2015).

### *2.6. Experimental procedure*

The study took place at the laboratories of the Department of Clinical Psychology and Psychotherapy of the Saarland University. Participation included three appointments: film presentation session (day 1), memory integration session (day 2), and follow-up session (day 8; see Fig. 2). Participants were assigned randomly to one of the two experimental conditions (memory integration of the “traumatic” film or the neutral film).



**Fig. 2:** Study design overview. All participants watched a “traumatic” and a neutral film including neutral sounds. During the memory triggering task (MTT) the neutral sounds from the film were presented again to trigger intrusive memories and conditioned fear. It was administered at four points of measurement (t1: after film presentation, t2: one day after film exposure, before memory integration, t3: after memory integration, t4: one week after film exposure). On the following day, participants were instructed to imagine and verbalize either the “traumatic” film or the neutral film. Participants documented every intrusive memory during the week following film exposure (intrusion diary).

### 2.6.1. Film presentation session (day 1)

After their arrival at the laboratories, electrodes for physiological measurements (electrocardiogram, skin conductance) were attached. Participants were subsequently presented with the three soundscapes of the memory triggering task (t0; CS+ cue condition, CS- cue condition, no-cue condition). This was done in order to assess whether the three conditions had pre-experimental differences in their potential to trigger physiological reactions. Afterwards, each participant saw the two film clips (neutral and traumatic). After presentation of both film clips, participants completed the first run of the memory triggering task (t1) to assess conditioned reactions to the film-associated sounds directly after the films. Before participants left the laboratory, they were reminded to record ambulatory intrusive memories with the electronic diary during the following week before they left the laboratory.

### 2.6.2. *Memory integration session (day 2)*

On the following day, participants returned to the laboratory. Electrodes for physiological measurements were again attached, and participants completed the second run of the memory triggering task (t2) to determine whether conditioned reactions to the film-associated sounds were still present one day after film exposure. Afterwards, they were instructed to complete a memory integration task either of the “traumatic” film or of the neutral film. After memory integration, participants completed the third run of the memory triggering task (t3) to examine the immediate effects of memory integration on conditioned reactions to the film-associated sounds and left the laboratory.

### 2.6.3. *Follow-up session (day 8)*

Seven days after film presentation, participants returned to the laboratory for the last time. They turned in the electronic diary, electrodes for physiological measurements were again attached, and they completed a final run of the memory triggering task (t4) to see whether conditioned reactions to the film-associated sounds were still observable one week after film presentation. Afterwards, participants received 56 Euros for their participation, and were offered to ask questions about the design and goals of the study.

### 2.7. *Apparatus and Physiological Recording*

Participants were seated in an electrically shielded room. Stimulus presentation and behavioral data acquisition were controlled by E-Prime 2.0 (Psychology Software Tools, Inc., Pittsburg, PA, USA). Acoustic stimuli were presented via shielded headphones at a constant volume across participants. To measure heart rate, a standard lead-II electrocardiogram (ECG) with two Ag/AgCl electrodes was used to collect a raw ECG signal with an ActiveTwo amplifier (BioSemi, Amsterdam, The Netherlands) at a sampling rate of 2048 Hz. R-waves were identified

automatically by ANSLAB (Wilhelm and Peyk, 2012) and edited manually for artifacts, false positives or non-recognized R-waves and transformed into instantaneous heart rates (HR). To measure skin conductance levels (SCL), two Ag/AgCl electrodes filled with isotonic electrode gel were attached to the proximal part of the palm of the participants' non-dominant hand (with an alternating current of 1 mA synchronized with the sampling frequency passed between the electrodes). The raw signal of electrodermal activity was collected using an ActiveTwo amplifier (BioSemi, Amsterdam, The Netherlands) at a sampling rate of 2048 Hz and decimated to 25 Hz before further analysis. It was then manually edited for artifacts, smoothed using a 1 Hz low-pass filter.

## 2.8. Statistical analyses

### 2.8.1. Memory triggering task

For each run of the memory triggering task (t1, t2, t3, t4), mean SCL and HR were calculated as the average across the whole phase (3 min) of each condition (CS+ cue condition, CS- cue condition, no-cue condition). Before the actual experiment, a habituation phase (t0) was completed, additionally examining potential pre-experimental differences in physiological reactions to the three cue conditions. No such differences were observed (see Table 1).

To examine the differences in intrusive memories and state anxiety as a reaction to CS+ versus CS-, repeated measures analyses of variance (ANOVA) were calculated separately for each point of measurement (t1, t2, t3, t4<sup>1</sup>) and each outcome measure (IMQ, STAI state anxiety) with the cue condition as the within-participant factor (CS+ cue condition, CS- cue condition, no-cue

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<sup>1</sup> As the two experimental groups (memory integration of the "traumatic" or neutral film) did not differ at point of measurement t3 and t4 with regard to all outcome variables of the MTT, the data of both groups were collapsed for this analysis.

condition). To examine the differential conditioning effects on physiological measures, repeated measures analyses of covariance (ANCOVA) were calculated separately for each point of measurement (t1, t2, t3, t4<sup>1</sup>) and each outcome measure (HR, SCL) with cue condition as the within-participant factor (CS+ cue condition, CS- cue condition, no-cue condition). To account for baseline differences, the respective physiological baseline measurement was included as a covariate.

Individual estimates of conditionability were calculated as the differential reaction to CS+ versus CS- separately for each outcome measure (IMQ, STAI state anxiety, HR, SCL) of the MTT directly after film presentation (t1). These indices of conditionability were correlated with subsequent ambulatory intrusions (frequency, duration, distress).

To examine differences between the memory integration group and the control group in the differential conditioning scores (CS+ minus CS-) repeated measures ANOVAs with point of measurement (t3, t4) as repeated factor and memory integration group as between subject factor were conducted separately for each outcome measure (IMQ, STAI state anxiety, HR, SCL).

### 2.8.2. *Ambulatory intrusive memories*

Individual *t*-tests were used to examine differences between the memory integration group and the control group in the frequency of intrusive trauma memories after memory integration exposure, as well as their duration and distress ratings. The alpha level for all analyses was set to .05 and significant main or interaction effects of ANOVAs were further explored using *t*-tests. For all ANOVAs and *t*-tests, effects sizes are reported as partial eta squared ( $\eta_p^2$ ) or Cohen's *d*, respectively. When the sphericity assumption was violated in ANOVAs, the Greenhouse-Geisser correction for repeated measures was applied with nominal degrees of freedom being reported.



Due to missing values, degrees of freedom varied across analyses. All statistical analyses were calculated using IBM SPSS Statistics 22 (IBM Corp., Armonk, NY, USA).

### 3. Results

#### 3.1. Validity of the film material

Participants reported significantly more negative emotions and higher subjective physiological arousal during presentation of the “traumatic” film as compared to the neutral film (see Table1). Participants also showed significantly enhanced physiological arousal as indexed by elevated skin conductance levels (SCL) and heart rate (HR) during presentation of the “traumatic” film as compared to the neutral film (see Table 1).

**Table 1:** Emotional and physiological reactions to the two film clips and ambulatory intrusive memories of the film clips.

	Reactions to the film clips		
	“traumatic” film	Neutral film	Interferential statistics
	<i>M (SD)</i>	<i>M (SD)</i>	
<b>PANAS – Negative</b>	24.02 (7.73)	5.60 (5.31)	$t(46) = 14.99, p < .001, d = 2.19$
<b>Subjective arousal</b>	23.5 (10.6)	7.3 (8.9)	$t(47) = 10.78, p < .001, d = 1.56$
<b>SCL</b>	8.865 (0.861)	8.690 (0.817)	$t(43) = 4.11, p < .001, d = 0.62$
<b>HR</b>	77.01 (13.69)	72.07 (11.58)	$t(42) = 4.78, p < .001, d = 0.73$

*Note:* PANAS – Negative: PANAS score for negative affect; Subjective arousal: subjective arousal rating after film presentation (“To what extent did the film cause physiological reactions (faster heartbeat, sweating etc.)?”, scale 0 – 100; 0 = not at all, 100 = extremely); SCL: skin conductance level given as  $\ln(1 + SCL)$  in  $\mu\text{S}$ ; HR: heart rate given as beats per minute.

#### 3.2. Conditioned intrusive memories and conditioned fear

##### 3.2.1. Intrusive Memory Questionnaire

As expected, intrusive memories as a conditioned response, assessed with the IMQ (including subjective intrusive trauma memory frequency and duration as well as distress through intrusive

trauma memories) differed significantly across groups in the memory triggering task, comprised of neutral soundscapes with CS+, CS-, or no faded in sound cues at all points of measurement (t1, t2, t3, t4; see Table 2). At all points of measurement participants reported more numerous, longer, and more distressing memories of the “traumatic” film during the CS+ cue condition as compared to the CS- and the no-cue condition (all  $ts(47) > 2.06$ ,  $ps < .05$ ,  $ds > 0.60$ ; see Table 2). The CS- cue condition, in turn, did not differ from the no-cue condition with regard to frequency, duration, or level of distress of memories of the “traumatic” film at all points of measurement (all  $ts(47) < 1.30$ ,  $ps > .20$ ,  $ds < 0.38$ ; see Table 2). This means that participants showed differential conditioning effects for intrusive trauma memories as a conditioned reaction and that these effects were still observable one day and one week after film presentation.

### 3.2.2. *State anxiety*

As expected, STAI state anxiety differed significantly by condition in the memory triggering task at all points of measurement (t1, t2, t3, t4; see Table 2). Participants reported more state anxiety during the CS+ condition than during the CS- and the no-cue condition for all points of measurement (all  $ts(47) > 2.78$ ,  $ps < .03$ ,  $ds > 0.81$ ; see Table 2). The CS- cue condition, in turn, did not differ from the no-cue condition with regard to state anxiety at all points of measurement (all  $ts(47) < 1.68$ ,  $ps > .10$ ,  $ds < 0.49$ ; see Table 2). This means that participants showed differential conditioning effects for state anxiety as a conditioned reaction and that these effects were still observable one day and one week after film presentation.

### 3.2.3. *Physiological parameters*

Contrary to our prediction, no significant differences between the three cue conditions (CS+ cue condition, CS- cue condition, no-cue condition) in SCL or HR during the memory triggering task

were observed at all points of measurement (see Table 2). These findings indicate that, counter to our prediction, no differential conditioning effects for SCL or HR were present.

**Table 2:** Results from intrusive memories, state anxiety, SCL, and HR during the memory triggering task after film presentation (t0, t1, t2, t3, t4).

	Memory triggering task			Interferential statistics
	CS+ condition	CS- condition	No-cue condition	
	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>	
<b>Day 1: Physiological baseline measurement (t0)</b>				
SCL	1.68 (0.71)	1.65 (0.69)	1.67 (0.73)	$F(2, 90) = 0.64, p = .53, \eta_p^2 = .01$
HR	73.82 (10.95)	74.03 (11.44)	73.87 (11.32)	$F(1.6, 72.4) = 0.93, p = .40, \eta_p^2 = .02$
<b>Post-film measurement (t1)</b>				
IMQ – Score	75.12 (30.40) <sup>a</sup>	58.92 (21.44) <sup>b</sup>	56.88 (24.12) <sup>b</sup>	$F(2, 94) = 15.65, p < .001, \eta_p^2 = .25$
IMQ - Frequency	4.49 (2.91) <sup>a</sup>	2.77 (2.09) <sup>b</sup>	2.56 (2.56) <sup>b</sup>	$F(2, 94) = 17.57, p < .001, \eta_p^2 = .27$
IMQ - Duration	26.61 (39.83) <sup>a</sup>	9.32 (18.00) <sup>b</sup>	7.35 (18.64) <sup>b</sup>	$F(1.5, 71.0) = 12.87, p < .001, \eta_p^2 = .22$
IMQ - Distress	36.77 (30.24) <sup>a</sup>	18.79 (25.03) <sup>b</sup>	19.31 (24.58) <sup>b</sup>	$F(2, 94) = 3.96, p < .05, \eta_p^2 = .08$
State anxiety	48.21 (13.90) <sup>a</sup>	45.02 (13.16) <sup>b</sup>	44.54 (11.88) <sup>b</sup>	$F(1.7, 78.5) = 8.53, p < .01, \eta_p^2 = .15$
SCL	2.11 (0.71)	2.11 (0.73)	2.12 (0.71)	$F(2, 86) = 1.69, p = .19, \eta_p^2 = .04$
HR	71.76 (10.71)	71.85 (10.61)	72.88 (11.17)	$F(2, 90) = 0.17, p = .85, \eta_p^2 = .01$
<b>Day 2: Pre memory integration measurement (t2)</b>				
IMQ – Score	62.55 (29.30) <sup>a</sup>	41.96 (18.04) <sup>b</sup>	40.13 (16.67) <sup>b</sup>	$F(2, 94) = 29.36, p < .001, \eta_p^2 = .39$
IMQ - Frequency	3.28 (2.70) <sup>a</sup>	1.40 (1.62) <sup>b</sup>	1.10 (1.39) <sup>b</sup>	$F(1.6, 76.6) = 26.43, p < .001, \eta_p^2 = .36$
IMQ - Duration	32.39 (46.19) <sup>a</sup>	15.14 (46.19) <sup>b</sup>	13.83 (28.21) <sup>b</sup>	$F(1.5, 71.0) = 12.87, p < .001, \eta_p^2 = .22$
IMQ - Distress	36.77 (30.24) <sup>a</sup>	18.79 (25.03) <sup>b</sup>	19.31 (24.58) <sup>b</sup>	$F(2, 94) = 15.21, p < .001, \eta_p^2 = .24$
State anxiety	40.23 (12.18) <sup>a</sup>	36.08 (10.04) <sup>b</sup>	35.81 (9.70) <sup>b</sup>	$F(1.3, 62.5) = 10.98, p < .01, \eta_p^2 = .19$
SCL	1.61 (0.74)	1.62 (0.69)	1.60 (0.69)	$F(2, 90) = 0.93, p = .40, \eta_p^2 = .02$
HR	78.46 (13.62)	77.85 (13.60)	78.03 (13.62)	$F(2, 92) = 0.02, p = .99, \eta_p^2 < .01$
<b>Post memory integration measurement (t3)</b>				
IMQ – Score	61.62 (25.83) <sup>a</sup>	46.44 (19.44) <sup>b</sup>	45.09 (20.71) <sup>b</sup>	$F(1.7, 81.9) = 21.42, p < .001, \eta_p^2 = .31$
IMQ - Frequency	3.08 (2.09) <sup>a</sup>	1.78 (1.79) <sup>b</sup>	1.46 (1.53) <sup>b</sup>	$F(2, 94) = 16.44, p < .001, \eta_p^2 = .26$
IMQ - Duration	26.85 (36.05) <sup>a</sup>	9.60 (16.98) <sup>b</sup>	12.35 (28.89) <sup>b</sup>	$F(1.5, 69.7) = 15.49, p < .001, \eta_p^2 = .25$
IMQ - Distress	36.35 (31.54) <sup>a</sup>	26.75 (30.04) <sup>b</sup>	24.06 (25.37) <sup>b</sup>	$F(2, 94) = 6.88, p < .01, \eta_p^2 = .13$
State anxiety	42.25 (11.50) <sup>a</sup>	39.92 (11.24) <sup>b</sup>	38.81 (10.93) <sup>b</sup>	$F(1.8, 83.5) = 9.39, p < .001, \eta_p^2 = .17$
SCL	2.11 (0.72)	2.09 (0.73)	2.09 (0.74)	$F(2, 90) = 0.27, p = .76, \eta_p^2 = .01$
HR	74.67 (12.35)	74.37 (12.85)	75.09 (12.57)	$F(1.3, 60.9) = 0.81, p = .45, \eta_p^2 = .02$
<b>Day 7: Follow-up measurement (t4)</b>				
IMQ – Score	45.27 (16.32) <sup>a</sup>	32.70 (9.01) <sup>b</sup>	33.32 (10.18) <sup>b</sup>	$F(1.7, 78.1) = 22.68, p < .001, \eta_p^2 = .33$
IMQ - Frequency	2.21 (1.95) <sup>a</sup>	0.81 (1.28) <sup>b</sup>	0.67 (0.97) <sup>b</sup>	$F(1.4, 67.5) = 27.91, p < .001, \eta_p^2 = .37$
IMQ - Duration	9.15 (11.52) <sup>a</sup>	2.23 (3.47) <sup>b</sup>	3.19 (5.15) <sup>b</sup>	$F(1.3, 60.2) = 14.73, p < .001, \eta_p^2 = .24$
IMQ - Distress	18.13 (23.73) <sup>a</sup>	6.50 (12.94) <sup>b</sup>	9.25 (19.39) <sup>b</sup>	$F(2, 94) = 6.94, p < .01, \eta_p^2 = .13$
State anxiety	35.71 (10.97) <sup>a</sup>	33.77 (9.82) <sup>b</sup>	33.96 (9.11) <sup>b</sup>	$F(1.4, 66.6) = 4.74, p < .05, \eta_p^2 = .09$
SCL	1.47 (0.64)	1.45 (0.65)	1.48 (0.64)	$F(2, 98) = 0.46, p = .63, \eta_p^2 = .01$
HR	77.10 (12.28)	76.71 (12.12)	76.49 (12.22)	$F(1.8, 77.4) = 0.18, p = .83, \eta_p^2 < .01$

*Note:* IMQ Score: composite scores of the IMQ in T-scores; state anxiety: assessed by STAI state; SCL: skin conductance level given as  $\ln(1 + SCL)$  in  $\mu S$ ; HR: heart rate given as beats per minute. As the two experimental groups (memory integration of the “traumatic” or neutral film) did not differ at point of measurement t3 and t4, data from both groups were collapsed for this analysis. a, b, different superscripts indicate that the conditions differed from each other at  $p < .05$  in post hoc tests.

### 3.3. Conditionability and ambulatory intrusive memories

To examine whether conditionability of intrusive memories (IMQ), state anxiety (STAI-S), and physiological arousal (SCL, HR) can predict later intrusive trauma memories, conditionability scores were calculated (CS+ minus CS-) separately for each variable.

Conditionability of intrusive memories (IMQ) was significantly correlated with the frequency, duration, and distress of subsequent ambulatory trauma intrusions (all  $r_s > .33$ ,  $p_s < .05$ ; see Table 3), indicating that the specific conditioned reactions to trauma-associated cues (CS+) were predictive of later intrusive memories in everyday life. No correlations, however, were observed for conditionability of state anxiety and physiological arousal (SCL, HR) (see Table 3).

**Table 3:** Correlations between conditionability (as indexed by differential effects on IMQ, STAI-S, HR, SCL) and ambulatory intrusive trauma memories.

	Ambulatory intrusive trauma memories		
	Frequency	Duration	Distress
	$r$ ( $p$ )	$r$ ( $p$ )	$r$ ( $p$ )
<b>Conditionability (CS+ minus CS-)</b>			
<b>IMQ – Score</b>	<b>.39 (.006)</b>	<b>.33 (.02)</b>	<b>.46 (.001)</b>
<b>State anxiety</b>	.12 (.42)	-.03 (.84)	.22 (.13)
<b>SCL</b>	-.02 (.92)	-.12 (.42)	.02 (.92)
<b>HR</b>	.09 (.54)	.08 (.58)	.12 (.41)

*Note:* All scores constitute differential conditioning scores (CS+ minus CS-) from the memory triggering task; IMQ Score: composite scores of the intrusive memory questionnaire for trauma intrusions in T-scores; state anxiety: assessed by STAI state anxiety scale; SCL: skin conductance level given as  $\ln(1 + SCL)$  in  $\mu S$ ; HR: heart rate given as beats per minute.

### 3.4. Effects of memory integration

Contrary to our hypotheses, the two exposure conditions did not differ with regard to differential conditioning effects for the IMQ-Score, STAI-S, SCL, or HR at both points of measurement after imaginal exposure ( $t_3$ ,  $t_4$ ; all  $p_s > .14$ ). No significant differences in frequency, duration, or distress of subsequent ambulatory trauma intrusions ambulatory intrusions between the two groups were observed (all  $p_s > .37$ ). Taken together, these findings indicate that in this study memory integration of the traumatic film had no beneficial effects on differential conditioning or intrusive memories as compared to memory integration of the neutral film.

#### 4. Discussion

This study reveals that associative learning contributes to ambulatory intrusive memories after an analogue trauma. Our findings support the assumption that conditioned associations between neutral stimuli and traumatic events play an important role in the development of intrusive memories of trauma (Duits et al., 2015). From a methodological perspective, we made it possible to study associative learning in the standard trauma film paradigm by experimentally controlling neutral sound stimuli (CSs) encountered during film presentation (UCS). These sound stimuli subsequently elicited intrusive memories and anxiety when presented again after film presentation, but also when presented again one or seven days after film presentation. Our work therefore opens up new possibilities for studying triggers of intrusive memories of trauma, which could enhance our understanding of PTSD.

In line with our prediction, originally neutral sounds that were associated with a traumatic film (and thus served as CS+) provoked more conditioned responses in form of intrusive memories and state anxiety when subsequently presented in a neutral background soundscape, as compared to sounds associated with a neutral film (CS- cue condition) or no additional sounds (no-cue condition). Our findings are therefore in line with a previous fear conditioning study, which demonstrated that presenting acoustic, conditioned trauma reminders during a neutral background soundscape can trigger intrusive memories and anxiety (Wegerer et al., 2013). Furthermore, the pattern of results observed was not only found directly after film presentation, but remained stable until the following day and was still present seven days after film presentation. Conditioned stimuli did retain their potential to trigger intrusive memories and anxiety for a timespan of at least one week, which is a very important extension of previous findings. The observed temporal stability of conditioning effects is in line with contemporary models of PTSD proposing that intrusive memories can be explained by associative learning

processes (Brewin, 2001; Ehlers & Clark, 2000; Foa et al., 1989; Keane et al., 1985; Rothbaum & Davis, 2003).

Surprisingly, peripheral physiological indicators of arousal (SCL, HR) for the three cue conditions (CS+, CS-, no-cue condition) did not differ significantly at any point of measurement (t1, t2, t3, t4). This stands in contrast to findings from Wegerer et al. (2013), who reported significant differences in SCL between the CS+ and the no-cue condition, and to a number of previous studies showing enhanced physiological reactivity to trauma reminders in trauma exposed participants (for a review see Pole, 2007).

In line with our prediction, the conditionability of intrusive memories (as indexed by the IMQ) was correlated with the frequency, duration, and distress of subsequent ambulatory trauma intrusions (assessed by means of the electronic diary): participants who acquired stronger differential conditioned intrusive trauma memories were more likely to experience ambulatory intrusive trauma memories on the days following the analogue trauma. This finding indicates that the conditionability of intrusive memories is related to the ambulatory occurrence of such memories, underlining the important role of conditioned reactions in the development of intrusive memories of trauma. No correlations, however, were observed for conditionability of state anxiety and physiological arousal.

Contrary to our prediction, the memory integration group showed neither a stronger reduction of intrusive trauma memories nor reduced state anxiety as compared to the control group. These findings indicate that early memory integration of the trauma film had no influence on intrusive trauma memories or conditioned reactions to trauma-associated stimuli. As conditioned intrusive trauma memories and anxiety were still observable one week after film presentation, the acquired associations may have been too robust to be impacted by a single memory integration session.

One limitation of our study is that we used an analogue design, so that it is not clear to what extent our results can be transferred to traumatic events in real life. Even though the film used in our study was very aversive, it is still a relatively mild stressor compared to traumatic events. Hence, the intrusive memories reported by our participants are not equivalent to intrusive memories after real-life trauma. As well, the frequency of intrusive memories in our sample was fairly small when compared to trauma exposed samples (Michael, Ehlers, Halligan, et al., 2005). Exposing participants to stronger stressors should, of course, lead to more intrusive memories, but ethical considerations set inevitable limits on the intensity of laboratory stressors.

A further limitation of the current study is that the sample was comprised of women only. We decided to include only women for several reasons: first, previous studies have observed significant gender differences in affective self-reports and physiological responses to emotional stimuli (Bianchin & Angrilli, 2012; Bradley et al., 2001; Kring & Gordon, 1998), so that including both genders would have added systematic variance to our outcome measures. Second, the prevalence of PTSD is higher among women (Perkonigg et al., 2000), so we expected the “traumatic” film clip to have a larger impact on women than on men. As we were interested in the memory processes underlying PTSD and its intervention methods and not in gender differences in this study, we decided to only include women. Future studies should extend our findings to both genders.

In conclusion, our experiment demonstrated that presenting neutral sound stimuli during a “traumatic” film leads to conditioned intrusive responses to these stimuli that remain stable over a time period of at least one week. Furthermore, the conditionability of intrusive trauma memories predicted later ambulatory intrusions of trauma memories. Our study therefore provides evidence for the assumption that intrusive trauma memories can at least partially be explained by conditioned responses to neutral stimuli that have been encountered during the trauma. However, no evidence was found for the assumption that early memory integration has

the effect of reducing these associations, which may be due to including only a single memory integration session. Future research should further examine the role of associative learning for memory integration to promote enhancements of this clinical intervention for PTSD patients.

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## Highlights

- Trauma-associated sounds elicit intrusive memories and anxiety after trauma film
- Conditioned intrusive memories are stable over one week
- Enhanced conditionability predicts subsequent ambulatory trauma intrusions

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