Contextual fear conditioning in humans using feature-identical contexts

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A B S T R A C T

Contextual fear conditioning studies in animals and humans found an involvement of the hippocampus and amygdala during fear learning. To exclude a focus on elements of the context we employed a paradigm, which uses two feature-identical contexts that only differ in the arrangement of the features and requires configural processing. We employed functional magnetic resonance imaging to determine the role of the hippocampus and neocortical areas during the acquisition of contextual fear in humans. For contextual fear acquisition, we paired one context (CS+) with an aversive electrical stimulus, whereas the other (CS−) was never followed by aversive stimulation. Blood oxygen level dependent activation to the CS+ was present in the insula, inferior frontal gyrus, inferior parietal lobule, superior medial gyrus and caudate nucleus. Furthermore, the amygdala and hippocampus were involved in a time-dependent manner. Psychophysiological interaction analyses revealed functional connectivity of a more posterior hippocampal seed region with the anterior hippocampus, posterior cingulate cortex and superior parietal lobule. The anterior hippocampus was functionally coupled with the amygdala and postcentral gyrus. This study complements previous findings in contextual fear conditioning in humans and provides a paradigm which might be useful for studying patients with hippocampal impairment.

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1. Introduction

In fear conditioning, an initially neutral conditioned-stimulus (CS) is paired with an aversive unconditioned stimulus (US) that evokes fear or anxiety responses. Repeated pairings of the CS with the US result in an association of both stimuli that causes the occurrence of the CS alone to elicit an emotional response. While cue conditioning requires only a single feature to be associated with the US, contextual conditioning demands the association of the US with a whole set of features. Consequently, these two variants of classical fear conditioning also differ in the way in which the CS–US association occurs on a behavioral and neural level. The dual-systems theory provides a mechanistic framework for contextual representations in the mammalian brain (Nadel & Willner, 1980; Rudy & O'Reilly, 2001). According to this account, a single stimulus is thought to be represented in the neocortex and bound into an association with a threatening event in the amygdala (Fanselow, 2010; Rudy, 2009). Several co-occurring

stimuli, in contrast, first need to be consolidated into a hierarchical, conjunctive representation which necessitates the binding capacity of the hippocampus (Rudy, 2009). This representation is then transferred to the amygdala to drive the associative process. However, studies showed that lesioning of the hippocampus shortly after the learned CS–US association severely impairs the expression of contextual fear, whereas damage to the hippocampus prior to conditioning has little effect (Maren, Aharonov, & Fanselow, 1997; Wiltgen, Sanders, Anagnostaras, Sage, & Fanselow, 2006). These findings have led to the hypothesis that if the hippocampus is damaged, single cues, which are stored in the neocortex, still can represent the context. This is referred to as 'elemental processing' as opposed to the hippocampus-dependent 'configural processing' (Iordanova, Burnett, Aggleton, Good, & Honey, 2009). Configural or relational learning theories state that the formation of the representation of context relies on the integration of multiple cues into a unified or configural representation and it is assumed that the hippocampus plays a major role in this process (Eichenbaum, 2004; Moses & Ryan, 2006; Nadel & Willner, 1980; Sutherland & Rudy, 1989). However, in rats, hippocampal damage only seems to affect performance in those configural learning paradigms that require discrimination between visual scenes containing common elements (Albasser et al., 2013; Dumont, Petrides, & Sziklas, 2007; Sanderson, Pearce, Kyd, & Aggleton, 2006). Albasser et al. (2013) suggest that stimuli with

common elements will be individually structured by binding together common cues in unique spatial ensembles. Hippocampal lesions can spare configural discriminations when item-location binding is not integral to the problem (Bussey, Warburton, Aggleton, & Muir, 1998; Sanderson et al., 2006; Saksida, Bussey, Buckmaster, & Murray, 2007). Amnesic patients compared to matched controls show deficits in reconstructing the spatial locations of a small array of objects after a short delay (Watson, Voss, Warren, Tranel, & Cohen, 2013). They were particularly impaired when two objects swapped places during the delay phase, which demanded object identity-to-relative-location bindings. A further study showed that hippocampal damage results in poor memory for the change in location of a single item embedded in a scene, even though the memory for the scene itself was intact (Hannula, Tranel, & Cohen, 2006). Similarly, Olson, Moore, Stark, and Chatterjee (2006) reported that amnesic patients had a specific deficit in remembering object-location conjunctions, while the memory for objects and individual locations was preserved. These results are consistent with the finding that hippocampal place fields show global remapping after the presentation of familiar cues in changed places (Leutgeb et al., 2005). In humans, previous contextual fear conditioning paradigms utilized virtual reality contexts (Alvarez, Biggs, Chen, Pine, & Grillon, 2008; Grillon, Baas, Cornwell, & Johnson, 2006), spatial picture contexts (Marschner, Kalisch, Vervliet, Vansteenwegen, & Büchel, 2008) or color background contexts (Lang et al., 2009; Pohlack et al., 2012a; Pohlack, Nees, Ruttorf, Schad, & Flor, 2012b) during fMRI. These studies did not focus on the question of elemental versus configural processing and thus did not employ stimulus material that included identical elements between the context scenes. This could lead to unclear results, especially in subjects with impaired hippocampal functioning, as these contextual stimuli could be processed without reverting to a configural, hippocampus-dependent strategy. To create an experimental conditioning scenario that requires configural processing we constructed a cue-array context paradigm that is comprised of two feature-identical picture stimuli, which are only differing in the arrangement of their context components. This paradigm should ensure that focusing on single elements is not a sufficient strategy to distinguish between the two context pictures and thus to predict the CS–US association. We expected that fear-related neocortical brain regions would be constantly active during acquisition, whereas learning-related regions in the medial temporal lobe should show an initial activation that would decrease over time (Büchel, Morris, Dolan, & Friston, 1998; Marschner et al., 2008). Furthermore, the coupling patterns of the hippocampus with other brain regions were of interest to delineate the contextual fear conditioning process, assuming that functional connections with regions involved in emotional (e.g. amygdala) as well as cognitive (e.g. parietal cortex) processing should emerge.

2. Materials and methods

2.1. Participants

Seventeen healthy young adults participated in the study after giving written informed consent (8 male, age range: 22–36; mean age: 28.5 ± 3.52 SD). They were all right-handed and reported no history of mental or neurological disorders. Two participants were excluded from further data analysis due to their inability to identify which of the two picture-stimuli was actually associated with an aversive stimulus, leaving 15 participants (7 male) for the fMRI analysis. Due to technical problems during recording of skin conductance responses (SCR), the data of one participant were discarded, reducing the number of participants for the SCR analysis

to 14 (6 male). All participants were German native speaking university students or graduates. The study was approved by the Ethics Committee of the Medical Faculty Mannheim and adhered to the Declaration of Helsinki.

2.2. Experimental design

The two context-picture stimuli were created using the virtual reality software NeuroVR (version 2.0; www.neurovr2.org) and depict a living room in which 4 elements (TV set on a cabinet, bookshelf, wall picture and a door) had a different spatial arrangement in picture one compared to picture two (Fig. 1). Three other elements (couch, chair and a floor lamp) remained stationary in both pictures. The experimental procedure in this event-related design consisted of three conditions: one picture that was never associated with an electric stimulus (CS−) and a second picture where a painful electric stimulus was pseudorandomly applied in 50 percent of the trials (CS+paired and CS+unpaired, respectively). The assignment of the pictures to CS+ and CS− was counterbalanced between participants. The condition CS+unpaired was created to investigate hemodynamic responses evoked by the CS+ without the confounding effects of the US. Pictures were presented for the duration of four seconds and appeared in a pseudo-randomized order with every picture being shown 40 times during the entire experimental run. The same stimulus (e.g. CS+) occurred maximally three times in a row and the US was never administered in two consecutive trials. Inter-stimulus intervals were randomly jittered between 8 and 12 s resulting in trials of 12, 13, 14, 15 and 16 seconds length (Fig. 2). As a US we used an electric stimulus, which was administered to the right thumb via a pair of surface electrodes and occurred within an interval of 0.5–3.5 s during the presentation of the CS+. US onset was randomized within the described interval to ensure that participants perceived the occurrence of the US as unpredictable, a prerequisite for inducing anxiety in aversive context conditioning (Grillon, Baas, Lissek, Smith, & Milstein, 2004). The US consisted of a train of 6 electric pulses that were applied in a frequency of 12.2 Hz over the duration of 480 ms. US intensity was individually adjusted to be aversive but not too painful. The magnitude of the stimulation was initially set at 80 percent of the difference between the individually assessed pain threshold and pain tolerance level. The electric stimulus of this magnitude was then administered to the subject's right thumb and had to be rated on painfulness and unpleasantness on a 9-point scale (from 1 = not painful/not unpleasant to 9 = very painful/very unpleasant). The magnitude of the stimulation was adjusted if ratings for painfulness and unpleasantness did not reach 7 or 8 points on both scales. Before the experiment started, participants were instructed to view the pictures attentively during the session while they would occasionally receive a painful stimulus. The net scanning time for a single subject session was 19 min. The experimental procedure included neither a habituation (presentation of CSs and US without pairing prior to acquisition) nor an extinction phase (presentation of CS+ and CS− without delivery of US during CS+ after the acquisition phase).

2.3. Skin conductance response (SCR)

Skin conductance was recorded continuously by two Ag/AgCl electrodes from the thenar and hypothenar of the left hand with a sampling rate of 5000 Hz. Before mounting of the electrodes, the skin was prepared with an isotonic saline solution (0.9 percent saline) and electrode paste was applied to the electrodes, which contained 0.5 percent saline in a neutral base. The signal was amplified using a BRAINAMP ExG MR device in combination with a GSR MR module (BRAIN PRODUCTS, Gilching, Germany).