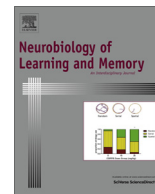




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## Odors as effective retrieval cues for stressful episodes

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## ABSTRACT

Olfactory information seems to play a special role in memory due to the fast and direct processing of olfactory information in limbic areas like the amygdala and the hippocampus. This has led to the assumption that odors can serve as effective retrieval cues for autobiographic memories, especially emotional memories. The current study sought to investigate whether an olfactory cue can serve as an effective retrieval cue for memories of a stressful episode. A total of 95 participants were exposed to a psychosocial stressor or a well matching but not stressful control condition. During both conditions were visual objects present, either bound to the situation (central objects) or not (peripheral objects). Additionally, an ambient odor was present during both conditions. The next day, participants engaged in an unexpected object recognition task either under the influence of the same odor as was present during encoding (congruent odor) or another odor (non-congruent odor). Results show that stressed participants show a better memory for all objects and especially for central visual objects if recognition took place under influence of the congruent odor. An olfactory cue thus indeed seems to be an effective retrieval cue for stressful memories.

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## 1. Introduction

Processing olfactory stimuli is a unique process in the mammalian brain. Olfactory stimuli are detected by olfactory neurons and are directly transferred to the olfactory bulb and from there directly, without thalamic gating, to the amygdala. The amygdala is directly connected to the hippocampus (Buck, 2000; Mouly & Sullivan, 2010; Wilson, Best, & Sullivan, 2004). Besides being involved in processing of olfactory information, the hippocampus is mainly involved in learning and memory processes, especially episodic memory (Moscovitch, Nadel, Winocur, Gilboa, & Rosenbaum, 2006; Nadel, Samsonovich, Ryan, & Moscovitch, 2000). The amygdala is mainly involved in the processing of emotional arousal and has a modulating function on memory processes (Cahill & McGaugh, 1998). Thus, by the fast and strong anatomical connection between brain structures processing emotion, memory, and olfactory information it is not surprising that odors appear to play a special role in memory, especially emotional memory processes. It has been shown that memories for odors are very long lasting and do not fade away as memories for e.g. pictures do (Engen,

1987). Furthermore, odors have been found to be effective retrieval cues. Aggelton and Waskett (1999) showed that odor exposure during retrieval enhanced memories for a museum visit where the same odors were present compared to other odors or no odors during retrieval. Odors also enhance context dependent memory when compared to visual cues (Pointer & Bond, 1998). Furthermore memories triggered by odors are older and more emotional than those triggered by verbal cues (Chu & Downes, 2002; Herz & Cupchik, 1995; Willander & Larsson, 2007). Especially odors eliciting memories of aversive events are more detailed, unpleasant, and arousing than memories elicited by verbal cues (Toffolo et al., 2012). When participants are in an anxious and stressed state shortly before an exam, odors can act as effective context retrieval cues which enhance memory (Herz, 1997).

Stress by itself is able to influence learning and memory processes. Stress induces an activation of the hypothalamic-pituitary-adrenal (HPA) axis leading to a release of glucocorticoids (GCs) acting predominately in the hippocampus, the amygdala, and prefrontal regions, all key regions for emotional memory processes (de Kloet, Joels, & Holsboer, 2005; Joels, Karst, DeRijk, & de Kloet, 2008; Ulrich-Lai & Herman, 2009). The direction of stress effects on memory is highly depending on the timing of the stressor. While stress during encoding and consolidation is enhancing memory performance, stress during the time of retrieval has an impairing effect. Additionally, material to-be-remembered has to be associated or bound to the stressor in order to be remembered

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better (Joels, Pu, Wiegert, Oitzl, & Krugers, 2006; Wolf, 2009). Most human studies have investigated effects of stress on memory for material which is often unrelated or only weakly related to the stressor and material was mostly learned shortly after or before stress induction (Schwabe, Bohringer, Chatterjee, & Schachinger, 2008; Smeets et al., 2009). We recently have shown that memory for a stressful episode follows a characteristic pattern itself (Wiemers, Sauvage, Schoofs, Hamacher-Dang, & Wolf, 2013b). We exposed participants either to a psychosocial laboratory stressor (Trier Social Stress Test; TSST) reliably inducing an activation of the HPA axis (Kirschbaum, Pirke, & Hellhammer, 1993) or a newly developed control condition (friendly-TSST; f-TSST) not activating the HPA axis (Wiemers, Schoofs, & Wolf, 2013a). During both conditions participants were exposed to visual objects which were either bound to the stressful situation (central objects) or which were not bound to it (peripheral objects). Consistent with literature from emotional memory research, central visual objects of a stressful episode were remembered better than central visual objects of a non-stressful episode. Furthermore, results from this study (Wiemers et al., 2013b) showed by receiver operating characteristics (ROC) analyses that especially the hippocampal-based retrieval process recollection (Sauvage, Fortin, Owens, Yonelinas, & Eichenbaum, 2008; Yonelinas, 2002) is influenced by stress. The process of familiarity is not influenced by stress. This fits to the dual process model of recognition memory which states that recollection is based on hippocampal processes while familiarity is based on perirhinal processes (Sauvage et al., 2008; Yonelinas, 2002). The effect of stress on only recollection might be attributable to the acting in of GCs in the hippocampus.

The current study sought to investigate whether an odor can serve as effective retrieval cue for memories of a stressful episode. Due to the direct and fast involvement of the amygdala and the hippocampus in olfactory processing and the involvement of exactly those regions in memory enhancing effects due to stress induced hormonal changes, we hypothesized that an odor would serve as especially effective retrieval cue for memories of a stressful episode. We additionally explored the contribution of recollection and familiarity to recognition memory.

## 2. Methods

### 2.1. Participants

Ninety-five healthy adults (48 males) between 18 and 32 years of age took part in the experiment. General exclusion criteria were former participation in the TSST, a Body Mass Index (BMI; weight in kg/height in m<sup>2</sup>) under 19 or over 30, being in medical treatment, taking medication influencing the HPA axis, and smoking. Pregnant or menstruating women and women taking hormonal contraception were excluded from participation as well, since it has been found that women taking hormonal contraception show a blunted cortisol response to the TSST (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Participants received a compensatory payment of 25€. The study was approved by the local ethical committee of the Faculty of Medicine of the Ruhr-University Bochum and the Declaration of Helsinki was followed.

### 2.2. Procedure

On the first day, participants sat in a waiting room, signed informed consent and afterwards performed two tasks irrelevant for current analyses (studying a wordlist and doing a picture story exercise). Fifty-five minutes after arrival participants rated their current affect by filling in the “Positive and Negative Affect Scale” (PANAS, pre; Watson, Clark, & Tellegen, 1988) and delivered the

first saliva sample (baseline). Next, participants were brought to a different room where they underwent the stress or control condition, group assignment was random. Stress was induced by a slightly modified version of the TSST, a public speaking task found to reliably induce a cortisol response (Kirschbaum et al., 1993). The friendly-TSST served as non-stressful control condition. It has been shown to not activate the HPA axis (Wiemers et al., 2013a). Both procedures are described more detailed below. During both procedures visual objects and an ambient odor were in the room. After the respective procedure participants were brought back to the waiting room where they delivered the second saliva sample (+1) and filled in the PANAS (post). After 15 min, participants delivered the next saliva sample (+15). The last saliva sample was taken 30 min after the end of the stress or control condition (+30). Afterwards participants were debriefed about the TSST but were never alerted that their memory for the stress or control condition would be assessed on the next day.

On day 2, approximately 24 h later, participants came back to the lab but this time to a different floor. In a waiting area in the hallway participants first filled in the PANAS and other questionnaires irrelevant for the current report. Next, a saliva sample (day2\_pre) was delivered. Then participants were seated into one out of two identical small test rooms which were equipped with a chair, a desk and a PC. In one room the congruent odor (the odor which was present in the TSST/friendly-TSST room the day before) was present in the other room the non-congruent odor was present. This retrieval odor assignment was random. Afterwards participants delivered a last saliva sample (day2\_post), did a short anosmia screening, and rated the odor for valence. Finally, participants were thanked, debriefed, and paid.

### 2.3. Material

#### 2.3.1. Salivary stress markers

Participants were advised to refrain from eating or drinking anything but water 1 h before testing and from doing excessive sports, drinking alcohol, or taking medication the day before. Saliva for hormonal assessment was sampled using Salivettes® (Sarstedt, Nuernbrecht, Germany) four times on the first testing day and twice about 24 h later on day 2. Cortisol was analyzed by an immunoassay (IBL, Hamburg, Germany). Inter- and intra-assay variabilities were below 10%. Additionally salivary Alpha Amylase (sAA) was analyzed as an indirect marker for sympathetic nervous system activity as described elsewhere (Rohleder & Nater, 2009). Since cortisol and sAA follow circadian rhythms (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004; Wolf, Convit, Thorn, & de Leon, 2002) all testing was carried out in the afternoon starting between 1 p.m. and 4.45 p.m. on the first day and starting between 11.30 p.m. and 5 p.m. the second day.

#### 2.3.2. Affect rating

Participants rated current affect on the “Positive and Negative Affect Scale” (PANAS; Watson et al., 1988), a five point scale with 20 items. Items can be subdivided resulting in one value for positive affect (PA) and one for negative affect (NA). We were only interested in changes of negative affect since it has been repeatedly shown that the TSST does not affect positive affect (Schoofs, Preuss, & Wolf, 2008; Wiemers et al., 2013a). Thus, in the following we will only report negative affect. Participants completed the PANAS twice on day 1 and once on day 2.

#### 2.3.3. Stress procedure

2.3.3.1. *Tsst*. To induce a hormonal stress reaction the Trier Social Stress Test (TSST) was used. It is a standardized psychosocial laboratory stressor leading to a robust activation of the HPA axis (Kirschbaum et al., 1993). Originally, it consists of a 5 min

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