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Electro-olfactogram Responses Before and After Aversive Olfactory Conditioning in Humans

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Abstract—The aim of the present study was to investigate whether repetitive aversive odor conditioning induced changes at the level of the peripheral olfactory system in humans. A total of 51 volunteers participated. A pair of indistinguishable odor enantiomers [(+)-rose oxide and (−)-rose oxide] were used as stimuli. During the pre-conditioning, participants' ability to discriminate between the two odors was assessed using a three-alternative, forced-choice discrimination test. In addition, electro-olfactograms (EOG) from the olfactory epithelium were recorded. Participants underwent three conditioning sessions on consecutive days. The experimental group received an electrical stimulus to the forearm only following (+)-rose oxide presentation, whereas its enantiomer sibling was never paired with the aversive stimulus; the control group did not receive any electrical stimulation. During the post-conditioning session, their ability to discriminate the two enantiomers was assessed again using the discrimination test and EOG recordings were obtained similarly to the pre-conditioning session. Results showed significant differences in the peripheral electrophysiological responses between the conditioned and the unconditioned stimulus, demonstrating contextually induced changes at the level of the first neuron in the olfactory system.

Key words: aversive conditioning, olfactory conditioning, electro-olfactogram, olfactory epithelium.

INTRODUCTION

The ability to associate a threat signal (e.g., the smell of rotten food) with its potential danger (i.e., food poisoning) is critical for safety and survival. A very common laboratory model to study this type of learning is represented by the Pavlovian fear conditioning (Pavlov, 1927). It involves a previously neutral stimulus (the conditioned stimulus, or CS+; e.g., a tone, light, or odor) which is associated with an aversive stimulus (the unconditioned stimulus, or US; e.g., an electrical stimulus), while a second neutral stimulus remains unpaired (CS-). After repeated pairings with the US, the individual learns to associate the CS+ with the presence of the aversive stimulus and therefore CS+ develops an emotionally salient conditioned response. Aversive olfactory conditioning, in particular, is a specific form of Pavlovian learning, which might shed light on aversive conditioning processes more than other forms of conditioning. Given the close anatomical and functional connections to brain areas strongly implicated in learning, memory and emotions [i.e., the limbic system; e.g., (Zald and Pardo, 1997)], olfaction might provide a tool to further elucidate aspects linked to the etiology and the treatment of fear-related disorders, such as post-traumatic stress disorder, multiple chemical sensitivity, and pre-treatment chemotherapy nausea (Parma et al., 2017)

Studies in animals – especially rodents – show that odor conditioning alters both the behavioral responses and the neural processing and morphology within the central olfactory system, especially the amygdala [for reviews, see: (Otto et al., 2000; Li, 2014)]. Interestingly, recent studies in mice reported that fear learning can induce plasticity even in the lower levels of olfactory hierarchy [i.e., olfactory sensory neurons (OSNs) and the olfactory bulb: e.g., (Jones et al., 2008; Kass et al., 2013)] suggesting that emotional information can be encoded already at the level of primary sensory processing. In a seminal paper, Jones et al. (2008) provided the first evidence that associative learning can affect primary sensory neurons in mammals, by demonstrating an increased odorant-specific representation in the main olfactory epithelium and in glomeruli within the olfactory bulb three weeks later. These changes have also shown

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Abbreviations: EOG, electro-olfactograms; fMRI, functional magnetic resonance imaging; ISI, interstimulus interval; OSNs, olfactory sensory neurons; rm-ANOVA, repeated-measures analysis of variance.

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to be reversed following extinction training specific to the conditioned odor stimulus (Morrison et al., 2015). Additionally, potentiated OSN responses in olfactory bulb glomeruli (Abraham et al., 2014) and an increased release of neurotransmitters from the OSNs (Kass et al., 2013) have been observed following olfactory conditioning. However, these changes in OSN odor representations were not observed in a very recent study (Chu et al., 2017) where mice were trained to discriminate between two very similar odorant mixtures.

Although evidence is yet scarce, studies on humans report that aversive olfactory conditioning modulates the neural processing of the conditioned odor in the primary olfactory (piriform) cortex (Li et al., 2008) and in the amygdala (Moessnang et al., 2013). To date, no study has reported a conditioning-dependent modulation in the OSNs in human participants. Nevertheless, psychophysical experiments have demonstrated that aversive olfactory conditioning may increase the sensitivity to a shock-predictive odorant (Åhs et al., 2013) that persists a few days (Parma et al., 2015). This result demonstrates that the previously reported aversive conditioning-dependent effects on OSNs in mice may generalize to humans. However, no studies have directly tested this hypothesis. A unique feature of the olfactory system is that the initial neural representations of external stimuli are experimentally accessible *in vivo*: OSNs are in direct contact with the environment and readily accessible (Zelano and Sobel, 2005). One method of studying OSNs entails the intranasal recording of the electro-olfactograms (EOG) (Knecht and Hummel, 2004) through the insertion of a tubular electrode into the nasal cavity. EOG represent the sum of generator potentials recorded from the olfactory epithelium in response to an olfactory stimulus which arises from the synchronous activity of OSNs (Getchell and Getchell, 1991; Knecht and Hummel, 2004), providing therefore important neuronal information from the peripheral olfactory level (Lapid and Hummel, 2013). They are typically recorded by placing a silver-chlorided electrode on the surface of the olfactory mucosa, under endoscopical guidance (Ottoson, 1955). The aim of the present investigation was to explore whether repetitive odor aversive conditioning might induce changes in the activity of OSNs thus reflecting a direct conditioning-dependent plasticity at the peripheral level. This was accomplished by recording EOG from the olfactory epithelium in human participants. In line with what was found in rodents [e.g., (Jones et al., 2008; Kass et al., 2013)] we expected to find alterations at the peripheral level after conditioning, indicating contextually induced plasticity.

EXPERIMENTAL PROCEDURES

Participants

A total of 51 participants (35 females) with a mean age of 25.2 years (SD: 3.9) took part in the study after providing informed written consent. All participants were in good general physical and mental health. None of the participants were currently taking any form of medication, or suffered from any form of hormonal, neurological, or autoimmune diseases, and none had suffered a head trauma leading to unconsciousness. Participants were instructed not to eat or drink anything but water one hour prior to testing and not to wear any perfume or scented products on the day of testing. Smokers were excluded in the study. Subjects received a moderate financial reward for participation in the study. All aspects of the study were performed in accord with the Declaration of Helsinki and approved by the Ethics Committee of the Medical Faculty at the TU Dresden (application number EK361082016).

Assessment of olfactory function

Prior to the electrophysiological measurements, normal olfactory function among participants was ascertained using the ‘‘Sniffin’ Sticks” test (Hummel et al., 1997). Felt-tip pens filled with odors were used to deliver the olfactory stimuli. For odor presentation the cap was removed by the experimenter for approximately 3 s and the pen’s tip was placed approximately 2 cm in front of both nostrils. Three different olfactory functions were assessed. First, odor thresholds were determined for phenyl-ethyl-alcohol (i.e., a rose-like odor) with 16 stepwise dilutions. Thresholds were measured using the single-staircase technique based on a 3AFC task. Second, odor discrimination was assessed over 16 trials. For each discrimination, three pens were presented, two containing the same odor and the third containing the target odorant (3AFC task). Third, odor identification was assessed by presenting 16 common odors, each presented with four verbal descriptors in a multiple forced-choice format (three distractors and one target). The interval between odor presentations was 20–30 s. A total score (Threshold-Discrimination-Identification: TDI) above 30.5 was considered to be within the normosmic range.

Odor stimuli and delivery

A pair of odor enantiomers, which are structural mirror images, were used as stimuli as they previously have been shown to be perceptually indistinguishable in humans [e.g., (Laska and Teubner, 1999; Li et al., 2008)]: (+)-rose oxide (Fisher Scientific, CAS 16409-43-1) and (−)-rose oxide (Sigma–Aldrich, CAS, 16409-43-1). Starting from concentrations used in the study of Li et al. (2008) at which enantiomers were indistinguishable [11.1% (+)-rose oxide and 8.3% (−)-rose oxide], we further diluted the odors with propylene glycol outside the olfactometer (Sigma–Aldrich, CAS 57-55-6). First, a lateralization task in an independent sample of 10 participants was run (Hummel et al., 2003) in order to determine the trigeminal activity of the two odorants. The odors were presented 20 times to the left or right nostril and if participants were unable to correctly localize the stimulated nostril the odorant was considered as not eliciting any major trigeminal activation. The information was needed to avoid behavioral discrimination based on the trigeminal component of the odor, and to obtain EOG responses based on olfactory and not on trigeminal stimulation. The final concentrations were 0.0275% (+)-rose