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Research report

Temporary inactivation of the medial and basolateral amygdala differentially affects TMT-induced fear behavior in rats

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Abstract

Trimethylthiazoline (TMT) is a component of fox feces and is thought to be a stimulus with innate fear-eliciting properties for rodents. Naive laboratory rats that are exposed to TMT display freezing behavior, a known behavioral sign of fear and anxiety. Early studies examining the neural basis of TMT-induced fear showed that the bed nucleus of the stria terminalis is important for this behavior. In contrast, the central and lateral nuclei of the amygdala does not seem to participate in the neural processing of TMT-induced fear. However, a study investigating c-fos expression in response to TMT-exposure revealed a strong activation of the medial as well as a weak activation of the basolateral amygdala. Therefore, the present study examined the effects of temporary inactivation of the medial and basolateral amygdala on TMT-induced freezing. Temporary inactivation was accomplished by local injections of the $GABA_A$ receptor agonist muscimol into the areas of interest. TMT-induced freezing was completely blocked by temporary inactivation of the medial amygdala. Temporary inactivation of the basolateral amygdala resulted in a delay of the onset of the freezing response to TMT. These results clearly demonstrate that the medial amygdala is crucial for TMT-induced freezing, whereas the basolateral amygdala seems to play a modulatory role in this type of fear behavior. Since the medial amygdala is also involved in the processing of cat odor-induced fear, the finding of the present study points towards a general role of the medial amygdala in the processing of predator odor-induced fear.

Keywords: Anxiety; Basolateral amygdala; Defensive behavior; Fear; Fox odor; $GABA_A$ receptor agonist; Muscimol; Predator odor; Trimethylthiazoline; Unlearned fear

1. Introduction

Fear is a behavioral system, which enables an animal to survive possible threatening situations [11]. It can be either expressed as a response to learned danger-predicting stimuli (conditioned or learned fear), or to stimuli with innate fear-eliciting properties (unconditioned or unlearned fear). While many studies have elucidated the neural basis of conditioned fear, the neural processing of unconditioned fear is much less well understood. In order to investigate the neural basis of unlearned fear, several studies took advantage of predator odor-induced fear responses.

Predator odors can be regarded as ecologically relevant signals for rodents and are innately capable of eliciting fear responses, i.e. specific defensive behaviors [16]. Complete predator odors [1; summarized in 6] as well as specific components of predator odors [summarized in [10]] have been used as species-specific stimuli to study fear responses in rodents. For example, trimethythiazoline (TMT), a component of fox feces, evokes a number of fear-related behaviors (e.g. freezing, risk assessment, startle potentiation, defensive burying) in rats that have never been exposed to TMT before [2,8,9,15,33,34]. The behavioral responses to TMT appear to be innate, since other novel and aversive odors (e.g. butyric acid, caproic acid, ethyl acetate or isoamyl acetate) fail to elicit such pronounced fear-related responses [14,26,33, Endres, unpublished observation].

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That is, rats do not generally show fear-like behavioral changes to novel and aversive odors.

A first investigation examining the neural basis of TMT-induced fear behavior by Wallace and Rosen [34] showed that the lateral amygdala, which is known to be critical for the processing of learned fear [3,17], does not seem to participate in the processing of TMT-induced fear behavior. The result of Wallace and Rosen was confirmed by a study of Fendt and colleagues [9] demonstrating that temporary inactivation of the lateral and central amygdala did not affect TMT-induced fear. Moreover, this study revealed that TMT-induced fear behavior is processed by the bed nucleus of the stria terminalis (BNST). Both studies quantified fear behavior by measuring freezing, a fear behavior that is characterized by a cessation of all movements except for those connected to respiration. Altogether, these studies indicate that the BNST seems to be crucial for TMT-induced fear-processing, whereas the central and the lateral amygdala do not seem to play an important role [9]. However, a recent study [4] investigating c-fos activation induced by exposure to TMT found that both the medial amygdala, which receives strong olfactory input [30], and the basolateral amygdala are activated. Moreover, it has been reported that both the medial and the basolateral nuclei of the amygdala are required for the neural processing of cat odor-induced fear behavior [19,28,31].

Therefore, we hypothesized that the medial and the basolateral nucleus of the amygdala may play a role in the processing of fear behavior in response to TMT-exposure. In order to test this hypothesis, we temporarily inactivated both nuclei by local microinjections of the $GABA_A$ receptor agonist muscimol and probed the effects of these injections on TMT-induced freezing.

2. Experimental procedures

2.1. Subjects

Thirty male Sprague-Dawley rats (Charles River GmbH, Sulzfeld, Germany) weighing 330–520 g at the time of the surgery were used. The animals were maintained at a 12:12 h light/dark cycle, food and water were available ad libitum. All experiments were performed in accordance with ethical guidelines for the use of experimental animals and were approved by the local animal care committee (ZP 4/02).

2.2. Surgery

The rats were anesthetized with ketamine/xylazine (9:1; 100 mg/kg, i.p.) and placed into a stereotaxic frame with blunt ear bars. Two stainless cannulae with an outer diameter of 0.7 mm (22 gauge) were implanted bilaterally into the brain aiming at the medial amygdala [3.1 mm caudal, ±3.2 mm lateral, 9.0 mm ventral with respect to Bregma [27]] and the basolateral amygdala [3.1 mm caudal, ±4.8 mm lateral, 8.5 mm ventral with respect to Bregma [27]]. The cannulae were fixed to the skull with dental cement and three anchoring screws. After the surgery and between the tests the cannulae were fitted with stylets (outer diameter: 0.4 mm; 22 gauge) in order to avoid occlusions of the cannulae. Before testing, the rats were given 4–6 days to recover from surgery.

2.3. Apparatus for odor exposure

To test TMT-induced freezing, the rats were placed into one of three identical exposure boxes (30 cm × 30 cm × 30 cm) made of PVC. The front doors of the exposure boxes were made out of Plexiglas in order to allow observation of the rats. The brightness inside the boxes was assessed by a lux-meter and was ∼50 lux. Each exposure box was connected to a generator supplying charcoal-filtered air via Teflon tubing. The outflow on the back wall of the exposure boxes was linked to the exhaust system. Electrically operated three-way Teflon valves allowed to direct the air stream either directly to the exposure boxes or through a glass bottle, which contained the odorant (5 μl of TMT [2,4,5-trimethylthiazoline; PheroTech Inc., Delta, Canada] on a piece of filter paper) or saline (control odor), and then to the boxes. Both, clean air- and air/odor-flows were regulated with needle valves and monitored by flow meters (17 l/min).

2.4. Procedure

Each rat was placed into one of the olfactory exposure boxes for 15 min once per day on three consecutive days to familiarize the animals with the boxes. In order to adapt the animals to the click sound of the valves controlling the air flow, the valves were operated after the fourth minute of each familiarization session.

On the following day, each animal received (in a pseudo-randomized manner) bilateral injections of either 2.2 nmol muscimol (dissolved in 0.25 μl saline) or saline alone into the medial or into the basolateral amygdala. The solutions were infused bilaterally at a rate of ∼0.1 μl/10 s. After injections, the injection cannulae (outer diameter: 0.4 mm; 27 gauge; extending 4 mm beyond the guide cannulae) were left in place for another 2 min to allow diffusion of the solution away from the injection cannulae. Each rat was just tested once per day. If an animal was treated with muscimol in the afternoon it was not tested in the morning of the following day.

Immediately after the infusions, the animals were put into the odor exposure boxes. All animals were tested four times in a pseudo-randomized order: saline injections and exposure to air, saline injections and exposure to TMT, muscimol injections and exposure to air, muscimol injections and exposure to TMT. The behavior of the animals in the boxes was videotaped for 15 min for later analysis. After 4 min, the Teflon valves were switched and either TMT, or still clean air was directed into the exposure boxes. After each TMT-session, the animals were placed into a cage, which was located in a fume hood for at least 2 h, and then returned to their home cages. After each experiment, the odor exposure chambers and the tubings were thoroughly washed with 70% ethanol and ventilated with clean air. After TMT sessions, the exposure boxes were ventilated with clean air for at least 15 h.

The videotapes from all experiments were analyzed by two observers who were blind to the condition of the animals. Freezing behavior (no movements except for the ones associated with respiration) was used as a measure of fear. The percentage of time spent freezing was calculated for each rat for every minute of each experimental session ([seconds of freezing/60] × 100).

2.5. Motor activity measurements

To test whether treatment-effects on freezing were a side effect on motor activity, we investigated the effects of muscimol injections on spontaneous motor activity in 19 of the rats used in the experiments described above. The motor activity measurements were carried out in a conventional housing cage (20 cm × 38 cm × 60 cm) once per day on two consecutive days 1 week after the last odor exposure experiment. Motor activity was registered by an activity measurement system using passive infrared sensors (TSE InfraMot, TSE systems, Bad Homburg, Germany). After injections (as above), each animal was placed into a cage in which the motor activity was tracked for 15 min. If an animal was treated with saline the first day, it was treated with muscimol the second day and vice versa. The activity was quantified by countersignals (arbitrary units), which provide a relative measure of duration and intensity of spontaneous motor activity.

2.6. Histology

After the experiments, the rats were killed with an overdose of Nembutal. The brains of the animals were dissected out of the skulls and stored in 20% glucose/8% paraformaldehyde solution. Coronal slices of the injection sites of a thickness of 60 μm were obtained with the help of a freezing microtome and Nissl-stained with thionine. Finally, the injection sites were drawn onto plates taken from a rat brain atlas [27].