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Short communication

Social modulation in extinction of aversive memories

Aleksandra Nowak, Tomasz Werka, Ewelina Knapska*

Department of Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Science, 3 Pasteur St., 02-093 Warsaw, Poland

HIGHLIGHTS

- We propose two models of social modulation of extinction memory retrieval.
- Extinguished fear response renews in the presence of a fearful conspecific.
- Extinguished avoidance response renews in the presence of a fearful conspecific.

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ABSTRACT

Return of fear after extinction is a considerable challenge for the efficacy of exposure-based therapies. Fear recovery is most often modeled in the laboratory by changing the experimental context and studied in isolated animals. Since social context is an important factor affecting behavior, the question arises how it influences the recovery of extinguished fear. Here we present two novel behavioral models that allow studying social modulation of extinction memory retrieval. We show that the presence of a fearful cage mate results in a robust renewal of freezing as well as avoidance responses that were previously successfully extinguished.

Fear-eliciting properties of a stimulus acquired through conditioning can be extinguished by a repeated presentation of the conditioned stimulus (CS) in the absence of the unconditioned stimulus [1]. Similarly, extinction of learned place avoidance behavior occurs when visiting the place is no longer punished [2]. However, such extinction process does not reflect unlearning of the original association, but results in a transient inhibition of fear. For example, extinguished fear responses may return after a change of context (renewal phenomenon) [3].

In recent years, increased interest in mechanisms underlying fear extinction has emerged, partly because it is a useful model for exposure-based therapies for the treatment of human anxiety disorders, such as phobias and post-traumatic stress disorder [4]. The return of fear after extinction is a considerable challenge for maintaining long-lasting fear suppression after exposure-based therapies [5]. Until recently, the fear extinction and recovery phenomena were studied only in isolated animals. However, since vicarious experience accounts of both etiology and extinction of phobias have been shown in humans [6,7], social modulation seems to be an important factor that can affect the efficacy of exposure based therapies.

Social modulation of fear and avoidance learning in animal models was shown before [8–11]; however until now there were no animal models allowing for studying vicarious modulation of aversive memories extinction. In the present study, we examined influence of a fearful conspecific's presence on the rate of retrieval of fear and place avoidance extinction memory.

In the first experiment we tested retrieval of fear extinction memory in the presence of a fearful conspecific. Male 2–3-month-old C57BL/6 male mice were housed in pairs, extensively handled for 3 weeks in order to minimize stress caused by an experimenter's presence and habituated to transport to experimental room and to experimental cage (in three 10-min sessions). Then, the mice were subjected to fear conditioning and extinction in the Panlab shuttle-box for mice (LE918), which was divided by a perforated transparent partition allowing the mice to see, hear and smell the neighbor, but not to contact him physically. The mice were trained and tested in the left or right part of the shuttle-box cage (for every animal the side of the cage was the same through the whole behavioral procedure). Sensory stimuli were adjusted to generate two distinct contexts (context A: room lights on, the cage cleaned with a 1% acid solution, the mice transported to this context in

* Corresponding author. Tel.: +48 22 5892 257; fax: +48 22 5892 490.
E-mail address: e.knapska@nencki.gov.pl (E. Knapska).

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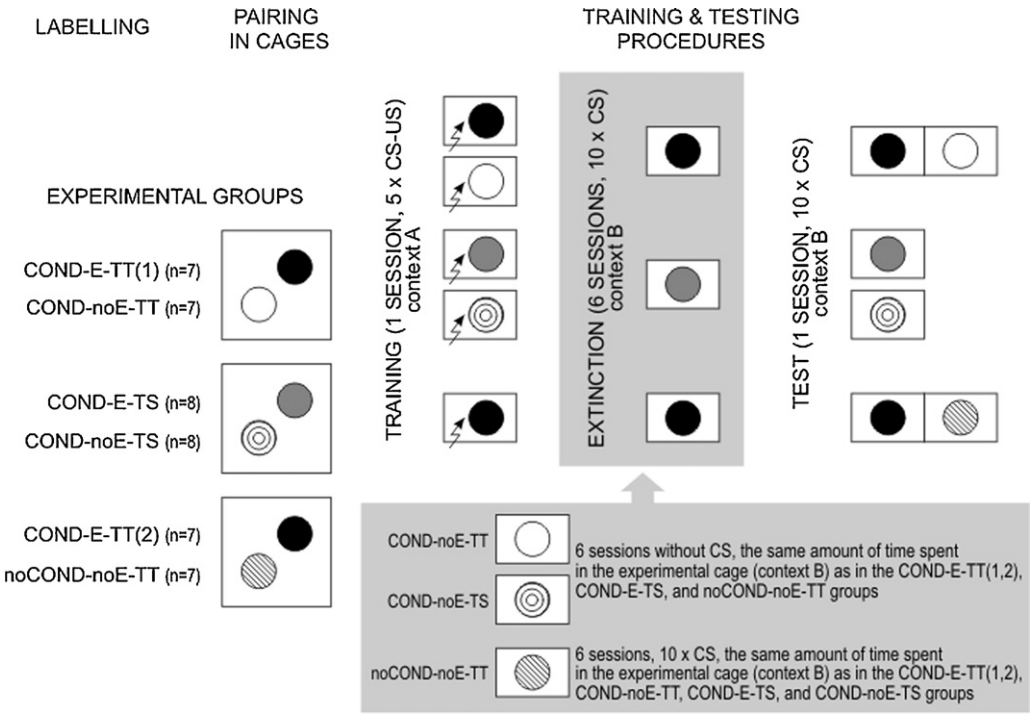

Fig. 1. Experimental design for social modulation of conditioned fear extinction model. The mice were housed in pairs. Both animals from each pair were separately subjected to cued fear conditioning (COND) or exposed to the auditory stimuli but not conditioned (noCOND). Subsequently, one mouse of the pair was subjected to six sessions of fear extinction (E). Another mouse from the pair was exposed to the experimental cage for the same amount of time without the CSs presentation (noE). On the following day, the mice were tested either together (TT) or separately (TS).

transparent plastic boxes; context B: room lights off, a 60 W red light provided illumination, the cage cleaned with a 1% ammonium hydroxide solution, the mice transported to this context in black plastic boxes). The freezing response was recorded by the camera placed in front of the cage and the computer system located in the adjoining room. The levels of freezing during training, extinction and test sessions were analyzed with BehaFreeze software and transformed to a percentage of total observations. The mice were divided into six groups (Fig. 1). Firstly, the animals were separately subjected to cued fear conditioning (COND: 5 CS–US associations, CS: 20 s, 85 dB, 5 kHz; US: footshock, co-terminated with CS: 1 s, 0.6 mA) or exposed to the auditory stimuli but not conditioned (noCOND). All mice were conditioned/exposed in context A. Subsequently, one mouse of the pair was subjected to six sessions of fear extinction (E: 10 CSs, context B). Another mouse from the pair was exposed to the experimental cage for the same amount of time without the CSs presentation (noE). On the following day, the mice were tested either together (TT) or separately (TS) by presenting them with 10 CSs in context B.

During fear conditioning all mice efficiently acquired freezing response to the CS. The subsequent fear extinction procedure significantly reduced the conditioned response (Fig. 2). However, the presence of a cage mate showing high freezing response resulted in robust renewal of fear in mice that previously successfully extinguished fear. These observations were confirmed by the statistical analysis. The levels of freezing during six extinction and one test sessions in the COND-E-TT(1 and 2), COND-E-TS and noCOND-noE-TT groups (see Fig. 1 for explanation of group labeling and Fig. 2 for behavioral data) were analyzed by three-way analysis of variance (ANOVA). A 4 (group) × 7 extinction and test sessions (session) ANOVA for repeated measures of the percentages of freezing response observed in consecutive 10 trials (trial) for each session revealed the group ($F_{(3,25)} = 80.73$, $P < 0.0001$), session ($F_{(6,150)} = 35.44$, $P < 0.0001$) and trial ($F_{(9,225)} = 9.19$, $P < 0.0001$) effects, as well as group × session ($F_{(18,150)} = 6.34$, $P < 0.0001$) and group × trial ($F_{(27,225)} = 2.63$, $P < 0.0001$) interactions. Results of further *post hoc* Duncan tests for these interactions indicated that the dynamics of freezing responses observed during extinction and test sessions was different in groups COND-E-TT(1), COND-noE-TT and COND-noE-TS in comparison to other groups ($P < 0.02$ or better). In the mice tested together with a partner with high level of fear, the freezing response significantly increased comparing to the last day of extinction, as well as comparing to the group tested separately. Since presence of unstressed, familiar mice in the cage had no effect on the level of freezing in the observers, the effect seems to be specific to the high freezing level of the demonstrator mice. This was confirmed by results of three-way ANOVA for freezing responses performed in consecutive 10 trials of the last extinction and test sessions in the COND-E-TT(1 and 2), COND-E-TS and noCOND-noE-TT groups. A 4 (group) × 2 (session) ANOVA for repeated measures of the percentage of freezing observed in extinction and test trials (trial) showed the group ($F_{(3,25)} = 5.10$, $P < 0.01$) and trial ($F_{(9,225)} = 2.30$, $P < 0.02$) effects, as well as group × session ($F_{(3,25)} = 3.99$, $P < 0.02$) interaction. An additional one-way ANOVA for percentage of freezing responses observed in all experimental groups in the test session yielded significant between-group differences ($F_{(5,60)} = 31.98$, $P < 0.0001$). Further *post hoc* Duncan tests showed that the level of freezing in the COND-E-TT(1) group was significantly higher than in the COND-E-TS, COND-E-TT(2) and noCOND-noE-TT groups ($P < 0.0001$ for all comparisons). Similarly, increased freezing response was observed in the groups that were not subjected to extinction procedure (COND-noE-TT and COND-noE-TS), and these groups also differed from the COND-E-TS, COND-E-TT(2) and noCOND-noE-TT groups ($P < 0.0001$). Relatively higher level of freezing was seen in the COND-E-TS group in comparison to the noCOND-noE-TT group ($P < 0.04$). Moreover, in all groups the percentage of freezing was analyzed for the pre-CS periods. The results show that the presence of a fearful conspecific resulted in the increased freezing not only in response to the CS but also to the experimental context. One-way ANOVA yielded