Behavioural Brain Research 238 (2013) 200–205

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

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.

ARTICLE INFO

Article history:
Received 13 June 2012
Received in revised form 16 October 2012
Accepted 18 October 2012
Available online 26 October 2012

Keywords:
Fear conditioning
Place avoidance
Extinction
Renewal
Empathy
Anxiety disorders

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).

0166-4328/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2012.10.031

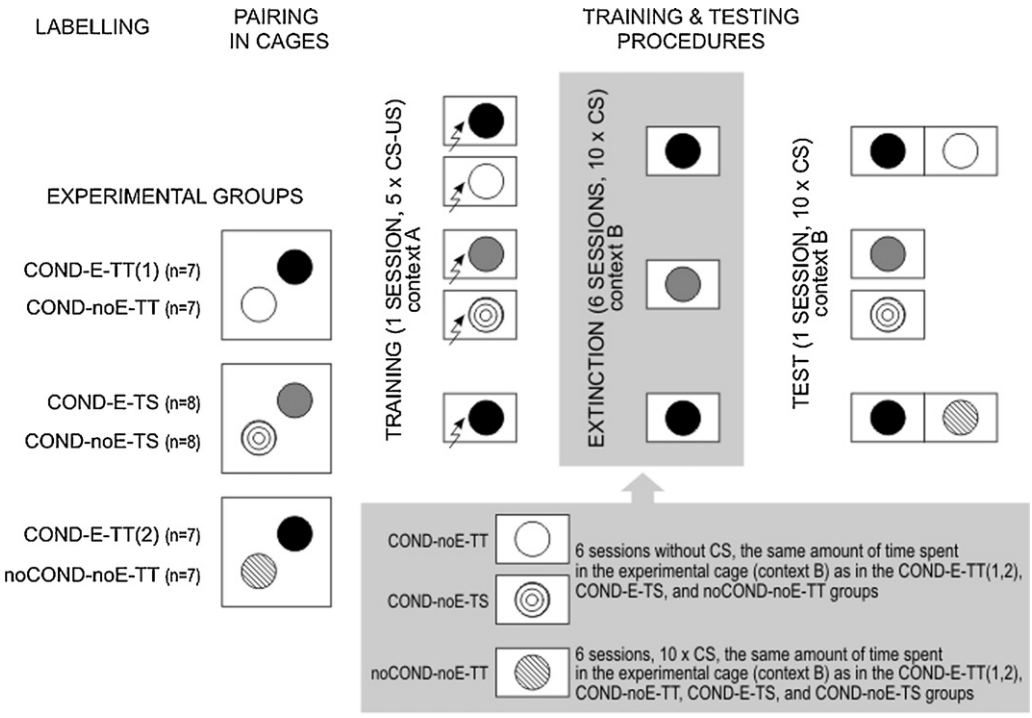

Fig. 1. Experimental design for social modulation of conditioned fear extinction model. The mice were housed in pairs. Both animals from each pair were separately subjected to cued fear conditioning (COND) or exposed to the auditory stimuli but not conditioned (noCOND). Subsequently, one mouse of the 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

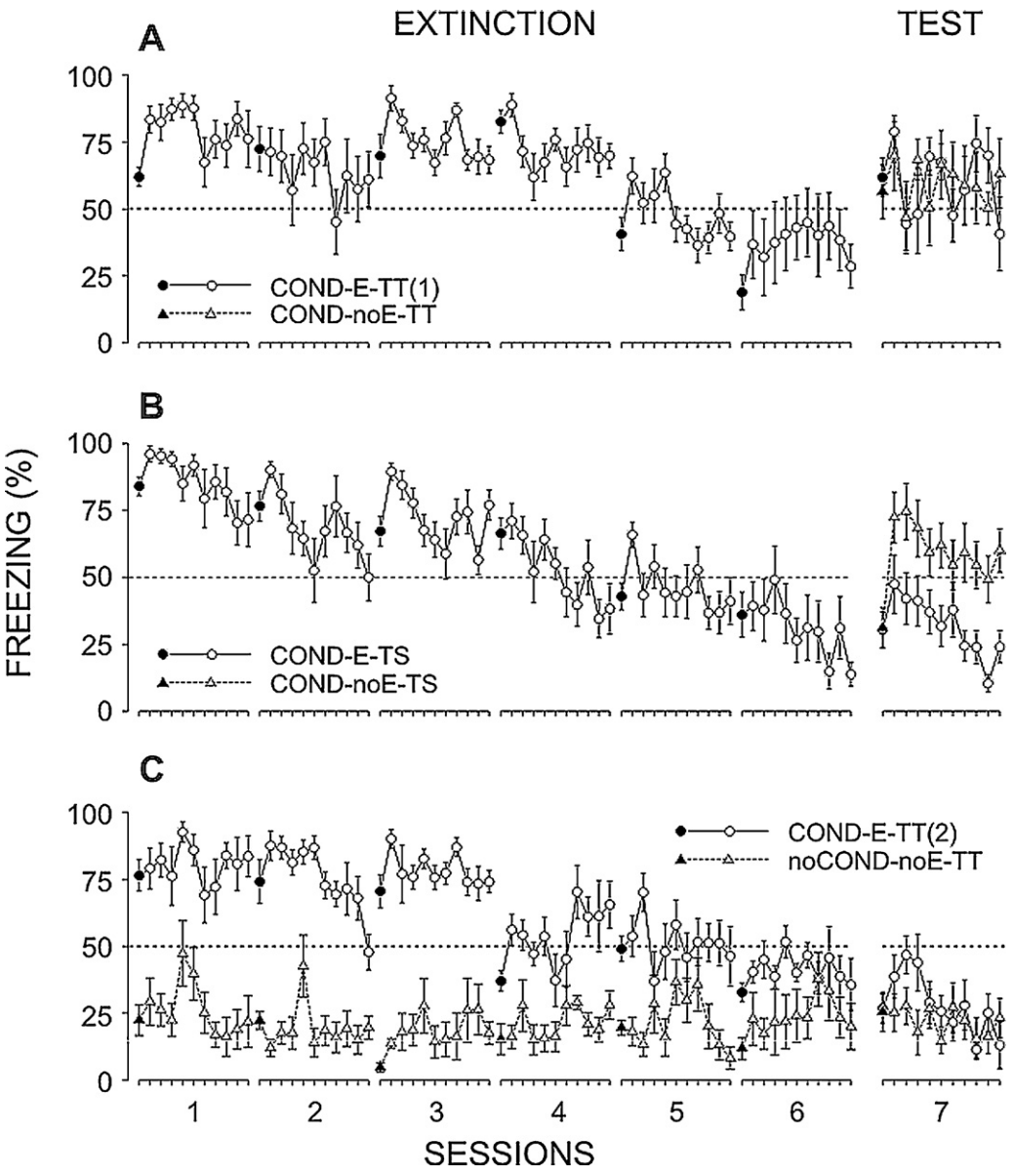

Fig. 2. Social modulation of conditioned fear extinction. The level of freezing was measured during six subsequent sessions of extinction and the test session. All mice subjected to fear conditioning and subsequent fear extinction significantly reduced the conditioned response (freezing) to the CS. Exposure to a fearful familiar conspecific resulted in renewal of conditioned fear in mice that were previously subjected to successful extinction procedure (A), comparing to mice tested separately (B) or tested with non-conditioned partner (C). Open circles and triangles – the level of freezing to the CS, filled circles and triangles – the level of freezing in the adaptation period (before the first CS was presented in a given session); error bars ± SEM.

group effect ($F_{(5,380)} = 3.93$, $P < 0.01$), and further *post hoc* Duncan tests showed that the level of freezing recorded in the pre-CS period in COND-E-TT(1) and COND-noE-TT groups was significantly higher than in other groups ($P < 0.04$ or better).

In the second experiment we examined the influence of conspecifics' behavior on renewal of avoidance responses. Male 2–3-month-old C57BL/6 male mice were trained and tested in the IntelliCage system [12]. The animals were subcutaneously injected with microtransponders (Trovan, ID-100), which emit a unique animal identification code when activated by a magnetic field. After 48 h the mice were introduced to the IntelliCage, which can be briefly characterized as follows (for a detailed description of the cage, see [12]). The cage was equipped in four operant learning chambers that fit into the corners of the housing cage. Access into the chamber was provided *via* a tubular antenna reading the transponder codes. The design restricted access to the learning chamber for a single mouse only. The chamber, equipped with a proximity sensor, contained two openings permitting access to drinking bottles. Aversive stimulation was delivered in forms of air-puffs directed to the head of the mouse through tubing controlled by electric valves. Each operant chamber was also equipped in signaling LEDs. In addition, the cage contained a sleeping shelter in the center on which the animals could climb to reach the food (*ad libitum*). The cage was controlled by a microcomputer recognizing visits in the operant chambers, nosepokes, and tube-lickings of individual mice, and delivering punishment (by entering the test chamber) according to preprogrammed schedules depending on the assignment of the mice to different test groups within the same cage. The system ran continuously for several days, behavioral activity of the mice was monitored from the experimenter office *via* Intranet.

The experiment was repeated twice (with 10 mice housed and trained together in the IntelliCage every time). The animals were kept under a 12:12 light–dark cycle. During all phases of the

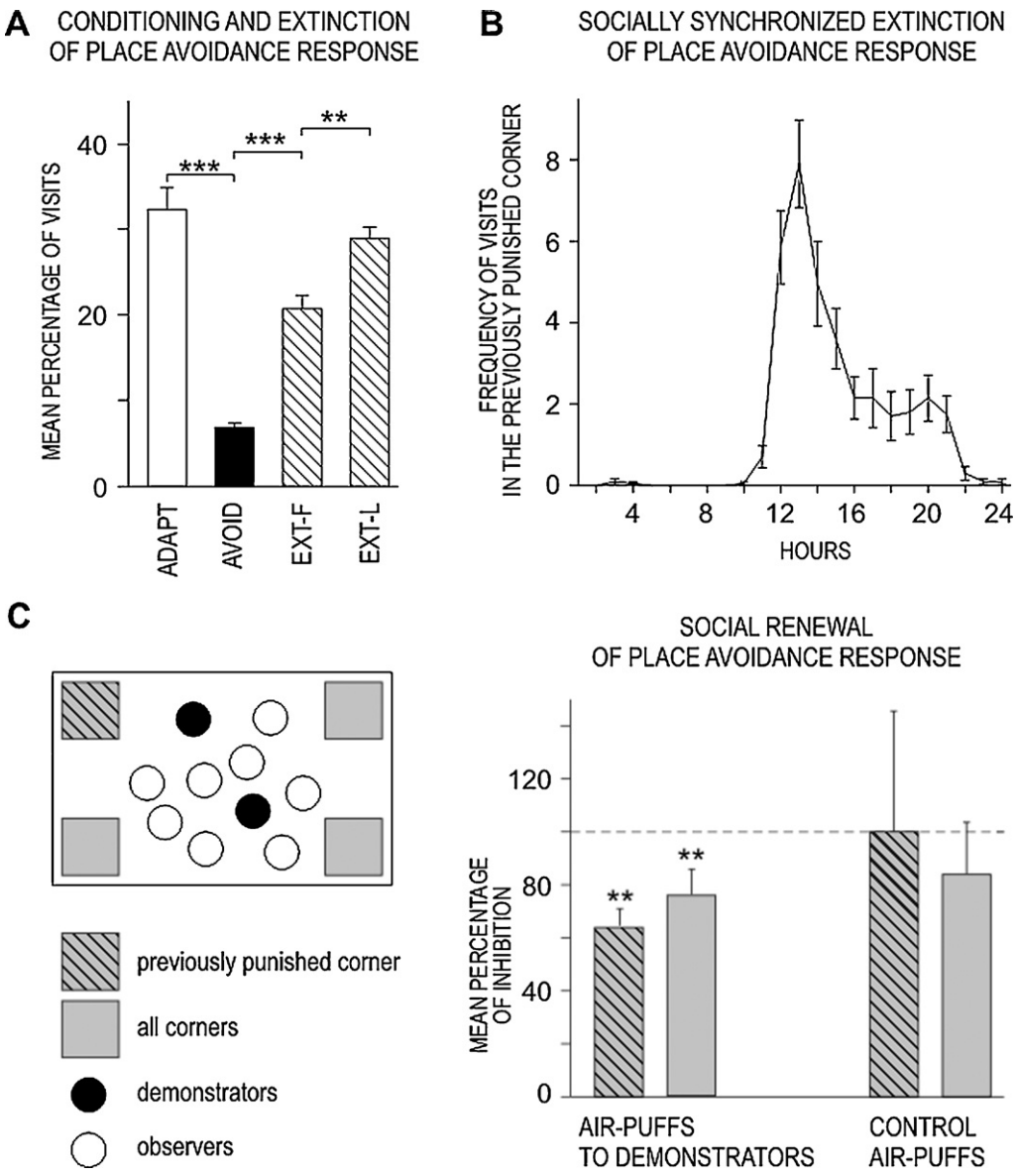

Fig. 3. Social modulation of place avoidance extinction. (A) The mice acquired and extinguished place avoidance response very efficiently. Mean percentage of visits in the corner in which air-puffs were applied during place avoidance learning, ADAPT – adaptation phase, AVOID – place avoidance learning, EXT-F – the first day of extinction, EXT-L – the last day of extinction. (B) Extinction of avoidance response was clearly synchronized between the animals in the cage. (C) The testing phase of the experiment. Subjecting demonstrators to air-puffs resulted in significant inhibition of visiting of both the previously punished corner and all other corners in the cage by observer mice. Such effect was not observed when the air-puffs were not directed at any mouse. Mean percentage of inhibition was calculated by comparing the frequency of visits during 3 h following application of the first air-puff to the frequency of visits observed in the control period of time (see text for details); $^{**}P < 0.01$; $^{***}P < 0.001$; error bars ± SEM.

training all animals had access to water in all four corners. Training procedure started with a 24-h adaptation to the cage. Then, for 12 h (the dark phase of the light–dark cycle, when mice are the most active) the animals received an air-puff (0.75 bar) accompanied by a blue light when entering the corner most preferred during the adaptation period (place avoidance learning). Subsequently, for 6 days the mice were subjected to the extinction procedure during which visits were not punished. When the avoidance response was extinguished, its social modulation was tested. During the test, 2 out of 10 mice in the cage ("demonstrators") were again subjected to the air-puffs and blue light when entering the corner a visit at which was punished during the place avoidance learning. Other mice ("observers") did not receive any air-puffs. The frequency of visits in the previously punished corner and in all corners of the cage was assessed in comparison to the frequency of visits in these corners during the same time of the day in the last day of extinction. Moreover, to control influence of an air-puff itself (not directed at any mouse), 3 air-puffs accompanied by a blue light in the previously punished corner were administered on the 4th day of extinction when none of the mice was present in this corner. The chosen number of air-puffs was based on the observation that in the place avoidance learning paradigm used in this study 1–3 air-puffs are required to acquire avoidance response. The frequency of visits in the previously punished corner and in all corners of the cage following administration of these air-puffs was assessed in comparison to the frequency of visits in these corners during the same time on the last day of extinction. Visiting of the previously punished corner reached a stable level and did not differ between the 4th and 6th extinction sessions.

The mice acquired and extinguished place avoidance response very efficiently (Fig. 3A). Interestingly, the extinction was clearly synchronized between the animals in the cage (Fig. 3B). The animals

ceased to visit the punished corner completely and then synchronically started to enter it. The number of visits significantly exceeded the number of visits observed for this corner in the adaptation phase during the analogous phase of the light–dark cycle. Subjecting demonstrators to air-puffs resulted in significant inhibition of visiting of both the previously punished corner and all other corners in the cage by observer mice (Fig. 3C). Such effect was not observed when the air-puffs were not directed at any mouse. These observations were confirmed by the statistical analysis. For all statistical comparisons the rate of visits was used (number of visits in one or all of the corners in the cage within a given period of time). Within-subjects analysis with Friedman ANOVA for the rate of visiting of the punished corner during the adaptation period, place avoidance learning, the first and the last extinction days showed the significant effect of the training phase: ($N = 15$, df = 3) = 33.32, $P < 0.001$. Further comparisons with Wilcoxon Matched Pairs Test yielded significant differences between the rate of visiting of the punished corner between the adaptation and place avoidance learning phases ($P < 0.001$), the place avoidance learning phase and the first extinction day ($P < 0.001$), as well as between the first and the last extinction days ($P < 0.01$). In the 3-h period following the first air-puff applied to the demonstrator mice, inhibition of the rate of visiting of the previously punished corner ($P < 0.01$), as well as all corners in the cage ($P < 0.01$) by the observer mice was shown (Wilcoxon Matched Pairs Test).

Using two novel behavioral models that allow studying social modulation of extinction memory retrieval, we showed that presence of a cage mate who shows high level of freezing or avoidance response results in a robust renewal of fear or avoidance responses in the mice that previously extinguished fear successfully.

In concert with our results, several studies have recently demonstrated that rats and mice respond to the distress experienced by a conspecific. These findings were interpreted as a simple form of empathy. For instance, modulation of pain sensitivity in mice produced by exposure to their cage mates in pain was reported [13]. In our previous study we also showed that a brief social interaction with a cage mate that had undergone an aversive learning experience increases emotional arousal and activates brain regions responsive to a direct experience with threatening stimuli [14]. The further studies demonstrated that interaction with a fearful conspecific may also promote learning and memory of an otherwise naive animal. For instance, it has been shown that both fear conditioning and avoidance learning are influenced by the presence of a fearful partner [9,15]. The present results extend our knowledge about social modulation of learned responses showing that fear extinction may be easily affected by the presence of a fearful conspecific.

In contrast to our results, Bredy and Barad [16] reported that exposing mice to a recently conditioned conspecific or a urinary chemosignal from shocked conspecifics facilitates fear extinction. The disparity between Bredy and Barad's and our results may stem from different behavioral paradigms used in both studies, a brief social interaction with a cage mate that has undergone an aversive learning experience and direct observation of a distressed conspecific, respectively. In the former case one can expect an increased arousal/vigilance that promotes learning [9], in the latter case rather emotional contagion effects. Such explanation is supported by the fact that in both behavioral paradigms of our study, behavioral responses of the observers mimicked the demonstrators' ones. We observed freezing response in the first model and general arrest of the visiting activity in the second model. Importantly, the modulatory effects depended on the presence of the fearful conspecific in the environment, thus confirming social aspects of the observed phenomena.

It has been previously shown that pair-exposure with a non-fearful conspecific to the CS associated with a footshock significantly reduced stress responses to this CS [17] and that pre-exposure to a non-fearful conspecific resulted in long-lasting context-specific impairments of fear conditioning [18]. Such mitigation of stress responses by signals from a conspecific are described as social buffering phenomenon. In our study, we did not observe a significant difference in the levels of fear response between the COND-E-TS and COND-E-TT(2) groups, which could be interpreted in terms of social buffering effect. This may be attributed to the fact that our experimental design precluded any direct contact between the trained animal and its non-fearful conspecific, thereby obstructing interactions between the animals. In addition, it is conceivable that a single appearance of a non-fearful conspecific is too weak a stimulus in comparison to contextual information (the experimental cage in which many training sessions were held) to affect the animal's behavior in a significant manner.

The two mouse models presented here can be useful for studying neuronal basis of the socially evoked fear recovery after extinction. Identifying the neural circuits underlying social modulation of extinction of classical and instrumental responses will allow determining whether the social component of modulation differ from contextual modulation observed in classical tests. It is conceivable that besides the brain structures that mediate fear extinction in isolated animals (*i.e.*, the amygdala, prefrontal cortex and hippocampus [1,4]), socially evoked renewal involve activation of additional parts of the brain such as the insular cortex and anterior cingulate cortex [19,20]. Moreover, the models described in this study may be useful for determining genetic aspects that influence the magnitude of social modulation of extinction memory [15]. Such knowledge would be very valuable for designing potential therapeutic strategies for patients, who, after all, do not live in social vacuum.

Acknowledgments

This work was supported by Polish Ministry of Science and Higher Education (COST Action BM0901 to E.K.) and a grant from Switzerland through the Swiss Contribution to the enlarged European Union; E.K. acknowledges support from the Foundation for Polish Science through its program HOMING. We thank Pawel Boguszewski for providing us with the BehaFreeze software.

References

[1] Maren S. Seeking a spotless mind: extinction, deconsolidation, and erasure of fear memory. Neuron 2011;70:830–45.
[2] Dubrovina NI, Tomilenko RA. Characteristics of extinction of a conditioned passive avoidance reflex in mice with different levels of anxiety. Neuroscience and Behavioral Physiology 2007;37:27–32.
[3] Bouton ME, Westbrook RF, Corcoran KA, Maren S. Contextual and temporal modulation of extinction: behavioral and biological mechanisms. Biological Psychiatry 2006;60:352–60.
[4] Milad MR, Quirk GJ. Fear extinction as a model for translational neuroscience: ten years of progress. Annual Review of Psychology 2012;63:129–51.
[5] Boschen MJ, Neumann DL, Waters AM. Relapse of successfully treated anxiety and fear: theoretical issues and recommendations for clinical practice. Australian and New Zealand Journal of Psychiatry 2009;43:89–100.
[6] Hygge S, Ohman A. Modeling processes in the acquisition of fears: vicarious electrodermal conditioning to fear-relevant stimuli. Journal of Personality and Social Psychology 1978;36:271–9.
[7] White Jr WC, Davis MT. Vicarious extinction of phobic behavior in early childhood. Journal of Abnormal Child Psychology 1974;2:25–32.
[8] Bruchey AK, Jones CE, Monfils M-H. Fear conditioning by-proxy: social transmission of fear during memory retrieval. Behavioural Brain Research 2010;214:80–4.
[9] Knapska E, Mikosz M, Werka T, Maren S. Social modulation of learning in rats. Learning and Memory 2010;17:35–42.
[10] Panksepp JB, Lahvis GP. Rodent empathy and affective neuroscience. Neuroscience and Biobehavioral Reviews 2011;35:1864–75.
[11] Riess D. Vicarious conditioned acceleration: successful observational learning of an aversive Pavlovian stimulus contingency. Journal of the Experimental Analysis of Behavior 1972;18:181–6.

[12] Knapska E, Walasek G, Nikolaev E, Neuhäusser-Wespy F, Lipp H-P, Kaczmarek L, et al. Differential involvement of the central amygdala in appetitive versus aversive learning. Learning and Memory 2006;13: 192–200.
[13] Langford DJ, Crager SE, Shehzad Z, Smith SB, Sotocinal SG, Levenstadt JS, et al. Social modulation of pain as evidence for empathy in mice. Science 2006;312:1967–70.
[14] Knapska E, Nikolaev E, Boguszewski P, Walasek G, Blaszczyk J, Kaczmarek L, et al. Between-subject transfer of emotional information evokes specific pattern of amygdala activation. Proceedings of the National Academy of Sciences of the United States of America 2006;103:3858–62.
[15] Chen Q, Panksepp JB, Lahvis GP. Empathy is moderated by genetic background in mice. PLoS ONE 2009;4:e4387.
[16] Bredy TW, Barad M. Social modulation of associative fear learning by pheromone communication. Learning and Memory 2009;16:12–8.
[17] Kiyokawa Y, Takeuchi Y, Mori Y. Two types of social buffering differentially mitigate conditioned fear responses. European Journal of Neuroscience 2007;26:3606–13.
[18] Guzmán YF, Tronson NC, Guedea A, Huh KH, Gao C, Radulovic J. Social modeling of conditioned fear in mice by non-fearful conspecifics. Behavioural Brain Research 2009;201:173–8.
[19] Jeon D, Kim S, Chetana M, Jo D, Ruley HE, Lin S-Y, et al. Observational fear learning involves affective pain system and Cav1.2 Ca^{2+} channels in ACC. Nature Neuroscience 2010;13:482–8.
[20] Thioux M, Keysers C. Empathy: shared circuits and their dysfunctions. Dialogues in Clinical Neuroscience 2010;12:546–52.