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Social buffering enhances extinction of conditioned fear responses in male rats

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HIGHLIGHTS

- Insufficient extinction training did not extinguish fear responses.
- Social buffering during insufficient extinction training suppressed fear responses.
- The effect of social buffering during extinction training was context specific.
- We concluded that social buffering enhanced extinction of fear responses.

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ABSTRACT

In social species, the phenomenon in which the presence of conspecific animals mitigates stress responses is called social buffering. We previously reported that social buffering in male rats ameliorated behavioral fear responses, as well as hypothalamic-pituitary-adrenal axis activation, elicited by an auditory conditioned stimulus (CS). However, after social buffering, it is not clear whether rats exhibit fear responses when they are re-exposed to the same CS in the absence of another rat. In the present study, we addressed this issue using an experimental model of extinction. High stress levels during extinction training impaired extinction, suggesting that extinction is enhanced when stress levels during extinction training are low. Therefore, we hypothesized that rats that had received social buffering during extinction training would not show fear responses to a CS, even in the absence of another rat, because social buffering had enhanced the extinction of conditioned fear responses. To test this, we subjected male fear-conditioned rats to extinction training either alone or with a non-conditioned male rat. The subjects were then individually re-exposed to the CS in a recall test. When the subjects individually underwent extinction training, no responses were suppressed in the recall test. Conversely, when the subjects received social buffering during extinction training, freezing and Fos expression in the paraventricular nucleus of the hypothalamus and lateral amygdala were suppressed. Additionally, the effects of social buffering were absent when the recall test was conducted in a different context from the extinction training. The present results suggest that social buffering enhances extinction of conditioned fear responses.

1. Introduction

In social species, stress responses induced by exposure to distressing stimuli can be ameliorated when an animal is exposed along with a conspecific animal(s). For example, the presence of a conspecific animal has been found to suppress corticosterone or cortisol release in response to a novel environment [1,2] or to predator-associated stimuli [3]. These phenomena are called social buffering [4].

In previous studies, we analyzed social buffering in male rats using fear conditioning. When fear-conditioned rats were exposed to an auditory conditioned stimulus (CS), they exhibited both behavioral fear responses and hypothalamic-pituitary-adrenal (HPA) axis activation. The presence of a non-conditioned unfamiliar male rat (associate) completely blocked these responses [5], suggesting that the fear-conditioned rat received social buffering from the associate rat. This social buffering of conditioned fear responses occurred even if the dyad was separated by a wire-mesh partition or by double wire-mesh partitions separated by 5 cm [6]. We identified several additional characteristics of social buffering. For example, we found that the presence of guinea pigs [6] or some strains of rats [7] did not induce social buffering, that familiar associates were more effective for social buffering than

Abbreviations: BA, basal amygdala; CeL, lateral division of the central amygdala; CeM, medial division of the central amygdala; CS, conditioned stimulus; HPA, hypothalamic-pituitary-adrenal; IL, infralimbic region of the prefrontal cortex; LA, lateral amygdala; pmOP, posteromedial olfactory peduncle; PVN, paraventricular nucleus of the hypothalamus.

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unfamiliar associates [8], that social buffering was similarly observed in female rats [9], and that associate-derived volatile olfactory signals can mediate social buffering [6,8,10]. In our investigations of the neural mechanisms underlying social buffering, we found that activation in the lateral amygdala (LA) in response to the CS was suppressed during social buffering [11]. We also found evidence that the posteromedial olfactory peduncle (pmOP) [12], most likely the posterior complex of the anterior olfactory nucleus within the pmOP [10], relays the olfactory signals responsible for social buffering from the main olfactory bulb to the amygdala. However, in all of our previous studies, rats were not re-exposed to the CS after the test. Therefore, whether rats that have experienced social buffering show fear responses when they are re-exposed to the CS in the absence of another rat remains unclear.

Extinction of conditioned fear responses appears to be a suitable experimental model for addressing this issue. In this model, fear-conditioned animals undergo extinction training 1 day after conditioning, in which they are repeatedly exposed to the CS. If the extinction training is sufficient, the animals will exhibit suppressed fear responses when they are re-exposed to the CS in a recall test on the following day, i.e., extinction of conditioned fear responses [13,14]. Thus, one possibility for assessing the effects of social buffering on subsequent fear responses to the CS would be to subject fear-conditioned rats to social buffering during extinction training and then conduct a recall test in the absence of another rat.

The stress levels of an animal during extinction training appear to affect the extinction of conditioned responses. For example, fear-conditioned rats showed little or no extinction of conditioned fear responses when they were stressed via foot shocks [15] or placement on an elevated platform immediately prior to extinction training [16]. These findings suggest that extinction will be enhanced when animals undergoing extinction training have low levels of stress. Based on our findings that social buffering ameliorated stress caused by an auditory CS, we hypothesized that rats that experienced social buffering during extinction training would not show fear responses to the CS in a recall test. Indeed, we would expect that social buffering would lower the stress status during extinction training and thus enhance extinction of conditioned fear responses.

To test this hypothesis, we subjected a group of rats to fear conditioning using an auditory CS. The rats underwent extinction training either alone or with an associate. The rats were then individually re-exposed to the CS in a recall test. The effects of social buffering during extinction training were evaluated by examining freezing behavior and HPA axis activity, as reflected by Fos expression in the paraventricular nucleus of the hypothalamus (PVN), during the recall test. Because the amygdala plays an important role in conditioned fear responses [17,18], we examined Fos expression in the amygdala to assess the underlying neural mechanisms (Experiment 1). Next, we assessed whether the effects of social buffering during extinction training could be observed even if the recall test was conducted in a different context than that of extinction training (Experiment 2).

2. Material and methods

2.1. Animals

All experiments were approved by the Animal Care and Use Committee of the Faculty of Agriculture at The University of Tokyo, according to guidelines that were adapted from the Consensus Recommendations on Effective Institutional Animal Care and Use Committees by the Scientists Center for Animal Welfare.

Experimentally naïve male Wister rats were purchased at 8 weeks of age from Charles River Laboratories Japan (Kanagawa, Japan). The rats were housed 2–4 per cage in a room with an ambient temperature of 24 ± 1 °C and a humidity of 45 ± 5%. The room had a 12-h light/12-h dark cycle (lights switched on at 08:00). Food and water were available ad libitum. In each cage, rats were assigned to be either subjects or associates (rat placed with the subject during extinction training), which ensured unfamiliarity between the subjects and associates. All rats were individually housed and handled for 5 min per day for 3 days before the conditioning day. All behavioral procedures were performed between 09:00 and 16:00.

2.2. Procedure

2.2.1. Experiment 1

Fear conditioning was performed in an acrylic conditioning box with a metal grid floor (28 × 20 × 27 cm) under a white light (Context A). The rats received seven repetitions of a 3-s auditory CS (8 kHz, 70 dB) that terminated concurrently with a 0.5-s foot shock (0.55 mA). We presented the CS twice before and twice after the conditioning procedure to measure pre- and post-conditioning freezing. The inter-trial interval was randomly varied between 90 and 220 s.

The subjects underwent extinction training either with (social situation) or without an associate (alone situation) 24 h after fear conditioning. Based on the post-conditioning freezing, the subjects were divided into extinction and no-extinction groups, which showed comparable freezing in each situation. The rats were placed in an acrylic extinction box with clean woodchip bedding (28 × 44 × 20.5 cm) under a dim red light (Context B). In the alone situation, the subjects in the extinction group (n = 9) received 24 CS presentations in the absence of other animals. In the social situation, the subjects in the extinction group (n = 9) underwent extinction training with an associate. We varied the inter-trial interval randomly between 60 and 120 s. For the rats in the no-extinction group (alone: n = 9, social: n = 9), we placed the subject and associate (in the social situation) in the extinction box for the same length of time as for the extinction group, without any CS presentation.

Twenty-four hours after extinction training, we conducted the recall test with the individual subjects in both situations in the same context as for the extinction training (Context B). The subjects were exposed to the CS twice with an interval of 90 s. After the test, the subjects were returned to their home cages.

Sixty minutes after the recall test [19], each subject was deeply anesthetized with sodium pentobarbital and intracardially perfused with saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed and immersed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer, and then placed in 30% sucrose/phosphate buffer for cryoprotection. We used the avidin-biotin-peroxidase immunohistochemistry method to detect Fos expression, as previously described [8,20]. Briefly, we collected six successive 30-μm sections containing the PVN (Bregma −1.80 mm) or LA, basal amygdala (BA), and lateral (CeL) and medial (CeM) division of the central amygdala (Bregma −2.76 mm). The sections were incubated with a primary antibody to c-Fos protein (1:8000; ABE457, Merck Millipore, Billerica, MA, USA) for 65 h and a biotinylated anti-rabbit secondary antibody (BA-1000, Vector Laboratories, Burlingame, CA, USA) for 2 h. The sections were then processed using the ABC kit (Vector Laboratories). Staining was developed by incubating the tissue in a diaminobenzidine solution with nickel intensification.

2.2.2. Experiment 2

Fear conditioning (Context A) and extinction training (Context B) were performed as described above with the exception that all subjects underwent extinction training in the social situation. The subjects then individually underwent the recall test in the same (Context B; same situation, extinction group: n = 8, no-extinction group: n = 8) or different context (Context C; novel situation, extinction group: n = 5, no-extinction group: n = 6) from that of extinction training. In context C, the recall test was conducted in a cylindrical acrylic box (28 × 28 × 25) with paper bedding under a white light.