



Social buffering ameliorates conditioned fear responses in the presence of an auditory conditioned stimulus



Yasushi Kiyokawa*, Yukari Takeuchi

Laboratory of Veterinary Ethology, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

HIGHLIGHTS

- Social buffering ameliorates fear responses primarily in the absence of a 3-s CS.
- Social buffering mitigates fear responses in the presence of a 20-s CS.
- Social buffering occurs regardless of the presence or absence of a CS.

ARTICLE INFO

Article history:

Received 19 July 2016

Received in revised form 21 September 2016

Accepted 26 October 2016

Available online 28 October 2016

Keywords:

Absence of a CS

Duration of fear responses

Exposure-type social buffering

Presence of a CS

Percentage of freezing

ABSTRACT

Social buffering is a phenomenon in which stress in an animal is ameliorated when the subject is accompanied by a conspecific animal(s) during exposure to distressing stimuli. Previous studies of social buffering of conditioned fear responses in rats have typically used a 3-s auditory conditioned stimulus (CS) as a stressor, observing stress responses during a specified experimental period. Because a 3-s CS is extremely short compared with a typical experimental period, freezing has thus been observed primarily in the absence of the CS. Therefore, it has been unclear whether social buffering ameliorates conditioned fear responses in the presence of the CS. To clarify this issue, the current study assessed the effects of social buffering on conditioned fear responses in the presence of a 20-s CS. We measured the percentage of time spent freezing during the 20-s period following the onset of the CS. When conditioned subjects were exposed to the 20-s CS alone, they exhibited a high percentage of freezing in the presence of the CS. The presence of another non-conditioned rat completely blocked this response. The same result was observed when freezing was observed primarily in the absence of the 3-s CS. In addition, we confirmed that the presence of an associate ameliorated conditioned fear responses induced by a 20-s CS or 3-s CS when the duration and frequency of fear responses was measured. These findings indicate that social buffering ameliorates conditioned fear responses in the presence of an auditory CS.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

When animals are exposed to distressing stimuli alongside their mother, mate, or a conspecific with which they have no sexual relationship, a wide variety of stress responses are attenuated. This phenomenon is known as exposure-type social buffering [1]. Social buffering induced by a conspecific(s) with no sexual relationship has been reported in a wide variety of social species, including mice [2], guinea pigs [3], sheep [4], pigs [5], goats [6], common marmosets [7], rhesus monkeys [8], and rats [9–11].

We have conducted a number of studies in our lab to investigate social buffering in rats using a fear conditioning paradigm. When a fear-conditioned rat is exposed to an auditory conditioned stimulus (CS)

alone, conditioned fear responses are typically observed, including increased freezing, decreased investigation and walking. However, the presence of an unfamiliar male rat (associate) has been found to completely block these responses, suggesting that social buffering can ameliorate conditioned fear responses [12]. Separating the subject and associate by 5 cm with a double wire-mesh partition had no effect on this social buffering [13,14]. Social buffering appears to be a biologically important phenomenon for rats because it has been observed in both male and female rats [15]. In addition, we observed social buffering between rats derived from the same colony [16] and found that it enhanced extinction of conditioned fear responses [17]. We also found that volatile olfactory signals released from associates [18,19] suppressed the activation of the lateral amygdala (LA) and induced social buffering [20,21], after being detected by the main olfactory epithelium of the subject [14].

In our previous studies, we observed duration and frequency of fear responses during a specific experimental period, regardless of the

* Corresponding author.

E-mail addresses: akiyo@mail.ecc.u-tokyo.ac.jp (Y. Kiyokawa), aytake@mail.ecc.u-tokyo.ac.jp (Y. Takeuchi).

presence or absence of the CS. These studies typically used a 3-s tone as the CS, which is short compared with the experimental period. Thus, the freezing response was observed primarily in the absence of the CS. In contrast, many studies using a fear conditioning paradigm have observed freezing in the presence of a CS duration of tens of seconds. A CS duration of 20-s has been particularly widely used [22–24]. To directly compare these two types of fear response, we have established a measure that can analyze them simultaneously [25]. In this experimental paradigm, freezing is observed during a 20-s period following the onset of the CS regardless of the presence or absence of the CS, making it possible to measure freezing in the presence of a 20-s CS, and freezing primarily in the absence of a 3-s CS. This direct comparison revealed that fear responses in the two conditions differ in intensity, resistance to extinction, and underlying neural mechanisms when the CS (3-s and 20-s) was paired with the same foot shock [25]. These findings raise questions about the effects of social buffering on conditioned fear responses. Although we have previously demonstrated that social buffering can ameliorate fear responses primarily in the absence of a 3-s CS, it remains to be clarified whether it also mitigates fear responses in the presence of a 20-s CS.

In previous studies reporting fear conditioning by-proxy, a fear-conditioned rat was exposed to a 20-s CS with a non-conditioned rat [26, 27]. In these studies, a peer rat was fear-conditioned to a 20-s CS while a subject rat remained in its home cage on day 1. On day 2, the fear-conditioned peer rat and non-conditioned subject rat were placed in a test box and exposed to a 20-s CS three times. When only the subject rat was placed in the test box and exposed to the 20-s CS on day 3, the subject showed freezing in the presence of the 20-s CS. This suggests the establishment of fear conditioning by-proxy. Although these studies did not observe freezing of the fear-conditioned peer rat on day 2, the establishment of fear conditioning by-proxy implies that the fear-conditioned peer rat showed freezing of substantial intensity, even if the non-conditioned subject rat was present in the same box. Based on these findings, we hypothesized that social buffering would be less effective or have no effect on fear responses in the presence of a 20-s CS.

The current study was conducted to test this hypothesis, by conditioning subject rats using a 20-s CS, or a 3-s CS. On the following day, subject rats were re-exposed to the same CS either alone or with an associate rat separated by a double wire-mesh partition. The effectiveness of social buffering was assessed by measuring the percent of freezing during the 20-s period following the onset of the CS.

2. Material and methods

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

2.1. Animals

Experimentally naïve male Wistar rats (aged 7.5 weeks) were purchased from Charles River Laboratories Japan (Kanagawa, Japan). Upon arrival, the rats were housed with 2–3 animals per cage in a room with an ambient temperature of 24 ± 1 °C, humidity of $45 \pm 5\%$, and a 12-h light/12-h dark cycle. Lights were switched on at 8:00. The rats were assigned to either a subject or associate role. Associate rats were exposed to the CS in the company of the subject. To maintain unfamiliarity between the subject and associate, cage mates were assigned to the same group. Food and water were available *ad libitum*. All rats were housed separately and were handled for 5 min daily, commencing 3 days before the conditioning day.

2.2. Fear conditioning

Fear conditioning was performed in an illuminated room between 9:00 and 15:00, as in our previous studies [25]. Each subject in the conditioned group was placed in an acrylic conditioning box ($28 \times 20 \times 27$ cm) for 20 min, where it received seven repetitions of either a 20-s or a 3-s tone (CS, 8 kHz, 70 dB), which terminated concurrently with a 0.9-mA or 0.35-mA foot shock (0.5 s), respectively. A previous study reported that a 3-s CS evoked stronger fear responses than a 20-s CS in the fear-expression test (see below) when the same intensity of foot shock was paired during fear conditioning [25]. Because the observed responses are residual intrinsic CS-induced responses after suppression by social buffering, we changed the intensity of the foot shock depending on the CS, to induce an equivalent intensity of responses in the fear-expression test when the subject was tested alone. We also prepared the non-conditioned subjects by presenting the tone and respective foot shocks separately during a 20-min period. The inter-trial interval randomly varied from 30 to 180 s. The subjects were returned to their home cages after fear conditioning.

2.3. Fear-expression test

A fear-expression test was performed 24 h after the fear conditioning, as described in our previous studies [25]. Two rectangular enclosures ($25 \times 25 \times 35$ cm) were placed on an acrylic board (45×60 cm) in a dark room illuminated by dim red light. Each enclosure had three acrylic walls, one wire mesh wall, and a wire mesh ceiling. The wire mesh wall was constructed from 1-cm² gauge mesh in the lower part (20 cm) and vertical bars spaced by 1-cm intervals in the upper part (15 cm), which prevented the rats from climbing up to the ceiling. Two enclosures were placed side-by-side so that the wire mesh walls in both enclosures were adjacent to one another, separated by a 5-cm gap. The acrylic board within each enclosure was covered in clean bedding.

In the Solitary situation, the subject was placed in one enclosure while the other enclosure was left vacant. In the Social situation, the subject was placed in one enclosure and an associate was placed in the other enclosure. After a 5-min acclimation period, either the 20-s (Solitary situation: non-conditioned, $n = 6$; conditioned, $n = 7$; Social situation: non-conditioned, $n = 6$; conditioned, $n = 7$) or 3-s (Solitary situation: non-conditioned, $n = 6$; conditioned, $n = 7$; Social situation: non-conditioned, $n = 6$; conditioned, $n = 6$) CS was presented five times at 2-min intervals during the 10-min experimental period. The behavior of the subjects during the acclimation and experimental periods was recorded with a video camera (DCR-TRV18; Sony, Tokyo, Japan) and an HDD-BD recorder (DMR-BW770; Panasonic, Osaka, Japan).

2.4. Data analyses and statistical procedures

Data are expressed as means \pm standard error of the means (SEM). The significance level was set at $P < 0.05$ for all statistical tests. A researcher who was blinded to the experimental conditions recorded the behaviors of the subjects, including the duration of freezing (immobile posture, with cessation of skeletal and vibrissae movement except in respiration), duration of investigation (sniffing towards another enclosure within 1 mm from the wire mesh, including poking of the snout towards the wire mesh and climbing up the wire mesh), and walking frequency (number of steps taken with the hind paws). Microsoft Excel-based Visual Basic software was used to record the duration and number of key presses, as in our previous studies [25].

In the acclimation period, the durations of freezing and investigation, and the frequency of walking were analyzed using a multivariate analysis of variance (MANOVA) followed by Fisher's protected least significant difference (PLSD) post hoc test. In the experimental period, fear responses were analyzed using two measures. The percentage of time

متن کامل مقاله

دریافت فوری ←

ISIArticles

مرجع مقالات تخصصی ایران

- ✓ امکان دانلود نسخه تمام متن مقالات انگلیسی
- ✓ امکان دانلود نسخه ترجمه شده مقالات
- ✓ پذیرش سفارش ترجمه تخصصی
- ✓ امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
- ✓ امکان دانلود رایگان ۲ صفحه اول هر مقاله
- ✓ امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
- ✓ دانلود فوری مقاله پس از پرداخت آنلاین
- ✓ پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات