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The strain of an accompanying conspecific affects the efficacy of social buffering in male rats

Kayo Nakamura [1], Akiko Ishii [1], Yasushi Kiyokawa *, Yukari Takeuchi, Yuji Mori

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

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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. We previously reported that in male Wistar rats, the presence of another Wistar rat mitigates conditioned fear responses to an auditory conditioned stimulus (CS). Subsequent analyses revealed several characteristics of this social buffering of conditioned fear responses. However, information regarding the specificity of accompanying conspecifics is still limited. In the present study, we assessed whether rats of other strains could induce social buffering in Wistar rats. When a fear-conditioned Wistar subject was re-exposed to the CS alone, we observed increased freezing and decreased investigation and walking, as well as elevated corticosterone levels. The presence of a Wistar, Sprague–Dawley, or Long–Evans rat blocked these responses, suggesting that social buffering was induced by these strains of rats. In contrast, a Fischer 344 rat did not induce social buffering in the Wistar subject. We further found that an inbred Lewis rat induced social buffering whereas a Brown Norway rat, a strain that has been established independently from Wistar rats, did not. These results suggest that the difference in origin, rather than the inbred or outbred status of the associate rat, seemed to account for the lack of social buffering induced by the F344 rats. Based on these findings, we conclude that strains of an accompanying conspecific can affect the efficacy of social buffering in rats.

Introduction

When animals are exposed to distressing stimuli alongside their mother, mate, or a conspecific with which there is no sexual relationship, a wide variety of stress responses are attenuated. This phenomenon is called exposure-type social buffering (Kiyokawa, 2015). Social buffering induced by a conspecific(s) with which there is no sexual relationship has been reported in a wide variety of social species, including sheep (da Costa et al., 2004), guinea pigs (Hennessy et al., 2008), and rats (Terranova et al., 1999).

Previously, we reported that accompaniment by an associate Wistar male rat with a subject Wistar male rat blocked conditioned fear responses and activation of hypothalamic–pituitary–adrenal axis in response to an auditory conditioned stimulus (CS) that had been paired with a foot shock during fear conditioning (Kiyokawa et al., 2007). 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 (Kiyokawa et al., 2009). Social buffering appears to be a biologically important phenomenon for rats because it was similarly observed in female rats (Ishii et al., 2016). Given that lesioning the main olfactory epithelium in subjects blocks social buffering (Kiyokawa et al., 2009) and that volatile olfactory signals from an associate alone can induce social buffering (Kiyokawa et al., 2014b; Takahashi et al., 2013), we suggest that olfactory communication between the dyad mediates social buffering of conditioned fear responses. In our investigations of the neural mechanisms underlying social buffering, we found that activation in the lateral amygdala in response to the CS was suppressed during social buffering (Fuzzo et al., 2015; Kiyokawa et al., 2007, 2014b; Takahashi et al., 2013). We also found that the posterior complex of the anterior olfactory nucleus relayed olfactory signals responsible for social buffering from the main olfactory bulb to the amygdala (Kiyokawa et al., 2012).

Research on social buffering induced by a mother or mate has implicated the specificity of the accompanying animal. For example, in guinea pigs, the presence of a mother, but not an unfamiliar female, induced social buffering in pre-weaning male pups (Hennessy et al., 2006; Hennessy and Morris, 2005). Similarly, the presence of a bonded female, but not an unfamiliar or familiar female, induced social buffering in adult male guinea pigs (Sachser et al., 1998). In addition to relationships between a mother and offspring or between mates, there are many

* Corresponding author.
E-mail addresses: kayopeche@gmail.com (K. Nakamura), 5202205938@mail.ecc.u-tokyo.ac.jp (A. Ishii), akiyo@mail.ecc.u-tokyo.ac.jp (Y. Kiyokawa), aytake@mail.ecc.u-tokyo.ac.jp (Y. Takeuchi), aymori@mail.ecc.u-tokyo.ac.jp (Y. Mori).
[1] These authors contributed equally to this work.

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types of conspecifics without family or sexual relationships. This implies that several factors can affect the efficacy of social buffering induced by a conspecific. Familiarity with a conspecific is one such factor. For instance, we demonstrated that a familiar associate produces a higher degree of social buffering compared with an unfamiliar associate in rats (Kiyokawa et al., 2014b). It is reasonable to hypothesize that the strain of the conspecific might be an additional factor. Although social buffering has been reported in Wistar (Hofer and Shair, 1978, 1980), Sprague–Dawley (SD) (Latane, 1969, 1972; Terranova et al., 1999), and Long–Evans (LE) rats (Taylor, 1981) (it has also been reported in "hooded" rats [File and Peet, 1980]), to the best of our knowledge, all previous studies used subjects and associates from the same strain. Given that rats show prosocial behavior towards conspecifics of the same, but not different, strains (Ben-Ami Bartal et al., 2014), it is possible that the strain of an accompanying conspecific can affect the efficacy of social buffering.

To assess this hypothesis, we assessed whether a Wistar, SD, LE, or Fischer 344 (F344) associate would induce social buffering of conditioned fear responses in Wistar subjects (Experiment 1). Among these, F344 associates are the only inbred rats that are genetically homogeneous (isogenic) as a result of sister–brother mating for >20 generations. In contrast, Wistar, SD, and LE associates are outbred rats that have genetic variations between individuals and are kept as closed colonies. Therefore, in Experiment 2, we used inbred Lewis (LEW) associates to assess whether inbred associates would induce social buffering in Wistar subjects. In addition to being the only inbred strain in our experiment, the F344 rats represented a strain that was established independently from Wistar rats (SD, LE, and LEW rats are derived from Wistar rats). To further investigate the effect of strains established independently from Wistar rats, we conducted an experiment with Brown Norway (BN) rats, which have this characteristic (Experiment 3). Specifically, we used BN rats as associates to assess whether rats established independently from Wistar rats could induce social buffering in Wistar subjects.

Material and methods

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 adapted from the *Consensus Recommendations on Effective Institutional Animal Care and Use Committees* by the Scientists Center for Animal Welfare.

Experimentally naïve male Wistar (aged 7.5 weeks), SD (aged 8 weeks), LE (aged 8 weeks), F344 (aged 9–10 weeks), LEW (aged 8 weeks), and BN (aged 9 weeks) rats were purchased from Charles River Laboratories Japan (Kanagawa, Japan). To ensure a similar body size, we ordered rats of different ages depending on strain. 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 ± 5%, and a 12-h light/12-h dark cycle (lights were switched on at 8:00). Food and water were available *ad libitum*.

Table 1
Behavioral responses during the acclimation period in Experiment 1.

Situation	Group	Freezing	Investigation	Walking
Alone	Non-conditioned	1.1 ± 1.1	32.0 ± 6.0	51.4 ± 7.8
	Conditioned	3.9 ± 3.9	35.5 ± 6.7	43.0 ± 7.4
Wistar	Non-conditioned	0 ± 0	79.5 ± 6.7	72.7 ± 8.6
	Conditioned	0 ± 0	84.7 ± 4.3	73.2 ± 4.4
SD	Non-conditioned	0 ± 0	88.1 ± 5.4	65.8 ± 7.8
	Conditioned	0 ± 0	75.0 ± 3.6	66.0 ± 6.5
LE	Non-conditioned	0 ± 0	86.9 ± 6.7	67.5 ± 8.5
	Conditioned	0 ± 0	84.1 ± 8.1	71.5 ± 9.1
F344	Non-conditioned	0 ± 0	72.2 ± 6.6	55.0 ± 9.6
	Conditioned	0 ± 0	65.4 ± 5.2	47.1 ± 3.3

Data are expressed as means ± standard error of the mean.

Experiment 1

Wistar rats were assigned to either subject or associate (the rat that was exposed to the CS with the subject) role. To maintain unfamiliarity between the subject and associate, cage mates were assigned to the same group. SD, LE, and F344 rats were used as associates only. All rats were housed separately and were handled for 5 min daily, commencing 3 days before the conditioning day.

Fear conditioning was performed in an illuminated room between 9:00 and 15:00, as described in our previous studies (Kiyokawa et al., 2007, 2014a). The subject in the conditioned group was placed in an acrylic conditioning box (28 × 20 × 27 cm) for 20 min, where it received seven repetitions of a 3-s tone (CS, 8 kHz, 70 dB) that terminated concurrently with a foot shock (0.5 s, 0.75 mA). We prepared the non-conditioned group by presenting the tone and foot shock separately during a 20-min period. The intertrial interval randomly varied from 30 to 180 s. The subjects were returned to their home cages after fear conditioning.

A fear-expression test was performed between 09:00 and 13:00, 24 h after the fear conditioning, as described in our previous studies (Kiyokawa et al., 2009, 2014a). Two rectangular enclosures (25 × 25 × 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-cm^2 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 the two enclosures were adjacent to one another with a 5 cm distance separating them. The acrylic board within the enclosures was covered in clean bedding.

In the Alone situation, the subject was placed in one enclosure while the other enclosure was left vacant (non-conditioned, $n = 8$; conditioned, $n = 8$). In the Wistar, SD, LE, and F344 situation, the subject was placed in one enclosure and a Wistar, SD, LE, or F344 associate was placed in the other enclosure, respectively (Wistar situation: non-conditioned, $n = 9$; conditioned, $n = 9$; SD situation: non-conditioned, $n = 9$; conditioned, $n = 8$; LE situation: non-conditioned, $n = 8$; conditioned, $n = 8$; F344 situation: non-conditioned, $n = 7$; conditioned, $n = 8$). After a 3-min acclimation period, a 3 s CS tone was presented five times at 1-min intervals during the first half of 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).

Experiment 2

Wistar and LEW rats were assigned to the subject and associate roles, respectively. All rats were housed separately and were handled for 5 min daily, commencing 3 days before the conditioning day.

Fear conditioning was performed as described in Experiment 1. The subject in the conditioned group received seven pairings of a 3-s tone (CS, 8 kHz, 70 dB) and a foot shock (0.5 s, 0.75 mA) while the tone and foot shock were presented separately to the subject in the non-conditioned group. The intertrial interval varied randomly from 30 to 180 s.

The fear-expression test was conducted as described in Experiment 1. In the LEW situation, the subject was placed in one enclosure and the LEW associate was placed in the other enclosure (non-conditioned,