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The benefits of social buffering are maintained regardless of the stress level of the subject rat and enhanced by more conspecifics

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ABSTRACT

Social buffering is a phenomenon in which the presence of an affiliative conspecific (associate) mitigates stress responses in a subject. We assessed the relationship between the stress level of subjects and the benefit of social buffering. In Experiment 1, subjects fear-conditioned using 0.15-mA, 0.45-mA, or 0.70-mA foot shocks were re-exposed to a conditioned stimulus (CS) either alone or with an associate on the day following fear conditioning. We found that behavioral responses were reduced by the presence of an associate. The intensity of this decrease was similar among all subjects. These results suggest that the intensity of social buffering was similar regardless of the stress level of the subject. The high stress subjects showed residual stress responses after receiving social buffering, indicating that the residual stress responses may have been resistant to social buffering. To further examine this, subjects fear-conditioned using 0.70-mA foot shocks were re-exposed to the CS either alone, with one associate, or with three associates in Experiment 2. We found that behavioral responses decreased as the number of associates increased. These results suggest that residual stress responses are further ameliorated when the number of associates increases. Therefore, the residual stress responses were also sensitive to buffering. Taken together, our data indicate that the benefits of social buffering are maintained regardless of the stress level of the subject rat and enhanced by more conspecifics.

1. Introduction

The presence of an affiliative conspecific, or cues associated with a conspecific, has been found to reduce stress responses to a wide variety of stimuli ranging from novel environments [1,2] to specific aversive stimuli [3–5]. This phenomenon is called “social buffering” [6]. Investigating social buffering may illuminate the origins of sociality in animals. That the stress response of one animal can be reduced by the presence of another animal may have contributed to the tendency to live in groups, thus leading to sociality in specific species. Social buffering is similarly observed in humans and improves human health as a part of the benefit of social support [7,8]. Thus, a better understanding of social buffering in non-human animal models may have enormous translational values. Ample studies have demonstrated that, in addition to social buffering via the mother or mate of an individual [9,10], buffering can be induced by other conspecifics in a variety of non-human species, including laboratory rats [4,11,12].

We have previously investigated social buffering induced by a conspecific other than the mother or mate of an individual using fear conditioning. When a fear-conditioned subject rat is re-exposed to an auditory or contextual conditioned stimulus (CS) alone, conditioned fear responses including increased freezing and hypothalamic-pituitary-adrenal axis activity are observed. However, the presence of an unfamiliar rat (associate) has been found to block these responses, suggesting that social buffering can ameliorate conditioned fear responses [11,13,14]. Subsequent analyses revealed that the addition of a double wire-mesh partition that separated the subject and associate by 5 cm had no effect on this social buffering [15,16]. Social buffering has been observed both between males and between females, indicating that it is a biologically important phenomenon in all rats [17]. Furthermore, we observed social buffering primarily between rats derived from the same colony [18] and found that it enhanced extinction of conditioned fear responses [19]. As a result of our investigations regarding the neural mechanisms of social buffering, we have delineated a circuit underlying this phenomenon. Specifically, a volatile olfactory signal detected at the main olfactory epithelium [16,20,21] activates the posterior complex of the anterior olfactory nucleus [21,22], which in turn suppresses the activation of the lateral amygdala in response to the CS [14,20,21,23].

To the best of our knowledge, previous investigations of social buffering used a single stressor, i.e., the stress level of the subjects was not manipulated. Therefore, the relationship between the stress level of the subjects and the benefit of social buffering has not been analyzed

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systematically. One possibility is that the benefit of social buffering is reduced because the high stress status of the subjects decreases the intensity of social buffering. In literature, substantial social buffering has been reported in subjects exposed to severe or even lethal stressors. For example, in rats, the odor of a predator (cat) is known to be a sever stressor [24]. When rats were exposed to cat fur, the presence of three conspecifics increased grooming, locomotor activity, and the number of contacts with the cat fur stimulus, and decreased Fos expression in the nuclei related to threat response [3]. In humans, assessing the benefit of social buffering on initial responses to severe stressors is accompanied by ethical challenges. However, retrospective studies have demonstrated the benefit of social support in individuals undergoing stressful life events. For example, people who received social support during stressful life events had a lower mortality rate [8]. In addition, patients with cancer [25], those undergoing dialysis [26], and individuals with massive burn injuries [27] had a lower mortality rate when they received higher levels of social support. Given that social buffering can also be evaluated as the enhancement of recovery from the adverse effects of stress [6,28] and that social buffering is a component of the benefits of social support [7,8], social buffering appears to take place in humans exposed to severe stressors. Based on these findings, we hypothesize that the intensity of social buffering will be similar regardless of the stress level of the subject.

High stress subjects show residual stress responses after receiving social buffering. Therefore, another possibility is that the benefit of social buffering is impaired because the high stress subjects show responses that are resistant to social buffering in addition to buffering-sensitive responses. To test this possibility, stronger social buffering must be presented to subjects. One possible method for accomplishing this is to increase the number of associates. Although this has not previously been assessed in rats or other rodents, a study using squirrel monkeys demonstrated that elevated cortisol levels in fear-conditioned subjects in response to a visual CS were slightly reduced in the presence of one associate and returned to pre-conditioning levels when five associates were present [29]. Based on this finding, we hypothesize that residual stress responses in high stress subject rats will decrease as the number of associates increases.

We conducted a series of experiments using rats to examine our hypotheses. In Experiment 1, we compared social buffering induced by one associate among subjects in low, middle, and high stress groups. We used a fear-conditioning paradigm to manipulate the stress status of the subject. Given that a higher foot shock intensity during the conditioning procedure is known to elicit a higher stress status in the fear-conditioned animal during CS re-exposure [30–32], we fear-conditioned the subjects to the CS with 0.15-mA, 0.45-mA, or 0.70-mA foot shocks. On the following day, these subjects were re-exposed to the CS either alone or with a non-conditioned associate. We assessed the efficacy of social buffering by measuring the behavioral responses of the subjects. We predicted that the presence of an associate would reduce behavioral responses in a similar manner among subjects with different stress levels. In Experiment 2, we compared social buffering induced by one vs. three associates using the subjects in the high stress group. The subjects were fear-conditioned to the CS with 0.70-mA foot shocks. On the following day, these subjects were re-exposed to the CS alone, with one non-conditioned associate, or with three non-conditioned associates. We predicted that three associates would lead to a greater reduction in behavioral responses compared with one associate.

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. A male experimenter (K.K.) cared for all the animals and conducted all the experiments.

2.1. Animals

Sixty (46 subjects and 14 associates) and 46 (26 subjects and 20 associates) experimentally naïve male Wistar rats (aged 7.5 weeks) were purchased from Charles River Laboratories Japan (Kanagawa, Japan) for Experiments 1 and 2, respectively. We used male rats because we expected them to show clearer behavioral responses compared with female rats [17], thus facilitating our assessment of our hypotheses. Upon arrival, the rats were housed in wire-topped, transparent cages (41 × 25 × 21 cm) with 2–3 animals per cage and placed on racks (about 30 lx) without any intention. The colony room had 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. The rats were assigned to either the subject or associate role. To maintain unfamiliarity between the subjects and associates, all the rats housed in each cage were assigned to the same role. Food and water were available ad libitum. As being housed with another rat after a conditioning procedure has been found to induce social buffering [14,33–35], all rats were housed individually 3 days before the conditioning day. During the individual housing period, the rats were handled for 5 min daily. Handling involved placing the rats one at a time on an experimenter's lap and gently petting their back and abdomen.

2.2. Procedures

2.2.1. Experiment 1

Fear conditioning was performed in an illuminated soundproof room during the light phase, specifically between 9:00 and 16:00, as described in our previous studies [36,37] (Supplemental Fig. 1). Each subject was placed in an acrylic conditioning box (28 × 20 × 27 cm) for 15 min, where it received five repetitions of a 3-s tone (CS, 8 kHz, 70 dB) that terminated concurrently with a 0.15-mA (0.15 group), 0.45-mA (0.45 group), or 0.70-mA (0.70 group) foot shock (0.5 s). 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 24 h after fear conditioning, as described in our previous study [16] (Supplemental Fig. 1). Two rectangular enclosures (25 × 25 × 35 cm) were placed on an acrylic board (45 × 60 cm) in a dark room illuminated by a dim red light. Each enclosure had three acrylic walls, one wire mesh wall, and a wire mesh ceiling. The lower section (20 cm) of the wire mesh wall was constructed from 1-cm^2 gauge mesh and the upper section (15 cm) was composed of vertical bars spaced 1 cm apart. This prevented the rats from climbing up to the ceiling. The two enclosures were placed side-by-side so that the wire mesh walls in the 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 Alone situation, one subject from the 0.15 (n = 9), 0.45 (n = 6), or 0.70 (n = 8) group was placed in one enclosure while the other enclosure was left vacant. In the Social situation, one subject from the 0.15 (n = 9), 0.45 (n = 6), or 0.70 (n = 8) group was placed in one enclosure and an associate was placed in the other enclosure. The rats first underwent a 5-min acclimation period that started when the subject was placed in the enclosure prior to the presentation of the first CS. Then, a 15-min experimental period began. During the first one third of the experimental period, the 3-s CS was presented five times at 1-min intervals. 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). All of the subjects were used only once. Some of the associates were used twice within an experimental day, with at least 30 min intervals between experiments. The order of the test conditions (intensity of foot shock and presence of an associate) was counterbalanced to reduce the effects of order.