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Social buffering reduces male rats' behavioral and corticosterone responses to a conditioned stimulus

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ABSTRACT

In social species, the presence of an affiliative same-sex conspecific ameliorates acute stress responses in threatening conditions. We previously found that the presence of an unfamiliar male rat separated by a wire mesh barrier blocks the behavioral responses and Fos expression in the paraventricular nucleus of the hypothalamus (PVN) in a male subject rat that had previously been exposed to an auditory conditioned stimulus (CS) paired with foot shocks. Based on the Fos expression in the PVN, we hypothesized that the presence of a conspecific ameliorated the hypothalamic–pituitary–adrenal (HPA) axis activation and induced social buffering of conditioned fear responses. The direct evidence for this hypothesis, however, is still lacking. To clarify this point, we exposed fear-conditioned and non-conditioned subjects to the CS either alone or with a conspecific separated by a wire mesh barrier. When the fear-conditioned subject alone was re-exposed to the CS, it exhibited increased freezing, decreased sniffing, and elevated corticosterone levels. In contrast, the presence of the conspecific suppressed these behavioral and HPA axis responses to a level similar to those observed in the non-conditioned subjects. These results suggest that the presence of a conspecific suppressed the behavioral responses and HPA axis activation to the CS. The present results provide direct evidence for the existence of social buffering of conditioned fear responses in male rats.

Introduction

Stress is a condition in which an animal's life is threatened by an uncontrollable and unpredictable stimulus (Koolhaas et al., 2011). Activation of the hypothalamic–pituitary–adrenal (HPA) axis is one of the primary stress responses. In the HPA axis response, parvocellular neurons in the paraventricular nucleus of the hypothalamus (PVN) release corticotrophin-releasing hormone that stimulates adrenocorticotropic hormone release from the anterior lobe of the pituitary, which in turn stimulates corticosterone release from the adrenal glands. Therefore, we can infer an animal's stress level by measuring the magnitude of the HPA axis response through corticosterone levels.

In mammals, the presence of a conspecific can attenuate stress. This effect was demonstrated through maternal buffering, where the mother's presence suppresses corticosterone secretion in response to a novel environment in preweaning, periadolescent, and adult male guinea pigs (Hennessy et al., 2002); the same effect was demonstrated with foot shocks in preweaning rats (Moriceau and Sullivan, 2006; Sullivan and Holman, 2010). Similarly, the presence of a same-sex conspecific can ameliorate stress in social buffering. For example, corticosterone secretion in response to a novel environment was suppressed in periadolescent rats (Terranova et al., 1999) and in adult sheep (da Costa et al., 2004) in the presence of a conspecific or stimuli associated with a conspecific, respectively. We previously reported that the behavioral responses to a conditioned stimulus (CS) in adult male rats were attenuated by the presence of another adult male rat (Kiyokawa et al., 2007). These effects persisted even if the dyad was separated by 2 wire mesh screens (Kiyokawa et al., 2009). Furthermore, we found that the conspecific released olfactory signals that mediated these effects (Takahashi et al., 2013), which were perceived by the main olfactory system (Kiyokawa et al., 2009). In another study, we revealed the neural pathway underlying these effects (Kiyokawa et al., 2012). Because Fos expression in the PVN was suppressed in our experimental model (Kiyokawa et al., 2004, 2007, 2009; Takahashi et al., 2013), we hypothesized that the presence of the conspecific suppresses HPA axis activation, which in turn suggested a decrease in the stress level of the subject. Based on this hypothesis, we have regarded this phenomenon as social buffering of conditioned fear responses.

In our previous studies, we focused on c-Fos immunohistochemistry rather than systemic hormone levels to determine the effects of the conspecific on HPA axis activity. However, the PVN contains both parvocellular and magnocellular neurons; while the activity of parvocellular neurons is related to HPA axis activation, that of magnocellular neurons is not related to HPA axis activation in response to acute stressors (Engelmann et al., 2004). Moreover, unlike the parvocellular neurons that secrete the corticotrophin-releasing hormone,

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those secreting oxytocin and vasopressin do not directly contribute to HPA axis activation in response to acute stressors (Gillies et al., 1982). Therefore, suppressed Fos expression in the PVN does not necessarily indicate that HPA axis activation is suppressed by the presence of another adult male rat.

To clarify this point, we directly assessed whether the presence of a conspecific suppresses HPA axis activation in response to a CS. While some rats were fear-conditioned, others were not, and on the following day, rats from both groups were exposed to the CS either alone or with a conspecific rat separated by 2 wire mesh screens. We observed behavioral responses during the test and corticosterone levels before and after the test in order to evaluate the stress level of the subject.

Material and methods

Animals

The experiments conducted for this study were approved by the Animal Care and Use Committee of the Faculty of Agriculture at The University of Tokyo, and were based on 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 Wistar rats (aged 8 weeks) were purchased from Charles River Laboratories Japan (Kanagawa, Japan). They housed 2 to 3 animals per cage in a controlled colony room with an ambient temperature of 24 ± 1 °C, humidity maintained at 45 ± 5%, and with food and water available ad libitum. The animals were maintained under a 12-h light/12-h dark cycle (lights switched on at 0800). Rats were assigned to either the subject group or the associate group in which rats were exposed to the CS with the subject. Cage mates were assigned to the same group in order to maintain unfamiliarity between the subject and the associate rats. All rats were housed separately and were handled for 5 min per day beginning 3 days before the conditioning day.

Fear conditioning

Fear conditioning was performed in an illuminated room between 0900 and 1300, as described in our previous studies (Kiyokawa et al., 2012, 2013; Takahashi et al., 2013). During conditioning, the subject in the conditioned group was placed in an acrylic conditioning box (28 × 20 × 27 cm) for 20 min, where 7 repetitions of a 3-s auditory tone (CS, 8 kHz, 80 dB) that terminated concurrently with a foot shock (0.5 s, 0.8 mA) were presented. We prepared the non-conditioned group by presenting the tone and foot shock separately during a 20-min period. The intertrial interval randomly varied between 30 and 180 s. The rats were returned to their home cage after the fear conditioning.

Fear-expression test and blood sampling

A fear-expression test was performed between 0900 and 1300, 24 h after the fear conditioning, and was performed using the procedures described in our previous studies (Kiyokawa et al., 2009, 2012). The test was conducted in 2 rectangular enclosures (25 × 25 × 35 cm) placed on an acrylic board (45 × 60 cm) in a room illuminated with a dim red light. Each enclosure was constructed of 3 acrylic walls, 1 removable wire mesh wall, and a wire mesh ceiling. Clean bedding was spread to cover the board encircled by the wall. The wire mesh wall consisted of 1-cm^2 gauge 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 for the 2 enclosures were adjacent to one another with a 5 cm distance between them. The subject tested in the alone situation was placed in one enclosure while the other enclosure was left vacant. In the dyad situation, the subject was placed in one enclosure and the associate was placed in the other enclosure. Subsequently, they were allowed a 5-min acclimation period that preceded the 10-min fear-expression test. During the test, the CS was presented 5 times for 3 s with 1-min intervals during the first half of the 10-min period. We made a small incision in the subject's tail and collected blood in heparinized syringes at the end of the acclimation period in order to measure the pretest value of corticosterone level. To measure the corticosterone level after the fear-expression test, blood was collected 10 min after the end of the test (an n of 6 in all groups). During this additional 10-min period, subjects remained in the test apparatus. Blood sampling was completed within 1–2 min of the intended time point. We recorded the rats with a video camera (DCR-SR300; Sony, Tokyo, Japan) and an HDD-BD recorder (DMR-BW770; Panasonic, Osaka, Japan) during the acclimation period and the fear-expression test to analyze their behaviors.

Enzyme immunoassay

Blood samples were centrifuged at 4 °C, and the plasma was stored at −80 °C for the enzyme immunoassay (EIA). The EIA for corticosterone was performed with the Corticosterone EIA Kit (Cayman Chemical Company, Ann Arbor, MI) according to the manufacturer's instructions. The intra-assay and inter-assay variations were 8.0% and 12.6%, respectively.

Data analyses and statistical procedures

The data are expressed as mean ± standard error of the mean (SEM); the significance level was set at $P < 0.05$ for all statistical tests. A researcher who was blind to the experimental conditions recorded the duration of the freezing behaviors (immobile posture, with cessation of skeletal and vibrissae movement except in respiration) and sniffing behavior (regular movement of vibrissae with exploring), and the frequency of walking (number of steps taken with the hind paws) by the subjects using Microsoft Excel-based Visual Basic software that records the duration and number of keyboard keys pressed, as previously described (Kiyokawa et al., 2012, 2013; Takahashi et al., 2013). The behavioral data recorded for the subjects during the acclimation period and the fear-expression test were analyzed by a two-way multivariate analysis of variance (MANOVA) followed by Fisher's protected least-significant difference (PLSD) post hoc test.

The plasma corticosterone level in each blood sample was measured in duplicate. The averaged data were analyzed using three-way analysis of variance (ANOVA) followed by Fisher's PLSD post hoc test.

Results

The results of statistical analyses are shown in Table 1. The behavioral responses during the acclimation period were significantly affected by the presence of an associate ($F(3,18) = 5.98$, $P < 0.01$). However, the effects of the conditioning procedure and the interaction between the 2 factors were not significant. A post hoc test revealed that the behavioral responses were not different between the conditioned and non-conditioned groups in the same situation (Table 2).

In contrast, the behavioral responses during the fear-expression test were significantly affected by the presence of an associate ($F(3,18) = 19.6$, $P < 0.01$) and the conditioning procedure ($F(3,18) = 9.68$, $P < 0.01$). The interaction between the 2 factors also was significant ($F(3,18) = 7.98$, $P < 0.01$). A post hoc test revealed that the conditioned group showed an increased freezing duration ($P < 0.01$) and a decreased sniffing duration ($P < 0.01$), and tended to decrease their walking frequency ($P = 0.0536$, Cohen's $d = 1.48$), as compared to the non-conditioned group when the test was conducted in the alone situation (Fig. 1). However, when the fear-expression test was conducted in the dyad situation, no significant differences in