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Research report

Physical interaction is not necessary for the induction of housing-type social buffering of conditioned hyperthermia in male rats

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HIGHLIGHTS

- Social housing after fear conditioning induces housing-type social buffering.
- Physical interactions were not necessary for the induction of social buffering.
- The induction of Fos expression by such social cohabitation was observed.
- These results provide information about the induction of social buffering.

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ABSTRACT

In social animals, housing with conspecific animals after a stressful event attenuates the subsequent adverse outcomes due to the event, and this has been called housing-type social buffering. We have previously found that housing-type social buffering attenuates the enhancement of hyperthermia and Fos expression in the paraventricular nucleus of the hypothalamus that occurs in response to an aversive conditioned stimulus in male rats. Here, we analyzed the role of physical interactions during social housing in the induction of housing-type social buffering. When a fear-conditioned subject was alone after the conditioning and then exposed to the conditioned stimulus, it showed behavioral, autonomic, and neural stress responses. However, social housing, during which physical interactions were prevented by wire mesh, attenuated these autonomic and neural stress responses, as has been seen in previous studies. These results suggested that physical interaction was not necessary for the induction of housing-type social buffering. With this social cohabitation model, we then found that social cohabitation increased Fos expression in the posterior complex of the anterior olfactory nucleus of the fear-conditioned subject. Social cohabitation also increased Fos expression in 11 brain regions, including the prefrontal cortex, the nucleus accumbens, the bed nucleus of the stria terminalis, and the medial, lateral, basal, and cortical amygdala. These results provide information about the neural mechanisms that induce housing-type social buffering.

1. Introduction

In social animals, two types of interactions with conspecifics can attenuate stress responses. One is the interaction when the animal is exposed to a stressor. For example, when animals are exposed to a novel environment or an aversive conditioned stimulus (CS), their behavioral, endocrine, and autonomic responses are attenuated if they can interact with one or more conspecific animals in the same test apparatus [1–4]. These phenomena are referred to as exposure-type social buffering [5]. The other is the interaction during the housing with a conspecific in the same cage after a stressful event. Studies have begun to suggest that such interactions can also attenuate subsequent stress responses due to a previous adverse event. For example, housing with a conspecific was found to prevent body weight loss [6] and increase of anxiety [7], both of which are changes that are typically seen in animals that are defeated by an aggressive conspecific. These phenomena are called housing-type social buffering [5].

Abbreviations: ANOVA, analysis of variance; AOP, posterior complex of the anterior olfactory nucleus; CS, conditioned stimulus; HPA, hypothalamus–pituitary–adrenal; PVN, paraventricular nucleus of the hypothalamus.

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We have previously found that these two types of social buffering differentially attenuate stress responses to aversive CS in male rats. When the subjects received repetitions of a tone that terminated concurrently with a foot shock, the tone served as an aversive CS and evoked freezing behavior, as well as enhanced hyperthermia and hypothalamus–pituitary–adrenal (HPA) axis activity, as indexed by an enhancement of Fos expression in the paraventricular nucleus (PVN) of the hypothalamus. However, when the fear-conditioned subject was socially housed with another rat immediately after the conditioning, the subject did not show autonomic and HPA axis responses, even if the subject alone was subsequently exposed to the CS, suggesting that housing-type social buffering ameliorates subsequent stress responses to the CS [5]. In contrast, the behavioral and HPA axis responses of the fear-conditioned subject was blocked when the subject was exposed to the CS with another rat in the same test apparatus, suggesting the existence of exposure-type social buffering that differentially mitigates stress responses to the CS compared to housing-type social buffering [5]. We further found that physical interactions between the dyad are not necessary for exposure-type social buffering because separation of the dyad with wire mesh did not interfere with exposure-type social buffering [8]. Based on this finding in exposure-type social buffering, we hypothesized that housing-type social buffering also does not require physical interactions between the dyad during social housing.

In our housing-type social buffering phenomenon, social housing suppresses subsequent stress responses, even if the subject alone is exposed to the CS. Therefore, the changes in the brain should occur during social housing, which subsequently induces housing-type social buffering. In order to address this issue, we first assessed a hypothesis that social housing interferes with memory consolidation processes that take place within 6 h after the conditioning and that play an important role in the expression of conditioned responses to the CS [9–11]. However, the finding that social housing after the consolidation process, that is, social housing that commenced 6 h after the conditioning, induces social buffering [12] does not support this hypothesis. Therefore, social housing might induce changes that are difficult to predict based on the literature. Brain mapping by c-Fos immunohistochemistry is a useful method that is used to obtain clues of unknown neural mechanisms. When we try to map the brain, the results become more informative if we minimize the irrelevant disturbances as much as possible. Although the neural activation that is induced by foot shocks during the conditioning procedure is the major obstacle that prevents us from mapping the brain, our previous finding that social housing that was started 24 h after the conditioning could also induce social buffering [12] enabled us to minimize this interference. Therefore, if we establish an experimental model in which the other disturbance is further minimized, the results would be more informative.

In the present study, we assessed the role of physical interactions during social housing in the induction of housing-type social buffering. Twenty-four h after the conditioning, a fear-conditioned subject was either housed alone or socially housed with a conspecific for 24 h. However, a wire-mesh partition between the dyad prevented physical interactions during this social housing. Then, the subject alone was re-exposed to the CS. Body temperature, freezing, and Fos expression in the PVN were examined in order to evaluate whether this social cohabitation could induce housing-type social buffering. Because this experimental model enabled us to minimize the disturbances by physical interactions during social housing, we next observed Fos expression in 30 regions of the brain in a second cohort of animals in response to social cohabitation with this experimental model.

2. Materials and methods

2.1. Animals

All experiments were approved by the Animal Care and Use Committee of the Faculty of Agriculture, University of Tokyo, based on guidelines adapted from the “Consensus Recommendations on Effective Institutional Animal Care and Use Committees” from the Scientist Center for Animal Welfare.

Experimentally naïve male Wistar rats were purchased from Charles River Laboratories Japan (Kanagawa, Japan). They were housed 3 animals per polycarbonate cage (28 cm × 44 cm × 20.5 cm) in an ambient temperature of 24 ± 1 °C and a humidity of 45 ± 5% in a controlled colony room with food and water available *ad libitum*. The animals were maintained under a 12-h light/12-h dark cycle (lights switched on at 08:00). Each rat was assigned as either the subject or the associate that was used for social housing with subjects, and cage mates were assigned to the same group in order to maintain unfamiliarity between the subject and the associate rats. Associate rats were housed individually in a colony room for 3 days before the day of social housing. During this period, the associates were handled 5 min per day. Each associate rat was used only once.

2.2. Experiments to assess the role of physical interaction

The general procedures were the same as those described in our previous study [12]. Briefly, 1 week before the conditioning day, all subjects were intraperitoneally implanted with a telemetry transmitter (TA10TA-F40; Data Sciences International, St. Paul, MN) under anesthesia with ether at 8 weeks of age. After the surgery, the subjects were housed individually and handled 5 min per day for 3 days before the conditioning day. Two days prior to the conditioning day, their home cage was moved from the colony room to an experimental room and kept on an antenna board (RLA1020 RPC-1; Data Sciences International) in a soundproof chamber (36 cm × 54 cm × 35 cm; Muromachi Kikai, Tokyo, Japan), in which they were maintained at a constant temperature (24 ± 1 °C) under a 12-h light/12-h dark cycle (lights on at 08:00).

Fear conditioning was performed in an illuminated conditioning room between 09:00 and 18:00. On the conditioning day, the subjects were brought into a conditioning room and placed in an acrylic box with a metal grid floor (28 cm × 20 cm × 27 cm). During a 15-min conditioning period, the subjects in the paired group received 5 repetitions of a 3-s tone (1 kHz, 80 dB) that terminated concurrently with a foot shock (0.5 s, 0.7 mA). We prepared the unpaired group by presenting the CS and foot shock separately during a 15-min period. The intertrial interval randomly varied between 60 and 240 s. After the conditioning, each subject was returned to their home cages in the soundproof chamber and kept there.

Social housing without physical interaction (social cohabitation) took place 24 h after the conditioning in a polycarbonate cage that was divided into 2 compartments (14 cm × 44 cm × 20.5 cm each) by wire mesh that consisted of a 1-cm^2 gauge. The subject was placed in 1 of 2 compartments, while the other compartment remained empty in the solitary situation (unpaired group, $n = 9$; paired group, $n = 8$) or an associate was placed in the other compartment at the same time in the social situation (unpaired group, $n = 8$; paired group, $n = 8$). Then, the cage was returned to the soundproof chamber and kept there.

The 60-min test was conducted 48 h after the conditioning. One min before the experimental period, subjects that showed stable baseline body temperatures were transported in its home cage to a table. Then, only the subject was placed in a polycarbonate test box (28 cm × 44 cm × 20.5 cm) with a punctured acrylic ceiling and