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Pair exposure with conspecific during fear conditioning induces the link between freezing and passive avoidance behaviors in rats

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A B S T R A C T

Social factor plays an important role in dealing with posttraumatic stress disorder related to excessive physiological fear response and insufficient fear memory extinction of the brain. However, although social circumstances occurred not only during contextual retrieval but also during fear conditioning, most previous studies focused on the advantageous aspects of social buffering in fear retrieval period. To demonstrate the association between fear responses and fear memory from social stimuli during fear conditioning, pair exposed rats with conspecific as social buffering were subjected to a fear conditioning of passive avoidance test to evaluate memory function and freezing behavior. Whereas single exposed rats showed the significant increase of freezing behaviors and passive avoidance behaviors compared to control rats, pair exposed rats showed significant alleviation of the freezing behaviors and passive avoidance behaviors compared to single exposed rats. Furthermore, we determined a significant correlation between freezing and passive avoidance behavioral alteration in pair exposed rats. Taken together, we suggest that pair exposure with conspecific during fear conditioning helps to cope with both freezing response and fear memory systems and their reciprocal interaction has a crucial potential as a resource for the relief of unreasonable stress responses in posttraumatic stress disorder.

1. Introduction

Social interaction and communication are essential not only for cooperation within a group, but also for protection from environmental threats; this is one of the most beneficial aspects of establishing a society. Social relationships and interpersonal behaviors exert regulatory influences on physiological, behavioral, and dispositional responses to emotionally charged situations (Uchino et al., 1996). Social interactions can either be a source of stress or provide a buffer against stress depending on the circumstances. Social transmission of information from fearful or stressed conspecific animals exacerbates fear or stress response, whereas social supportive experience from non-fearful ones lessens the stress responses, a phenomenon referred to as ‘social buffering’ (Davitz and Mason, 1955; Kiyokawa et al., 2004; Kiyokawa et al., 2006; Knapska et al., 2006; Langford et al., 2006).

In rodent studies, the presence of conspecific has abundant physical and physio-psychological health benefits such as improving wound healing, anxiety and extended plasma corticosterone level and that called from diverse research papers as a social buffering (Glasper and Devries, 2005; Hennessy et al., 2009; Kikusui et al., 2006). Social buffering has been suggested as an important coping strategy of animals and humans (Clapp and Gayle Beck, 2009; Kiyokawa et al., 2007). In the previous studies, fear-conditioning test is commonly performed to understand the biological effects of posttraumatic stress disorder (PTSD) in rodents and human, contextual or conditioned fear-conditioning test using electric foot-shock is used in rodent (Knight et al., 2004; Maren et al., 2013; Milad et al., 2007). In general, after 24 h, when a rodent exposed to the same context or conditioning, its shocked experience induced fearful emotional state are shown as a freezing behavior and it is generally used as an indicator to measure a posttraumatic response of rodents (Eskandarian et al., 2013; Johansen et al., 2011). Interestingly, the presence of conspecific alleviated the electric foot-shock-induced freezing behavior, hyperthermia and activation of the amygdala in response to fear stimuli in rats (Baum, 1969; Fuzzo et al., 2015; Kiyokawa et al., 2007).

Although social circumstances occurred not only during contextual retrieval but also during fear conditioning, most previous studies focused on the advantageous aspects of social buffering in fear retrieval period and there is little known about the effect of pair exposure with conspecific during fear conditioning on association between fear responses and fear memory from social stimuli. In this study, we determined whether the pair exposure with conspecific

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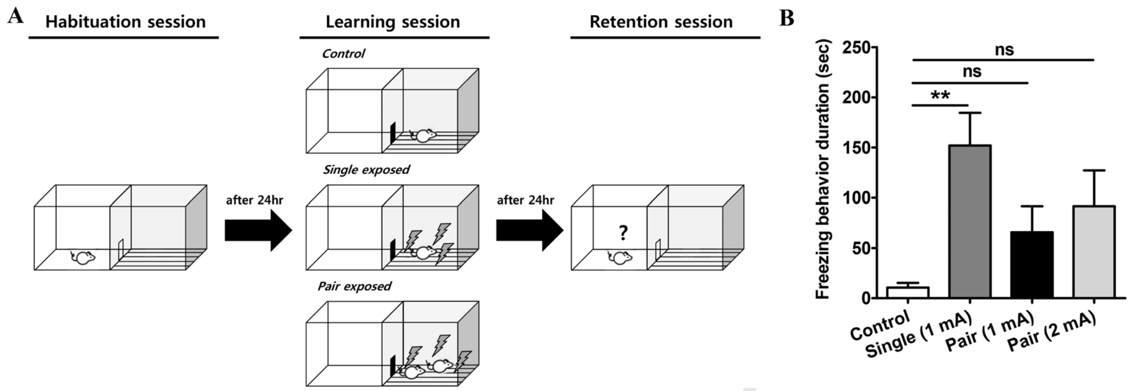

Fig. 1. Experimental procedures and alteration of freezing behaviors for assigned experimental groups. (A) Illustration showing the experimental procedures for the rats assigned to the each of the following experimental groups: the control group containing rats that received no electric foot-shock, the single exposed group containing rats that received electric foot-shock alone and the pair exposed group of rats that received electric foot-shock with conspecifics. (B) In the retention session, altered physiological states were expressed as the freezing behavior duration of rats. There were statistically significant differences among four groups by one-way ANOVA ($F(3, 27) = 4.28$; $P < 0.05$). Whereas single exposed rats showed a significant increase in freezing behavior duration compared to those of the control group, there were no significant differences among the pair exposed (1 mA and 2 mA) and control groups (Control, 10.50 ± 4.84 s, $n = 6$; Single (1 mA), 152.0 ± 32.49 s, $n = 8$; Pair (1 mA), 65.75 ± 25.92 s, $n = 8$; Pair (2 mA), 91.67 ± 35.49 s, $n = 6$; $^{**}P < 0.01$, Newman–Keuls post hoc test). Data are expressed as mean ± S.E.M.

during fear conditioning induces social buffering or social contagion. To evaluate the effect of social buffering, we examined the effect of pair exposure by using a passive avoidance test to assess the link between freezing behaviors and fear memory and learning in rats.

2. Materials and methods

2.1. Animals

For the experiments, male Sprague-Dawley rats were obtained from Orient Bio (Seongnam, Korea). All experiments were conducted in accordance with the Dankook University ethics committee's guidelines for the care and use of laboratory animals (Dankook University IACUC: DKU-14-003). During the test session, rats were individually housed in Plexiglas cages (46 cm × 23 cm × 20 cm) with wood bedding. All of the treating and caring for the experiment progressed in a temperature and humidity controlled (23 ± 1 °C; 45 ± 5%) sound-insulated room. Illumination was maintained in a regular 12:12-h light–dark cycle (lights were on from 09:00 to 21:00) and the rats were provided with free access to food and water.

2.2. Passive avoidance test

For the passive avoidance test procedure, all rats were conditioned between postnatal days 21 and 26 and introduced into the shuttle box, in a sound insulated room, over three consecutive days; they were adapted in the conditioning room for over 30 min before every treatment. The shuttle box (Jeung Do Bio & Plant, Seoul, Korea) consisted of two compartments, a white and a black room (24.5 cm × 25 cm × 25 cm, each) divided by the sliding door (8 cm × 9.5 cm). The electric foot-shock is delivered through the floor composed of 22 grids (grid interval: 1.1 cm; grid diameter: 0.4 cm). For delivering the 1 mA electric foot-shock, the shuttle box respectively supplies 80 V of power supplies to each grid. Each grid is in parallel and this power supply and resistance of grids are sufficient to deliver regular 1 mA electric foot-shock to rat regardless the difference of internal resistances of rat. Three sessions were performed. Rats were assigned to control, single exposed and pair exposed groups (Table 1 and Fig. 1A).

For the habituation session (Day 1), rats were placed into the white room (230 lux) of the shuttle box and the sliding door was opened to allow the rats to freely explore the white and black rooms (50 lux) of the shuttle box for 5 min. In the learning session (Day 2), assigned rats were placed into the white room but the sliding door was closed. All behaviors of animals in learning session were recorded by a camcorder during 50 s (HMX-H304BD, Samsung Electronics Co., Ltd., Suwon, Korea). The behaviors of rats were quantified as walking, immobile sniffing, climbing/rearing, immobility, self-grooming and social exploration following previous studies (Casarrubea et al., 2015; Trezza et al., 2010) (Table 2).

After 1 min from start of learning session, the sliding door was opened. When the rat passed into the black room with all four paws, the sliding door was closed and the subject rat received an unavoidable continuous electric foot-shock of 1 mA for 5 s. Each of the two subject rats in the pair exposed group were conditioned together and the electric shock was delivered after both rats had passed into the black room. Rats were removed after receiving electric shock. Control rats were returned to their individual home-cage, 1 min after the sliding door opening. In the retention session (Day 3), after 24 h had passed, assigned rats were placed into the white room. After 1 min, the sliding door was opened and the activities of the animals, their passive avoidance behaviors were recorded. From the recordings, the freezing behavior duration of fear, the black room preference, the step-through latency and the transition numbers to the black room were derived. Freezing behavior is described as an immobile posture with no movement, except for the respiration. The freezing behavior duration, the transition numbers to the black room and the black room preference were analyzed during the 5 min after the opening of the sliding door. The step-through latency was expressed as the spent time from the opening of the sliding door until all four paws of the rat were completely passed into the black room. The shuttle box was washed with 70% ethanol and wiped dry with a paper towel between each conditioning test.

2.3. Data analysis

Data were presented as mean ± standard error of the mean (S.E.M.) and analyzed by one-way analyses of variance (ANOVA),

Table 1
Description of the experimental groups.

Abbreviation	Description
Control	Subject rat conditioned without electric shock alone
Single exposed	Subject rat conditioned with electric shock alone
Pair exposed	Two subject rats conditioned with electric shock together