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Can Rats Feel Stress of Their Cage Mates?: An Experimental Aanalysis Using Schedule-con-trolled Behavior

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1. Introduction

Social cognition, particularly "empathy" in animals, has recently become an intensely investigated topic in comparative cognition (for example, see de Waal, 2009). In earlier research, Church (1965) reported suppression of operant behavior in rats that observed a conspecific receiving electric shock. This suppression soon disappeared, but was sustained when the observing rats also had experienced the electric shock. Watanabe and Ono (1985) confirmed this type of "empathy" in pigeons, and reported that an anxiolytic drug (diazepam) dose-dependently reduced the suppressive effects (Inagawa and Watanabe, 1991).

More recently, Kavalier et al. (2003) found increased burying behavior and levels of corticosterone in mice that observed conspecifics exposed to biting flies. Likewise, Chen et al. (2009) reported freezing behavior induced by observing tone-shock conditioning of conspecifics in C57/Bl mice but not in Balb/c mice. C57 mice also showed more pain response when they received a formalin injection together with their cage mates (Langford et al., 2006). Thus, exposure to conspecifics receiving aversive experiences induced or enhanced corresponding behaviors in animals that observed those experiences. Finally, in rats, exposure to a mate that had received electric shock activated the amygdala of observers (Knapska et al., 2006).

When a cage mate was removed from a cage, ate a food with a particular flavor, and then was returned to the cage, a rat that had remained in the cage preferred the food with that novel flavor to food with the other flavor (Galef et al., 1984). Thus, rats obtained some information from the cage mates, even without directly observing eating behaviors. This does not mean that the rats "told" something about their experience to the cage mates, but exposure to the combination of a living rat and a flavor somehow resulted in preference for that flavor in observers.

Here, we examine effects of social information on schedule-controlled behavior in rats. Pairs of rats (one demonstrator, one observer) shared a cage; both of them were separately trained on a Fixed Ratio schedule. The demonstrator was moved to an operant chamber, received the training, and then was returned to the cage. Later, the observer was moved to another operant chamber and received the same training. In the experimental session, the demonstrator received restrict stress instead of operant training, and then was returned to the cage.

The first question is whether a rat can discriminate between a cage mate that received the stress and one that did not receive stress. The second question is the effect of the exposure on the stress-received cage mate. If the rats are able to discriminate such an experience of a cage mate, then the exposure of the demonstrator to the aversive experience should affect the subsequent operant behavior of the observer; probably some suppression should occur, because the exposure should cause "fear" in the observer.

2. Method

2.1. Subjects

Eight experimentally naive male Wister rats were used. They were approximately 12 weeks of age at the start of experiment. The rats were housed in pairs for more than three weeks under reversed 12:12-h light conditions. Room temperature was maintained at 25 degrees centigrade. All the rats were maintained at 80% of their free feeding weights. Water was available continuously.

2.2. Apparatus

Two operant chambers $(29.5 \times 23.5 \times 23.5 \text{ cm}; \text{MED Associates, Georgia, VT; ENV-008})$ were used. One was used for the demonstrator, and the other for the observer. A retractable lever was positioned on the left side of the front panel (3.5 cm from the sidewall and 6.5cm above the floor). A food cup was centered on the front panel through which a pellet dispenser could deliver 45-mg food pellets. Experimental conditions were controlled by the MED-SKED system.

The restriction apparatus was an acrylic tube (diameter 55mm, length 200mm) with holes for respiration. Room temperature was likewise maintained at 25 degrees centigrade.

2.3. Procedure

Rats were trained to press a lever by successive approximation, and then trained on FR5 (Fixed Ratio 5) for a few days, finally on FR10. After the animals showed a steady state of responding, experimental sessions started. One of each pair of rats was randomly assigned as demonstrator and the other as observer. In baseline sessions, the demonstrator was moved to the experimental chamber and received FR10 until 50 reinforcements, and then it was returned to the cage. After 30 min together in the cage, the observer was moved to an experimental chamber different from that used for the demonstrator, and received FR10 until it obtained 50 pellets.

Every fourth session was an experimental session. The observer received 3 hr restrict stress, and then received FR10 training in the control condition. This condition is control condition of own stress. The subjects were inserted into a tube for restriction -- a tube located in a separate room, so that the other rats could not see the restrict process. In the observing condition, the demonstrator, instead of the observer, received the restrict stress and was returned to the cage (the demonstrator did not receive FR10 training in this condition). These four-session blocks were repeated five times in each condition. The procedure is illustrated in Figure 1.

2.4. Data analysis

Response rate in the baseline session just before the baseline session (third session of four) and that in the experimental session (fourth session) were used for analysis. First the rate of responding was used for analysis.



Figure 1. Design of the training. D and O indicate Demonstrator and Observer respectively. In baseline session, D receives FR training and then O receives the same training. In experimental session, D receives restrict stress for 3-hrs and the O receives FR training. In control condition (not presented in the figure), O receives the restrict stress and then receives FR training.

But due to individual differences in this rate, a ratio (obtained by dividing the rate of responding in the experimental session by that in the third baseline session) was used in a two-factor ANOVA (in which control vs. observing condition and number of repetitions were used as factors).

3. Results

Figure 2 presents mean response rates of each observer in the third baseline session just before the experimental session, and in the control and observing conditions. The condition in which the observers experienced stress did not affect the performance of the same observers under FR10; there was no change of response rate between the baseline and experimental sessions. On the other hand, every observer showed an increase in responding rate after observation of the demonstrator that received the restraint stress.

As shown in Figure 2, the animals exhibited considerable individual differences in response rate, and also exhibited day-to-day fluctuations. Figure 3 shows the mean of the ratio obtained by dividing response rate in each experimental session by that in the baseline session just before the experi-



Figure 2. Response rate of observers in baseline and experimental sessions. Left: response rates in the control condition. The stress of the observer dose not affect its response rate after the stress. Right: response rates in experimental condition. Exposure to demonstrator that experienced stress causes increment of response rate of the observer. Each dot indicates individual.

mental session. Clearly, the ratio increased after observation of the stress-receiving demonstrator (the observing condition), whereas the observers' own experience of the restraint stress did not affect the ratio (the control condition). A two-factor ANOVA revealed that the control vs. experimental condition factor was significant (F (1/29)=11.08 P<0.005), but neither the session factor nor the interaction had any significant effect (F (4/29)=1.07 and F (4/29)=0.41, respectively).



Figure 3. Relative change of response rate of observers. The vertical axis indicates mean ratio dividing response rate in experimental session by response rate in baseline session. Closed circle and open circle indicate the experimental and control conditions respectively. Clearly, observing stressed demonstrator increases observer's response rate.

Thus, we can conclude that when one rat observed a cage mate experiencing stress the observer's response rate increased, but the observer's own experience of the stress did not affect the response rate after the experience.

4. Discussion

The present results demonstrated two things. First, rats can discriminate events experienced by their cage mates. They showed different behavior depending on whether the cage mate received training with food reward or received restrict stress. Second, rats responded differently to their own experience and to the same experience of their cage mate. Interestingly, their own experience of restrict stress did not affect their operant behavior controlled by FR schedule, but experience of the same stress to the cage mate facilitated their behavior.

The facilitative effect of the restraint stress of the cage mate was unexpected. As we described in the Introduction, we predicted a suppressive effect. There are, however, several possible explanations. The first explanation is cognitive one. Information obtained from the cage mate should be limited. It is not realistic to assume that the demonstrator transmitted details of their experience to the observer. The demonstrator would presumably provide information about some aversive experience, but no more than that. Thus, the information should be about an experience that was uncertain or unpredictable. On the other hand, the observer's own experience was certain, and pertained to an event in the past. Uncertain aversiveness in the future may induce more fear than one's own aversive experience in the past (the past is, of course, known and thus no longer threatening). The facilitative effect thus perhaps reflects an increased response rate induced by the strong fear. A reversed explanation is also possible. Weak shock facilitates operant behavior of pigeons, whereas strong shock does not (Bloomfeld, 1971). Observing the stress-receiving cage mate perhaps facilitates responding because observing results in weak fear, whereas one's own experience of the stress does not facilitate the response, because one's own experience causes strong fear.

The second explanation is a behavioral one based on direct interaction between the demonstrator and the observer. The restrict stress often induced aggressive behavior after the release. Such aggressive behavior may induce hyper responding of the observer under FR schedule.

The last explanation is a physiological one: "Alarm pheromone" from the demonstrator may induce hyperactivity in the observer. In the control condition, the stress of the observer should also induce the production of alarm pheromone, but we would not expect one's pheromone production to have much effect on oneself. Kiyokawa et al. (2006) reported that rats placed in a box in which their cage mates received electric shock showed more freezing, rearing, and sniffing than control rats; such behavior should be mediated by the alarm pheromone.

The present experiment did not determine the mechanism of the facilitative effect, but these explanations can be tested in future research.

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