Chem. Senses 30: 513–519, 2005

doi:10.1093/chemse/bji044
Advance Access publication June 16, 2005

Alarm Pheromone that Aggravates Stress-induced Hyperthermia is Soluble in Water

Yasushi Kiyokawa[1,2], Takefumi Kikusui[1], Yukari Takeuchi[1] and Yuji Mori[1]

[1]Laboratory of Veterinary Ethology, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan and [2]Research Fellow of the Japan Society for the Promotion of Science, 1-8, Chiyoda-ku, Tokyo 102-8472, Japan

Correspondence to be sent to: Yasushi Kiyokawa, Laboratory of Veterinary Ethology, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. e-mail: kiyokawa-ns@umin.ac.jp

Abstract

We previously reported that stressed male Wistar rats released alarm pheromone from the perianal region, which aggravated stress-induced hyperthermia and increased Fos expression in the mitral/tufted cell layer of the accessory olfactory bulb in recipient rats. In this study, we attempted to obtain this pheromone in water using these responses as bioassay parameters. Water droplets were collected from the ceiling of a box in which no animal was placed, or from a box in which an anesthetized donor rat was given electrical stimulation to either the neck or perianal regions in order to induce neck odor or alarm pheromone release, respectively. Then we placed one of the three kinds of water-containing filter papers on the wall of a recipient's home cage and observed heart rate, body temperature and behavioral responses, as well as Fos expression in the main and accessory olfactory bulbs of the recipient. The water collected from the box containing the alarm pheromone was found to generate a reproduction of all of the responses seen in the animal that had been directly exposed to alarm pheromone in our previous studies. These results suggest that the alarm pheromone is soluble in water.

Key words: accessory olfactory bulb, alarm pheromone, appeasing pheromone, solvable substance, stress-induced hyperthermia, vomeronasal system

Introduction

Chemical communication plays an important role in various social interactions among mammals, including sexual (Vandenbergh, 1973), territorial (Eichmann and Holst, 1999), and maternal behaviors (Leon and Moltz, 1971). When produced by a member of a species, alarm pheromone communicates the presence of danger to others of that same species (Pfeiffer, 1963), thus alarm pheromone is thought to be important for increasing the overall fitness of a species. Following an initial report published in 1968 demonstrating that rats could distinguish between the odors released from stressed and non-stressed conspecifics (Valenta and Rigby, 1968), little progress has been made in understanding alarm pheromone communication among rodents in detail, in spite of reports of the effects of alarm pheromone in several experimental models (Courtney *et al.*, 1968; Stevens and Koster, 1972; Mackay-Sim and Laing, 1980; Abel and Bilitzke, 1990).

We previously reported that the alarm pheromone released from male rats led to an enhancement of behavioral responses, rises in body temperature, and increases in Fos expression in the mitral/tufted cell layer (M/T) of the accessory olfactory bulb (AOB) (Kikusui *et al.*, 2001). We then found that this alarm pheromone could be divided into two categories, namely, those that modify recipient behavior and are released in a testosterone-dependent manner and those that aggravate an acute rise in body temperature, i.e. stress-induced hyperthermia (SIH), and are released in a testosterone-independent manner (Kiyokawa *et al.*, 2004b). In addition, it was revealed that the former type of alarm pheromone was released from the whisker pad, whereas the latter was released from the perianal region of the donor (Kiyokawa *et al.*, 2004a). Considering that the importance of propagating notification of a dangerous situation to a family or to group members is not limited to males, and that the intensity of SIH reflects the animal's anxiety status (Lecci *et al.*, 1990; Olivier *et al.*, 2003), it appears reasonable to postulate that the testosterone-independent pheromone that aggravates SIH is biologically more important than the other testosterone-dependent pheromone. We have therefore decided to focus more attention on this category of alarm pheromone, i.e. the testosterone-independent type, in our subsequent investigations, and we

found that this type of pheromone increased Fos expression in the vomeronasal pathway, as well as in several stress-related nuclei in the brain of recipient animals (Kiyokawa *et al.*, 2005). However, in order to conduct further studies of alarm pheromone communication in rats, it became necessary to obtain the pheromone in carriers. Based on a previous report demonstrating that alarm pheromone effects could be observed in a forced swimming test paradigm (Abel and Bilitzke, 1990), it appeared reasonable to hypothesize that the alarm pheromone is soluble in water. If this were indeed the case, then it follows that water could be used as a pheromone carrier.

To test this hypothesis, we prepared three types of water samples collected from the ceiling of a small box containing one of the following: alarm pheromone, neck odor or no odor. Then we exposed male rats carrying a telemetry transmitter to one of these three types of water using filter paper, and we measured the behavioral and autonomic responses of the animals, as well as Fos expression in both the main olfactory bulb (MOB) and the AOB; these parameters have previously been used as indices of alarm pheromone effects in our previous studies (Kikusui *et al.*, 2001; Kiyokawa *et al.*, 2004a,b, 2005).

Materials and methods

Recipient animals

Experimentally naive male Wistar rats were purchased from Clea Japan (Tokyo, Japan) at 8 weeks old. The rats were housed 2–3 animals per cage under constant temperature (24 ± 1°C) and humidity (45 ± 5%) for 6 days until they were implanted with a telemetry transmitter. Food and water were available *ad libitum*, and the animals were kept under a 12 h light/12 h dark cycle (lights on at 08.00) throughout the experiment. The animals were cared for in accordance with 'Policies Governing the Use of Live Vertebrate Animals', set by the University of Tokyo, and based on *The Public Health Service Policy on Humane Care and Use of Laboratory Animals* (revised in 1985) and the *National Institutes of Health's Guide for the Care and Use of Laboratory Animals*. Recipient rats were implanted with a telemetry transmitter (TA11CTA-F40, Data Sciences International, St Paul, MN) intraperitoneally under anesthesia with sodium pentobarbital (50 mg/kg i.p. Nembutal, Abbott Laboratories, North Chicago, IL) 10–11 days before the experiment. After surgery, the animals were housed individually in a polycarbonate standard rat cage (28 × 44 × 18 cm) on an antenna board (RLA1020 RPC-1; Data Sciences International) in a soundproof chamber (36 × 54 × 35 cm; Muromachi Kikai, Tokyo, Japan) located in a room maintained at a constant temperature (22 ± 1°C) under a 12 h light/12h dark cycle (lights on at 08.00). The cage bedding was not changed after surgery in order to render it the home cage, and all recipient rats were handled for 5 min per day, beginning 6–7 days before the experiment.

Preparation of water samples

We prepared adult male Wistar rats as pheromone donors and an acrylic box (20 × 20 × 10 cm) as the pheromone box. Approximately 5 ml of purified water, prepared on the morning of the experimental day, was sprayed on the ceiling of the pheromone box. An anesthetized donor rat (50 mg/kg i.p. Nembutal, Abbott Laboratories) bearing two intradermal needles (27 G) for electrical stimulation of the neck or perianal region was placed into the box for 15 min. During this period, the donor rats received 15 times of electrical stimulation (10 V for 1 s) generated by an electronic stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan) and an isolator (SS-104J, Nihon Kohden) at 1 min intervals to either the neck or perianal region. The electrical stimulation to the perianal region induced the alarm pheromone release that aggravated the SIH response in the other rats, whereas stimulation to the neck region was conducted in an attempt to provide a similar amount of olfactory stimuli which affected neither SIH nor behavioral responses (Kiyokawa *et al.*, 2004a). After being stimulated in this manner, the donor rat was removed and the water droplets on the ceiling were collected using forceps and two sheets of filter paper (5 × 5 cm). Care was taken to ensure that nothing, e.g. the donor's body, the electrical cord or the experimenter's hands, came into direct contact with the water droplets on the ceiling, until after the samples had been collected in the filter paper. Water droplets collected from the box in which no animal had been placed were used as a vehicle control. The pheromone donors were used 2–3 times, with at least a 1 week interval between uses, and the pheromone box was washed in hot water with a cleanser and wiped with a paper towel before each use.

Sample exposure

After the preparation of water samples, one of the three types of filter paper was brought into the room in which the recipients were maintained after the surgery. The recipients showing a stable baseline, i.e. a body temperature of less than 37.5°C and a heart rate of <350 beats/min, were used for the experiment. The wire mesh ceiling of the recipient's home cage was replaced with punctured acrylic board and two sheets of filter paper were placed on both sides of the walls simultaneously (Figure 1). Then, the home cage was replaced onto an antenna board in the soundproof chamber and was kept there for 30 min. The recipient rats were randomly assigned to one of three groups according to the type of water sample they had been exposed to, i.e. alarm pheromone (n = 8), neck odor (n = 9) and control (n = 8) groups. At this stage of the experiment, the behavior of the recipient rat was video-recorded (DCR-TRV18; SONY, Tokyo, Japan) through a window in the wall of the chamber, and body temperature and heart rate were transmitted via the antenna board placed under the home cage (mentioned above); the values obtained were recorded by a data acquisition system

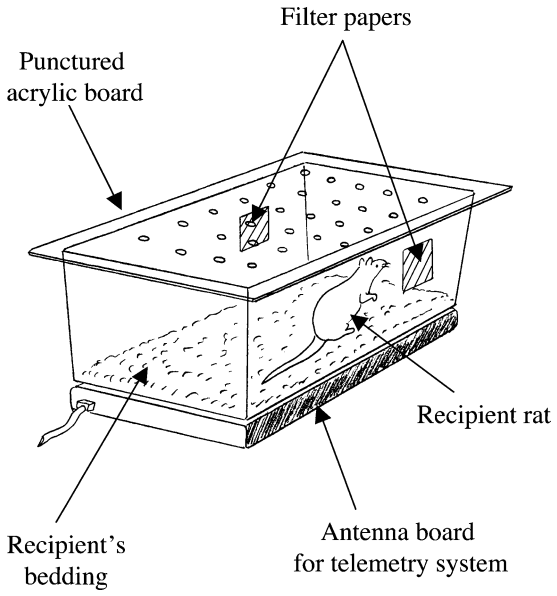

Figure 1 Schematic diagram of the test apparatus used in this study. The recipient rat was exposed to water sample in its home cage.

(Dataquest LabPRO 3.10; Data Sciences International). All of the sample exposure trials were conducted between the hours of 09.00 and 18.00.

Fos immunohistochemistry

After a 30 min exposure period, each recipient rat was anesthetized deeply with sodium pentobarbital (Nembutal; Abbott Laboratories) and perfused intracardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The brain was removed and immersed overnight in the same fixative and then was placed in 30% sucrose/PB for cryoprotection. The avidin–biotin–peroxidase method was used for the immunohistochemical analyses, which were performed in a manner described in our previous study (Kiyokawa *et al.*, 2005). Briefly, six successive sagittal sections of the AOB were cut at 30 μm using a freezing microtome. The second and fifth of these sections were stained with Cresyl Violet in order to confirm the location of the nucleus, and the remaining sections were used for free-floating immunohistochemistry. The sections were incubated with primary antibody to Fos protein (Ab-5, Calbiochem, La Jolla, CA; diluted 1:7500) for 65 h, with anti-rabbit second antibody (VECTASTAIN elite ABC kit, Vector Laboratories, Burlingame, CA) for 2 h. Then, the sections were processed with the elite ABC kit and developed using diaminobenzidine solution with nickel intensification.

Data analysis and statistical procedures

Data analysis was performed with Stat View J 5.0 software (SAS Institute, Cary, NC; no longer available). The data were expressed as means ± SEM, and the significance level was set at $P = 0.05$ for all statistical tests.

The behavior of the recipients was analyzed by a researcher who was blind to the experimental conditions using Microsoft Excel-based Visual Basic software for the data collection. The number of steps taken with the hind paws (walking), and the duration of digging, grooming, rearing, sniffing, freezing, resting and contact were recorded during the 30 min experimental period. 'Digging' was defined as follows: when the rat would dig at or into the bedding with the forelimbs or nose; similarly, 'contact' was defined as the rat making direct contact with the filter paper, regardless of its mode of attachment on the wall, including chewing or the use of vibrissae. For the definitions of other behaviors, see our previous studies (Kikusui *et al.*, 2001; Kiyokawa *et al.*, 2004c). All behavioral data were statistically analyzed by multiple analysis of variance (Hotelling's trace).

Body temperature and heart rate were recorded continuously, and the values were stored as the average obtained for a 5 s period in each minute. The individual baseline values were defined as the averaged body temperature and heart rate values recorded in the home cage during the 5 min period just prior to the measurement. The SIH and heart rate response results were expressed as the change from baseline for the group comparison, and these data were analyzed by two-way repeated measures analysis of variance (ANOVA) followed by Fisher's PLSD *post hoc* test. To achieve a more precise comparison, we also analyzed heart rate response each minute by one-way ANOVA followed by Dunnett's *post hoc* test.

For the immunohistochemical analysis, four sections in each recipient were captured using a microscope equipped with a digital camera (DP-12, Olympus, Tokyo, Japan) and the number of Fos-immunoreactive cells was counted unilaterally by a researcher who was blind to the experimental groups using NIH image 1.63 software. The AOB was divided into the M/T and the granule cell layer (GR), and the immunoreactive cells in each layer were analyzed. Because the boundary between the mitral cell layer and GR was ambiguous in the MOB, the immunoreactive cells were counted inclusively in its dorsal anterior region, as was also the case in our previous study (Kikusui *et al.*, 2001; Kiyokawa *et al.*, 2005). The mean values of the density (number of cells per square millimeter) were calculated for each rat and were analyzed by one-way ANOVA followed by Dunnett's *post hoc* test.

Results

All recipient rats showed autonomic as well as behavioral responses, as moving their home cage and replacing the ceiling served as stressors. However, the behavioral responses were not found to differ among the three groups [$F(16,28) = 0.692$, $P = 0.778$]. All behavioral data are shown in Table 1.

Table 1 Behavioral responses of recipient rats

Behavior	Control (n = 8)	Neck odor (n = 9)	Alarm pheromone (n = 8)
Grooming	460 ± 54	436 ± 35	467 ± 63
Digging	73.6 ± 38.9	66.5 ± 27.3	115 ± 63
Rearing	323 ± 21	321 ± 32	330 ± 30
Walking	169 ± 13	188 ± 19	179 ± 12
Sniffing	437 ± 25	441 ± 37	462 ± 38
Freezing	1.64 ± 1.06	0.43 ± 0.33	0.85 ± 1
Resting	368 ± 108	314 ± 125	317 ± 98
Contact	196 ± 35	211 ± 41	228 ± 52

Walking is shown as frequency (number of steps); all other behaviors are shown as duration (s). Data are expressed as means ± SEM. The number of subjects is given in parentheses.

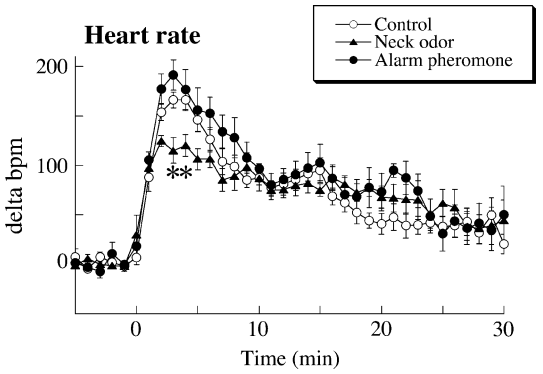

Figure 2 Time-dependent changes in the heart rate of recipient rats that had been exposed to water collected from a box in which alarm pheromone (alarm pheromone: n = 6) or neck odor (neck odor: n = 8) was released from an anesthetized donor rat. The water collected from a box in which no animal had been placed was used as a control stimulus (control: n = 8). *P < 0.05 as compared with the control group by one-way ANOVA followed by Dunnett's *post hoc* test (mean ± SEM).

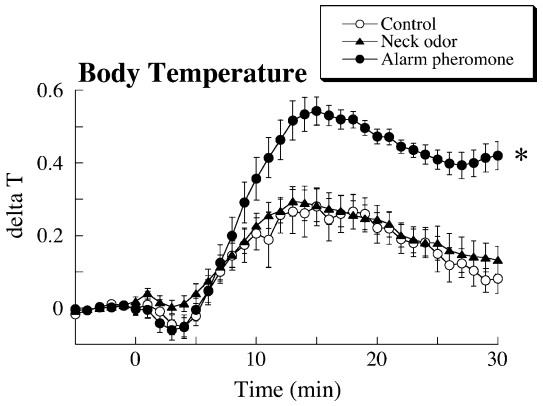

Figure 3 Time-dependent changes in the body temperature of recipient rats that were either exposed to the water collected from a box in which alarm pheromone (alarm pheromone: n = 8) or neck odor (neck odor: n = 9) was released from an anesthetized donor rat. The water collected from a box in which no animal had been placed was used as a control stimulus (control: n = 8). *P < 0.05 as compared with the control group by two-way repeated measures ANOVA followed by Fisher's PLSD *post hoc* test (mean ± SEM).

The animals lacking heart rate data due to technical problems were excluded from the analysis (neck odor, n = 1; alarm pheromone, n = 2). The baseline heart rate values were virtually identical among the three groups (alarm pheromone: 320 ± 8; neck odor: 317 ± 6; control: 319 ± 6); this response changed significantly over time [F(35,665) = 48.1, P < 0.01]. The heart rate response did not differ among the three groups [F(2,665) = 1.31, P = 0.293]; however, the interaction between these two factors was found to be significant [F(70,665) = 6.91, P < 0.01]. The statistical analyses for each time point revealed that the heart rate response differed among the three groups from 2 to 4 min after the exposure [2 min: F(2,19) = 7.51, P < 0.01; 3 min: F(2,19) = 10.4, P < 0.01; 4 min: F(2,19) = 4.93, P < 0.05], and the *post hoc* test revealed that the neck odor group showed significantly attenuated heart rate response 3 and 4 min after exposure, as compared with the values seen in the control group (neck odor versus control: P < 0.05) (Figure 2).

As regards the baseline body temperature, no differences were seen among the three groups (alarm pheromone: 37.253 ± 0.041; neck odor: 37.192 ± 0.037; control: 37.206 ± 0.058); however, the SIH value was significantly different among the three groups [F(2,770) = 12.8, P < 0.01]. Body temperature also changed significantly over time [F(35,770) = 75.7, P < 0.01] and the interaction between these two factors was found to be significant [F(70,770) = 6.91, P < 0.01]. The *post hoc* test revealed that the alarm pheromone group showed an aggravation of the SIH, as compared with the results obtained in the control group (alarm pheromone versus control: P < 0.01; neck odor versus control: P = 0.516) (Figure 3).

Fos protein-immunoreactive cells were observed in the AOB in all three groups (Figure 4). The numbers of immunoreactive cells in the M/T and GR were significantly different among the three groups [M/T: F(2,22) = 6.28, P < 0.01; GR: F(2,22) = 4.13, P < 0.05]. The *post hoc* test revealed that both the alarm pheromone and neck odor groups had a significantly increased number of Fos protein-immunoreactive cells in the M/T, as compared with the number observed in the control group (alarm pheromone versus control: P < 0.05; neck odor versus control: P < 0.05) (Figure 5, top), whereas only alarm pheromone groups showed increased number of Fos protein-immunoreactive cells in the GR of the AOB (alarm pheromone versus control: P < 0.05) (Figure 5, bottom). In contrast to the AOB, no group difference was seen in the number of Fos protein-immunoreactive cells in the MOB [alarm pheromone: 282 ± 25; neck odor: 272 ± 15; control: 246 ± 11, F(2,22) = 1.05, P = 0.366].

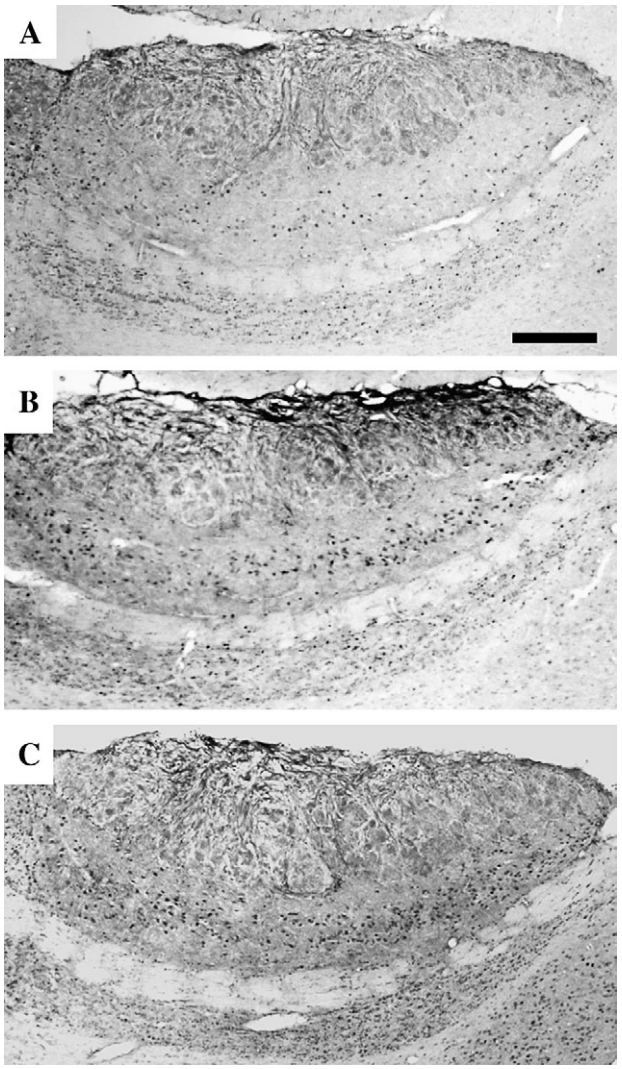

Figure 4 Photomicrographs of Fos-immunoreactive cells in the accessory olfactory bulb. The expression of Fos protein was observed in rats that were either exposed to the water collected from a box in which no animal had been placed **(A)**, or in which neck odor **(B)** or alarm pheromone **(C)** had been released from an anesthetized donor rat. The rostral portion is shown on the left, and the horizontal bar indicates 200 μm.

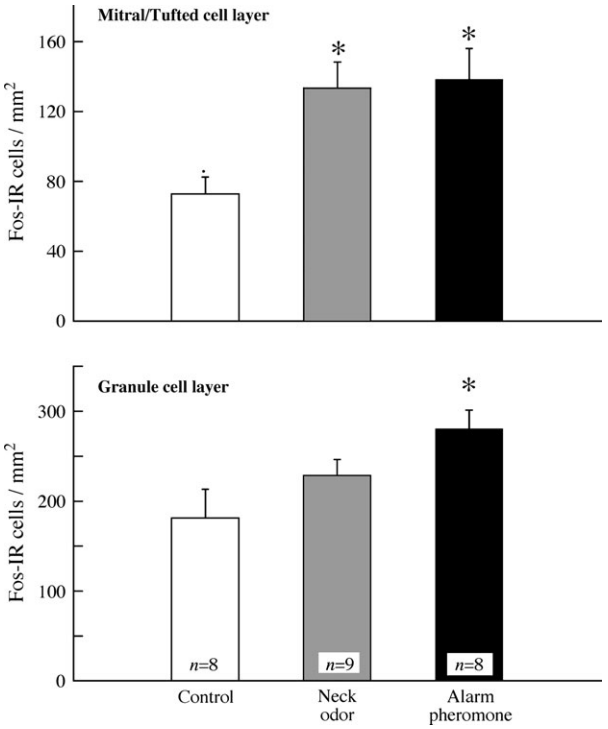

Figure 5 The mean (+ SEM) density of immunoreactive (IR) cells against Fos protein in the mitral/tufted cell layer (top) and granule cell layer (bottom) of the accessory olfactory bulb of rats that were either exposed to water collected from a box in which no animal had been placed (control), or in which alarm pheromone (alarm pheromone) or neck odor (neck odor) had been released from an anesthetized donor rat. $*P < 0.05$ as compared with the control group by one-way ANOVA followed by Dunnett's *post hoc* test.

Discussion

In the present study, the water collected from a box containing alarm pheromone was found to exert an influence on the autonomic stress response (as shown by aggravated SIH) and increased Fos expression in the M/T and GR of the AOB in recipient rats, whereas it did not affect heart rate, behavioral responses or Fos expression in the MOB, in comparison to the values of the responses evoked by water only. All of these responses were identical to those seen in recipients that had been directly exposed to alarm pheromone in our previous studies (Kikusui *et al.*, 2001; Kiyokawa *et al.*, 2004a, 2005). These results suggest that a sufficient amount of alarm pheromone for evoking an autonomic response was obtained in the water droplets, and that recipient rats can perceive the water-soluble alarm pheromone via the vomeronasal system.

Another important, but unexpected, finding was that the water collected from the box containing neck odor attenuated heart rate responses via the vomeronasal system, as assessed by Fos expression in the M/T of the AOB. To the best of our knowledge, this is the first finding demonstrative of the notion that the same-sex adult conspecific's odor can attenuate the stress response in another rat via a process that appears to be mediated by the vomeronasal system. The finding that the odor released from the body surface of a conspecific was perceived by the vomeronasal system of a recipient was consistent with a previous finding showing that the AOB neurons were activated when mice investigated the face and head of an anesthetized conspecific animal (Luo *et al.*, 2003). This 'appeasing pheromone' might play some role in the gregariousness seen in stressed rats or in the stress-attenuating effects of accompanying conspecifics (known as social buffering). It has been reported that when rats were stressed by an open-field test or noise, they showed high levels of gregariousness or chose to interact with a conspecific in a T-maze, respectively (Latane, 1969; Taylor,

1981). In addition, it is known that the presence of conspecific animals can attenuate stress responses including behavioral, autonomic, neurochemical and hormonal responses (Stanton *et al.*, 1985; Thorsteinsson *et al.*, 1998; Hennessy *et al.*, 2002; Kiyokawa *et al.*, 2004c).

Although these two pheromones appeared to be perceived by the vomeronasal system, only alarm pheromone increased Fos expression in the GR of the AOB. One possible explanation for this finding is that alarm pheromone activates noradrenergic (NA) system in the brain. The nucleus locus coeruleus (LC) is known as the major source of NA innervation of the brain and ~40% of these neurons project to the olfactory bulb including the GR of the AOB (Shipley *et al.*, 1985; McLean *et al.,* 1989). Therefore, if the NA system was activated by the exposure to alarm pheromone, this might have subsequently induced neural activation in the GR of the AOB. The previous findings that the exposure to alarm pheromone increased Fos expression in the LC of the recipient rats (Kiyokawa *et al.,* 2005) and that the activation of NA system raised body temperature (Beckman, 1970) support this view. In this connection, if the exposure to appeasing pheromone were to have a property to suppress the NA system in recipient rats, then the different pattern of the neural activity observed in the GR of the AOB following the exposure to the neck odor might be attributed to it. Further study will be necessary to test this hypothesis.

The hypothesis that the alarm pheromone is soluble in water is also supported by reports demonstrative of alarm pheromone effects in forced swimming test paradigms (Abel and Bilitzke, 1990). When rats are placed in a cylinder containing water, they initially swim energetically, but eventually become immobile, making a minimal number of movements to keep their heads above water level (Borsini and Meli, 1988). Forced swimming tests use the immobility time as a parameter, and decreases in this immobility time in rats is thought to reflect a state of increased fear or anxiety (Hawkins *et al.*, 1978; O'Neill and Valentino, 1982; Abel and Bilitzke, 1990). Rats have been shown to release alarm pheromone in water while swimming, which shortened the immobility time among subsequent rats, even if the pheromone-containing water was diluted by 25% (Abel, 1991). Considering that alarm pheromone was found to evoke a variety of anxiety responses in each of these paradigms (e.g. SIH in this study, decreased immobility time in the forced swimming paradigm), it is conceivable that the alarm pheromone could be classified as a modulator pheromone (McClintock, 2002), i.e. the alarm pheromone increases anxiety in recipients, rather than evoking a stereotyped response.

In summary, the water collected from a box containing alarm pheromone led to a reproduction in rats of all of the same responses as those seen in recipients exposed directly to alarm pheromone, thus suggesting that alarm pheromone is soluble in water. Although at this moment no information is available regarding how alarm pheromone is dissolved in water, e.g. here, small volatile molecules having high affinity to water were dissolved after being released into the ambient air, or less volatile but water-soluble molecules were released as invisible mist and reached the interior of water droplets on the ceiling, we believe that the present results will facilitate further investigation into communication by alarm pheromone. In particular, these findings suggest that pheromone effects can be investigated with minimal confounding effects due to novelty stress, as recipient rats need not share the same location with donors. Furthermore, it should become possible to identify responsible molecule(s) by analyzing water samples using the appropriate technology and/or *in vitro* experimental models.

Acknowledgements

This study was supported by Grants-in-aid for Scientific Research (14760187 and 15GS0306) from the Japan Society for the Promotion of Science (JSPS) and by Research Fellowships of the JSPS for the Promotion of Science for Young Scientists (11563).

References

Abel, E.L. (1991) *Gradient of alarm substance in the forced swimming test*. Physiol. Behav., 49, 321–323.

Abel, E.L. and **Bilitzke, P.J.** (1990) *A possible alarm substance in the forced swimming test*. Physiol. Behav., 48, 233–239.

Beckman, A.L. (1970) *Effect of intrahypothalamic norepinephrine on thermoregulatory responses in the rat*. Am. J. Physiol., 218, 1596–1604.

Borsini, F. and **Meli, A.** (1988) *Is the forced swimming test a suitable model for revealing antidepressant activity?* Psychopharmacology (Berl.), 94, 147–160.

Courtney, R.J., Reid, L.D. and **Wasden, R.** (1968) *Suppression of running times by olfactory stimuli*. Psychon. Sci., 12, 315–316.

Eichmann, F. and **Holst, D.V.** (1999) *Organization of territorial marking behavior by testosterone during puberty in male tree shrews*. Physiol. Behav., 65, 785–791.

Hawkins, J., Hicks, R.A., Phillips, N. and **Moore, J.D.** (1978) *Swimming rats and human depression*. Nature, 274, 512–513.

Hennessy, M.B., O'Leary, S.K., Hawke, J.L. and **Wilson, S.E.** (2002) *Social influences on cortisol and behavioral responses of preweaning, periadolescent, and adult guinea pigs*. Physiol. Behav., 76, 305–314.

Kikusui, T., Takigami, S., Takeuchi, Y. and **Mori, Y.** (2001) *Alarm pheromone enhances stress-induced hyperthermia in rats*. Physiol. Behav., 72, 45–50.

Kiyokawa, Y., Kikusui, T., Takeuchi, Y. and **Mori, Y. (** 2004a) *Alarm pheromones with different functions are released from different regions of the body surface of male rats*. Chem. Senses, 29, 35–40.

Kiyokawa, Y., Kikusui, T., Takeuchi, Y. and **Mori, Y.** (2004b) *Modulatory role of testosterone in alarm pheromone release by male rats*. Horm. Behav., 45, 122–127.

Kiyokawa, Y., Kikusui, T., Takeuchi, Y. and **Mori, Y.** (2004c) *Partner's stress status influences social buffering effects in rats*. Behav. Neurosci., 118, 798–804.

Kiyokawa, Y., Kikusui, T., Takeuchi, Y. and **Mori, Y.** (2005) *Mapping the neural circuit activated by alarm pheromone perception by c-Fos immunohistochemistry*. Brain Res., 1043, 145–154.

Latane, B. (1969) *Gregariousness and fear in laboratory rats*. J. Exp. Soc. Psychol., 5, 61–69.

Lecci, A., Borsini, F., Volterra, G. and **Meli, A.** (1990) *Pharmacological validation of a novel animal model of anticipatory anxiety in mice*. Psychopharmacology (Berl.), 101, 255–261.

Leon, M. and **Moltz, H.** (1971) *Maternal pheromone: discrimination by preweanling albino rats*. Physiol. Behav., 7, 265–267.

Luo, M., Fee, M.S. and **Katz, L.C.** (2003) *Encoding pheromonal signals in the accessory olfactory bulb of behaving mice*. Science, 299, 1196–1201.

Mackay-Sim, A. and **Laing, D.G.** (1980) *Discrimination of odors from stressed rats by non-stressed rats*. Physiol. Behav., 24, 699–704.

McClintock, M.K. (2002) *Pheromones, odors, and vasanas: the neuroendocrinology of social chemosignals in humans and animals*. In Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E. and Rubin, R.T. (eds), Hormones, Brain and Behavior. Academic Press, San Diego, CA, pp. 797–870.

McLean, J.H., Shipley, M.T., Nickell, W.T., Aston-Jones, G. and **Reyher, C.K.** (1989) *Chemoanatomical organization of the noradrenergic input from locus coeruleus to the olfactory bulb of the adult rat*. J. Comp. Neurol., 285, 339–349.

O'Neill, K.A. and **Valentino, D.** (1982) *Escapability and generalization: effect on 'behavioral despair'*. Eur. J. Pharmacol., 78, 379–380.

Olivier, B., Zethof, T., Pattij, T., van Boogaert, M., van Oorschot, R., Leahy, C., Oosting, R., Bouwknecht, A., Veening, J., van der Gugten, J. and **Groenink, L.** (2003) *Stress-induced hyperthermia and anxiety: pharmacological validation*. Eur. J. Pharmacol., 463, 117–132.

Pfeiffer, W. (1963) *Alarm substances*. Experientia, 19, 113–123.

Shipley, M.T., Halloran, F.J. and **de la Torre, J.** (1985) *Surprisingly rich projection from locus coeruleus to the olfactory bulb in the rat*. Brain Res., 329, 294–299.

Stanton, M.E., Patterson, J.M. and **Levine, S.** (1985) *Social influences on conditioned cortisol secretion in the squirrel monkey*. Psychoneuroendocrinology, 10, 125–134.

Stevens, D.A. and **Koster, E.P.** (1972) *Open-field responses of rats to odors from stressed and nonstressed predecessors*. Behav. Biol., 7, 519–525.

Taylor, G.T. (1981) *Fear and affiliation in domesticated male rats*. J. Comp. Physiol. Psychol., 95, 685–693.

Thorsteinsson, E.B., James, J.E. and **Gregg, M.E.** (1998) *Effects of video-relayed social support on hemodynamic reactivity and salivary cortisol during laboratory-based behavioral challenge*. Health Psychol., 17, 436–444.

Valenta, J.G. and **Rigby, M.K.** (1968) *Discrimination of the odor of stressed rats*. Science, 161, 599–601.

Vandenbergh, J.G. (1973) *Acceleration and inhibition of puberty in female mice by pheromones*. J. Reprod. Fertil. Suppl., 19, 411–419.

Accepted May 23, 2005