

Pretreatment with CP-154526 Blocks the Modifying Effects of Alarm Pheromone on Components of Sexual Behavior in Male, but not in Female, Rats

Tatsuya Kobayashi, Yasushi Kiyokawa, Yukari Takeuchi and Yuji Mori

Laboratory of Veterinary Ethology, University of Tokyo, 1-1-1 Yayoi Bunkyo-ku, Tokyo 113-8657, Japan

Correspondence to be sent to: Yasushi Kiyokawa, Laboratory of Veterinary Ethology, University of Tokyo, 1-1-1 Yayoi Bunkyo-ku, Tokyo 113-8657, Japan. e-mail: akiyo@mail.ecc.u-tokyo.ac.jp

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Abstract

We previously demonstrated that an alarm pheromone released from male donor Wistar rats evoked several physiological and behavioral responses in recipient rats. However, the pheromone effects on social behavior were not analyzed. In the present study, we examined whether the alarm pheromone affects sexual behavior in male or female rats. When a pair of male and female subjects was exposed to the alarm pheromone during sexual behavior, the ejaculation latency was elongated, the number of mounts was increased, and the hit rate (number of intromissions/number of mounts and intromissions) was decreased in the male subject. In contrast, female sexual behavior was not affected by the alarm pheromone. When we exposed only the male or female subject of the pair to the pheromone just before sexual behavior, the results were similar: the pheromone effects were evident in male, but not in female, subjects. In addition, when we pretreated with corticotropin-releasing factor (CRF) antagonist (CP-154526) before exposing the male subject to the alarm pheromone, the pheromone effects were attenuated in a dose-dependent manner. These results indicate that the alarm pheromone modifies male, but not female, components of sexual behavior and that CRF participates in the effects.

Key words: copulation, corticotropin-releasing factor, sex differences

Introduction

Chemical communication plays an important role in various types of social interactions among mammals, including sexual (Vandenbergh 1976), territorial (Nakamura et al. 2007), and maternal behavior (Leon and Moltz 1971). When produced by a member of a species, the alarm pheromone communicates the presence of danger to others of that same species. Thus, the alarm pheromone is thought to be important for increasing the overall fitness of a species.

We previously showed that foot-shocked male Wistar rats release an alarm pheromone, which aggravates stress-induced hyperthermia (SIH) in pheromone-exposed rats (Kikusui et al. 2001) via the vomeronasal system (Kiyokawa et al. 2007). This pheromone is released from the perianal region of the donor rat (Kiyokawa et al., 2004a) in a testosterone-independent manner (Kiyokawa et al. 2004b). In addition to its volatility (Inagaki et al. 2009), the alarm pheromone has been shown to be water soluble because water droplets collected from the ceiling of a box in which the alarm pheromone was released reproduced all the responses observed in recipients directly exposed to the

pheromone (Kiyokawa et al. 2005a). This pheromone solution allows us to observe pheromone effects in different experimental paradigms and evokes several responses, such as aggravated SIH in the home cage (Kiyokawa et al. 2005a, 2007), increased defensive and risk assessment behaviors in a modified open-field test (Kiyokawa et al. 2006), and enhanced acoustic startle reflex (ASR) (Inagaki et al. 2008, 2010). However, although the effects of this pheromone had been examined in individual animals, its effects on social behavior had not been analyzed.

Among the wide variety of social behaviors, reproduction is one of the most important activities in organisms. Sexual behavior is known to be suppressed by danger signals. For example, exposure to predator odor decreased the proportion of sexual behavior occurrences (Bian et al. 2005), reduced sex organ weights and testosterone levels in male rodents (Ronkainen and Ylonen 1994; Vasilieva et al. 2000), and disturbed the estrous cycle in female rodents (Koskela et al. 1996; Apfelbach et al. 2001). In addition, exposure to aversively conditioned odors decreased the

proportion of sexual behavior occurrences in male rats (Lawrence and Kiefer 1987). Our previous findings had suggested that the alarm pheromone informs other rats of danger (Kiyokawa et al. 2006). Therefore, we hypothesized that the alarm pheromone should suppress sexual behavior.

Corticotropin-releasing factor (CRF) is a 41-amino acid polypeptide released from cells in the paraventricular nucleus (PVN) of the hypothalamus. The PVN has an important role in the stress response. For example, increased CRF messenger RNA (mRNA) expression in the PVN has been found with a wide variety of stressors, including foot shock (Imaki and Vale 1993), restraint (Imaki et al. 1995), swimming (Harbuz and Lightman 1989), and immobilization (Imaki et al. 1992). CRF is also known to affect several behavioral responses. For example, intracerebroventricular (ICV) administration of CRF decreased time spent in the open arms of an elevated plus maze test (Jones et al. 1998), enhanced ASR (Swerdlow et al. 1986), reduced exploration in an open field, and increased defensive withdrawal into a small chamber (Takahashi et al. 1989). In addition to its effects on individual animals, CRF also has an important role in social behavior, including sexual behavior. For example, ICV administration of CRF to male rats produced a dose-dependent suppression of male sexual behavior (Sirinathsinghji 1987). Considering that the presentation of alarm pheromone increased Fos expression in the PVN (Kiyokawa et al. 2005b) and that the pheromone effects on ASR were blocked by pretreatment with the CRF antagonist (Inagaki et al. 2010), we also hypothesized that CRF is involved in the alarm pheromone effect on sexual behavior if the pheromone suppresses sexual behavior in rats.

To test these hypotheses, we assessed the effects of the alarm pheromone on sexual behavior in male and female rats. In Experiment 1, we observed the effects of the alarm pheromone on both male and female subjects during sexual behavior. The male subject was exposed to the alarm pheromone for 5 min in its home cage and then the female subject was placed in the male subject's home cage. Subsequent sexual behavior was observed during the first 2 copulation periods. In Experiment 2, we tried to specify whether the pheromone affected the male or female subject because the pheromone had remained in the environment during the entire experimental period in Experiment 1. Therefore, in Experiment 2, we presented the pheromone only to the male or only to the female subject in its home cage and recovered the pheromone before the beginning of sexual behavior. In Experiment 3, we observed the effects of CP-154526 on the pheromone effects in the male subject.

Materials and methods

Animals

Ninety-four sexually naive male Wistar Imamichi rats 7.5 weeks of age and 94 sexually naive female Wistar Imamichi

rats 8.5 weeks of age were purchased (Institute for Animal Reproduction). Animals were provided with water and food *ad libitum* and kept on a 12 h light:dark cycle (lights turned off at 20:00). The colony room was maintained at a constant temperature (24 ± 1 °C) and humidity (40–45%). Animals were housed in pairs in wire-topped transparent cages (410 × 250 × 180 mm) with wood shavings for bedding. This study was approved by the Animal Care and Use Committee of the Faculty of Agriculture, The University of Tokyo.

Preparation of water samples

Before the experiment, we prepared water samples according to an established method that has been previously described in detail (Kiyokawa et al. 2005a). We prepared adult male Wistar Imamichi rats (12–16 weeks old) as pheromone donors and sprayed purified water (5 mL) on the ceiling of an acrylic box (200 × 200 × 100 mm). Each donor rat was anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally [i.p.]) (Somnopenyl; Schering-Plough Animal Health), and intradermal needles (27G) for electrical stimulation were placed in the neck or perianal region. Each rat was placed in the box for 5 min and was given 15 electrical stimulations (10 V for 1 s) at 20-s intervals to either the neck or the perianal region. The electrical stimulation to the perianal region induced alarm pheromone release, whereas stimulation to the neck region was conducted in an attempt to provide a similar amount of olfactory stimuli that affected neither autonomic nor behavioral responses (Kiyokawa et al. 2004a). After the rats were stimulated in this manner, the water droplets on the ceiling were collected in a conical polypropylene tube using a glass bar and Pasteur pipette. Each sample of water was stored at 4 °C and used within the same day. The box was washed with a cleaner in hot water and wiped with a towel prior to each use. The donor rats were used twice with at least 2 weeks between uses.

Procedure for Experiment 1

All subjects were handled for 5 min per day beginning 2 days before the experiment. One day before the experimental day, male subjects were housed individually and were acclimatized to the experimental apparatus and room for 30 min. Female subjects were also acclimatized to the experimental room for 30 min.

The experiment was conducted at 20:30 in the male subject's home cage under dim red light. On the experimental day, the cages containing subjects were moved to the experimental room 150 min before the experiment. The stainless steel cage top of the male subject's home cage was then replaced with punctured acrylic board, and light in the experimental room was turned off 30 min before the experiment. At the beginning of the experiment, 2 sheets of filter paper (5 × 5 cm) containing water samples (750 µL each) were placed on the wall of the male subject's home cage for 5 min, and the female subject was placed in the cage. Sexual

behavior was video recorded for 60 min, which was sufficient for the 2 copulation periods (see Data Analysis and Statistical Procedures). We divided 19 subject pairs into 2 groups depending on the type of water sample to which they were exposed: Control ($n = 9$) or Pheromone ($n = 10$).

Procedure for Experiment 2

All subjects were handled for 5 min per day beginning 2 days before the experiment. One day before the experimental day, both male and female subjects were housed individually and were acclimatized to the experimental apparatus and room for 30 min.

Experiment 2 was performed as described in Experiment 1 with one exception: the water sample was presented only to the male or only to the female subject before the sexual behavior. In this experiment, water samples were dropped on 2 sheets of filter paper (5×5 cm) that were slipped between 2 acrylic plates ($120 \times 60 \times 3$ mm), one of which had 18 nine-mm-diameter holes. The acrylic plates holding the filter papers were then placed in the subject's home cage and left there for 5 min. After the sample presentation, the plates were removed, and the female subject was placed into the male subject's home cage. Sexual behavior was video recorded for 60 min.

We divided 36 subject pairs into 4 groups depending on the pheromone target and water sample, that is, male–Control ($n = 9$), male–Pheromone ($n = 9$), female–Control ($n = 9$), and female–Pheromone ($n = 9$).

Procedure for Experiment 3

All subjects were handled, housed individually, and acclimatized to the experimental apparatus as described in Experiment 1.

On the experimental day, the subjects were treated as in Experiment 1. However, in this experiment, a vehicle (saline containing 0.5% tragacanth gum powder; Wako Pure Chemical Industries) or a single dose of CP-154526 (10 or 30 mg/kg dissolved in the vehicle; Pfizer) was administered i.p. to male subjects 60 min before the experiment. Presentation of the water sample and observation of sexual behavior were then conducted as described in Experiment 2.

We divided 39 subject pairs into 4 groups depending on the water sample and the dose of CP-154526; that is, Control and CP 0 mg/kg ($n = 9$), Pheromone and CP 0 mg/kg ($n = 10$), Pheromone and CP 10 mg/kg ($n = 12$), and Pheromone and CP 30 mg/kg ($n = 8$).

Data Analysis and Statistical Procedures

Sexual behavior during the first 2 copulation periods was analyzed by a researcher who was blind to the experimental conditions. A pair of rats usually begins sexual behavior by investigating one another's face and anogenital regions. Estrous female rats display proceptive behaviors, including darts and hops (abrupt moving and jumping), ear wiggling,

and solicitations (headwise orientation to the male followed by an abrupt runaway). These behaviors trigger mounts (pelvic thrusting from the rear of the female rat without penile insertion) and intromissions (deeper pelvic thrusting from the rear of the female rat with penile insertion) from the male rat. When the male rat mounts, the female rat shows lordosis (arching of the back, dorsiflexion of the tail, and extension of the neck). After an adequate number of intromissions, the male rat ejaculates. Then, male rat grooms and rests during the postejaculation interval (PEI), which may last for 6–10 min before the male rat resumes intromission. The period in which this group of events occurs is defined as the copulation period, which is repeated 6–7 times until the male rat reaches sexual satiety.

We observed the following measures of sexual behavior during the first 2 copulation periods for the male: mount latency, intromission latency (time from introduction of the female to the first mount or intromission), ejaculation latency (time from the first intromission to ejaculation in a copulation period), number of mounts, and number of intromissions (number of mounts or intromissions that were needed for an ejaculation in a copulation period). Female measures of sexual behavior were the number of lordoses during the first 2 copulation periods and the number of darts, hops, and solicitations during the first 15 min. In addition, the hit rate (ratio between the number of intromissions and the sum of mounts plus intromissions), PEI, and lordosis quotient (ratio between the number of lordoses and the sum of mounts plus intromissions) were calculated. The data are expressed as means \pm standard error of the mean (SEM), and significance was set at $P < 0.05$ for all statistical tests. In Experiments 1 and 2, all data were analyzed by one-way analysis of variance (ANOVA). In Experiment 3, all data were analyzed by one-way ANOVA followed by Dunnett's post hoc test.

Results

Experiment 1

In the first copulation period, exposure to the alarm pheromone increased the number of mounts ($F_{1,17} = 7.38$, $P < 0.05$), decreased the hit rate ($F_{1,17} = 5.68$, $P < 0.05$), and increased the ejaculation latency ($F_{1,17} = 6.85$, $P < 0.05$) (Figure 1). The alarm pheromone did not affect the number of intromissions ($F_{1,17} = 2.34$, $P = 0.145$), PEI ($F_{1,17} = 2.58$, $P = 0.126$), mount latency ($F_{1,17} = 0.21$, $P = 0.655$), or intromission latency ($F_{1,17} = 0.13$, $P = 0.724$) in male subjects nor did it affect the lordosis quotient ($F_{1,17} = 0.04$, $P = 0.838$), number of darts and hops ($F_{1,17} = 0.05$, $P = 0.823$), or number of solicitations ($F_{1,17} = 0.16$, $P = 0.698$) in female subjects (Tables 1 and 2). In the second copulation period, the alarm pheromone did not affect any measures of sexual behavior in either male or female subjects, including the number of mounts ($F_{1,17} = 0.20$, $P = 0.658$), hit rate ($F_{1,17} = 1.68$, $P = 0.212$), ejaculation

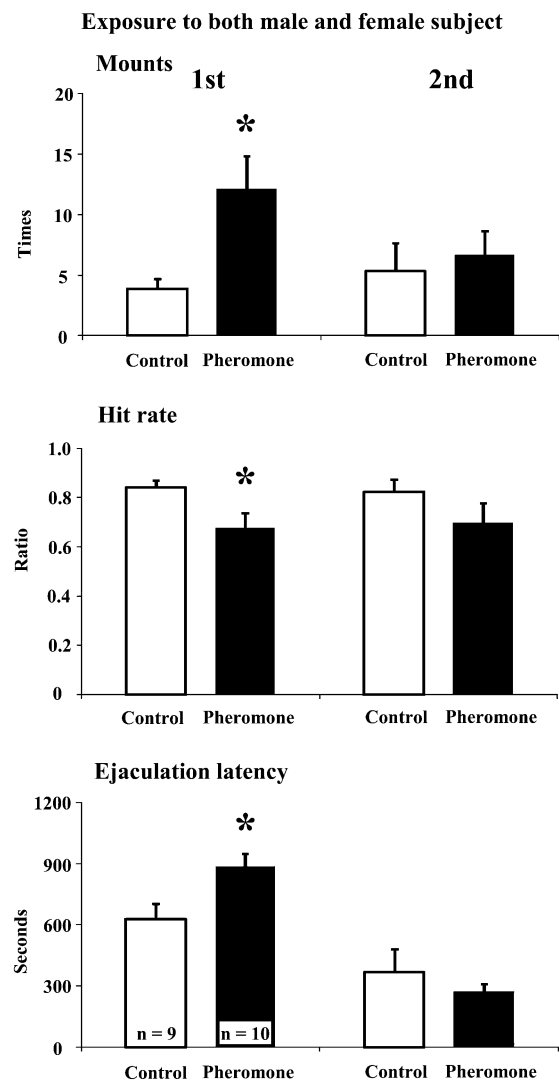


Figure 1 The number of mounts, hit rate, and ejaculation latency of male subjects in the first and second copulation periods in Experiment 1. A pair of subjects was exposed to the water collected from the box in which either the alarm pheromone (Pheromone) or a control odor from the neck region (Control) was released. * $P < 0.05$ compared with the control group by ANOVA (mean \pm SEM).

latency ($F_{1,17} = 0.70$, $P = 0.415$), number of intromissions ($F_{1,17} = 2.68$, $P = 0.120$), or PEI ($F_{1,17} = 1.03$, $P = 0.323$) in male subjects and the lordosis quotient ($F_{1,17} = 0.04$, $P = 0.835$) in female subjects (Figure 1 and Table 1).

Experiment 2

When male subjects were exposed to the alarm pheromone, results showed an increased number of mounts ($F_{1,16} = 16.52$, $P < 0.001$) and decreased hit rate ($F_{1,16} = 15.05$, $P < 0.01$) in the first copulation period (Figure 2). The alarm pheromone did not affect the ejaculation latency ($F_{1,16} = 0.69$, $P = 0.417$), number of intromissions ($F_{1,16} = 3.78$, $P = 0.070$), PEI ($F_{1,16} = 0.42$, $P = 0.526$), mount latency ($F_{1,16} = 1.75$, $P = 0.204$), or intromission latency ($F_{1,16} = 1.15$, $P = 0.299$) in male subjects nor did it affect the lordosis quotient ($F_{1,16} = 0.05$, $P = 0.824$), number of darts and hops ($F_{1,16} = 0.25$, $P = 0.626$), or number of solicitations ($F_{1,16} = 0.02$, $P = 0.893$) in female subjects (Figure 2, Tables 3 and 4). In the second copulation period, alarm pheromone did not affect any measures of sexual behavior in either male or female subjects: the number of mounts ($F_{1,16} = 3.93$, $P = 0.065$), hit rate ($F_{1,16} = 3.24$, $P = 0.091$), ejaculation latency ($F_{1,16} = 4.41$, $P = 0.052$), number of intromissions ($F_{1,16} = 1.61$, $P = 0.222$), or PEI ($F_{1,16} = 0.10$, $P = 0.754$) in male subjects, and the lordosis quotient ($F_{1,16} = 1.24$, $P = 0.283$) in female subjects (Figure 2 and Table 3).

When we presented alarm pheromone only to female subjects just before sexual behavior, it did not affect any measures of sexual behavior in either male or female subjects in both the first and second copulation periods. In the first copulation period, no effects were seen in the number of mounts ($F_{1,16} = 0.81$, $P = 0.381$), hit rate ($F_{1,16} = 0.80$, $P = 0.385$), ejaculation latency ($F_{1,16} = 0.13$, $P = 0.722$), number of intromissions ($F_{1,16} = 3.38$, $P = 0.085$), PEI ($F_{1,16} = 1.57$, $P = 0.229$), mount latency ($F_{1,16} = 0.91$, $P = 0.354$), or intromission latency ($F_{1,16} = 1.02$, $P = 0.328$) in male subjects, and the lordosis quotient ($F_{1,16} = 0.07$, $P = 0.788$), number of darts and hops ($F_{1,16} = 2.89$, $P = 0.108$), or number of solicitations ($F_{1,16} = 0.60$, $P = 0.452$) in female subjects (Figure 2, Tables 4 and 5). In the second copulation period, no effects were seen in the number of mounts ($F_{1,16} = 0.66$, $P = 0.428$), hit rate

Table 1 Measures of sexual behavior of the subjects that were exposed to alarm pheromone during the sexual behavior in Experiment 1

	First copulation period		Second copulation period	
	Control (n = 9)	Pheromone (n = 10)	Control (n = 9)	Pheromone (n = 10)
Number of intromissions	19.6 \pm 1.9	23.7 \pm 1.9	18.3 \pm 3.9	12.0 \pm 1.1
PEI	431 \pm 18	401 \pm 8	506 \pm 25	478 \pm 12
Mount latency	75 \pm 27	61 \pm 18	— ^a	— ^a
Intromission latency	76 \pm 27	64 \pm 18	— ^a	— ^a
Lordosis quotient	0.950 \pm 0.028	0.943 \pm 0.020	0.956 \pm 0.020	0.963 \pm 0.024

Data are expressed as means \pm SEM. The number of subjects is given in parentheses.
^aNot analyzed. Mount latency and intromission latency in the second copulation period is synonymous with PEI in the first copulation period.

($F_{1,16} = 1.41$, $P = 0.253$), ejaculation latency ($F_{1,16} = 0.79$, $P = 0.386$), number of intromissions ($F_{1,16} = 1.00$, $P = 0.332$), or PEI ($F_{1,16} = 0.53$, $P = 0.476$) in male subjects, and the lordosis quotient ($F_{1,16} = 1.20$, $P = 0.290$) in female subjects (Figure 2 and Table 5).

Table 2 Measures of sexual behavior of female subjects during the first 15 min of Experiment 1

Pair of rats was exposed to the pheromone		
	Control ($n = 9$)	Pheromone ($n = 10$)
Number of darts and hops	100.2 \pm 8.0	97.9 \pm 6.5
Number of solicitations	12.8 \pm 2.1	14.0 \pm 2.3

Data are expressed as means \pm SEM. The number of subjects is given in parentheses.

Experiment 3

In the first copulation period, the number of mounts ($F_{3,35} = 2.95$, $P < 0.05$), hit rate ($F_{3,35} = 5.48$, $P < 0.01$), ejaculation latency ($F_{3,35} = 3.26$, $P < 0.05$), and PEI ($F_{3,35} = 4.20$, $P < 0.05$) were significantly affected by the treatment. The treatment did not affect the number of intromissions ($F_{3,35} = 2.15$, $P = 0.1119$), mount latency ($F_{3,35} = 2.51$, $P = 0.0747$), or intromission latency ($F_{3,35} = 2.23$, $P = 0.1020$) in the male subjects nor did it affect the lordosis quotient ($F_{3,35} = 0.66$, $P = 0.5836$), number of darts and hops ($F_{3,35} = 1.43$, $P = 0.2503$), or number of solicitations ($F_{3,35} = 1.74$, $P = 0.1762$) in the female subjects (Tables 6 and 7). Post hoc test results indicated that alarm pheromone increased the number of mounts ($P < 0.05$) and decreased the hit rate ($P < 0.01$) in male subjects. These pheromone effects were dose dependently attenuated by the pretreatment of CP-154526 (Figure 3). The pretreatment with high-dose CP-154526 (30 mg/kg)

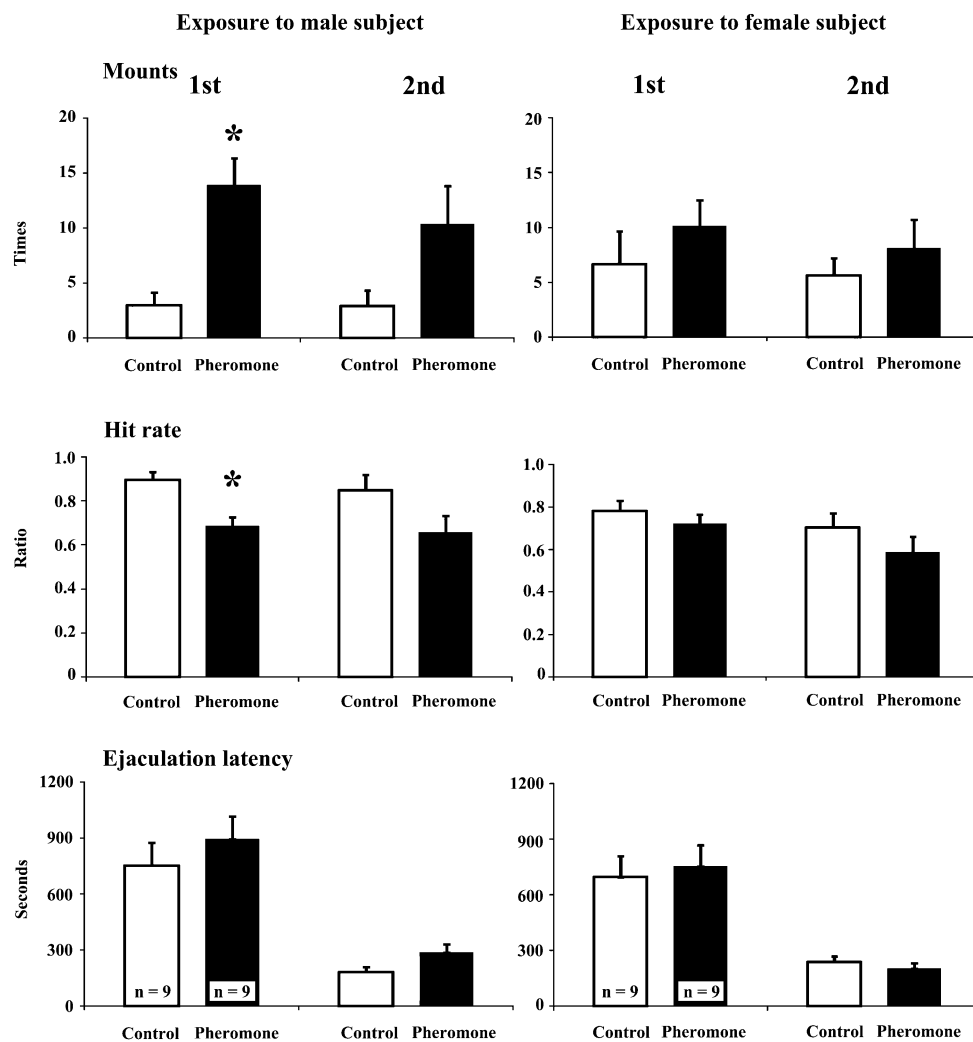


Figure 2 The number of mounts, hit rate, and ejaculation latency of male subjects in the first and second copulation periods in Experiment 2. Either the male or female subject was exposed to the water collected from the box in which either the alarm pheromone (Pheromone) or a control odor from the neck region (Control) was released. * $P < 0.05$ compared with the control group by ANOVA (mean \pm SEM).

Table 3 Measures of sexual behavior of subjects in Experiment 2 in which male subject was exposed to alarm pheromone

	First copulation period		Second copulation period	
	Control (<i>n</i> = 9)	Pheromone (<i>n</i> = 9)	Control (<i>n</i> = 9)	Pheromone (<i>n</i> = 9)
Number of intromissions	21.4 ± 2.4	27.6 ± 2.0	10.1 ± 1.1	14.3 ± 3.1
PEI	395 ± 3	383 ± 18	467 ± 17	475 ± 21
Mount latency	109 ± 42	50 ± 15	— ^a	— ^a
Intromission latency	117 ± 43	67 ± 17	— ^a	— ^a
Lordosis quotient	0.946 ± 0.019	0.951 ± 0.012	0.981 ± 0.010	0.947 ± 0.029

Data are expressed as means ± SEM. The number of subjects is given in parentheses.

^aNot analyzed. Mount latency and intromission latency in the second copulation period is synonymous with PEI in the first copulation period.

Table 4 Measures of sexual behavior of female subjects in the first 15 min of Experiment 2

	Male was exposed to the pheromone		Female was exposed to the pheromone	
	Control (<i>n</i> = 9)	Pheromone (<i>n</i> = 9)	Control (<i>n</i> = 9)	Pheromone (<i>n</i> = 9)
Number of darts and hops	99.7 ± 9.4	93.2 ± 9.0	77.9 ± 6.4	96.6 ± 8.9
Number of sollicitations	10.9 ± 2.0	11.1 ± 1.4	9.0 ± 1.7	7.3 ± 1.4

Data are expressed as means ± SEM. The number of subjects is given in parentheses.

Table 5 Measures of sexual behavior of subjects in Experiment 2 in which female subject was exposed to alarm pheromone

	First copulation period		Second copulation period	
	Control (<i>n</i> = 9)	Pheromone (<i>n</i> = 9)	Control (<i>n</i> = 9)	Pheromone (<i>n</i> = 9)
Number of intromissions	17.3 ± 2.3	23.3 ± 2.3	11.2 ± 2.2	8.8 ± 1.2
PEI	405 ± 9	428 ± 16	501 ± 12	515 ± 15
Mount latency	33 ± 9	47 ± 11	— ^a	— ^a
Intromission latency	117 ± 56	60 ± 14	— ^a	— ^a
Lordosis quotient	0.989 ± 0.007	0.986 ± 0.008	0.983 ± 0.008	0.952 ± 0.027

Data are expressed as means ± SEM. The number of subjects is given in parentheses.

^aNot analyzed. Mount latency and intromission latency in the second copulation period is synonymous with PEI in the first copulation period.

increased PEI ($P < 0.05$) in the same time (Table 6). In the second copulation period, the treatment significantly affected the number of mounts ($F_{3,35} = 3.36$, $P < 0.05$), hit rate ($F_{3,35} = 2.97$, $P < 0.05$), and PEI ($F_{3,35} = 3.28$, $P < 0.05$). The treatment did not affect ejaculation latency ($F_{3,35} = 2.73$, $P = 0.0588$) or number of intromissions ($F_{3,35} = 1.15$, $P = 0.3418$) in male subjects nor did it affect the lordosis quotient ($F_{3,35} = 1.02$, $P = 0.3959$) in female subjects (Figure 3 and Table 6). Post hoc test results indicated that alarm pheromone increased the number of mounts ($P < 0.05$) and decreased the hit rate ($P < 0.05$) in male subjects (Figure 3). These pheromone effects were again attenuated by the CP-154526 pretreatment (Figure 3). In the second copulation period, both low ($P < 0.05$) and high ($P < 0.05$) pretreatment doses of CP-154526 increased PEI (Table 6).

Discussion

When a pair of subjects was exposed to the alarm pheromone (Experiment 1), male subjects showed an elongated ejaculation latency, increased number of mounts, and decreased hit rate. These results suggest that alarm pheromone suppressed male sexual behavior. Presenting the pheromone only to the male subject evoked the same modifications in the number of mounts and hit rate (Experiment 2), suggesting that alarm pheromone affected the male subjects. In addition, pretreatment with CP-154526, a CRF antagonist, dose dependently blocked these modifications induced by the alarm pheromone (Experiment 3). These results suggest that CRF plays an important role in the modifications of sexual behavior by alarm pheromone. All these results suggest that the alarm

Table 6 Measures of sexual behavior of male subject in the first and second copulation period in Experiment 3 in which male subject was pretreated CP-154526

		0 mg/kg Control (n = 9)	0 mg/kg Pheromone (n = 10)	10 mg/kg Pheromone (n = 12)	30 mg/kg Pheromone (n = 8)
Number of intromissions	First	20.3 ± 2.4	15.9 ± 1.4	20.8 ± 1.8	15.3 ± 2.4
	Second	10.2 ± 0.8	8.7 ± 1.2	11.2 ± 1.1	11.1 ± 1.1
PEI	First	417 ± 12	408 ± 12	445 ± 14	480 ± 21*
	Second	481 ± 8	507 ± 16	528 ± 11*	537 ± 15*
Mount latency	First	142 ± 64	32 ± 5	202 ± 70	47 ± 10
	Second	— ^a	— ^a	— ^a	— ^a
Intromission latency	First	164 ± 83	39 ± 9	214 ± 72	52 ± 9
	Second	— ^a	— ^a	— ^a	— ^a
Lordosis quotient	First	0.980 ± 0.012	0.964 ± 0.020	0.985 ± 0.007	0.988 ± 0.012
	Second	0.991 ± 0.009	0.944 ± 0.028	0.941 ± 0.023	0.956 ± 0.024

Data are expressed as means ± SEM. The number of subjects is given in parentheses.

^aNot analyzed. Mount latency and intromission latency in the second copulation period is synonymous with PEI in the first copulation period.

**P* < 0.05 with Dunnett's post hoc test as compared with 0 mg/kg—Control group.

Table 7 Measures of sexual behavior of female subjects in the first 15 min of Experiment 3

	0 mg/kg Control (n = 9)	0 mg/kg Pheromone (n = 10)	10 mg/kg Pheromone (n = 12)	30 mg/kg Pheromone (n = 8)
Number of darts and hops	90.3 ± 9.7	84.2 ± 8.9	82.4 ± 3.9	104.6 ± 10.7
Number of solicitations	12.9 ± 1.4	10.4 ± 1.1	13.3 ± 1.4	15.0 ± 1.6

Data are expressed as means ± SEM. The number of subjects is given in parentheses.

pheromone induces CRF secretion, which in turn modifies components of sexual behavior in male subjects.

From the present results, it can be hypothesized that alarm pheromone suppresses components of sexual behavior in male subjects. Because the main purpose of male sexual behavior is to ejaculate, which is achieved by repeated intromissions, whether the same modifications of the other components of sexual behavior are considered enhancing or suppressing depends on the accomplishment of these components of sexual behavior. Therefore, it is presently unclear whether alarm pheromone enhanced or suppressed the number of mounts and hit rate because 2 important measures, that is, ejaculation latency and number of intromissions, were not consistently suppressed. However, we still assume that alarm pheromone has suppressive effects on the components of sexual behavior based on its CRF dependency. In the present study, the modifications of the number of mounts and hit rate by the alarm pheromone were blocked by the pretreatment with the CRF antagonist, suggesting that CRF modified these components of sexual behavior. Activation of the CRF system is suggested to suppress sexual behavior in male rats. For example, one report indicated that ICV injection of CRF elongated the ejaculation latency and

increased the number of intromissions, accompanied by an increased number of mounts (Sirinathsinghji 1987). In addition, several stressors, including water immersion, foot shock, and immobilization, are known to activate CRF secretion as assessed by the corticosterone concentration in plasma and to simultaneously elongate ejaculation latency, increase the number of mounts, and decrease the hit rate (Retana-Marquez et al. 2003; Retana-Marquez et al. 2009), although the causal linkage between the 2 phenomena remains to be elucidated. Therefore, considering its CRF dependency, it is conceivable that alarm pheromone has suppressive effects on components of sexual behavior in male rats. The suppressive effect on the ejaculation latency observed in Experiment 1 also supports this hypothesis. However, we cannot deny an alternative possibility that alarm pheromone initially enhances components of sexual behavior due to the lack of its clear suppressive effects on the ejaculation latency or the number of intromissions. The restriction of the pheromone effects to the first copulation period in Experiments 1 and 2 also supports this possibility.

Another important finding in this study is that the alarm pheromone did not affect sexual behavior in females. It is possible that high levels of endogenous estrogen and

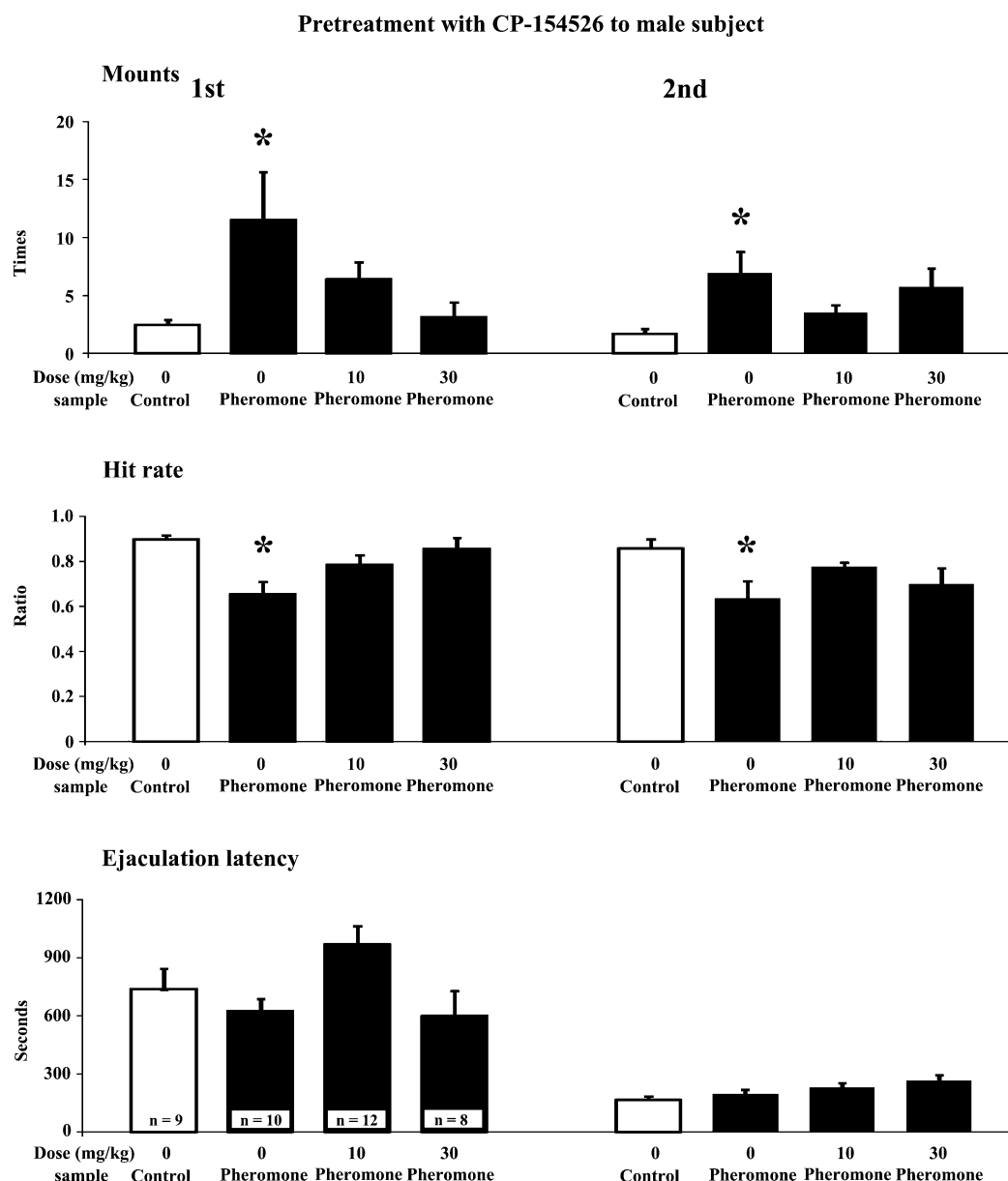


Figure 3 The number of mounts, hit rate, and the ejaculation latency of male subjects in the first and second copulation periods in Experiment 3. The male subject was pretreated with CP-154526 and exposed to the water collected from the box in which either the alarm pheromone (Pheromone) or a control odor from the neck region (Control) was released. * $P < 0.05$ compared with the control 0 mg/kg group by one-way ANOVA followed by Dunnett's post hoc test (mean \pm SEM).

progesterone override the pheromone effects. In this study, all female subjects were proestrous when estrogen and progesterone levels were high compared with other phases of the estrous cycle. Estrogen and progesterone are known to have a suppressive effect on CRF secretion. For example, estrogen administration to ovariectomized (OVX) rats decreased CRF mRNA expression in the PVN (Paulmyer-Lacroix et al. 1996) and attenuated foot shock-induced Fos expression in the PVN (Gerrits et al. 2005). In addition, both estrogen and progesterone subcutaneous injections attenuated the enhanced ASR induced by ICV administration of

CRF (Toufexis et al. 2004). Moreover, subcutaneous injection of estrogen and progesterone blocked the suppression of female sexual behavior induced by a restraint (White and Uphouse 2004) that increased CRF mRNA expression and Fos expression in the PVN (Imaki et al. 1995). Therefore, it is conceivable that proestrous female subjects did not respond to the alarm pheromone because high levels of estrogen and progesterone interfered with the induction of CRF secretion in this study.

In Experiment 3, CP-154526 treatment unexpectedly increased PEI; the reason for this increase is unclear.

Considering that PEI was not affected by the ICV administration of CRF (Sirinathsinghji 1987), whereas several stressors that increased the corticosterone concentration in plasma increased PEI (Retana-Marquez et al. 2003; Retana-Marquez et al. 2009), we expect that the PEI was independent from the CRF system. In addition, it is unclear whether this phenomenon is a common side effect of CP-154526 administration or specifically observed in this study which, to our knowledge, is the first study to observe the effect of CP-154526 on male sexual behavior. Therefore, further study is needed to clarify why the administration of CP-154526 elongated the PEI in this study.

In the present study, we cannot exclude the following possibilities. First, the female rats may not have had the ability to respond to the alarm pheromone that was derived from the male rats. This possibility seems unlikely because an OVX female rat showed defensive and risk assessment behavior in the modified open-field test in our preliminary observations. Second, we may have overlooked alterations in female behavior induced by alarm pheromone. However, most of the measures of sexual behavior in the female subjects were not altered by the pheromone, suggesting that the alarm pheromone has negligible effects on female sexual behavior.

In summary, we first found that male sexual behavior was suppressed when a pair of subjects was exposed to the alarm pheromone during sexual behavior. We next found that alarm pheromone affects male, but not female, subjects. Finally, we found that the pheromone effects were attenuated by the pretreatment with CP-154526 in a dose-dependent manner. On the basis of its CRF dependency, we hypothesize that the alarm pheromone suppresses male, but not female, components of sexual behavior. This study provides new information on how pheromones mediate sexual behavior in rats.

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