Chem. Senses 29: 555–563, 2004 doi:10.1093/chemse/bjh054

Importance of Learning in the Response of Ewes to Male Odor

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Abstract

Exposure of anestrous ewes to a ram or its odor results in the activation of the luteinizing hormone (LH) secretion leading to reinstatement of cyclicity in most females. Sexual experience and learning have been suggested as important factors to explain the variability of the female responses. In experiment 1, we compared the behavioral and endocrine responses of four groups of anestrous females that differed in age (young or adult) and previous exposure to males [naive (no exposure) or experienced (courtship behavior for young and numerous mating for adults)]. Age did not seem to affect the LH response to males or their odor. In contrast, sexual experience was a critical factor: the proportion of females exhibiting an LH response to male odor was significantly higher in experienced than in naive ewes. Sexual experience affected the response to male odor, but did not have an effect on responses to the male himself. A second experiment investigated whether the LH response to male odor could result from an associative learning process. Accordingly, we tested the effectiveness of a conditioned stimulus (lavender odor) previously associated with the male, in inducing the endocrine response. The results indicate that the odor of lavender activated LH secretion only in ewes that have been previously exposed to scented males. This demonstrates that ewes are able to learn the association between a neutral odor and their sexual partner.

Key words: luteinizing hormone, olfactory cues, sheep, social stimuli

Introduction

Social stimuli are known to influence reproductive behavior and neuroendocrine function in a variety of vertebrate species. In mammals, olfactory cues can mimic most effects of the interactions between conspecifics or between sexual partners.

Physiological changes induced by sexual chemosignals are frequently stereotyped responses (Keverne, 1986). In several species, male odors accelerate puberty in young females that have never been in contact with males, for example: mouse (Vandenbergh, 1969); prairie vole (Carter *et al.*, 1980); hamster (Reasner and Johnston, 1988); and cow (Izard and Vandenbergh, 1982). In hamsters, chemosignals in female vaginal secretions induce an increase of testosterone in sexually naive males (Pfeiffer and Johnston, 1994). Naive male mice also reflexively release luteinizing hormone (LH) and testosterone pulses after encountering a female or her urinary pheromones (Macrides *et al.*, 1975; Maruniak and Bronson, 1976). In some cases, chemosignals may also induce stereotyped behavioral responses. For example, sexually naive males hamster are attracted to female vaginal secretions and attempt to copulate with anaesthetized surrogates scented with these secretions (Murphy, 1973; Macrides *et al.*, 1977, 1984).

However, most behavioral responses elicited by sexual pheromones are facilitated by previous sexual experience. In many species, chemical signals serve as sexual attractants, induce sexual arousal, appetitive and precopulatory postures and mating behavior but these responses are preferentially expressed by sexually experienced individuals. In adult male rats, mice and dogs, sexual experience results in a preference for estrous over nonestrous females odors (Carr *et al.*, 1965; Doty and Dunbar, 1974; Hayashi and Kimura, 1974). In mice, the presentation of female urine elicits vocalizations only in sexually experienced males (Dizinno *et al.*, 1978). The role of learning in the development of behavioral response to olfactory cues has been also demonstrated using conditioning paradigms. A conditioned cue (a neutral odor associated with mating with a receptive female) induces sexual partner preferences (Kippin and Pfaus, 2001a,b) and elicits males vocalizations (Nyby *et al.*, 1978).

The role of experience is not limited to the treatment of social and sexual olfactory cues. On the contrary, learning processes are generally required for the identification of environmental odors that can be either associated with a reward or an aversive stimulus (Schoenbaum *et al.*, 1998; Tronel and Sara, 2002). These artificial learned odors are principally detected by the main olfactory system, which is usually considered as a general molecular analyser of an enormous variety of odorants (Mori *et al.*, 2000).

In rodents, chemosignals inducing stereotyped physiological or behavioral changes are essentially detected and integrated by the accessory olfactory system (Keverne, 1983, 1999; Brennan, 2001). Lesion of the vomeronasal organ (VNO) impairs dramatically the responses to conspecific odors (Wysocki and Lepri, 1991). This blockage mainly concerns the endocrine responses that are generally preprogrammed, whereas sexual experience can compensate for the behavioral effects of VNO destruction. In male hamsters, lesion of the VNO abolished copulatory behavior only in sexually naive animals (Meredith, 1986). Destruction of the VNO of male mice eliminates vocalizations in response to female urine but only in sexually naive individuals (Bean, 1982).

During the non-breeding season, the ram or its fleece induce an immediate increase in plasma LH by ewes (Knight *et al.*, 1983; Martin *et al.*, 1983). In sheep, in contrast to rodents, male pheromone seems essentially detected by the main olfactory system (Cohen-Tanoudji *et al.*, 1989; Gelez and Fabre-Nys, 2004). Furthermore, preliminary data suggest that the endocrine response induced by male odor may be affected by sexual experience and could involve learning mechanisms (Cohen-Tanoudji, unpublished data). This phenomenon, commonly called the ‘male effect’, constitutes a major factor in the control of reproductive events in ungulates, with physiological and ecological relevance and provides an original model for studying the actions of olfactory cues.

The aim of our work was to assess the importance of learning in anestrous ewes’ responses to ram odor. To achieve this, we adopted two strategies. In experiment 1, we compared the behavioral and endocrine responses of females differing in sexual experience (naive or experienced) and age (young or naive). In experiment 2, we examined the capacity of a neutral conditioned stimulus (lavender odor) which had been previously associated with an adult male, to induce an LH increase.

Materials and methods

Experiment 1

Animals

We used 49 Ile-de-France ewes in anestrus that differed in age and sexual experience: young (1 year old) sexually naive ewes (n = 7); young (1 year old) sexually experienced ewes (n = 19); adult (2–5 years old) sexually naive ewes (n = 13); and adult (2–5 years old) sexually experienced ewes (n = 10).

The young sexually experienced ewes were in contact with males for 2 weeks and only received male courtship but not mating. The adult sexually experienced ewes were multiparous. The two groups of sexually naive females were never previously exposed to males or their odors.

The females were housed by groups (four or five ewes per groups) in indoor pens which had not previously contained rams, under a natural photoperiod. They were fed daily with a constant diet of straw, maize, lucerne pellets and mineral supplements and had free access to water. The ewes were diagnosed as seasonally anovulatory by persistent low concentrations of circulating progesterone (<1 ng/ml) in weekly progesterone assays, indicating the absence of a functional corpus luteum. These assays were performed with a method adapted from Terqui and Thimonier (1974).

Six sexually experienced adult, Ile-de-France rams, were used as stimuli. Fleece of 10 other Ile-de-France or Romanov rams and 10 Ile-de-France ewes was collected during the breeding season and stored at –20°C.

LH assay

Blood samples were centrifuged and plasmas were stored at –20°C. Concentrations of LH were measured in duplicate samples of 100 µl plasma by the radioimmunoassay (RIA) method of Pelletier *et al.* (1968) as modified by Montgomery *et al.* (1985). The sensitivity of the assay was 0.16 ± 0.05 ng/ml (four assays) standard 1051-CY-LH (i.e. 0.31 ng/ml NIH LH-S1). The intra-assay and inter-assay coefficients of variation were 4.4 and 10.3%, respectively.

Behavioral data

For each stimulation, quantitative and qualitative data were recorded. During exposure to the fleece, we recorded the number of olfactory investigations of the fleece, the number of urine emissions, defecations, bleats, movements and the position of the animal in the pen: immobile near or far from the fleece (Gelez and Fabre-Nys, 2004). During male stimulation, we analysed both female and male sexual behaviors as previously described (Banks, 1964; Fabre-Nys and Venier, 1987). We recorded the number of male and female anogenital sniffs, male and female head sniffs, male nudges followed by female immobilization, male nudges followed by female movements and male mount attempts.

Experimental protocol

During the anestrous period, all females were isolated from males for at least 1 month and were habituated to manipulation and bleeding during 1 week. They received a catheter in the jugular vein that permits the collection of blood samples. Afterwards, the four groups of females were randomly exposed to female fleece (control situation) or male fleece, then to males. The stimulation consisted in introducing in the females’ pens an open box containing the fleece or a sexually active male. At least 2 or 3 days separated each stimulation. For each stimulation, blood samples were collected every 15 min over 5 h. After 1 h, the fleece or the males were introduced in the females’ pens.

Experiment 2

Animals

We used 26 sexually naive young Ile-de-France ewes that were 10 months old at the beginning of the experiment. Females were housed by groups in indoor pens and were fed daily with a constant diet of straw, maize, lucerne pellets and mineral supplements and had free access to water.

Behavioral data

As in experiment 1, quantitative and qualitative data were recorded: the number of olfactory investigations of the fleece, urine emissions, defecations, bleats, movements and the position of the animal in the pen: immobile near or far from the fleece.

Experimental protocol

During the breeding season, the ewes were placed for 2 weeks with: males scented with lavender (MALELAV, $n = 12$); males not scented with lavender (MALE, $n = 7$); or females scented with lavender (FEMLAV, $n = 7$).

Each morning, the animals stimulus were perfumed with 200 ml of 1% lavender solution applied on the fleece. They then remained in contact with the females. The concentration of the lavender solution was chosen according to the results of preliminary trials in which six females were allowed to choose between a trough scented with lavender and containing food, a second empty unscented trough. Three concentrations of lavender solution were tested: 0.1, 1 and 10%. According to the results of the test choice, the concentration 1% was the concentration detected but not repulsive for ewes, and subsequently used.

After the 2 weeks of the odor conditioning procedure, all females were isolated from the males. Two months later, during the anestrous period, all females were habituated to manipulation and bleeding for 1 week. They received a catheter in the jugular vein. Afterwards, the three groups of ewes were randomly exposed to unscented female fleece or female fleece scented with 1% lavender solution. At least 2 or 3 days separated the two stimulus presentations. For each stimulation, blood samples were collected every 15 min over 5 h. After 1 h, an open box containing the fleece was introduced in the females' pens.

Data analysis

Behavioral responses

For each group, the behavioral data were compared between the control situation (exposure to female fleece) and the other stimulus conditions, and with the non-parametric Wilcoxon test for related samples. For each stimulus, the behavioral data were compared between groups with the Mann–Whitney *U*-test. Two-tailed tests were used for all the analyses.

Endocrine responses

The increase of LH pulsatility was used to identify the female response (Martin *et al.*, 1986). The pulses of LH were defined as a rise of LH according to the following criteria established by Goodman and Karsch (1980): (i) both the increase and subsequent decrease in concentration had to exceed the sum of the assay errors appropriate for the concentrations at the onset and the peak of the pulse; (ii) the increase had to occupy no more than two sampling intervals and the decline had to begin within two sampling intervals of attainment of the peak.

Ewes were considered as responsive to the stimulus if they presented an increased frequency of LH pulses relative to the control situation (exposure to female fleece). Proportions of responsive ewes were compared with the χ^2 test.

The response latency corresponds to the interval between the stimulus presentation and the first LH pulse. The LH maximum was the highest LH level during the stimulation period. For each group, these three physiological parameters (number of pulses, response latency and LH maximum) were compared between the control situation and the other stimuli with the Wilcoxon test. These parameters were also compared using between groups Mann–Whitney *U*-test.

To assess the effect of age and sexual experience, we used a two factors analysis of variance (ANOVA).

One-tailed tests were used since preliminary data provide a basis for predicting an effect of sexual experience on endocrine responses. For all analyses, statistical significance was considered when $P < 0.05$.

All experimental procedures were performed in accordance with the local animal regulation (Authorization No. 006259 of the French Ministry of Agriculture, in accordance with ECC directive).

Results

Experiment 1

Response to male odor

Behavioral responses. In both groups of experienced ewes, the number of olfactory investigations and the number of times they 'ate' the fleece were significantly higher during exposure to the male fleece rather than the female fleece ($P < 0.05$, Figure 1A). The naive adult ewes also displayed a significantly higher number of olfactory investigations of the male fleece ($P < 0.05$) whereas the young naive ewes smelled similarly the male and female fleeces (Figure 1A).

The sexually experienced ewes (young and adult) urinated significantly more during exposure to the male fleece than during exposure to the female fleece ($P < 0.05$, Figure 1B). This was not the case for the naive females, although the naive adult ewes tended to urinate more in the presence of the male fleece ($P = 0.07$, Figure 1B).

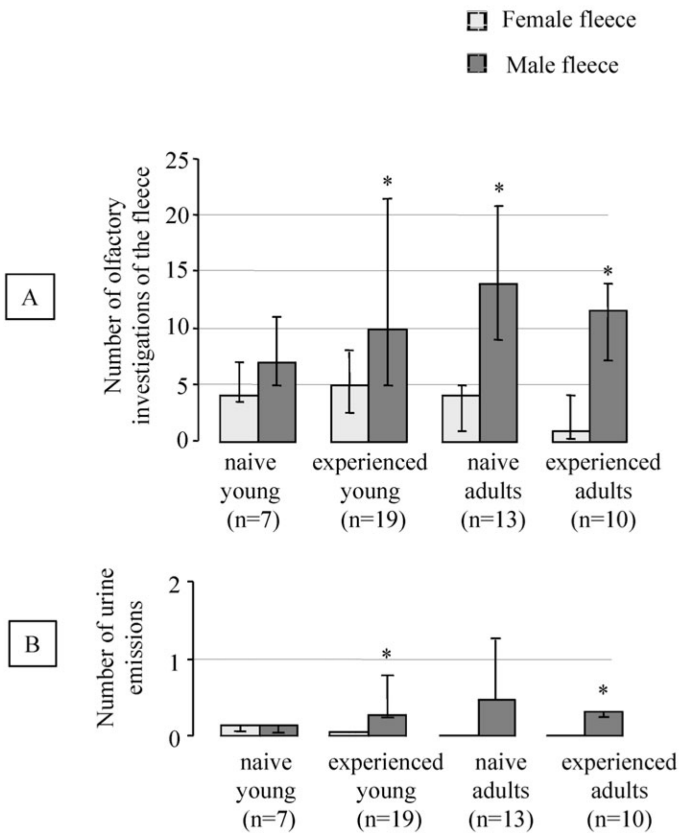

Figure 1 Number of olfactory investigations of the fleece **(A)** and number of urine emissions **(B)** during exposure to the female and male fleeces (median ± interquartile range). *Different between both stimuli in the same group ($P < 0.05$, Wilcoxon).

Other quantitative or qualitative behavioral data (bleats, movements, defecations, time spent near the fleece) did not significantly differ among two stimuli nor 4 groups of females.

Endocrine responses. The proportions of ewes exhibiting an increase of LH in response to the male odor differed significantly among four groups of females. The proportion of responsive females was significantly higher in experienced (24/29) than in naive ewes (8/20, $P < 0.05$, Table 1). The significant effect of experience was confirmed by the comparison of these proportions between groups of the same age (Table 1).

Sexual experience had a significant effect on LH responses [$F(4,25) = 4.15$, $P < 0.05$]. The experienced ewes, regardless of their age, exhibited a significantly greater number of LH pulses during exposure to the male odor than during exposure to the female fleece ($P < 0.05$, Table 1). No significant increase of LH pulses was detected in naive females. Furthermore, the ram odor elicited a significantly higher number of LH pulses in the two groups of experienced ewes than in the groups of naive ewes ($P < 0.05$, Table 1). The response latency was also significantly shorter for the experienced ewes ($P < 0.05$, Table 1).

Response to male

Behavioral responses. The young ewes investigated the male significantly more than did the experienced females (number of olfactory investigation of the male head or anogenital part, $P < 0.05$, Figure 2A). The number of male nudges followed by female avoidance was significantly higher in young than in adult ewes ($P < 0.05$, Figure 2B). Experienced adults ewes remained immobile following male nudges significantly more often than ewes of the three other groups ($P < 0.05$, Figure 2B).

Endocrine responses. The proportion of ewes exhibiting an increase of LH in response to males did not differ significantly among four groups of females. During exposure to males, the number of LH pulses, the response latency and the maximum concentration of LH did not differ significantly among four groups (Table 1).

For all groups of females the number of LH pulses and the maximum concentration of LH were significantly higher during exposure to males than during exposure to the female fleece ($P < 0.05$, Table 1).

The responses to the males and their fleece were also compared. In both groups of naive ewes, the actual male induced a significantly higher number of LH pulses than did the presentation of only male fleece ($P < 0.05$, Table 1). Furthermore, the naive females presented a significantly shorter response latency in the presence of males than in the presence of their fleece ($P < 0.05$, Table 1). In contrast, no differences were observed in experienced ewes responses to these two stimuli.

Experiment 2

Behavioral responses

All females of the MALELAV and MALE groups were stimulated by males during the conditioning period in the breeding season. However, because of their young age, none of them was receptive and accepted mating. Rather, they were only exposed to male courtship: olfactory investigations, nudges and attempts to mount.

During the anestrous period, the females that have been previously exposed to lavender (MALELAV and FEMLAV groups) displayed significantly more olfactory investigations oriented to the scented than the unscented fleece ($P < 0.05$, Figure 3A). In contrast, the ewes of the MALE group investigated both fleeces in a similar manner. Only the MALELAV ewes tended to urinate more during exposure to the lavender scented fleece than in presence of the unscented fleece ($P = 0.06$, Figure 3B).

Endocrine responses

The proportion of ewes exhibiting an increase of LH in response to the lavender odor differed significantly among three groups of females ($P < 0.05$, Table 2). The proportion of responsive females was significantly higher in the MALELAV group than in the MALE group ($P < 0.05$,

Table 1 LH responses of young and adult sexually naive or experienced ewes during exposure to a male or male odor

	Young naive (*n* = 7)	Young experienced (*n* = 19)	Adult naive (*n* = 13)	Adult experienced (*n* = 10)
Proportion of responsive females				
Male odor	3/7	15/19[a]	5/13	9/10[a]
Male	7/7	16/19	9/13	9/10
Response latency (min)				
Male odor	135 ± 37	94 ± 17[a]	147 ± 23	93 ± 14[a]
Male	60 ± 18[b]	92 ± 15	87 ± 16[b]	74 ± 22
Number of pulses				
Control	0.14 ± 0.14	0.16 ± 0.09	0.69 ± 0.13	0.10 ± 0.10
Male odor	0.71 ± 0.28	1.68 ± 0.35[a,c]	0.92 ± 0.24	2.10 ± 0.50[a,c]
Male	2.43 ± 0.37[b,c]	2.0 ± 0.25[c]	2.38 ± 0.33[b,c]	2.50 ± 0.48[c]
LH maximum (ng/ml)				
Control	0.5 ± 0.1	0.9 ± 0.3	3 ± 0.9	0.9 ± 0.4
Male odor	4.9 ± 1.5[c]	4.6 ± 1[c]	4.6 ± 1.6	5.3 ± 0.7[c]
Male	6.8 ± 1.4[c]	7.2 ± 1.0[c]	5.7 ± 0.8[c]	9. ± 2.7[c]

[a] Different between sexually naive and sexually experienced ewes in groups of the same age ($P < 0.05$, χ^2 or Mann–Whitney).
[b] Different from male odor in the same group ($P < 0.05$, χ^2 or Wilcoxon).
[c] Different from controls in the same group ($P < 0.05$, Wilcoxon).

Table 2). The proportion of responsive ewes did not differ between the MALELAV FEMLAV groups.

The ewes of the MALELAV group showed a significantly greater number of LH pulses and a high LH maximum during exposure to the scented fleece than during exposure to the unscented fleece ($P < 0.05$, Table 2). No differences were found for the ewes of the FEMLAV and the MALE groups.

Discussion

Our results show that a large proportion of sexually naive ewes, which have never previously been in contact with a male, do not exhibit an endocrine response during their first exposure to ram fleece. The fact that experienced ewes are more sensitive to the male odor demonstrates that sexual experience facilitates the response to this chemosignal. This markedly differs from other species. Indeed, in most mammals, the effects of olfactory cues on neuroendocrine secretions are independent of previous sexual experience and do not require learning mechanisms. The male odor accelerates sexual maturation in young females which have never been in contact with males, for example: mouse (Vandenbergh, 1969); prairie vole (Carter *et al.*, 1980); hamster (Reasner and Johnston, 1988); and cow (Izard and Vandenbergh, 1982). Sexually naive males exhibit a reflexive increase of testosterone during their first exposure to female urine or vaginal secretion, for example: mouse (Maruniak and Bronson, 1976) and hamster (Pfeiffer and Johnston, 1994). The male effect in sheep therefore constitutes the first demonstration that the action of an olfactory message eliciting a physiological response, is facilitated by previous experience.

The response to the ram odor may result in part from a reflex processes since some naive females are able to exhibit an endocrine response. However, the facilitatory effect of sexual experience indicates the involvement of more complex mechanisms. Our results show that a neutral conditioned stimulus can mimic the effect of the ram odor, suggesting that the LH response may be due to associative olfactory learning. It is possible that ewes have to learn the association between their sexual partner and its odor to assign a 'meaning' to the male chemosignal. Similar conditioning paradigms have been extensively used to reproduce behavioral changes induced by chemosignals. For example, when mating occurs with female scented with an artificial odor, subsequent exposure to this odor induces vocalizations by male mice (Nyby *et al.*, 1978) and rats develop a preference for sexual partner treated with this scent (Kippin and Pfaus, 2001a,b). In contrast, conditioned endocrine responses are difficult to obtain (Keverne, 1986), and they have only been demonstrated in one study. Graham and Desjardins (1980) showed that male rats exhibit an increase in circulating levels of LH and testosterone in response to an artificial odor (methylsalicylate) which had been previously paired with a receptive female during mating. An important difference between this work and ours is that the young ewes in our experiments were not receptive during the conditioning procedure and they did not accept mating. This

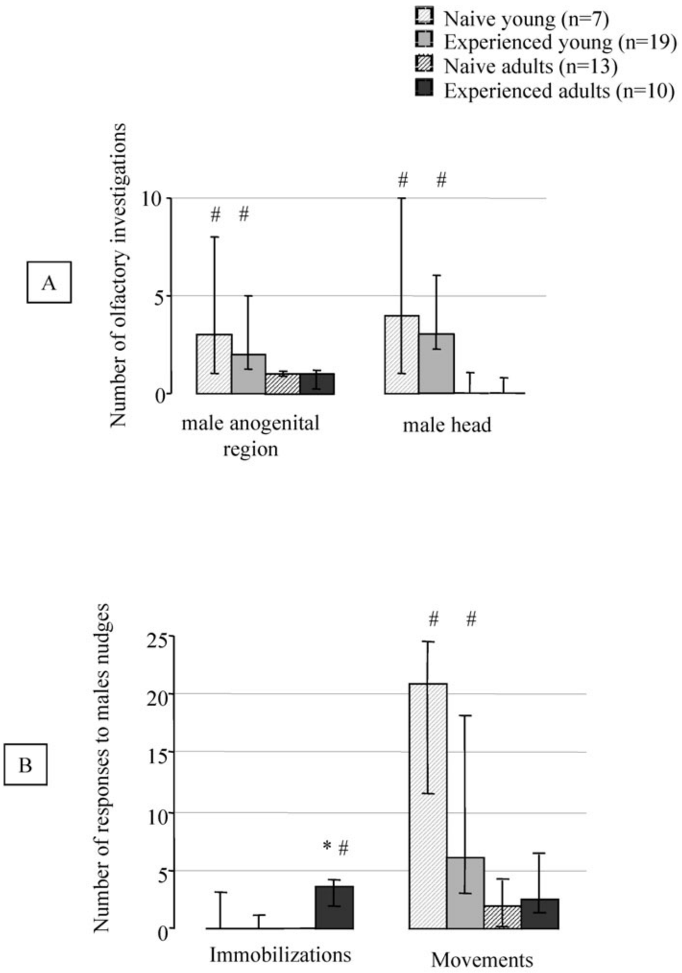

Figure 2 Number of olfactory investigation of the anogenital region and the head of the male **(A)** and female responses to males nudges (immobilization or movement, **B**; median ± interquartile range). Different between young and adults ewes in groups of the same sexual experience ($P < 0.05$, Mann–Whitney). *Different between naive and experienced ewes in groups of the same age ($P < 0.05$, Mann–Whitney).

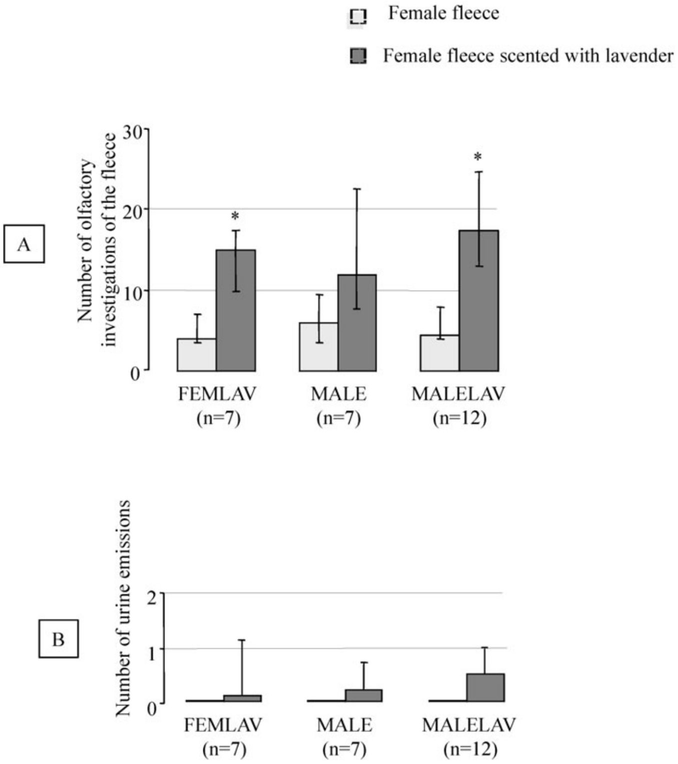

Figure 3 Number of olfactory investigations of the fleece **(A)** and number of urine emission during exposure to the unscented female fleece (CONTROL) and the female fleece scented with 1% lavender solution (LAVENDER) (median ± interquartile range). *Different from controls in the same group ($P < 0.05$, Wilcoxon).

same difference exists for other forms of associative olfactory learning occurring in natural conditions. In female mice, the memorization of the odor of the stud male at the time of mating leads to pregnancy block when the female is subsequently exposed to urinary pheromones of a strange male (Brennan and Keverne, 1997). In sheep, learning the newborn lambs' odor enables the mothers to recognize and accept suckling only their own offspring (Lévy *et al.*, 1991). In these examples, learning processes are triggered by the vaginocervical stimulation arising from either mating or parturition, resulting in major synaptic changes in the olfactory bulbs (Kendrick *et al.*, 1992; Keverne *et al.*, 1993; Brennan *et al.*, 1995). Our results show that in the context of the male effect, sexual experience limited to males' courtship appears sufficient for responsiveness to the ram odor, suggesting that vaginal stimulation is not indispensable to learn the meaning of the male olfactory cue. What ewes exactly learn and associate during these incomplete sexual interactions remains an open question.

In the presence of males, naive ewes exhibited an LH response similar to those of the experienced females. In our protocol, all ewes were first exposed to the male fleece then to males. It is possible that the first olfactory stimulation facilitated the subsequent response to males themselves. However, a more likely explanation is that males constitute a sufficiently powerful stimulation that does not require prior sexual experience.

In some species, the attractiveness of odor of the sexual partner is not dependent on learning, for example: hamster (Johnston, 1974) and mouse (Jemiolo *et al.*, 1991; Moncho-Bogani *et al.*, 2002). Our results show that the ram odor does not possess innate attractive properties, since naive young ewes displayed similar olfactory investigations of both male and female fleeces. However, the naive adult ewes smelled the male fleece significantly more than the female fleece. This result may be explained by the past experience of the females. The naive adult ewes were housed with several groups of conspecifics and had been in contact with the odors of numerous different females. Thus, they had a greater 'social experience' than the young naive ewes that only encountered the females of their living group. Therefore, for the naive adult ewes that are habituated to meet

Table 2 LH responses of three groups of females: FEMLAV (previously exposed to ewes scented with lavender), MALE (previously exposed to unscented males) and MALELAV (previously exposed to males scented with lavender), during exposure to female fleece (control) and during exposure to female fleece scented with 1% lavender solution

	FEMLAV ($n = 7$)	MALE ($n = 7$)	MALELAV ($n = 12$)
Proportion of responsive females	2/7	0/7	$6/12^{a}$
Response latency (min)	184 ± 32	223 ± 17	162 ± 25
Number of pulses			
Control	0.14 ± 0.14	0.28 ± 0.18	0.08 ± 0.08
Lavender	0.28 ± 0.18	0.14 ± 0.14	$0.92 \pm 0.29^{a,b}$
LH maximum (ng/ml)			
Control	0.5 ± 0.1	0.8 ± 0.4	1.0 ± 0.4
Lavender	2.6 ± 1.1^{a}	0.6 ± 0.4	2.7 ± 0.9^{b}

[a]Different from the MALE group ($P < 0.05$, χ^2 or Mann–Whitney).
[b]Different from controls in the same group ($P < 0.05$, Wilcoxon).

unfamiliar female odors, the male fleece may be perceived as a really novel odor eliciting a particular interest. In contrast, for the young females, both fleeces may represent novel odors. The difference between young and adult naive females may also be due to different emotional and/or attentive state. In previous work, we showed that naive adult ewes acquired a level of proceptivity (corresponding to the time spent in interaction with males in a test choice) similar to that of experienced adults, more rapidly than young ewes (Gelez *et al.*, 2004a). The young ewes did not exhibit lower learning capacity than adult females, but higher levels of stress that may explain their difficulty in focusing their attention on males (Gelez *et al.*, 2004a). In anestrous young ewes, a similar reaction might occur during exposure to the male fleece and could mask the attractiveness of this cue. This explanation may also apply to the endocrine responses. Only the ewes exhibiting a low level of stress during exposure to the male fleece might present an endocrine response, and the facilitative action of sexual experience could result in a lower stress response to the olfactory cue.

In contrast to to naive females, the experienced ewes urinated more during exposure to the ram fleece than during exposure to the female fleece. Furthermore, the MALELAV females that have been previously exposed to males perfumed with lavender, urinated more in presence of the scented than the unscented fleece. These results suggest that the emission of urine is not a pre-programmed response but rather a response to an identified stimulus. The emission of urine in the presence of the ram or the lavender odor suggests that the females learned the association between the ram and its odor or between the ram and the artificial odor. In ungulates, the emission of urine by females is frequent during sexual interactions and is commonly followed by male flehmen (Banks, 1964; Ladewig *et al.*, 1980). In most breeds, the females urinate more in response to male courtship when they are in anestrus rather than in estrus (Stevens *et al.*, 1982; Bland and Jubilan, 1987). In this manner, females may signal to the males their physiological state since rams are able to distinguish between urine of anestrous and estrous females (Blissitt *et al.*, 1990). In another breed of sheep (Romanov) and in goats, however, females urinate more in the presence of males when they are in estrus (Fabre-Nys, personal observation). This behavior can serve to attract males and stimulate their sexual arousal, and may considered a sign of proceptivity similar vaginal marking by females hamster (Johnston, 1979). This same explanation is not applicable to our results since in the context of the male effect, ewes are in anestrus. The exact significance of female urine emission remains therefore unclear.

The involvement of learning processes in both endocrine and behavioral responses to the ram odor may related to the neural mechanisms mediating detection and integration of this chemosignal and the respective roles of both olfactory systems. In rodents, the neural pathway supporting the action of olfactory cues inducing neuroendocrine changes, involve direct connections with a limited number of synapses between the accessory olfactory bulb and the hypothalamic structures (Keverne, 1983; Brennan and Keverne, 1997). This pathway that bypasses cognitive centers of the brain, like the cortical areas, may account for pre-programmed and stereotyped responses. In contrast, the ram odor acts primarily through the main olfactory system: the destruction of the olfactory epithelium or the inactivation of the cortical amygdala, first relay of the main olfactory bulb, completely blocks the LH response to male odor (Gelez *et al.*, 2004b; Gelez and Fabre-Nys, 2004). The demonstration of learning processes is consistent with the preponderant role of the main olfactory system in the action of the ram odor, since cortical structures belonging to this pathway can proceed to an elaborate cognitive processing of the olfactory message. It is interesting to note that all other chemosignals that are integrated by the main olfactory system and require learning mechanisms, concern only behavioral responses, for example: pig (Dorries *et al.*, 1997) and ferret (Kelliher *et al.*, 1998). In comparison, the ram odor constitutes an original model of action of a primer pheromone. This may correspond to a strategy of reproduction to adapt efficiently the ewes' endocrine state to their changing environmental conditions, since in artiodactyls contrary to laboratory rodents, the risk of predation accentuates the importance of the reproductive events synchronization between sexual partners.

Acknowledgements

The authors thank Richard Porter for his correction of English and his valuable comments on the manuscript and Odile Moulin for her help with the illustrations.

References

Banks, E.M. (1964) *Some aspects of sexual behavior in domestic sheep, Ovis aries.* Behavior, 23, 249–279.

Bean, N.J. (1982) *Olfactory and vomeronasal mediation of ultrasonic vocalizations in male mice*. Physiol. Behav., 28, 31–37.

Bland, K.P. and **Jubilan, B.M.** (1987) *Correlation of flehmen by male sheep with female behaviour and oestrus.* Anim. Behav., 35, 735–738.

Blissitt, M.J., Bland, K.P. and **Cottrell, D.F.** (1990) *Discrimination between the odours of fresh oestrous and non-oestrous ewe urine by rams.* Appl. Anim. Behav. Sci., 25, 51–59.

Brennan, A. (2001) *The vomeronasal system.* Cell Mol. Life Sci., 58, 546–555.

Brennan, P.A. and **Keverne, E.B.** (1997) *Neural mechanisms of mammalian olfactory learning*. Prog. Neurobiol., 51, 457–481.

Brennan, P.A., Kendrick, K.M. and **Keverne, E.B.** (1995) *Neurotransmitter release in the accessory olfactory bulb during and after the formation of an olfactory memory in mice*. Neuroscience, 69, 1075–1086.

Carr, W.J., Loeb, L.S. and **Dissinger, M.L.** (1965) *Responses of rats to sex odors.* J. Comp. Physiol. Psychol., 59, 370–377.

Carter, C.S., Getz, L.L., Gavish, L., McDermott, J.L. and **Arnold, P.** (1980) *Male-related pheromones and the activation of female reproduction in the prairie vole (*Microtus ochrogaster*).* Biol. Reprod., 23, 1038–1045.

Cohen-Tanoudji, J., Lavenet, C., Locatelli, A., Tillet, Y. and **Signoret, J.P.** (1989) *Non-involvement of the accessory olfactory system in the LH response of anoestrous ewes to male odor.* J. Reprod. Fertil., 86, 135–144.

Dizinno, G., Whitney, G. and **Nyby, J.** (1978) *Ultrasonic vocalizations by male mice (*Mus musculus*) to female sex pheromone: experiential determinants.* Behav. Biol., 22, 104–113.

Dorries, K.M., Adkins-Regan, E. and **Halpern, B.P.** (1997) *Sensitivity and behavioral responses to the pheromone, androstenone, are not mediated by the vomeronasal organ in domestic pigs*. Brain Behav. Evol., 49, 53–62.

Doty, R.L. and **Dunbar, I.** (1974) *Attraction of beagles to conspecific urine, vaginal and anal sac secretion odors*. Physiol. Behav., 12, 825–833.

Fabre-Nys, C. and **Venier, G.** (1987) *Development and use of a method for quantifying female sexual behavior in ewes*. Appl. Anim. Behav. Sci., 17, 289–304.

Gelez, H. and **Fabre-Nys, C.** (2004) *The 'male effect' in sheep and goats: a review of the respective role of the two olfactory systems*. Horm. Behav., in press.

Gelez, H., Lindsay, D.R., Blache, D., Martin, G.B. and **Fabre-Nys, C.** (2004a) *Role of experience in the neuroendocrine control of ewes' sexual behavior.* Horm. Behav., 45, 190–200.

Gelez, H., Archer, E., Chesneau, D., Magallon, T. and **Fabre-Nys, C.** (2004b) *Inactivation of the olfactory amygdala prevents the endocrine response to male odour in anoestrus ewes*. Eur. J. Neurosci., 19, 1581–1590.

Goodman, R.L. and **Karsch, F.J.** (1980) *Pulsatile secretion of luteinizing hormone: differential suppression by ovarian steroids*. Endocrinology, 107, 1286–1290.

Graham, J.M. and **Desjardins, C.** (1980) *Classical conditioning: induction of luteinizing hormone and testosterone secretion in anticipation of sexual activity*. Science, 210, 1039–1041.

Hayashi, S. and **Kimura, T.** (1974) *Sex-attractant emitted by female mice.* Physiol. Behav., 13, 563–567.

Izard, M.K. and **Vandenbergh, J.G.** (1982) *The effects of bull urine on puberty and calving date in crossbred beef heifers.* J. Anim. Sci., 55, 1160–1168.

Jemiolo, B., Xie, T.M. and **Novotny, M.** (1991) *Socio-sexual olfactory preference in female mice: attractiveness of synthetic chemosignals*. Physiol. Behav., 50, 1119–1122.

Johnston, R.E. (1974) *Sexual attraction function of golden hamster vaginal secretion*. Behav. Biol., 12, 111–117.

Johnston, R.E. (1979) *Olfactory preferences, scent marking and 'proceptivity' in female hamster*. Horm. Behav., 13, 21–39.

Kelliher, K.R., Chang, Y.M., Wersinger, S.R. and **Baum, M.J.** (1998) *Sex difference and testosterone modulation of pheromone-induced neuronal Fos in the Ferret's main olfactory bulb and hypothalamus*. Biol. Reprod., 59, 1454–1463.

Kendrick, K.M., Lévy, F. and **Keverne, E.B.** (1992) *Changes in the sensory processing of olfactory signals induced by birth in sheep*. Science, 256, 833–836.

Keverne, E.B. (1983) *Pheromonal influences on the endocrine regulation of reproduction*. Trends Neurosci., 6, 381–384.

Keverne, E.B. (1986) *Non-olfactory chemoreceptors of the nose: recent advances in understanding the vomeronasal and trigeminal systems.* Chem. Senses, 11, 119–133.

Keverne, E.B. (1999) *The vomeronasal organ.* Science, 22, 716–720.

Keverne, E.B., Lévy, F., Guevara-Guzman, R. and **Kendrick, K.M.** (1993) *Influence of birth and maternal experience on olfactory bulb neurotransmitter release.* Neuroscience, 56, 557–565.

Kippin, T.E. and **Pfaus, J.G.** (2001a) *The development of olfactory conditioned ejaculatory preferences in the male rat. I.* Nature of the unconditioned stimulus*. Physiol. Behav., 73, 457–469.

Kippin, T.E. and **Pfaus, J.G.** (2001b) *The nature of the conditioned response mediating olfactory conditioned ejaculatory preference in the male rat*. Behav. Brain Res., 122, 11–24.

Knight, T.W., Tervit, H.R. and **Lynch, P.R.** (1983) *Effects of boar pheromones, ram's wool and presence of bucks on ovarian activity in anovular ewes early in the breeding season*. Anim. Reprod. Sci., 6, 129–134.

Ladewig, J., Price, E.O. and **Hart, B.** (1980) *Flehmen in male goats: role in sexual behavior.* Behav. Neural Biol., 30, 312–322.

Lévy, F., Gervais, R., Kindermann, U., Litterio, M., Poindron, P. and **Porter, R.** (1991) *Effects of early post-partum separation on maintenance of maternal responsiveness and selectivity in parturient ewes.* Appl. Anim. Behav. Sci., 31, 101–110.

Macrides, F., Bartke, A. and **Dalterio, S.** (1975) *Strange females increase plasma testosterone levels in male mice.* Science, 189, 1104–1106.

Macrides, F., Johnson, P.A. and **Schneider, S.P.** (1977) *Responses of the male golden hamster to vaginal secretion and dimethyl disulfide: attraction versus sexual behavior*. Behav. Biol., 20, 377–386.

Macrides, F., Clancy, A.N., Singer, A.G. and **Agosta, W.C.** (1984) *Male hamster investigatory and copulatory responses to vaginal discharge: an attempt to impart sexual significance to an arbitrary chemosensory stimulus*. Physiol. Behav., 33, 627–632.

Martin, G.B., Scaramuzzi, R.J. and **Lindsay, D.R.** (1983) *Effect of the introduction of rams during the anoestrous season on the pulsatile secretion of LH in ovariectomized ewes*. J. Reprod. Fertil., 67, 47–55.

Martin, G.B., Oldham, C.M., Cognié, Y. and **Pearce, D.T.** (1986) *The physiological responses of anovulatory ewes to the introduction of rams—a review.* Livest. Prod. Sci., 15, 219–247.

Maruniak, J.A. and **Bronson, F.H.** (1976) *Gonadotropic responses of male mice to female urine.* Endocrinology, 99, 963–969.

Meredith, M. (1986) *Vomeronasal organ removal before sexual experience impairs male hamster mating behavior*. Physiol. Behav., 36, 737–743.

Moncho-Bogani, J., Lanuza, E., Hernandez, A., Novejarque, A. and **Martinez-Garcia, F.** (2002) *Attractive properties of sexual pheromones in mice. Innate or learned?* Physiol. Behav., 77, 167–176.

Montgomery, G.W., Martin, G.B. and **Pelletier, J.** (1985) *Changes in pulsatile LH secretion after ovariectomy in Ile-de-France ewes in two seasons*. J. Reprod. Fertil., 73, 173–183.

Mori, K., von Campenhausen, H. and **Yoshihara, Y.** (2000) *Zonal organization of the mammalian main and accessory olfactory systems.* Philos. Trans. R. Soc. Lond. B Biol. Sci., 355, 1801–1812.

Murphy, M.R. (1973) *Effects of female hamster vaginal discharge on the behavior of male hamsters*. Behav. Biol., 9, 367–375.

Nyby, J., Whitney, G., Schmitz, S. and **Dizinno, G.** (1978) *Postpubertal experience establishes signal value of mammaliam sex odor.* Behav. Biol., 22, 545–552.

Pelletier, J., Kann, G., Dolais, J. and **Rosselin, G.** (1968) *Dosage radio-immunologique de l'hormone lutéinisante plasmatique chez le mouton: mise au point de la technique de dosage*. C. R. Hebd. Séanc. Acad. Sci., 266, 2291–2294.

Pfeiffer, C.A. and **Johnston, R.E.** (1994) *Hormonal and behavioral responses of male hamsters to females and female odors: roles of olfaction, the vomeronasal system, and sexual experience.* Physiol. Behav., 55, 129–138.

Reasner, D.S. and **Johnston, R.E.** (1988) *Acceleration of reproductive development in female Djungarian hamsters by adult males*. Physiol. Behav., 43, 57–64.

Schoenbaum, G., Chiba, A.A. and **Gallagher, M.** (1998) *Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning*. Nat. Neurosci., 1, 155–159.

Stevens, K., Perry, G.C. and **Long, S.E.** (1982) *Effect of ewe urine and vaginal secretions on ram investigative behaviour*. J. Chem. Ecol., 8, 23–28.

Terqui, M. and **Thimonier, J.** (1974) *New rapid radioimmunologic method for estimation of plasma progesterone. Application to early diagnosis of gestation in the ewe and goat.* C. R. Hebd. Séanc. Acad. Sci., 279, 1109–1112.

Tronel, S. and **Sara, S.J.** (2002) *Mapping of olfactory memory circuits: region-specific c-fos activation after odor-reward associative learning or after its retrieval.* Learn. Mem., 9, 105–111.

Vandenbergh, J.G. (1969) *Male odor accelerates female sexual maturation in mice.* Endocrinology, 84, 658–660.

Wysocki, C.J. and **Lepri, J.J.** (1991) *Consequences of removing the vomeronasal organ.* J. Steroid Biochem. Mol. Biol., 39, 661–669.

Accepted May 2, 2004