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Development of social familiarity in ewes

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ABSTRACT

We assessed the development of individual discrimination resulting from direct contact/familiarization in ewes. Unfamiliar ewes were introduced during 6, 24 or 72 h in groups of already familiarized ewes. At the end of this contact period, the development of social recognition with the animal that has been introduced was assessed using two different types of tests: a Y-maze preference test and a delayed paired close encounters test where tested ewes are successively and randomly interacting with the familiar animal and an unfamiliar conspecific. The results of both tests showed that ewes developed a recognition of the familiar animal in comparison to a completely unfamiliar female. However, this preference was evidenced after 24 h of contact when using the paired close encounters test whereas it appears only after 72 h when using the Y-maze test, suggesting that the paired close encounters test is a more sensitive methodology to assess the development of social familiarization. The importance of estrogens, in the formation of social familiarization was also evaluated. To this end, social recognition in the paired close encounters test was compared between ovariectomized animals receiving estrogen implants or not. Despite significant high levels of estradiol in estrogen implanted females, no major differences in recognition appeared between groups, suggesting that in our conditions estrogens do not have major influence on social familiarization.

1. Introduction

Familiarization with individuals resulting from direct exposure to their sensory signatures is the primary mechanism mediating social recognition in many vertebrate species. Previous studies have determined that familiarity plays an important role in the development of social recognition in sheep, a species living in large flocks where the ability to recognize conspecifics is highly developed. For example, in the context of mother–young relationship, ewes develop a recognition of their own lamb within a few hours of contact after lambing [1, 2]; in addition, lambs also respond selectively to their mothers and to agemates of their own group after a few days of contact [3, 4]. A very efficient recognition also exists between adult sheep. Ewes are able to discriminate familiar conspecific on the basis of various sensory cues. For example, sheep discriminate between frontal photographs of different breeds of sheep or between male and female [5]. In similar conditions, sheep also learn significantly faster to discriminate between frontal views of familiar conspecific faces compared with unfamiliar ones [6]. This recognition memory is very enduring since sheep can remember up to 50 different conspecific faces over 2 years [7]. Despite the existence of this efficient recognition between adults, its developmental time course has never been explored in details. In this context, the first series of experiments reported in this manuscript is an initial attempt to assess the development of individual discrimination resulting from direct contact/familiarization in ewes.

In addition, although results are sometimes inconsistent, it has been previously reported in rodents that gonadal hormones, particularly estrogens, can exert influences on social preference and social recognition [8, 9]. It has been demonstrated that mice lacking the estrogen receptor α (ERα) show impaired social recognition [10]. In agreement with these results, it has been also reported that long-term estrogens treatment improves social recognition in ovariectomized female rats [11, 12]. In sheep, social preferences of ewes in a two choice tests between faces of male and female widely differ depending on whether the animals are in estrus vs anestrus physiological status. While females in anestrus display a strong preference for face of female conspecific, the same animals show a profound shift in their social preference in the estrus condition, with a marked preference for the face of a ram [6]. In the context of sheep offspring recognition, the recognition processes are established during a short-time window characterised by the fact that the ewe is primed by estrogen and progesterone during the late gestation period and experience vaginocervical stimulation as a result of giving birth. Even if a role for other gonadal hormones cannot be discarded, these data suggest an influence of estrogens on the establishment of social preferences. Indeed, if lamb olfactory memory formation can be induced by artificial vaginocervical stimulation in non parturient ewes, this could

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only occur after ewes have been artificially primed with estrogens [13]. As a whole these results suggest that estrogens could play a main role in social memory formation in sheep. Therefore, in an additional step, we tested whether estrogens can modulate the establishment of social familiarization towards conspecifics (paired close encounters and Y-maze test).

2. Material and methods

2.1. Animals

The animals used were Ile-de-France (IDF; n = 114) ewes and a crossbreed between Romanov × Ile-de-France ewes (RIF; n = 47). The animals were aged between 2 to 6 years old. The RIF animals were used as unfamiliar animals because they had never been in contact with IDF ewes due to the fact that breeding procedures for different breeds are conducted separately in our experimental farm. Ewes of both breeds were kept permanently indoors in separate barns and fed with dehydrated lucerne, maize, straw, and a supplement of vitamins and minerals; they had free access to water. The ewes were individually identifiable by the use of ear tags. In each experience, ewes were randomly assigned to experimental groups and were tested only once to avoid possible habituation to the test. All experimental procedures were performed in accordance with local animal regulations (authorization no. A 37073, French Ministry of Agriculture) and with European Council Directive of 24 November 1986 (86/609/EEC).

The experiments were performed in spring, during the anestrus season, at the INRA station in Nouzilly, France. To be sure that all animals were in a similar and physiologically constant state, ewes were diagnosed as seasonally anovulatory by showing persistent low concentrations of circulating progesterone (<1 ng/ml) in weekly progesterone assays, indicating the absence of a functional corpus luteum. These assays were routinely performed with a method adapted from Terqui and Thimonier [14].

2.2. Development of social familiarization: general experimental procedure

At the beginning of the experiments, pairs (n = 2) of IDF ewes were introduced into pens (10 m^2). Pens were visually isolated from each other. After a further 10 days familiarization period between these two IDF ewes, an unfamiliar RIF ewe was introduced within each pair of IDF ewes. Therefore, groups of three females were formed, each comprised of two IDF ewes and one RIF ewe. After various durations following the introduction of the RIF ewe (independent groups with 6 h, n = 32; 24 h, n = 32; or 72 h of contact, n = 30), IDF animals were tested for social preference in two types of tests where their behavior was scored when interacting with the RIF familiar ewe and another unfamiliar RIF ewe, belonging to another group of three animals.

In these tests, our working hypothesis is that ewes will respond preferentially to their familiar conspecific (the RIF ewe introduced in their pen) in comparison to a unfamiliar animal as this has been already demonstrated in various situations [1, 15]. This type of discriminative interactions is used as a basis for inferring that the preferred animal is recognized (e.g. [16–18]). Social recognition is thus defined operationally as observable differential interactions or selective responsiveness among particular conspecifics.

2.2.1. Testing procedure: paired close encounters test

The first testing procedure used is a paired close encounters test which has previously been found to be an effective and simple method to assess discrimination between familiar vs unfamiliar conspecifics in sheep [4, 19]. Each ewe was tested twice: ewe was once paired with a familiar RIF stimulus ewe while during the other situation the ewe was tested with a RIF stimulus ewe with which she had no prior contact (unfamiliar RIF ewe). At the beginning of each test, the tested ewe was removed from its pen and introduced into a cage measuring 2 m × 2 m situated in a separate barn. After 2 min, a stimulus RIF ewe (either the familiar or the unfamiliar ewe) was introduced in the cage. The number of bleats emitted by each ewe during a 5 min period was then recorded with digital counters; other aggressive (head butts) and investigation (sniffings) behaviors were also recorded (sniffing behavior was defined as a movement of orientation of the nostrils towards the anogenital region or the head of a conspecific). At the end of the test, the ewes were immediately returned to their respective pens. Around 1 to 2 h later, the subject ewes were again tested but with a partner of the opposite category, i.e. with an unfamiliar ewe if they had been paired with a familiar partner for the first test and vice versa. Testing order was counter-balanced across all subject ewes, i.e. half of the animals were first tested with a familiar partner then later paired with an unfamiliar ewe, while the remaining half of the animals were tested in the reverse order. Based upon previous experiments performed in lambs using similar procedures [19], it was hypothesized that ewes paired with a familiar partner would emit fewer distress bleats as well as fewer aggressive and olfactory investigation behavior (head butts and sniffings) than when paired with an unfamiliar partner.

2.2.2. Testing procedure: simultaneous preference test in a Y-maze

The same subject animals were observed when exposed to two simultaneously present RIF ewes in a Y-maze consisted of a triangular enclosure (10 × 10 × 6 m), delimited by 1 m high, solid-metal barriers [1]. Two individual pens (2 × 1 m) made of fine wire mesh were located at each arm of the base of the enclosure and contained either the familiar or an alien ewe. Opposite these arms, a starting pen (2 × 1 m) served to hold the ewe before releasing her into the testing enclosure. The testing area was divided into three main zones by string: two 1 m-wide contact zones in front of the two stimuli ewe pens and a neutral zone. In each case, the unfamiliar RIF ewe had been housed in a different yard than the tested ewe. The position of familiar and unfamiliar ewes was reversed at each test. The ewe was left in the starting pen (2 × 1 m) for 1 min, giving her the opportunity to see and hear both stimulus ewes before being released. In all cases, both stimulus ewes had bleated before the tested ewe was released. Two experimenters recorded the total time spent in the contact zone near each stimulus ewe during 5 min. The tested ewe was scored as being in a contact zone when its four feet were entirely inside the string boundary. When the 5 min trial ended, the tested ewe was immediately returned to its own pen. The left and right location of the familiar and unfamiliar stimulus ewes was randomly determined for each test. To avoid neophobic reactions during the test, ewes were habituated to the experimental device for 5 min during 3 consecutive days prior the beginning of the experiment [1].

In each group of three animals, both IDF ewes were tested but only as experimental animals; therefore these IDF ewes were used only once in each type of test (paired close encounters and Y-maze test). RIF animals were used as stimulus animal: these animals were only used in a limited number of tests (4 times in each type of test) so that the potential effect of prior experience as a stimulus animal had only minor impact on subject's subsequent behavior. The order of each type of test (paired close encounters and Y-maze test) was counter-balanced with half of the animals being tested in the paired close encounters test first and the other half being tested with the Y-maze test first.

2.3. Influence of estrogens on social familiarization

Sexually mature Ile-de-France ewes (n = 20) were ovariectomized at least 1 month before experimentation. One week before the study, half of the animals (n = 10) were implanted s.c. with a 16-cm Silastic capsule containing 17β-estradiol (Sigma, St-Louis, MO, USA) to maintain a high but physiological circulating concentrations of estrogens (peak follicular phase concentration, approx. 10–15 pg/ml, [20]). This