Preference of 12-h-old kids for their mother goat is impaired by pre-partum-induced anosmia in the mother

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We investigated whether kids were able to discriminate their own mother from an alien one in a two-choice test on the day of birth when they had access to acoustic, visual and olfactory cues from their mother, and whether this discrimination depended on the selective maternal behaviour of the mother (i.e. exclusive nursing of own kids). When given the choice between their own mother and an alien equivalent dam, 8-h-old kids did not show a significant preference for their dam, whereas 12- and 24-h-old kids did. When given the choice between their own and an alien mother that were both non-selective because they had been rendered peripherally anosmic by irrigation of the nostrils with zinc sulphate, 12-h-old kids did not show a significant preference for their mother. These results are similar to those reported in sheep and may suggest that the contrast of behaviour between their own and an alien mother existing in normosmic does is important for discrimination of dams by kids at this age. Finally, testing 8-h-old kids in a smaller enclosure resulted in some improvement of their performance, although they still failed to display a significant preference for their mother. On the whole, kids are able to discriminate between their own and an alien mother goat as early as previously reported in lambs. The impairment of this ability when mothers are anosmic and not selective suggests that acceptance behaviours displayed by the mother may serve as one of the cues orientating the choice of the kid when given the choice between intact mothers. Finally, the present results do not suggest the existence of fundamental differences in the establishment of a preference for the mother between lambs, which are followers, and kids, which are hiders.

Keywords: anosmia, maternal behaviour, mother recognition, neonates

Introduction

In ungulates, two main types of mother–young relationships have been described, depending on the behaviour of the neonate. On the one hand, some species give birth to neonates that start to follow their mother as soon as she moves from the birth site, and which are therefore classified as ‘followers’ (Lent, 1974). In other species, neonates do not follow their mother when she goes foraging. Rather, for the first few days they lie down and hide, waiting for the mother to return for nursing, hence their classification as ‘hiders’. Sheep belong to the first category, and the system of mother–young recognition has been extensively studied in this species (Lévy et al., 1996; Kendrick et al., 1997; Nowak et al., 2001). In contrast, work on goats, especially regarding the neonate, is rather scarce and little information is available concerning the establishment of maternal recognition by the newborn kid. In the sheep, the neonate develops a preference for its dam within 12 to 24 h (Nowak et al., 1987; Val-Laillet et al., 2004), initially on the basis of the acceptance behaviours displayed by the mother (Terrazas et al., 2002), followed later by true recognition of her individual physical features. Thus, lambs become able to choose between their mother and an alien dam from a distance of several metres from day 3 of age onwards (Nowak, 1990) and can recognise her bleats (Sébe et al., 2007).

Goats, in contrast to sheep, belong to the hider category, whether under feral or domestic conditions (Lent, 1974; Lickliter, 1985). Studies concerning the discrimination of the kid by its dam have shown that the mechanisms involved are similar to those found in sheep (Romeyer et al., 1993, 1994a and 1994b; Poindron et al., 2003). In contrast, it is not known whether the hiding behaviour of kids is associated with marked differences in their discrimination abilities compared with that of lambs. In particular, it is not

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known whether the hiding behaviour of kids is associated with a later display of discrimination of the dam than in lambs. There is some evidence that kids can show a preference for their mother at 2 days of age (Lickliter and Heron, 1984; Addae et al., 2000) but not earlier, whereas lambs already discriminate their dam at 12 h (Nowak et al., 1987; Terrazas et al., 2002; Val-Laillet et al., 2004). While this difference may be related to the fact that kids hide and lambs do not, it may also depend on other factors. Thus in sheep, breed is well known to influence the early ability of the mother and of the neonate to recognise each other (Shillito-Walser et al., 1982; Nowak et al., 1987). It cannot be excluded that the same applies in goats, especially since Addae et al. (2000) suggested that mothers of the breed they studied had poor learning abilities. Indeed, they found that recognition was not clearly established before 48 h post partum, whereas in a recent study using a similar testing situation we found that crossbred dairy-type goats already recognised their kids at 4 h post partum (Poindron et al., 2003). Therefore, we investigated whether 8-, 12- and 24-h-old kids displayed a preference for their mother in a two-choice test and, if so, whether this initial preference depended on differences in maternal selective behaviour between the own and the alien dam, as reported in sheep (Terrazas et al., 2002). To assess this question, we studied the response of 12-h-old kids given the choice between two anoxic mother goats, both of which displayed acceptance behaviours, since peripheral anosmia induced before parturition by irrigation of the nostrils with zinc sulphate prevents the development of maternal selectivity (Romeyer et al., 1994a; Hernández et al., 2004). Finally, we also investigated whether the size of the testing pen could influence the display of an early preference for the mother; we compared the performances of 8-h-old kids tested in a pen similar to that used for lambs, with that of kids tested in a smaller pen and placed closer to the dams at the beginning of the test.

Material and methods

General conditions of study and management before the test

The experiment was carried out in June 2000 and April 2001 at the experimental unit of the Institute of Neurobiology (UNAM), located on the grounds of the farm belonging to the Veterinary Faculty of the Autonomous University of Querétaro (Mexico). Different animals were used each year. The subjects were 87 kids (45 females and 42 males; 12 singles, 49 twins and 26 triplets) and their 47 multiparous Mexican mixed-breed mothers (dairy type). The animals were kept under intensive conditions at a density of approximately 10 m² per mother, with a section of the pens provided with a sheltered area for protection from sun and rain. Animals were fed lucerne hay, minerals and concentrate pellets (18% crude protein; Generaleche, Purina®) according to their gestation status, and water ad libitum. Oestrus was synchronised in the does using vaginal sponges (fluorogestone acetate, 45 mg; Intervet®), Holland) for 19 days, followed by an injection of pregnant mare serum gonadotropin (Folligon, Intervet®), 300 IU intramuscular. Females were fecundated by active bucks (Ferreira et al., 2000; Poindron et al., 2003; Lévy et al., 2004 for review). It is important to note that in the present paper, maternal selectivity refers specifically to this aspect of maternal recognition, which relies on maternal olfaction. Indeed, while anoxic ewes and goats are able to recognise their young in a two-choice tests by sight and/or hearing, they do not display rejection behaviours towards alien young that intent to suck (Ferreira et al., 2000; Poindron et al., 2003; Lévy et al., 2004). Also, it is important to note that in the present study, in all cases the kids had full access to visual, acoustic and olfactory cues from the dams.

As the number of subjects studied in 2000 was only about 60% of the number originally planned (12 at 8 h, 7 at 12 h, 9 at 24 h with intact mothers and 11 at 12 h with anoxic mothers), the experiment was repeated the following year. The maintenance conditions and feeding of the

Experimental groups and testing procedure

To test the ability of kids to discriminate between their own mother and an alien equivalent one, we used a two-choice test paradigm that had already been used previously with success in lambs and kids (Lickliter and Heron, 1984; Nowak et al., 1987; Addae et al., 2000; Awotwi et al., 2000). Kids were given the choice between their mother and an alien equivalent goat having given birth at about the same time, each mother being placed in one corner of a triangular (experiments 1 and 2, 1st and 2nd year) or rectangular (experiment 3, 2nd year) testing pen. The 1st year, the aim of the study was to test the ability of kids to discriminate between their mothers in three age groups (8, 12 and 24 h) and to investigate if their performance would be impaired by the experimental suppression of maternal olfaction before parturition, as reported in sheep (Terrazas et al., 2002). In both species, pre-partum-induced anosmia induced surgically by olfactory bulb ablation, nerve severing or destruction of the olfactory mucosa by zinc sulphate prevented the establishment of exclusive nursing of the own young by its mother that relies on olfactory recognition by the dam (see Lévy et al., 2004 for review). It is important to note that in the present experiment, maternal selectivity refers specifically to this aspect of maternal recognition, which relies on maternal olfaction. Indeed, while anoxic ewes and goats are able to recognise their young in a two-choice tests by sight and/or hearing, they do not display rejection behaviours towards alien young that intent to suck (Ferreira et al., 2000; Poindron et al., 2003; Lévy et al., 2004). Also, it is important to note that in the present study, in all cases the kids had full access to visual, acoustic and olfactory cues from the dams.

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animals, the location of living and testing pens, as well as the testing and observation procedures were the same in both years. In addition, results from the 1st year suggested that 8-h-old kids failed to show a preference for their mother. Since kids may be less active than lambs due to their hiding tendency, in the 2nd year we completed the initial groups and we constituted an additional group to verify whether the lack of preference could be due to the size of the testing pen being too large, associated with a low mobility of the kids. In this latter group, 8-h-old kids were tested in a smaller pen.

**Discrimination of intact/selective mothers by their kids.** Twenty-two kids (3 singles, 12 twins and 7 triplets) were tested 8 h after birth, 11 (1 single, 7 twins, 3 triplets) at 12 h and 18 (3 singles, 10 twins, 5 triplets) at 24 h, using different animals in each group, balancing the order of testing between the three groups over time as evenly as possible. The testing pen was located in a roofed building adjacent to the holding pens of the pregnant and post-parturient animals. The tested subjects could only see the two mothers in the testing pen, but acoustic isolation from the rest of the flock was not possible. The testing pen consisted of a triangular area (6 m × 6 m × 6 m), delimited by a 1-m-high metal barrier (Figure 1). Thirty minutes before the test, the two stimulus mothers to be used in the test and their kids were introduced in the central part of the testing pen for habituation, and remained there until the time of test. The alien mother was an unrelated female from the same living pen, having given birth at most 24 h before the mother of the tested kid. For the test, all kids except the one to be tested were removed and kept in a pen outside the building and out of the mothers’ sight. The two mothers were placed in their respective holding zone by one experimenter (Figure 1), the side of the own mother being alternated between tests and unknown to the two observers recording the behaviour of the kid. The kid to be tested could not see in which pen its dam was placed. The holding pens for the mothers were made of open-work metal panels allowing the animals fully to see and hear each other, as well as smell each other when at close quarters. Also in all cases, even though the exact number of vocalisations of the two stimulus mothers could not be recorded, it was noted that both goats were repeatedly bleating during the test, thus ensuring that kids had access to vocal cues. The kid was then placed in the starting zone of the pen and kept there for 30 s, so it could see and hear the does, before being released for the 5-min test. Two trained observers, using preformatted recording sheets and chronometers, recorded the following four variables. (a) Latency to leave the waiting zone (s). If it took the kid longer than 30 s to leave this zone, it was gently pushed out with a broom and given a 31-s latency. (b) Latency to reach a mother: time at which the kid first reached a contact zone after leaving the waiting zone. Kids that did not reach a contact zone during the test were given a latency of 301 s and not included in the analyses of this variable. (c) Identity of mother (left or right) that the kid reached first. (d) Time spent in each contact zone (s). The kid was considered to be in a given zone when it has at least the two front feet in that zone.

The proportions of kids leaving the waiting pen or reaching a mother, as well as the latency to leave the waiting pen, provided information on the general activity of the kids and was analysed in all kids. The proportion of kids that reached their mother first and the time spent near the mothers gave indications about their discrimination ability and were analysed only in kids that reached at least one dam during the test.

**Discrimination of anosmic/non-selective mothers by their 12-h-old kids.** Nineteen 12-h-old kids (2 singles, 11 twins, 6 triplets) born from anosmic goats were tested in the pen shown in Figure 1. In this group, each kid was given the choice between its own and an alien equivalent mother, both of which had been rendered anosmic 1 year before this study by irrigation of the nostrils with a 1.5% solution of zinc sulphate. It is well documented that in ewes and does, anosmia induced by zinc sulphate during pregnancy prevents the establishment of olfactory recognition of the neonate by its mother and the development of selective maternal care. As a consequence, in both species, anosmic mothers display maternal acceptance towards an alien young as well as towards their own young (Romeyer et al., 1994a; Hernández et al., 2004; Lévy et al., 2004). The goats had been rendered peripherally anosmic by irrigation of the olfactory mucosa with a 1.5% zinc sulphate solution 1 year before this study, according to the method of Romeyer et al. (1994a). All females were anosmic and non-selective at the time of the test, as assessed by behavioural tests performed just after the two-choice tests (see Hernández et al., 2002).
for details of anosmia and selectivity tests). In the present paper, we used the term anosmia, on the grounds that zinc-sulphate-treated animals were behaviourally anosmic to the food preference and maternal selectivity tests used to evaluate the efficacy of the treatment. For animal care and experimental treatments, including anosmia, guidelines A37801 for animal experimentation of the French Ministry of Agriculture were used. Anosmia did not induce observable changes in the general behaviour of the females, their body weight or the body weight of the kids. All testing procedures and recorded variables were the same as with intact mothers.

Influence of the size of the testing pen on maternal discrimination in 8-h-old kids. Seventeen 8-h-old kids (3 singles, 9 twins, 5 triplets) were tested in a pen that consisted of a rectangular corral 4 m wide × 3 m deep, the kids being placed at a distance of 1.5 m from the contact zones (Figure 2). As there was no waiting zone, the test started as soon as the kid was placed in the testing pen. Other variables were the same as for the other groups. Allocation of the 17 kids to the test small pen group and of the 10 kids tested the 2nd year in the large pen was balanced over time at the rate of about one kid tested in the large pen every 3rd test.

Data analysis
The results are presented as medians and quartiles. Within each group, data were compared with the Wilcoxon test, while the comparisons between groups were carried out with Kruskal–Wallis or Mann–Whitney U-tests. Between-group comparisons of proportions were carried out using the χ² and Fisher’s exact probability tests. The binomial test was used to test the hypothesis of a choice at random. Bilateral probabilities were used, except for the binomial test. Possible differences in performances due to sex of the kids or litter size were tested globally and within each group with Mann–Whitney U-tests. As no significant effects of these variables were found (P > 0.17 in all cases), these variables were not taken into account in further analyses. Statistics were carried out using the statistical package Systat 10 of Statistical Packages for the Social Sciences (2000).

Results

Preference for the mother on the 1st day of life
The proportion of kids that did not enter a contact zone was significantly higher in 8-h-old kids than in the two other groups (Table 1). Among the kids that reached a mother, the latency to reach a doe did not differ significantly between groups (Table 1). The proportions of kids that reached a mother but had to be pushed out of the starting area did not differ between groups (Table 1). In the groups of kids tested 8 and 12 h after birth, the proportion of kids that reached their mother first did not differ from a random choice (binomial test, P = 0.19 and 0.17, respectively). However, kids tested 24 h after birth reached their mother first more frequently than expected by chance (binomial test, P = 0.023). The proportion of first correct choice also

![Figure 2](https://doi.org/10.1017/S17517311107000626) Published online by Cambridge University Press
The proportion of kids reaching a mother tended to be higher in the small pen than in the large one (Fisher’s exact test, \( P = 0.09 \); Table 1). The latency taken by kids to reach a mother did not differ significantly from that found in the large pen (Mann–Whitney \( U = 67, P = 0.38 \); Table 1). For those kids that reached a mother, the proportion of them reaching their mother first still did not differ from a random choice (binomial test, \( P = 0.60 \)) or from the proportion found in the large pen (Fisher’s exact test, \( P = 0.45 \); Table 1). Nonetheless, the total time spent with the two dams tended to be higher for kids tested in the small pen (209 s [156–255], v. 110 s [72–212]; Mann–Whitney \( U = 48.5, P = 0.07 \)). Also, kids that reached a dam tended to spend more time near their mother in the small pen (134 s [47–233] v. 40 s [0–115.5]; Mann–Whitney \( U = 48.5, P = 0.07 \)), whereas this was not the case for the time near the alien dam (26 s [0–85] v. 51 s [12–97]; Mann–Whitney \( U = 66, P = 0.35 \)). Besides, in the small pen, there was no statistical difference between the time kids spent with their own mother and with the alien doe (own: 134 s [47–233]; alien: 26 s [0–85]; Wilcoxon \( z = –1.48, P = 0.14 \); Figure 3).

However, the percentage of time spent near the own mother relative to the total time spent near the two stimulus females was significantly higher than for kids tested in the large pen (84% [45–100] v. 32% [0–93]; Mann–Whitney \( U = 45.5, P = 0.04 \)).

Preference for the mother by 12-h-old kids when both dams were anosmic and non-selective

The proportion of 12-h-old kids tested with anosmic mothers that did not reach a dam during the test tended to be higher than for kids tested with intact mothers, even though they were gently pushed with a broom (Fisher’s exact test, \( P = 0.10 \); Table 1). Among kids that reached a goat during the test, the latency to reach a doe did not differ from that found in kids tested with intact mothers (Table 1). The proportion of these same kids that had to be pushed out of the starting area did not differ from that found in kids tested with intact dams (Fisher’s exact test, \( P = 0.39 \)) and the proportion of kids reaching their own mother first did not differ from a random choice (binomial test, \( P = 0.27 \); Table 1). The total time spent by kids in the contact zones near the two dams did not differ from that found for kids tested with intact mothers (226 s [191.5–249.5]; Mann–Whitney \( U = 36, P = 0.18 \)). Also, the time spent near their own mother (135 s [33.5–193.5]) did not differ significantly from that spent near the alien dam (70 s [0–149]; Wilcoxon \( z = 0.71, P = 0.48 \)), contrary to the results of kids tested with intact mothers (Figure 3). Nevertheless, none of the studied variables differed significantly between the group of kids tested with two intact mothers and the group tested with two anosmic mothers (Mann–Whitney \( U \)-tests, \( P \geq 0.18 \) in all cases).

Discussion

Three main conclusions can be drawn from the results of the present study. First, kids can already display a preference for their own mother at 12 h of age in a two-choice situation, whereas this does not seem yet to be the case at 8 h for a majority of kids, even when they are placed relatively close to the does (2 m). Second, the fact that discrimination is impaired when kids are tested with two anosmic and non-selective dams suggests that the behaviours characterising maternal acceptance play some role in the initial preference shown by 12-h-old kids for their mother. Third, these results do not support the hypothesis that the time at which early recognition is established...
Maternal recognition by 12-h-old kids

differs between newborn lambs and kids, despite the fact that lambs are followers and kids are hiders.

The results obtained in 12- and 24-h-old kids indicate without ambiguity that kids are able to discriminate their mother at those ages, even though they only do so at close quarters. This is at variance with the findings of Addae et al. (2000) and Awotwi et al. (2000) in dwarf African goats. The most likely explanation for this difference is that the slow learning abilities mentioned for the mothers in their studies (Addae et al., 2000) also applied for the kids. The possibility that some differences in breed exist between the dwarf African goat and the type of goats we used is also supported by the response of the animals to separation. Indeed, both in the present experiment and in a previous study of the recognition of the kid by the mother in the same type of goats (Poindron et al., 2003), animals were highly responsive to the testing situation, despite the fact that mothers and kids had been separated from each other only for a few minutes before the test. In contrast, in the studies of Addae et al. (2000) and Awotwi et al. (2000), animals did not display sufficient motivation in the test with less than 2 h of separation.

Whether kids are able to discriminate their mother before 12 h of age is not clear. The high proportion of kids that failed to reach a mother at 8 h in the large pen may suggest that discrimination is not yet established at that time, but it is also possible that this poor performance is related to the hiding behaviour of the kids. Thus, the tendency for more kids to reach a mother (regardless of her identity) in the small pen than in the large pen and to spend more time with both dams suggests that seeing the dam from a short distance is important to elicit an approaching response in kids. In addition, testing kids in a small pen seemed to facilitate the display of a preference for those kids that did reach a mother, as these kids tended to spend more time with their own dam than with the alien one. It is possible that because of their hiding behaviour, neonates do not have a strong motivation to walk towards a mother from a distance of several metres. Also, the improvement of the discrimination performances of the kids during the 1st day of life could depend on their motor activity, which at 8 h may still be insufficient for this type of test. Nonetheless, it must be noted that for kids that did reach a mother, latencies to do so did not differ depending on the age of the kids, suggesting that more than motor ability it is the response to the presence of the dams that may change with age. Besides, the criteria used in the present experiment and the experimental testing situations may not have been the most adequate to evidence this discrimination in kids of that age. Hence, we cannot exclude the possibility that 8-h-old kids may discriminate between their own and an alien doe at this early age. Additional studies including other criteria of a preference for the mother in very young kids would help clarify this point.

The impairment of the preference for the own mother in 12-h-old kids, given the choice between two anosmic dams, is similar to the results reported for lambs (Terrazas et al., 2002). The behaviour of anosmic does was not monitored in the present study during the test or at parturition. Therefore, it cannot be excluded that the impairment of the performance of kids tested with anosmic mothers may be partly caused by some early disturbance of maternal behaviour, which in turn would affect the development of the kid’s preference, rather than by the behaviour of the goats during the test. Further studies are warranted to clarify this and it would be particularly interesting to verify whether the latency to first nursing and early frequencies of suckling are affected by anosmia, given the important role of the first suckling activity of the neonate for its bonding to the dam in sheep (Nowak et al., 1997). Nonetheless, in sheep, the results of Terrazas et al. (2002) show that if both the lamb’s own mother and an alien mother are anosmic, they do not behave differently. Furthermore, it is well established that anosmic goats display similar acceptance behaviours towards alien kids as intact goats do towards their own kid (Romeyer et al., 1994a; Hernández et al., 2004). Therefore, our present results suggest that newborn kids first develop a preference for behaviours indicative of maternal acceptance, before developing the ability to recognise individual characteristics of their dam, as it is the case in lambs. However, this phase of preference for acceptance behaviours is probably of short duration, since 24-h-old kids already chose their dam from a distance, which presumably depends on the recognition of individual visual and/or acoustic maternal cues. Which behaviours of the mother may serve as a basis for discrimination has not been investigated in the present study. Results in sheep indicate that maternal vocalisations (Terrazas et al., 2002), the motor behaviour of the female and her posture during the test (Nowak et al., 2001) may serve as a basis for discrimination. Also, studies in sheep indicate that this early preference of the neonate for its mother depends on a learning process, rather than on a spontaneous preference for some key stimuli from the ewe (Nowak et al., 1997; Val-Laillet et al., 2004). While the sensory cues used by the neonate to make its choice remain to be identified, it is clear that olfaction does not play a major role in the present two-choice test situation. If this had been the case, kids would have been able to discriminate their mother from the alien doe on the basis of olfactory cues, regardless of the fact that both dams displayed acceptance behaviours as a consequence of maternal anosmia.

As a whole, the results of the present study do not support the hypothesis that newborn kids are slower in developing an early preference for their mother than lambs. Therefore, the following or hiding behaviour of the neonate does not seem critical in that respect. Two main factors may explain this lack of difference. First, the pressure to establish rapidly a preferential mother–young bond ensuring proper maternal investment is probably similarly high in the two species. Indeed, sheep and goats are both gregarious and present seasonal synchronised breeding leading to a high probability of numerous births concentrated over a short period. Second, in both species there is an initial
phase of intense interacting between the mother and her neonate(s) that lasts several hours and with a similar behavioural repertoire and pattern in sheep and goats (Hersher et al., 1963). Therefore, factors possibly common to the two species, other than the hiding or following strategy of the neonate, are certainly involved during this early period. For example, a very general factor that may stimulate early learning in the neonate is the experience of labour itself, which has recently been found to facilitate early olfactory exposure learning in human babies, possibly through the stimulation of catecholamines release (Lagerkrantz, 1996; Wang et al., 1999; Varendi et al., 2002).

Another important factor for the establishment of early preferences is the sucking activity of the neonate (Hall, 1987). In particular, in the lamb the development of the initial preference for the mother depends on an associative process involving the first nursing bouts (Val-Laillet et al., 2004). Therefore, it would be worthwhile to investigate whether association during early nursing also occurs in kids, given the similarity of the immediate post-partum care of the neonate in the two species.

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