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Involvement of central muscarinic receptors in social and nonsocial learning in sheep

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Abstract

Within 12 h following parturition, ewes develop visual and auditory recognition of their lamb. To investigate whether central cholinergic muscarinic transmission plays a specific role in this social learning, we studied the effects of a muscarinic antagonist on neonate recognition via visual/auditory cues and acquisition of a nonsocial visual discrimination. Injections of scopolamine (100 μg/kg; a muscarinic antagonist crossing the blood–brain barrier) after birth did not affect maternal behavior but impaired visual/auditory recognition of the offspring. Recognition impairment did not occur in mothers treated with methylscopolamine (100 μg/kg; a peripheral muscarinic antagonist), indicating that central muscarinic transmission is specifically involved in this social learning. Similar doses of scopolamine strongly delayed learning of a nonsocial, visual discrimination task in comparison to either control or methylscopolamine-treated ewes. Performance on this task was not affected when scopolamine treatment was applied after learning, demonstrating that central muscarinic receptors are necessary for acquisition but not for retrieval. These results suggest that the central muscarinic transmission participates in the establishment of visual/auditory recognition of conspecifics. Moreover, activation of central muscarinic receptors is critical for learning regardless of the social properties of the stimulus.

Keywords: Acetylcholine; Scopolamine; Social learning; Maternal behaviour; Sheep; Visual discrimination

1. Introduction

In mammals, the central cholinergic system is involved in cognitive functions including learning and memory processes (Jaffard and Micheau, 1994; Everitt and Robbins, 1997; Hasselmo, 1999; Harder et al., 1998). Whereas the cholinergic influence was largely investigated in nonsocial learning tasks, only a few experiments have related the importance of this system in social memory. Systemic injection of a centrally acting cholinergic muscarinic antagonist, scopolamine, impaired short-term memory of conspecifics in rats (Anglade et al., 1999; Perio et al., 1989; Soffié and Lamberty, 1988) and mice (Winslow and Camacho, 1995) and prevented the formation of offspring-recognition memory in sheep (Ferreira et al., 1999; Lévy et al., 1997). Nevertheless, these types of recognition memories are based on experiments that deal only with olfactory recognition, and no one has investigated the effect of cholinergic drugs in nonolfactory social learning. On the other hand, several studies have found disruption by scopolamine treatment of the learning of visual or auditory discrimination tasks using nonsocial stimuli (Harder et al., 1998; Rudy, 1996).

In sheep, recognition of conspecifics is mediated by different sensory modalities (Ferreira et al., 2000; Kendrick, 1998; Peirce et al., 2000). In particular, ewes are able to rapidly and individually recognise their offspring with olfactory and nonolfactory cues, i.e., visual and auditory cues. Learning the lamb's smell, which mediates selective suckling behaviour, develops within 2–4 h postpartum (Poindron and Le Neindre, 1980; Lévy et al., 1995a), whereas visual and auditory recognition allows location of the lamb at a distance and is evident after 12 h of mother–young contact (Ferreira et al., 2000; Terrazas et al., 1999). Moreover, establishment of the visual and auditory recognition of the offspring is independent of the memorisation of olfactory cues of the lamb as revealed by a similar devel-

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opment of visual and auditory recognition in anosmic and intact ewes (Ferreira et al., 2000). To date, neurobiological studies have focused on the lamb olfactory memory with particular attention on the central cholinergic system. Indeed, systemic injections of the central muscarinic antagonist, scopolamine hydrobromide (100 μg/kg), during the first hours postpartum impaired lamb olfactory recognition by interfering with acquisition (Lévy et al., 1997) and post-acquisition processes (Ferreira et al., 1999) but not with the retrieval or the expression of this memory (Ferreira et al., 1999; Lévy et al., 1997). Injections of the same dose of scopolamine methylbromide, which does not cross the blood–brain barrier, were ineffective therefore demonstrating the involvement of central, but not peripheral, muscarinic transmission in the establishment of individual lamb odour memory. Concerning the formation of visual and auditory memory of the lamb, the neurochemical basis involved has never been explored. According to the independence of both types of offspring recognition, it may differ from olfactory recognition.

The present study was thus designed to investigate the involvement of the central cholinergic system in the visual and auditory recognition of the lamb. For this purpose, the effect of central versus peripheral blockade of muscarinic transmission during the formation of lamb visual and auditory memory (i.e., the first 12 h postpartum) was investigated in a nonolfactory recognition test performed at 12 h postpartum. Moreover, because social memory could have a neural basis distinct from other forms of memory (Ferguson et al., 2000; Everts and Koolhaas, 1997), the effects of scopolamine were compared with those obtained on a nonolfactory instrumental discrimination learning using two visually distinct buckets. This task was chosen because of its dependence on the integrity of the central cholinergic system in rodents and primates (Ellen et al., 1986; Harder et al., 1998; Ridley et al., 1984).

2. Materials and methods

2.1. Animals and housing

Animal care and all procedures were in accordance with authorisation A 37801 of the French Ministry of Agriculture and ECC directive 86/609/EEC. Seventy-eight adult Île-de-France ewes were used. They were permanently housed in sheds and fed with dehydrated lucerne, maize, straw, and a supplement of vitamins and minerals. They had free access to water.

2.2. Visual and auditory lamb recognition

Subjects were 50 multiparous parturient ewes aged 2 to 5 years (weight range after birth: 60–70 kg). Reproductive function was fully synchronised with vaginal sponges impregnated with progesterone and injections of pregnant mare-stimulating gonadotrophins to induce ovulation, and dexamethasone (16 mg Dexadreson, Intervet, on Day 144 of gestation) to induce parturition. Parturition occurs within a period of 36 h beginning 36 h after the dexamethasone injections. This method of inducing birth has not been found to affect the establishment of maternal behaviour (Poindron et al., 1979).

One week before parturition, females were housed in individual pens (2 × 1 m) and accustomed to human presence and handling. After parturition, the ewe and her offspring were left together in the individual pen where the animals were allowed to interact freely and the presence of maternal behaviour was verified. Four hours after parturition, four to six ewes and their lambs were put together in a larger pen (3 × 8 m) to allow some degree of mother–young separation and mother–alien interactions. They remained there until the time of testing, i.e., 12 h after parturition.

From birth to 10 h postpartum, ewes received an intramuscular injection every 2 h, i.e., six injections per ewe of 5 ml of either isotonic saline (L for lamb experiment, Lcontrol group, $n = 16$), scopolamine methylbromide (Sigma, St. Louis, MO) at 100 μg/kg (Lmscop group, $n = 14$), or scopolamine hydrobromide (Sigma) at 100 μg/kg (Lscop group, $n = 20$). The drugs were dissolved in 5 ml of isotonic saline and the dose used was chosen according to previous experiments (Ferreira et al., 1999; Lévy et al., 1997). The injection protocol used allows the maintenance of a constant concentration of the antagonist during the first 12 h of mother–young contact because it has been reported that the plasma half-life of scopolamine is about 120 min after intramuscular injection (Ali-Melkkila et al., 1993).

Visual and auditory recognition were assessed with a nonolfactory test performed at 12 h postpartum in the same testing conditions as those described in previous studies (Ferreira et al., 2000; Terrazas et al., 1999). The testing pen consisted of a triangular enclosure (10 × 10 × 10 m) delimited by 1-m-high solid metal barriers. Two individual pens (2 × 1 m) made of widely spaced bars were located at each corner of the enclosure's base and contained either the familiar or an alien lamb, with the side on which the own lamb was placed changed at every test. Alien lambs were of matching size and age and came from a different pen than the subject ewe. In the case of twins, only one of the familiar lambs was tested. A barrier, made of widely spaced bars, was situated 1 m in front of the individual pens. This prevented the ewes from having access to the lamb's olfactory cues because it has been shown that the individual odour of the lamb cannot be discriminated at greater than 0.25 m (Alexander, 1978; Alexander and Shillito, 1977; Shillito-Walser and Alexander, 1980; see also Terrazas et al., 1999 for an extensive discussion of this point). In this condition, primiparous ewes, which develop a rapid olfactory recognition of the lamb at suckling (within 2–4 h after parturition), are completely unable to discriminate between their own lamb and an alien at 12 h postpartum (Keller et al., in preparation). This rules out the involvement of olfactory cues from the lambs in this two-choice test and indicates that mothers rely exclusively on visual and/or auditory cues to recognise their lamb. In the third corner, a starting pen (2 × 1 m) served to hold the ewe for 30 s in the testing enclosure, giving her the opportunity to see and hear both lambs before being released. The testing area was divided into three main zones: two 1-m-wide contact zones in front of the two lamb pens and a neutral zone. According to previous experiments (Ferreira et al., 2000; Terrazas et al., 1999), three relevant behavioural parameters were recorded by two experimenters during the 3-min test: latency to reach a contact zone, the total time spent in the contact zone near each lamb, and the total time spent looking at each lamb regardless of the position of the ewe in the testing area. Looking was defined as the rear–front axis of the head of the ewe being directly orientated towards a lamb. With these data, we calculated:

- the percentage of time spent in the contact zone near the familiar lamb as defined by [(time spent near the familiar lamb/time spent near both lambs) × 100].
- the percentage of time spent looking at the familiar lamb performed by similar calculation.

Moreover, for a synthetic overview of the results, each ewe was categorised as showing a preference for the familiar lamb when the mean of both percentages (time in the contact zone and looking time) was $\geq 60\%$. This criterion usually allows a clear characterisation of lamb recognition by mothers (Ferreira et al., 2000; Terrazas et al., 1999).

2.3. Instrumental visual discrimination task

Twenty-eight cycling adult ewes, aged 3 to 6 years (weight range: 55–65 kg), different from those used in the visual and auditory recognition experiment were submitted to the visual discrimination task. Cycling ewes were preferred to parturient animals because mothers cannot be separated from their lambs without major behavioural disturbances and the presence of the lamb could interfere with the learning performance.

For this task, two visually distinct buckets were presented and the sheep obtained a food reward when the appropriate bucket was selected. Ewes were tested in a rectangular room (11 × 7.5 m) comprising resident pens and a test pen (4 × 4 m). Movements between the residence and the test pens were possible through a starting cage (0.7 × 1.4 m) from which ewes could observe both buckets situated in the test pen. Guillotine doors allowed animals to move between the residence pen, the starting cage and the test pen. Observations were made from a hidden platform (2 m high) on the left side of the test pen from which the experimenter could open the guillotine doors.

A 4-day period of habituation preceded the 6-day training period to allow ewes to become familiar with the different pens and the movement of the guillotine doors. The day before and during the 6-day training period, ewes received only 80% of their maintenance requirements to ensure a high level of food motivation. Each training day, individual ewes were given 15 trials in two blocks of eight (between 9:00 and 11:00 a.m.) and 7 consecutive trials (between 2:00 and 4:00 p.m.). In a block, trials were approximately 10 min apart. During each trial, two buckets different in form and colour were presented: one round and red and the other square and grey. Previous experiments indicated that sheep may have a colour vision because of their ability to discriminate colour, especially red and grey (Alexander and Shillito-Walser, 1978; Alexander and Stevens, 1979). Pelleted cereal was set down in both buckets to avoid choice based on food-odour detection. Food reinforcement was only accessible in one bucket (the correct bucket); the other one contained wire mesh over the food. Cereal was wet to facilitate feed intake in treated ewes since scopolamine induces drying of mouth mucous. Only the round, red bucket was used as the correct one in this experiment since sheep performed similarly on the visual discrimination task regardless of which bucket was rewarded (pilot study). The left/right position of the buckets varied randomly across trials.

For each trial, we recorded the latency to reach a bucket and the identity (correct or incorrect) of the first bucket chosen. Ewes that first chose the rewarded bucket (correct choice) were allowed 5 s of feeding, whereas the animal was immediately removed from the testing pen by an experimenter in case of an incorrect first choice. If no choice was made after 2 min in the test pen, the trial was stopped. With these parameters, we calculated for each sheep:

- the percentage of correct choice for each day defined as [(number of correct choices/number of trials) × 100]; thus, chance level is 50%.
- the number of trials to reach a criterion of 80% correct choices.

To investigate the involvement of muscarinic transmission in the learning of the visual discrimination, 20 min before each block of eight (morning) and seven (afternoon) trials of the 6 days of testing, ewes received an intramuscular injection of 5 ml of either isotonic saline (B for bucket experiment, Bcontrol group, $n = 8$), scopolamine methylbromide at 100 μg/kg (Bmscop group, $n = 10$) or scopolamine hydrobromide at 100 μg/kg (Bscop group, $n = 10$). This injection protocol allows the maintenance of a constant concentration of the antagonist during the block of trials (which lasted 70–90 min). Moreover, the importance of the activation of central muscarinic receptors in the retrieval of information was assessed by submitting ewes of the Bcontrol group to one additional day of testing (Day 7) under scopolamine hydrobromide treatment.

2.4. Statistical analyses

Because of the lack of normality of the data, statistical analyses of all behavioural parameters were realised with two-tailed nonparametric tests. Intragroup comparisons were performed by Friedman and Wilcoxon signed ranks tests for ordinal data. Intergroup comparisons were analysed by Kruskal–Wallis and Mann–Whitney U tests for ordinal data and Fisher's exact probability test for nominal data. In the visual discrimination task, the sign test compared for each day the percentage of correct choices to random choices. All analyses were performed with the statistical package Systat 5.03 (Evanston, IL), and in all cases P values less than .05 were considered significant. Results are presented in terms of median and interquartile ranges.

3. Results

3.1. Effect of scopolamine on visual and auditory lamb recognition

All of the ewes showed full maternal behaviour towards their own lamb with acceptance behaviours (maternal bleats and licking) and no rejection behaviours observed. Moreover, when lambs attempted to suck, all the mothers accepted their suckling without udder refusals. It indicated that the scopolamine treatment did not disturb maternal behaviour as previously demonstrated (Lévy et al., 1997).

During the visual/auditory recognition test, no significant differences between groups were found in the latency to reach a contact zone and in the total time spent in both contact zones and in looking at both lambs. Nevertheless, the percentage of time spent near the familiar lamb in the Lscop group was significantly lower than in the Lcontrol group (Mann–Whitney U test, $P<.05$; Fig. 1A). No differences between groups were detected in the percentage of time spent looking at the familiar lamb.

The percentage of ewes showing a clear preference for the familiar lamb was 82% (13 of 16) in the Lcontrol group, 71% (10 of 14) in the Lmscop group, and only 50% (10 of 20) in the Lscop group. This proportion was marginally lower in the Lscop group than in the Lcontrol group (Fisher's exact probability test, $P=.08$; Fig. 1B).

3.2. Effect of scopolamine on visual discrimination performance

Absence of choice was very low and latencies were generally less than 10 s. For each group, there was a significant improvement in the percentage of correct choices over the 6 days of training (Friedman test, $P<.01$; Fig. 2A) but the evolution of accuracy was different between groups. The percentage of correct choices in the Bmscop and Bcontrol groups differed significantly from random choice for the first and the second day of training, respectively (sign test, $P<.05$), whereas in the Bscop group, this difference appeared only on Day 5 (sign test, $P<.01$). The performance was significantly lower in the Bscop group compared to the Bcontrol group on Days 2 to 5 and to the Bmscop group on Days 2 and 3 ($P<.05$; Fig. 2A). Moreover, it did not differ between the Bcontrol and Bmscop groups.

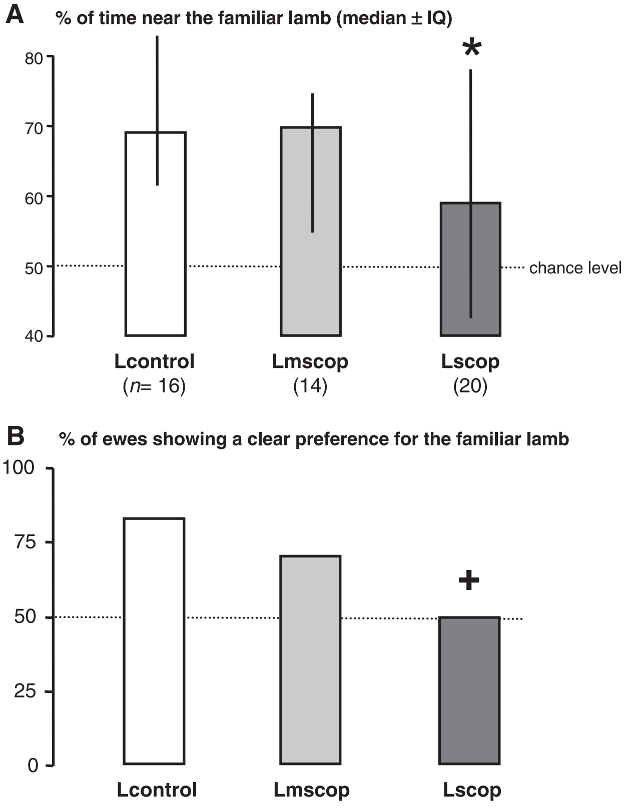

Fig. 1. Effect of intramuscular injections of saline (Lcontrol), methylscopolamine (100 μg/kg; Lmscop), or scopolamine (100 μg/kg; Lscop) on the percentage of time spent by ewes in the contact zone near the familiar lamb (A) and the percentage of ewes showing a clear preference for the familiar lamb (B) in a two-choice nonolfactory test performed between the familiar and an alien lamb at 12-h postpartum. See the Materials and methods section for the definition of preference used in (B). Horizontal lines represent interquartile ranges. $^{*}P<.05$: Lscop versus Lcontrol groups (Mann–Whitney U test). $^{+}P=.08$ (Fisher's exact probability test).

The number of trials to reach the criterion (80% correct choices) was significantly higher in the Bscop group (75 [60–90]) than in the Bcontrol and Bmscop groups (45 [30–45] and 37.5 [30–45], respectively; Mann–Whitney U test, $P<.01$; Fig. 2B). The Bcontrol and Bmscop groups did not differ from each other. At the end of the 6 days of testing, all animals of the Bcontrol and Bmscop groups and 8 of 10 of the Bscop group reached the criterion.

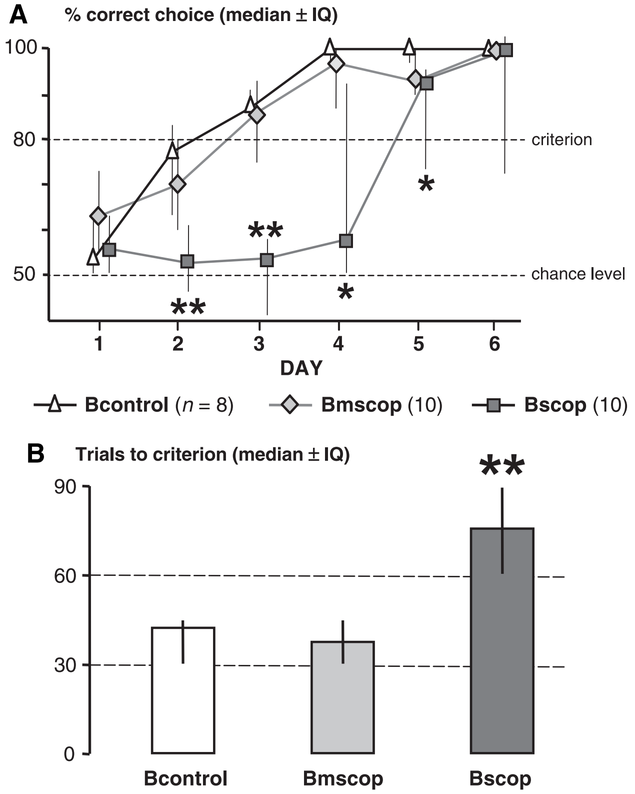

Fig. 2. Effect of intramuscular injections of saline (Bcontrol), methylscopolamine (100 μg/kg; Bmscop), or scopolamine (100 μg/kg; Bscop) on the evolution of the percentage of correct choice (A) and the number of trials to reach the criterion of 80% correct choices (B) in an instrumental visual discrimination learning. Horizontal lines represent interquartile ranges. * $P<.05$ as compared to Bcontrol group and ** $P<.01$ as compared to both the Bcontrol and Bmscop groups (Mann–Whitney *U* test).

When ewes of the Bcontrol group were injected with scopolamine on Day 7, they made 100% correct choices, indicating that a central muscarinic transmission is not required for retrieval of a previously learnt discrimination (data not shown).

4. Discussion

The present results demonstrate that scopolamine treatments impaired nonolfactory lamb recognition by mothers. They also show that similar pharmacological challenges disturbed the acquisition, but not the retrieval, of an instrumental, visual discrimination task. In addition, methylscopolamine did not induce behavioural deficits in both tasks indicating that activation of central, but not peripheral, muscarinic transmission is critical.

4.1. Effects of scopolamine on visual and auditory lamb recognition

Blockade of central muscarinic receptors during the first 12 h postpartum impaired the visual and auditory recognition of the lamb. This deficit cannot be attributed to a general disturbance of lamb nursing because scopolamine-treated ewes displayed full maternal behaviour towards their own lambs as it was previously described (Ferreira et al., 1999; Lévy et al., 1997). In addition, during the test, behavioural parameters such as the latencies to reach the contact zones and the total time spent near both lambs or looking at both lambs did not differ between groups indicating that motor, sensorial, or motivational processes were not affected by the drugs.

One can argue that impairment of the visual/auditory lamb recognition could be the consequence of the disturbance of lamb olfactory recognition induced by scopolamine (Lévy et al., 1997). Nevertheless, formation of the visual and auditory recognition memory is independent of learning the olfactory cues of the lamb as demonstrated by a similar development of visual and auditory recognition of the lamb in intact ewes and in animals that did not develop olfactory recognition (Ferreira et al., 2000). Therefore, scopolamine treatments impaired the visual and auditory recognition of the lamb by acting directly on the processing of visual and auditory cues. However, only 50% of ewes were impaired by scopolamine treatments. Differences in sensitivity between subjects is commonly related in rodents and primates following systemic or intracerebral administration of scopolamine (for instance, see Savage et al., 1996; Ravel et al., 1994). The ineffectiveness of scopolamine to induce recognition deficits in all animals, already reported in sheep (Ferreira et al., 1999; Lévy et al., 1997), could result from an insufficient dose used. Nevertheless, preliminary studies indicated that a higher dose of scopolamine (200 μg/kg) induced side effects like motor disturbances, suggesting that the dose used was optimal (see Lévy et al., 1997). Another explanation could be the time of testing. The important duration of the scopolamine treatment (12 h) before the test could allow other systems such as the noradrenergic system to compensate for the muscarinic deficits. Indeed, the noradrenergic system is activated after parturition in sheep (Lévy et al., 1993, 1995b) and interaction between cholinergic and noradrenergic systems has been reported in learning and memory processes (for reviews, see Decker and McGaugh, 1991; McGaugh and Cahill, 1997). Because we have recently shown in intact ewes that the visual and auditory lamb recognition is already present at 8 h postpartum (Terrazas et al., 1999), a two-choice test at this early stage of discrimination could better prevent eventual compensation and might reveal recognition impairment in a greater number of treated mothers.

4.2. Effects of scopolamine on visual discrimination task

Control animals rapidly learned to discriminate between two visually distinct buckets to obtain the reward. After 4 days of training, their performances were optimal, i.e., virtually all first choices were correct for each ewe. Moreover, the fact that their performance level was at chance during the first 15 trials demonstrates that both buckets were equally attractive at the beginning of the experiment. Visual discrimination learning was previously performed with success in sheep using different kinds of food (Edwards et al., 1997), different shapes (Baldwin, 1981; Kendrick et al., 1996) or different faces of conspecifics (Kendrick et al., 1995, 1996; Peirce et al., 2000). Interestingly, results obtained in visual discrimination tasks in sheep are in agreement with those reported in laboratory animals commonly used, i.e., rodents and primates (e.g., rats: Wible et al., 1992; Murray et al., 1995; primates: Ridley et al., 1999; Zola-Morgan et al., 1989). In spite of some different methodological procedures, these experiments showed that simple visual discrimination required a smaller amount of trials, between 30 and 60, to reach the 80% correct choices criterion, whereas more than 100 trials were needed for more complex discriminations (e.g., using, black objects). Taken together, these results confirm the importance of visual cues in ungulates and indicate that sheep constitute an alternative experimental model to rodents and primates for studying the neural mechanisms involved in visual learning.

The blockade of central muscarinic receptors by scopolamine impaired acquisition of this simple visual discrimination task in sheep, whereas the performance of methylscopolamine-treated animals was similar to that of the control group. These results confirmed those obtained in other species where activation of central, but not peripheral, muscarinic receptors is involved in visual discrimination learning of objects (Harder et al., 1998; Ridley et al., 1984). At the end of the 6 days of training, control animals tested on the well-learnt discrimination task performed normally under scopolamine. Indeed, all the ewes made 100% correct choices. These results, in accordance with other studies in rodents and primates (Ellen et al., 1986; Ridley et al., 1984), demonstrated that activation of muscarinic receptors is not critical for the retrieval of a previously learnt visual discrimination task. They further indicated that scopolamine-treated ewes were able to normally discriminate visual stimuli, to give adequate motor responses, and that their attentional processes were not affected contrary to that previously detected in visual discrimination in rats (Drinkenburg et al., 1995; Jones et al. 1995).

4.3. Comparison of the effect of scopolamine between social and nonsocial tasks

These experiments showed that activation of central, but not peripheral, muscarinic receptors are involved in the acquisition of both social and nonsocial tasks. Similarly, we have recently demonstrated that extensive immunolesions of the basal forebrain cholinergic system impaired offspring recognition as well as visual discrimination learning in sheep (Ferreira et al., 2001). Thus, activation of muscarinic receptors by basal forebrain cholinergic neurones may be a general feature of learning and memory of social and nonsocial tasks.

Nevertheless, the cholinergic modulation could be exerted in different brain areas and/or in interaction with particular neurotransmitter systems depending on the social characteristics of the stimuli. The oxytocinergic system could be specifically involved in social memory. Indeed, intracerebral infusion of oxytocin antagonist impaired social but not object recognition in rats (Everts and Koolhaas, 1997), and oxytocin gene mutation in mice abolished social memory, whereas other forms of memory, such as spatial memory, remained intact (Ferguson et al., 2000). In sheep, oxytocin is necessary for the onset of maternal behaviour (Kendrick, 2000) and oxytocin infusion in the olfactory bulb of parturient sheep increases the release of acetylcholine in this structure (Lévy et al., 1995b). Thus, interactions between cholinergic and oxytocinergic systems might be a part of a neural system specific to lamb recognition memory. Whether or not the oxytocinergic system can be specifically related to the cholinergic modulation of social memory and thus, lamb recognition, remains an important question to be addressed.

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