Male behavioral response to the urine odor of females in lesser mouse lemur (*Microcebus murinus* Miller, 1777)(Cheirogaleidae, Primates)[1]

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ABSTRACT: Chemical signals play an important role in *Microcebus murinus* Miller, 1777 social communication, a representative species of prosimians. It presents the major and vomeronasal olfactory systems and uses mainly urine for chemical marking. I studied the effect of females' urine on the behavior of 14 males in 3 group conditions: intact, vomeronasalectomized (VNx) and bulbectomized (Bx). Stimuli presented were pro-estrus female's urine, post-estrus female's urine, distilled water and female's presence. The groups were submitted to two phases: familiarization in the cage (3 days), and experimental stage (4 days) when each stimulus was presented for 30 min once a day. Results showed that intact males could discriminate different chemical stimuli; VNx males continued to discriminate stimuli using the major olfactory system but not as efficiently as intact animals; Bx animals did not perform any olfactory discrimination. The reduction in olfactory discrimination by VNx and Bx males may have been due to a central action of bulb activity.

Key Words: Urine odor, Chemical communication, Prosimian, *Microcebus*, Lesser mouse lemur.

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INTRODUCTION

In mammals, chemoreception plays an important role in various functions, among them feeding and reproduction. In feeding behavior, olfaction plays a role in the detection of food sources. In the sexual domain, many mammals use olfaction in the search for sex partners and in the detection of the phase of the reproductive cycle, with important physiological and behavioral implications (BROSSUT, 1996; MOLINA *et al.*, 2000).

For communication by the chemical route, mammals utilize some secretions produced by glands in the facial, thoracic and anogenital regions and/or excretions (urine, feces, and saliva) (ALBONE, 1984). Mammals usually employ two systems for the reception of chemical stimuli, the major olfactory system and the accessory olfactory system (STEPHAN *et al.*, 1982, ALBONE, 1984; SCHILLING, 1987; BHATNAGAR & MEISAMI, 1998).

The major olfactory system is formed at the peripheral level by sensory cells located on the surface of the nasal mucosa. The axons of the cells are linked to the homolateral major olfactory bulb (MOB) (ALBONE, 1984; SCHILLING, 1970, 1987; STEPHAN *et al.*, 1982). Projections are emitted from the bulb to the mesencephalon, hypothalamus and piriform cortex. This system is responsible for the detection of highly volatile chemical substances (ALBONE, 1984; SCHILLING, 1970, 1987; STEPHAN *et al.*, 1982).The vomeronasal system if formed by the vomeronasal organ which emits fibers of the vomeronasal nerve that form synapses in the accessory olfactory bulb (AOB) (SCHILLING, 1987; MEISAMI & BHATNAGAR, 1998; WHORMANN-REPENNING & BERGMANN, 2001). The vomeronasal projections reach the brain centers that control sex physiology and behavior, the medial preoptic area and the median hypothalamus (MEREDITH, 1980; MOLINA *et al.*, 2000). The vomeronasal system is involved in the detection of chemical substances of low volatility (SCHILLING, 1970).

In general, the more corticalized a species, the more it uses visual and auditory signals, with a much lower influence of signals of the pheromonal type. However, enormous differences exist in the extent of corticalization and in the development of the different sensory spheres of primates (JOLICOEUR *et al.*,

1984). Primates essentially are visual animals, although prosimians, and the more primitive and nocturnal species in particular, have maintained well-developed chemoreceptor organs and a system of social communication in which chemical signals play an important role (SCHILLING, 1979, 1990; MOLINA *et al.*, 2000).

Microcebus murinus Miller, 1777, is a highly representative species of an ancestral primate (RUMPLER & DUTRILLAUX, 1991). This species has a major olfactory system and a vomeronasal system of the insectivore type (SCHILLING, 1970, 1980, STEPHAN *et al.*, 1982), using urine as a marking material, since it did not develop specialized glands (SCHILLING, 1990). Urine is a mixture of substances originating from multiple organs to which microbial fermentation in the urinary tract and secretions form the accessory glands of the reproductive tract are added (ALBONE, 1984). This mixture reflects physiological and psychological variations depending on numerous internal factors as well as social and ecological factors (SCHILING, 1980). In the lesser mouse lemur, urine is dispersed through two types of urine markings, rhythmic micturition and urine washing. These modes of dispersal are effective and economical once the animal doesn't need to urinate repeatedly in different parts of its environment, instead, the urine is spread by feet and hands and also urinating dripping at regular intervals (SCHILLING, 1990).

It has been shown that the urine of the lesser mouse lemur selectively stimulates the olfactory chemoreceptor centers, the MOB in the volatile form and the AOB in the liquid form (SCHILLING, *et al.*, 1990). Urine contains volatile substances that act like pheromones of the primer type, they provoke physiological responses without any previous behavioral conditioning. Indeed, as soon as captive lesser mouse lemurs are heterosexually grouped at the time of seasonal sexual activation, the males develop a hierarchy in such a way that the dominant males will have normal plasma testosterone concentration, whereas subordinate individuals will undergo a reduction of sex hormones (PERRET, 1985, 2002; ANDRÈS *et al.*, 2001, 2002). This inhibition is of the pheromonal type since isolated males submitted to the odor of a dominant male present a fall in plasma testosterone levels which does not occur when they are submitted to the odor of non-dominant males (PERRET

& SCHILING, 1987). Similarly, the urine of females contains pheromones that stimulate the sexual function of males. The odor of the urine of a female in pro-estrus is sufficient to increase testosterone levels in males (PERRET, 1992).

The destruction of the vomeronasal organ or of the olfactory bulb reduces the hormonal response of males to seasonal breeding activation (PERRET, 1992; SCHILLING & PERRET, 1992, HOUOT & SCHILLING, 2002), as well as certain behaviors linked to this activity such as aggressiveness, hierarchy and marking (OSORIO DA CRUZ, 1991). *M. murinus* are dependent on photoperiodic variations (PETTER-ROUSSEAUX, 1975; SCHILLING, 1980; PERRET, 1992, 1997, PERRET *et al.* 1998). In captivity, an artificial photoperiodic schedule is imposed consisting of three months of short days (8 hrs of light per day) corresponding to sexual inactivity, followed by five months of long days (14 hrs of light per day) corresponding to breeding season (PERRET, 1992).

In view of the sensitivity to chemical signals and of the functional importance of chemoreceptor centers, the objective of the present study was to investigate the impact of vomeronasalectomy and bulbectomy on the behavioral responses of males submitted to stimuli originating from females of the same species. This work investigates the behavioral aspects of the response to olfactory chemical stimulus, in order to complement a previous study on the same species which emphasized more specifically on physiological aspects (SCHILLING 1980; SCHILING & PERRET 1992; AUJARD, 1997).

MATERIAL AND METHODS

Subjects

Twenty-eight adult *M. murinus* specimens, 14 males and 14 females born at the Laboratoire d'Ecologie Générale, Museum National d' Histoire Naturelle (France) were used in the present study. General captivity conditions (photoperiod, temperature, and feeding) were identical for all groups used and were described by Perret (PERRET, 1980). All animals were sexually experienced.

Mouse lemurs have a sexually active seasonally. This season corresponds to long-day photoperiod (PETTER-ROSSEAUX, 1975). In our laboratory the animals were submitted to an artificially accelerated photoperiod, 5 months of long days (14 h light: 10 h dark) followed by 3 months of short days (8 h light:16 h dark). All animals were tested during their period of sexual activation, between the 8th and 14th week after the beginning of the long photoperiod. This period corresponds to a plateau in sexual activity (PERRET, 1992), when the frequency of reproductive behavior reaches a maximum level and remains stable throughout the remainder of the cycle.

The males were divided into three experimental groups: i) intact male group (INT), a group of 6 intact males and females; ii) vomeronasalectomized male group (VNx), 5 vomeronasalectomized males and 5 intact females. Surgery was performed 8 months before the experimental period and consisted of the destruction of the vomeronasal organ through the palatine route; iii) bulbectomized male group (Bx), 3 bulbectomized males and 3 intact females. Bulbectomy was performed by aspiration 24 months before the experimental period, with elimination of the MOB and AOB.

Before the experiments, the animals were kept in heterosexual groups consisting of two sub-groups of 3 males/3 females (intact males), one sub-group of 3 males/3 females and 2 males/2 females (vomeronasalectomized males), one group of 3 males/3 females (bulbectomized males) in order to reduce intra-group agonism.

The cages for the sub-groups had the same basic structure (3 x 2 x 2 m), and presented a nest-box/animal. Besides, there were many branches linking the different parts of the cage and nest-box, that enriched the environment and were used for locomotion in the area.

Experimental cage

The experimental cage (38.0 x 46.5 x 29.0 cm) was made of plexiglas and was divided into two arenas by a transparent plexiglas wall (C) with perforations which permitted visual and olfactory contact with the opposite side, where the stimuli were presented (fig. 1). A removable perch (E) connecting the entrance

(F) to the arena to the plexiglas wall was fixed inside each arena. The perches were fitted with supports for the liquid stimulus (water or urine) (D).

During the observation period the animals were kept in home cages (B) connected to the experimental arena, which was maintained under constant ventilation (A). Since these are nocturnal animals, they were observed under attenuated red light (3 lux) using a videocamera connected to a videocassette recorder for later analysis of each observation section.

Stimuli

Urine from a female in pro-estrus (PR). Urine was collected during the period of vulvar swelling until the opening of the vagina. During this period, females are quite attractive to males, although they are receptive only during a period of 2-4 h when vaginal opening occurs (PERRET, 1980).

Urine from a female in post-estrus female (PS). Urine was collected after estrus, i.e., after vaginal closure, a period when females are no longer attractive to males.

Distilled water (DW). Control stimulus.

Female presence (F). Ten females in post-estrus (PS) and 4 in pro-estrus (PR); 5 PS and 1 PR were in the intact males; 4 PS and 1 PR were in the group of the VNx males; and 1 PS and 2 PS were in the group of Bx males. The estrous phases occurred during the study.

The urine presented to males was collected from animals belonging to other colony groups. After collection, samples from different donors were mixed, taking into account the phase of the estrous cycle of females. This pool of stocked urine was divided into 0.2 ml aliquots and frozen at -20°C until the time for use. Distilled water samples were similarly treated. Each urine or distilled water sample was thawed 3 min before use and placed in glass containers for exposure to males.

Experimental conditions

At the beginning of each week, a male and a female were taken at random from each heterosexual group and placed in home cages (fig. 1).

The experiments were conducted over a period of one week and consisted of two phases:

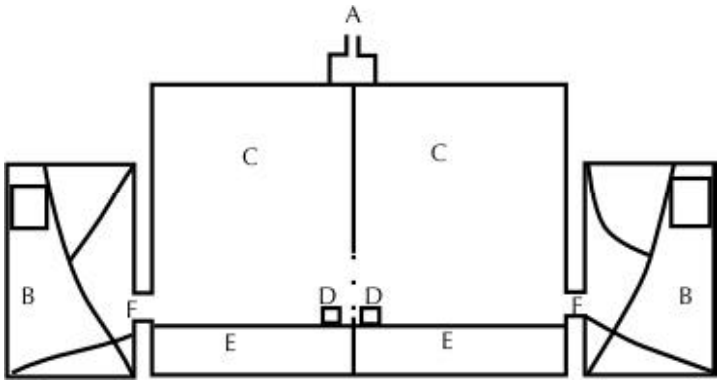

Figure 1. Cage where the male and female were kept during experimental sessions. A - ventilation; B - home cage; C - Experimental Arena; D - place where olfactory stimuli were located; E - shelf which linked arena's door to chemical stimulo; F - access door to arena.

Phase I. No recording was performed and no stimulus was presented during the first 3 days but the animals were allowed to freely explore the experimental arena during periods of time progressively closer to real observation time: on the first day the animals had access to the arena during their period of activity (10 h). On the second day, the period of access to the box was reduced to 5 h and on the third to 2 h. The objective of this procedure was to habituate the animals with the experimental conditions.

Phase II. Over a period of 4 days, each male was observed during two different periods: one hour after the beginning of the dark cycle and one hour before the beginning of the light cycle corresponding to the reported for wild animals (MARTIN, 1973). Two stimuli were presented over a period of 30 min each with a 10-min interval between presentations (5 min to change the stimulus and 5 min with no intervention). Since there was no significant difference between the experiments developed in the beginning or in the end of the dark cycle or between days, the results were pooled (Kruskal-Wallis one-way analysis test, $p>0.05$).

Table 1 shows the sequence of stimulus presentation to which the males were submitted. It can be seen that at the end of 16 presentations each male had been challenged 4 times with

Table 1. Sequence of stimuli presentation. (FM - Female, PR - pro-estrus urine, PS - post-estrus urine, DW - distillated water).

Experimental Phase (Days)			
I	II	III	IV
PS	PR	PR	PS
FM	FM	FM	FM
PR	DW	PS	DW
DW	PS	DW	PR

one of the stimuli defined above. The sequence of presentation was determined at random and was the same for all animals.

For each experiment the door that gave access to the observation cage was opened 5 min before the recording. Thus, the animals had access to the observation cage for 35 min but were recorded only during the last 30 min. Since the odor of *Microcebus* urine is persistent, the cages and especially the observation arena were washed after each series of experiments. Similarly, the glass vessels containing the urine samples were left in a mixture of chloroacetic acid, washed and dried before being used again 24 h later.

Recorded variables

The following items were recorded for each male with the aid of a chronometer:

Presence in the arena. Time spent in the experimental arena during the 30 min of recording, as well as the topographic division of the exploration in the arena.

Smelling. This behavior was recorded when the animal placed its muzzle at a distance of 2 cm or less from the substrate. The smelling behavior was divided into two types: smelling directed stimuli (female or urinary stimuli) or at the environment. Smelling the environment was considered to occur when the animal smelled the perch, the floor or the walls of the experimental arena.

Licking . This behavior was recorded when the animal directly licked the liquid stimulus or when it placed its paw inside the urine/distilled water before licking it. As done for the previous behavior, licking was divided into two types: licking the stimulus and licking the environment. Licking the environment was

considered to occur when the animal licked the perch, the floor or the walls of the experimental arena.

Scent marking: i) Urine washing - the animal rests on the homolateral limbs, places the contralateral forepaw on the urinary meatus, urinates on it and reaches the hindpaw, rubbing it several times before resting it on the support (SCHILLING, 1970). ii) Rhythmic micturition - the animal deposits urine on the substrate in the form of small drops in a continuous manner or not, while moving about (SCHILLING, 1970). iii) Anogenital - rubbing the anogenital area against an object in the experimental arena. iv) Salivating - rubbing the corners of the mouth and/or biting the substrate.

Data analysis

Data were analyzed statistically by the Friedman's rank test and the Mann-Whitney test (SIEGEL, 1979) for inter-group comparison and by the Kruskal-Wallis one-way analysis of variance and the Wilcoxon signed-rank test for intra-group comparison. The Spearman correlation coefficient was used to determine the correlation between the different behaviors for each group (SIEGEL, 1979). For the smell marking data, the chi-square test was used both for inter and intra-group comparisons (SIEGEL, 1979).

RESULTS

Time of permanence in the arena

Intact males spent more time in the experimental arena during the presentation of the liquid stimuli (urine and water) than the VNx and Bx males ($p<0.05$) but this phenomenon did not occur when they were in the presence of a female. VNx and Bx males presented a similar activity in response to all stimuli ($p>0.05$) (fig. 2).

However, the time of permanence in the experimental arena by the males from the various groups was not affect by the type of stimulus presented ($p>0.05$). Intact males spent about 350 s per stimulus out of a total of 3600 s, and VNx and Bx males spent about 200 s per stimulus (fig. 2). Indeed, some VNx and Bx males spent a lot of time motionless in the arena, looking

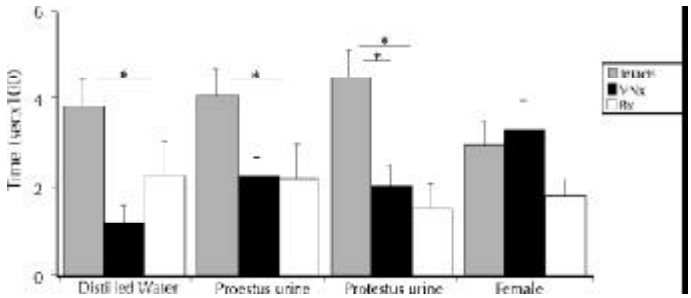

Figure 2. Time the animal stayed at the experimental arena according to different stimuli (mean-SD) (DW - distillated water; PR - pro-estrous female's urine; PS - post-estrous female's urine; FM - female present on the arena). U test, *p<0,05

at the cage with no apparent objective. Usually these animals stayed motionless close to the entrance to the arena.

The animals entered the arena in different ways. Upon the first presentation of a stimulus, the animals hesitated before entering the arena, sniffing and looking in all directions starting from the entrance door. After this first entrance, intact and VNx males approached the chemical stimulus (urine and water) or the female. Bx males, however, did not show this behavior.

For the PR, PS and FM stimuli, intact males stayed closer to the urinary stimuli or to the female, whereas during the presentation of DW, the distribution of activity in the arena was more homogeneous ($p<0.05$). Bx males showed very little exploratory activity, remaining motionless most of the time close to the entrance to the experimental arena, regardless of the type of stimulus presented. Furthermore, since the animals were allowed to enter or leave the arena at will during the experimental session, it could be seen that Bx males clearly avoided the female, returning to their cages when the females entered the opposite arena. VNx males presented an intermediate exploratory activity between intact and Bx males, exploring more or less uniformly the entire arena, regardless of the stimulus presented ($p>0.05$).

Sniffing

Intact males always presented higher mean stimulus-sniffing frequencies than VNx or Bx males ($p<0.05$) and similar environment sniffing behavior except for the PR and FM stimuli

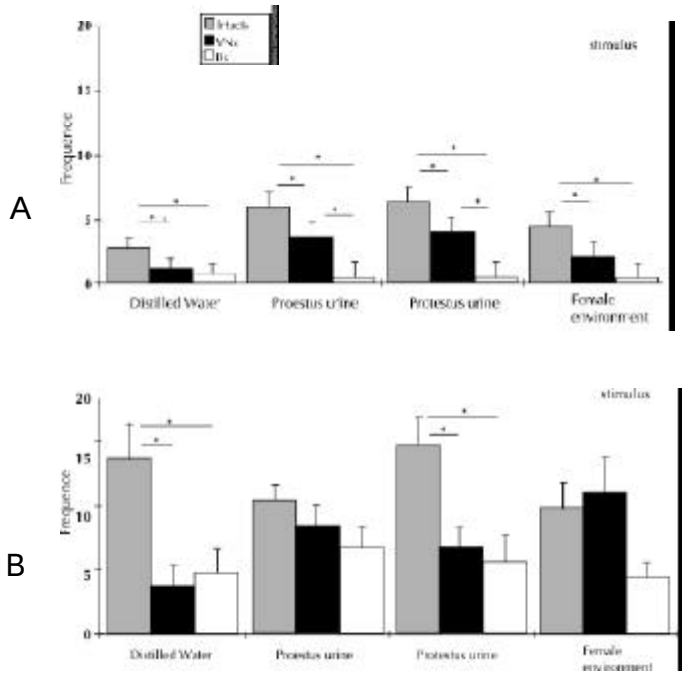

Figure 3. Sniffing behavior a different stimuli (A) and on the neighboring environmrnt (B) during the experimental phase. (mean ± SD) Abbreviatrions as in Figure 2 U test, *p<0,05.

(fig. 3). On the other hand, VNx and Bx males presented similar sniffing frequencies for the stimuli and for the environment. However, when urinary stimuli were presented VNx males sniffed more than Bx males (P<0.05) (fig. 3).

We also noted that intact and VNx males presented a higher frequency of urine sniffing than distilled water sniffing (p<0.05 for both groups). However, intact males sniffed the urine of females in pro-estrous more than the females themselves (p<0.05), whereas VNx males sniffed the urine of females in post-estrous more than the females (p<0.05) (fig. 3). Bx males presented little sniffing behavior, regardless of the stimulus.

Finally, the frequency of environmental sniffing did not differ significantly between the various types of stimuli within each group (p>0.05) (fig. 3). Environmental sniffing was always more frequent than stimulus sniffing regardless of the male group tested (about 3:1). However, a positive correlation between

sniffing the stimulus and sniffing the environment was detected both for intact and VNx males ($p<0.05$).

Marked differences in the expression of sniffing behavior were observed between the three groups of males. Generally, sniffing the stimulus started after the animal had spent some time in the arena, a characteristics common to the three groups of males. However, when they first entered the arena, intact males frequently headed towards the stimulus and started to sniff it, and sometimes to lick it. With respect to sniffing the female presented as a stimulus, muzzle-to-muzzle contact was always observed., whereas contact between the muzzle and another part of the female's body was quite rare. Only three times did one of the intact males sniff the lateral part of the body and once the anogenital region of the female.

Intact and VNx males sniffed the environment without a specific localization within the observation arena. In contrast, Bx males almost always sniffed the part closest to the entrance to the arena since, as mentioned earlier, they always remained close to the entrance. The environmental sniffing behavior was positively correlated with time spent inside the observation arena in all 3 groups ($p<0.05$).

Licking

Only the behavior of licking liquid stimuli (PR, PS and DW) was observed. Neither the FM stimulus nor any other part of the arena was licked during the test sessions. The absence of female licking was due to the fact that, even though the separating barrier permitted licking behavior, the males and females avoided each other.

Intact males showed licking behavior more frequently than VNx and Bx males ($p<0.05$) during the presentation of liquid stimuli (fig. 4). VNx males showed quite low licking frequencies and Bx males never showed this behavior. Comparison of the licking behavior observed for different groups of stimuli did not differ significantly in intact or VNx males separately ($p>0.05$).

The licking behavior shown by intact and by VNx males was preceded by the behavior of sniffing the stimulus or the environment close to the stimulus, behaviors that were positively correlated ($p<0.05$). On some occasions the males did not lick the chemical stimulus directly but placed one of

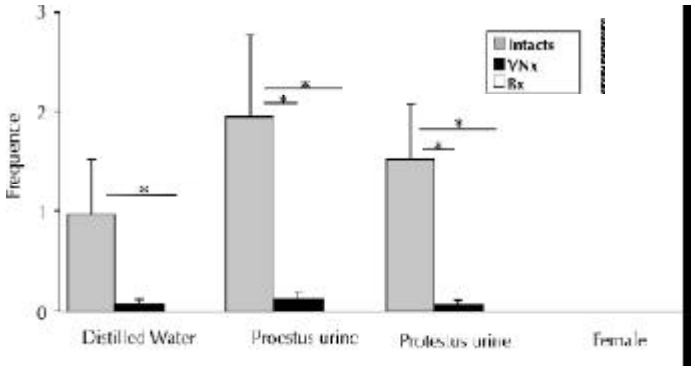

Figure 4 - Liching behavior on different stimuli presented to the animal. (mean + SD) Abbreviations as in Figure 2 . U test, *p<0,05.

the forepaws inside the vessel containing the stimulus and then licked their paw.

Marking

In all groups, the mean marking frequency was low regardless of the type of stimulus, although urine washing was the predominant marking in all male groups. This type of marking represented 80% of all markings in all groups, except for intact and VNx males during the presentation of the FM stimulus. In general, during an observation session the animals used only one or at most two types of markings, which usually were urine washing associated or not with another type.

The two types of urine provoked an increase in urine washing behavior in intact males compared to the remaining stimuli ($p<0.01$), whereas VNx males showed an increase only during the presentation of a female in pro-estrous ($p<0.01$) (fig. 5). The number of urine washings performed by Bx males was always low, regardless of the stimulus, whereas VNx males always exhibited a marking behavior similar to that of intact males.

The markings were not correlated with any behavior recorded in the present study. However, as observed for sniffing, the markings performed by Bx males were close to the entrance to the arena, whereas the markings performed by other males were distributed homogeneously around the arena.

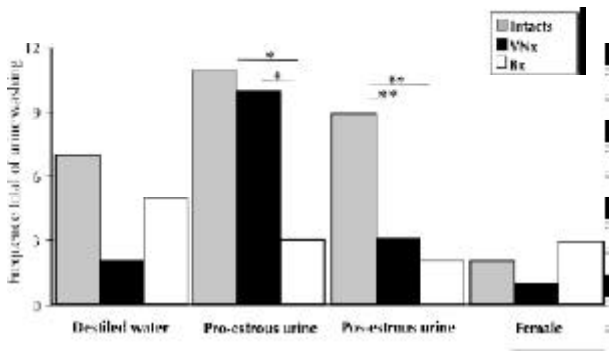

Figure 5 - Frequency total of urine washing mark in relation to each of the stimuli presented. Abbreviations as in Figure 2. *χ^2=15.19 p<0.001,**χ^2=24.93 p<0.001

DISCUSSION

We noted that lesser mouse lemur males presented an increased frequency of sniffing and licking behavior as soon as they were placed in the presence of urinary stimuli from females of their own species. This same type of response was reported for males of the same species submitted to stimulation with the urine of resting males or males engaged in sexual activity (OSÓRIO DA CRUZ, 1991). Using operant conditioning techniques, SCHILLING (1980) showed that *Microcebus coquerelli* Grandidier, 1867, males can discriminate between the urine from different individuals of the same sex, the urine from individuals of the opposite sex, and urine samples of different ages obtained from the same individual.

These behavioral modifications in the presence of urine stimuli have also been reported in rodents. HURST (1989, 1990a-c) described the behavioral responses of mice (*Mus domesticus* Linnaeus, 1766) in the presence of different olfactory stimuli. The author demonstrated that mice investigate and mark more when they are submitted to odors of familiar individuals than when they are submitted to the odor of unfamiliar mice. These animals can also discriminate between the urine of males and females, between different age ranges and between different hierarchical positions.

PETITJOHN (1977), in a study of Mongolian gerbils (*Meriones unguiculatus*, Milne-Edwards, 1867), showed that the

males spend a longer time close to a site impregnated with the urine of a female in estrous than at a site impregnated with the urine of females in post-estrous. In addition, females prefer the urine of congeners to the urine of animals of other species.

However, in the present study the presentation of stimuli from females in pro-estrous or post-estrous was not accompanied by a marked preference for the urine of sexually attractive females, as determined mainly by the time spent in the arena by the animal. On the contrary, the males sniffed perceptibly more the urine of females in post-estrous. Perhaps this fact reflects the need of males to detect any possible change in the reproductive status of the female towards the receptive phase, which is more easily identifiable, although these same males exhibited a higher frequency of marking in the presence of both urine stimuli.

HOUOT & SCHILLING (2002) found similar results after testing the urine from females along ovarian cycle. According to these authors, males visited females' urine in the follicular phase more frequently than other types of urine; the least frequently visited urine was collected during estrus. They explain the results by suggesting that during ovulation, other signals are important for female's attractiveness. The pool of urine that was used in our experiments included urine from different phases grouped as pro-estrous and post-estrus (see methodology).

In nature, *Microcebus* males are described as solitary, with a home range overlapping the territories of three or four females (MARTIN, 1973; PAGES-FEUILLADE, 1989, RADESPIEL, 2000, KAPPELER *et al.*, 2002). Thus, the males must identify at any time if one or more females are sexually receptive based on their markings, including urinary ones (EBERLE & KAPPELER, 2002).

Indeed, the frequency of the marking behavior varies in time and space in lesser mouse lemurs. Practically absent during the period of sexual rest, this behavior appears at the beginning of the reproductive season and it maximum frequency occurs at the time of female pro-estrous (SCHILLING & PERRET, 1987). On the other hand, this behavior is also linked to social status (PERRET, 1992) and depends in part on the sex hormone levels of the male (SCHILING *et al.*, 1984). When the animals are held in captivity the markings play a preponderant role in social dominance. In nature, these markings have been described as having a "delimiting" function with respect to the home range

(SCHILLING, 1979; CHARLES-DOMINIQUE, 1977). Since the observation periods followed the establishment of social dominance or of the home range, the marking behavior may have remained at the basal level with absence of competition.

The significantly lower frequencies of the sniffing and licking behaviors and even the duration of the time spent inside the arena when the males were exposed to the presence of a female seem to be opposite to the frequencies obtained for the urinary signals. Indeed we would expect males to react with a general increase in all the behaviors recorded when placed in the presence of a female. However, we observed a fairly regular behavior of female avoidance on the part of the males. In general, prosimian females are dominant over males (RICHARD & NICOLL, 1987, RADESPIEL & ZIMMERMANN, 2001) and are more aggressive after the period of vaginal opening (estrous). In the present study, most of the females utilized (10/14) were in unreceptive post-estrous.

Lesser mouse lemur males appeared to be more motivated to explore urinary signs than distilled water, used as a neutral stimulus. Although the discrimination of the estrous condition of the females is less clear, it seems to really exist since we observed greater olfactory exploration of the environment in particular when females in pro-estrous and post-estrous were present.

As a whole, these behaviors with respect to the urinary signals disappeared or were quite reduced after total bulbectomy. Indeed, bulbectomized males presented reduced mean frequencies for all behaviors recorded. In these males, bulbectomy was performed in such a way as to remove the MOB and AOB. Thus, even though they still possessed the peripheral olfactory system (olfactory epithelium, vomeronasal organ, nerve endings and the nerves linking these organs to the bulbs) they could not receive any olfactory stimulation at the central level. HOUOT & SCHILLING (2002) described that bulbectomized *M. murinus* don't show differentiated responses to females' urine in the various phases of ovarian cycle which are in agreement with our results..

Aside from the loss of olfactory capacity, destruction of the olfactory bulbs leads to behavioral modifications in certain mammalian species. BANDLER JR. & CHI (1972) reported

behavioral changes in "killer rats" which stopped killing mice and frogs after bilateral bulbectomy because of the lack of olfactory recognition of the "victim" animal. MURPHY (1976) also reported that male hamsters (*Mesocricetus auratus* Waterhouse, 1839) are quite aggressive with their congeners when territorial disputes arise. After bilateral bulbectomy, these aggressive behaviors are completely eliminated, whereas they persist after unilateral bulbectomy. LIEBENAUER & SLOTNICK (1996) also reported similar data for CF-1 strain mice and also stated that two olfactory bulbectomized male mice moved away from one another when they met.

The bulbectomized males used in the present experiments exhibited little activity, remaining motionless close to the entrance to the arena and running away from the presence of a female. These facts may reflect a "general inhibition" of the exploratory behaviors of males after general bulbectomy, probably caused by the absence of olfactory information.

In countless species of mammals, bulbectomy also has important consequences for sexual development (HEIMER & LARSSON, 1967; EDWARDS, 1974; BEAUCHAMP *et al.*, 1977). EDWARDS & DAVIS (1997) performed surgical deafferentation of the olfactory bulbs of male rats which made the animals anosmic and reduced their sexual performance during copulation, substantially reducing the frequency of erections when olfactory clues from receptive females were presented to them. Bulbectomy affects certain physiological parameters, and sex hormone levels in particular. It has been demonstrated that bulbectomized lesser mouse lemur males have reduced testosterone levels (OSÓRIO D A CRUZ, 1991) since the olfactory bulb interferes with the mechanisms of regulation linked to photoperiodicity (PERRET, 1992; SCHILLING & PERRET, 1992).

The data obtained for VNx animals show that these males presented a greater frequency of sniffing behavior in the presence of urine than in the presence of distilled water or of a female. However, the licking behavior not only was greatly reduced but also remained unchanged in the presence of the different stimuli.

As described, the vomeronasal organ is responsible for the perception of weakly volatile components that are present in urine or of high molecular weight substances that do not reach

the olfactory epithelium (WYSOCKI, 1982). In the present study, the absence of licking behavior in VNx males reflects the absence of the receptor organ. In several mammals, the presence of an acquired behavior frequently persists even after ablation of the receptor organ. However, BEAUCHAMP *et al.* (1985) studied the effect of time after vomeronasalectomy on the behavioral response to olfactory stimulation in *Cavia porcellus* Erxleben, 1777, males and demonstrated that head bobbing as a behavioral response to female urine, which is normally observed in intact males, progressively declined with time in vomeronasalectomized males due to a progressive destruction of the organization of the accessory olfactory system.

This phenomenon may have occurred in the animals tested here, since the interval between surgery and the experiments was 8 months, a period apparently sufficient for the disappearance of licking behavior as a form of communication. We observed no compensatory increase in sniffing behavior in VNx animals. On the contrary, sniffing frequency was significantly reduced, as also observed by OSÓRIO DA CRUZ (1991), although the number of urine markings was elevated when individuals from this group were in the presence of urine from a female in pro-estrous (AUJARD, 1997).

The general reduction in the activity of VNx and Bx males may have depended on a central action of bulb activity. Indeed, both VNx and Bx lead to a reduction of sex hormone levels (SCHILLING & PERRET, 1992). In conclusion, intact males showed changes in the frequency of olfactory and exploratory behaviors according to the type of stimulus, whereas VNx and Bx males showed little activity linked to the olfactory sphere.

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