



Research Article

## STUDY ON THE INFLUENCE OF DIFFERENT SCENT SOURCES THAT ATTRACTS OPPOSITE AND SAME SEX OF SOFT-FURRED FIELD RAT *MILLARDIA MELTADA* (GRAY, 1837)

S. Kalaiyarasan<sup>1</sup> and A. Amsath<sup>2\*</sup>

<sup>1</sup>Department of Zoology, Government Arts College (Autonomous), Kumbakonam-612 001, Tamil Nadu, India

<sup>2</sup>Department of Zoology, Khadir Mohideen College, Adirampattinam-614 701, Tamil Nadu, India

**Article History:** Received 15<sup>th</sup> June 2016; Revised 15<sup>th</sup> June 2016; Accepted 25<sup>th</sup> June 2016; Published 30<sup>th</sup> June, 2016

### ABSTRACT

The present study was carried out to evaluate the ability of to discriminate soft-furred field rat *Millardia meltada* rat odor from different reproductive phases, with a view toward detecting the estrous phase. Experiments were also carried out to establish the relationship between the behavioral analyses were carried out in a Y-maze apparatus, in which the soft furred field rat were acclimatized in before Y maze apparatus. The number and duration of visits, and grooming behavior by male responders towards the gland samples were recorded. Intact male rat showed a higher response towards glands samples. These results suggest that rat have the ability to discriminate the different scent gland odour. The grooming behavior shown by males in response to scent glands may be taken as key parameters to produce specific odors that probably involve both intra specific and inter specific communication.

**Keywords:** *Millardia meltada*, Scent glands, Behavioral analyses, Discrimination, Communication.

### INTRODUCTION

Nocturnal habits and dark living environments have led to the evolution of olfaction as a major method of communication in rodents (Robertson *et al.* 1993). Among mammals chemical signals can send powerful messages with behaviour modulating effects that may be of considerable social importance. The study of pheromone cueing systems in relation to complex behaviours has been hampered by the lack of identification of specific compounds functioning as behaviour modifiers. Pheromones, like chemical signals, are detected by special receptor neurons in the olfactory system. The major difference between pheromones (species-specific) and other chemical signals (inter-specific) is in the output: when processed by the brain, chemical signals result in the sensation of smell, whereas pheromone signals trigger a unique characteristic behavioural or physiological response (Ben-Ari, 1998). Mammalian pheromones are found to be involved in many reproductive behaviours, such as sexual attraction (Kannan *et al.*, 1998), interference with puberty, oestrous cycle and pregnancy (Dominic, 1991), as well as

social behaviours namely territorial marking (Doty, 1980; Prakash and Idris, 1992), individual identification (Poddar-Sarkar and Brahmachary, 1999) and initiation of aggression (Mugford and Nowell, 1971). The major sources of physiologically and behaviourally important chemical cues are the secretions of specialized scent glands (Mykytowycz, 1970; Adams, 1980; Balakrishnan and Alexander, 1985; Johnston, 1990; Kannan and Archunan, 1997a; Kennaugh *et al.*, 1997), urine (Hurst, 1990) and faeces (Mykytowycz, 1970; Asa *et al.*, 1985).

### MATERIALS AND METHODS

Adult male and female rat soft furred-field rat *Millardia meltada* (Swiss strain) were housed separately in polypropylene cages (40×25×15 cm) with 2 cm of rice husk lining the bottom as bedding material and were provided pellet food (Hindustan Lever Ltd., Bangalore) and water *ad libitum* in accordance with the guidelines for animal care by the Institutional Animal Ethics Committee (Ref. No.: BDU/IAEC/2012/71/28.03.2012). The rats

\*Corresponding author: Dr. A. Amsath, Associate Professor, Department of Zoology, Khadir Mohideen College, Adirampattinam-614 701, Tamil Nadu, India, Email: aamsath@gmail.com, Mobile: +91 9524582977.

maintained under standard laboratory conditions (12:12 hr D: L; Temperature  $24\pm 2^{\circ}\text{C}$ ). The bedding material was changed before every odor preference test. The sexually matured 12 weeks old rats were used to test odor preference. They were divided into three groups of 15 animals each. During the odor discrimination study, the amount of time spent and number of visits by test animals in behaviors such as grooming, body rubbing, and licking scent glands samples were observed.

#### Determination of number and duration of visits

Odor preference was tested in a covered 'Y' maze ( $150\times 15\times 15$  cm) apparatus made of tin sheeting. The lateral sides of the test apparatus were closed with glass plates, whereas the top was covered with iron mesh (Kannan and Archunan, 2001). This apparatus had a facility to provide food and water ad libitum. The apparatus was about 80 cm long by 15 cm wide. The two choice arms were 75 cm long. During behavior analysis, distilled water was placed in the right arm. The sample was presented in a small container (5 ml) having several openings and placed in left arm (Archunan, 2003). The soft-furred rats were released into the central chamber to begin in a common place. The duration and the number of visits exhibited by responders were observed. Test animals were allowed to acclimatize for 15 min in the apparatus; the actual behavioral assessment began after this period. When a responder came very close (at least 2 cm) to a sample, this was recorded as one visit. The time spent by the responder from the time it approached a sample (within at least 2 cm) until it moved away from the sample was also recorded.

#### Assessment of behaviour

The test animal was released in to the control chamber of "Y" maze apparatus in which water was placed (right arm) in one arm and in the other arm (left arm) the scent glands extract of various stages was kept as per the experimental procedure. The responder soft-furred field rat were released in to the middle arm to begin in common place and the frequencies of various behaviors such as duration, number of visits body rubbing, self grooming, and licking were observed. The rats were allowed to acclimatize for 5 minutes in the "Y" maze apparatus before observation. Active behavioral assessment was begun after acclimatization of 5 minutes following the release of test animals. The number and duration of visits to each arm, frequency of licking, grooming and body rubbing behaviors were recorded in 5 minutes at their maximum acting period i.e, 2000 and 2400 h under red light. Such Observation was repeated as many as times for six day in each rat. The frequency and duration of various behavioral activities were recorded with the help of a stop watch. Duration the interval of each test, the "Y" maze apparatus was thoroughly washed and dried.

Body rubbing, self-grooming, and licking, were observed. The rats were allowed to acclimatize for 5 minutes in the 'Y' maze apparatus before observations. Tests were conducted with each sample for 30 minutes. Actual behavioral assessment was begun after

acclimatization of 5 minutes following the release of the test animals.

## RESULTS

#### Number of visits

All female responders visited the different samples. A significant difference in the frequency of visit by the responders was noticed between different samples. The females visited the preputial gland sample more times when compared to all other gland samples. The frequency of visit between the responders significantly varied towards the samples (Table 1).

#### Duration of visits

Both males and females displayed a preference for the scented slides over the control (waster) slide. There was a significant difference between the responders exposed to various scent sources namely cheek (male and female), armpit (male and female), flank (male and female), preputial (male) and clitoral (female) odours. Of these five scent sources, the preputial odour was found to be more attractive, as the responders spent more time with these scents than the remaining sources Table 5. The male or female devoted more time in investigation the preputial scents of the opposite sex. The preputial odour of the male and clitoral odour of the female were less attractive to the same sex responder as compared with the opposite sex.

The flank and cheek gland secretions were also greatly preferred by the opposite sex and the responder spent appreciable time to investigate the scents of flank gland (Table 1). In the case of responders exposed to same sex flank gland odour, the time devotion was comparatively less. The responder spent less time to investigate scents of armpit gland secretion than the other scent gland odours (Table 1). The variation among the scent sources and attractiveness differed significantly and further the interaction between scent sources and attractiveness was also significant.

#### Self Grooming, Body rubbing and licking Behaviour

The scent gland secretions induced several types of behavioural responses such as grooming, licking, and body rubbing in the conspecifics of the soft-furred rats.

**Self Grooming behavior:** The rat exhibited self-grooming movements when the rat was introduced to the odour of all the selected scent sources of the same as well as opposite sexes (Figure 1). The preputial gland induced greater level of genital grooming movements in opposite sex than in the same sex, male to female, male to male, female to male and female to female. By contrast, the flank, armpit and cheek glands induced more non-genital grooming activities in the opposite sexes: male to female and female to male.

**Body rubbing:** The male rat rubbed more frequently on the scented slide as well as on the corner of the cage when introduced to the odour of same sex. Flank and cheek glands induced more body rubbing behavior (Figure 2) both in males and females than the other scent sources.

**Body Licking:** The male and female respondents spent more time in body licking activities (Figure 3) when exposed to preputial odour of opposite sexes. When compared to the licking behaviour expressed by the responders exposed to scents of same sex, the flank gland

extract elicited the maximum response male to male and female to female. The armpit and cheek gland extracts of same and opposite sexes showed variation in licking behaviour among opposite and same sex responders.

**Table 1.** Influence of different scent sources that attract the opposite/ same sex of soft furred rat, *Millardia meltda*.

Scent gland Sample	Sex of the responder	Number of visits (30 min/test)	Duration of visits (Sec/30 Min)
Control	Male	49 ± 0.81	20.1 ± 0.8
	Female	55 ± 0.78	30 ± 1.5
Male Cheek gland	Male	50 ± 0.74	40.3 ± 0.8
	Female	51 ± 0.63	35.2 ± 0.4
Female Cheek gland	Male	39 ± 0.6	28.1 ± 1.2
	Female	42 ± 0.71	30 ± 1.4
Male Armpit gland	Male	46 ± 0.72	48.2 ± 1.5
	Female	43 ± 0.65	30.2 ± 1.4
Female Armpit gland	Male	40 ± 0.68	40.5 ± 1.2
	Female	40 ± 0.53	45 ± 0.5
Male Flank gland	Male	42.3 ± 0.55	35.5 ± 0.4
	Female	39.8 ± 0.62	28.2 ± 1.4
Female Flank gland	Male	41 ± 1.43	38 ± 1.2
	Female	38 ± 0.62	41 ± 0.8
Male Preputial gland	Male	59 ± 1.70	55 ± 1.9
	Female	79.3 ± 0.60	68.2 ± 0.8
Female Clitoral gland	Male	48.2 ± 1.4	42 ± 1.9
	Female	51.5 ± 1.2	49 ± 1.6

Values are expressed in Mean ± SE. Those means in the same vertical column that are not marked with the same superscript letters are significantly different at (P<0.05).



**Figure 20.** Body rubbing.



**Figure 21.** Self Grooming.



**Figure 22.** Body Licking.

## DISCUSSION

The present study revealed that the responders spent more time towards the odour of opposite sex irrespective of the scent glands. The results of present experiments are consistent with previous reports (Pandey and Pandey, 1984; Kannan and Archunan, 1998, 2001) that the female showed a strong preference and spent more time with the male preputial homogenates. The attractant function of the preputial secretion has been attributed to be a releaser pheromone. The present findings gain support from the contribution of other workers (Edwards and Binhorn, 1986; Ferkin, *et al.*, 1994; Drickamer, 1995; Edwards, *et al.*, 1996) that the sexually active rats, mice and voles prefer sexually receptive females. A sexually motivated male spends time with a female appropriate to his motivational state of sexual motivation (Edwards *et al.*, 1996). Further, the present investigation indicates that the duration of visits made by the responders and the responsiveness of male to female and female to male odour was varied according to the nature of scents/odours. The male and female responders spent greater time to preputial followed by urine and faecal odours. Gawienowski *et al.* (1976) indicated that male rats are attracted to preputial extract and urine of sexually receptive females.

In the case of flank gland, the responders devoted more time to visit the flank odours of same sex than the opposite sexes. This contribution is correlated to the results obtained by Johnston (1981) in hamsters that the flank glands are comparatively more attractive to the same sex rather than opposite sex. Therefore, the flank gland is mainly involved in the species identification, territorial marking and individual recognition (Johnston, 1990).

Odours are extremely important for rodents and other mammals for many types of behavioural communication (Gosling 1985). In the present study, all the scent glands were attracted the odours males and females. Such results were observed in rats (Carr *et al.*, 1965, 1970; Lydell and Doty, 1972; Pfaff and Pfaffman, 1979), sheep (Lindsay, 1965), dogs (Beach and Gilmore, 1949) and hamsters (Johnston, 1980).

The cheek and armpit glands showed more or less equal attraction towards the same as well as opposite sexes. It is evident that the cheek rubbing is the form of active scent marking in terrestrial squirrels and rats (Armitage, 1976; Owings, *et al.*, 1977; Bakker, *et al.*, 1996). The cheek odours are used mainly to mark territorial boundaries. Similar observations were made on the

arboreal squirrels (Benson, 1980; Ferron, *et al.*, 1986). All the reports are in consistent with the present results, which stated that the cheek gland odours are found to be attractive to both male and female rats. This may be due to their active involvement in maintaining social behaviours. Balakrishnan and Alexander (1980) reported that during social interaction shrews of both sexes rely considerably on the odour of cephalic region.

## CONCLUSION

The present investigation on volatile nature of scent glands of soft-furred field rat revealed the significant influences on the same and opposite sex. The results further suggest that produces specific odors that probably involve both intra specific and inter specific communication.

## ACKNOWLEDGEMENTS

The first author is thankful to the UGC for the award of Rajiv Gandhi National Fellowship for this work and also to the Principal and Head of the Department of Zoology, Government Arts College (Autonomous), Kumbakonam for the facilities provided to carry out this work.

## REFERENCES

- Adams, M.G., 1980. Odour-producing organs of mammals. *Symp. Zool. Soc. London*, 45, 57-86.
- Archunan, G., 2003. Odour discrimination study using 'Y' and '+' maze apparatus. In: *Lectures on Recent Trends in Ethology and Behavioural Sciences*. (ed) John Thomas, K., Irinjalakuda, Kerala, pp. 92-96.
- Armitage, K.B., 1976. Scent marking by yellow-bellied marmots. *J. Mammal.*, 57, 583-584.
- Asa, C.S., Peterson, E.K., Mech, L.D. and Seal, U.S., 1985. Deposition of anal sac secretions by captive wolves (*Canis lupus*). *J. Mammal.* 66, 89-99.
- Bakker, J., Ophemert, J.V. and Slob, A.K., 1996. Sexual differentiation of odour and partner preference in the rat. *Physiol. Behav.*, 40, 489-494.
- Balakrishnan, M. and Alexander, K.M., 1980. Olfactory inhibition of scent marking in the Indian musk shrew, *Suncus murinus viridescens* (Blyth). *Bonn. Zool. Beitr.*, 31, 2-15.
- Balakrishnan M. and Alexander, K.M., 1985. Sources of body odours in some Indian mammals. *Indian Rev. Life Sci.*, 71, 257-258.



- Beach, F.A. and Gilmore, R.W., 1949. Response of male dogs to urine from females in heat. *J. Mammal.*, 30, 391-392.
- Ben-Ari, E.T., 1998. Pheromones: What's in a name? *Bioscience*, 48, 505-511.
- Benson, B.N. 1980. Dominant relationships, mating behaviour and scent marking in fox squirrels. *Mammalia*, 44, 143-160.
- Carr W.J., Krames, L. and Costango, D.J., 1970. Previous sexual experience and olfactory preference for novel versus original sex partners in rats. *J. Comp. Psychol.*, 71, 216-222.
- Carr, W.J., Loeb, L.S. and Dissinger, M.L., 1965. Responses of rats to sex odours. *Comp. Psychol.*, 59, 210-211.
- Dominic, C.J., 1991. Chemical communication in animals. *J. Sci. Res. (Banaras Hindu University)*, 41, 157-169.
- Doty, R.L., 1980. Scent marking in mammals. In: Denny, M.R. (Ed.). *Comparative Psychology: Research in Animal Behaviour*. Wiley, New York, pp. 385-399.
- Drickamer, L.C., 1995. Odours in traps: Does most recent occupants influence capture rates for house mice. *J. Chem. Ecol.*, 21, 541-555.
- Edwards, D.A. and Binhorn, L.C., 1986. Preoptic and midbrain control of sexual motivation. *Physiol. Behav.*, 37, 329-335.
- Edward, D.A. Walter, B. and Liang, P. 1996. Hypothalamic and olfactory control of sexual behaviour and partner preference in male rats. *Physiol. Behav.*, 60, 1347-1354.
- Gawienowski, A.M., Denicola, D.B. and Stacewicz-sapuntzakis, M., 1976. Androgen dependence of a marking pheromone in rat urine. *Norm. Behav.*, 7, 401-405.
- Gosling, L.M., 1985. The even toad ungulates: Order Artiodactyla, In: *Social odours in mammals*. Brown, R.E. and Macdonald, D.W. (Eds.), Clarendon Press, Oxford, pp. 550-618.
- Hurst, J.L., 1990. Urine marking in population of wild house mice (*Mus domesticus*) communication between the sexes. *Anim. Behav.*, 40(2), 233-243.
- Johnston, R.E., 1980. Responses of male hamsters to the odors of females in different reproductive states. *J. Comp. Physiol. Psychol.*, 94, 894-904.
- Johnston, R.E., 1981. Attraction to odours in hamsters: An evaluation of methods. *J. Comp. Physiol. Psychol.*, 95, 951-960.
- Johnston, R.E., 1990. Chemical communication in golden hamsters: from behaviours to molecules and neural mechanisms. In: Dewsbury, D.A. (ed.) *Contem Psychol*. Signaur Press, New York, pp. 381-409.
- Kannan, S. and Archunan, G. 1997. Biochemical variations of male scent markers alter the attractiveness in the female rats, *Rattus norvegicus*. *Acta Physiol.*, 85(2), 175-181.
- Kannan, S. and Archunan, G., 1998. Effect of gonadectomy on scent glands pheromonal orientation and grooming behaviour in rats, *Rattus norvegicus*. *J. Exp. Zool. India*, 149-158.
- Kannan, S. and Archunan, G., 2001. Chemistry of clitoral gland secretions of the laboratory rat: assessment of behavioural response to identified compounds. *J. Biol. Sci.*, 26, 247-252.
- Kannan, S., Krishnan, M., Tarvinder, K.T., Vinodkumar, S. 2007. Raising antibody against mouse sex pheromone binding protein in rabbit. *J. Biosains*, 18, 13-21.
- Kannan, S., Bharathiraja, C. and Krishnan, M., 2008. Regulatory mechanism of sex pheromone and intracellular calcium level in V2Rx receptor expressing cells. *Curr. Sci.*, 95, 397-400.
- Kennaugli, J.H., Chapman, D.I. and Chapman, N.G., 1977. Seasonal changes in the prepuce of adult follow deer (*Dama dama*) and its probable function as a scent organ. *J. Zool.*, 183, 301-310.
- Lindsay, D.R., 1965. The importance of olfactory stimuli in the mating behaviour of the ranj. *Aniin. Behav.*, 13, 75-78.
- Lydell, K. and Doty, R.L. 1972. Male rat odour preferences for female urine as a function of sexual experience, urine age and urine sources. *Norm. Behav.*, 3, 205-212.
- Mugford, R.A. and Nowell, N.W., 1971. The reputial gland as a source of aggression promoting odour in mice. *Physiol. Behav.*, 6, 247-249.
- Mykytowycz, R., 1970. Role of skin glands in mammalian communication. In: *Advances in chemoreception I Communication by chemical signals* (Eds.) J.W. Jonhson, D.G. Moulton and A. Turk. New York, Appleton Century crofts, pp. 327-360.

- Owings, D.H., Borliert, M. and Virginia, R., 1977. The behaviour of California ground squirrels. *Anim. Behav.*, 25, 221-230.
- Pandey, S.D. and Pandey, S.C., 1984. Effect of an androgen on attractive function of preputial glands in the wild mouse. *Physiol. Behav.*, 35, 851-854.
- Pfaff, D.W. and Pfaffmann, C., 1979. Behavioural and electro physiological responses of male rats to female rats urine odours. In: *Olfaction and Taste III*. Pfaffmann, C. (Ed.). Rockefeller University Press, New York, pp. 258-267.
- Poddar-Sarkar, M., Brahmachary, R.L., 1999. Can free fatty acids in the tiger act as an individual finger print? *Curr. Sci.*, 76, 141-142.
- Prakash, I. and Idris, M., 1992. Scent marking behaviour. In: *Rodents in Indian Agriculture*, Prakash, I. and Ghosh, P.K. (Eds.), Scientific publishers, India, 32, 445-460.
- Robertson, D., Beynon, R. and Evershed, R., 1993. Extraction, characterization, and binding analysis of two pheromonally active ligands associated with major urinary protein of house mouse (*Mus musculus*). *J. Chem. Ecol.*, 19, 1405-1416.