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Response of *Rattus rattus* towards Salivary Gland Extract of conspecifics

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ABSTRACT: Understanding the role of scent glands and their pheromones in communicative patterns in pest species could open the doors for pest management. House rat (*Rattus rattus)* is one of the major commensal rodent pests worldwide. Salivary glands are reported to be pheromonal in nature and play role in social and sexual communication in some species. Therefore, current investigation was carried out to study the response of *Rattus rattus* towards salivary gland extract of conspecifics of different sexes and maturity groups and to understand the role of salivary glands in social and sexual communication. From mature and immature male and female groups of rats (4 groups), 50% extract from mandibular, sublingual, and parotid glands was prepared individually in a solvent mixture of dichloromethane and n-hexane (1:1, v/v) and was exposed to all four groups under bi-choice conditions during the treatment period of seven days in laboratory cages. Results revealed that there was no repellent/attractant effect of salivary glands extract of immature male and female rats towards different maturity groups and sexes. Sexually mature rats were, however, responsive towards only mandibulargland extracts of sexually mature rats of both sexes. Therefore, in the future, pheromones present in the mandibular gland of mature house rats could be identified and further used in the preparation of the pheromonal traps to improve the trapability of rats for managing pest populations.

Keywords: Salivary glands, House rat, pheromones, communication.

INTRODUCTION

House rat, *Rattus rattus* is the most abundant, widely distributed, highly adaptable, and cosmopolitan commensal rodent species worldwide which causes huge losses to the food stuffs and other stored items.10-15% of damage in paddy godowns is due to house rats only (Kambarajan *et al.,* 2004). House rat is the prominent pest of poultry farms in India as these farms provide predator-free environment along with a constant nutritious food supply in the form of eggs, newly born chickens etc. (Hussain *et al.,* 2006).

Farmers prefer to use acute rodenticides as these are fast-acting but persistent use of these rodenticides leads to the development of poison aversion and bait shyness in rats. Rodenticides persist in the environment for a longer period and harm non-target species also (Geduhn *et al*., 2014) therefore, cannot be used regularly (Selvaraj and Archunan 2002). In such circumstances, more advanced rat pest management strategies taken from natural systems are required that are environmentally benign, target-specific, and humane.

All animals have to coordinate their activity with other members of their species if they are to survive and reproduce. This requires some form of communication, which for the majority of animals involves the use of chemical signals, known as pheromones (Rodriguez 2005). In many mammalian species, individuals use different sources of odors/scents to communicate such as feces, urine, vaginal secretions, flank glands, ear glands, saliva, feet glands, etc. (Gray *et al.,* 1984).

Saliva and salivary glands are thought to contain pheromonal substances. Several potential chemosignals have been identified in the saliva of different species, including the well-known sexual attractant pheromones of boars (Loebel *et al.,* 2000). Signaling pheromones 3-androstenol and 5alpha-androstenone in boar saliva play a major role in the overall effect of the male on puberty attainment in gilts (Booth, 1984). Cheek glands secretions are also reported to be involved in the attraction of both male and female rats. Response of male to female scent was comparatively greater than that of female to male cheek gland secretions in *Rattus norvegicus* (Kannan and Archunan 2001). This might be due to the active involvement of androgens in the pheromonal communication in rats (Kannan and Archunan 1998). Cheek glands secretions of sexually mature male and female lesser bandicoot rats have also been reported to attract the opposite sex (Kannan and Archunan 1999). The presence of non-volatile pheromones in saliva comes from findings of mouse saliva containing high concentrations of lipocalins, which are very similar to the major urinary proteins (MUPs) (Marchese *et al.,* 1998; Pelosi 1998). Like urinary proteins, lipocalins are synthesized in the liver as well as in the submaxillary, lachrymal, sublingual, parotid, and mammary glands under the control of various developmental and hormonal stimuli (Shahan *et al.,* 1987), lipocalins are also testosterone-dependent, which signal the presence of a reproductively active

male (Brennan and Kendrick 2006) and are strongly implicated in intraspecies chemical communication (Spinelli *et al.*, 2002).

Success in the field of insect pheromones for their management lured the scientists to work on animal pheromones. Sex attractant pheromones are used as trap lure to attract and capture mice (Musso *et al.,* 2017; Takács *et al.*, 2016, 2017). Understanding the role of the salivary gland in social/sexual communication would confirm its pheromonal nature. In the future, chemical analysis of the salivary glands could be done to determine the chemical compounds involved in social and sexual communication which can be further used as trap lures to attract and capture rats for their management.

Therefore, the present study was carried out to assess the response of house rats towards salivary gland secretions of conspecifics of different age groups and sexes.

MATERIALS AND METHODS

The current investigation was carried out at Animal House Laboratory and Rodent Research Laboratory, Department of Zoology, Punjab Agricultural University, Ludhiana located at an intersection of 30˚55'N parallel of latitude and 75˚54' E line of longitude.

A. Rats' collection and maintenance

Rats were captured from stores, grocery stores, and poultry farms in and around Ludhiana for the current research. Rats were individually acclimatized in laboratory cages before the start of the experiment, with WSO bait (Cracked wheat, powdered sugar, and vegetable oil; 96: 2: 2) and water provided *ad libitum*. Animals were used and maintained as per the guidelines of the Institutional Animal Ethics Committee. Approval of Institutional Animal Ethics Committee Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana was obtained for the usage of animals. Before starting the experiment, rats were weighed and divided into four groups of healthy and sexually mature male and female (body weight above 150 gm) and immature male and female rats (body weight below 150 gm) (n=6, each group).

B. Preparation of salivary gland extract (mandibular, sublingual and parotid)

Males and females of both mature and immature groups of both sexes of rats were also sacrificed and their mandibular (Fig. 1A), sublingual (Fig. 1B), and parotid glands (Fig. 1C) were excised and weighed. 50% extract of each salivary gland was prepared by homogenizing each gland individually in a solvent mixture of n-hexane and dichloromethane (1:1v/v) with a glass homogenizer under ice-cold conditions. Immediately after homogenization, the supernatant was filtered out in clean sterile glass vials. Few drops of polyethylene glycol were added to the extract for odor fixation and vials were sealed and stored at – 20°C.

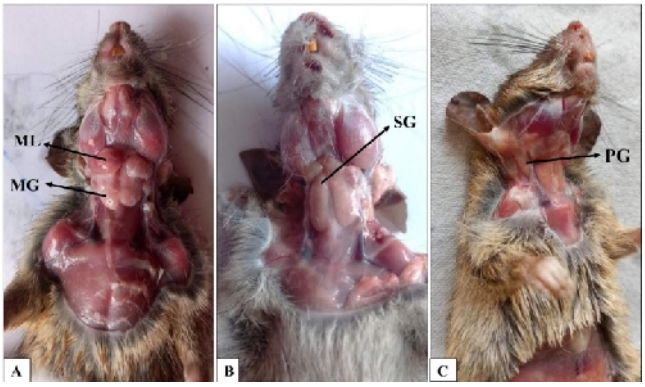

Fig. 1. (A)- Mandibular gland (MG), Mandibular lymph node (ML); (B)- Sublingual gland (SG); (C)- Parotid gland (PG) in house rat.

C. Exposure of rats of different maturity groups and sexes to mandibular, sublingual, and parotid glands extracts

Rats were fed on weighed plain WSO bait during the pre-treatment period and water was provided *ad libitum.* After the pre-treatment period, rats of different age groups and sexes were exposed individually to extracts of different salivary glands collected from different groups of rats for 7 days under bi-choice conditions. A cotton swab soaked with 0.5ml of 50% salivary gland extracts was placed in the clean plastic vials and each vial was tied on the one corner of the joined cages and that cage was considered as a treated side while the other side was considered as an untreated side. Two bowls each containing 20g of plain WSO bait were placed diagonally to each other on the treated and untreated sides of the cages. After the treatment period, rats were again fed on plain WSO bait to record the post-treatment bait consumption. Daily bait intake was recorded after every 24 hours during the pre-and post-treatment period of three days and treatment period of seven days. The formula used to calculate daily bait consumption is given below:

Daily bait consumption (g/100 g body weight) =

$$\frac{\text{Daily consumption of bait by rat}}{\text{Weight of rat (g)}} \times 100$$

D. Statistical analyses

Values were calculated as mean ± SE. Data collected for the dependent variable (consumption of bait from plain and treated side) using factorial completely randomized design was subjected to analysis of variance using Proc GLM procedure of statistical software SAS 9.4. Tukey's multiple comparison method was applied to compare the significant difference among different treatments, groups, and days at P≤0.05.

RESULTS AND DISCUSSION

A. Exposure of rats of different maturity groups and sexes to 50% mandibular, sublingual, and parotid glands extracts of immature male and female house rats

All four groups were exposed individually to 0.5ml of 50% extract from each gland of immature male and female house rats. There was a significant difference in the body weight between mature and immature rats of both sexes (Tables 1 & 2) and there was a non-significant difference in overall average consumption of plain WSO bait between pre-and post-treatment periods. There was also a non-significant difference in the daily and overall mean bait consumption between plain and treated sides and among all the four groups during the treatment period. These results, therefore, indicated neither attractant nor repellent effects of mandibular, sublingual, and parotid gland extracts of immature male and female rats towards mature and immature rats of both sexes. During the present study, the non-responsive behavior of rats towards salivary gland extracts of immature rats might be due to the low level of free testosterone and estradiol in the body of immature rats, which are responsible for altering the composition of the scent gland extracts. Present studies revealed that the immature female rats show irregularity in the estrous cyclicity, which might be due to the low level of FSH, LH, and estrogen in immature rats. The composition of the scents of the opposite sexes is altered by circulating free testosterone and estradiol from a physiological standpoint, which is responsible for the attraction/repulsion towards the rats of opposite/same sexes. Males and females need these steroid hormones to produce smells that were appealing to opposite-sex conspecifics. The attraction towards scents from opposite-sex conspecifics is influenced by the receiver's hormonal milieu. Individuals with low gonadal steroid titers, do not emit odors that are alluring to the opposite sex (Petrulis 2013).

B. Exposure of rats of different maturity groups and sexes to 50% mandibular, sublingual, and parotid gland extracts of mature female house rats

All four groups of rats were exposed to 0.5ml of 50% extracts of different glands of mature female house rats during the treatment period under bi-choice conditions. There was a significant difference in the body weight between mature and immature rats of both sexes and a non-significant difference in overall average consumption of plain WSO bait between the pre-and post-treatment periods (Table 3). In the case of mandibular gland extract of mature female rats, daily bait consumption from the plain and treated sides differed non significantly in the immature and mature male and female groups of rats. However, overall bait consumption differed non-significantly in immature male and female rats but differed significantly (P≤ 0.05) in the mature male and female groups of rats being significantly (P≤ 0.05) high on the treated side in the mature male rats (4.38±0.31) and significantly (P≤ 0.05) low on the treated side in mature female rats (4.10±0.22). These results indicated the attractant and repellent effect of mandibular gland extract of mature female rats towards opposite and same-sex respectively of mature rats but these effects were not very significant**.**

However, in the case of sublingual gland extract of mature female rats, there was a non-significant difference in the mean and overall bait consumption between plain and treated sides, between mature male and female groups, and between immature male and female groups of rats. These results, therefore, indicated that sublingual gland extract of mature female house rats has neither attractant nor repellent effect on different sexes and maturity groups of rats. Sublingual gland extract of mature female rats might not be used in social communication.

In the case of parotid gland extract of mature female rats, daily bait consumption from the plain and treated sides differed non significantly in the immature and mature male and female groups of rats. Overall average bait consumption from plain and treated sides also differs non-significantly in mature male, immature male, and female groups of rats. However, in the case of mature females, overall bait consumption was significantly (P≤ 0.05) high on the plain side (5.15±0.16) as compared to the treated side (4.43±0.20) indicating a slight repellent effect on the parotid gland extract in mature female rats.

C. Exposure of rats of different maturity groups and sexes to 50% mandibular, sublingual, and parotid gland extracts of mature male house rats

All four groups of rats were exposed to mandibular, sublingual, and parotid gland extracts of mature male house rats individually under bi-choice conditions for seven days. Results (Table 4) showed that there was a significant difference in the body weight between mature and immature rats of both sexes. There was a non-significant difference in the consumption (g/100gmbw) of plain WSO bait between pre-and post-treatment periods with all the 3 extracts of salivary glands.

In the case of mandibular gland extract of mature male rats, daily mean bait consumption from plain and treated sides differed non-significantly in immature male, female, and mature female groups of rats but differed significantly in the mature male group of rats. In the case of the mature male group of rats, daily mean bait consumption was significantly (P≤ 0.05) high on the plain side on day 1 and 2 as compared to the treated side. These results indicated the significant repellent effect of mandibular gland extract of mature male rats towards the same sex for 2 days.

Table 1: Exposure of rats of different maturity group and sexes to 0.5ml of 50% mandibular, sublingual and parotid glands extract of immature male house rat

Exposure of rats of different maturity group and sexes to 0.5ml of 50% immature male mandibular gland extract											
Groups (n=6 rats)	**Body weight**	**Treat-ments**	**Consumption of bait (g/100gm bw)**								
			Pre-treatment Period	**Treatment period**							**Post-treatment period**
				Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Average**	
Mature Male	188.33±6.60*	P	8.25±0.02[1]	5.41±0.36	5.51±0.39	4.63±0.65	4.43±0.72	4.57±0.58	4.56±0.68	5.45±0.28	4.94±0.17[AB]	9.41±0.21[1]
		T		5.70±0.52	5.24±0.35	4.47±0.61	4.16±1.17	3.35±0.52	4.68±0.53	4.05±0.50	4.52±0.27[AB]	
Immature Male	135.19±1.45**	P	5.43±0.19[2]	4.57±0.74	4.48±0.62	3.32±0.70	5.33±0.73	4.87±0.43	4.82±0.53	4.51±0.30	4.56±0.22[AB]	5.59±0.22[2]
		T		4.60±0.77	3.84±0.88	5.06±0.94	3.66±0.58	3.50±0.30	3.75±0.27	4.11±0.87	4.07±0.20[B]	
Mature Female	176.33±1.91*	P	8.59±0.21[1]	4.08±0.80	4.39±0.51	6.16±0.38	5.50±0.47	6.04±0.48	5.46±0.79	5.96±0.55	5.37±0.28[A]	9.10±0.31[1]
		T		4.29±0.87	4.67±0.70	5.14±0.16	3.47±0.63	4.35±0.46	3.06±0.56	5.33±0.57	4.33±0.29[AB]	
Immature Female	135.83±1.40**	P	5.55±0.11[2]	4.35±0.68	5.20±0.42	4.55±0.75	4.96±0.49	3.71±0.43	4.34±0.51	4.46±0.49	4.51±0.17[AB]	6.01±0.13[2]
		T		4.33±0.34	3.57±0.30	4.17±0.81	3.58±0.41	4.19±0.27	3.33±0.68	4.32±0.46	3.93±0.15[B]	
Exposure of rats of different maturity group and sexes to 0.5ml of 50% immature male sublingual gland extract												
Mature Male	188.33±6.60*	P	8.42±0.18[1]	5.19±0.37	5.56±0.52	5.13±0.35	5.16±0.27	5.36±0.89	5.02±0.30	5.00±0.53	5.20±0.07[A]	9.04±0.25[1]
		T		4.92±0.79	3.68±0.43	4.21±0.50	4.47±0.59	4.28±0.46	4.47±0.70	3.87±0.50	4.27±0.14[ABC]	
Immature Male	135.19±1.45**	P	5.04±0.24[2]	4.78±0.65	5.30±0.63	4.56±0.51	4.67±0.55	4.57±0.70	3.91±0.46	3.80±0.36	4.51±0.18[ABC]	5.27±0.11[2]
		T		3.69±0.66	3.18±0.65	3.81±0.61	4.05±0.51	4.65±0.64	4.06±0.59	3.56±0.28	3.86±0.16[BC]	
Mature Female	176.33±1.91*	P	8.35±0.43[1]	5.65±0.56	4.93±0.64	5.77±0.35	5.58±0.37	5.75±0.65	3.71±0.82	5.08±0.42	5.21±0.26[A]	8.79±0.32[1]
		T		4.76±0.63	4.33±0.44	4.75±0.39	5.76±0.28	3.18±0.76	4.90±0.73	5.33±0.68	4.72±0.29[AB]	
Immature Female	135.83±1.40**	P	5.53±0.33[2]	4.23±0.66	4.35±0.69	3.61±0.82	4.24±0.92	3.36±0.70	3.87±0.34	3.50±0.73	3.88±0.14[BC]	5.47±0.09[2]
		T		3.36±0.77	3.38±0.59	3.86±0.32	3.48±0.56	4.00±0.50	3.12±0.42	3.13±0.44	3.48±0.12[C]	
Exposure of rats of different maturity group and sexes to 0.5ml of 50% immature male parotid gland extract												
Mature Male	188.33±6.60*	P	8.40±0.11[12]	4.17±0.28	4.87±0.54	4.95±0.66	3.73±0.41	5.02±0.39	4.81±0.31	4.52±0.69	4.58±0.16[A]	9.46±0.14[1]
		T		4.87±0.50	4.20±0.32	4.13±0.49	4.00±0.28	3.18±0.28	3.80±0.63	3.63±0.20	3.97±0.18[AB]	
Immature Male	135.19±1.45**	P	6.47±0.13[3]	4.77±0.40	3.46±0.63	4.41±0.48	3.32±0.50	4.06±0.39	3.31±0.51	2.94±0.24	3.75±0.23[B]	5.06±0.24[3]
		T		4.30±0.45	3.79±0.38	3.81±0.77	4.17±0.53	4.16±0.32	4.41±0.16	4.28±0.24	4.13±0.08[AB]	
Mature Female	176.33±1.91*	P	8.86±0.38[2]	5.37±0.44	4.35±0.33	3.41±0.45	4.55±0.32	5.01±0.11	4.83±0.16	4.91±0.81	4.63±0.22[A]	9.11±0.19[12]
		T		4.66±0.74	4.25±0.31	5.30±0.26	4.25±0.40	3.31±0.25	3.40±0.42	4.56±0.64	4.25±0.25[AB]	
Immature Female	135.83±1.40**	P	5.08±0.12[3]	4.25±0.53	3.86±0.26	3.97±0.50	4.23±0.35	3.11±0.40	4.11±0.24	2.60±0.37	3.73±0.22[B]	5.66±0.18[3]
		T		2.84±0.23	3.24±0.33	3.98±0.31	3.36±0.48	4.24±0.45	3.24±0.84	3.89±0.63	3.54±0.17[B]	

[Values are Mean±SE; P: Plain side; T: Treated side; *,** denotes significant (P≤ 0.05) difference in body weight of different groups of rats; [1,2,3]denotes significant (P≤ 0.05) difference in overall average bait consumption among different groups of rats during pre- and post-treatment period; [A,B,C]denotes significant (P≤ 0.05) difference in overall average bait consumption among different groups of rats during the treatm ent period . Mean values with no superscript along the columns and rows indicate non-significant difference at P≤ 0.05]

Table 2: Exposure of rats of different maturity group and sexes to 0.5ml of 50% mandibular, sublingual and parotid glands extract of immature female house rat.

Exposure of rats of different maturity group and sexes to 0.5ml of 50% immature female mandibular gland extract											
Groups (n=6 rats)	**Body weight**	**Treat-ments**	**Consumption of bait (g/100gm bw)**								
			Pre-treatment period	**Treatment period**							**Post-treatment period**
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Average	
Mature Male	188.33±6.60*	P	9.77±0.15[1]	6.15±0.79	5.08±0.53	5.64±0.89	4.27±0.80	5.42±0.56	3.51±1.23	4.43±0.97	4.93±0.32[A]	9.58±0.25[1]
		T		5.14±0.35	6.04±0.50	5.30±0.45	5.29±0.80	4.12±1.33	6.66±0.52	5.23±0.61	5.40±0.28[A]	
Immature Male	135.19±1.45**	P	7.27±0.24[2]	3.91±0.94	2.46±0.77	5.51±0.82	3.62±0.59	4.84±0.51	3.02±1.20	4.44±1.22	3.97±0.37[AB]	7.38±0.23[2]
		T		3.35±0.40	3.51±0.59	2.87±0.66	4.93±1.30	4.77±0.60	4.36±1.17	3.70±0.98	3.93±0.27[AB]	
Mature Female	176.33±1.91*	P	10.24±0.26[1]	5.74±1.04	6.92±1.11	4.16±0.81	3.89±0.38	4.54±0.59	6.96±0.78	5.05±0.31	5.32±0.44[A]	10.20±0.43[1]
		T		3.37±0.53	4.54±0.68	2.77±0.61	4.29±0.25	5.30±0.78	5.30±0.78	5.27±0.40	4.41±0.35[AB]	
Immature Female	135.83±1.40**	P	6.49±0.18[2]	5.43±2.14	3.68±0.39	3.57±0.87	3.39±1.11	3.23±0.80	4.36±1.07	3.88±1.02	3.93±0.26[AB]	6.15±0.36[2]
		T		3.21±0.33	3.33±0.14	3.22±0.85	4.31±0.89	3.22±1.37	3.34±1.10	4.21±0.39	3.55±0.17[B]	
Exposure of rats of different maturity group and sexes to 0.5ml of 50% immature female sublingual gland extract												
Mature Male	188.33±6.60*	P	9.02 ± 0.58[1]	5.2±0.52	5.82±0.33	4.77±0.50	4.92±0.90	4.75±0.28	3.78±0.84	6.21±0.32	5.06±0.28[AB]	9.19±0.47[1]
		T		3.25±0.72	4.08±0.76	5.13±0.69	3.75±1.12	6.06±0.75	5.60±0.87	4.23±0.51	4.59±0.36[ABC]	
Immature Male	135.19±1.45**	P	6.96±0.34[12]	3.70±0.89	2.65±0.61	2.49±0.60	2.60±0.51	3.66±1.12	3.58±0.74	3.55±0.89	3.17±0.62[C]	6.28±0.33[2]
		T		2.56±0.55	2.66±0.87	2.60±0.88	2.71±1.25	4.28±0.51	4.28±0.64	4.92±0.44	3.43±0.36[C]	
Mature Female	176.33±1.91*	P	8.73±0.21[12]	6.85±0.96	6.14±1.02	4.96±0.78	5.37±0.42	5.37±0.69	5.42±1.09	4.32±0.73	5.49±0.28[A]	9.12±0.15[1]
		T		4.89±0.93	4.72±0.69	5.37±0.59	4.78±0.93	5.57±0.64	3.46±0.68	6.11±1.13	4.98±0.29[AB]	
Immature Female	135.83±1.40**	P	6.38±0.36[2]	4.10±0.44	4.13±1.04	3.62±0.51	3.71±0.74	3.37±0.64	3.22±1.02	3.50±0.60	3.67±0.12[BC]	7.18±0.50[12]
		T		4.79±1.31	3.61±0.61	5.26±1.32	3.35±1.22	3.37±0.45	4.47±1.11	4.59±0.66	4.21±0.27[ABC]	
Exposure of rats of different maturity group and sexes to 0.5ml of 50% immature female parotid gland extract												
Mature Male	188.33±6.60*	P	8.90±0.19[1]	6.11±0.66	4.43±0.63	5.59±0.83	3.38±0.26	4.08±0.23	5.17±0.63	5.51±0.80	4.90±0.34	8.52±0.26[1]
		T		4.15±0.98	4.67±0.41	5.52±1.56	5.84±0.70	4.33±0.64	4.49±0.42	4.66±0.65	4.81±0.22	
Immature Male	135.19±1.45**	P	6.01±0.17[23]	3.89±1.01	3.05±0.48	4.29±0.27	3.81±0.24	4.05±0.76	4.31±0.63	5.63±0.55	4.15±0.27	5.70±0.18[2]
		T		3.21±0.73	4.54±0.64	3.07±0.41	3.20±0.82	4.65±0.66	4.32±0.79	4.50±1.06	3.93±0.25	
Mature Female	176.33±1.91*	P	8.98±0.05[1]	6.19±0.84	3.88±0.45	5.86±1.66	4.45±0.37	4.16±0.65	4.15±0.37	4.18±0.54	4.70±0.32	8.04±0.02[12]
		T		5.15±0.72	5.42±1.16	5.01±0.70	4.89±0.52	5.47±0.44	4.05±0.71	4.24±0.77	4.89±0.19	
Immature Female	135.83±1.40**	P	6.26±0.35[23]	3.71±0.58	3.85±0.63	2.33±0.68	3.83±0.64	4.44±0.38	3.19±0.63	4.95±0.55	3.76±0.29	6.27±0.29[23]
		T		4.44±0.42	3.21±0.37	3.70±0.86	3.21±0.45	3.58±0.50	3.81±0.84	5.56±0.71	3.93±0.29	

[Values are Mean±SE; P: Plain side; T: Treated side; *,** denote significant (P≤ 0.05) difference in body weight of different groups of rats; ^{1,2,3} denotes significant (P≤ 0.05) difference in overall average bait consumption among different groups of rats during pre- and post-treatment period; ^{A,B,C} denotes significant (P≤ 0.05) difference in overall average bait consumption among different groups of rats during the treatment period ; Mean values with no superscript along the columns and rows indicate non-significant difference at P≤ 0.05]

Table 3: Exposure of rats of different maturity group and sexes to 0.5ml of 50% mandibular, sublingual and parotid glands extract of mature female house rat.

Exposure of rats of different maturity group and sexes to 0.5ml of 50% mature female mandibular gland extract												
Groups (n=6 rats)	**Body weight**	**Treat-ments**	**Consumption of bait (g/100gm bw)**									
			Pre-treatment period	**Treatment period**								**Post-treatment period**
				Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Average**	
Mature Male	188.33±6.60*	P	9.22±0.11[1]	3.78±0.42[abcd]	3.2±0.13[bcd]	1.59±0.33[d]	2.26±0.30[dc]	5.24±0.39[abc]	4.21±0.46[abcd]	4.01±0.33[abcd]	3.47±0.32[C]	8.75±0.03[1]
		T		4.10±0.22[abcd]	4.11±0.33[abcd]	3.50±0.27[abcd]	4.42±0.44[abcd]	4.03±0.52[abcd]	4.19±0.44[abcd]	6.36±0.75[a]	4.38±0.31[AB]	
Immature Male	135.19±1.45**	P	6.52±0.09[2]	3.12±0.72[bcd]	4.10±0.54[abcd]	4.75±0.44[abc]	3.60±0.40[abcd]	3.61±0.33[abcd]	2.87± 0.33[bcd]	3.76±0.51[abcd]	3.69±0.22[BC]	6.64±0.12[2]
		T		4.11±0.46[abcd]	4.86±0.23[abc]	3.62±0.59[abcd]	3.49±0.30[abcd]	4.88±0.37[abc]	3.12±0.23[bcd]	3.75±0.19[abcd]	3.98±0.24[BC]	
Mature Female	176.33±1.91*	P	9.43±0.23[1]	4.68±1.06[abc]	5.38±0.38[ab]	5.14±0.97[abc]	5.21±0.61[abc]	4.89±0.62[abc]	5.25±0.59[abc]	5.50±0.40[ab]	5.15±0.10[A]	8.81±0.54[1]
		T		5.22±0.79[abc]	4.00±0.53[abcd]	3.70±0.77[abcd]	4.08±0.30[abcd]	4.15±0.65[abcd]	3.15±0.25[bcd]	4.40±0.34[abcd]	4.1±0.22[BC]	
Immature Female	135.83±1.40**	P	6.56±0.05[2]	4.33±0.22[abcd]	3.82±0.18abcd	3.21±0.23[bcd]	3.10±0.34[bcd]	4.37±0.96[abcd]	2.72±0.28[bcd]	2.72±0.16[bcd]	3.47±0.25[C]	5.87±0.18[2]
		T		3.47±0.62[abcd]	4.07±0.42[abcd]	3.06±0.48[bcd]	4.58±0.41[abcd]	3.06±0.48[bcd]	4.20±0.33[abcd]	3.69±0.27[abcd]	3.73±0.20[BC]	
Exposure of rats of different maturity group and sexes to 0.5ml of 50% mature female sublingual gland extract												
Mature Male	188.33±6.60*	P	8.61±0.05[1]	4.97±0.34	4.85±0.54	5.28±0.42	5.27±0.42	4.11±0.32	4.80±0.31	5.35±0.39	4.95±0.15[A]	8.51±0.20[1]
		T		4.79±0.73	4.12±0.41	3.49±0.39	4.18±0.44	4.42±0.42	4.75±0.41	4.04±0.43	4.26±0.16[ABC]	
Immature Male	135.19±1.45**	P	5.73±0.17[2]	3.91±0.47	3.80±0.26	3.91±0.19	3.93±0.45	3.80±0.19	3.91±0.31	3.81±0.29	3.87±0.02[BC]	6.47±0.38[2]
		T		3.81±0.42	4.03±0.46	4.05±0.54	4.78±0.48	4.17±0.37	3.69±0.57	3.92±0.15	4.06±0.12[BC]	
Mature Female	176.33±1.91*	P	8.57±0.47[1]	5.27±0.42	5.01±0.42	4.45±0.44	4.24±0.53	5.20±0.47	4.16±0.54	4.06±0.24	4.62±0.18[AB]	8.64±0.44[1]
		T		3.33±0.40	4.62±0.40	4.06±0.35	4.25±0.40	3.40±0.48	4.47±0.64	4.44±0.55	4.08±0.18[BC]	
Immature Female	135.83±1.40**	P	6.38±0.15[2]	4.35±0.58	4.35±0.44	3.50±0.31	3.85±0.49	3.88±0.36	3.51±0.40	3.72±0.51	3.88±0.12[BC]	6.72±0.06[2]
		T		3.38±0.44	3.86±0.48	4.71±0.52	3.86±0.40	4.11±0.14	3.49±0.55	3.26±0.45	3.81±0.17[C]	
Exposure of rats of different maturity group and sexes to 0.5ml of 50% mature female parotid gland extract												
Mature Male	188.33±6.60*	P	9.29±0.16[23]	5.00±0.20[ab]	5.29±0.41[ab]	4.81±0.18[abc]	4.840.31[abc]	5.00±0.28[ab]	4.89±0.15[ab]	4.89±0.18[ab]	4.96±0.06[AB]	9.24±0.14[12]
		T		4.70±0.63[abcd]	4.43±0.50[abcd]	4.29±0.20[abcd]	4.32±0.50[abcd]	4.63±0.25[abcd]	4.54±0.24[abcd]	4.54±0.31[abcd]	4.49±0.05[ABC]	
Immature Male	135.19±1.45**	P	6.75±0.21[3]	4.66±0.29[abcd]	3.43±0.22[abcd]	3.68±0.19[abcd]	3.93±0.55[abcd]	3.81±0.27[abcd]	3.92±0.25[abcd]	3.44±0.16[abcd]	3.84±0.15[DC]	6.67±0.38[3]
		T		3.31±0.33[abcd]	3.54±0.29[abcd]	3.69±0.31[abcd]	4.41±0.35[abcd]	4.54±0.28[abcd]	4.31±0.44[abcd]	4.18±0.74[abcd]	4.00±0.17[DC]	
Mature Female	176.33±1.91*	P	9.47±0.23[3]	4.54±0.17[abcd]	4.82±0.23[abc]	5.68±0.37[a]	5.48±0.31[ab]	5.60±0.37[ab]	5.09±0.50[ab]	4.82±0.19[abc]	5.15±0.16[A]	9.17±0.27[3]
		T		4.82±0.31[abc]	5.10±0.37[ab]	3.68±0.38[abcd]	4.43±0.41[abcd]	3.57±0.35[abcd]	4.75±0.59[abc]	4.64±0.54[abcd]	4.43±0.20[BC]	
Immature Female	135.83±1.40**	P	6.93±0.26[3]	3.61±0.48[abcd]	3.47±0.36[abcd]	3.37±0.17[abcd]	3.24±0.28[bcd]	3.51±0.31[abcd]	3.37±0.24[abc]	3.87±0.70[abcd]	3.49±0.07[D]	7.22±0.16[3]
		T		4.11±0.41[abcd]	4.12±0.41[abcd]	4.24±0.20[abcd]	4.61±0.63[abcd]	4.23±0.31[abcd]	2.47±0.30[cd]	2.36±0.43[d]	3.73±0.32[D]	

[Values are Mean±SE; P: Plain side; T: Treated side; *,** denotes significant (P≤ 0.05) difference in body weight of different groups of rats; ^{1,2,3} denote significant (P≤ 0.05) difference in overall average bait consumption among different groups of rats during pre- and post-treatment period; $^{a\text{-}d}$ denotes significant (P≤ 0.05) difference in average daily bait consumption among days and groups during the treatment period; $^{A\text{-}D}$ denotes significant (P≤ 0.05) difference in overall average bait consumption among different groups of rats during the treatment period Mean values with no superscript along the columns and rows indicate non-significant difference at P≤0.05]

Table 4: Exposure of rats of different maturity group and sexes to 0.5ml of 50% mandibular, sublingual and parotid glands extract of mature male house rat.

Exposure of rats of different maturity group and sexes to 0.5ml of 50% mature male mandibular gland extract												
Groups (n=6 rats)	**Body weight**	**Treat-ments**	**Consumption of bait (g/100gm bw)**									
			Pre-treatment period	**Treatment period**								**Post-treatment period**
				Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Average**	
Mature Male	188.33±6.60*	P	8.48±0.01[1]	6.94±0.57[ab]	7.07±0.63[a]	6.62±0.31 [abc]	3.79±0.45 [abc]	4.72±0.32 [abc]	5.82±0.66 [abc]	4.52±0.56 [abc]	5.64±0.45[A]	8.32±0.01[1]
		T		2.11±0.55[c]	2.42±0.46 [bc]	4.42±0.79 [abc]	4.40±1.23 [abc]	3.63±0.71 [abc]	5.32±0.43 [abc]	4.73±0.69 [abc]	3.86±0.42[B]	
Immature Male	135.19±1.45**	P	5.92±0.15[2]	4.23±0.58[abc]	5.65±0.48 [abc]	5.31±0.45 [abc]	2.08±1.00[c]	3.80±0.99 [abc]	3.52±0.55 [abc]	4.10±0.33 [abc]	4.10±0.42[B]	6.02±0.19[2]
		T		2.84±0.51 [abc]	4.76±0.31 [abc]	4.14±0.16 [abc]	2.07±1.31[c]	3.57±1.14 [abc]	4.36±1.17 [abc]	4.59±1.59 [abc]	3.76±0.34[B]	
Mature Female	176.33±1.91*	P	8.37±0.11[1]	3.94±0.40 [abc]	3.46±0.32 [abc]	3.71±0.38 [abc]	3.98±0.38 [abc]	4.75±0.72 [abc]	5.12±0.51 [abc]	5.23±0.70 [abc]	4.32±0.25[B]	8.19±0.12[1]
		T		7.10±0.31[a]	7.00±0.27 [ab]	6.53±0.52 [abc]	6.30±0.65 [abc]	5.33±0.70 [abc]	5.33±0.70 [abc]	5.42±0.48 [abc]	6.14±0.27[A]	
Immature Female	135.83±1.40**	P	6.22±0.09[2]	4.42±0.68 [abc]	3.65±1.14 [abc]	2.15±1.68[c]	3.48±1.13 [abc]	3.11±0.83 [abc]	4.49±1.09 [abc]	4.87±1.19 [abc]	3.74±0.32[B]	5.90±0.11[2]
		T		4.220.34 [abc]	4.56±0.44 [abc]	4.06±0.96 [abc]	3.88±0.65 [abc]	3.27±1.38[abc]	3.36±1.08 [abc]	3.18±0.74 [abc]	3.79±0.18[B]	
Exposure of rats of different maturity group and sexes to 0.5ml of 50% mature male sublingual gland extract												
Mature Male	188.33±6.60*	P	9.330.33[1]	6.44±1.48	5.07±0.68	3.28±0.61	3.36±0.23	3.54±0.54	3.78±0.76	3.48±0.62	4.13±0.41	9.05±0.23[1]
		T		4.04±1.19	4.83±1.03	3.86±0.81	3.53±0.45	3.38±0.77	2.94±1.33	2.55±0.47	3.59±0.26	
Immature Male	135.19±1.45**	P	6.44±0.08[2]	5.99±0.47	3.88±0.89	4.03±1.44	4.55±0.96	4.37±0.92	3.42±0.59	3.92±0.50	4.30±0.28	6.92±0.27[2]
		T		3.07±0.28	4.21±0.87	3.54±0.44	4.14±0.77	4.52±1.16	3.65±0.83	3.54±0.72	3.81±0.17	
Mature Female	176.33±1.91*	P	9.43±0.21[1]	4.49±1.17	5.41±0.92	3.77±0.77	3.25±0.55	4.19±0.95	2.83±0.80	4.50±1.67	4.06±0.30	8.99±0.14[1]
		T		3.43±0.44	4.44±0.54	4.63±1.59	5.81±1.66	4.12±0.69	4.98±1.02	4.98±0.60	4.63±0.26	
Immature Female	135.83±1.40**	P	6.54±0.07[2]	3.93±1.62	3.53±0.40	3.81±1.15	4.97±1.49	5.23±1.53	5.12±1.57	5.34±1.18	4.56±0.26	6.27±0.04[2]
		T		4.64±0.80	4.72±1.66	4.91±0.74	4.68±1.50	3.74±1.31	3.07±1.36	5.15±1.01	4.41±0.25	
Exposure of rats of different maturity group and sexes to 0.5ml of 50% mature male parotid gland extract												
Mature Male	188.33±6.60*	P	9.21±0.47 12	5.16±0.90	4.15±0.94	4.38±0.46	3.48±0.96	4.480.45	4.21±0.39	5.26±1.07	4.44±0.21	9.33±0.15[1]
		T		4.66±0.84	4.670.56	3.96±1.37	4.70±0.74	4.18±0.66	4.58±0.66	4.98±0.44	4.53±0.12	
Immature Male	135.19±1.45**	P	6.38±0.06[3]	3.28±1.31	4.56±0.85	5.76±1.18	4.75±0.77	4.55±0.66	4.56±0.43	4.24±1.15	4.52±0.25	6.09±0.11[3]
		T		5.41±0.47	4.20±1.16	3.78±0.80	4.19±1.12	5.09±0.45	4.79±1.50	4.65±0.62	4.58±0.20	
Mature Female	176.33±1.91*	P	8.44±0.29[2]	5.43±0.58	4.23±0.42	4.86±0.51	5.59±0.48	5.12±0.48	5.48±1.00	5.08±0.89	5.11±0.16	9.22±0.18[12]
		T		5.50±1.11	4.58±1.03	5.51±0.48	3.44±0.33	3.07±1.15	3.97±0.66	5.09±0.81	4.45±0.34	
Immature Female	135.83±1.40**	P	6.54±0.15[3]	4.89±0.80	3.21±0.98	4.22±1.09	4.83±1.14	3.53±0.71	4.37±0.93	5.95±0.79	4.42±0.32	5.94±0.07[3]
		T		4.76±0.28	4.78±0.43	4.64±0.70	4.79±1.03	5.29±0.97	5.61±0.65	4.400.93	4.89±0.14	

[Values are Mean±SE; P: Plain side; T: Treated side; *,** denotes significant (P≤ 0.05) difference in body weight of different groups of rats; ^{1,2,3} denotes significant (P≤ 0.05) difference in overall average bait consumption among different groups of rats during pre- and post-treatment period;$^{a-c}$ denotes significant (P≤ 0.05) difference in average daily bait consumption among days and groups during the treatment period; ^{A,B} denotes significant (P≤ 0.05) difference in overall average bait consumption among different groups of rats during the treatment period . Mean values with no superscript along the columns and rows denotes non-significant difference at P≤0.05

Overall mean bait consumption from plain and treated sides differed non-significantly between immature male and a female group of rats. However, on the treated side, it was significantly ($P \leq 0.05$) low for mature male rats (3.86±0.42) and significantly ($P \leq 0.05$) high for mature female rats (6.14±0.27). Results, therefore, revealed the attractant/repellent effect of the mandibular gland of mature male rats towards opposite and same-sex respectively, but these effects were not very significant. In the case of both sublingual and parotid gland extract of mature male rats, daily mean bait consumption differs non-significantly from day 1 to day 7, among groups and between treatments during the treatment period. There was also a non-significant difference in overall mean bait consumption between plain and treated sides in all the four groups of rats. These results indicated neither repellent nor attractant effects of sublingual and parotid gland extract of mature male rats towards same and opposite sexes of immature and mature rats.

Comparison of data on the effectiveness of salivary gland extract of mature male and female rats against animals of different maturity groups and sexes revealed slight attractant/repellent effects of mandibular gland extracts of mature male and female rats towards mature rats of both sexes and mature females were slightly repelled from parotid gland extract of mature female rats indicating the presence of pheromones in low titer in mandibular and parotid gland secretions which might be playing a secondary role in sexual communication in mature rats. Cheek glands secretions were also reported to attract both male and female rats. Response of male to female scent was comparatively greater than that of female to male cheek gland secretions in *Rattus norvegicus albinus* (Kannan and Archunan 2001). This might be due to the active involvement of androgens in the pheromonal communication in mature rats (Kannan and Archunan 1998). Cheek glands secretions of sexually mature male and female lesser bandicoot rats have also been reported to attract the opposite sex (Kannan and Archunan 1999). Laukaitis *et al.* (2005) observed the expression of many androgen binding proteins (Abps) in various glands including the lacrimal and salivary glands. It was reported that Salivary Abp mediates the role of mate preference and saliva might be involved in sexual communication (Wickliffe *et al.*, 2002) indicating the presence of pheromones in the salivary glands. Other than sexual communication, cheek gland secretions were also reported to be involved in marking activities of mammalian species such as mice, rats, and squirrels (Kannan and Archunan 1999). During the present study, salivary glands extract of mature male and female rats were not attracted to immature rats. It might be due to the reason that scents of sexually receptive, opposite-sex conspecifics are typically not attractive to receivers with low circulating titers of gonadal hormones (Petrulis, 2013).

CONCLUSION

From this research, we concluded that mature and immature rats of both sexes do not show any response toward salivary gland extract of immature rats of both sexes. Mature rats were more responsive to mandibular gland extract of mature male and female rats. These results indicated that mandibular gland extract of mature male and female house rats may play a role in social and sexual communication. During present study, we have confirmed the pheromonal nature of the mandibular gland of the mature male and female house rats.

FUTURE SCOPE

In future, chemical analysis of mandibular gland of mature male and female rats can be done to identify the chemical compounds involved in social and sexual communication which can be further used as trap lures to increase the trapability of the rodent pest, house rat.

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