Rat cheek gland compounds: Behavioural response to identified compounds

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Cheek gland secretions from sexually mature and reproductively active male and female laboratory rats (Wister strain), Rattus norvegicus albinus were analysed by gas chromatography/mass spectroscopy. Alkanes, aliphatic acids esters and alcohols were identified in the secretions. Cheek gland preparation from males contained predominantly three compounds, viz. d-n-octyl phthalate (I); 1,2-benzene dicarboxylic acid butyl (2-ethyl hexyl) ester (II); and 1,2 benzene dicarboxylic acid (2-methylpropyl) ester (III) whereas in the cheek gland of females two compounds, viz. 1,2-benzene dicarboxylic acid (2-methylpropyl) ester (I) and 2,6,10-decatrien-1-o, 3,7,11-trimethyl-(Z, E) (II) were the major fractions. The identified compounds were tested for odour preferences. Compounds II and III of males and I and II of females attracted both male and female conspecifics. By contrast, compound I from males only attracted females. The level of attraction also varied from compound to compound. The results suggest that cheek gland secretions have pheromonal functions.

Chemical signals are involved in olfactory communication in many animals. Among mammals, olfactory communication has been especially well studied, primarily focusing on scent gland secretions \( ^{15,18} \), urinary pheromones \( ^{6,7} \), faeces \( ^{4-10} \) and vaginal secretions \( ^{11} \). Such odorous substances can send powerful messages that modulate social and reproductive behaviours of mammals. The study of perceptual mechanisms of pheromonal cueing has been hampered by the lack of identification of specific compounds functioning as conspecific chemical cues. Only for a few species of mammals such as mice, hamsters and elephants the behavioural, chemical and neurobiological aspects of pheromonal communication related to sexual reproduction have been investigated \( ^{14,15} \). Laboratory rat has a variety of known and potential sources of scents such as integumentary glands including preputial, cheek, armpit and flank glands and excretory substances namely urine, faeces and vaginal secretion \( ^{13,14} \). A variety of functions for secretions of skin glands have been described, such as territorial marking, maintenance of social and reproductive behaviours, individual recognition and evocation of fighting behaviour and regulation of ovulation in humans \( ^{15,17} \). Scent glands are comparatively larger and more active in males than in females. In several mammalian species, the scent gland development and the activities are androgen-dependent \( ^{15,18} \). Volatile compounds are predominant in scent gland secretions; non-volatile compounds are less evident. These volatile and non-volatile compounds are easily detected by the main or accessory olfactory systems of the responding animals \( ^{10,21} \). The cheek gland secretions are deposited on substrates including branches of trees, or food items, or on the body itself. Chemical identification of mammalian urinary pheromones have been achieved in mice \( ^{22-24} \), tiger \( ^{25} \) and elephant \( ^{12} \). Though sebaceous glands play an important role in the maintenance of social and reproductive behaviours in mammals, especially in rodents, adequate information is not available about the chemistry of their secretions. In the present study, an attempt to determine the chemical nature of cheek gland secretions was determined. The behavioural importance of the identified compounds was ascertained in an effort to delineate their functions.

**Materials and Methods**

Sexually mature and reproductively active adult male and female laboratory rat (Wister strain), Rattus norvegicus albinus weighing 200 to 250 g were purchased from the King’s Institute, Chennai and housed individually in polypropylene cages (40×25×15 cm) with 2.0 cm husk as bedding material. The bedding material was changed twice a week. Rat feed (Hindustan Lever Ltd, Bangalore) and water was provided ad libitum. Animals were maintained on a 14L : 10D photoperiodic schedule in
a climate controlled environment with a temperature of $25^\circ \pm 3^\circ$ C. Lights were on from 0600 to 1800 hrs.

Twenty-five females in estrus and 25 male rats were sacrificed by cervical dislocation under mild diethyl ether anesthesia. At autopsy, cheek glands were dissected out under a dissection microscope and placed in double distilled solvent mixture (n-hexane and dichloromethane 1:1 ratio v/v) and ground well separately for about 10 min with glass homogeniser under ice cold condition. The supernatant was filtered through silica gel (50-60µ mesh size) and collected in a glass vial and sealed with air tight screw type cap made up of glass (Borosil). The sample vials were stored at -20°C until they were used for analysis.

GC-MS analysis was made in a Shimadzu QP5000 (Japan) instrument under computer control at 70 eV. Using ammonia as reagent gas at 95-eV (Ref. 24) performed chemical ionization. The identified compounds were fractionated by the method of Pause et al. 26.

The extracted samples (20mL) were distilled for 5-10 min at room temperature (30°C) under a vacuum of 0.2 torr. The distillate was condensed by cooling with liquid nitrogen. These distillates were restored to their original volume (2mL) vacuum condensation.

Y-maze odour preference test was conducted by following the procedure of Ferkin and Seamon 27. Three individuals were (randomly taken from a pool of 20 rats) used for each behaviour analysis. Fresh samples were used for each trial. The behaviour was assessed for 15 min with the identified compounds and the solvent mixture was used as control. Both male and females were tested. The time taken for visiting each fraction was recorded. The data were analyzed by student’s $t$ test 28.

Results

The gas chromatographic study revealed the presence of eleven different compounds in male cheek gland extract. Three compounds constituted the major part of the extract. In the male cheek gland, the identified compounds were di-n-octyl phthalate (I); 1,2-benzene dicarboxylic acid butyl (2-ethyl hexyl) ester (II); and 1,2 benzene dicarboxylic acid (2-methylpropyl) ester (III). In the female cheek gland, only two compounds were observed, namely 1,2-benzene dicarboxylic acid (2-methyl propyl) ester (I); and 2,6,10 dodecatrien-1-01,3,7,11-trimethyl- (Z,E) (II). Compound I of the female was also identified in the male cheek gland (Figs 1, 2).

The odour preference test revealed that male cheek gland extract attracted both male and female conspecifics. Compound I of male was more attractive to females than to males whereas, compound II and III attracted both male and female rats (Table 1). Compound I and II of female cheek gland have strongly attracted both males and females and female responders spent significant amount of time with compound I and II of the female cheek gland. Among the two major fractions of female cheek gland, compound II was significantly more attractive to both males and females than compound I.

<table>
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<tr>
<th>Responders</th>
<th>I</th>
<th>I</th>
<th>II</th>
<th>II</th>
<th>III</th>
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<tr>
<td></td>
<td>SS BS</td>
<td>SS BS</td>
<td>SS BS</td>
<td>SS BS</td>
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<td>30.60±3.00</td>
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<td></td>
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<td></td>
<td>Female cheek gland extract</td>
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<td>364.66±4.57#</td>
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</tr>
</tbody>
</table>

SS—Scented slide (Solvent + Solute); and BS—Blank slide/ control slide (Solvent only)

* Opposite sex attractant; and # both sex attractant

**Male cheek gland extract:** Compound I (Di-n-octyl phthalate); compound II (1,2-benzene dicarboxylic acid butyl (2-ethyl hexyl) ester); and compound III (1,2-benzene dicarboxylic acid (2-methylpropyl) ester).

**Female cheek gland extract:** Compound I (1,2-benzene dicarboxylic acid (2-methylpropyl) ester); and compound II (2,6,10 dodecatrien-1-01,3,7,11-trimethyl- (Z,E)).

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**Table 1—Behavioural responses of male and female laboratory rats to compounds (I-III) identified from cheek gland extract (15 min odour preference tests)**

[Values are mean ± SE of 6 observations]
Discussion
The present study has revealed that male and female cheek gland extracts consist of eleven major compounds (see GC and Mass spectra Figs 1,2). Earlier chemical studies in mammalian species, such as mouse29,30, hamster31, tiger25 and elephant32 showed that pheromones consist of group of compounds, and the major compound are of greater biological significance. Dodecyl propionate is identified in the preputial glands of female rat pups and is involved in attracting other members33. The dorsal gland of Tayassu tajacu (collared red peccary) contains some volatile fractions which are involved in the maintenance of social behaviour44.

The results of the behavioural tests with identified compounds in the cheek gland are given in Table 1. The behavioural importance of cheek gland secretions are well documented in bandicoot rats24, black tailed deer35, arboreal squirrels36 and shrews37. These studies suggest that the secretary activity of cheek glands and the frequency of scent marking are higher in adult males than in females and the secretions elicit social

Fig. 1 — Capillary column (with a film thickness of 0.25 m) chromatogram of the rat cheek gland extract. Each compound-giving rise to a signal is analyzed by mass spectrometry (Coupled GC-MS, Shimadzu, Japan). In mass spectra, ion relative abundance is plotted against ion ratio of charge to mass (m/e). All mass spectra were analyzed and the chemical structures determined by comparison with computerized databases.
interactions in both the sexes. The present findings demonstrate that cheek gland secretions are involved in the attraction of both male and female rat. The response of male to female scent was comparatively greater than that of the female to male cheek gland secretions. This may be due to the active involvement of androgen in pheromonal communication in rats.

Kannan recently reported that male rat preputial gland contains a mixture of volatiles including di-n-octyl phthalate. Di-n-octyl phthalate is also identified in the male cheek gland of laboratory rats. Further, the female responders devote more time investigating this compound. Hence, this compound may also be involved in sexual attraction between males and females. Earlier reports are consistent with this view. Since di-n-octyl phthalate is found only in male cheek glands and is a powerful attractant for females it may be considered as an important pheromonal compound in the rat.

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