

## CHAPTER 23

## CHEMICAL COMMUNICATION IN DASYURID MARSUPIALS

*C.L. Toftegaard<sup>A,B</sup> and A.J. Bradley<sup>B</sup>*

<sup>A</sup>Department of Medical Physiology, The Panum Institute, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen K, Denmark.

<sup>B</sup>School of Biomedical Sciences, Department of Anatomy and Developmental Biology, The University of Queensland, Brisbane, Queensland 4072, Australia. Email: A.Bradley@mailbox.uq.edu.au

This chapter gives a brief overview of studies that have been carried out to describe the way in which dasyurid marsupials communicate by chemical means. This involves the production of chemical substances from both cutaneous scent glands and glands associated with the reproductive tract, the dispersal of these substances by various morphological adaptations, and the detection of these airborne chemicals (pheromones) by the special sensory system, the vomeronasal organ (VNO). Gas chromatography coupled with mass spectroscopy GC-MS is increasingly being applied to identify substances used by animals in chemical communication. While many of the structures used by animals to detect pheromones have been known for many years, it is only now with the availability of sensitive techniques such as functional magnetic resonance imaging (fMRI) that we are able to visualise regional changes in brain activity in temporal sequence in response to these pheromones.

**INTRODUCTION**

Within the last two decades, the role of olfaction in the mediation and control of reproductive behaviour and in modulation of reproductive physiology in many eutherian species has been extensively studied. In many species, chemosignals or pheromones of male origin may alter the timing of adult oestrus cycles and accelerate the onset of puberty in females (Vandenbergh 1969, Carter et al. 1980, Drickamer 1983), and numerous studies have described the effect of female urinary pheromones on testosterone, LH and gonadotropin levels in males of many species (Clancy et al. 1988, Waring et al. 1996). Although the complexity of social communication in marsupials appears fully equivalent to that observed in ecologically similar eutherian taxa

(Gansglosser 1982, Fadem 1986), it is not well understood and remains relatively unexplored. Pheromonal effects on ovulation and oestrus have been recorded in only four marsupial species: the woolly opossum *Caluromys philander* (Perret and M'Barek 1991), the grey short-tailed opossum *Monodelphis domestica* (Fadem 1985, 1987), and the dasyurids *S. crassicaudata* (Smith et al. 1978), and *A. stuartii* (Scott 1986). Fadem (1987) reports that adult female grey short-tailed opossums normally remain in anoestrus when housed in single-sex groups, and only enter oestrus when exposed to male pheromones (Fadem 1989b, Stonerook and Harder 1992). In contrast, Scott (1986) found that isolated female *A. stuartii* ovulate synchronously when placed with grouped females suggestive of an oestrus-stimulated

female chemosignal. Based on the findings of Scott (1986) and the observations of Cockburn and Lazenby-Cohen (1992) that male *A. stuartii* may use olfactory cues to locate females during the mating period, further studies are warranted to explore the importance of olfactory cues in reproduction and social communication in this species.

The brains of eutherian and metatherian mammals differ very little in both their structure and function (Johnston 1977). As is the case in eutherian mammals, marsupial sensory systems are well-developed, particularly the olfactory bulb which is prominent in all marsupials (Croft 1982). Furthermore the nasal cavity of marsupials is covered with extensive areas of olfactory epithelium, and may contain a functionally intact vomeronasal organ (Croft 1982) that is believed to be specialised for reception of chemosignals (Jacobson 1811, Meredith and O'Connell 1979, Taniguchi et al. 1992a, 1992b).

Because of several problems associated with the use of the term *pheromone*, the term *semiochemical* is now more commonly used in studies of chemical communication. A pheromone is generally regarded as a substance that elicits a stereotypic response whatever the circumstances associated with its release and reception. Such a substance may have dual effects, acting as an attractant to members of one sex and at the same time eliciting an aggressive response from members of the other sex. A *semiochemical* encompasses any form of chemical communication, from a single compound to a complex mixture. While the chemical may be an odour, it does not have to be. The response that an individual makes to the semiochemical will depend upon the context, physiological state and previous experience of the recipient (Mykytowycz 1972). Semiochemistry is thus much broader than the concept of a pheromone with the former broad term including the latter (Albone 1984).

### TAXONOMIC REASSESSMENTS

Taxonomic reassessments of genus *Antechinus* are relevant to several earlier studies of dasyurid marsupials in eastern and SE Australia. Studies of *A. stuartii* carried out at Mount Glorious in SE Queensland would now be regarded as studies of *A. subtropicus* because of a recent reassessment (Van Dyck and Crowther 2000) while studies of *Antechinus stuartii* from SE Australia, such as those ecophysiological studies conducted near Warburton (Bradley et al. 1980), would now be regarded as investigations of *A. agilis* in the light of a reassessment by Sumner and Dickman (1998). Behavioural studies carried out on *A. stuartii* in forests near Canberra would be regarded as studies of *A. flavipes*.

In spite of the apparent morphological and genetic differences, most researchers would agree that apart from slight differences in the timing of reproduction all these species share a common life history strategy. To avoid confusion in references to *Antechi-*

*nus* in this chapter, the new species names will be included in brackets after the species names used in the original citation.

### SCENT-MARKING BEHAVIOUR

Scent marking and subsequent olfactory investigation of chemosignals are fundamental components of social interactions within the majority of mammalian species. In mammals, a variety of scent marking mechanisms are found ranging from urine, faeces, vaginal secretions and saliva to specialised glands such as the temporal gland of elephants, supraorbital and inter-digital glands of many ungulates, anal glands of carnivores, and the sternal gland of the marsupial sugar glider (Strahlendorff 1987, Stoddart et al. 1994). Research into the chemistry of mammalian pheromones, which builds on the success of chemical investigations of insect pheromones, has mainly focused on social conditions which influence sexual maturation and reproduction in rodents (Drickamer 1984, Jemiolo et al. 1994), but is now increasingly employed in a wide range of other species (e.g. tigers, beavers, moles, badgers and primates).

Within the Metatheria, little is known about the chemistry of chemosignals although the involvement in reproduction and social organisation is well documented (Fadem 1989a, Fadem et al. 1989). Biggins (1979, 1984) reported on the importance of chemical communication to the brushtail possum *Trichosorus vulpecula*. Woolhouse and co-workers (1994) have described a possible paraoal and sternal gland pheromone in the *Trichosorus vulpecula* which may act as an intraspecific attractant. Furthermore, partially racemic compounds have been identified as possum urinary metabolites (Carman and Klika 1992), the function of which, however, is unknown. To our knowledge, only one study has identified chemical compounds with possible pheromonal effects in a dasyurid marsupial. In *Antechinus subtropicus* from Mount Glorious in SE Queensland males possess both a sternal gland and a cluster of paraoal glands which are believed to produce chemical signals for intraspecific communication (Toftegaard 1999). Females also possess paraoal glands, the function of which is unknown, but they may also serve in chemical communication.

Using a solid phase microextraction technique, urinary volatiles from male, female and castrated *A. subtropicus* were extracted and analysed using GC-MS. Fourteen volatile compounds were identified, some of which were gender-specific (Table 1).

The GC-MS profile of the males were distinguished by two pyrazine compounds (2,6-dimethylpyrazine and 2-ethenyl-6-methylpyrazine) and a series of methyl ketones which were not detected in the profiles of females, nor in the castrate (Toftegaard, Moore and Bradley 1999). Urine from females, however, contains several aldehydes not present in the profile of males. The apparent sexual dimorphism in urinary constituents such as pyrazine derivatives in *A. subtropicus* is of particular interest because such compounds

**Table 1** Structure of volatile compounds identified in urine of *Antechinus stuartii* using GC-MS.

| Urinary volatile            | Mol. Wt. (Da) | Elemental formula                                 | Present in males | Present in females | Present in castrates |
|-----------------------------|---------------|---|------------------|--------------------|----------------------|
| 2,6-dimethylpyrazine        | 108.14        | C <sub>6</sub> H <sub>8</sub> N <sub>2</sub>      | X                |                    |                      |
| 2-ethenyl-6-methylpyrazine  | 120.15        | C <sub>7</sub> H <sub>8</sub> N <sub>2</sub>      | X                |                    |                      |
| 2-heptanone                 | 114.19        | C <sub>7</sub> H <sub>14</sub> O                  | X                | X                  |                      |
| 2-octanone                  | 128.22        | C <sub>8</sub> H <sub>16</sub> O                  | X                |                    |                      |
| 2-nonanone                  | 142.24        | C <sub>9</sub> H <sub>18</sub> O                  | X                |                    |                      |
| 2-decanone                  | 156.27        | C <sub>10</sub> H <sub>20</sub> O                 | X                |                    |                      |
| 2-hexanone                  | 100.16        | C <sub>6</sub> H <sub>12</sub> O                  | X                |                    |                      |
| 2-undecanone                | 170.29        | C <sub>11</sub> H <sub>22</sub> O                 | X                |                    |                      |
| Nonanal                     | 142.24        | C <sub>9</sub> H <sub>18</sub> O                  |                  | X                  |                      |
| Decanal                     | 156.27        | C <sub>10</sub> H <sub>20</sub> O                 |                  | X                  |                      |
| Undecanal                   | 170.30        | C <sub>11</sub> H <sub>22</sub> O                 |                  | X                  |                      |
| Benzaldehyde                | 106.13        | C <sub>7</sub> H <sub>6</sub> O                   |                  |                    |                      |
| Decanol                     | 158.29        | C <sub>10</sub> H <sub>22</sub> O                 |                  |                    |                      |
| 2,4-dithiapentane           | 108.23        | C <sub>3</sub> H <sub>8</sub> S <sub>2</sub>      | X                |                    | X                    |
| N-butyl benzene sulfonamide | 213.30        | C <sub>10</sub> H <sub>15</sub> NO <sub>2</sub> S | X                | X                  | X                    |
| limonene                    | 136.24        | C <sub>10</sub> H <sub>16</sub>                   | X                | X                  |                      |

have also been reported in tree shrews *Tupaia belangeri* (Strahlen-dorff 1987) where male-specific 2,5-dimethylpyrazine was identified. The presence of this compound was interpreted to be directly related to the activation of male chinning behaviour. The identification of urinary pyrazines as key regulatory components in reproduction has shown that long-term exposure of female mice to male-specific 2,5-dimethylpyrazine may inhibit their overall reproductive fitness (Jemiolo and Novotny 1993). Pyrazines have been found in the adrenal gland of mice (Novotny et al. 1986), and adrenalectomy, but not ovariectomy, eliminates the biological activity of excreted urine (Drickamer and McIntosh 1980). Finally, it has been proposed that tetraethylpyrazine and 2,5-dimethylpyrazine may modulate the gonadotropin releasing hormone and/or gonadotropic hormone release from the hypothalamic-pituitary axis in juvenile rats (Yamada et al. 1989). The role of these sexually dimorphic urinary constituents in social interactions and during the life history of *A. subtropicus* is not yet fully understood. It may be that male-specific pyrazines serve as female attractants during the breeding period or conspecific male aggression signals. Further studies are needed to address the involvement of urinary pheromones during social interactions in *A. subtropicus*.

Olfactory stimuli in the form of pheromones, semiochemicals or 'social odours', may represent specific metabolites in the animals' waste products such as urine or faeces, or may be released from specialised glandular regions on the body. Chemosignals may be actively deposited by rubbing the glandular area against the substrate known as scent marking, or passively through indiscriminate liberation. Scent glands have been described in

15 of the 19 orders of mammals (Thiessen and Rice 1976). Within the Metatheria, scent glands have been identified in the sugar glider *Petaurus breviceps* (Stoddart and Bradley 1991), *Phascogale calura* (Bradley 1997), and grey short-tailed opossum *Monodelphis domestica* (Fadem and Schwartz 1986, Fadem et al. 1989, Fadem 1990). Several different forms of scent marking behaviour have been reported in dasyurids (Table 2).

Most species studied are known to use cloacal dragging to deposit chemosignals. Sternal and chin rubbing are also frequently used marking mechanisms.

Sexual dimorphism has been reported in marking behaviour and physiology. During the mating period, for example, male *A. subtropicus* secrete copiously from their cutaneous sternal glands (Bradley pers. obs.), and have been found to frequently scent mark their nesting area using cloacal rubbing (Braithwaite 1974). Although females of this species also possess cloacal glands, they do not appear to engage in marking behaviour. In *Planigale maculata*, only males use sternal marking (Van Dyck 1979), and in *Sminthopsis crassicaudata*, only males use cloacal marking in female-male encounters (Ewer 1968a).

#### MORPHOLOGICAL ADAPTATIONS TO PHEROMONE DISPERSAL

The hair covering the surface of specialised integumentary glands located around the body of various mammals are known to serve special functions. They may occur in the form of well-developed tufts as in the case of the flank gland of the shrew (Balakrishnan 1987), and the tarsal and metatarsal glands of

**Table 2** Modes of chemical deposition used by dasyurids in olfactory communication. (+) indicates observed; (-) shown not to occur.

| Species                          | Urine dribble | Gloacal drag | Chin rub | Cheek rub | Sternal rub | Reference                               |
|----------------------------------|---------------|--------------|----------|-----------|-------------|---|
| <i>Planigale maculata</i>        |               | +            | +        |           |             | Van Dyck 1979                           |
| <i>Planigale tenuirostris</i>    |               | +            |          |           | +           | Andrew and Settle 1982                  |
| <i>Ningau</i> spp.               |               | +            | +        |           |             | Fanning 1982                            |
| <i>Antechinus flavipes</i>       | +             | +            |          |           | +           | Ewer 1968                               |
| <i>Antechinus stuartii</i>       |               | +            | +        |           | +           | Rigby 1972, Braithwaite 1974            |
| <i>Sminthopsis crassicaudata</i> | -             | +            |          |           | +           | Ewer 1968a+b                            |
| <i>Sminthopsis macroura</i>      | +             | +            |          |           |             | Van Dyck 1979                           |
| <i>Antechinomys laniger</i>      |               | +            | +        |           |             | Happold 1972, Eisenberg and Golani 1977 |
| <i>Dasyercus cristicauda</i>     |               | +            |          |           |             | Ewer 1968b, Sorenson 1970               |
| <i>Dasyuroides byrnei</i>        | +             | +            | +        |           |             | Sorenson 1970, Aslin 1974               |
| <i>Dasyurus viverrinus</i>       | +             |              |          |           | +           | Eisenberg and Golani 1977               |
| <i>Sarcophilus harrisii</i>      | +             | +            |          |           | +           | Ewer 1968b, Eisenberg and Golani 1977   |

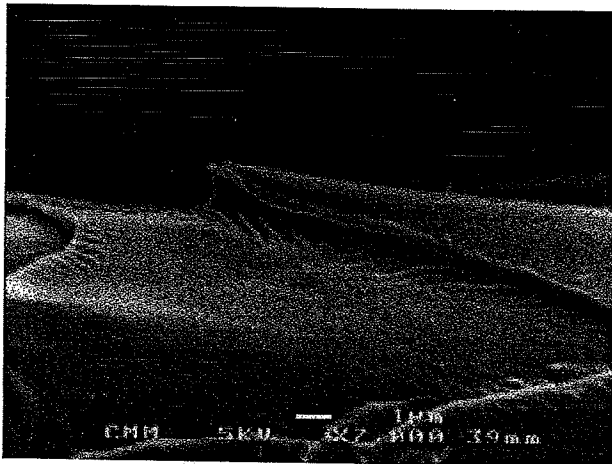
deer (Müller-Schwarze et al. 1977) functioning as visual stimuli during intraspecific interactions, or they may be structurally different from hairs of other body regions and modified for holding and releasing materials of olfactory relevance. Such structural modifications have been reported in the Crested rat, *Lophiomys imhausi* (Stoddart 1979) and some species of pteropodid and molossid bats (Hickey and Fenton 1987). The term 'osmetrichia' has been proposed for structurally specialised mammalian scent hair (Müller-Schwarze et al. 1977) that is capable of retaining material containing olfactory information.

As far as we are aware, the presence on the hair surface of specialisations for holding material that may provide chemical signals in social communication has not been described in dasyurid marsupials. Bradley (1997) has discussed the apparent importance of the sternal gland and its secretions in *Phascogale calura* in which the sternal gland of males becomes very active when the plasma testosterone concentration rises during the breeding period. In ovariectomised females of another marsupial species the grey short-tailed opossum *Monodelphis domestica* the development of suprasternal glands and chest marking may be stimulated by testosterone administration but not by oestradiol (Fadem 1990). Similarly in castrated males testosterone, but not oestradiol, stimulated chest marking (Fadem et al. 1989a, b). This androgen dependence of cutaneous scent gland function has also been described (Stoddart and Bradley 1991; Stoddart, Bradley and Mallick 1994) in the sugar glider *Petaurus breviceps*.

The production of sternal gland secretion in *A. stuartii* (*A. agilis*), but also in *A. subtropicus* in SE Queensland, is at a maximum at the time of breeding coinciding with male peak androgen concentration (Bradley et al. 1980) however individual differences are apparent. In light of this relationship between onset of breeding and activity of the gland and the apparent

morphological difference between hair covering the sternal gland and hair from other body regions, the hair of the sternal gland was studied using the scanning electron microscope (SEM) (Toftegaard and Bradley 1999). In this study comparisons were made between the surface ultrastructure of sternal gland hair and hair from the dorsum of sexually intact males ( $n = 3$ ), castrated males ( $n = 2$ ) and females ( $n = 3$ ) to determine whether hairs that overlie the scent producing gland would show surface specialisation to promote scent retention. Morphological differences were evident between the sternal gland hairs and back hairs of the intact males that had active sternal glands. The specialised sternal gland hairs were only about one-third of the length of the control hairs from the dorsal body region in contrast with the relatively flat surface of the long dorsal hair created by the smooth, even cuticular scales. The scales of the sternal gland hairs project outward from the cortex, creating angled chambers between the scales.

At magnifications of  $\times 7000$  numerous ridges and grooves were found lining the distal edges of the scales (Fig. 1); however, only hairs from the sternal gland of the two actively secreting males had large numbers of these grooves. Hairs taken from both the sternal and dorsal areas of females lacked visible grooves and no comparable grooves were identified on the sternal hairs of the castrate males (hairs examined 6 months after castration) and males which were not actively secreting. The scales from hair of actively secreting males appeared very efficient in retaining lipid material. Some sternal hairs were still covered with sebum after a period of 72 hours in solvent, thus making examinations of grooves difficult. The general appearance of the hair shaft changed from root to tip, the scales becoming elongate and narrowly conical towards the tip. This pattern was identical for all hair samples examined.



**Figure 1** Scanning electron micrograph of a sternal gland hair from an actively secreting male showing the pattern of arrangement of cuticular angular scale 'chambers' displaying distal ridge-like grooves. x7000 (white scale bar = 1  $\mu$ m).

The present study revealed that the specialised fur overlying the male sternal gland surface exhibits structural modifications in the form of rough keratinised chambers which act as reservoirs for the glandular secretions. Structurally these modifications resemble the sebum-storing flank gland hairs of the musk shrew, *Suncus murinus viridescens* (Balakrishnan 1987) and common shrew, *Sorex araneus* L. (Kapischke and Mühle 1988), which have been recognised as important instruments in scent-marking in these species. However, this specialisation is found only in the sternal gland area of the male *A. stuartii*, in contrast to the musk shrew in which both sexes possess the modification.

Pheromones have been chemically identified in the ventral gland sebum of the jird *Meriones tristrami* (Kagen et al. 1983) and *Meriones unguiculatus* (Thiessen et al. 1974). From ongoing chemical and behavioural studies in our lab we suggest that male *A. stuartii* may discharge a continuous, but highly volatile scent from the sternal gland during the breeding season for which the glandular sebum acts as a carrier. The presence of these modified scent gland hairs as special sebum storing chambers may facilitate the continuous availability of the volatile pheromone as well as delay its oxidation and degradation upon exposure to air. Since the chemical substances of olfactory relevance are of low molecular weight (C. Moore pers. comm.), it is proposed that the lipid secretions produced by the sternal glands and retained in the modified hair chambers act as a medium for olfactory information required for intraspecific communication during the breeding season. While cloacal marking is common during behavioural encounters (Braithwaite 1974) male *A. stuartii* do not appear to use their sternal gland for scent-marking. It is proposed that the glandular sebum contains a pheromone that may act as a status signal during the breeding season, which occurs in

many mammal species (Boero 1995). We propose that the functional significance of the sternal hair modifications is to stabilise and delay oxidation of this pheromone, an effect that has been suggested by Allen (1975; 1982) to occur with paracloacal secretions in *Trichosurus vulpecula*.

Quite clearly the hair overlying the sternal gland in *Antechinus stuartii* possesses surface features that would appear to assist both in the storage and propagation of odour.

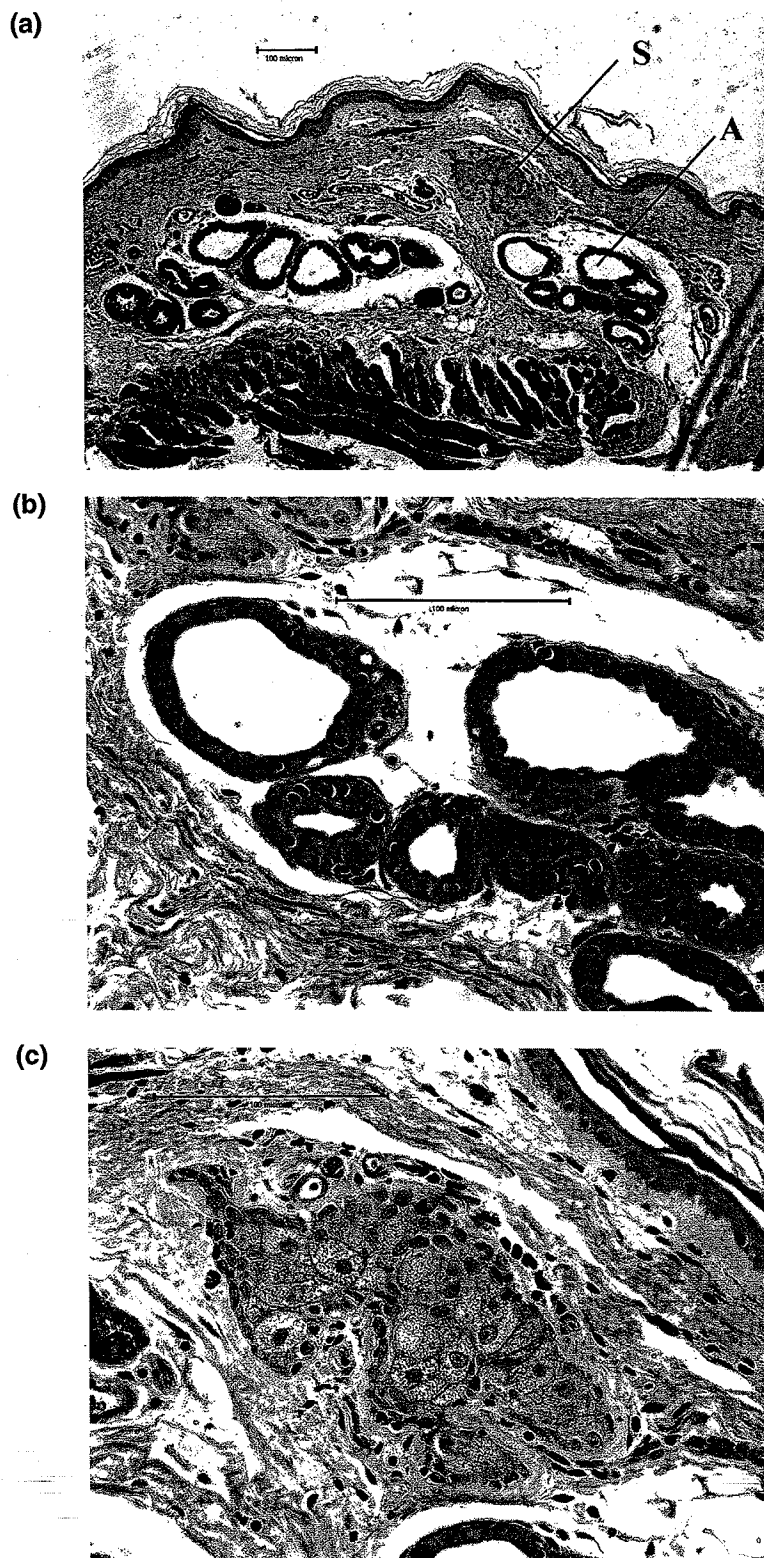
### STERNAL GLAND MORPHOLOGY AND CONTROL

All adult *A. stuartii* males possess a sparsely haired patch of skin approximately 3 mm in diameter in the sternal region which is covered by a thin oily film. This is particularly prominent during the breeding period in September. Histological examination of biopsies (Bradley and Stoddart 1991) taken from the sternal gland (Fig. 2a) revealed that this organ consists of two distinct layers of glandular tissue (Fig. 2a), a deep layer of apocrine tissue (Fig. 2b) overlain by a layer of holocrine sebaceous tissue (Fig. 2c). The sebaceous secretion appears colourless when produced by the gland; however, with prolonged oxidation, an orange staining of the fur surrounding the glandular tissue is observed.

The sternal gland of adult male *A. stuartii* contains both apocrine and sebaceous glandular tissues, the latter of which may be under androgen control (Toftegaard and Bradley 1999). This is in agreement with a study by Dixon (1976) on the Greater Galago *Galago crassicaudatus crassicaudatus*, in which it was found that low levels of testosterone are insufficient to maintain sebaceous glands and result in atrophy of the entire sternal gland. The low plasma testosterone levels observed in the castrate male *A. stuartii* are presumably caused by the continuous secretion of androgens from the zona reticularis of the adrenal cortex.

Sebaceous gland secretions may act as a vehicle for the volatile substances produced by the apocrine glands (Mykytowycz 1972). This type of cooperative action has been suggested for civetone, the crude secretion from the glands of the civet cat. This substance is well known for its use as a fixative by perfumers to extend the valuable components of the perfume. It acts as a *fixative*, binding to the molecules with high volatility and, in so doing, slowing their release rate. This ensures that the more ephemeral odours produced by the animal persist in the scent for much longer than would otherwise be expected (Whitten 1969).

Experiments on different mammalian species using castration, and castration followed by subsequent hormone replacement, have shown that androgens greatly increase sebaceous gland size, whereas oestrogen may reduce them (Dryden and Conway 1967, Jannett 1978, Bradley and Stoddart 1993, Helder and Freymuller 1995, Romo et al. 1996). The same has been demonstrated in paracloacal glands (Bradley and Stoddart 1993). In the marsupial sugar glider *Petaurus breviceps*, plasma androgens



**Figure 2** Longitudinal section through a gland biopsy core sample taken from a male *A. substriatus* (a) showing the two distinct glandular zones (A = apocrine tissue zone, S = sebaceous tissue zone). (b) apocrine tissue. (c) sebaceous tissue. [Scale bars = 100  $\mu\text{m}$ ].

have been found to play an essential role in the development and activity of cutaneous scent-producing glands (Stoddart and Bradley 1991, Mallick et al. 1994, Stoddart et al. 1994). Although such correlations between androgens and gland activity have yet to be described in dasyurids, the production of sternal gland secretions in male *A. stuartii* (*A. agilis*) is at a maximum with the onset of the mating period, which coincides with a peak androgen concentration in the blood of males (Bradley et al. 1980).

Androgen replacement treatment increases sebaceous gland size, mitotic activity and lipogenesis (Ebling et al. 1971, Stoddart and Bradley 1991) in many mammalian species. Although the apocrine tissue within the side glands of the shrew *Suncus murinus* (Dryden and Conway 1967) and the skin glands of the hare and rabbit have been found to respond in the same way to the withdrawal and replacement of testosterone, this was not observed in male *A. subtropicus*. In this species only apocrine cell height was observed to increase following testosterone implantation, whereas apocrine cell nuclear diameter was found to decrease as the plasma testosterone concentration increased.

Knowledge of the specific behavioural contexts in which the sternal gland of *A. subtropicus* is used is at present limited. It is known, however, that specialised osmetrichia are found (Toftegaard and Bradley 1999) overlying the sternal gland that may prevent rapid oxidation of the gland exudate thus prolonging the release of a chemical signal. From studies on the sugar glider, *Petaurus breviceps* (Stoddart and Bradley 1991), it is known that the frontal gland secretions may contribute to the whole-body odour which, when released by dominant males, can effect the elevation of plasma cortisol and catecholamine levels as well as heart rate in subordinates. Unlike sugar gliders, *A. stuartii*, have no distinct hierarchical system; however, social communication during lekking displays at the time of breeding may be facilitated by whole-body odour in which sternal gland secretions may play a vital part. The fact that testosterone levels increase prior to the onset of the breeding season (Cockburn and Lazenby-Cohen 1992) supports the theory that sternal gland secretions may be involved in the triggering of sexual behaviour.

#### PERCEPTION OF CHEMOSIGNALS – OLFACTORY SYSTEMS

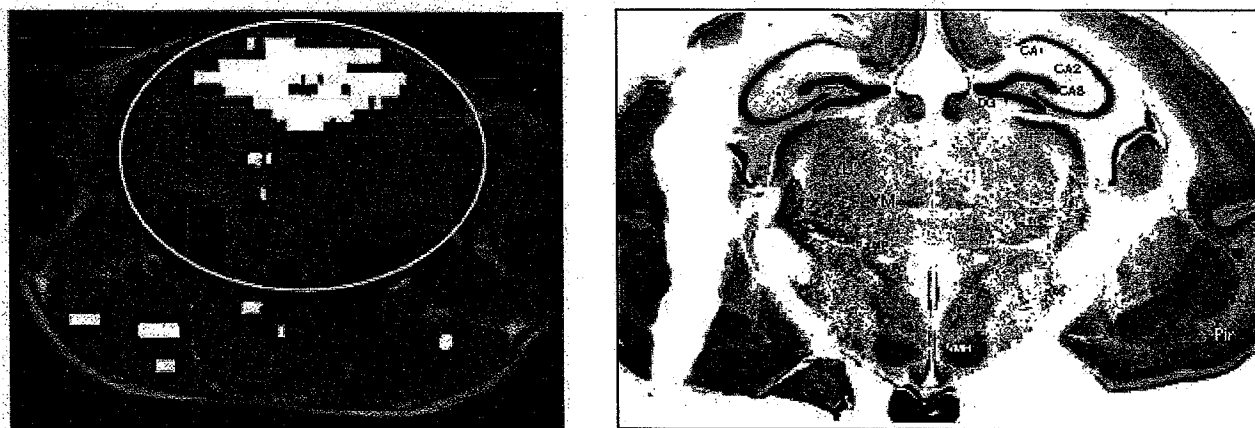
Two systems are currently recognised as perceiving chemosensory input: the main olfactory system and the accessory olfactory system. The main olfactory system is suggested to serve a general function as a 'molecular' analyser for environmental chemicals without predetermined meaning. In this capacity, it would serve a role in the association between odours and contexts. The accessory olfactory system, which consists of chemoreceptor neurons in the vomeronasal organ, and their central pathway through the accessory olfactory bulb, amygdala and basal forebrain, has been implicated in pheromone detection and chemical communication in several species (Pfeiffer and Johnston 1993, Jackson and

Harder 1996). Although used extensively in the literature, the proposed segregation of function between the main and accessory olfactory system is not absolute (Meredith 1991).

First described by Jacobson (1811), the vomeronasal organ (VNO) has been shown to be of particular importance in the mediation of chemical signals such as pheromones, and play a crucial role in biologically significant functions related to feeding, and more specifically breeding (Johnston and Rasmussen 1984, Johnston 1985, Pfeiffer and Johnston 1993). VNOs are two paired crescent-shaped tubular chemosensory organs that run caudally in the upper palate at the base of the nasal septum. Depending on the particular species, they open either to the nasal cavity, the mouth or both (Schilling et al. 1990). The VNO is encased by either a bony or cartilaginous capsule, and contains a specialised neuroepithelium that differs from the main olfactory epithelium in that the apical portions of the bipolar receptor cells are lined with microvilli instead of cilia. The VNO is derived embryologically from the olfactory placode, and vomeronasal sensory neurons, like olfactory neurons, regenerate continuously throughout life (Liman 1996). The olfactory nerves of the main olfactory system amalgamate in the main olfactory bulb, from which projections are sent to the primary olfactory cortex, the nucleus of the lateral olfactory tract, the olfactory tubercle, and the periamygdaloid region. In contrast, the vomeronasal nerves containing axons of bipolar neurons project to the accessory olfactory bulb, from which secondary neurons extend into the bed nucleus of the stria terminalis, the medial amygdala, and the hypothalamus, thereby facilitating direct pheromonal influences on reproductive physiology and behaviour (Wysocki and Meredith 1991, Kennedy and Anholt 1997). Relatively few studies have described the anatomy and function of the accessory olfactory system in metatherian species (Kratzing 1978, Shamamah-Lagnado and Negrao 1981, Kratzing 1982a, b, 1984; Jackson and Harder 1996), and information regarding VNO anatomy and functional significance in chemical communication in dasyurids is at present lacking.

#### NEW DIRECTIONS IN OLFACTORY RESEARCH

Our knowledge of the central neural mechanisms of marsupial olfaction is limited because of the lack of objective methods to evaluate it. Positron emission tomography (PET) is one useful method for investigating brain structure-function relationships. However, since PET uses radioactive substances, subjects may suffer from radiation overexposure. The advent of magnetic resonance imaging (MRI) has enabled researchers to use a non-radioactive high-resolution *in vivo* method to study the auditory, visual, motor and sensory regions of the mammalian brain. Recent studies of human olfactory function (Koizuka et al. 1994, Yousem et al. 1997, Sobel et al. 1998) have demonstrated that it is possible to use MRI to detect alterations of brain activation. This technique of functional MRI (fMRI) is particularly



**Figure 3** White pixels indicate signal activation in coronal section through the brain of a female *A. subtropicus* during exposure to male urine. The brain tissue is circled in white. Tissue lying outside this circle is bone and soft tissue. Activation is evident in the frontal (area 1–3) cortex, hippocampal fissures (CA1–3), dentate gyrus (DG), anterior paraventricular, ventrolateral (VL) and ventromedial thalamic (VM) nuclei, and paraventricular (PaMP) nucleus of the hypothalamus. A corresponding coronal histological section through the brain of *Antechinus subtropicus* is indicated on the right-hand side. Note that section on the right-hand side contains only brain tissue and is equivalent to the tissue outlined on the left-hand side.

useful because it is possible to tune the instrument to detect different target molecules. General brain activity can be inferred by looking at differences in the number of oxygen molecules with a defined tissue before and after a particular experimental treatment. Regional changes in vascular perfusion may be indicated within the hippocampus and hypothalamus (Fig. 3) of an isoflurane anaesthetised marsupial mouse *Antechinus subtropicus* (Toftegaard et al. 2002).

This picture of altered brain activity was obtained by computing the difference between images collected approximately 70 seconds apart before and after the presentation of a pheromonal stimulus (urinary odour) to the experimental subject. Concern that global vascular change may occur in the brain of an anaesthetised animal and obscure the effect ascribed to the pheromone may be answered by pointing out that pixels indicated in red highlight differences between two otherwise identical brain images.

This technique shows particular promise when it is realised that fMRI can be arranged to target specific and discrete neurotransmitter molecules such as glutamate. This makes it possible to monitor animals during longitudinal experimental studies in which subjects may be investigated using a non-invasive, non-destructive technique. In addition to the intrinsic scientific merit of monitoring progressive change in individuals, the use of fMRI also enables experimental designs using fewer animals. The benefit for animal ethics in this conservative approach is obvious.

#### FUTURE DIRECTIONS

The use of functional MRI promises to revolutionise the study of chemical communication in mammals. In combination with

standard methods for describing cytoarchitecture, such as tract tracing, fMRI allows researchers to describe the temporal sequence of changes that occur in response to the presentation of an odour of biological significance. In *A. subtropicus* 2,4 dimethylpyrazine, that appears in the urine of intact males, has been shown to cause a significant physiological response and it is likely that other compounds may also cause physiological change. Undoubtedly different species will be shown to produce their own unique chemical signatures that can be detected using GC-MS. In many cases these semiochemicals are readily available from commercial sources. When compounds are not commercially available, research arms of some large companies can often supply small quantities for testing (e.g. pyrazines sourced from the tobacco industry). The future seems very promising for studies of the role of chemical communication in behaviour in marsupials. Studies carried out using marsupial models in the US, New Zealand and Australia promise to significantly advance our knowledge in this area of biology during the next few years and the intrinsic and practical benefits will certainly become more apparent both for metatheria and eutheria. While the dasyurid marsupials provide interesting models for the role of pheromones in the pituitary-adrenocortical axis and pathological change, studies of Petauridae and Phalangeridae, in which clear social structures exist, promise to provide important new information in the future.

#### ACKNOWLEDGEMENTS

We thank the Zoological Society of London for permission to use the scanning electron micrograph of osmetrichia.



## REFERENCES

- Albone, E.S., Eglinton, G., Walker, J.M., & Ware, G.C. (1974), 'The anal sac secretion of the red fox (*Vulpes vulpes*); its chemistry and microbiology. A comparison with the anal sac secretion of the lion (*Panthera leo*)', *Life Sci*, **14**:387-400.
- Allen, N.T. (1975), 'A new mechanism for scent dispersal by the anal glands of the Possum, *Trichosurus vulpecula*', unpublished Honours thesis, Zoology Department, University of Western Australia, Perth.
- Allen, N.T. (1982), 'A study of the hormonal control, chemical constituents, and functional significance of the paracloacal glands in *Trichosurus vulpecula*', unpublished MSc thesis, Zoology Department, University of Western Australia.
- Balakrishnan, M. (1987), 'Sebum-storing flank gland hairs of the musk shrew, *Suncus murinus viridescens*', *J Zool Lond*, **213**:213-20.
- Biggins, J.G. (1979), 'Olfactory communication in the brush-tailed possum *Trichosurus vulpecula* Kerr, 1792 (Marsupialia: Phalangeridae)', unpublished PhD thesis, Department of Zoology, Monash University, Melbourne.
- Biggins, J.G. (1984), 'Communications in possums: a review', in *Possums and Gliders* (eds. A.P. Smith, & I.D. Hume), pp. 35-57, Australian Mammal Society & Surrey Beatty & Sons, Sydney.
- Boero, D.L. (1995), 'Scent-deposition behaviour in the alpine marmot (*Marmota marmota* L): Its role in territorial defence and social communication', *Ethology*, **100**:26-38.
- Bolliger, A., & Whitten, W.K. (1948), 'The paracloacal (anal) glands of *Trichosurus vulpecula*', *Proceedings of the Royal Society of New South Wales*, **82**:36-43.
- Bradley, A.J. (1997), 'Reproduction and life-history in the Red-tailed phascogale, *Phascogale calura* (Marsupialia: Dasyuridae): The adaptive-stress senescence hypothesis', *J Zool Lond*, **241**:739-55.
- Bradley, A.J., McDonald, I.R., & Lee, A.K. (1980), 'Stress and mortality in a small marsupial (*Antechinus stuartii*, Macleay)', *Gen & Comp Endocrinol*, **40**:188-200.
- Bradley, A.J. & Stoddart, D.M. (1991), 'A non-destructive small scale biopsy method for obtaining repetitive tissue samples from skin glands in a marsupial', *Aust Mammal*, **14**:151-3.
- Bradley, A.J., & Stoddart, D.M. (1993), 'The dorsal paracloacal gland and its relationship with seasonal changes in cutaneous scent gland morphology and plasma androgen in the marsupial sugar glider (*Petaurus breviceps*; Marsupialia: Petauridae)', *J Zool Lond*, **229**:331-46.
- Braithwaite, R.W. (1974), 'Behavioural changes associated with the population cycle of *Antechinus stuartii* (Marsupialia)', *Aust J Zool*, **22**:45-62.
- Carman, R.M., & Klika, K.D. (1992), 'Partially racemic compounds as Brushtail Possum urinary metabolites', *Aust J Chem*, **45**:651-7.
- Carter, C.S., Getz, L.L., Gavish, L., McDermott, J.L., & Arnold, P. (1980), 'Male-related pheromones and the activation of female reproduction in the prairie vole *Microtus ochrogaster*', *Biol Reprod*, **23**:1038-45.
- Clancy, A.N., Singer, A.G., Macrides, F., Bronson, F.H., & Agosta, W.C. (1988), 'Experiential and endocrine dependence of gonadotropin responses in male mice to conspecific urine', *Biol Reprod*, **38**:183-91.
- Cockburn, A., & Lazenby-Cohen, K.A. (1992), 'Use of nest trees by *Antechinus stuartii*, a semelparous lekking marsupial', *J Zool Lond*, **226**:657-680.
- Dixon, A.F. (1976), 'Effects of testosterone on the sternal cutaneous glands and genitalia of the male Greater Galago (*Galago crassicaudatus crassicaudatus*)', *Folia Primatol*, **26**:207-13.
- Drickamer, L.C. (1983), 'Male acceleration of puberty in female mice (*Mus musculus*)', *J Comp Psychol*, **97**:191-200.
- Drickamer, L.C. (1984), 'Urinary chemosignals from mice (*Mus musculus*): Acceleration and delay of puberty in related and unrelated young females', *J Comp Psychol*, **89**:414-20.
- Drickamer, L.C., & McIntosh, T.K. (1980), 'Effects of adrenalectomy on the presence of a maturation-delaying pheromone in the urine of female mice', *Horm Behav*, **14**:146-52.
- Dryden, G.L., & Conway, C.H. (1967), 'The origin and hormonal control of scent production in *Suncus murinus*', *J Mammal*, **18**:420-8.
- Ebling, F.J., Ebling, E., McCafferty, M., & Skinner, J. (1971), 'The response of the sebaceous glands of the hypophysectomized castrated male rat to 5 alpha-dihydrotestosterone, androstenedione, dehydroepiandrosterone and androsterone', *J Endocrinol*, **51**:181-90.
- Ewer, R.F. (1968a), 'A preliminary survey of the behaviour in captivity of the dasyurid marsupial, *Sminthopsis crassicaudata* (Gould)', *Zf. Tier-psychol*, **25**:319-65.
- Fadem, B.H. (1985), 'Evidence for the activation of female reproduction by males in a marsupial, the gray short-tailed opossum (*Monodelphis domestica*)', *Biol Reprod*, **33**:112-16.
- Fadem, B.H. (1986), 'Chemical communication in gray short-tailed opossums (*Monodelphis domestica*) with comparisons to other marsupials and with reference to monotremes', in *Chemical Signals in Vertebrates IV. Ecology, Evolution and Comparative Biology* (eds. D. Duvall, D. Müller-Schwarze, & R.M. Silverstein), pp. 587-607, Plenum Press, New York.
- Fadem, B.H. (1987), 'Activation of estrus by pheromones in a marsupial: Stimulus control and endocrine factors', *Biol Reprod*, **36**:328-32.
- Fadem, B.H. (1989a), 'The effects of pheromonal stimuli on estrus and peripheral plasma estradiol in female gray short-tailed opossums (*Monodelphis domestica*)', *Biol Reprod*, **41**:213-17.
- Fadem, B.H. (1989b), 'Sex differences in aggressive behavior in a marsupial, the Gray Short-Tailed opossum (*Monodelphis domestica*)', *Aggres Behav*, **15**:435-41.
- Fadem, B. (1990), 'The effects of gonadal hormones on scent marking and related behaviour and morphology in female gray short-tailed opossums (*Monodelphis domestica*)', *Horm Behav*, **24**:459-69.
- Fadem, B.H., & Schwartz, R.A. (1986), 'A sexually dimorphic supresternal scent gland in gray short-tailed opossums (*Monodelphis domestica*)', *J Mammal*, **67**:205-8.
- Fadem, B.H., Erienne, G.S., & Karen, L.M. (1989), 'The hormonal control of scent marking and precopulatory behavior in male gray short-tailed opossums (*Monodelphis domestica*)', *Hormones & Behavior*, **23**:381-92.
- Gansglosser, V.U. (1982), 'Social structure and communication in marsupials', *Zool Anz*, **209**:294.
- Helder, J., & Freymuller, E. (1995), 'A morphological and ultrastructural study of the paracloacal (scent) glands of the marsupial *Metachirus nudicaudatus* Geoffroy, 1803', *Acta Anat*, **153**:31-8.
- Hickey, M.B., & Fenton, M.B. (1987), 'Scent dispersing hairs (Osmetricia) in some pteropodidae and molossidae (Chiroptera)', *J Mammal*, **68**:381-4.

- Jackson, L.M., & Harder, J.D. (1996), 'Vomeronal organ removal blocks pheromonal induction of estrus in Gray Short-Tailed opossums (*Monodelphis domestica*)', *Biol Reprod*, **54**:506–12.
- Jacobson, L. (1811), 'Description anatomique d'un organe observé dans les mammifères', *Ann Mus Hist Nat (Paris)*, **18**:412–24.
- Jannett, F.J. (1978), 'Dosage response of the vesicular, preputial, anal, and hip glands of the male vole, *Microtus montanus*, to testosterone propionate', *J Mammal*, **59**:772–79.
- Jemiolo, B., & Novotny, M. (1993), 'The long-term effect of a urinary chemosignal on reproductive fitness in female mice', *Biol Reprod*, **48**:926–9.
- Jemiolo, B., Gubernick, D.J., Yoder, M.C., & Novotny, M. (1994), 'Chemical characterization of urinary volatile compounds of *Peromyscus californicus*, a monogamous biparental rodent', *J Chem Ecol*, **20**:2489–500.
- Johnston, R.E. (1977), 'The causation of two scent marking behaviors in female golden hamsters (*Mesocricetus auratus*)', *Anim Behav*, **25**:317–27.
- Johnston, R.E. (1985), 'Olfactory and vomeronasal mechanisms of communication', in *Taste, Olfaction and the Central Nervous System* (ed. D.W. Pfaff), New York, Rockefeller University Press, 346 p.
- Johnston, R.E., & Rasmussen, K. (1984), 'Individual recognition of female hamsters by males: Role of chemical cues and of the olfactory and vomeronasal systems', *Physiol Behav*, **33**:95–104.
- Kagan, R., Ikan, R., & Haber, O. (1983), 'Characterization of a sex pheromone in the jird (*Meriones tristrami*)', *J Chem Ecol*, **9**:775–83.
- Kapischke, H., & Mühle, H. (1988), 'Zur morphologie der seitendrüsenaare bei der waldspitzmaus (*Sorex araneus*) (Mammalia, Insectivora, Soricidae)', *Zool Abhandl*, **44**:71–4.
- Kennedy, S.W., & Anholt, R.R.H. (1997), 'Pheromone regulated production of inositol-(1, 4, 5)-triphosphate in the mammalian vomeronasal organ', *Endocrinol*, **138**:3497–504.
- Koizuka, I., Yano, H., Nagahara, M., Mochizuki, R., Seo, R., Shimada, K., Kubo, T., & Nogawa, T. (1994), 'Functional imaging of the human olfactory cortex by magnetic resonance imaging', *Otolaryngol*, **56**:273–75.
- Kratzing, J.E. (1978), 'The olfactory apparatus of the bandicoot (*Isodon macrourus*): Fine structure and presence of a septal olfactory organ', *J Anat*, **125**:601–13.
- Kratzing, J.E. (1982), 'The anatomy of the rostral nasal cavity and vomeronasal organ in *Tarsipes rostratus* (Marsupialia: Tarsipedidae)', *Aust Mammal*, **5**:211–19.
- Kratzing, J.E. (1984), 'The anatomy and histology of the nasal cavity of the koala (*Phascolarctos cinereus*)', *J Anat*, **138**:55–65.
- Liman, E.R. (1996), 'Pheromone transduction in the vomeronasal organ', *Curr Opin Neurobiol*, **6**:487–93.
- Mallick, J., Stoddart, D.M., Jones, I., & Bradley, A.J. (1994), 'Behavioral and endocrinological correlates of social status in the male sugar glider (*Petaurus breviceps* Marsupialia: Petauridae)', *Physiol Behav*, **55**:1131–4.
- Meredith, M. (1991), 'Sensory processing in the main and accessory olfactory systems: Comparisons and contrasts', *J Steroid Biochem Molec Biol*, **39**:601–14.
- Meredith, M., & O'Connell, R.J. (1979), 'Efferent control of stimulus access to the hamster vomeronasal organ', *J Physiol*, **286**:301–16.
- Müller-Schwarze, D., Volkman, N.J., & Zemanek, K.F. (1977), 'Osmetrichia: Specialized scent hair in black-tailed deer', *J Ultrastruct Res*, **59**:223–30.
- Mykytowycz, R. (1972), 'The behavioural role of the mammalian skin glands', *Naturwissenschaften*, **59**:133–9.
- Novotny, M., Jemiolo, B., Harvey, S., Wiesler, D., & Marchlewska-Koi, M. (1986), 'Adrenal-mediated endogenous metabolites inhibit puberty in female mice', *Science*, **231**:722–5.
- Perret, M., & M'Barek, S.B. (1991), 'Male influence on oestrous cycles in female woolly opossums (*Caluromys philander*)', *J Reprod Fert*, **91**:557–66.
- Pfeiffer, C.A., & Johnston, R.E. (1993), 'Hormonal and behavioral responses of male hamsters to females and female odors: Roles of olfaction, the vomeronasal system, and sexual experience', *Physiol Behav*, **55**:129–38.
- Romo, E., de Miguel, M.P., Arenas, M.I., Frago, L., Fraile, B., & Paniagua, R. (1996), 'Histochemical and quantitative study of the cloacal glands of *Triturus marmoratus marmoratus* (Amphibia: Salamandridae)', *J Zool Lond*, **239**:177–86.
- Schilling, A., Serviere, J., Gendrot, G., Perret, M. (1990), 'Vomeronasal activation by urine in the primate *Microcebus murinus*: A 2 DG study', *Exp Brain Res*, **81**:619–25.
- Scott, M.P. (1986), 'The timing and synchrony of seasonal breeding in the marsupial, *Antechinus stuartii*: Interactions of environmental and social cues', *J Mammal*, **67**:551–60.
- Shammah-Lagnado, S.J., Negro N. (1981), 'Efferent connections of the olfactory bulb in the opossum (*Didelphis marsupialis aurita*): A Fink-Heimer study', *J Comp Neurol*, **201**:51–63.
- Smith, M.J., Bennett, J.M., & Chesson, C.M. (1978), 'Photoperiod and some other factors affecting reproduction in female *Sminthopsis crassicaudata* (Marsupialia: Dasyuridae) in captivity', *Aust J Zool*, **26**:449–63.
- Sobel, N., Prabhakaran, V., Desmond, J.E., Glover, G.H., Goode, R.L., Sullivan, E.V., & Gabrieli, J.D.E. (1998), 'Sniffing and smelling: Separate subsystems in the human olfactory cortex', *Nature*, **392**:282–6.
- Stoddart, D.M. (1979), 'A specialised scent-releasing hair in the Crested rat *Lophiomyia imhausi*', *J Zool Lond*, **189**:553.
- Stoddart, D.M., & Bradley, A.J. (1991), 'The frontal and gular dermal scent organs of the marsupial sugar glider (*Petaurus breviceps* Waterhouse)', *J Zool Lond*, **225**:1–12.
- Stoddart, D.M., Bradley, A.J., & Mallick, J. (1994), 'Plasma testosterone concentration, body weight, social dominance and scent-marking in male marsupial sugar gliders (*Petaurus breviceps*; Marsupialia: Petauridae)', *J Zool Lond*, **232**:595–601.
- Stonerook, M.J., & Harder, J.D. (1992), 'Sexual maturation in female gray short-tailed opossums, *Monodelphis domestica*, is dependent upon male stimuli', *Biol Reprod*, **46**:290–4.
- Stralendorff, F.V. (1987), 'Partial chemical characterization of urinary signalling pheromone in tree shrews (*Tupaia belangeri*)', *J Chem Ecol*, **13**:655–79.
- Sumner, J., & Dickman, C.R. (1998) 'Distribution and identity of species in the *Antechinus stuartii* – *A. flavipes* group (Marsupialia: Dasyuridae) in south-eastern Australia', *Aust J Zool*, **46**:27–41.
- Taniguchi, K., Matsusaki, Y., Ogawa, K., & Saito, T.R. (1992a), 'Fine structure of the vomeronasal organ in the common marmoset (*Callithrix jacchus*)', *Folia Primatol*, **59**:169–76.
- Taniguchi, K., Taniguchi, K., Arai, T., & Ogawa, K. (1992b), 'Enzyme histochemistry of the olfactory and vomeronasal sensory epithelia in the golden hamster', *J Vet Med Sci*, **54**:1007–16.
- Thiessen, D.D., Regnier F.E., Rice, M., Goodwin, M., Isaacks, N., & Lawson N. (1974), 'Identification of the ventral scent marking pheromone

- one in the male Mongolian gerbil (*Meriones unguiculatus*)', *Science*, **184**:83-4.
- Thiessen, D., & Rice, M. (1976), 'Mammalian scent gland marking and social behavior', *Psychol Bull*, **83**:505-39.
- Toftegaard, C.L. (1999) 'Morphological and endocrine correlates of chemical communication during the life history of *Antechinus stuartii* (Macleay)', PhD thesis, University of Queensland, Brisbane, Australia
- Toftegaard, C.L., & Bradley, A.J. (1999), 'The structure of specialised osmetrichia in the brown antechinus, *Antechinus stuartii* (Marsupialia: Dasyuridae)', *J Zool Lond*, **248**:27-33.
- Toftegaard, C.L., Moore, C., & Bradley, A.J. (1999) 'Chemical characterisation of urinary pheromones in the brown antechinus, *Antechinus stuartii* (Marsupialia: Dasyuridae)', *J Chemical Ecology*, **25**:527-35.
- Toftegaard, C.L., McMahon, K.L., Galloway, G.J., & Bradley, A.J. (2002), 'Processing of urinary pheromones in *Antechinus stuartii* (Marsupialia: Dasyuridae): Functional magnetic resonance imaging of the brain', *J Mammal*, **83**:71-80.
- Vandenbergh, J.G. (1969), 'Male odor accelerates female sexual maturation in mice', *Endocrinol*, **84**:658-60.
- Van Dyck, S. (1979), 'Behaviour in captive individuals of the dasyurid marsupial *Planigale maculata* (Gould 1851)', *Mem Qld Museum*, **19**:413-31.
- Van Dyck, S., & Crowther, M.S. (2000) 'Reassessment of Northern representatives of the *Antechinus stuartii* complex (Marsupialia: Dasyuridae): *A. subrtopicus* sp.nov., & *A. adustus* new status', *Mem Qld Museum*, **45**:611-35.
- Waring, C.P., Moore, A., & Scott, A.P. (1996), 'Milt and endocrine responses of mature male Atlantic salmon (*Salmon salar* L.) parr to water-borne testosterone, 17, 20 beta-dihydroxy-4-pregnen-3-one 20 sulfate, and the urines from adult female and male salmon', *Gen Comp Endocrinol*, **103**:142-9.
- Whitten, W.K. (1969), 'Mammalian pheromones', in *Olfaction and Taste III* (ed. C. Phaffmann), pp. 252-7, Rockefeller University Press, New York.
- Woolhouse, A.D., Weston, R.J., & Hamilton, B.H. (1994), 'Analysis of secretions from scent-producing glands of Brushtail Possum (*Trichosurus vulpecula* Kerr)', *J Chem Ecol*, **20**:239-53.
- Wysocki, C.J., & Meredith, M. (1991), 'The vomeronasal system', in *Neurobiology of Taste and Smell* (eds T.E. Finger, & W.L. Silver), pp 125-50, John Wiley & Sons, New York.
- Yamada, K., Itoh, R., & Otha, A. (1989), 'Influence of pyrazine derivatives on the day of vaginal opening in juvenile female rats', *Japan J Pharmacol*, **49**:529-30.
- Yousem, D.M., Williams, S.C.R., Howard, R.O., Andrew, C., Simmons, A., Allin, M., Geckle, R.J., Suskind, D., Bullmore, E.T., Brammer, M.J., & Doty, R.L. (1997), 'Functional MR imaging during odor stimulation: Preliminary data', *Radiology*, **204**:833-8.