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Female attraction to male scent and associative learning: the house mouse as a mammalian model

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Many territorial mammals invest heavily in competitive scent marks that advertise their location, identity and current social and physiological status. Here we review the behavioural and molecular components of scent marking in house mice, *Mus musculus domesticus*, that influence female attraction to males and discuss how pheromone-induced learning among females and differential scent investment among males both influence female attraction to specific scent owners. Although mouse urine scents contain numerous sex-specific and individual-specific components, female attraction to spend more time near urine from males depends on contact with an involatile protein pheromone, darcin. This is an atypical major urinary protein (MUP) expressed only by males. On contact, this pheromone acts as a highly potent stimulus for associative learning, such that females learn similar attraction to the individual male's airborne odour associated with darcin; they also learn attraction to spatial cues where the pheromone was encountered. This targets female attraction to both the odour and location of individual male scent mark owners. However, the concentration and quality of airborne volatiles emitted from scent marks influence approach and contact with male scents. Under competitive pressure, males invest heavily in refreshment of scent marks at a high rate and deposit a high concentration of MUPs that bind urinary volatiles and extend the duration of volatile release. Females also gain information from airborne volatiles, including the social and infection status of the owner, which can alter their attraction to contact his scent. The ability of females to learn about individual males from their scent marks means that most decisions about preferred males are likely to be made before females are ready to mate. We are just starting to understand how different information in male scents is integrated in making these decisions.

Scent communication is widely used for sexual communication and attraction across the animal kingdom. Some of the most well-known examples include the simple species- and sex-specific mating attractant pheromones emitted by moths to attract the opposite sex, often over large distances (e.g. Butenandt, Beckmann, Stamm, & Hecker, 1959; Howard & Blomquist, 2005; Karlson & Butenandt, 1959; Witzgall, Kirsch, & Cork, 2010). However, chemical signals play much wider roles in mate assessment and selection that underpin sexual attraction (e.g. see Johansson & Jones, 2007; Wyatt, 2014). Among terrestrial mammals scent marks deposited around an animal's home area or territory are widely used to advertise an individual's identity, location and current status to other conspecifics in the locality (Gosling & Roberts, 2001; Johnson, 1973). These scents are highly complex, typically consisting of many hundreds of components that encode information about the animal's species, sex and individual identity as well as a broad range of information about the owner's current reproductive status, social status and health, all things that might influence their attractiveness to potential mates. This provides other animals using the area or visiting a scent-marked site with ample opportunity to learn information about individual scent owners, their spatial locations and defended territories that may be used in current and future mate choice decisions (Drea, Vignieri, Kim, Weldele, & Glickman, 2002; Hurst & Beynon, 2004; 2013; Vogt, Zimmerman, Kölliker & Breitenmoser, 2014).

The best-studied model of mammalian communication is the house mouse, *Mus musculus domesticus*, for which studies have been able to integrate behavioural, molecular and neurophysiological approaches under strictly controlled laboratory and semi-natural conditions to understand the functions and mechanisms of scent signalling. Although house mouse social structure can vary across different habitats, given a choice most house mice live in agricultural or human-built environments where food resources

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are concentrated (Barnard, Hurst, & Aldhous, 1991; Berry, 1981; Bronson, 1979). Adult males typically defend small territories, which may cover only a few square metres in high-density populations. Successful territory owners ensure that their own scent marks predominate throughout their defended area by continually and deliberately depositing their urine in many small streaks and spots as they move around the territory. Discovery of competing scents from other males provokes not only aggression towards the owners but also a rapid and elevated countermarking response, resulting in a particularly high rate of scent marking at borders between neighbouring male territories (Hurst & Beynon, 2004). Adult females typically range over several male territories, even though they may choose to nest in the territory of one particular resident male according to the quality and protection offered by available nest sites (Rich & Hurst, 1998; Wolff, 1985). Females mate with the owners of these scent-marked territories, although they frequently mate with more than one male, visiting selected males when ready to mate (there is little opportunity for male coercion under free-ranging conditions, with males mating only within their own territories). Here, we review the mechanisms that underlie female sexual attraction to male scents in this well-studied system and discuss the implications of an important new mechanism of pheromone-induced associative learning that we recently discovered for targeting female sexual attraction to specific individual males of high quality.

MECHANISM OF SCENT ATTRACTION

Investigation and Processing of Scents

Mammalian scent marks are multicomponent signals that contain both volatile components, which can become airborne and are gradually lost from the scent source, and nonvolatile (or much less volatile) components that can often persist in the environment for an extended period of time. On detecting an airborne scent that is unfamiliar or has not been encountered for some time, animals typically approach the scent source to investigate further at close contact, spending longer sniffing to process information when the scent is more unfamiliar and distinct from scents recently encountered (Todrank & Heth, 2003). This general investigatory response is not sex specific though, reflecting a general motivation to gain further information about whether scent cues come from the same or opposite sex. Airborne volatiles are detected through the main olfactory system as air is taken into the nasal cavity during normal breathing and active sniffing. However, most terrestrial vertebrates (except catarrhine primates and birds) also have an accessory olfactory system that is activated only on close contact with scents. Activation of a vascular pump (or active flehmen response in species such as ungulates or felids) is required to deliver molecules, in solution, from the scent source to the vomeronasal organ which is sited in a blind-ended mucus-filled capsule at the base of the nasal cavity (Breer, Fleischer, & Strotmann, 2006; Halpern & Martinez-Marcos, 2003). While odorant receptors (ORs) in the main olfactory epithelium are typically broadly tuned to detect a wide range of social and nonsocial odours alongside more specialized receptors sensitive to volatile amines (TAARs, Liberles & Buck, 2006), vomeronasal receptors respond more specifically to particular lipophilic low molecular weight odorants (V1Rs), nonvolatile peptides and proteins (V2Rs) and N-formyl peptides that are produced largely by bacteria (FPRs) (Dulac & Axel, 1995; Herrada & Dulac, 1997; Matsunami & Buck, 1997; Riviere, Challet, Fleggue, Spehr & Rodriguez, 2009; Ryba & Tirindelli, 1997). During close contact investigation, scents may be processed in parallel through the main and accessory olfactory systems, both of which are necessary to fully process the entire scent cue (Restrepo, Arellano, Oliva, Schaefer, & Lin, 2004; Spehr et al., 2006), with inputs from the two systems converging at the level of the amygdala (Brennan & Kendrick, 2006). However, the main olfactory system is likely to be particularly important for the detection of airborne scents at a distance from the source and for recognition when scents require further close contact investigation, while the accessory olfactory system appears to be particularly important for detection of nonvolatile components held within scent marks or on an animal's body (Luo, Fee, & Katz, 2003); these components include proteins and peptides but also low molecular weight ligands that are bound to involatile molecules or have not yet evaporated from the scent source.

Attraction to Male Scents among Female House Mice

When female house mice encounter unfamiliar urinary scents from adult male or female conspecifics, they are stimulated to approach and investigate both. However, following brief close contact investigation, females reliably prefer to spend more time near scents from intact adult males than near those from females or castrated males. This attraction to male scents is shown by both sexually experienced and naïve females, and by laboratory mice and wild-stock house mice, even when naïve females have never previously encountered scent from an adult male (Moncho-Bogani, Lanuza, Hernandez, Novejarque, & Martinez-Garcia, 2002; Ramm, Cheetham, & Hurst, 2008; Roberts et al., 2010). Indeed, wild-stock female house mice are attracted to spend time near male odours even when these are placed in open areas where wild mice normally choose to spend very little time (Roberts et al., 2010). However, if direct contact with scent is prevented, females fail to spend any more time near airborne volatiles from male urine versus female urine (Moncho-Bogani et al., 2002), whether or not females are naïve to male odours or sexually experienced (Ramm et al., 2008). This is somewhat surprising given that there are a large number of differences in volatiles that emanate from male and female mouse urine. Simple discrimination tests show that females readily discriminate these urinary volatile differences (e.g. Martel & Baum, 2009). None the less, normal female mice fail to show any inherent attraction to spend time near androgen-dependent volatiles beyond their normal investigation of an unfamiliar odour. This suggests that females detect an androgen-dependent signal on contact that is essential to stimulate attraction to spend more time near a male's scent, most likely perceived through the accessory olfactory system. Consistent with this, lesion of the accessory olfactory bulbs eliminates female attraction on contact with male soiled bedding (Martinez-Ricos, Agustin-Pavon, Lanuza & Martinez-Garcia, 2008). It should be noted, though, that studies using laboratory mice in which hormonal levels of female subjects have been artificially manipulated through ovariectomy and chronic treatment with oestradiol implants have found differential attraction to urinary volatiles from intact males compared with either females or castrated males, even without direct contact with male urine (reviewed by Baum, 2012). Currently, the relevance of this to mouse behaviour under natural conditions is difficult to interpret, but variation in female sensitivity to sex-specific cues during normal hormonal cycling deserves further research. None the less, adult females are attracted to spend time near male urine not only when in oestrus and ready to mate but also at random stages with respect to the oestrous cycle (Moncho-Bogani et al., 2002) and continue to be attracted even through pregnancy (Fig. 1); however, prepubertal females avoid unfamiliar adult male urine scents (Drickamer, 1989; Lanuza et al., 2014) while 2–3-week-old juveniles secrete a peptide, ESP22, in tear fluids that inhibits adult male mating behaviour (Ferrero et al., 2013).