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Pheromones: The Scent of a Male

There has been an enduring fascination with discovering biological odours which can evoke behavioral and physiological responses in mammals. New findings in goats have now identified a key molecule involved in the effect male odours have on female reproductive cycles.

Keith M. Kendrick

Despite extensive evidence for biologically active pheromones in invertebrate species the search for them in mammals has met with less success. The term ‘pheromone’ was first introduced over 50 years ago [1] to describe airborne communication molecules by which individual animals could evoke robust and specific physiological/behavioral effects in recipient individuals of the same species. Pheromones can either have a ‘releaser’ function by promoting rapid behavioral effects, such as sexual or aggression responses, or act as ‘primers’ by facilitating longer term physiological changes, which may also ultimately influence behavior.

While there is reasonable evidence that biological odours can influence both physiology and behavior in a number of mammalian species, it is clear that their effects are more variable and generally less specific than in invertebrates [2,3]. As such their actions do not really conform to the strict definition of pheromones, particularly in terms of being single molecules having both species and functional specificity and whose effects are not influenced by learning. Nevertheless, the term has been adopted widely in mammalian olfactory research despite this caveat. In mammals the main focus has been on identifying pheromones influencing reproductive physiology and behavior, notably the control of puberty, ovulation and sexual attraction and receptivity. Putative signaling pheromones have also been localized to a variety of different biological sources such as urine, tears, skin glands, wool, saliva and vaginal secretions. In mice, for example, the major urinary protein pheromones in male urine, 2-sec-butyl-4,5-dihydrothiazole, 3,4-dehydro-exo-brevicomin, α- and ß-farnesene, and 6-hydroxy-6-methyl-3-heptanone from the preputial gland all appear to have an influence on oestrus synchronization and acceleration of puberty in females [4]. The peptide exocrine gland-secreting peptide 1 (ESP1) released in the tear fluid of male mice also enhances female sexual receptivity [5]. In hamsters, female vaginal secretions contain dimethyl disulfide which in association with a carrier molecule, aphrodisin, facilitates male sexual responses [6]. In pigs, androstenone (5α-androst-16-en-3-one) and androstenol (5α-androst-16-en-3-ol) in boar saliva facilitate sexual receptivity in females [7].

One of the areas where identification of pheromones has proved elusive has been in relation to the impact that male odours have on female reproductive cycles in seasonally breeding species such as sheep and goats. Odours from the wool of rams and from the head and sebaceous glands of bucks can stimulate ovulatory cycles in seasonally anoestrus females and thereby prolong the breeding season. This has become known as the ‘male effect’ and is testosterone dependent. In fact this primer pheromone effect is not restricted to restoring ovulatory cycles but can also influence their duration and synchronicity (see [8]). In keeping with a less rigorous definition of pheromone specificity in mammals relevant odours from the two species can influence each other to some extent. Also other non-olfactory sociosexual stimuli from the male, such as sexual vigour, are influential. Further, the number of females already ovulating modulates the strength of the male effect [8] so female-produced pheromones may act synergistically with those produced by males to induce/synchronize ovulation.

A key problem which has hindered identification of the ‘male-effect pheromone’, and indeed other mammalian pheromones, has been the lack of robust biomarkers to test the efficacy of the many different potential candidate molecules. In this issue of *Current Biology*, Murata *et al.* [9] have utilized such a biomarker to identify a key novel odourant molecule from the skin and sebaceous glands of the male goat responsible for influencing female reproductive cycles. This group has previously established this biomarker using recordings of the electrical activity from the hypothalamic region containing the small population of gonadotrophin releasing hormone (GnRH) neurons responsible for stimulating pulsatile release of luteinizing hormone (LH) from the anterior pituitary and subsequently ovarian function [10,11].

By a systematic exploration of different extracts from samples collected from the head of the male using gas chromatography and mass spectroscopy they have identified a small number of ethyl-branched aldehydes and ketones which reliably increased electrophysiological activity. In particular, 4-ethyloctanol proved to be the most influential single molecule, although a cocktail of 18 compounds including this was the most effective, suggesting that a number of other components also contribute. The authors have yet to demonstrate directly whether 4-ethyloctanol alone

can actually stimulate LH release and ovulatory cycles, although the robust nature of their biomarker inspires reasonable confidence that it might. We also currently do not know how species-specific responses to 4-ethyloctanol are, although when it is oxidized to 6-ethyloctanol this gives rise to the characteristic 'goaty' smell.

So how are these pheromones detected and processed by the mammalian brain to influence female reproduction? Mammalian species have two olfactory systems (Figure 1), the vomeronasal and main olfactory systems [12]. These were originally considered to be independent of one another, with the vomeronasal system thought to be the one responsible for pheromonal effects having specialized receptors and a direct projection route to hypothalamic regions controlling reproduction. However, it is increasingly recognized that the two systems have overlapping and possibly complementary functions in processing odourant molecules both in terms of some common receptors, such as V1Rs [13], and interactions within shared processing regions in the brain, such as the amygdala [14]. Indeed, evidence suggests that the majority of mammalian pheromones identified to date influence both systems to a varying degree, although often the main olfactory system plays the major role. In terms of the signaling pathway through which 4-ethyloctanol or other candidate pheromones influence reproductive physiology this is also beginning to be more clearly understood. As can be seen in Figure 1, a primary target for olfactory projections routed via the amygdala is neurons in the arcuate/median eminence region of the hypothalamus containing the neuropeptide kisspeptin [15]. It is known that these neurons also contain neurokinin B and dynorphin receptors (termed KNDy neurons) and blockade of neurokinin B receptors prevents the male effect [11]. Kisspeptin neurons play an important role in modulating hypothalamic GnRH neurons, and may indeed be the site of the so-called GnRH 'pulse generator' which regulates their characteristic phasic discharge and resultant pulsatile release of LH from the anterior pituitary that influences gonadal function [15]. What is often overlooked, however, is that the GnRH neurons themselves have other extensive neural connections and may perform a

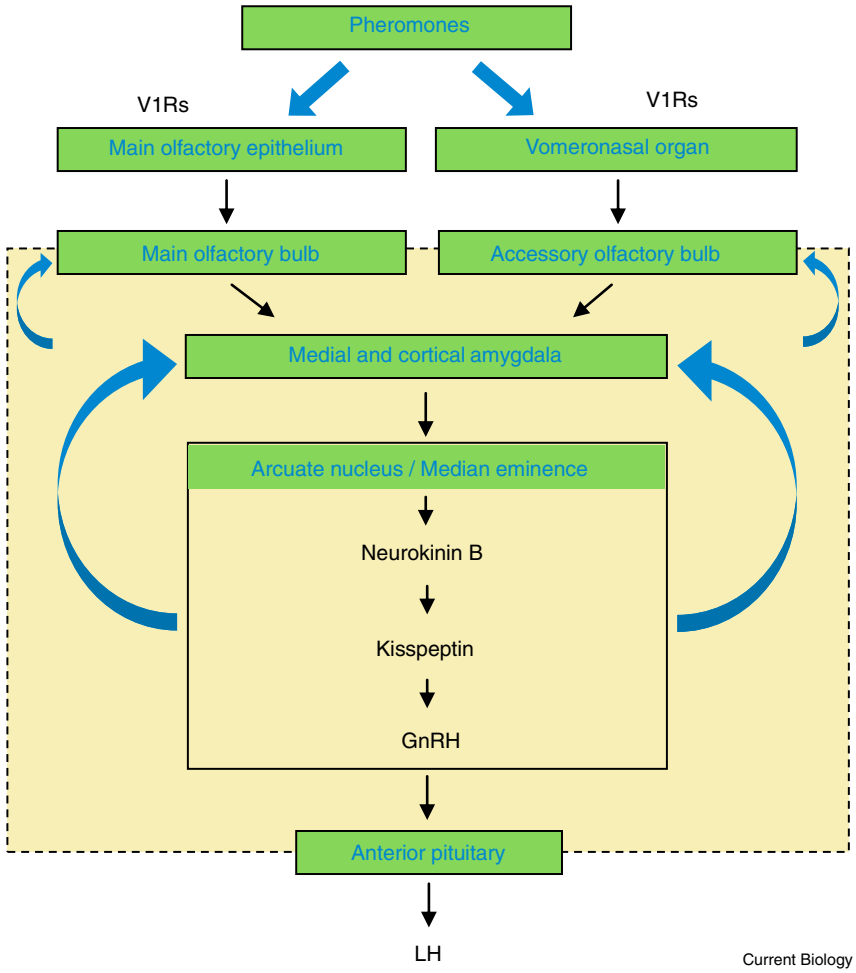

Figure 1. Proposed neural signaling pathways for the 'male effect' pheromone.

Pheromones are suggested to bind to similar receptors in both the main olfactory epithelium and vomeronasal organ (possibly V1Rs). The main olfactory bulb projection plays the major role, although both this and the vomeronasal system can interact at the level of the medial and cortical amygdala. Projections from the amygdala target neurokinin B receptors on kisspeptin neurons responsive to neurokinin B. Kisspeptin release then targets GnRH neurons via Kiss1r receptors, and subsequent GnRH release promotes luteinizing hormone (LH) release from the anterior pituitary, which then influences ovarian function. Importantly, the GnRH neurons can also feedback to the amygdala and thence to the olfactory bulbs, providing an integrated and flexible feedforward and feedback control system.

centrifugal feedback function via the amygdala which in turn may also feedback information to the olfactory bulbs [16]. As such this represents a highly integrated and complex feedback-controlled neural processing system capable of being modified by experience and is rather far from being a simple automated unidirectional system envisaged as mediating stereotyped functional responses to pheromones.

Human males may also be capable of influencing the synchronization and length of female ovulatory cycles through axillary gland (under arm) secreted pheromones. A possible candidate molecule for this is androstadienone, although this remains to be fully established [17]. As in goats pheromones from human females can also contribute to synchronization of ovulatory cycles [18]. However, evidence suggests that the vomeronasal system is unable to respond to putative pheromones [19] and functional effects may exclusively involve the main olfactory system.

Thus, an important take-home message from this field of research attempting to identify signaling pheromones in mammalian species is that while many more may ultimately be identified, their functional effects are

unlikely to be as dramatic as is often presupposed. The evolution of the mammalian olfactory processing system and its connections with hypothalamic and other regions controlling reproductive physiology and behavior have resulted in a far more flexible and integrated system than that for processing pheromones in invertebrates. We are unlikely, therefore, to discover mammalian pheromones which drive an inevitable and unconscious behavioral or physiological reaction such as that portrayed, for example, in Roald Dahl's celebrated short story *Bitch* [20] which features a fictional odourant cocktail capable of driving men to exhibit unconscious intense and insatiable sexual responses to women.

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Climate Change: A Hybrid Zone Moves North

A shifting zone of hybridization between two chickadee species helps us understand the proximate mechanisms driving species responses to climate change.

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In association with climate change, the northern range limits of northern species are moving northward [1]. These species southern range limits also appear to be moving northward, albeit at a slower rate and with greater heterogeneity between species [2,3]. While the ultimate reason may well be climate change, the more proximate mechanisms are difficult to determine. How much can range shifts be attributed to changes in the available resources (such as a longer growing season or different kinds of food) plus competition for these resources [4]?

One reason why it is so difficult to determine the role of competition is that when resource quality or quantity gradually varies over space, theory predicts that the species will show a large overlap in their geographical range. One species may be superior at one end of the gradient and the other at the other end so that the two species mutually set each other's range limit, but over an extensive area, each species can persist alongside the other by consuming a different portion of the available resources [5] (Figure 1). The gradual spatial turnover from one species to the other makes it difficult to empirically demonstrate competition as the ultimate cause of the range limits of each species. It should make it even more difficult to assess drivers of range expansions and contractions under climate change.

Hybridization between species changes this dynamic. A small amount of cross-mating between a pair of species can considerably narrow the overlap between them [5] (Figure 1). This is essentially because any individual that hybridizes leaves no offspring, and hence has zero fitness. The result is a steep cline across which the two species regularly interact, making it easier to directly evaluate species interactions in range movements. Further, in a hybrid zone, the southern limit of one species is tied to the northern limit of the other, so the two boundaries move at the same rate. This coordinated change in range movements, plus the close proximity of the parental forms, implies studies on moving hybrid zones have much to offer to our understanding of species