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Mammalian Pheromones: From Genes to Behaviour

Dispatch

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Knocking-out selected genes for receptors of the vomeronasal organ has been found to impair specific aspects of pheromone-induced behaviour in the mouse. This is not unexpected; less predictable is the finding that deleting the gene for a vomeronasal-organ-specific ion channel causes gender blindness.

Moving from gene regulation to behavioural output requires a relatively amenable and simple experimental system. In the context of pheromones and behaviour, work on invertebrates has helped to elucidate the complexity of pheromone signals, a finding made tractable by the simplicity of the stereotyped behavioural response [1]. Indeed, the very definition of pheromone was formulated from studies of insect behaviour [2]. In some respects, trying to shoe-horn mammalian behaviour into similar stimulus–response stereotypes has proved a handicap to progress in understanding mammalian pheromones. While it is true that many important aspects of mammalian behaviour depend on olfactory cues — mate choice, mother–infant recognition, feeding behaviour, territorial recognition, aggressive interactions — they do not simply depend on this one sensory modality [3]. Moreover, each of these behavioural contexts is frequently influenced by past experiences, the outcome of which is determined by the complexity of the mammalian brain [4].

The discovery of genes that code for olfactory receptors has not simplified this complexity. The identification of more than 1000 olfactory receptor genes [5] provided the molecular basis for understanding how a vast repertoire of odour discriminations could be generated, but failed to provide a tractable platform from which to decipher the odour signal -ing codes that initiate behaviour. Most mammals, however, have a second chemosensory system in the vomeronasal organ (VNO), which is enclosed in a cartilaginous capsule on the medial surface of the nasal septum [6]. The VNO neurons convey the pheromone signal directly to parts of the brain's limbic system — the amygdala and several hypothalamic regions — involved in primary motivated behaviour and neuroendocrinology [7].

Pheromone signals have long been known to change endocrine states selectively advantageous to their biological context [8]. Secondary to these endocrine events are changes in behaviour, and lesions of the VNO certainly cause behavioural changes [9]. Such behavioural changes are not simple to interpret, however, because of parallel endocrine changes and because the lesions do not produce all-or-none effects. In the hamster, for example, male sexual behaviour is severely impaired in 40% of males with sectioned VNO nerves — but not with males paired with naturally cycling females or males that are sexually experienced [10]. Sexual experience has also been shown to be important for vomeronasal activation of ultrasonic responses of male mice to females [11], and maternal experience is important for vomeronasal activation of female mouse aggression to intruders [12].

It was not until the genes encoding likely odorant/pheromone receptors were cloned and characterised that it became clear the VNO itself has a complexity and organisation commensurate with the multiplicity of behavioural functions. The sequencing and analysis of the cloned receptor genes revealed two superfamilies of about 140 and 160 genes — the first being the V1r superfamily discussed below — which are expressed in the VNO and encode proteins distinct from the main olfactory receptor families [13]. Now, four important papers [14–17] have been published reporting results which clearly implicate the VNO as playing an integral part in directly initiating behavioural responses to pheromones.

In their recent study, Mombaerts and colleagues [14] investigated the effects of selectively excising a cluster of 16 V1r genes from the mouse genome, by using 'chromosome engineering cassettes' targeted to chromosome 6. The V1r superfamily consists of about 137 genes, which can be grouped in 12 phylogenetic families [15]. The eliminated cluster contains most of the V1ra and V1rb families, representing about 12% of the complete superfamily. That the authors observed effects of this deletion of V1r genes on behaviour at all is itself interesting, but perhaps more important is the selectivity of the behavioural effects relative to those caused by gross anatomical lesions of the VNO.

Del Punta *et al.* [14] found that elimination of the V1r gene cluster, unlike lesioning of the VNO, produces no effects on male ultrasonic responses to females and no impairment of male aggressive behaviour. Changes in male testosterone levels in response to females are also not impaired. Sexually naive male mice often mount males and females, but with experience male mounting declines and selective female mounting increases. Males lacking the VNO cluster were found to differ, showing an overall lower sexual interest in both males and females, which does not change with sexual experience. Mutant females show normal maternal behaviour, but they are impaired in maternal aggression to a strange male intruder. No behaviour was completely eliminated in this mutant mouse, but females are slow to respond with aggression and males are sexually sluggish, despite having testosterone levels in the normal range.

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Table 1. Behaviour impairments, relative to wild type, caused by genetic or surgical VNO lesions.

	V1rab cluster deletion	Surgical lesions	TRP2 ion channel deletion
Male ultrasonic responses	No effect	Eliminated to females by sexually naïve males	Emitted in response to either sex
Male aggression	No effect	Reduced amount; latency of onset longer and variable	Initiation completely eliminated
Male sexual behaviour	Few mounted; decreases with experience	Fewer mounted; latency increased; frequency reduced and variable	High levels observed
Male mounting of males	Initially lower but with subsequent tests no different		High levels observed
Gender discrimination	NA	Unaffected with experienced males	Both sexes equally attractive
Female aggression	Reduced dependent on experience	Reduced depending on experience	Initiation completely eliminated
Male territorial marking	NA	Not reduced	Absence of gender discrimination in territorial marking
Testosterone levels	Not affected	Fails to increase on exposure to females	Not affected

As might be predicted, the impairments resulting from the genetic lesion are not as global as those caused by anatomical lesions, but within those categories of behavioural impairment common to both procedures, there are remarkable similarities (Table 1). Both procedures reduce, but fail to eliminate, male copulatory behaviour. Indeed, if a mutant male successfully copulated with a female, then it displayed a level of sexual activity comparable to that of wild-type males in the components of sexual activity and their latency. Hence, the ability to perform sexually is normal, while the ability to engage sexual activity is impaired.

Similarly, post-partum female aggression is not eliminated by either complete VNO lesions or deletion of the V1ra/V1rb receptor genes, but in both cases the mouse is slower to activate the behaviour and there is less of it. Nevertheless, within seven minutes of the interaction starting, the mutants are not different in their aggression scores from controls. Again, the ability to engage the behaviour is impaired, but its performance is normal. One interpretation of these findings is that the V1r(ab) family of receptors represent part of a broader signal, the partial impairment of which delays the onset of behaviour and retards its performance. But as complete lesions of the VNO produce similar behavioural effects, a more plausible interpretation is that the neurons which express the V1r(ab) receptors code for responses to specific pheromones, the deletion of which is partially compensated for by using other sensory modalities, including the main olfactory receptors.

That these findings might have been predicted confirms the strengths of this approach to dissect -ing behavioural complexity. What is perhaps less predictable is the recently discovered contrast between the phenotypes of mice with complete VNO lesions and those with an equally complete lack of vomeronasal receptor signaling because of deletion of gene encoding the Trp2 cation channel [16,17]. In these *trp2*$^{-/-}$ mutants, male–male aggression and female post-partum aggression are eliminated. The males mount males as much as they mount females, and this does not decline with sexual experience. Mounting is robust, but equally distributed to males and females even when given a choice. Moreover, mutant males are not selective in their ultrasound vocalisations to females, and these emissions are equally intense when directed to males.

In the absence of aggression, mutant males fail to form dominance hierarchies with other males and continue to urine mark as if the other male was a female. If attacked, the mutant mice can reciprocate aggression, but they fail to initiate it to the appropriate sex. In essence, all behavioural components are performed at normal levels, the main difference being the failure of each sex to determine the sexual identity of a member of the opposite sex.

These findings are remarkably different from those on mice with V1r gene deletions or complete VNO lesions, where the impairments are quantitative, rather than all-or-none sex-dependent responses (Table 1). The *trp2*$^{-/-}$ mice are truly gender blind, despite the fact that their gonadal hormone levels are perfectly normal. Genetic manipulations such as these add a new dimension to our way of thinking about pheromones and behaviour. The fact that the *trp2*$^{-/-}$ mutant is motivationally normal, but fails to engage gender-specific behaviour, suggests that the VNO input may influence the development of dimorphic behaviour (unlike mice that are lesioned as adults, the *trp2*$^{-/-}$ mutants are without VNO signalling capacity at the critical period post-natally when sexual differentiation of the brain occurs). It would certainly be interesting to examine the sexually dimorphic regions of the forebrain that receive a VNO input and regulate these motivated behaviours [18].

These studies have provided a platform from which the functioning of the VNO can be taken from gene expression to behaviour. Mombaerts' group [14] has already identified a group of three ligands to which the Vr1a/Vr1b-expressing neurons respond, as revealed by electrophysiological recordings of field potentials from the VNO sensory epithelium. Further ligands found in urine have been identified which activate other Vr1 receptors, and these have been topographically mapped to regions of the VNO [19]. Electrophysiological

recordings have shown that the detection threshold for these ligands is remarkably low (10^{-7} M) and, unlike most other sensory neurons, they do not adapt under prolonged stimulus exposure [20]. Hence we now have the technologies to realistically address important questions as to how the VNO influences behaviour both developmentally and functionally.

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