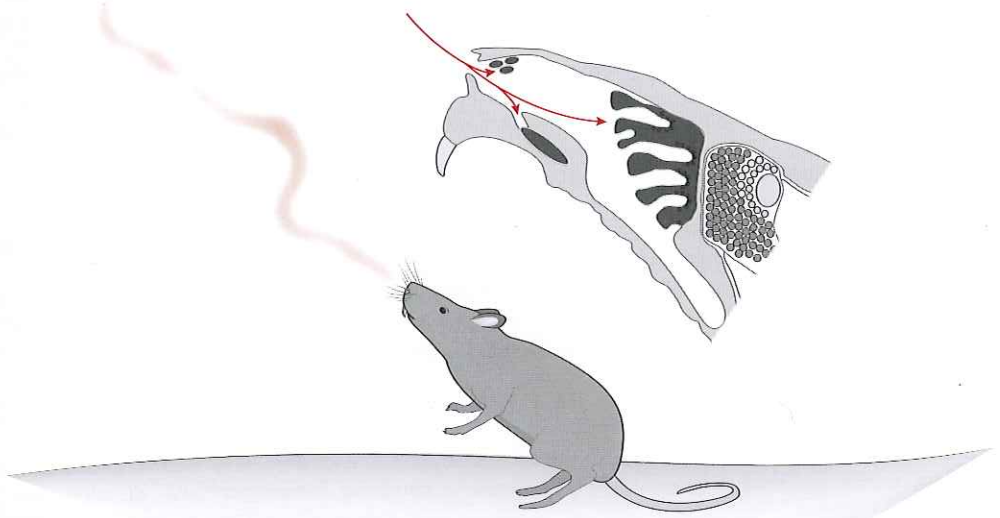
CHAPTER 2

Olfaction



The Neuroethology of Predation and Escape, First Edition. Keith T. Sillar, Laurence D. Picton and William J. Heitler.

Olfaction is the technical term for the process of perceiving smells. More specifically, for terrestrial animals, olfaction is the perception of chemicals carried in air and is, therefore, a form of long-range chemoreception. Chemoreception is the most ancient of all senses. Even bacteria have extensive and sophisticated mechanisms for detecting and responding to the chemical composition of their environment (Hazelbauer *et al.*, 2008), so it is not surprising that chemoreception plays a crucial role in animal life.

In mammals, the sense of smell is different from the sense of taste (gustation), which refers to short-range perception of chemicals liberated from food particles that are dissolved in saliva and detected by taste buds[1]. For other animals, the distinction may be less clear. Obviously, for aquatic species, the chemicals are all carried in water, and the difference is more to do with the distance from the source of the chemicals – it's a smell if it comes from a long way away; it's a taste if it's food that has actually been captured and is being chewed up[2].

Odours propagate from a source in two ways. In still air or water, it is by simple diffusion. In this mode, the concentration decreases rapidly with distance from the source, but the absolute concentration is directly related to this distance, which may be useful information. In moving air or water, the odour is carried downstream in a plume which can travel a considerable distance, but the concentration gradients within the plume are chaotic and contain little information. However, if the receiver can sense the direction of the wind or water current, then they immediately know the general direction of the odour source and just have to move upstream to find it, zigzagging if necessary to remain within the plume.

'To smell' is one of those confusing verbs in the English language that can have two meanings – you can *perceive* smells or *generate* them. It is almost impossible for an animal not to smell in the sense of *emitting* an odour. Evaporation of volatile compounds from the skin of a terrestrial animal inevitably broadcasts information about that animal – its identity, its sex, its hormonal status, its diet, and so on. Given that this is unavoidable, it is not surprising that smell has evolved as a means of intra-specific communication. Many animals use specialised glands to produce chemical odours that are used as signals to conspecifics, including trail markers, territory markers, indicators of sexual and social status, and so on. Such olfactory signals are known as **pheromones**.

However, olfactory signals cannot easily be targeted – they are available to any animal that possesses a suitable receptor system to detect them. It is therefore also not surprising that olfactory 'eavesdropping' has developed, and this is where the topic becomes highly relevant to predator and prey behaviour. A predator that can detect the scent of prey while it is still out of sight and earshot gets early notification of possible lunch upwind, while an animal that can detect a hidden predator from its smell reduces its chances of becoming that lunch. Olfactory signals such as these, that are detrimental to the wellbeing of the odour emitter, are

[1]The overall flavour of food results from a combination of taste and smell; food activates taste buds directly but, as we exhale, air passes over food in our mouth and picks up volatile chemicals which activate olfactory receptors in the nose. Flavour can also be detected through trigeminal nerve **chemesthesis**, in which material such as chili peppers or menthol activates non-chemosensory neurons, such as pain or temperature receptors. In contrast, when we 'sniff the air', we inhale, and chemicals in the air originating from the outside world are drawn over the olfactory receptors.

[2]One remarkable exception to this is the star-nosed mole, a semi-aquatic mammal which uses an ingenious mechanism to literally sniff underwater. The animal blows out a series of bubbles from its nose, which gather up odour particles from the surrounding water. The bubbles are then sniffed back up, enabling the mole to use its olfactory receptors to sample the water for potential prey odours (Catania, 2006).

known as **kairomones**, and these are the inevitable 'evil twin' of pheromones. Of course, the same signal can play either role, depending on the intentions of the receiver. Also, of course, non-pheromone odours, such as sweat or exhaled breath, can also act as kairomones.

In this chapter, we concentrate on olfaction in vertebrates – mainly mammals – and how they use this sense to detect potential predators and prey. There is also a large amount known about invertebrate olfactory systems, and we do mention some of the relevant studies here, but others will appear in later chapters, such as that on molluscs (Chapter 12). This choice has mainly been made on the basis of space constraints, and the need to maintain focus on stories with direct relevance to predation and predator avoidance.

2.1 Mechanisms of olfaction

2.1.1 Detection and specificity

In essence, **olfactory receptors** (ORs) in vertebrates resemble metabotropic post-synaptic receptors[3]. The activating molecule binds to the extracellular domain of a membrane-bound receptor in the **olfactory sensory neuron** (OSN), which possesses hair-like cilia which protrude into the lumen of the nasal cavity. Olfactory receptor binding activates a **G-protein coupled cascade** that ultimately results in the opening of ion channels, and leads to membrane depolarisation and afferent spikes (Figure 2.1). As in visual receptors, the key advantage of this sort of second-messenger system is that it allows massive biochemical amplification so that a single odour molecule activating a single receptor can lead to the opening of a large number of ion channels. This in turn means that some odours can be detected at extremely low (attomolar – 10^{-18}M) concentrations (Mayer and Mankin, 1990).

Many animals can detect an extremely wide range of different smells. It has recently been estimated, for example, that humans are capable of distinguishing over one trillion different odours (Bushdid *et al.*, 2014). Ligand-receptor binding is stereospecific, so how can this be accomplished? This question has been extensively studied, especially in mammals, and some of the findings were so important that they led to the award of the 2004 Nobel Prize to two of the key players in the field, Richard Axel and Linda Buck (see Firestein (2005) for an informative history of that research).

Part of the answer is that, in mammals, the largest gene family in the entire genome is devoted to coding for olfactory receptors; mice and rats have more than a thousand such genes[4]. Each olfactory sensory neuron expresses just one allele of one of these olfactory genes and, hence, just a single receptor type. However, this line labelling is not enough in itself; mammals can detect many more smells than there are genes. The next part of the answer is that each receptor binds a specific conformational motif of the odour molecule, such as a functional group, but different odorants can contain multiple motifs. This means that specific odour molecules can activate more than one receptor type, while specific receptor types can respond to more than one odorant (Figure 2.2). The number of possible 'codes' formed by combinations of line activation is, therefore, immense, and it is this which is thought to enable mammals to distinguish such a wide range of odours (Malnic *et al.*, 1999).

[3]In invertebrates, many olfactory receptors are also metabotropic, but some are ionotropic (i.e. the binding of the odour molecule to the receptor directly opens an ion channel).

[4]It is worth pausing for a moment to mentally compare this with the visual system, in which our entire sense of colour is based on the relative activity of just three receptor types.

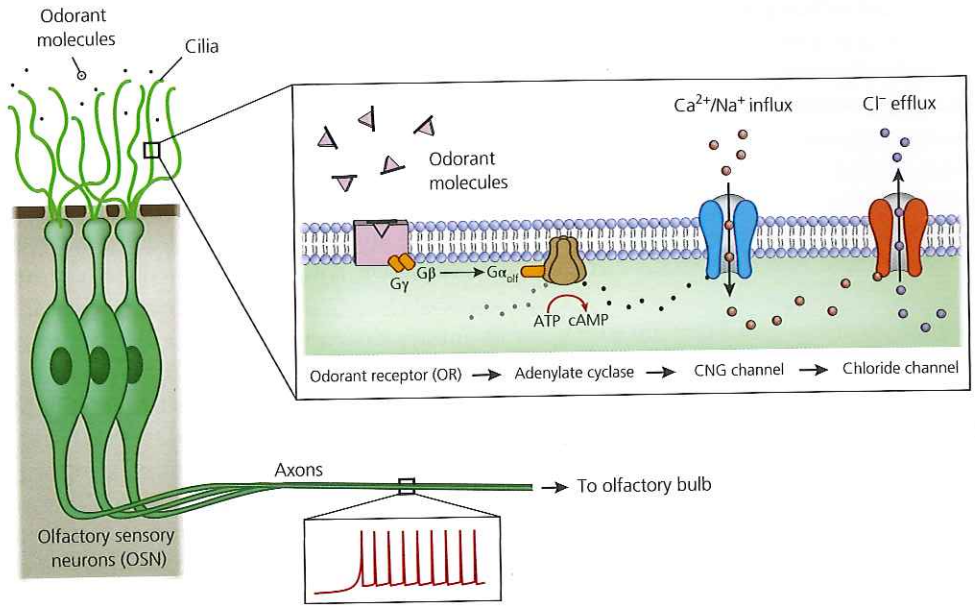


Figure 2.1 Olfactory sensory neurons and an example of a typical mammalian odorant signalling pathway. An odorant molecule binds to an odorant receptor (OR) embedded in the membrane of an olfactory sensory neuron (OSN). OR activation results in the G-protein coupled activation of adenylate cyclase, which increases intracellular levels of the second messenger cyclic-AMP (cAMP). cAMP, in turn, opens cyclic nucleotide-gated (CNG) channels, allowing the entry of calcium (Ca^{2+}) and sodium (Na^{+}) into the cell. This causes a small direct depolarisation, but the main effect is that the increased intracellular Ca^{2+} leads to the opening of calcium-dependent chloride (Cl^{-}) channels. The intracellular Cl^{-} concentration is unusually high in OSNs, so opening Cl^{-} channels leads to an efflux of Cl^{-} and depolarisation of the OSN.

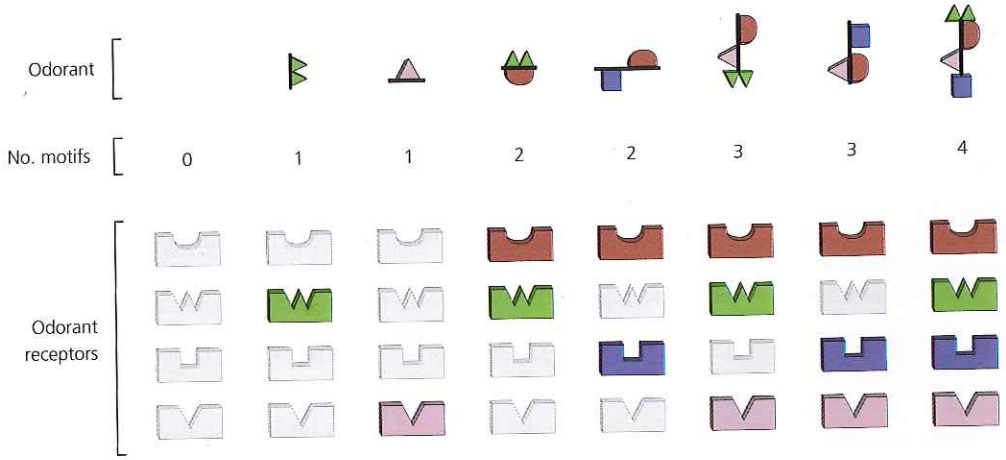


Figure 2.2 Combinatorial coding of odours. Each olfactory sensory neuron expresses a single odorant receptor type (row), which binds a single specific conformational motif, and each odour molecule (column) can contain one or more motifs. Four receptor types can therefore unambiguously distinguish between 15 possible odour molecules through different combinations of activity, of which just seven are shown in the diagram. As the number of receptor types increases, the number of possible codes increases dramatically – with 1000 different receptors, it is effectively unlimited. Adapted from Malnic *et al.* (1999).

2.1.2 Olfactory sub-systems

The olfactory epithelium lining the nasal cavity of mammals is divided into different anatomical subsystems, including the **main olfactory epithelium** (MOE) in the dorsal nasal cavity, the **vomeronasal organ** (VNO) located in the anterior portion of the palate between the nose and mouth, the septal organ, and the recently-discovered **Grueneberg ganglion** (GG) located near the tip of the nose (Figure 2.3). All of these are bilaterally symmetrical paired structures. Sensory neurons in the MOE are mainly ciliated in structure, while those of the VNO are microvillar, and there are differences in the receptor signalling mechanisms between these two subsystems[5]. Traditionally, the MOE is thought to mediate 'canonical'

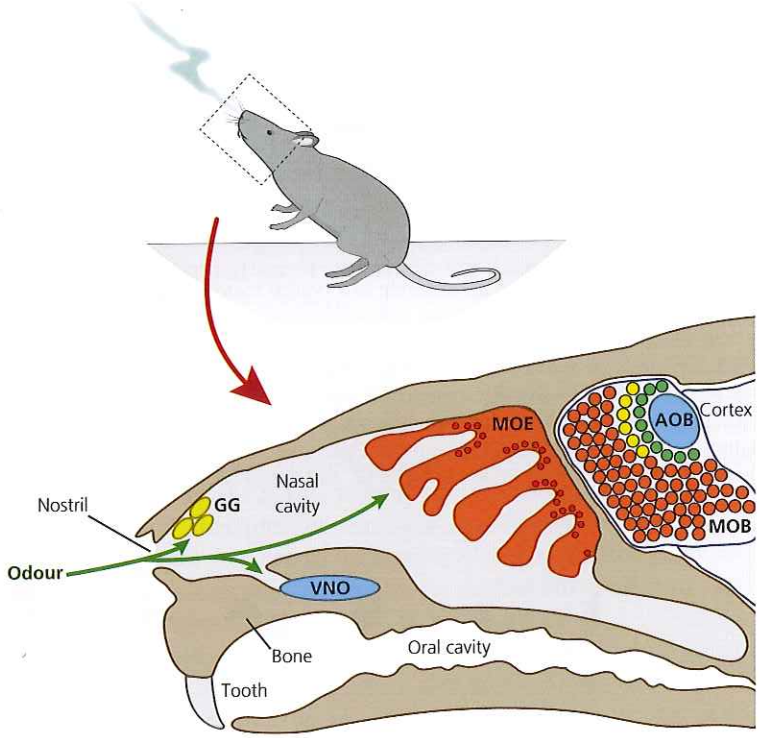


Figure 2.3 Anatomy of mammalian olfactory subsystems. Sensory neurons in the main olfactory epithelium (MOE; orange) project to glomeruli in the main olfactory bulb (MOB; orange circles). Neurons in the vomeronasal organ (VNO; blue) project to the accessory olfactory bulb (AOB; blue). Neurons in the Grueneberg ganglion (GG; yellow) project to the hemi-necklace in the MOB (yellow circles). Specialised neurons in the MOE detecting odours indicating metabolic status (not discussed in the text) project to necklace glomeruli in the MOB (green circles). The septal organ subsystem is not shown, because little is known of its specific function. Adapted from Mombaerts (2004) with permission from *Nature*.

[5]In the MOE, the signalling pathway is that shown in Figure 2.1. Outflow of negative charge is the main cause of the receptor generator potential in MOE neurons (Wong *et al.*, 2000). The VNO pathway involves receptors from two different gene families – V1R and V2R. These activate the phospholipase C (PLC) pathway, leading to the production of inositol triphosphate (IP_3), diacylglycerol (DAG) and arachidonic acid (AA). DAG and AA activate two separate channels – the transient receptor potential channel 2 (TRPC2) and an unidentified cation channel – both of which mediate calcium inflow. Thus, inflow of positive charge is the main cause of the receptor generator potential in VNO neurons (Zhang *et al.*, 2010).

olfaction – the detection of small, volatile odorants that tell the animal about its general environment, which give rise to conscious awareness of smells, and which can trigger memories and learned behaviours. The VNO, on the other hand, is thought to respond to larger molecules such as pheromones, and to trigger innate 'instinctive' responses (Dulac and Torello, 2003), although this subsystem-function pairing is by no means absolutely clear cut (Baxi *et al.*, 2006). The GG seems to be specialised for the detection of alarm pheromones (and also cold temperature – its location at the tip of the nose making it rather well suited to both these functions).

2.1.3 Brain processing

The sensory neurons from these subsystems all project to the first brain receiving station, the **olfactory bulb** (OB) (Figure 2.4). Here, the axons converge on each side onto about 2000 discrete clusters of neurons known as **glomeruli**. The axons of all the sensory neurons in the MOE expressing one particular receptor all converge onto just one or two of the glomeruli in the **main olfactory bulb** (MOB). This high convergence (at least 1000 : 1) ensures both high sensitivity and enhanced signal-to-noise ratio. VNO afferents expressing a particular receptor project to a larger, but still restricted, set of 6–30 glomeruli in the **accessory olfactory bulb** (AOB) (Wagner *et al.*, 2006). The AOB has anterior and posterior regions, receiving afferents from apical and basal regions of the VNO, respectively, where the sensory neurons express receptors from different gene classes. GG neurones send axons to the 'hemi-necklace' glomeruli in the caudal MOB.

The very specific convergence pattern, combined with a network of lateral inhibition between glomeruli, means that odours form a sort of map in the olfactory bulb, in which different odours activate a specific pattern of glomeruli (but note that this map has no relation to the geography of the outside world). Within a particular glomerulus, sensory neurons synapse onto relay neurons (mitral and tufted cells), which carry olfactory information to deeper brain regions (Figure 2.4). There are also projections back from deep brain regions to the OB, which presumably modulate and refine the OB output. MOB glomeruli, activated from the MOE, project relay neurons to the **primary olfactory cortex**, which includes the piriform cortex and the lateral amygdala. These inputs do not go through the thalamus, which is the usual relay station for sensory input to the cortex. Receptor line-labelling is maintained in this projection – each relay neuron is activated by a single glomerulus which, in turn, is activated by sensory neurons expressing a single receptor type. In the cortex, the information is combed to achieve olfactory 'object recognition' (Howard *et al.*, 2009).

In contrast, glomeruli in the AOB which are activated by the VNO project to the **limbic system**, including the vomeronasal-amygdala and hypothalamus, which fits with the role of the VNO in triggering innate 'emotional' behaviour, such as mating and aggression. VNO relay neurons have less distinct line labelling, with each relay neuron being activated by several glomeruli.

With this general background, let us now consider the role of olfaction specifically in the context of predators and prey.

2.2 Olfactory tracking and localisation

For a predator, one of the most obvious uses of olfaction is to track down prey and, particularly, prey that has already been damaged in an initial attack (Figure 2.5). Dogs are famously good at this, and since their domestication from the grey wolf tens of thousands

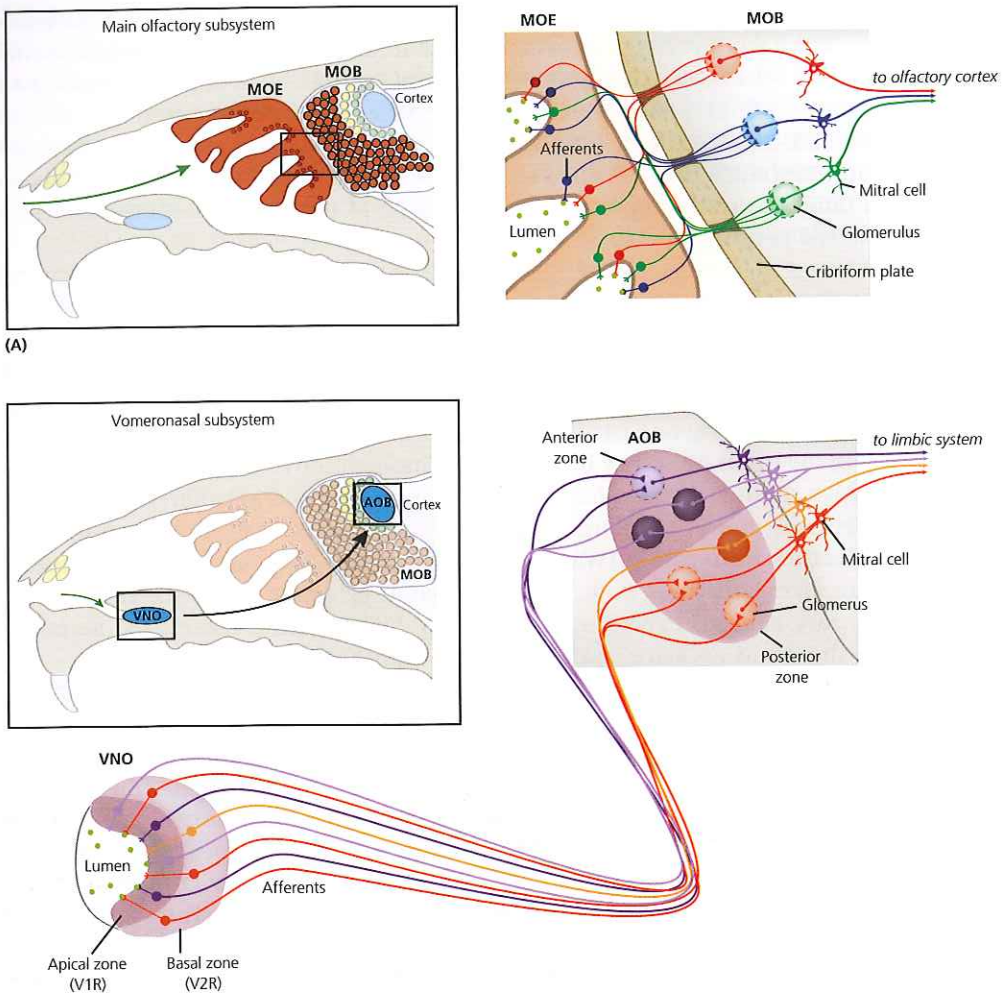


Figure 2.4 Basic circuitries for initial processing in two mammalian olfactory subsystems. **A**: The main olfactory epithelium (MOE) pathway. Each afferent in the canonical main olfactory epithelium (MOE) expresses a single receptor type (red, blue, green) and all of the afferents that express this receptor project to a single glomerulus at the main olfactory bulb (MOB). Mitral cells receive input from single glomeruli and carry information into the olfactory cortex. There are genetic and additional functional differences between receptors in the dorsal and ventral MOE which are not shown in the diagram. **B**: The vomeronasal organ olfactory pathway. Afferents in the vomeronasal organ express single receptors from at least two gene families (V1R, purple and V2R, orange), which are segregated into apical and basal regions of the organ. They project to glomeruli in the accessory olfactory bulb (AOB), where the V1R afferents terminate at the anterior zone and the V2R terminate at the posterior zone. Mitral cells carry information from the AOB into the limbic system, but the mapping is not clear-cut (see text; Kobayakawa *et al.*, 2007). Adapted from Mombaerts (2004) with permission from *Nature*.

of years ago, many have been selectively bred for this purpose. Scent hounds, such as bloodhounds or beagles, have a highly-folded MOE, with a surface area of up to 380 cm^2, which is more than one hundred times greater than that of a human. They also typically have long drooping ears and rather 'slobbery' jowls, both of which have been claimed to

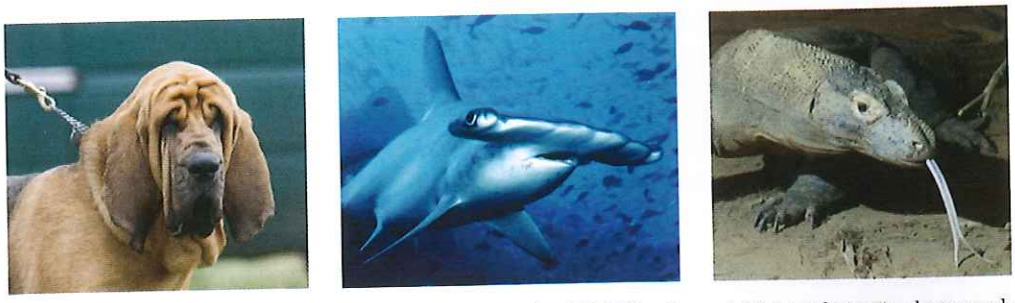


Figure 2.5 Olfactory trackers. Left: a bloodhound from the 2008 Bloodhound Club trials (UK); photograph courtesy of John Leslie, Flickr, CC-BY-2.0 licence. Centre: a hammerhead shark from Cocos Island, Costa Rica; photograph: Barry Peters, Flickr, CC-BY-2.0 licence. Right: a Komodo dragon (*Varanus komodoensis*) sticks out its tongue at the Cincinnati Zoo. Photograph courtesy of Mark Dumont.

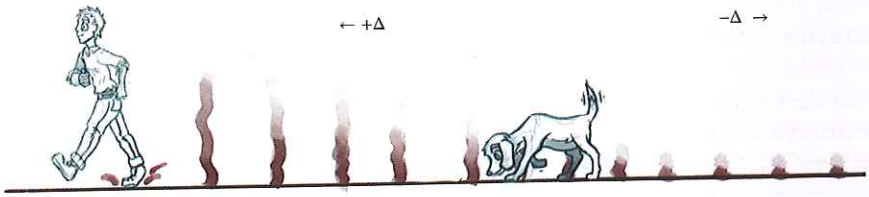


Figure 2.6 Directional tracking. Scent trails increase in concentration in the direction that the trail-layer was moving (Cartoon: Thomas Heitler).

help trap scent particles near the nose – although we are not aware of any rigorous investigation of this. Specialist tracker dogs have noses which are at least 10 000–100 000 times more sensitive than humans to some odorants (Walker *et al.*, 2006), and they seem particularly sensitive to butyric acid, which is a component of sweat. This may underlie the well-documented ability of some bloodhounds to follow trails even when they are several days old.

Scent hounds are not only able to detect very small odour concentrations, but also very small *changes* in odour concentration. This enables experienced scent hounds to determine the direction that the trail creator was going in if they come across an old trail. They do this by comparing the changes in ground scent strength in the two directions (Steen and Wilsson, 1990). The scent is laid down by the quarry with each step that it takes, but then immediately starts to dissipate, which means that more recent steps have a stronger scent than older steps (Figure 2.6). Thus, if the scent gets weaker as the hound searches along the trail, then it is moving away from the quarry – but if it gets stronger, then it is moving towards the quarry. The dogs can learn this and switch direction if appropriate.

This has nothing to do with moving towards scent diffusing from the body of the quarry itself – the scent that the dog smells has been laid on the ground and is diffusing up from the trail. If the trail is more than just a few minutes old, the directional differences become very small, which again emphasises the excellent olfactory ability of these specialist trackers.

Specialist trackers detect scent trails on the ground, but many odorants are simply carried by diffusion in the air. Animals can detect the origin of such an airborne odour by comparing the olfactory input coming into the two nostrils. Rats sample the air by sniffing

at about 7–8 Hz, and they can usually distinguish between left and right-sourced odours within one or two sniffs. If either nostril is blocked, they lose this ability. Delivering separately-timed odour pulses to the two nostrils while recording from MOB neurons indicates that the rats can distinguish time-of-arrival differences of as little as 50–100 ms if correctly synchronised with a sniff (Rajan *et al.*, 2006). This inter-nasal time difference pales into insignificance compared to the microsecond timing differences that the auditory system can detect (which we will discuss later, in Chapter 4), but it is nonetheless quite impressive for a sense of smell.

It suggests that rats have the potential ability to localise an odour source using a **stereo-based timing mechanism** if the odour is windborne, so long as the airflow over the nostrils is less than about 10 cm s^{-1}. However, rats also use a concentration-based discrimination mechanism, which requires a neural computation comparing the concentration of left and right inputs. There is no airway communication between the nasal passages of the two nostrils, and no neural crossover at the OB or relay neuron level, so the inputs are segregated right up to the cortex. Here, neurons in the **pars externa of the anterior olfactory nucleus** (AONpE; the most rostral part of the olfactory cortex) receive excitatory input from the ipsilateral nostril and inhibitory input from the contralateral nostril. These E-I neurons are thus ideal for comparing odour strengths at the two nostrils (Kikuta *et al.*, 2010).

Furthermore, the E-I response seems to be specific to a single odour category. The excitatory response can be elicited by a mixture of odours, but only one element of that mixture elicits the inhibitory response for a particular AONpE neuron. The inhibition seems to be essentially subtractive. An odour presented to the ipsilateral nostril alone elicits a large response in these neurons, and this is progressively diminished as increasing concentrations of the same odour are presented to the contralateral nostril. The onward central projection of these neurons is not yet known, but they provide a neuronal basis for concentration-dependent localisation, which could enable the rat to determine the position of a potential predator or prey.

Mammals are not the only animals to use a stereo-based mechanism for olfactory tracking/localisation. Snakes and other reptiles famously have forked tongues (or, at least, some do), and they flick the tongue in and out to sample the environment by picking up olfactory chemicals. Because the tongue is forked, chemicals are sampled at two points simultaneously. However, the tongue itself does not have olfactory receptors. Instead, the odour molecules are brought back into the mouth in the retraction phase of the flick and deposited near the openings of the bilaterally-paired VNOs. The forked tongue thus acts almost like paired nostrils, but delivers olfactory information to the left and right VNOs, rather than the MOEs.

For many reptiles, the main chemical cues delivered by this method come from the relatively narrow scent trails laid by either prey or conspecifics, so the forked tongue acts as a trail edge-detector that can keep the reptile on the track of its objective (Schwenk, 1994). Such trail-following is important in finding mates, but is also extensively used in predation (e.g. Kirschenbaum *et al.*, 1986).

Many reptiles are venomous, but the venoms can take some time to act. This means that the predator needs to be able to follow the trail of the envenomated victim, so as to realise the benefit of resources it invested in the attack. The largest reptile of all, the Komodo dragon, uses its forked tongue in combination with head-swinging to detect the direction of potential prey at a range of up to about 4 km. It can also follow the trail of

injured but not yet dead prey[6], and can detect prey brought down by another dragon so that it can join the feast.

So far, we have only considered terrestrial animals, but aquatic olfaction is also immensely important, and sharks are a well-known example. Phrases like 'the smell of blood in the water' and 'feeding frenzy' may be clichés, but they are still true – sharks can detect the smell of blood from potential food in the water, and they can get quite excited by it. Not only can they smell the food, but they can also track it down and find the source. One technique they use in moving water is simply to swim upstream (**rheotaxis**), presumably on the basis that the smell is being wafted downstream by the current. Sharks detect the direction of water flow using sensitive lateral line mechanoreceptors and this, combined with the smell, enables them to find a food source even in complete darkness (Gardiner and Atema, 2007). In fact, some fish can follow the flavoured eddies left in the wake of live prey trying to escape, again using a combination of nasal and lateral line receptors.

However, sharks also use timing information for odour localisation. They are able to detect differences in the time of arrival of odours at the two nostrils of as little 100 ms (similar to rats), and will turn in the direction of the nostril that gets the first whiff of the smell (Gardiner and Atema, 2010). Concentration differences seem to play no part in this orienting response – the later-arriving smell may have a much higher concentration, but the animal still turns towards the side stimulated first. This makes sense, because odour concentration gradients are quite chaotic in a turbulent flow, so having a sensitive detector that reacts to the first arrival is arguably a better way of determining source direction than comparing concentrations. Increasing nostril separation will increase the time difference and will allow better discrimination, and this may have contributed to the evolution of the spectacular head shape of hammerhead sharks (Figure 2.5, middle)[7].

2.3 Pheromones and kairomones

2.3.1 Alarm pheromones

When under actual or potential attack by a predator, many animals emit an **alarm pheromone** (AP) as a means of signalling danger[8] to other members of the same species (reviewed in Verheggen *et al.*, 2010). The original purpose of the chemical may have been simple defence (a repugnatorial **allomone**), or perhaps the release was an unavoidable physiological response to stress, but over time they have evolved into specialised **semiochemicals** that can benefit both the emitter and conspecific receivers.

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[6]Large prey items such as buffalo may take up to a week to succumb after being bitten by a Komodo dragon, but there is some debate as to whether the fatality is due to sepsis from bacteria in the dragon's mouth, sepsis from general environmental bacteria, or the result of specific Komodo dragon venom. Whatever the cause, a dragon's ability to follow wounded prey from its scent trail is undoubtedly advantageous to the dragon (Smithsonian Institute Fact Sheet: Komodo Dragon (http://nationalzoo.si.edu).

[7]The head shape also improves depth perception by increasing the binocular separation of the eyes (McComb *et al.*, 2009).

[8]Damaged plants may also emit alarm pheromones that attract predators of the herbivores doing the damage (although this seems to be small-scale – for example, attracting parasitoid wasps to attack leaf-eating caterpillars, rather than lions to attack zebras!).

Many well-known examples are found amongst the social insects. For instance, ants that are attacked may emit a species-specific AP which can affect conspecifics in a concentration-dependent manner. Nearby ants receiving the signal at high concentration will head upwind and join in the defence, thus aiding the signal emitter. More distant ants getting the message at a lower concentration are not attracted to it, but display heightened vigilance, thus presumably receiving benefit themselves. However, it is not a cost-free process. Predators such as spiders can detect the signal as well, and may be attracted to it in the hope of joining in the melee and getting some easy pickings (Allan *et al.*, 1996). As mentioned in the introductory section, when a receiving animal uses an olfactory message for its own purposes against the best interests of the signal emitter, the signal is called a **kairomone**.

Recent research is starting to reveal some of the neurobiology underlying predation-related pheromone responses in mammals, particularly rodents. Stressed mice release an AP which causes a fear response in other mice, characterised by freezing in place or crouching low to the ground, which presumably reduces their chances of being seen (behavioural crypsis). The AP is detected by olfactory receptors in the GG – which, being at the very tip of the nose, is well-placed to act as a 'sentry' early warning system. If the ganglion is ablated by cutting the sensory axons between the GG and their target **hemi-necklace glomeruli** in the MOB (Figure 2.3), the AP no longer elicits a fear response, although the general olfactory ability of the mouse is not impaired (Brechbühl *et al.*, 2008). A key volatile chemical component of the AP has recently been identified as **2-sec-butyl-4, 5-dihydrothiazole** (SBT) (Brechbühl *et al.*, 2013).

2.3.2 Predator odours

The mouse alarm pheromone SBT shares structural features with a pungent odour molecule found in fox faeces – **2,4,5-trimethylthiazoline** (TMT)[9], which also activates GG neurons and which also triggers a fear response in mice (Brechbühl *et al.*, 2013). It thus seems likely that the alarm pheromone SBT has evolved as a chemical mimic of the fox odour TMT, with the latter thus acting as a kairomone. However, in addition to activating hemi-necklace glomeruli via the GG, the fox odour TMT also activates glomeruli scattered throughout the MOB, via the MOE, and the function of these different pathways has been investigated by genetically ablating specific regions of the MOE (Figure 2.7; Kobayakawa *et al.*, 2007).

If sensory neurons in the dorsal zone of the MOE are ablated, then glomeruli fail to develop properly in the dorsal MOB, and the mouse does not show the innate fear response to TMT shown by the wild type (Figure 2.7, purple pathway); neither does it show the **hypothalamic-pituitary-adrenal** (HPA) axis-mediated endocrine alarm response that the odour triggers in the wild type. It thus seems that parallel activation of *both* the GG and dorsal-zone MOE/MOB are necessary to elicit the full fear response to TMT. However, in the absence of either pathway, the mouse can still *detect* TMT, since it can be trained to respond to it (Figure 2.7, pink pathway). In other words, the mouse still knows that there is a predator around, but it just doesn't worry about it!

Predators are carnivores by definition, and a carnivorous diet might be expected to produce components in urine which are not present in herbivores, which could be very useful to prey as kairomones. Although TMT is useful to the prey of foxes, it is relatively

[9]Both are heterocyclic compounds containing nitrogen and sulphur in the ring.

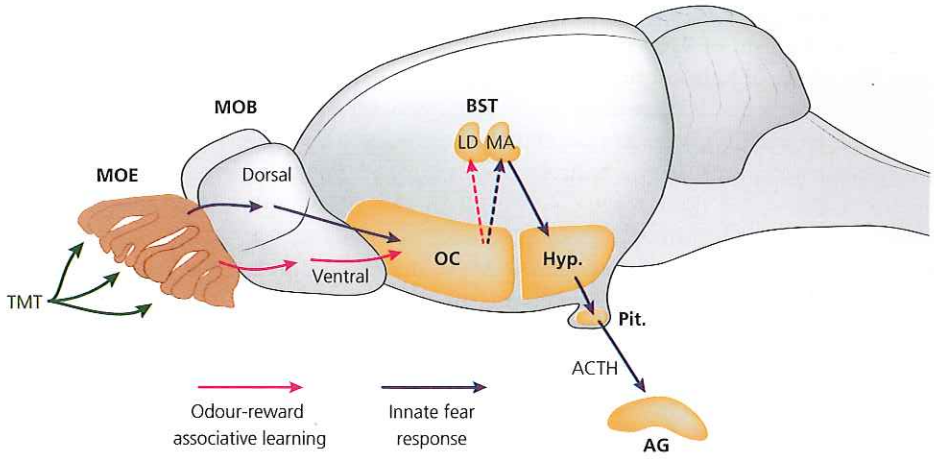

Figure 2.7 Pathways from the MOE in the mouse mediating the responses to the fox odour TMT. Dorsal and ventral olfactory sensory neurons in the MOE activate dorsal and ventral domains in the MOB, mediating the innate fear and associative responses respectively. The MOB projects to the olfactory cortex (OC), from where the innate fear circuitry is activated via the medial aspect (MA) of the bed nucleus of the stria terminalis (BST) and the HPA axis, while the associative response is activated via the lateral division (LD) of the BST. The dashed lines indicate that there are many aspects of the circuit which are still uncertain. Hyp – hypothalamus; Pit – pituitary gland; AG – adrenal gland; ACTH – adrenocorticotropic hormone. Adapted from Kobayakawa *et al.* (2007).

fox-specific and is not widely produced by predators in general. In contrast, another chemical, **2-phenylethylamine** (PEA), is present in urine from a wide range of carnivores, but is either absent or has a low concentration in urine from herbivores. It does, indeed, act as a kairomone for rodents (Ferrero *et al.*, 2011). PEA activates multiple olfactory receptors in the MOE in both dorsal and ventral regions, and induces classic aversion behaviour, but this does not seem to require activity in the VNO subsystem, since it works even in VNO-deficient mutants. It seems to be a key component in urine for triggering aversion to carnivores in general, since lion urine is a powerful trigger for avoidance behaviour, even in laboratory mice and rats who never encounter them, while giraffe urine has no significant effect. However, if the PEA is removed from the lion urine, it no longer has any significant effect either.

2.3.3 Dual purpose signals: the MUP family

A different set of signalling chemicals has been discovered which belongs to the **major urinary protein** (MUP) family. As the name suggests, these are proteinaceous components of urine. What makes them particularly interesting is that they have evolved a dual function in mice. Most mammals encode just a single MUP of unknown function (although they are allergenic to humans). However, mice and a few other species have an expanded MUP gene family, and have evolved receptors that can detect MUPs from other species as well as their own. Male mice excrete a MUP which acts as an aggression-inducing pheromone when detected by male conspecifics (Chamero *et al.*, 2007). However, homologous but species-specific MUPs from natural mouse predators, such as rats, cats and even snakes, elicit the innate fear response in both male and female mice (Papes *et al.*, 2010).

The MUP detectors are thought to reside in the VNO, since mutants lacking the primary transduction mechanism of VNO neurons do not respond either to the conspecific pheromone or to the predator kairomone. In fact, a mutant mouse will walk right up to a rat and sniff it without showing any apprehension, when a wild type mouse in an equivalent situation would be cowering in the corner. Furthermore, in wild type mice, MUPs directly activate VNO sensory neurons, as indicated by increased c-Fos[10] expression in both the afferents themselves and their target glomeruli in the AOB, but this is absent in the mutants. VNO-mutant mice show normal avoidance of smells such as burning wood or fox faeces (TMT), which are detected through the MOE and/or GG, so they have not lost aversion to all danger – just the danger from certain predators.

There is an interesting, but unexplored, wrinkle in MUP chemistry. MUPs have a β-barrel conformation, with a small central hydrophobic binding pocket which carries organic ligands. The mouse aggression-promoting MUP primarily binds SBT, which, as we have seen earlier, is the main component of the mouse alarm pheromone. The SBT can be removed from the pocket, and the protein component alone still acts as a fully-functional aggression-inducing factor, but its close association with the alarm pheromone seems likely to be more than a coincidence.

The mouse MUP pheromone and the rat MUP kairomone elicit different behaviours in receiver mice. Both are detected by neurons in the VNO, but by different sub-populations. It is not known how this is sorted out in the CNS, but it is known that the pheromone response is context-dependent – aggression is only triggered by mouse MUP in the presence of another male mouse, while the fear response is triggered just by the rat MUP, even in the absence of an actual predator. Aggression and fear responses may seem to be mutually exclusive and opposite reactions but, in fact, they are both exemplars of the 'fight-or-flight' response and, as such, may share common elements. The VNO signalling may activate a stress response, with the balance between the outcomes determined by the ensemble of receptors and the context of the signal. Another interesting question is whether the rat MUP, which acts as a kairomone to mice, acts as a pheromone to other rats. It seems likely that this is the case but, so far as we know, this has not been demonstrated.

At this stage, the reader may be getting confused as to which signalling chemicals activate which olfactory subsystems, which behaviours are initiated by which chemicals and, indeed, whether there are actually any rules as to how this is all organised. The answer seems to be a bit like the Pirate Code in the movies[11] – 'more guidelines than actual rules' (Table 2.1). Receptors in the VNO tend to bind heavier molecules of low volatility, and these tend to be pheromones which trigger innate behaviours, while the lighter volatile molecules which bind to receptors in the MOE are often general environmental smells, which elicit learned behaviours and memories[12]. However, both intra- and inter-specific chemical communication involves multiple complex pathways with redundant signalling, and without a simple one-to-one correspondence between signalling molecule, receptor subsystem and behaviour.

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[10]c-Fos is an **immediate early gene** which can be used experimentally as a proxy measure of neuronal activity, because its expression is increased when neurons increase their activity.

[11]For instance, the film *Pirates of the Caribbean: The Curse of the Black Pearl* (2003) Directed by Gore Verbinski. USA: Walt Disney Pictures.

[12]Smell is an excellent trigger of memories in humans. A famous (although sadly unread by us) seven-volume novel by Marcel Proust apparently starts with memories brought on by the smell of a biscuit.

Table 2.1 Some mammalian semiochemicals. This is not at all a complete list, but it summarises the key chemicals mentioned in this chapter.

Chemical	Category	Receptor system	Behaviour elicited	Reference
SBT	Mouse alarm pheromone	GG	Innate fear response	Brechbühl *et al.*, 2008 Brechbühl *et al.*, 2013
TMT	Fox kairomone	GG	Innate fear response in rodents	Brechbühl *et al.*, 2013
TMT	Fox kairomone	MOE → MOB (dorsal zones)	Innate fear response in rodents	Kobayakawa *et al.*, 2007
TMT	Fox kairomone	MOE → MOB (ventral zones)	Conditioned fear response in rodents	Kobayakawa *et al.*, 2007
PEA	General carnivore kairomone	MOE	Innate fear response in rodents	Ferrero *et al.*, 2011
MUP	Mouse pheromone	VNO	Male-male aggression in mice	Chamero *et al.*, 2007
MUP	Cat, rat, snake kairomone	VNO	Innate fear response in mice	Papes *et al.*, 2010
Light MW urine	Mouse pheromone	VNO	Male-male aggression in mice	Chamero *et al.*, 2007
Unidentified	Mouse pheromone	MOE	Male-male aggression in mice	Wang *et al.*, 2006

For instance, there are at least three different pheromones promoting male-male aggression in mice: the MUP described above; a light molecular weight but otherwise unidentified component of mouse urine, which activates a different set of VNO neurons (Chamero *et al.*, 2007); and an unidentified component that acts through the MOE rather than the VNO (Wang *et al.*, 2006). It may not be the way an engineer would have designed it, but it is what evolution has come up with over the course of about half a billion years.

2.3.4 Parasites: when kairomones go bad!

There are usually three participants in a pheromone/kairomone communication network: the animal that emits the signal; the intended signal receiver which treats the signal as a pheromone; and the eavesdropper which treats the signal as a kairomone. Sometimes an insidious and microscopic fourth participant joins the network – a parasite. This can turn the tables on the eavesdropper.

Toxoplasma gondii is an intracellular protozoan parasite that infects warm-blooded vertebrates such as birds and mammals. It reproduces in the intestine of cats and other felines, and is shed as oocysts in their faeces. These get eaten by ground-foraging vertebrates, including mice and rats. Human infection is also common, due to the close association between humans and domestic cats[13]. Once a rodent has ingested the *Toxoplasma*, it undergoes a surprising change in behaviour. There is an increase in locomotor activity and general arousal which, in itself, increases the chances of the rodent encountering a predator, but the most dramatic change is in the response to feline kairomone (Webster, 2001). Instead of being frightened by the smell of cat urine, it actually becomes attracted to it, which means that it is likely to approach the cat and get eaten, thus completing the parasite's life cycle. The effect is highly specific to cat urine; aversive reactions to other predator

[13]It is estimated that about 25% of all humans are infected worldwide.

odours are unaffected (Vyas *et al.*, 2007). What causes this reversal in the response to the cat kairomone?

Once *Toxoplasma* has been eaten by the rat, it can invade any nucleated cell type of the host, but it has the particularly effective strategy of penetrating the immune-reactive **dendritic cells** (DCs)[14] that the host uses to try to counter the infection. *Toxoplasma* then induces the DCs to start producing the neurotransmitter GABA which, in turn, activates GABA autoreceptors on the DCs – and, for some unknown reason, this induces the DCs to become hypermigratory (Fuks *et al.*, 2012). As a result, the infected DCs spread throughout the entire body of the rat, including the central nervous system, rather than just heading to their normal targets which are the lymph nodes (where the adaptive immune response would help ensure pathogen clearance).

The parasite spreads throughout the brain tissue of an infected rodent, but it seems to concentrate particularly in the limbic-amygdalar regions that process 'emotional' responses, and which receive VNO-induced input directly from the AOB. In normal rats, exposure to the smell of cat urine increases activity in the 'defensive' part of this region, while exposure to a conspecific opposite-sex odour increases activity in the 'reproductive' part of the region. However, in infected rats, the cat urine increases activity in *both* regions, with the reproductive region being as highly stimulated by the cat urine as it would have been by a real opposite-sex stimulus (House *et al.*, 2011). It is obviously not possible to know what the infected rat is consciously experiencing, but the neurophysiological evidence suggests that it must be highly conflicted. It seems to be simultaneously frightened by, and sexually aroused by, the smell of a cat – with the latter likely to prove a literally fatal attraction.

The actual mechanism by which the parasite alters the neural activity is not known, but chronic *Toxoplasma* infection causes an increase in overall brain levels of dopamine (Stibbs, 1985; Flegr *et al.*, 2003), and dopamine elevation is commonly associated with neural reward and arousal systems. Treatment with dopamine antagonists, such as the anti-psychotic drug haloperidol, reduces the attraction of infected rats to feline kairomone, so elevated dopamine may be the linking factor causing the re-rooting of the limbic information flow. Given this dopamine connection and the high level of human toxoplasmosis, there is a considerable interest in the possibility that some instances of human affective disorders such as schizophrenia might, in fact, be due to *Toxoplasma* infection altering brain catecholamine levels (Webster *et al.*, 2006).

2.4 Summary

We humans tend to think of smell and taste as less significant forms of perception compared to, say, vision and hearing but, for many animals, chemoreception is the main way in which they get information about their surroundings. It was probably the first perceptual mechanism to evolve in the history of life, and even single-celled organisms can show very clear responses to external chemical signals. Many animal species devote a considerable amount of genetic resources to encoding olfactory receptors. Individual receptors respond to individual odour motifs, but such motifs occur on a range of odour molecules, so each receptor responds to any odour containing the appropriate motif. Furthermore, individual

[14]Despite their name, dendritic cells are part of the immune system, not the nervous system (the name reflects their morphology). The discovery of their role in the adaptive immune response led to the award of the Nobel Prize to Ralph Steinman in 2011.

odour molecules contain a range of different motifs and thus activate a range of receptors. The result is a massive number of combinatorial possibilities, enabling animals to distinguish, in theory, an almost limitless number of different odours. Many olfactory receptors activate a second-messenger intracellular cascade which allows considerable amplification, and which can make animals sensitive to even very low concentrations of odour.

Most odours fall into one of two categories: volatile 'canonical' odours, which provide information about the general environment, and to which the receiver responds with learned, associative behaviours; and heavier semiochemical odours, which carry an intra- or inter-specific message, and which elicit innate, genetically-programmed behaviours. The different types of odour may be detected by different olfactory sub-systems containing anatomically and physiologically distinct receptors. There is a lot of co-evolution of semiochemicals, with pheromones which benefit the producer and conspecifics becoming kairomones that harm the producer and benefit another species, and vice versa. Predators can use prey kairomones to detect and track them, while prey can use predator kairomones to avoid them. Animals that are under actual or threatened predator attack can also use pheromones to alert conspecifics to the danger. Finally, some parasites can alter prey behaviour, so that they actually become sexually attracted to the smell of a predator!

Abbreviations

AOB	accessory olfactory bulb
AONpE	pars externa of the anterior olfactory nucleus
AP	alarm pheromone
CNG	cyclic nucleotide-gated
DC	dendritic cell
GG	Grueneberg ganglion
HPA	hypothalamic-pituitary-adrenal
MOB	main olfactory bulb
MOE	main olfactory epithelium
MUP	major urinary protein
OB	olfactory bulb
OR	olfactory receptor
OSN	olfactory sensory neuron
PEA	2-phenylethylamine
SBT	2-*sec*-butyl-4,5-dihydrothiazole
TMT	2,4,5-trimethylthiazoline
VNO	vomeronasal organ

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