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The Chemical Stimulus and Its Detection

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One of the most difficult aspects of studying the chemical senses is that researchers do not know which properties of the stimulus are relevant for detection and perception. In this article, we explain what we do and do not know about the features of chemical stimuli, the ways in which these features are detected and coded by chemosensory organs and the central nervous system of tetrapod vertebrates, and the characteristics of a stimulus that might make it likely to stimulate a particular chemosensory organ.

Before sensory stimuli can affect an animal's physiology or behavior (i.e., be "perceived"), they have to be transduced into electrical signals and processed in the central nervous system. In the visual system, the relevant parameters of light are its wavelength and intensity, which are transduced by specialized photoreceptor cells in the retina and, after processing in the central nervous system, perceived as color and brightness, respectively. Many vertebrates also have two classes of photoreceptor cells that are differentially sensitive to light of different intensities, the rod and cone cells, enabling the visual system to operate in both bright and dim light. Furthermore, the photosensitive pigments (opsins) in these cells are sensitive to light within a limited range of wavelengths; an animal with two or more classes of photoreceptor cells with different opsins will be able to perceive color (Reuter and Peichl, chapter 10 in this volume). Water differentially attenuates light of different wavelengths, and these changes are reflected in the differences in spectral tuning of opsins seen in animals that live at different depths (e.g., Fasick and Robinson, 2000). Thus, the properties of light and the ways in which sensory specializations are tuned to these properties are well understood. (For a detailed discussion of the properties of visual stimuli and the ways in which they differ in air and water, see chapters 8 and 9 in this volume by Kröger and Kröger and Katzir).

In contrast to our detailed understanding of visual stimuli and the sensory ecology of the visual system, we know surprisingly little about chemosensory stimuli. Do chemical stimuli possess regular features, analogous to wavelength and intensity, that are coded by the nervous system? If so, how do changes in a given feature affect perception? Do animals have differentially sensitive chemosensory receptor cells, or chemosensory organs, for detecting stimuli with different features? Finally, how do air and water alter the features or availability of chemical stimuli?

Most tetrapods have three anatomically separate chemosensory organs: a gustatory (taste) organ, an olfactory (smell) organ, and a vomeronasal (accessory olfactory or smell) organ. The central projections of these three organs are anatomically distinct through several synapses, and this anatomical distinction is used to recognize and differentiate the chemosensory systems in diverse vertebrates. The organs may detect largely nonoverlapping sets of chemical stimuli, but information from the three organs is integrated within the central nervous system so that animals can make appropriate decisions about what to eat, flee from, fight with, or mate with.

In general, tetrapods employ the three chemosensory systems according to a rough temporal and informational hierarchy (e.g., Schwenk, 1995). The olfactory system is capable of detecting chemicals at a distance from their source. Once an animal has detected a potentially interesting odorant using its olfactory system, it may then investigate the source further using its close-range chemosensory organs, the tongue (taste buds) and vomeronasal organ. We still do not know why some chemicals preferentially stimulate the taste system instead of the vomeronasal organ, or why others stimulate the vomeronasal but not olfactory organ.

In general, the different chemosensory organs detect compounds with differing behavioral significance. The taste system is specialized for sampling possible food items before they are ingested and responds to compounds that are dissolved in fluids and do not become airborne to any significant degree: sugars, protons, salts, proteins, and amino acids, as well as diverse molecules that taste bitter and serve to warn of the presence of potential toxins. The olfactory and vomeronasal systems may serve less specialized functions. Although some researchers have suggested that the vomeronasal system could be specialized for detection of pheromones, the olfactory system mediates some pheromonal effects, and the vomeronasal system responds to nonpheromonal stimuli such as prey odorants and artificial chemicals with no inherent biological significance (reviewed in Baxi et al., 2006). The vomeronasal system also has been suggested to mediate unlearned responses to odorants (Meredith, 1986; Halpern, 1987), but this intriguing idea has not received much experimental attention.

If the olfactory system is specialized for detecting chemicals at a distance, what features of a chemical determine whether it will move from the source, or remain in place? In terrestrial environments, we might expect that a simple parameter of the chemicals themselves, such as volatility, might play a role. However, most biological sources of odorants contain water or lipids, and in all tetrapod chemosensory systems chemical stimuli must pass through a fluid covering the sensory epithelium before they are transduced by sensory neurons. Therefore, we might expect that other chemical parameters, such as solubility or polarity, might also be important in determining which organ detects a given stimulus. We consider each of these potentially important chemical qualities in turn.

VOLATILITY

The volatility of a given chemical depends on several factors, including its molecular weight. Within any chemical series, vapor pressure (and therefore volatility) decreases rapidly with increasing molecular weight. Early perfumers believed molecular weight to be the sole determinant of volatility, and that chemicals larger than about 500 daltons are too heavy to become airborne; nevertheless, volatility depends on several factors such as the length of side chains.

Volatility has been suggested to play a key role in determining which chemosensory organ detects a given stimulus. Most prominently, the olfactory epithelium has been hypothesized to detect volatile odorants, and the vomeronasal organ to detect nonvolatiles (for a thorough discussion, see work by Baxi et al. [2006]). This hypothesis is founded on the observation that some tetrapods have specialized behavioral or physiological mechanisms to promote access of nonvolatile molecules to the vomeronasal organs. For example, in squamate reptiles (lizards and snakes), the vomeronasal organs are sequestered from the nasal cavities, and chemical access is limited to two tiny openings in the anterior palate. Scent molecules are picked up by the mucus-covered surface of the tongue tip and physically delivered to the vomeronasal orifices in the mouth during a behavior known as tongue-flicking (reviewed in Halpern, 1992; Schwenk, 1995). The nasal epithelium, on the other hand, is stimulated by presumably volatile odorants that enter the nose relatively passively during breathing (Dial and Schwenk, 1996). In some rodents and other mammals, the vomeronasal organs are surrounded by vascular, contractile tissue that acts as a pump, drawing nonvolatile molecules into the organs along with the mucus surrounding their openings in the nose (Meredith, 1994). Nuzzling, nose-pressing, and licking are common mammalian behaviors that potentially introduce nonvolatile chemicals into the oral and nasal cavities. A peculiar lip-curling behavior known as *flehmen* is evident in artiodactyls and some carnivorans and is thought to facilitate suctioning of fluids from the mouth through palatal openings into the vomeronasal organ (reviewed in Wyatt, 2003). In contrast, the olfactory epithelium of most mammals is located in the posterodorsal region of the nasal cavity, a location suggesting that only volatile molecules would have access to the sensory receptor neurons.

Several problems complicate this tidy scenario. First, nonvolatile compounds, and even large particles such as viruses, can gain access to the olfactory epithelium (Mori et al., 2005). Conversely, there is no evidence suggesting that volatile compounds cannot gain access to the vomeronasal organ. The volatility hypothesis would be saved if one could demonstrate that "inappropriate" stimuli that gain access to the vomeronasal or olfactory organs are not transduced, but relevant data are lacking. Some studies demonstrate that volatile compounds can stimulate vomeronasal receptor neurons in vitro (e.g., Leinders-Zufall et al., 2000), but the results of in vivo studies are equivocal (e.g., Luo et al., 2003).

A second problem involves the presence of odorant binding proteins within the mucus in the nasal cavity (Baxi et al., 2006). In short, the importance of volatility may be significantly mitigated by the presence of large, nonvolatile carrier or transport proteins, particularly if odorants frequently or always arrive at the sensory epithelium in a bound state. Although some studies indicate that volatile compounds stimulate the vomeronasal system only in the presence of carrier proteins (e.g., Guo et al., 1997), other studies suggest that the volatile compounds and their carriers may be transduced by the vomeronasal neurons and then processed independently in the central nervous system (e.g., Brennan et al., 1999).

A final problem arises from the observation that some aquatic tetrapods, such as amphibians, possess a vomeronasal system (Reiss and Eisthen, chapter 4 in this volume), and volatility is irrelevant in water. Indeed, large molecules that are nonvolatile in air can be highly soluble in water if they are polar and, therefore, could be available to any of the three chemosensory systems (Wilson, 1970). As such, molecules of varying sizes and properties may have equal access to the olfactory and vomeronasal epithelia in aquatic tetrapods, such as amphibians, in which the vomeronasal epithelium is not sequestered (for discussion of this issue in marine turtles, see chapter 5 by Schwenk in this volume). Thus, the significance of volatility in determining which chemosensory organ detects a given stimulus is unclear, particularly for aquatic organisms.

SOLUBILITY

Chemical stimuli must cross a watery mucus layer before contacting receptor cells in taste buds, or in the olfactory or vomeronasal epithelium. Thus, one might expect that solubility plays a role in determining whether a chemical gains access to chemosensory receptor cells, and it may also determine which sensory organ is stimulated. As with volatility, the importance of solubility is complicated and poorly understood.

In some cases, odorant molecules probably diffuse across the mucus barrier directly to the sensory receptor cells. However, the mucus in the nasal cavity contains odorant binding proteins, which may function in transporting hydrophobic molecules across the mucus barrier to the sensory epithelium (Bignetti et al., 1987; Vogt, 1987). Nevertheless, the odorant binding proteins may bind odorants nonselectively, in which case hydrophobicity is irrelevant (Tegoni et al., 2000).

Although one might also expect that in aquatic environments chemical stimuli must be soluble to be detected, this does not appear to be the case. For example, the magnificent tree frog, *Litoria splendida*, produces a hydrophobic peptide pheromone. The molecule's hydrophobicity appears to be an important aspect of its function, as it causes the pheromone to disperse much more rapidly than would occur through simple diffusion (Apponyi and Bowie, 2005). Indeed, dispersal rate of chemical signals may be at a premium in aquatic environments owing to the diffusion kinetics of chemicals in water versus air. The time interval between chemical release and fade-out is 10,000 times greater in water; that is, a chemical signal takes much longer to fill the same volume of space in water compared to air (Wilson, 1970). For organisms inhabiting still waters, low rates of diffusion could pose a significant problem for chemical signaling. Therefore, the rapid spreading afforded by nonsoluble, hydrophobic chemical signals could be advantageous to aquatic organisms, a possibility that may strike some as nonintuitive.

POLARITY

Polarity is a virtually unexplored attribute of potential chemical signals in vertebrates. Its possible significance in the context of aquatic species is obvious in that it relates to chemical solubility: polar molecules are usually highly soluble in water and, therefore, potentially available in aquatic habitats. Indeed, as polarity increases, vapor pressure (volatility) decreases and solubility in water increases. High solubility owing to molecular polarity can offset the relatively slow diffusion rate in water versus air by greatly increasing the emission rate of a chemical source in water (Wilson, 1970). Further, although larger molecules are generally less volatile and less soluble than smaller molecules, even very large molecules, such as proteins, are soluble in water if they are polar. Thus, large, polar chemicals such as peptides that would make poor signals in terrestrial environments might provide an important source of chemical information to animals in aquatic habitats. This is known to be the case for some aquatic invertebrates and amphibians (Wilson, 1970; Kikuyama et al., 2002).

SENSORY TRANSDUCTION

The means by which odorants bind to and activate receptors, as well as the basis of receptor selectivity, are important unsolved problems in chemosensory research. One of the earliest models of olfactory transduction was Amoore's stereochemical model. Chemicals with similar odors, such as those that smell "fruity" or "floral," were postulated to have a similar shape and fit only into a subset of receptors, like a lock and key. The binding of a particularly shaped molecule to a receptor would then signal the presence of a particular class of molecule (Amoore, 1963). This idea stimulated much productive research, which ultimately proved the model false. Our current understanding is that two molecules that we perceive as similar in odor might bear no structural similarity. Nevertheless, both behavioral and electrophysiological work demonstrate that in some cases the processing of a stimulus depends on length of a side chain or other geometric features of a stimulus (Kent et al., 2003), suggesting that shape may not be completely irrelevant to perception.

A different model of olfactory processing suggests that odorant receptors detect molecular vibrations of stimulus compounds (Turin, 2002). The basic predictions of the theory do not stand up to experimental scrutiny (Keller and Vosshall, 2004).

CONCLUDING COMMENTS

This brief review demonstrates that we understand little about the parameters of chemical stimuli that are coded by chemosensory systems. No simple relationship predicts which chemosensory system will be stimulated, or in what fashion, by a particular stimulus. We therefore cannot make any broad generalizations about how the functional domains of the three tetrapod chemosensory systems are sorted according to the features of chemical stimuli, nor how these domains might differ between terrestrial and aquatic species.

To illustrate the difficulty we have in segregating the chemosensory systems, consider the apparently straightforward question of taste versus olfaction. We cannot define these systems based on the chemicals they respond to, as one might suppose; rather, we distinguish them on the basis of their anatomy and central nervous system coding. From empirical work, we know that taste receptor cells are involved in feeding behavior and usually respond to proximate chemical cues released during physical contact with a chemical source, whereas olfactory receptors can respond to chemicals released by distant sources. Further, information from the two systems is combined to give rise to the perception of “flavor,” which depends to a large degree on olfactory cues emanating from items in the oral cavity; thus, the same food item gives rise to both gustatory and olfactory stimuli. Overall, our understanding of the functional attributes of the system is based on empirical observation and not predictions based on first principles.

When it comes to distinctions between the olfactory and vomeronasal systems, we are at even more of loss, given the anatomical similarity between them. The functional dichotomy of sensitivity to low versus high molecular weight stimuli, respectively, may be true under some circumstances, but it is not a perfect distinction. Perhaps the more important question is one of accessibility. After all, one must consider not only whether a particular sensory receptor cell can respond to or transduce a particular class of chemicals, but the circumstances in which the cell will be exposed to those chemicals.

In mammals, squamate reptiles, and marine turtles, the vomeronasal epithelium is sequestered, and unlike the olfactory epithelium, its cells are not readily stimulated during normal breathing. Rather, stimulation requires a distinct action such as tongue-flicking in squamates, vomeronasal suction in rodents, flehmen in artiodactylans and carnivorans, or some other kind of physical contact with a chemical source. As such, the animal has the opportunity to sample a particular, localized source of chemicals, as well as to sample classes of chemicals not readily available in the surrounding air or water, such as nonvolatile or nonsoluble molecules. This is not to say that such molecules cannot also stimulate the olfactory epithelium or that volatile and/or soluble molecules cannot stimulate the vomeronasal receptors—only that the vomeronasal system may be used facultatively, actively, and selectively to add a new level to the sensory information an animal gathers about its environment. In amphibians and most turtles, in which the vomeronasal epithelium is broadly exposed within the nasal cavities (see Reiss and Eisthen, chapter 4 in this volume; Schwenk, chapter 5 in this volume), this hypothesis is harder to support, and the segregation of functional domains even more ambiguous.

A second question of accessibility relates to whether tetrapods flood their nasal cavities with water when submerged. For example, crocodylian and squamate reptiles evince adaptations for closing the nostrils during submersion, excluding water from the olfactory chamber, whereas aquatic turtles actively pump water into and out of the nasal cavities (Schwenk, chapter 5 in this volume). Thus, water-based olfaction is possible only in the latter taxon. Perhaps this behavioral difference relates to the fact that crocodylians lack a vomeronasal epithelium and in squamates it is inaccessible from the nasal cavity, whereas in turtles it is more or less exposed. Schwenk (chapter 5 in this volume) suggested that pulsing water through the nasal chambers in turtles represents vomeronasal stimulation rather than olfaction (a trapped air bubble, in fact, might prevent immersion of the dorsally located olfactory epithelium).

Finally, we note that the chemical parameters of volatility, solubility, and polarity to some extent determine the universe of potential chemosensory stimulants in any given environment, and that these chemical qualities suggest that quite different sets of biologically relevant chemicals may be available to a given species in air versus water. However, most aquatic tetrapods spend time in both terrestrial and aquatic environments, and aquatic specialization of the chemical senses, particularly the olfactory system, might come with insupportable fitness consequences. Furthermore, the long terrestrial history preceding even the most aquatic of extant tetrapods suggests a historical phenotypic burden that may be difficult to circumvent. The chemical senses of aquatic tetrapods might therefore exhibit far less adaptive specialization than we might expect.

In conclusion, the relevant properties of chemical stimuli seem straightforward, and one might assume that volatility, solubility, and polarity would play critical roles in determining which chemical stimuli are transduced, and by which chemosensory system. Furthermore, one might assume that these same properties would determine what kinds of compounds serve as chemical stimuli in air versus water. However, one only needs to consider that short-range pheromones used by brown algae tend to be nonpolar, volatile, hydrophobic hydrocarbons that become suspended in water but do not dissolve in it (Pohnert and Boland, 2002) to realize that we are a long way from predicting the relevant features of chemical stimuli in any environment or for any group of organisms.

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