

ARCHIVING ODORS

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"nam unguentum dabo, quod meae puellae
donarunt Veneres Cupidinesque,
quod tu cum olfacies, deos rogabis
totum ut te faciant, Fabulle, nasum."

Gaius Valerius Catullus (c. 84 BCE - c. 54 BCE)

In an ode addressed to his friend Fabullus, the Roman poet Catullus speaks of a fragrance so pleasing that "when you smell it you will beg the gods to make you all nose". Would that the recipe for such a scent had been transmitted through the ages! Even today, however, it is not possible to document chemical composition with adequate fidelity to reconstruct an odor perfectly.

Catullus writes that the gods of love gave the perfume to his girlfriend. Suppose such gods existed and could list the ingredients of its aroma. The list would contain hundreds -- perhaps thousands -- of chemical structures and their relative proportions. Very likely many of the structures would stand for compounds that are currently unknown, but they could be synthesized in the laboratory. Would that knowledge permit me to reproduce the odor? This chapter argues that the answer remains uncertain. The current state of chemical knowledge can neither account for why an odor smells the way it does nor what determines its intensity. The recipe for replicating a sensory experience -- what is essential and what is superfluous -- remains obscure.

The sense of smell challenges chemical understanding. On the one hand, given the structure of a new molecule a chemist can predict its spectroscopic properties over a wide domain of electromagnetic frequencies. A mixture ordinarily displays a spectrum that superimposes the spectra of its individual components, unless they physically interact with each other. In the chemical senses, on the other hand, perceptions of mixtures often cannot be inferred from their constituents, even though the components do not interact at the molecular level. Moreover, no one can reliably predict the organoleptic properties (taste or smell) of a new molecule from its structure. Even if that were possible, the English language does not offer a vocabulary with which to describe new smells, except by analogy to odors that are already familiar.

The poverty of descriptors means that, in talking about olfactory stimuli, many people allude to direct experiences. These allusions call upon memories of characteristic odors of familiar objects, which represent "unitary percepts". A unitary percept stands for a specific whole that resists straightforward decomposition in terms of the perceptions of its individual components. A smell often represents the response to a chemical mixture of dozens of different compounds, no one of which corresponds to a dominant signal carrier. As an example, when I mention the aroma of coffee, the reader brings to mind a set of odors that unambiguously fall into a clearly recognizable category; but none of those odors corresponds to any pure organic compound yet discovered [Czerny, Mayer, and Grosch (1999)]. Available evidence does not support the thesis that unitary

percepts form as simple superpositions of responses to individual chemical constituents [Livermore and Laing (1998)]. Although the context of an odor often confuses people when they try to identify it [Engen (1991)], unitary percepts seem nevertheless to constitute fundamental entities in discussing olfaction.

Chemists traditionally classify organic molecules according to functional groups. A systematic study found that chemists do moderately well at correlating functional group with odor [Brower and Schafer (1975)]. Their near-perfect performance in identifying divalent sulfur compounds must be discounted, however, since that functional group has a rich oxidation chemistry in the presence of air, which produces impurities that have a characteristic stench. The reader can confirm the effect of these impurities by contrasting commercial dimethyl sulfide with a sample that has been freshly washed with saturated aqueous mercuric chloride to remove di- and polysulfides and mercaptans! The correlation for other functional groups demonstrates an apparent but imperfect connection between the categories of molecules and categories of smells.

In speaking of a molecule a chemist calls to mind a structure, which often stands for a collection of equilibrating tautomers or conformational isomers. I think of molecules in terms of the notion of pure compounds. The equation $A + B \rightarrow C$ refers to the reaction of pure A with pure B, regardless of the fact that C may stand for a mixture of products. The notion of a pure compound depends upon context: in one circumstance the molecule called "ethyl acetoacetate" designates a substance purified by distillation and available from a commercial vendor, which is in fact a mixture of interconverting tautomers. In another circumstance the same name explicitly denotes the keto tautomer only. Olfaction recognizes unitary percepts instead of pure compounds (although some pure compounds turn out to correspond to unitary percepts). The names for unitary percepts have a degree of latitude comparable to the names of molecules.

Surely the science of chemistry will some day reconcile the sense of smell with a reductionist approach in terms of pure compounds. In the interim this chapter seeks to raise two questions related to the one posed in the opening paragraph:

- i.* What can another organism detect by means of olfaction?
- ii.* How might an odor be archived so as to convey it to posterity?

These questions address the issue of reproducibility of sensation. Unless we take the position that all sensory experiences are unique, some criterion must be advanced to assess whether two stimuli are perceived as similar. Ideally this criterion should apply to other air-breathing vertebrates as well as ourselves, particularly since we can envisage controlled experiments that cannot be ethically be performed on human subjects. Once the possibility of such a criterion has been established, the question of how to replicate a smell becomes meaningful. In the discussion below I shall differentiate between the nouns used to designate olfactory stimuli (odor, odorant, aroma, fragrance, *etc.*) and the one used to denote the perception (smell).

What is olfaction?

All living creatures respond to chemicals in their environment. There can be little doubt that other mammals use their noses in much the same way as humans do. Drawing parallels between our sense of smell and that of another terrestrial species (which may have sensory modalities that we lack) requires a set of guidelines for assessing features of chemoreception that correspond to human olfaction. These guidelines seek to segregate olfaction from other chemical senses, in order to discuss how other animals respond to chemical stimuli. Future investigation may prove the impossibility of such a compartmentalization, so I shall attempt to circumscribe olfaction rather than to define it.

Any operational definition of olfaction must pertain to the behavioral responding of an organism to volatile compounds. Though olfaction may correlate with electrical responses of nerves, which can be measured independently of responding, behavior has to be considered as the primary standard for deciding whether an organism perceives an odor. This postulate permits one to differentiate olfaction from the enormous variety of other responses that living creatures exhibit to chemicals in their environment. The set of compounds that elicit behavior does not coincide perfectly with the set that produces electrical activity, even in comparatively primitive terrestrial vertebrates [Mason, Stevens, and Rabin (1980); Dorries, White, and Kauer (1997)]. Given a discrepancy between two observables -- behavior on the one hand and electrophysiology on the other -- the former trumps the latter.

Consider the following propositions, which provide additional boundaries that separate olfaction from other types of chemoreception:

1. The stimuli have appreciable vapor pressures;
2. The association between stimulus and response is learned;
3. Severing the first cranial nerve on both sides eliminates the behavior (Or, more precisely, olfaction designates those features of responding that bilateral nerve transection abolishes);
4. The organism can discriminate different molecules and can discriminate different stimulus intensities without necessarily confusing intensity with the qualitative features of an odor.

This list embraces much of what I believe to be true of olfaction in human beings and is put forth as a set of necessary conditions for the analogous chemical sense in other vertebrate species. Given the range of biological diversity, it probably does not represent a set of conditions sufficient to define olfaction. In discussing these four guidelines I shall make reference not only to my own perceptions of odors, but also to published reports about people who display peculiarities in their sense of smell. Some are anosmic (*i.e.* "odor blind"), while at least one may be said to be "odor deaf".

The stimuli have appreciable vapor pressures

In the present context, a compound can be defined as having an appreciable vapor pressure if it has a normal boiling point or sublimation temperature below 350°C. Some compounds

decompose before they boil at atmospheric pressure, but normal boiling points (or sublimation temperatures) can be extrapolated from vapor pressures at lower temperatures. This definition excludes all molecules with electric charges and also zwitterions such as amino acids. Limiting olfaction to the sensory detection of volatile compounds does not mean stimuli must necessarily be presented in the gas phase. Olfactory receptor neurons are naturally immersed in a fluid layer (mucus, in the case of mammalian noses) and do not come into direct contact with vapors. Plate 1 reproduces a scanning electron micrograph of the olfactory mucosa of an adult tiger salamander (*Ambystoma tigrinum*). The receptor neurons of this amphibian morphologically resemble those of virtually every other animal, from tiny nematodes to human beings. In tiger salamanders (unlike many mammals) the receptor neurons cluster in distinct patches, separate from other types of cells. This species has been widely studied, because the surface represented in the micrograph is readily accessible to the experimenter without the necessity of dissection.

Several cilia attach to the outer end of each receptor neuron, so that (as the photomicrograph shows) the surface of the olfactory mucosa looks like a tangle of spaghetti. The receptor surface is ordinarily covered with mucus (to a depth of 10-100 μm). (In order to prepare the tissue for microscopy, the mucus layer was removed by washing with a solution of EDTA). Adult tiger salamanders are the only cold-blooded, terrestrial vertebrates that have yet been trained in the laboratory to respond behaviorally to vapors from reagent-grade chemicals. Extensive experimentation has been reported on the electrical responses of their receptor neurons and brains to chemical stimuli. The olfactory mucosa responds electrically to volatiles presented either in an airstream or in aqueous solution [Arzt, Silver, Mason, and Clark (1986)]. At this point there is no reason to suspect that these animals could not be trained to respond behaviorally to aqueous presentations, though such experiments have yet to be reported.

The distinction between volatile and nonvolatile corresponds roughly to the separation between smell and taste in human beings, but this pair of distinctions may not be perfectly congruent. I cannot smell glycerine, sodium chloride, quinine, and dilute sulfuric acid, but they taste sweet, salty, bitter, and sour. By contrast, many volatile organic compounds produce a burning sensation when placed on the tongue without eliciting the sensation of taste. Most of the flavor of food comes from olfaction (as you can demonstrate by holding your nose while you eat), but there might be volatiles that can be detected by taste, as well as nonvolatile compounds that might elicit olfactory responses when their dust impinges on the olfactory mucosa.

If volatile molecules have to diffuse through a layer of mucus that is at least 10 μm thick before reaching the neuron, molecular diffusion implies a temporal resolution no less than 0.1 second. In other words, fluctuations of odorant concentrations will not be detectable if they occur on a timescale much less than 0.1 second. This conclusion derives from the value of the diffusion constant for small molecules in solution (about 0.001 mm^2 per second), which shows little dependence upon the identity of the solvent or of the solute. This time constant represents a intrinsic constraint that results from the physics of the stimulus and its receptor.

The vapor pressure criterion pertains especially to animals that possess sensory apparatus that humans lack. Many mammals have a morphologically distinct chemosensory organ called the vomeronasal organ (VNO) [Meredith (1998)], which is vestigial in human beings. The VNO appears to respond to nonvolatile chemical stimuli that arrive as airborne particles. The VNO may also have the capacity to detect volatile stimuli, and the remaining guidelines help differentiate its inputs from olfaction.

Association between stimulus and response is learned

Many natural languages contain words that describe the painful sensations associated with harsh chemical stimuli. Strong vinegar is pungent. So is ammonia. Most people recoil from them without having learned to do so. The pain can be sensed elsewhere than in the nose. Fumes from strong vinegar or ammonia sting the eyes. Therefore pungency refers to sensations that have similar effects on other sensitive tissues. Pungency and other sensations that are not localized to the nose, such as the thermal "feel" of menthol, must be viewed as separate from olfaction, particularly since congenitally anosmic human subjects can detect and discriminate among odorants with such properties [Laska, Distel, and Hudson (1997)]. The "common chemical sense" comprises this set of chemosensory inputs.

The English language contains few words to describe smells that do not allude to specific examples. One often hears "That smells like ...", and even the vocabulary of perfumers is filled with similes such as "floral" or "woody". The word "putrid", however, has a more general meaning. But it denotes a hedonic judgment in addition to its descriptive function. Even a slight acquaintance with the variety of world cuisine suffices to refute any claim that humans agree about what is putrid and what is not.

There is no evidence that small children classify odors as smelling good or bad (apart from the irritation produced by pungent odors, which, I assert, does not constitute olfaction) [Engen (1978); Mennella and Beauchamp (1998)]. They certainly distinguish between familiar and unfamiliar and form hedonic categories on that basis. At some stages of development, for instance, nearly all children display neophobia, an aversion to unfamiliar stimuli. But I submit that the olfactory associations, which represent a prominent feature of the adult sense of smell, represent a set of entirely learned responses and that there are no odors that are intrinsically pleasant or repellent (except insofar as they produce pain) [Engen (1988)]. In any event, I wish to exclude from olfaction any components of smell that elicit responding that has not been learned.

Pheromones are examples of compounds that evoke behavior that has not been acquired by associative learning. For instance, during their estrus domestic sows respond to the volatile steroid androstenone in a characteristic fashion. Androstenone constitutes a pheromone for swine, which appears to be detected via the olfactory system [Dorries, Adkins, and Halpern, 1997]. It is possible that humans may also respond to volatile steroids as olfactory stimuli that cannot be consciously perceived [Sobel et al (1999)]. The above criteria of olfaction do not include this type of responding. In choosing to exclude pheromone detection from the definition of olfaction I am

following Engen [1978; 1988; 1991], who views pheromones as airborne hormones, which represent a category of chemical messengers physiologically distinct from olfactory stimuli.

Bilateral transection of the first cranial nerve abolishes olfaction

Olfaction represents a subset of the sense of smell, which this chapter seeks to circumscribe by means of four propositions put forth as guidelines. Of these guidelines the first is chemical and the second behavioral. The third of the guidelines addresses the relationship between anatomy and behavior. Applying it requires that the organism possess a well developed central nervous system (CNS). The CNS of terrestrial vertebrates includes a spinal cord and a brain, from which emanates a set of cranial nerves. The first cranial nerve is often called the olfactory bulb. If the connections between the nose and the olfactory bulb are completely severed, the ability to sense and to discriminate among volatile stimuli do not necessarily vanish utterly. Those capabilities that are completely lost, however, include olfaction.

Regeneration of the olfactory nerve after transection occurs in many species. Subsequent reacquisition of olfactory-mediated behavior does not take place without reconditioning. Hamsters, for example, lose their ability to discriminate odors when the olfactory nerve is cut, but they recover their ability to smell over a period of a few weeks. Even if trained to discriminate a pair of odors prior to surgery, they need to relearn the task postoperatively. Relearning the familiar pair of odors follows the same time course as learning a new pair of odors [Yee and Costanzo (1998)].

In tiger salamanders bilateral olfactory nerve transection (abbreviated ONX) greatly reduces -- but does not totally abolish -- discriminative responding to airborne chemical stimuli [Mason, Meredith, and Stevens (1981); Mason, Clark, and Morton (1984); Mason, Leong, Plaxco, and Morton (1985)]. The brain of this amphibian has been well explored by anatomists [Herrick (1948)], and the animals' responding to the vapors of pure organic compounds fulfills the guidelines discussed so far [Mason and Stevens (1981); Dorries, White, and Kauer (1997)]: the discriminative capacity destroyed by ONX is a learned behavior. In many species, though, the first cranial nerve also transmits impulses from the VNO, so anatomy does not, by itself, necessarily segregate olfaction from other chemosensory modalities. Therefore, in addition to the other guidelines, an additional one must be included in order to account for what is known from studies of human subjects.

Olfaction discriminates different molecules and different stimulus intensities

A widely accepted dictum holds that humans enjoy three chemical senses -- the common chemical sense (which records irritation, pungency, and other "feels"), taste, and smell. A recent study of human subjects who lack the last (anosmics) has shown that they can still distinguish among volatile chemical stimuli by means of the first [Laska, Distel, and Hudson (1997)]. For several odorants the anosmic subjects' verbal responses mirror those of people with a normal sense of smell (normosmics). However, the anosmics do not distinguish ethanol and propanol easily. I find that those alcohols smell quite different, but (to extrapolate from the published data) an

experimenter could prepare concentrated solutions of the two such that an anosmic person could not differentiate them, while a normosmic person could.

While this insight may not appear very profound, its implications warrant some reflection. Some molecules convey similar odors -- citral (from lemongrass) and limonene (from lemon peel) smell the same to me -- but I do not know of many examples where two compounds with different odors become confused with one another when their relative concentrations are adjusted. That sort of problem does often occur, though, in electronic instrumentation in chemical laboratories: a signal from an impurity can be misinterpreted as coming from the analyte. The comparative rarity of interferences of this kind in olfaction argues against any simple analogy between the nose and a spectrophotometer.

The ability to discriminate intensity differences raises a very complicated issue, since we do not know what physical parameter determines the strength of an olfactory stimulus. My subjective impression of an odor is that it fades with time, even when the concentration of stimulus does not change. This phenomenon (known as adaptation) has been studied for over a century [Stuiver (1958)]. Olfaction must register something different from the ambient concentration of an odoriferous chemical; otherwise the sensation would remain constant. Single-celled organisms (such as bacteria) swim towards attractive chemical stimuli by responding to the change in concentration with time [Koshland (1972); Koshland, Goldbeter, and Stock (1982)]. In other words, they exhibit rate-sensitive detection. Do humans respond in a similar fashion?

Some years ago we modeled the properties that olfaction should display if intensity is coded for humans in the same way as for a simple model of bacterial chemotaxis [Nachbar and Morton (1981)]. Recent experimental studies appear to confirm that human olfaction does operate via rate-sensitive detection [Baek, Linforth, Blake, and Taylor (1999)]. The implications of our model include the expectation that more rapid transit of an odorant pulse through the nose should result in a greater perceived intensity, as has been demonstrated in the laboratory by examining the consequences of increasing airflow [Rehn (1978)] or decreasing nasal resistance [Hornung, Chin, Kurtz, Kent, and Mozell (1997)]. For a fixed set of airflow characteristics, our model also predicts a dependence of perceived intensity upon concentration that fits neither of the commonly used 2-parameter mathematical scaling functions -- Stevens's Law and the Beidler Equation -- perfectly, but gives approximately the same goodness of fit to either expression. Despite the substantial differences between those two mathematical functions our expectation has also been recently confirmed [Chastrette, Thomas-Danguin, and Rallet (1998)], although a 3-parameter expression based on the Hill Equation appears to fit the experimental relationship between concentration and perceived intensity better than either of the 2-parameter fits. In any event, perceived intensity is not a simple function of concentration. Studies of insect chemoreception have been interpreted in terms of two different types of detectors -- flux detectors and concentration detectors [Kaissling (1998)] -- but our model accounts for the same results without demanding that sort of dichotomy.

If we cannot quantify stimulus intensity in terms of some well understood physical parameter, still less can we discuss odor quality. From the organic chemist's standpoint the sense of smell remains a mystery. Undoubtedly the flavor and fragrance industry has amassed a huge body of pertinent data, which remains proprietary. But information that stays unavailable does not equate to scientific knowledge.

Patient H.M., an odor-deaf subject

The ability to discriminate different molecules constitutes a criterion for olfaction. Since, as mentioned above, anosmic persons can tell some pairs of odors apart based on nonolfactory cues, an experimenter must choose with care the compounds for study. β -Phenethyl alcohol has an odor that many people find reminiscent of roses, and vapors from dilute solutions are widely accepted as an olfactory stimulus that does not interact with other chemosensory modalities in humans [Betcher and Doty (1998)]. Consider a human subject who can detect β -phenethyl alcohol with the same sensitivity as normosmics and can also detect *n*-butanol (another alcohol often used for testing olfactory sensitivity [Hummel, Sekinger, Wolf, Pauli, and Kobal (1997)], which has an odor very different from that of β -phenethyl alcohol) with normal acuity. Suppose this subject cannot distinguish the two odors. How can an experimenter assess whether the subject exhibits the sense called olfaction?

This question arises in the case of H.M., a patient who underwent experimental brain surgery in the 1950's to alleviate a severe epileptic disorder. Many experimenters have published postsurgical studies of case H.M., since he has lost the ability for declarative learning while retaining the capacity for procedural learning. Tests of H.M.'s sense of smell have documented that he has normal acuity but cannot tell one odor from another [Eichenbaum, Morton, Potter, and Corkin (1983)]. He can describe what he smells in some detail, but the descriptions do not correlate with the stimulus. Descriptions of the same odor vary widely from one presentation to another and show no obvious trend when compared to his descriptions of different odors. When given verbal cues, H.M. can identify pieces of fruit with his hands without looking at them. When given olfactory cues, he selects haphazardly.

H.M. has demonstrated that he can consciously differentiate chemical vapors, but not by means of olfaction. In a randomized series of trials, I presented H.M. with the odor of a dilute solution of β -phenethyl alcohol or of *n*-butanol. I then asked him to choose which of two flasks -- one a "target" and the other a "distractor" -- contained the same odor. One of those flasks contained β -phenethyl alcohol solution and the other *n*-butanol solution. In half of the trials, the target odor solution had the same concentration as the initial presentation, and the distractor was more concentrated. In the other half the distractor solutions had the same concentrations as the initial presentations, and the target odor solutions were more concentrated. Thus we had four possibilities for the presented stimulus and the target: dilute β -phenethyl alcohol with a dilute target; dilute β -phenethyl alcohol with a more concentrated target; dilute *n*-butanol with a dilute target; and dilute *n*-butanol with a more concentrated target. Unscrambling the results of 40 trials I found that H.M.

had scored a perfect 10/10 in matching dilute β -phenethyl alcohol with a dilute target, but that he had performed at random (half right answers and half wrong) for the other three matches. I rationalize his performance as based on the ability to match perceived intensities. Suppose dilute β -phenethyl alcohol represents a "weak" stimulus, but the other three -- more concentrated β -phenethyl alcohol or *n*-butanol at either concentration -- are perceived as equally "strong". Then H.M.'s successes would correspond to his ability to match "weak" with "weak", and his failures represent an inability to distinguish among the "strong".

H.M. clearly understands how to discriminate chemical stimuli. His impairment limits only his recognition of odors, but no other aspect of his sense of smell. He is "odor deaf", by analogy to stroke victims who can read and write and retain an intact sense of hearing but cannot recognize words aurally (and are said to be "word deaf" [Takahashi et al. (1992)]). Based on the evidence discussed so far, H.M.'s behavior does not satisfy the fourth of the guidelines for olfaction.

Could one devise a strategy for differentiating *n*-butanol from β -phenethyl alcohol using H.M. as a detector, without confounding odor quality with odor intensity? An affirmative answer comes from H.M.'s adaptation to odors. When H.M. sniffed *n*-butanol for a period of time, his experimentally measured sensitivity to both odors decreased, but adaptation attenuated his sensitivity to β -phenethyl alcohol much less than his sensitivity to *n*-butanol. Similarly, sniffing β -phenethyl alcohol for a period of time attenuated his sensitivity to that odorant (self adaptation) but did not affect his sensitivity to *n*-butanol significantly (no cross adaptation). The selectivity of his adaptation demonstrates that H.M. indeed uses olfaction to detect odors. Moreover, his adaptation (like that of normosmics) occurs in the CNS, as detailed in the following discussion.

The experiments with H.M. employed a battery of tests. One set of experiments measured his sensitivity by means of a technique derived from signal detection theory [Corbit and Engen (1971)], in which I asked H.M. to sniff 20 presentations of dilute odorant solution randomly interspersed with 20 presentations of odorless blank. The odor was so faint as to make it hard to tell it apart from blank. Figure 1 compares some of the data for H.M. with a male normosmic (P.D.) matched for age and race. After each presentation I asked H.M. whether he could smell an odor. His pattern of responding was the same as that of normosmics: sometimes he gave affirmative responses to blanks (false alarms, symbolized by open symbols in Figure 1), but he did not always respond affirmatively to the dilute sample (correct affirmatives are symbolized by solid symbols in Figure 1).

The circles in Figure 1 symbolize responses to *n*-butanol without adaptation. Using both nostrils H.M. gave 28 correct answers out of 40 presentations. Using just one nostril he gave 26 correct answers. The important datum is not the total number of correct answers, but rather the distribution of false alarms (open symbols) and correct affirmatives (solid symbols), from which can be extracted a measure of his signal-to-noise ratio (d') for olfactory detection. H.M.'s one-nostril d' was virtually the same as his two-nostril (bilateral) d' , in agreement with a recent report that normosmics' bilateral sensitivity is the same as the sensitivity of their more sensitive nostril

Figure 1. Comparison of an odor-deaf patient (H.M.) with an age-matched normosmic (P.D.) in responding to randomized presentations of weak samples of *n*-butanol (*solid symbols*) inter-spersed with blank (*open symbols*). Circles denote unadapted condition; squares denote adaptation to *n*-butanol; triangles represent adaptation to β -phenethyl alcohol (P.D. only).

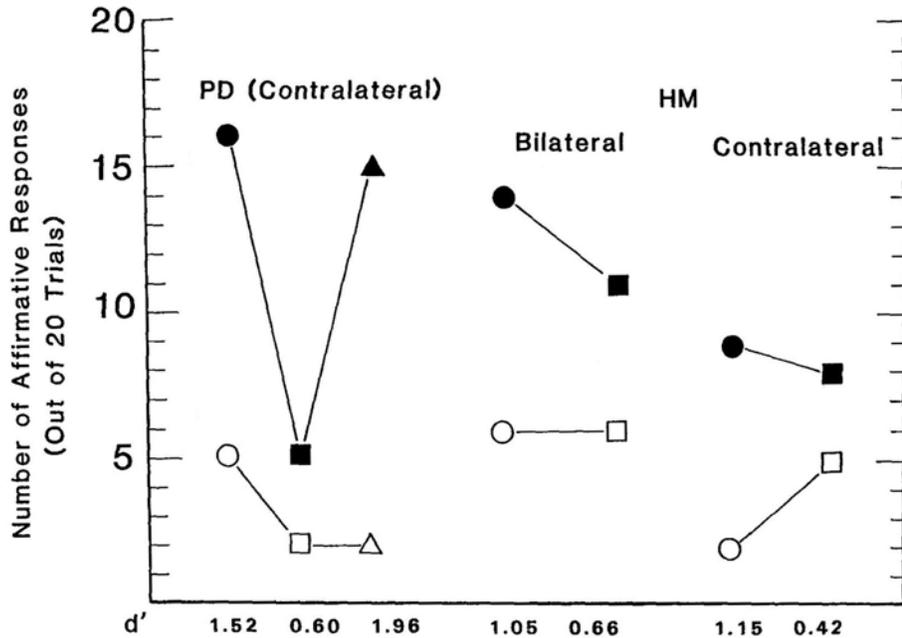
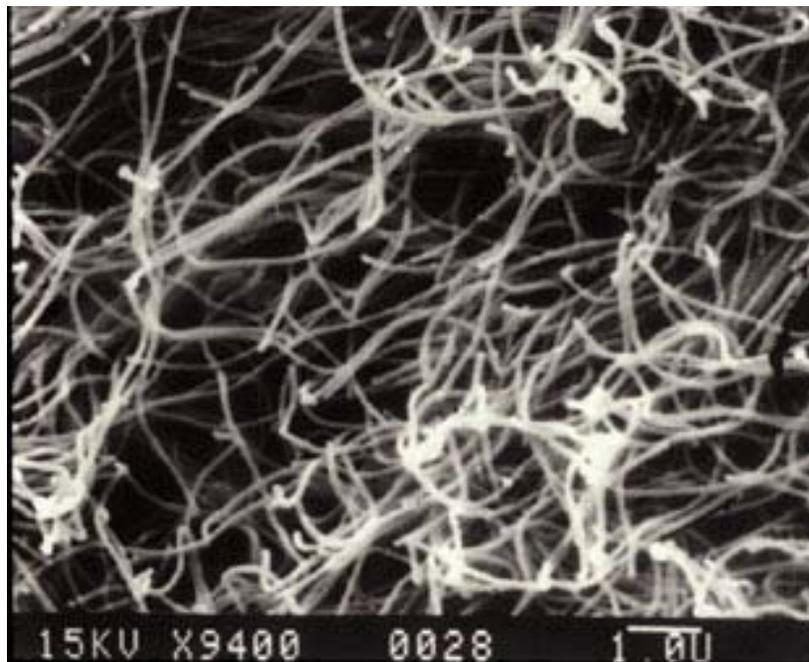


Plate 1. Scanning electron micrograph of the surface of olfactory mucosa of a tiger salamander. Viewed from this aspect, the end of each individual neuron has an area of approximately $1 \mu\text{m}$, but all that can be seen are the cilia ($0.2 \mu\text{m}$ in diameter and approximately $10 \mu\text{m}$ long), of which each neuron has a few projecting from its dendritic knob. The pale line at bottom right indicates the $1 \mu\text{m}$ scale.



[Betcher and Doty (1998)]. H.M.'s sensitivity to *n*-butanol was lower than that of P.D., but the difference between them is well within the normal range of human variation.

When P.D. sniffed a more concentrated solution of *n*-butanol in the opposite nostril, his one-nostril sensitivity decreased (symbolized by squares in Figure 1). But when he subsequently sniffed concentrated β -phenethyl alcohol in the opposite nostril, his sensitivity was slightly greater than in the unadapted condition (symbolized by triangles). Thus, P.D. exhibited contralateral self adaptation to *n*-butanol without any cross adaptation to β -phenethyl alcohol. It was not possible to test H.M. as thoroughly as P.D., but, as Figure 1 summarizes, his contralateral self adaptation to *n*-butanol did not differ significantly from his bilateral self adaptation, and both were essentially the same as P.D.'s contralateral self adaptation.

In human beings a complete septum separates the two nostrils, which isolates them from one another aerodynamically. Therefore contralateral adaptation represents an effect that does not result from saturation of binding sites in olfactory receptor neurons, but must instead correspond to a phenomenon of the brain [Stuiver (1958)]. H.M. and P.D. exhibit self adaptation that occurs in the CNS rather than in the peripheral nervous system.

This result illustrates the difference between a learned response and a conscious one. H.M. learned to respond to *n*-butanol -- had I not asked for his judgment after each presentation, he would not have evinced a consistent behavioral pattern. Had I not told him whether he was right or wrong after each time he responded, he would have answered essentially at random, as I discovered in a separate set of trials. The same is true of normosmic subjects, since the odorant is so highly diluted that reinforcement greatly increases their d' values. However, H.M. lacks the ability to recognize odors. When asked to make conscious choices he confuses odor quality with intensity. He retains the physiological capacity, since his pattern of adaptation cannot be distinguished from that of a normosmic. His peripheral and central nervous systems function well enough that the following tactics could ascertain from his behavior whether an unknown odorant is β -phenethyl alcohol or *n*-butanol: (1) measure d' for the unknown; (2) adapt to *n*-butanol and remeasure d' for the unknown; (3) adapt to β -phenethyl alcohol and remeasure d' for the unknown. This procedure, albeit cumbersome, will identify the unknown odorant as the one for which adaptation caused a more profound decrement of d' . Hence, H.M. does indeed meet the fourth of the guidelines for displaying olfaction.

Since H.M. cannot consciously identify odors or remember them, an experimenter would have difficulty asking him to assess the degree of similarity of two smells. But it is possible to pose that problem to experimental animals. One can train an air-breathing vertebrate to respond to a given odorant and then ask how frequently it gives the same response to a different one. This kind of generalization has been studied [Mason, Johri, and Morton (1987), for example]. Investigations of the olfactory abilities of non-human species also permit a comparison of behavior with the electrical responses of the nervous system. The outcome of such investigations illustrates the limits of current understanding.

Olfaction in non-mammalian vertebrates: behavior *versus* electrophysiology

H.M. has convinced me that he uses olfaction to detect odors, without the necessity of probing the consequences of olfactory nerve transection, an obviously impossible experiment to perform on a human being. To some extent, my conviction arises from introspection: H.M. behaves as I would imagine myself doing, were I unable to recognize or remember odors. Similarly, the belief that many mammals use olfaction derives in part from outward behaviors, such as sniffing, which mimic our own. Anthropocentric interpretation of these behaviors might deceive, however. A rabbit's quivering nostrils could serve to bring nonvolatile stimuli to the VNO rather than volatiles to olfactory receptors.

Interpretation becomes even more complicated for non-mammalian vertebrates, such as amphibians and birds, which do not exhibit stimulus-seeking behaviors that resemble those of human beings. For such species olfactory nerve transection (ONX) provides a pivotal piece of evidence in determining whether an animal uses olfaction to guide its actions. Classical conditioning associates a stimulus with a reinforcement. ONX eliminates the responding, presumably by blocking perception of the stimulus. For instance, tiger salamanders avoid bright light. If presented with an airstream containing an odorant, a salamander will not usually evince any response. If a flash of bright light follows the delivery of odorant, the animal will learn to avoid the odorant. Over a period of about one week a salamander will learn to avoid 8-9 conditioned presentations out of 10 [Mason, Stevens, and Rabin (1980); Mason, Johri, and Morton (1987)]. Animals do not avoid odors that are not followed by reinforcement, and their behavior extinguishes if reinforcement is discontinued. Sham surgery does not affect their responding, nor does ONX abolish their responding to bright light.

A more rapid conditioning paradigm measures galvanic skin response instead of avoidance [Dorries, White, and Kauer (1997)]. Tiger salamanders have been conditioned to respond to *n*-butanol by both methods. Tiger salamanders cannot be conditioned to respond to camphor by either method, despite the fact that their peripheral neurons and olfactory bulbs exhibit pronounced electrical activity in response to this odorant. While camphor might conceivably exert some peculiar anesthetic effect, the disjunction between behavior and electrophysiology implies that experimenters have not yet identified the nerve impulses that code for olfactory information.

ONX produces general anosmia. While no surgical procedure is known that produces selective hyposmia (specific odor blindness), chemical treatments can do so. If presentations of cyclohexanone are followed by reinforcement but presentations of *n*-butanol are not, the salamanders avoid the former but do not respond to the latter. After having been conditioned in this fashion a salamander will avoid cyclopentanone, demonstrating that the animal perceives cyclopentanone as being more like cyclohexanone than like *n*-butanol. Animals can be trained to avoid two different odorants, *e.g.* cyclohexanone and dimethyl disulfide. Lavage of the receptor mucosa with a solution of cyclohexanone temporarily diminishes the frequency with which animals respond to that odorant, but does not affect their responding to dimethyl disulfide. Lavage of the

receptor mucosa with dimethyl disulfide diminishes the frequency with which the animals respond to dimethyl disulfide, but does not affect their responding to cyclohexanone [Mason and Morton (1982)]. These partial impairments represent selective hyposmias, and their duration depends on the concentration of the lavage solution. By contrast, lavage with *n*-butanol has no significant effect on responding to either cyclohexanone or to dimethyl disulfide. Finally, ONX drastically reduces responding to both odorants.

Lavage produces a temporary impairment in animals analogous to adaptation in humans. One can run experiments on animals, though, that cannot be done on human subjects. Lavage with dilute (1 mM) ethyl acetoacetate does not affect tiger salamanders' responding, nor does lavage with more concentrated (50 mM) sodium cyanoborohydride. Performed sequentially, however, those two lavages produce the same selective hyposmia as does lavage with cyclohexanone, and they also impair the animals' generalization to cyclopentanone. This result is congruent to the chemical specificity of that sequence of reagents [Mason, Clark, and Morton (1984); Mason, Leong, Plaxco, and Morton (1985)], even though salamanders do not appear to be able to smell ethyl acetoacetate. However, electrophysiology shows no differential alteration in the electrical responses of the receptor neurons or of single neurons in the olfactory bulb as a consequence of lavages. The electrical responses of the receptor mucosae of immobilized animals to airborne cyclohexanone [*cf.* Arzt, Silver, Mason, and Clark (1986)] were found to be significantly reduced by lavage for two days after treatment, as were the electrical responses to dimethyl disulfide, but after three days both had recovered completely [Schafer and Winegar (1988)]. Since the selective behavioral impairment lasted nearly a week, no correlation could be established between behavior and electrical activity. Similarly, a total of 52 individual neurons in the olfactory bulb were examined over a period of 6 days. Before treatment, 60% of the cells responded to dimethyl disulfide only, none to cyclohexanone only, 20% to both odorants, and 20% to neither odorant. Six days after treatment, only 10-20% of the cells responded to dimethyl disulfide only, 20-30% responded to both odorants, and a few percent responded to cyclohexanone only.

Monitoring the electrical activity of the nervous system does not appear to give results that accord with the behavior of conditioned animals. On the one hand, electrical responses are observed for volatile chemical stimuli that tiger salamanders apparently do not smell. On the other hand, no selective attenuation of their electrical responses takes place under conditions that reproducibly impair their behavioral responding to one odor but not to another.

Olfactory Coding: Still a Mystery

If I can ascertain what another organism detects via olfaction, then I can perform experiments upon it, which cannot be performed on human subjects. The objective of such experiments -- to find out how odor is coded -- has yet to be achieved. Suppose the olfactory code were unraveled. Reproducing an odor would become a matter of replicating the pattern of neural responses without having to duplicate the chemical stimulus (much as cinematography appears to

reproduce color without necessarily matching the complete spectroscopic profile of the original scene [Robertson (1992)].

Can a method for archiving an odor -- so that it can be passed on to posterity with fidelity -- be developed without such knowledge? Arguably the answer is yes, though discovering the molecular mechanism of coding would simplify that task considerably.

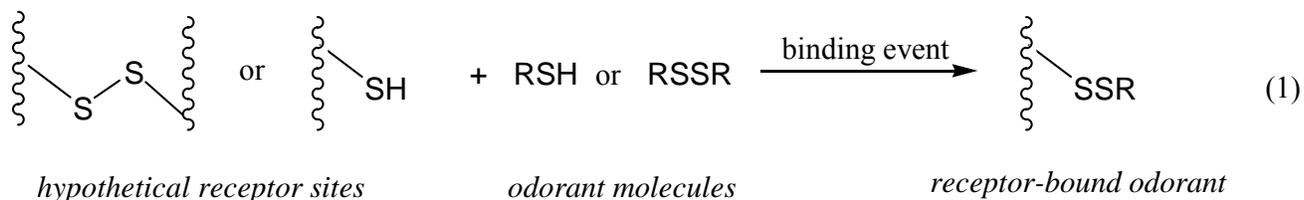
If the odors of specific objects translate into unitary percepts, which constitute the basic entities in linguistic descriptions of olfaction, then the question follows as to whether these unitary percepts take shape at the level of the receptor neurons or in the olfactory bulb or elsewhere in the brain. That question remains unanswered, as of this writing. Since the sense of smell does not correlate perfectly with externally monitored patterns of electrical response from the receptor neurons or the olfactory bulb, the nature of olfactory coding remains unknown. Outside the laboratory unitary percepts rarely equate to pure compounds. Two vocabularies coexist, one of smells (which varies from individual to individual and which refers to other inputs besides olfaction) and the other of chemical structures.

Theories abound, which relate those two vocabularies, but no one of them has emerged as predominant. Many of the theories suppose the existence of specific receptor sites on the surface of the receptor neurons. One hypothesis posits a set of odors of specific objects (*e.g.* camphor, sperm, urine, fish) that correspond to pure compounds and represent fundamental submodalities [Beets (1982)]. Another (based on molecular biology) proposes dozens -- perhaps hundreds -- of different types of cell surface receptor proteins, each of which is tuned to a specific odorant compound or class of compounds [Buck (1996); Zhao *et al.* (1998)].

While the molecular mechanisms of olfaction remain unknown at present, two sets of assumptions seem likely to be true if olfactory receptor proteins do indeed exist. First, olfactory receptor sites ought to bind odorant molecules quite loosely; that is, with dissociation constants $K_d \geq 10^{-7}M$ with reference to the odorant concentration in the surrounding mucus. If this be not true, then receptors should become easily saturated. Furthermore the on- and off-rates for binding must be fast, leading to what has been termed "fast and loose" binding [Mason and Morton (1984)]. Secondly, high sensitivity does not require tight binding. As originally noted for rate-sensitive detectors [Paton (1961)], a given type of receptor site can exhibit useful responses for a wide domain of concentrations below its dissociation constant.

These speculations present a meager collection of precepts about what the nose should do. No less meager is the list of what the nose cannot do. Olfaction cannot rely entirely on emission or absorption of electromagnetic radiation by isolated odorant molecules, since some (but not all) optical isomers have different odors [Friedman and Miller (1971); Laska and Teubner (1999)]. Nor can the characteristic stench of organosulfur compounds depend on the reaction depicted in equation 1, since lavage of the olfactory mucosa of experimental animals with iodoacetamide or with methylmercury hydroxide followed by iodoacetamide (which would irreversibly modify the

hypothesized receptor site) does not affect their responding to dimethyl disulfide [Mason, Clark, and Morton (1987)].



The paucity of acknowledged impossibilities hampers understanding of how chemical structure translates into a sequence of nerve impulses. Not until experiment falsifies many more plausible suggestions can a coherent theory of olfactory coding take shape.

Reconstructing Odors

In perfumery a mixture that smells different from its components is an example of an "accord" [Calkin and Jellinek (1994)]. In this respect olfaction exhibits "synthetic processing", just as color vision cannot distinguish a mixture of red and green light from yellow light [Robertson (1992)]. In another respect olfaction exhibits "analytic processing": human subjects can name individual unitary percepts in a mixture of object odors, even though each object odor is a complex mixture of pure compounds [Livermore and Laing (1998)]. Not surprisingly, chemists have chosen to develop instrumentation whose output lends itself exclusively to analytic processing.

A spectrometer and an organ of perception both function in the same general way: a detector converts an input into an electrical signal, which is then processed. Despite this similarity between contemporary scientific apparatus and the nose, one cannot easily draw further analogies between them. Mass spectrometry (MS) illustrates a technique whose sensitivity rivals that of human olfaction. When combined with gas chromatography (GC), GC/MS is currently the method of choice for analyzing unknown mixtures of volatile compounds. Curiously, no set of first principles exists for predicting the fragmentation pattern that a new compound will give in its MS. In that sense, GC/MS resembles the sense of smell: the instrument conveys a large amount of information, which chemical understanding cannot yet fully comprehend. However, GC retention times and MS fragmentation patterns can be archived on a computer and reproduced at any time in the future. This cannot yet be accomplished for odors.

For archiving odors no instrumental or electronic method presently matches the ability of a trained perfumer. An accord depends upon the proportions of its components and is highly sensitive to the presence of minor components. GC/MS analyzes common flavors and fragrances, each of which contains hundreds of chemically distinct constituents, but does not necessarily record sufficient information for an odor to be reconstructed from pure compounds. A component that

appears by GC/MS to represent a negligible fraction of a mixture might nevertheless play an important role in its odor.

The perfumer's art passes from generation to generation via transmission from master to apprentice. But how is one to know whether a scent (particularly one from a natural source) has changed over the span of years? One point of view holds that archiving an odor simply requires more information, such as GC/MS might obtain, with ever greater resolution in separating the constituents and ever greater sensitivity in characterizing the trace components. This approach is consistent with the notion that each of the 10^7 - 10^8 receptor neurons transmits its own pattern of response to a chemical stimulus, a "holistic code" [Lettvin and Gesteland (1965)] in which each receptor cell responds to most (if not all) chemicals. That means that the nose sends a huge volume of information to the brain, a large fraction of which may prove redundant. Since there is no way of recognizing *a priori* the redundant part, even the most thorough GC/MS analysis might not contain any more information than the nose actually transmits to the olfactory bulb. In other words, from this point of view solving the problem of archiving odors resides in pursuing chemical analysis to an increasing degree of refinement. The two methods of profiling an odor -- holistic coding postulated for the nervous system *versus* separation and analysis of the constituents by a chemist -- acquire very different kinds of information in very different ways, but approach the same limit.

An alternative viewpoint holds that the key to archiving an odor involves simulating the way the olfactory bulb handles inputs, rather than the brute force method outlined above. Developing a biomimetic "artificial nose" [Dickinson, White, Kauer, and Walt (1998)] therefore hinges upon finding a suitable processing algorithm. The artificial detectors themselves can have "imperfect selectivity", just as do the receptor neurons in the holistic coding model [Hirschfeld (1986)]. After connecting an array of such detectors in parallel to an appropriate computer, the system "learns" to recognize mixtures. The Hirschfeld model does not require any knowledge of chemical structure nor even any understanding of how chemicals interact with the detectors. Current investigations of "artificial noses" based on this model explore the time course of the array's response as an additional source of information, but it remains to be seen if an array with $\leq 10,000$ detectors (the number of glomeruli in the olfactory bulb, the junction boxes that receive the inputs from receptor neurons) can discriminate odor intensity as well as odor quality without confusing the two. If so, the outputs of a collection of Hirschfeld detectors could be stored in a computer. While such a database would not tell how to reconstruct an odor, it would provide a comparison standard for evaluating the similarity of one smell to another.

How do odors travel?

What does the cartoon in Figure 2 depict? The cartoon in Figure 3?

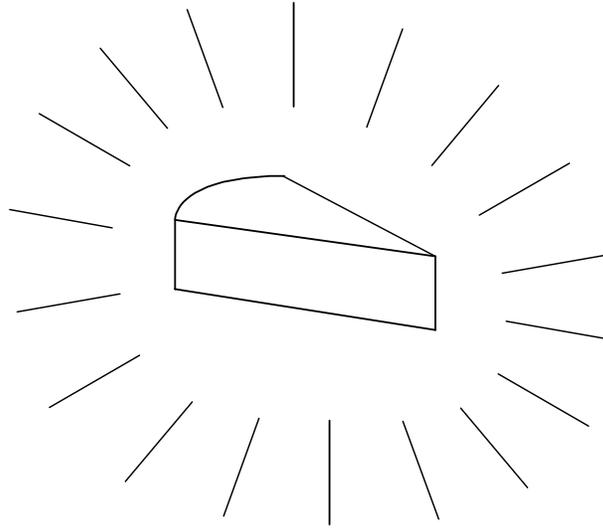


Figure 2. What does this depict?

I drew the cartoon in Figure 3 on my computer and asked my teenaged daughter what it looked like. She immediately replied, "Stinky cheese." Then I asked her what Figure 2 represented. "A shining cheese." When I showed Figure 2 to a group of students none of them thought of an odor emanating from the central wedge, and most of them thought of something luminous -- one student called it a picture of "golden cake". Then I asked them Figure 3 depicted, and none thought of luminosity. About half gave answers that related to smell: *e.g.* "aroma wafting from a piece of pie".

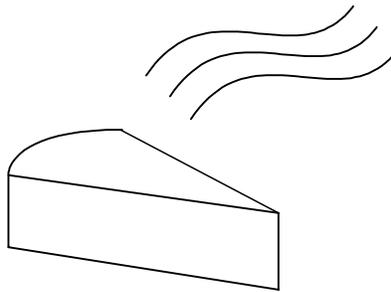


Figure 3. What does this depict?

Straight lines radiate from the center of Figure 2 uniformly in all directions -- isotropically, as a physicist might say. This universally symbolizes rays of light. By contrast, many viewers interpret the wavy lines in Figure 3 as symbolizing odor. The picture of an odor shows it drifting in a variegated, nonuniform pattern -- aeolotropically, to borrow a term from 19th century physics.

The difference between Figures 2 and 3 represents the distinction between light, which radiates from its source, and vapors, which are transported via convection. If molecular motions alone conveyed odor it would diffuse isotropically, but so slowly that would take an hour to travel a few centimeters. Instead, odors are carried as plumes in currents of air. How could it happen that, despite the invisibility of these plumes, they are recognizable as symbolic of odors? Could it be that olfaction senses the heterogeneity of the stimulus, as it is ordinarily presented? And that, as a consequence, we comprehend odors as travelling by convection -- like smoke or waves of heat -- rather than by passive diffusion?

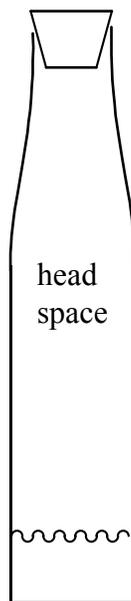
Animals that are celebrated for their keen sense of smell -- dogs and rats, for example -- have whiskers that exhibit astonishing sensitivity to slight displacements. To these species the structure of air currents is not invisible. A considerable portion of their brains has evolved to process information from their whiskers (or vibrissae), and one may plausibly suggest that their olfactory sensitivity derives, at least in part, from the ability to monitor the structure of air and to situate a scent within the currents that eddy about their snouts [Cain, Mason, and Morton (1985)]. If, even in the absence of vibrissae, human olfaction can nevertheless sense the heterogeneity of odors, this adds another complexity to our experience of smell. As argued above, diffusion through mucus limits the temporal resolution of olfaction to about 0.1 second. If humans have the capacity to sense whether a scent is well mixed with air or arrives in a plume, that ability would have to depend on fluctuations that take place on such a timescale.

Catullus's nose

The opening paragraphs of this chapter imagined that the gods of love might itemize the ingredients of the aroma that Catullus praised. Would it prove adequate for them to give a head space analysis (that is, the chemical composition of the vapors in equilibrium with the odor source, as drawn schematically in Figure 4 for a liquid in a bottle)? Could Catullus's nose apprehend any difference between a sample of air that replicated the head space and the fragrance that so pleased him?

If human olfaction can discern the filamentous character of scent plumes in air, then a headspace analysis, no matter how complete, might not suffice to reconstruct the fragrance. The distribution of molecules would play a role, as would the rate with which they are replenished after they have been depleted by sniffing. If, on the other hand, olfaction (independent of other sensory inputs) cannot differentiate a heterogeneous stimulus from one that has been well mixed with air, then a complete chemical analysis could serve to archive odors.

Figure 4. Head space refers to the vapors in equilibrium with the other contents of the vessel.



Let me relate a piece of anecdotal evidence that suggests the dependence of olfaction upon the way that odors arrive at the nose. One of my students had been a subject in numerous signal detection experiments using weak solutions of β -phenethyl alcohol in water as a stimulus, in which she was inhaling its vapors in a continuous sniff of about 1-2 seconds duration. She consistently averred that the solution did not possess any floral odor whatsoever. Then one afternoon she happened to walk by an open bottle containing a somewhat more concentrated solution and unexpectedly caught a whiff of it. Suddenly she perceived the smell of roses. Since she was about a meter away from the open bottle it is hard to believe that more molecules entered her nose during that breath than she had been inhaling during any of her previous exposures to β -phenethyl alcohol. Was her revelation due to a difference in how her nose transmitted information to her brain? Could odor quality depend on whether you take a sniff or catch a whiff?

The sense of smell often elicits a sense of epiphany that other senses do only rarely [Laird (1935)]. Does this depend on whether you smell a scent plume or an odorant well mixed with air? Experiment can address that issue. Were we to probe the olfactory abilities of rats or dogs temporarily deprived of their vibrissae (difficult experiments, which have not, to the author's knowledge, been reported), considerable insight would be gained. An experimenter can design detection tasks under more rigorously controlled conditions for animals than for humans, so as to rule out alternative sensory inputs. However, people give a much more detailed report of their perceptions. Continued interplay between studies of humans and of animals stands at the center of piecing together a molecular picture of chemoreception.

Chemical understanding interprets phenomena in terms of the positions and movements of atoms and the forces acting upon them. The Oxford English Dictionary defines phenomenon as

"That of which the senses or mind directly takes note; an immediate object of perception." This chapter has argued that behavioral performance permits an assessment of what another creature can detect by means of olfaction alone, thus extending the notion of "senses or mind" beyond the realm of the human. The belief that all terrestrial vertebrates share similar olfactory mechanisms implies that these will ultimately prove susceptible to chemical understanding. The confirmation of such understanding will be the ability to archive odors.

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