45 Roelofs, W.L., and Comeau, A., Sex pheromone perception: synergists and inhibitors for the red-banded leaf roller attractant. J. Insect Physiol. *17* (1971) 435–448.
46 Roelofs, W.L., Glass, E.H., Tette, J., and Comeau, A., Sex pheromone trapping for red-banded leaf roller control: theoretical and actual. J. econ. Ent. *63* (1970) 1162–1167.
47 Roelofs, W., Hill, A., and Cardé, R., Sex pheromone components of the red-banded leaf roller, *Argyrotaenia velutinana* (Lepidoptera: Tortricidae). J. chem. Ecol. *1* (1975) 83–89.
48 Schneider, D., Electrophysiological investigations of insect olfaction, in: Olfaction and Taste I. pp. 85–103. Ed. Y. Zotterman. Pergamon Press, Oxford 1963.
49 Steinbrecht, R.A., and Gnatzy, W., Pheromone receptors in *Bombyx mori* and *Antheraea pernyi*. I. Reconstruction of the cellular organization of the sensilla trichodea. Cell Tissue Res. *235* (1984) 25–34.
50 Vetter, R.S., and Baker, T.C., Behavioral responses of male *Heliothis virescens* in a sustained-flight tunnel to combinations of seven compounds identified from female sex pheromone glands. J. chem. Ecol. *9* (1983) 747–759.
51 Wilson, E.O., Introduction, in: Communication by Chemical Signals. pp. 1–3. Eds J.W. Johnston Jr, D.G. Moulton and A. Turk. Appleton-Century-Crofts, New York 1970.

0014-4754/86/030232-10$1.50 + 0.20/0

Bacterial chemotaxis and vertebrate olfaction

by S. J. Kleene

University of Cincinnati Medical Center, Anatomy and Cell Biology, ML 521, Cincinnati (Ohio 45267, USA)

Key words. Olfaction; bacterial chemotaxis; stimulus; receptors; transduction; response assay.

Our understanding of the biochemistry of vertebrate olfaction is very limited. Since so many biochemical systems have been conserved across evolution, we might hope to learn something about olfaction by examining the chemical recognition systems of less advanced organisms. Bacterial chemotaxis is one such system that is understood in some detail.

It is my intention here to assess bacterial chemotaxis and vertebrate olfaction from as common a perspective as is possible. The discussion is restricted to those properties where comparisons between the two systems can be made. Chemoreception and initial events in stimulus transduction offer the greatest possibilities in this regard. In a sense I will be trying to compare single-celled bacteria with single olfactory receptor neurons. Olfaction could also be related to chemotaxis in slime molds, protozoans, various invertebrates, leukocytes, and so forth, but I have chosen to make a detailed comparison with the bacterial system.

Even though the discussion of chemotaxis has been confined to two species of bacteria, it is far from complete. There exist many more comprehensive reviews[5,61,62,74,75,84,124]. For other reviews of vertebrate olfactory reception, see Lancet[80] or Cagan and Kare[24].

Bacterial chemotaxis

1. The organisms

The most enlightening studies of bacterial chemotaxis have been done with two closely related species of enteric bacteria, *Escherichia coli* and *Salmonella typhimurium*. The single cell of either organism is rod-shaped, about 1 μm in diameter and 1–2 μm long. From each cell protrude 6–8 flagella, each of 5–10 μm length. Certain properties of these organisms have contributed greatly to their successful study. Generation times in liquid culture are commonly 0.3–2 h, with 2 ml of growth medium yielding 10^9 cells. Results from such cultures may be treated as averages of enormous numbers of identical single organisms. Highly developed methods for selecting mutants and transferring their genetic defects have resulted in collections of strains with widely varying chemotactic defects. The experimenter can manipulate the extracellular environment at will by centrifuging the bacteria and resuspending them in a new medium. The cells are not fragile, and the rights of experimental bacteria are rarely called into question.

The involvement of transmembrane potential is difficult to study in such small cells. Pharmacologically produced giant *E. coli* cells can be penetrated with intracellular microelectrodes[40], but this method has not yet been applied to the study of chemotaxis.

2. Assays of response

Microscopy has enabled description of the behavioral responses of single bacteria to chemical stimuli. In an unchanging environment, the cell alternates at random between two swimming states[18,85]. In one state, the several flagella, rotating counterclockwise[81], work together as a bundle to propel the cell smoothly ahead[16,17,121]. In the other state, the flagella rotate clockwise[81]. Due to the helical sense of the flagella, this causes the bundle to fly apart[86,87], and the cell 'tumbles' in place. When smooth swimming resumes, it will be in a new, randomly chosen direction[18]. The cell is able to migrate toward or away from the source of a chemical by modulating the proportion of time it spends in each state. If an episode of smooth swimming is bringing the cell closer to the source of an attractant, smooth swimming is prolonged (i.e. tumbling is suppressed)[18,85]. If the cell is going away from the attractant, it will soon tumble[85] and try swimming in a new direction. The responses to attractants and repellents are opposite, and so the cell can also migrate away from a repellent source[134]. It is able to integrate simultaneous stimuli[134,135], although the response is not always the exact

sum of the responses to the stimuli presented individually[114]. The chemotactic response is 'all-or-none' rather than graded. The cell spends virtually all of its time in one of two extreme states, smooth swimming (counterclockwise flagellar rotation) or tumbling (clockwise rotation).

Several ways exist to quantitate this response to chemical stimulation. For a single cell, one can monitor the direction of flagellar rotation[19,81] or the three-dimensional swimming pattern[15,83,125]. Chemotaxis en masse can be measured by counting the bacteria that enter a capillary containing an attractant or leave one containing a repellent[4]. Rings of bacteria migrating chemotactically across an agar surface can be formed under certain conditions[2].

3. *Stimulus substances*

Most of the chemicals which attract *E. coli* and *S. typhimurium* are freely water-soluble compounds ranging in molecular weight from 75 (glycine) to around 360 (disaccharides). Using the capillary assay method[4], experimenters have determined threshold concentrations for chemotactic responses. Among the most effective attractants, given with threshold molarities for detection of the chemotactic response in *E. coli*[7,93], are L-aspartate (6×10^{-8} M), L-serine (3×10^{-7} M), D-galactose (1×10^{-6} M), D-glucose (3×10^{-6} M), and maltose (3×10^{-6} M). These are all metabolizable by the bacteria, but nutritive value is neither necessary nor sufficient for attractiveness[3]. Oxygen is also an attractant[2].

Structural modifications usually decrease the attractiveness of stimulus compounds. Thus α-aminoisobutyrate (a serine analogue) and 2-deoxy-D-glucose (a glucose analogue) are attractants, but have higher threshold molarities (2×10^{-5} M and 10^{-4} M, respectively)[7,93]. Stereoisomers of good attractants are usually less effective: L-glucose (10^{-3} M) and L-galactose (10^{-2} M) are very weak attractants[7,93].

Repellents[135] include aliphatic fatty acids and alcohols, some hydrophobic amino acids, indole, skatole, benzoate, Co^{2+}, and Ni^{2+}. Threshold molarities range from 10^{-6} M for indole and skatole to 10^{-1} M for methanol[135]. pH below 6.5 or above 7.5 also repels[135]. Although many repellents are toxic, toxicity is neither necessary nor sufficient for a repellent effect[135].

The chemotactic effects of substances not found in the natural bacterial environment have seldom been studied, with the exceptions of the structural analogues already mentioned. Some ionophores and uncouplers or inhibitors of electron transport mimic attractants or repellents[61]. Recently the sweet-tasting protein 1-acetylthaumatin was found to be an attractant for *E. coli*[12].

4. *Definition of stimulus*

The presence of a stimulus substance is not sufficient to elicit a chemotactic response. A bacterium modulates its swimming only in response to a *change* in the concentration of a chemoeffector[85,134]. In particular, it has been shown that the cell responds to temporal changes in attractant or repellent concentration[23,85]. When an attractant is enzymatically generated in a spatially uniform manner in a suspension of bacteria, the cells swim smoothly for prolonged periods[23]. Very rapid mixing of an attractant into the cell suspension produces the same result[85]. In nature, the bacteria experience temporal concentration gradients as they swim through spatial gradients formed by diffusion from chemical sources.

Immediately after the presentation of such a temporal stimulus, the bacteria show an altered swimming pattern[23,85]. Increasing attractant or decreasing repellent concentration causes prolonged smooth swimming for as long as several minutes. For a given stimulus substance, the duration of the response is proportional to the magnitude of the concentration change[19,85,125]. Decreasing attractant or increasing repellent causes tumbling; this response is typically shorter than the smooth swimming response[19,85]. After the initial response, the cell behavior adapts: despite the continued presence of the altered environment, the bacteria resume random alternation between the two swimming states[19,85,125].

5. *Stimulus receptors*

Three types of studies[3,5] have been used to classify the receptors involved in bacterial chemotaxis: taxis mutants, competition between stimuli, and receptor inducibility.

The chemotaxis assays described above can be used to recognize specific taxis mutants. For example, mutants defective in L-aspartate taxis remain at the point of inoculation on an aspartate-agar plate, while wild-type cells chemotactically migrate away as they consume the aspartate[93]. Such mutants are found to be defective in taxis not only to aspartate, but also to glutamate[93], suggesting that the two amino acids share a chemoreceptor.

The capillary assay method[4] can be used to study competition between two chemical stimuli. For example, bacteria can be suspended in a solution containing aspartate, to which they soon adapt. In this condition, they are unable to detect a spatial gradient of glutamate issuing from a capillary, since the common chemoreceptor is already saturated by bound aspartate[93]. Spatial gradients of glycine are still detected, and so it is concluded that glycine binds to a different receptor.

Finally, taxes to some attractants are inducible[3,7]. If bacteria are grown on D-fructose, for example, their response to fructose is elevated 9-fold[7]. This increase is due to increased synthesis of a fructose chemoreceptor.

Several bacterial chemoreceptor proteins have been identified. Glucose[60], galactose[60], ribose[9], and maltose[59] bind to soluble proteins found in the space between the outer and inner membranes of the cell[9,59,60]. Dissociation constants are $\sim 10^{-6}$ M. Other sugars apparently use the enzymes II of the phosphotransferase system as chemoreceptors[6]; each of these is a sugar-specific, membrane-associated protein. All of the above-mentioned attractants are nutrients for wild-type bacteria, and the binding proteins are essential both for chemotaxis and for transport of the sugar into the cell. Recently two proteins, Tsr and Tar, have been identified as the chemoreceptors for serine and aspartate, respectively[29,63,140]. Tsr and Tar are not involved in transport processes. They do, however, also serve as stimulus transducers and are discussed further below. No repellent-specific receptors have been found.

6. Stimulus transduction

A good deal is known about the biochemical mechanism of chemotactic stimulus transduction. Particularly well-studied are the methylation and demethylation of a set of integral cytoplasmic membrane proteins called 'transducers'[124]. These proteins, individually named Tsr, Tar, and Trg (or MCP I, II, and III, respectively), are methylated to a constant level in an unchanging environment[73]. Addition of an attractant causes an increase in the methylation level; this new level is maintained as long as the attractant concentration is stable[51,73]. Removal of a repellent has the same effect[124]. Addition of a repellent or removal of an attractant[51,73] causes a rapid demethylation to a lower level which is maintained until the next change in environment. The time for the new methylation level to be reached correlates well with the duration of the transient swimming response[51]. Decreases in methylation level happen much faster than increases[51], just as swimming responses to repellents are much briefer than those to attractants[19,85].

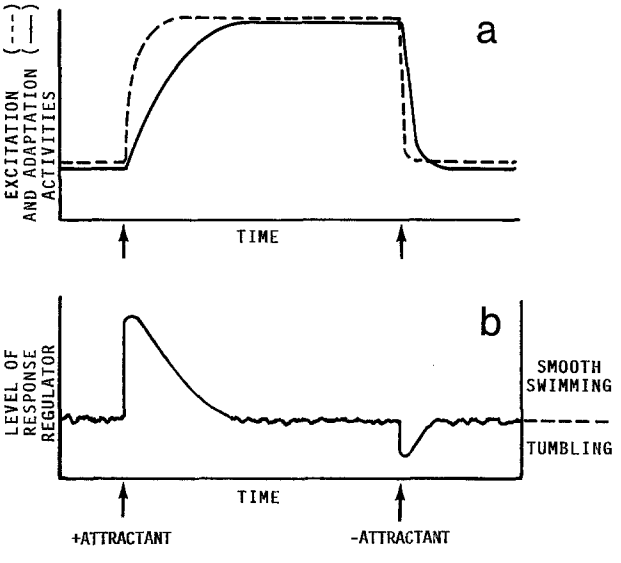
Integration of sensory input begins at the level of transducer methylation[122,123]. The methylation of each of the three transducers can be influenced by more than one stimulus substance[51,72,123]. Increased methylation of the Tar protein, for example, can result from binding of aspartate to Tar itself[29,63,140] or from binding of a maltose-maltose binding protein complex to Tar[71,113]. Neither of these interactions has much effect on methylation of the Tsr or Trg transducers.

There is strong evidence that the effects of some chemical stimuli are not mediated by stimulus-specific receptors[68,111]. Lipophilic weak acids (e.g. acetate, salicylate) are repellents[134,135], while lipophilic weak bases (e.g. ammonia, methylamine) are attractants[93]. The Tsr transducer and relatively high concentrations of the stimulus compounds are required to elicit these chemotactic responses[135]. Apparently the lipophilic bases and acids cause chemotactic responses by increasing and decreasing, respectively, the intracellular pH[68,111]. Thus the Tsr transducer can be influenced not only by specific stimulus-receptor binding, but also by changes in intracellular pH.

It is worth noting here the pitfalls involved in classifying chemoreceptors even in the relatively simple bacterial system. Both competition between stimuli and mutant phenotypes may reflect effects at the receptor level, the transducer level, or both. Thus a mutant now known to lack the Tar transducer was at first thought to be missing a simple aspartate-specific receptor. Later the mutant was found to lack maltose taxis as well[110]. Mutants lacking maltose taxis but not aspartate taxis were found; these lack a maltose-specific chemoreceptor. As another example, galactose can block taxis to ribose, not by competing for a shared chemoreceptor, but by saturating the methylation of the shared Trg transducer[126]. Study of the purified galactose and ribose chemoreceptors enabled this deduction; the stimulus competition experiments alone were misleading. Finally, it should always be kept in mind that the effects of some stimuli, such as the lipophilic acids and bases, may not be mediated by specific receptors at all.

One model of excitation and adaptation in bacterial chemotaxis[74,124] is shown in the figure. Fig., *a* depicts the levels of an excitation process (dashed curve) and an adaptation process (solid curve) during addition and removal of an attractant. (This could just as well represent removal and then addition of a repellent). The curve in fig., *b* shows the level of a 'response regulator'; this level is determined by the ratio of the two activities of fig., *a*. In the initial adapted state (i.e. absence of environmental changes), each activity of fig., *a* is constant. Thus the ratio represented by fig., *b* is also nearly constant, although it is shown with some random fluctuations. During this time, the cells randomly alternate between smooth swimming and tumbling as the level of response regulator fluctuates around a threshold value. When an attractant is added (fig., first arrow), the two activities of fig., *a* each increase, but at different rates. The ratio of these activities (response regulator, fig., *b*) rises ('excitation'), and the cells exhibit only smooth swimming. As the two processes of fig., *a* approach a common level again, their ratio returns downward ('adaptation'). Despite the continued presence of attractant, a new adapted state is reached, and the cells alternate swimming modes once again. Removal of attractant (fig., second arrow) similarly results in a transient excitation of opposite polarity, causing the cells to tumble. Altered swimming behavior lasts only while the two activities are out of balance; as soon as the slower process catches up, the cell's behavior adapts.

Several questions remain unanswered. The process represented by the dashed curve in fig., *a* has not yet been identified. There is some evidence[20] that the intracellular level of cyclic GMP or some similar compound fits this part of the model. The solid curve of fig., *a* correlates with the methylation of any one of the three transducer proteins, but how their combined information is finally integrated into a single cellular response is not yet known. Likewise no one knows how the identified chemical processes can physically influence the direction of flagellar rotation. The intervening signal could be a change in transmembrane potential. Addition of either attractants



Model of excitation and adaptation in bacterial chemotaxis, redrawn from Springer et al.[124] with permission. See text for details.

or repellents produces a transient hyperpolarization of the cell membrane[38,130]. The effect is absent in the non-chemotactic mutants tested.

Vertebrate olfaction

1. The organisms

As subjects for experimental study, vertebrates are less accommodating than bacteria. The possibility of averaging results from 10^9 organisms is nonexistent, and well-characterized mutants are scarce. Because the olfactory receptor cells are part of an epithelium, the experimenter has limited ability to manipulate the extracellular environment.

Heterogeneity is a major experimental variable at several levels. Genetic differences among animals are usually extensive. Within the olfactory epithelium of a single animal are several cell types, and efficient methods for segregating the cells by type are not yet known. Even the receptor neurons of a given epithelium are a heterogeneous population. These cells are constantly turning over[53–55,96], and the cell properties vary with maturity.

Studies of stimulus reception naturally focus on those parts of the olfactory receptor neurons in close contact with the nasal airspace. (See Graziadei[52] for a review of olfactory morphology.) Each neuron sends to the surface of the epithelium a dendritic process with a knob-shaped ending. In most vertebrates, cilia protrude from each knob into the mucus. These cilia are composed of microtubules enclosed in an extension of the cell's plasma membrane[82]. Evidence discussed below indicates that these cilia bear chemoreceptors. Olfactory cilia are not to be confused with bacterial flagella. The latter are helical protein homopolymers which are not membrane-enclosed and do not bear chemoreceptors[120]. The flagella propel the bacterium by rotating like propellers, rather than by beating[17,121].

2. Assays of response

2a. Human perception. Our own perception is a response commonly used to investigate olfaction. Early workers often put odorants into classes such as 'spicy' and 'floral' based on human perception. To conclude that 'spicy' and 'floral' odors have distinct molecular receptors, though, is unwarranted. It may be that one odorant diffuses across the surface of the olfactory epithelium at a different rate from the other; a different spatiotemporal pattern of receptor cell activity may then result in a different perception[8,98,99]. An odorant could interact with several classes of receptor molecules in such a way that the relative occupancy of each class within its useful concentration range determines the perceived odor quality. Indole, for example, is described as 'jasmine-like' at low concentrations but 'fecal' at higher levels[94]. The presence of trigeminal nerve endings in the olfactory cavity also complicates human perception. Perceived odor qualities are often combinations of olfactory and trigeminal sensations[137]. Thus inferences about receptor mechanisms based on human perception must be made with extreme caution.

2b. The electro-olfactogram. The initial effects of olfactory stimuli at the receptor level are reflected by the electro-olfactogram (EOG)[104,105]. This is a negative potential measured in the sensory region of the olfactory epithelium in response to a presentation of odorant-containing air. The EOG has the properties expected of a summated receptor potential[104,105]: it originates in the apices of the receptor neurons, its amplitude increases with stimulation level, and it correlates with (but does not depend on) neuronal impulse activity. Since the EOG is the summated output of many receptor cells, inferences cannot be made about single neurons. Odorant-induced surface potentials having different polarity or temporal patterns than the negative-going EOG have also been observed (see Gesteland[43]), but their origins are less clear. The EOG may partly arise from cells which do not transmit information to the brain. In the rat embryo, the EOG develops before synapses between receptor neurons and the olfactory bulb are detectable[45]. In adult frog[53–55] and rat[96], the population of receptor cells continually turns over. There may be immature neurons in adult animals that contribute to the EOG before forming synaptic connections with the brain.

2c. Action potentials. The pattern of impulses of a receptor neuron is its ultimate output, just as the pattern of flagellar rotation is the output of a bacterium. The impulse response of a single neuron can be measured by penetrating the epithelium with a microelectrode[13,44,48,88,102,119,133]. The pattern of impulses may then be followed as a function of stimulus presentation. To date, this response provides the only measure of olfactory response at the single-cell level. It is not a simple 'all-or-none' response. Stimulation may cause increased frequency of action potentials, decreased frequency, or sequential patterns involving both of these.

It is also possible to measure the summated action potentials of many receptor neurons by recording from the olfactory nerve or from small strands of the nerve[97,136].

3. Stimulus substances

As identified by human perception, there is an enormous number of olfactory stimulus substances. They range in molecular weight from 17 (ammonia, also a trigeminal stimulus) to around 290 (odorous steroids). The odorants normally encountered by terrestrial vertebrates must be sufficiently volatile that at least a few molecules[35] can contact the nasal mucosa. Fish olfactory organs, on the other hand, are normally bathed in solutions and are sensitive to amino acids[58,127,129] and bile salts[37] in solution. Frogs, too, give EOG responses to non-volatile substances, including sugars[47] and L-amino acids[47,78], if solutions of them are placed on the olfactory mucosa. Most odorants are lipid-soluble, but in water they may be very soluble (e.g. ethyl acetate) or sparingly soluble (e.g. limonene).

Stereoisomers may or may not have different perceived odors. Highly purified R-(−)-carvone ('spearmint') and S-(+)-carvone ('caraway') are easily distinguished[42,115]. Positional isomers, diastereomers, and enantiomers of some steroids are also distinguishable[103]. Enantiomeric

pairs of some other substances (e.g. 2-octanol and camphor[42]) are not distinguishable.

Thresholds for human detection of many odorants have been estimated[39]. They range from 2×10^{-2} M for ethane to 10^{-10}–10^{-12} M for musk xylene and some sulfides and mercaptans. Each such threshold is the concentration of odorant in the vapor phase presented to the mucosa. Odorant concentrations in the mucus itself are generally unknown, although approximations have been made by theoretical[21] and experimental[66] methods.

4. Definition of stimulus

No very precise definition of an olfactory stimulus is possible yet. The topological complexity of the nasal cavity hampers smooth flow of odorant, especially in mammals. Much of the presented odorant may be adsorbed by non-olfactory tissues. Partitioning of the odorant through the mucus layer and into the cell membrane is an important variable in olfactory perception[57,77], but the kinetics of this process are not well characterized.

At the point an odorant leaves its source, the stimulus can be defined operationally. Mozell et al.[100] found that variation of any of the stimulus presentation parameters (total number of odorant molecules, volume of air used to deliver the stimulus, and duration of stimulus) causes a change in the receptor cell output. Here the output was measured as the summated action potentials of many frog receptor neurons. Even in these experiments, however, the kinetics of stimulus presentation at the cell surface level are uncertain.

Most experimenters stimulate the olfactory mucosa by injecting odorant for a second or two into a continuous stream of deodorized air. Initially, the injection results in an increase in odorant concentration which no doubt reaches the cell surface. Then the odorant injection is stopped, but the deodorized air continues to flow, and one expects the concentration at the receptors to decrease. Mucosal transport processes might hasten this process, while specific binding or solution of the odorant in the mucus or plasma membrane would retard it. After a brief presentation of *n*-butanol, the EOG does in fact indicate a rapid decrease in odorant concentration at the receptors[104], although most of the odorant remains in the mucosa for at least 30 min[65]. It is interesting to note that such a 'square wave' presentation would be seen by a bacterium as two stimuli in sequence. Addition of an attractant, for example, would be seen as an attractant stimulus, and its subsequent removal would be a repellent stimulus.

One might hope that continuous flow of odorant would eventually cause a steady state concentration at the cell surface to be reached, although the time for this equilibrium to occur would be difficult to measure. The EOG seen under such stimulation shows an immediate phasic response, followed by a tonic response which is stable as long as the odorant stream is applied[104]. Two mechanisms may be at work, one which adapts and one which does not[104,105]. Measuring impulses under such conditions is more complicated. Repeated nerve firing during continuous stimulation is often followed by a quiescent period which may be due to depolarization block of action potential generation[43,133]. This should not be regarded as adaptation, since a blocked cell is incapable of responding to additional stimuli. Whether the output of an olfactory receptor neuron adapts in the sense that bacterial behavior adapts remains an open question. There can be no doubt, however, that human olfactory perception shows adaptation[76].

5. Stimulus receptors

5a. Location of receptor sites. It has long been supposed that stimulus reception occurs at the apices of the olfactory receptor neurons. The latency of the olfactory response in the tiger salamander has been taken to suggest that the receptors are on the olfactory knobs or the proximal portions of the cilia[49]. Other recent evidence supports this idea in the frog[1,22]. Lavage of the epithelium with dilute Triton X-100 deciliates the receptor neurons and virtually eliminates the EOG response. The EOG returns concurrently with regeneration of the proximal parts of the cilia. Deciliation affects impulse generation in the frog as well. Soon after deciliation, the neurons show the normal pattern of occasional action potentials, but this pattern is not influenced by odorous stimuli. As the cilia begin to regenerate, the impulse pattern again changes with stimulation (G.D. Adamek and R.C. Gesteland, personal communication). In frogs, cilia increase the receptor cell area at the mucosal surface 40-fold[90] and apparently contain the bulk of the chemoreception sites. In catfish, the EOG amplitude is still 50–60% of normal after deciliation[27], suggesting that the knobs or microvilli of these receptor cells may bear many of the receptors. The neurons of vomeronasal organs[10,56] are not ciliated at all, and many fish have both ciliated and non-ciliated olfactory receptor neurons[67,143]. It seems likely that olfactory knobs, microvilli, and cilia all may bear chemoreception sites.

5b. Stimulus-receptor specifities. It is difficult to imagine that there is a specific receptor molecule for each of the many odorants we can discriminate. On the other hand, our ability to distinguish some pairs of stereoisomers does suggest the existence of specific odorant-receptor mechanisms. Many of the theories of olfactory perception have been reviewed[32,95]. Some do not require the presence of stimulus-specific receptors at all. Davies[32], for example, postulates that lipophilic odorants can penetrate and puncture the plasma membrane bilayer. Ions may flow across this wound before it reseals, depolarizing the cell and contributing to the generator potential. It is not clear how this model accounts for perceived differences in stereoisomers.

Much effort has been devoted to demonstrating the existence of stimulus-specific olfactory receptor molecules. The techniques used to classify bacterial chemoreceptors have not yet revealed much about olfactory chemoreception. The mutants available are humans[11] and mice[109,142] with specific anosmias. Classes of odorants for which anosmics have raised detection thresholds may be defined, but whether the lesions causing the anosmia are at the initial stages of stimulus reception and transduction is unknown. The bacterial competition experiments have their counterparts in studies of olfactory cross-adapta-

tion, measured by either human perception[76] or with the EOG[14]. The results suggest very complicated distributions of receptor sites and types. There is one significant difference between the bacterial competition and olfactory cross-adaptation methods. In the latter, the first stimulus is usually terminated before the second begins. Bacteria, however, can recover very quickly when a stimulus is removed. A state of adaptation to the first stimulus can be maintained only by the compound's continued presence; the second stimulus is then presented on top of the first.

Ligand binding studies have provided evidence for specific olfactory receptor molecules in some cases. Amino acids bind ($k_D \sim 10^{-6}$ M) to plasma membranes of olfactory epithelium[25] and to olfactory cilia[112], both from trout. The amount and stereospecificity of ligand binding correlate well with the effectiveness of each stimulus in inducing impulses in the trout cells. Similar binding of amino acids to isolated catfish olfactory neurons has been demonstrated[26]. These studies are noteworthy because they demonstrate not only odorant binding, but also correlations between binding and stimulatory effectiveness.

A steroid binds with high affinity ($k_D \sim 10^{-9}$ M) to extracts of sheep whole olfactory epithelium[36]. The particular steroid is a pheromone detected by sows; its effect on sheep is unknown. High-affinity binding ($k_D \sim 10^{-9}$ M) of camphor to a fraction of olfactory mucosa has also been shown[41]. Certain pyrazine odorants bind strongly to a protein from bovine olfactory epithelium[107,108], but the function of this binding is not yet certain.

Chemical modifications of olfactory epithelium offer some insight into the nature of the receptor molecules. Stimulus-specific protection from chemical inactivation has been widely studied. Getchell and Gesteland[46] found that treatment of frog olfactory mucosa with the thiol-modifying reagent *N*-ethylmaleimide (NEM) causes irreversible reduction of the EOG amplitude. If the odorant *n*-ethyl butyrate is also present during the NEM treatment, however, the EOG responses to *n*-ethyl butyrate and *n*-methyl butyrate are protected from inactivation by NEM. The responses to other odorants tested were abolished. There are two explanations[33,46,91] for the protection results. The protecting odorant, bound to a stimulus-specific proteinaceous receptor, may protect that receptor from chemical inactivation. Only EOG responses mediated by that type of receptor molecule will survive. On the other hand, the odorant may affect entire receptor cells to which it binds, as by depolarization, and prevent the reagent from binding to those cells' membranes. Any stimulus specificity observed would then be a consequence of the uneven distribution of molecular receptor types across the population of receptor cells. Interpretation of these experiments is further complicated by the finding that NEM causes dissociation of the olfactory epithelium[69]. Other protection studies have been conducted using mersalyl[91,117] (an impermeant thiol-specific reagent), alkylating agents[30], enzymatic iodination[117], and the lectin concanavalin A[118,141] as inactivating agents. Stimulus-specific protection is sometimes observed and sometimes not, and a simple interpretation of the results is not yet possible. It is interesting that concanavalian A reduces the EOG amplitude; this suggests that some of the many glycoproteins found on olfactory cilia may be active in chemoreception[28].

Other variations of the chemical modification theme exist. Chemically reactive odorant analogues (affinity labels and photoaffinity labels) are being investigated[34,92]. An antibody to an anisole-binding protein from dog olfactory epithelium has been found to depress the EOG response in mice[50]. It reduces the EOG to most stimuli tested.

A less direct approach to the classification of odorant receptors has been advanced by Holley and co-workers (ref. 119 and others cited there). In a typical study, recordings were made of impulses from 76 frog olfactory neurons while each neuron was stimulated with a defined set of 20 odorants. It was found, for example, that most of the neurons either responded to both cineole and camphor or to neither, suggesting that these two odorants belong to a class which shares a common receptive site. Multidimensional analyses of these and other results[119] have identified five such classes of odorants. It was not practical to test each odorant at several concentrations, and so all were tested at levels presumed to be well above threshold.

In a population of genetically defined bacteria grown under a given condition, each cell can be assumed to possess the same set of chemoreceptors as the next. The distribution of receptive sites across the population of olfactory neurons is apparently much more complicated. Numerous studies of the impulses fired by single olfactory receptor neurons in response to various odorants[13,44,88,102,119] have shown that the full range from broad to narrow selectivity is found. Statistical analyses of the data from Holley's group[119] do not yet allow a simple classification of receptor cells on the basis of stimulus selectivity. It seems that for most pairs of receptor neurons, it is possible to find some stimulus that will excite one but not the other. This spatial heterogeneity of receptive site distribution is also demonstrable at a broader level. The ratio of EOG amplitudes in response to a given pair of odorants varies across the surface of the mucosa[31,79,101,132].

6. Stimulus transduction

Little is known about how an odorous stimulus, once detected, is transduced into impulse generation. Generation of the receptor potential (EOG) is almost certainly one of the earliest transduction steps. Its underlying ionic basis is still controversial. Tucker and Shibuya[138] reported that removal of Na^+ has little effect on the phasic component of the EOG in the box turtle. Takagi et al.[131], however, found that in frogs the EOG requires Na^+ and K^+. Suzuki's study of the lamprey[128] found that odorant-induced impulses require the presence oc Ca^{2+}, but not Na^+ or K^+, at the mucosa. Most recently, Yoshii and Kurihara[144] reported that the presence at the mucosa of any one of 12 cations suffices to allow stimulus-induced impulses in the carp. They suggest that binding of cations to the mucosal surface causes an interfacial potential which spreads electrotonically to the axon hillock. This model requires no transmembrane receptor currents.

A more conventional model would suggest that stimulus reception results in state transitions in one or more ionic

channels of the receptor cell membrane. An odorant-sensitive channel which conducts K^+ has been found in a homogenate of rat olfactory epithelium[139]. Whether the channel originates in the receptor neurons is unknown, but it is absent in homogenates of non-olfactory epithelium. Membrane patch recording methods[116] are also being applied to the study of channels in isolated olfactory neurons[89].

Recent evidence points to an involvement of cyclic nucleotides in olfactory transduction. Pace et al.[106] have found that adenylate cyclase from frog olfactory cilia is activated by odorants. The activation is tissue- and ligand-specific and requires GTP.

Conclusions

At first glance, several distinctions seem obvious between the bacterial and olfactory chemoreception systems:

1. Bacteria respond to a well-defined set of chemical stimuli; the number of odorants we can perceive seems endless.
2. Bacterial attractants are generally ionic; odorants are most often hydrophobic.
3. The bacterial chemoreception and transport systems are closely linked; most odorants are of little nutritive value.
4. Most bacterial attractants bind to stimulus-specific receptors; there is evidence both for and against the existence of specific olfactory receptors.
5. The chemotactic response is a simple 'on-off' response; olfactory impulses are much more complex.

Given such differences, the search for homologies between the two systems might seem to be a case of wild goose taxis by chemoreceptions scientists.

However, each of these distinctions is superficial, as follows:

1. If the weak attractants and repellents are included, the number of bacterial chemoeffectors grows considerably. The thresholds of others may be below the detection limits of the chemotaxis assays, and many have never been tested. We are more likely to recognize a weak odorant than a weak bacterial attractant, and it is easier to sniff 1000 potential odorants than to perform 1000 capillary assays.
2. It is clear, at least in some vertebrates, that some ionic compounds can be effective olfactory stimuli if they are allowed to contact the mucosa. Most of the hydrophilic odorants have never been tested as bacterial stimuli.
3. The apparently strong link between bacterial chemoreception and transport was broken when the Tsr and Tar proteins were identified as the serine and aspartate receptors. Serine and aspartate transport systems are independent of Tsr and Tar.
4. The evidence amassed so far suggests that olfactory chemoreception may involve both specific receptor mechanisms and non-stimulus-specific perturbations. The bacterial transducers are influenced in each of these ways. Excitation mediated by these transducers can result from binding of an attractant to the transducer, binding of an attractant-receptor complex to the transducer, or change in intracellular pH caused by a stimulus substance.
5. It is not unreasonable to regard impulse firing, like bacterial chemotaxis, as a transition between two states. One state leads to an increase in firing rate, while the other causes inhibition of impulses. Although complex patterns involving both of these states have been observed after stimulation, they may more commonly result from strong laboratory stimuli than from those encountered in nature. The two-state model of chemotaxis may reveal itself to have the same complexity as impulse patterns once examination of the flagellar rotor with better spatial and temporal resolution is possible. In the meantime, the two-state model is a valuable working hypothesis in bacterial chemotaxis.

What can be learned from the experiences of researchers studying bacterial chemotaxis? Their abilities to manipulate the extracellular environment and to present well-defined stimuli made many advances possible. Such experiments led to the discoveries that methionine and ATP are required for chemotaxis, which in turn led to the successful search for methylations[73]. Methods exist for producing suspensions of isolated cells from olfactory epithelia[26,64,70]. It should be possible to deliver well-defined stimuli in solution to these cells without the complications of a mucous layer. Recently developed membrane patch recording methods[116] will probably result in detailed descriptions of the transmembrane events occurring in isolated receptor neurons.

Chemical modifications of the mucosal surface offer excellent opportunities for investigating receptor specificity. The bacterial studies make it clear, though, that a given phenotype can be caused by genetic or chemically-induced lesions at any of several points. Only through the use of several independent lesioning agents can the chemoreceptive hierarchy be described with much confidence.

Given the different methods used so far to study bacterial chemotaxis and vertebrate olfaction, any analogy between the two is tenuous at best. Even so, a knowledge of the bacteriologists' methods and interpretations can only help in unraveling the reception chemistry of single olfactory receptor neurons. Ultimately, of course, the analogy's usefulness will end when we ask how all those single neurons work together to create a perception.

Acknowledgments. I am grateful to Bert Ph. M. Menco, Michael S. Lidow, Julius Adler, Robert C. Gesteland, Robert C. MacDonald, Daniel B. Kurtz, and Martin S. Springer for helpful discussions and literature references. This work was supported by National Institutes of Health grants NS18490 and NS14663 and by National Science Foundation grants BNS-8117075 and BNS-8316827.

1 Adamek, G.D., Gesteland, R.C., Mair, R.G., and Oakley, B., Transduction physiology of olfactory receptor cilia. Brain Res. *310* (1984) 87–97.
2 Adler, J., Chemotaxis in bacteria. Science *153* (1966) 708–716.
3 Adler, J., Chemoreceptors in bacteria. Science *166* (1969) 1588–1597.
4 Adler, J., A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by Escherichia coli. J. gen. Microbiol. *74* (1973) 77–91.
5 Adler, J., Chemotaxis in bacteria. A. Rev. Biochem. *44* (1975) 341–356.
6 Adler, J., and Epstein, W., Phosphotransferase-system enzymes as chemoreceptors for certain sugars in Escherichia coli chemotaxis. Proc. natn. Acad. Sci. USA *71* (1974) 2895–2899.
7 Adler, J., Hazelbauer, G.L., and Dahl, M.M., Chemotaxis toward sugars in Escherichia coli. J. Bacteriol. *115* (1973) 824–847.

8 Adrian, E.D., Sensory discrimination with some recent evidence from the olfactory organ. Br. med. Bull. *6* (1950) 330–333.
9 Aksamit, R.R., and Koshland, D.E. Jr, Identification of the ribose-binding protein as the receptor for ribose chemotaxis in Salmonella typhimurium. Biochemistry *13* (1974) 4473–4478.
10 Altner, H., and Müller, W., Elektrophysiologische und elektronenmikroskopische Untersuchungen an der Riechschleimhaut des Jacobsonschen Organs von Eidechsen (Lacerta). Z. vergl. Physiol. *60* (1968) 151–155.
11 Amoore, J.E., Specific anosmia and the concept of primary odors. Chem. Senses *2* (1977) 267–281.
12 Barreau, C., and van der Wel, H., The effect of thaumatins on the chemotactic behavior of Escherichia coli. Chem. Senses *8* (1983) 71–79.
13 Baylin, F., Temporal patterns and selectivity in the unitary responses of olfactory receptors in the tiger salamander to odor stimulation. J. gen. Physiol. *74* (1975) 17–36.
14 Baylin, F., and Moulton, D.G., Adaptation and cross-adaptation to odor stimulation of olfactory receptors in the tiger salamander. J. gen. Physiol. *74* (1979) 37–55.
15 Berg, H.C., How to track bacteria. Rev. scient. Instrum. *42* (1971) 868–871.
16 Berg, H.C., Dynamic properties of bacterial flagellar motors. Nature *249* (1974) 77–79.
17 Berg, H.C., and Anderson, R.A., Bacteria swim by rotating their flagellar filaments. Nature *245* (1973) 380–382.
18 Berg, H.C., and Brown, D.A., Chemotaxis in Escherichia coli analyzed by three-dimensional tracking. Nature *239* (1972) 500–504.
19 Berg, H.C., and Tedesco, P.M., Transient response to chemotactic stimuli in Escherichia coli. Proc. natn. Acad. Sci. USA *72* (1975) 3235–3239.
20 Black, R.A., Hobson, A.C., and Adler, J., Involvement of cyclic GMP in intracellular signaling in the chemotactic response of Escherichia coli. Proc. natn. Acad. Sci. USA *77* (1980) 3879–3883.
21 Bostock, H., Diffusion and the frog EOG, in: Transduction mechanisms in chemoreception, pp. 27–36. Ed. T.M. Poynder. Information Retrieval Ltd., London 1974.
22 Bronshtein, A.A., and Minor, A.V., The regeneration of olfactory flagella and restoration of the electroolfactogram after treatment of the olfactory mucosa with Triton X-100. Tsitologiya *19* (1977) 33–39.
23 Brown, D.A., and Berg, H.C., Temporal stimulation of chemotaxis in Escherichia coli. Proc. natn. Acad. Sci. USA *71* (1974) 1388–1392.
24 Cagan, R.H., and Kare, M.R., (Eds) Biochemistry of taste and olfaction, Academic Press, New York 1981.
25 Cagan, R.H., and Zeiger, W.N., Biochemical studies of olfaction: binding specificity of radioactively labeled stimuli to an isolated olfactory preparation from rainbow trout (Salmo gairdneri). Proc. natn. Acad. Sci. USA *75* (1978) 4679–4683.
26 Cancalon, P., Isolation and characterization of the olfactory epithelial cells of the catfish. Chem. Senses *3* (1978) 381–396.
27 Cancalon, P., Effect of salts, pH and detergents on the catfish olfactory mucosa, in: Olfaction and taste VII, pp. 73–76. Ed. H. van der Starre. IRL Press Ltd., London 1980.
28 Chen, Z., and Lancet, D., Membrane proteins unique to vertebrate olfactory cilia: Candidates for sensory receptor molecules. Proc. natn. Acad. Sci. USA *81* (1984) 1859–1863.
29 Clarke, S., and Koshland, D.E. Jr, Membrane receptors for aspartate and serine in bacterial chemotaxis. J. biol. Chem. *254* (1979) 9695–9702.
30 Criswell, D.W., McClure, F.L., Schafer, R., and Brower, K.R., War gases as olfactory probes. Science *210* (1980) 425–426.
31 Daval, G., Leveteau, J., and MacLeod, P., Electro-olfactogramme local et discrimination olfactive chez la grenouille. J. Physiol., Paris *62* (1970) 477–488.
32 Davies, J.T., Olfactory theories, in: Handbook of sensory physiology, vol. 4, pt. 1, pp. 322–350. Ed. L.M. Beidler. Springer, Berlin 1971.
33 Delaleu, J.C., and Holley, A., Modification of transduction mechanisms in the frog's olfactory mucosa using a thiol reagent as olfactory stimulus. Chem. Senses *5* (1980) 205–218.
34 Delaleu, J.C., and Holley, A., Investigations of the discriminative properties of the frog's olfactory mucosa using a photoactivable odorant. Neurosci. Lett. *37* (1983) 251–256.
35 DeVries, H., and Stuiver, M., The absolute sensitivity of the human sense of smell, in: Sensory communication, pp. 159–167. Ed. W.A. Rosenblith. Wiley & Sons, New York 1960.
36 Dodd, G., and Persaud, K., in Cagan and Kare, op. cit., pp. 333–357.
37 Døving, K.B., Selset, R., and Thommesen, G., Olfactory sensitivity to bile acids in salmonid fishes. Acta physiol. scand. *108* (1980) 123–131.
38 Eisenbach, M., Raz, T., and Ciobotariu, A., A process related to membrane potential involved in bacterial chemotaxis. Biochemistry *22* (1983) 3293–3298.
39 Fazzalari, F.A., (Ed.) Compilation of odor and taste threshold values data. American Society for Testing and Materials, Philadelphia 1978.
40 Felle, H., Porter, J.S., Slayman, C.L., and Kaback, R.H., Quantitative measurements of membrane potential in Escherichia coli. Biochemistry *19* (1980) 3585–3590.
41 Fesenko, E.E., Novoselov, V.I., Mjasoedov, N.F., and Sidorov, G.V., Molecular mechanisms of olfactory reception. III. Binding of camphor by rat and frog olfactory mucosa components. Stud. Biophys. *73* (1978) 71–84.
42 Friedman, L., and Miller, J.G., Odor incongruity and chirality. Science *172* (1971) 1044–1046.
43 Gesteland, R.C., Phyisology of olfactory reception, in: Frog neurobiology, pp. 234–249. Eds R. Llinás and W. Precht. Springer, Berlin 1976.
44 Gesteland, R.C., Lettvin, J.Y., Pitts, W.H., and Rojas, A., Odor specificities of the frog's olfactory receptors, in: Olfaction and taste I, pp. 19–34. Ed. Y. Zotterman, Pergamon Press, London 1963.
45 Gesteland, R.C., Yancey, R.A., and Farbman, A.I., Development of olfactory receptor neuron selectivity in the rat fetus. Neuroscience *7* (1982) 3127–3136.
46 Getchell, M.L., and Gesteland, R.C., The chemistry of olfactory reception: stimulus-specific protection from sulfhydryl reagent inhibition. Proc. natn. Acad. Sci. USA *69* (1972) 1494–1498.
47 Getchell, T.V., The interaction of the peripheral olfactory system with nonodorous stimuli, in: Olfaction and taste III, pp. 117–124. Ed. C. Pfaffmann. Rockefeller University Press, New York 1969.
48 Getchell, T.V., Analysis of unitary spikes recorded extracellularly from frog olfactory receptor cell and axons. J. Physiol., London *234* (1973) 533–551.
49 Getchell, T.V., Heck, G.L., DeSimone, J.A., and Price, S., The location of olfactory receptor sites. Biophys. J. *29* (1980) 397–412.
50 Goldberg, S.J., Turpin, J., and Price, S., Anisole binding protein from olfactory epithelium: evidence for a role in transduction. Chem. Senses *4* (1979) 207–214.
51 Goy, M.F., Springer, M.S., and Adler, J., Sensory transduction in Escherichia coli: role of a protein methylation reaction in sensory adaptation. Proc. natn. Acad. Sci. USA *74* (1977) 4964–4968.
52 Graziadei, P.P.C., The ultrastructure of vertebrates olfactory mucosa, in: The ultrastructure of sensory organs, pp. 267–305. Ed. I. Friedmann. North Holland/American Elsevier, Amsterdam 1973.
53 Graziadei, P.P.C., Cell dynamics in the olfactory mucosa. Tissue Cell *5* (1973) 113–131.
54 Graziadei, P.P.C., and DeHan, R.S., Neuron regeneration in frog olfactory system. J. Cell Biol. *59* (1973) 525–530.
55 Graziadei, P.P.C., and Metcalf, J.F., Autoradiographic and ultrastructural observations on the frog's olfactory mucosa. Z. Zellforsch. mikrosk. Anat. *116* (1971) 305–318.
56 Graziadei, P.P.C., and Tucker, D., Vomeronasal receptors in turtles. Z. Zellforsch. *105* (1970) 498–514.
57 Greenberg, M.J., Dependence of odor intensity on the hydrophobic properties of molecules. A quantitative structure odor intensity relationship. J. agric. Fd Chem. *27* (1979) 347–352.
58 Hara, T.J., Olfactory responses to amino acids in rainbow trout (Salmo gairdneri). Comp. Biochem. Physiol. *44A* (1973) 407–416.
59 Hazelbauer, G.L., The maltose chemoreceptor of Escherichia coli. J. Bacteriol. *122* (1975) 206–214.
60 Hazelbauer, G.L., and Adler, J., Role of the galactose-binding protein in chemotaxis of Escherichia coli toward galactose. Nature New Biol. *230* (1971) 101–104.
61 Hazelbauer, G.L., and Harayama, S., Sensory transduction in bacterial chemotaxis. Int. Rev. Cytology *81* (1983) 33–70.
62 Hazelbauer, G.L., and Parkinson, J.S., Bacterial chemotaxis, in: Microbial interactions, pp. 59–98. Ed. J.L. Reissig. Chapman and Hall, London 1977.
63 Hedblom, M.L., and Adler, J., Genetic and biochemical properties of Escherichia coli mutants with defects in serine chemotaxis. J. Bacteriol. *144* (1980) 1048–1060.
64 Hirsch, J.D., and Margolis, F.L., Cell suspensions from rat olfactory neuroepithelium: biochemical and histochemical characterization. Brain Res. *161* (1979) 277–291.

65 Hornung, D. E., and Mozell, M. M., Odorant removal from the frog olfactory mucosa. Brain Res. *128* (1977) 158–163.

66 Hornung, D. E., Mozell, M. M., and Serio, J. A., Olfactory mucosa/air partioning of odorants, in: Olfaction and taste VII, pp. 167–170. Ed. H. van der Starre. IRL Press Ltd., London 1980.

67 Ichikawa, M., and Ueda, K., Fine structure of the olfactory epithelium in the goldfish, Carassius auratus. Cell Tissue Res. *183* (1977) 445–455.

68 Kihara, M., and Macnab, R. M., Cytoplasmic pH mediates pH taxis and weak-acid repellent taxis of bacteria. J. Bacteriol. *145* (1981) 1209–1221.

69 Kleene, S. J., and Gesteland, R. C., Dissociation of frog olfactory epithelium with N-ethylmaleimide. Brain Res. *229* (1981) 536–540.

70 Kleene, S. J., and Gesteland, R. C., Dissociation of frog olfactory epithelium. J. Neurosci. Meth. *9* (1983) 173–183.

71 Koiwai, O., and Hayashi, H., Studies on bacterial chemotaxis. IV. Interaction of maltose receptor with a membrane-bound chemosensing component. J. Biochem. *86* (1979) 27–34.

72 Kondoh, H., Ball, C. B., and Adler, J., Identification of a methyl-accepting chemotaxis protein for the ribose and galactose chemoreceptors of Escherichia coli. Proc. natn. Acad. Sci. USA *76* (1979) 260–264.

73 Kort, E. N., Goy, M. F., Larsen, S. H., and Adler, J., Methylation of a membrane protein involved in bacterial chemotaxis. Proc. natn. Acad. Sci. USA *72* (1975) 3939–3943.

74 Koshland, D. E. Jr, A model regulatory system: bacterial chemotaxis. Physiol. Rev. *59* (1979) 811–862.

75 Koshland, D. E. Jr, Biochemistry of sensing and adaptation in a simple bacterial system. A. Rev. Biochem. *50* (1981) 765–782.

76 Köster, E. P., Adaptation and cross-adaptation in olfaction. Ph. D. thesis, University of Utrecht, the Netherlands. Bronder-Offsett, Rotterdam 1971.

77 Koyama, N., and Kurihara, K., Effect of odorants on lipid monolayers from bovine olfactory epithelium. Nature, London *236* (1972) 402–404.

78 Kruzhalov, N. B., Natural amino acids as olfactory stimuli for some amphibians, in: Sensory systems: olfaction and taste, pp. 60–72 (in Russian). Nauka, Leningrad 1980.

79 Kubie, J., Mackay-Sim, A., and Moulton, D. G., Inherent spatial patterning of responses to odorants in the salamander olfactory epithelium, in: Olfaction and taste VII, pp. 163–166. Ed. H. van der Starre. IRL Press Ltd., London 1980.

80 Lancet, D., Vertebrate olfactory reception. A. Rev. Neurosci. *9* (1986), in press.

81 Larsen, S. H., Reader, R. W., Kort, E. N., Tso, W.-W., and Adler, J., Change in direction of flagellar rotation is the basis of the chemotactic response in Escherichia coli. Nature *249* (1974) 74–77.

82 Lidow, M. S., and Menco, B. Ph. M., Observations on axonemes and membranes of olfactory and respiratory cilia in frogs and rats using tannic acid-supplemented fixation and photographic rotation. J. Ultrastruc. Res. *86* (1984) 18–30.

83 Lovely, P., Dahlquist, F. W., Macnab, R., and Koshland, D. E. Jr, An instrument for recording the motions of micro-organisms in chemical gradients. Rev. scient. Instrum. *45* (1974) 683–686.

84 Macnab, R. M., Bacterial motility and chemotaxis: the molecular biology of a behavioral system. Crit. Rev. Biochem. *5* (1978) 291–341.

85 Macnab, R. M., and Koshland, D. E. Jr, The gradient-sensing mechanism in bacterial chemotaxis. Proc. natn. Acad. Sci. USA *69* (1972) 2509–2512.

86 Macnab, R. M., and Koshland, D. E. Jr, Bacterial motility and chemotaxis: light-induced tumbling response and visualization of individual flagella. J. molec. Biol. *84* (1974) 399–406.

87 Macnab, R. M., and Ornston, M. K., Normal-to-curly flagellar transitions and their role in bacterial tumbling. Stabilization of an alternative quaternary structure by mechanical force. J. molec. Biol. *112* (1977) 1–30.

88 Mathews, D. F., Response patterns of single neurons in the tortoise olfactory epithelium and olfactory bulb. J. gen. Physiol. *60* (1972) 166–180.

89 Maue, R. A., and Dionne, V. E., Ion channel activity in isolated murine olfactory receptor neurons. Biophys. J. *45* (1984) 266a.

90 Menco, B. Ph. M., Qualitative and quantitative freeze-fracture studies on olfactory and nasal respiratory structures of frog, ox, rat, and frog. Cell Tissue Res. *207* (1980) 183–209.

91 Menevse, A., Dodd, G., and Poynder, T. M., A chemical-modification approach to the olfactory code. Biochem. J. *176* (1978) 845–854.

92 Menevse, A., Dodd, G. H., Poynder, T. M., and Squirrell, D., A chemical modification approach to the olfactory code. Biochem. Soc. Trans. *5* (1977) 91–194.

93 Mesibov, R., and Adler, J., Chemotaxis toward amino acids in Escherichia coli. J. Bacteriol. *112* (1972) 315–326.

94 Moncrieff, R. W., The chemical senses, 3rd edn, p. 565. CRC Press, Cleveland 1967.

95 Ibid., pp. 568–600.

96 Moulton, D. G., Çelebi, G., and Fink, R. P., Olfaction in mammals – two aspects: proliferation of cells in the olfactory epithelium and sensitivity to odors, in: Taste and smell in vertebrates, pp. 227–246. Eds G. E. Wolstenholme and J. Knight. J. A. Churchill, London 1970.

97 Mozell, M. M., Olfactory mucosal and neural responses in the frog. Am. J. Physiol. *203* (1962) 353–358.

98 Mozell, M. M., Evidence for a chromatographic model of olfaction. J. gen. Physiol. *56* (1970) 46–63.

99 Mozell, M. M., and Jagodowicz, M., Chromatographic separation of odorants by the nose: retention times measured across in vivo olfactory mucosa. Science *191* (1973) 1247–1249.

100 Mozell, M. M., Sheehe, P. R., Swieck, S. W. Jr, Kurtz, D. B., and Hornung, D. E., A parametric study of the stimulation variables affecting the magnitude of the olfactory response. J. gen. Physiol. *83* (1984) 233–267.

101 Mustaparta, H., Spatial distrubution of receptor-responses to stimulation with different odours. Acta physiol. scand. *82* (1971) 154–166.

102 O'Connell, R. J., and Mozell, M. M., Quantitative stimulation of frog olfactory receptors. J. Neurophysiol. *32* (1969) 51–63.

103 Ohloff, G., Maurer, B., Winter, B., and Giersch, W., Structural and configurational dependence of the sensory process in steroids. Helv. chim. Acta *66* (1983) 192–217.

104 Ottoson, D., Analysis of the electrical activity of the olfactory epithelium. Acta physiol. scand. *35*, suppl. *122* (1956) 1–83.

105 Ottoson, D., The electro-olfactogram, in: Handbook of sensory physiology, vol. 4., pt. 1, pp. 95–131. Ed. L. M. Beidler. Springer, Berlin 1971.

106 Pace, U., Hanski, E., Salomon, Y., and Lancet, D., Odorant-sensitive cyclase may mediate olfactory reception. Nature *316* (1985) 255–258.

107 Pelosi, P., Baldaccini, N. E., and Pisanelli, A. M., Identification of a specific olfactory receptor for 2-isobutyl-3-methoxypyrazine. Biochem. J. *201* (1982) 245–248.

108 Pevsner, J., Trifiletti, R. R., Strittmatter, S. M., and Snyder, S. H., Isolation and characterization of an olfactory receptor protein for odorant pyrazines. Proc. natn. Acad. Sci. USA *82* (1985) 3050–3054.

109 Price, S., Specific anosmia to geraniol in mice. Neurosci. Lett. *4* (1977) 49–50.

110 Reader, R. W., Tso, W.-W., Springer, M. S., Goy, M. F., and Adler, J., Pleiotropic aspartate taxis and serine taxis mutants of Escherichia coli. J. gen. Microbiol. *111* (1979) 363–374.

111 Repaske, D. R., and Adler, J., Change in intracellular pH of Escherichia coli mediates the chemotactic response to certain attractants and repellents. J. Bacteriol. *145* (1981) 1196–1208.

112 Rhein, L. D., and Cagan, R. H., Biochemical studies of olfaction: isolation, characterization, and odorant binding activity of cilia from rainbow trout olfactory rosettes. Proc. natn. Acad. Sci. USA *77* (1980) 4412–4416.

113 Richarme, G., Interaction of the maltose-binding protein with membrane vesicles of Escherichia coli. J. Bacteriol. *149* (1982) 662–667.

114 Rubik, B. A., and Koshland, D. E. Jr, Potentiation, desensitization, and inversion of response in bacterial sensing of chemical stimuli. Proc. natn. Acad. Sci. USA *75* (1978) 2820–2824.

115 Russell, G. F., and Hills, J. I., Odor differences between enantiomeric isomers. Science *172* (1971) 1043–1044.

116 Sakmann, B., and Neher, E., (Eds) Single-channel recording. Plenum Press, New York 1983.

117 Shirley, S., Polak, E., and Dodd, G., Chemical-modification studies on rat olfactory mucosa using a thiol-specific reagent and enzymatic iodination. Eur. J. Biochem. *132* (1983) 485–494.

118 Shirley, S., Polak, E., and Dodd, G., Selective inhibition of rat olfactory receptors by concanavalin A. Biochem. Soc. Trans. *11* (1984) 780–781.

119 Sicard, G., and Holley, A., Receptor cell responses to odorants; similarities and differences among odorants. Brain Res. *292* (1984) 283–296.

120 Silverman, M., Building bacterial flagella. Q. Rev. Biol. *55* (1980) 395–408.
121 Silverman, M., and Simon, M., Flagellar rotation and the mechanism of bacterial motility. Nature *249* (1974) 73–74.
122 Silverman, M., and Simon, M., Chemotaxis in Escherichia coli: methylation of che gene products. Proc. natn. Acad. Sci. USA *74* (1977) 3317–3321.
123 Springer, M.S., Goy, M.F., and Adler, J., Sensory transduction in Escherichia coli: two complementary pathways of information processing that involve methylated proteins. Proc. natn. Acad. Sci. USA *74* (1977) 3312–3316.
124 Springer, M.S., Goy, M.F., and Adler, J., Protein methylation in behavioral control mechanisms and in signal transduction. Nature *280* (1979) 279–284.
125 Spudich, J.L., and Koshland, D.E. Jr, Quantitation of the sensory response in bacterial chemotaxis. Proc. natn. Acad. Sci. USA *72* (1975) 710–713.
126 Strange, P.G., and Koshland, D.E. Jr, Receptor interactions in a signalling system: competition between ribose receptor and galactose receptor in the chemotaxis response. Proc. natn. Acad. Sci. USA *73* (1976) 762–766.
127 Sutterlin, A.M., and Sutterlin, N., Electrical responses of the olfactory epithelium of Atlantic salmon (Salmo salar). J. Fish Res. Bd. Can. *28* (1971) 565–572.
128 Suzuki, N., Effects of different ionic environments on the responses of single olfactory receptors in the lamprey. Comp. Biochem. Physiol. *61A* (1978) 461–467.
129 Suzuki, N., and Tucker, D., Amino acids as olfactory stimuli in freshwater catfish, Ictalurus catus (Linn.). Comp. Biochem. Physiol. *40A* (1971) 399–404.
130 Szmelcman, S., and Adler, J., Change in membrane potential during bacterial chemotaxis. Proc. natn. Acad. Sci. USA *73* (1976) 4387–4391.
131 Takagi, S.F., Wyse, G.A., Kitamura, H., and Ito, K., The roles of sodium and potassium ions in the generation of the electro-olfactogram. J. gen. Physiol. *51* (1968) 552–578.
132 Thommesen, G., and Døving, K.B., Spatial distribution of the EOG in the rat; a variation with odour quality. Acta physiol. scand. *99* (1977) 270–280.
133 Trotier, D., and MacLeod, P., Intracellular recordings from salamander olfactory receptor cells. Brain Res. *268* (1983) 225–237.
134 Tsang, N., Macnab, R., and Koshland, D.E. Jr, Common mechanism for repellents and attractants in bacterial chemotaxis. Science *181* (1973) 60–63.
135 Tso, W.-W., and Adler, J., Negative chemotaxis in Escherichia coli. J. Bacteriol. *118* (1974) 560–576.
136 Tucker, D., Olfactory, vomeronasal and trigeminal receptor responses to odorants, in: Olfaction and taste I, pp. 45–69. Ed. Y. Zotterman. Pergamon Press, London 1963.
137 Tucker, D., Nonolfactory responses from the nasal cavity: Jacobson's organ and the trigeminal system, in: Handbook of sensory physiology, vol. 4, pt. 1, pp. 151–181. Ed. L.M. Beidler. Springer, Berlin 1971.
138 Tucker, D., and Shibuya, T., A physiological and pharmacologic study of olfactory receptors, in: Cold Spring Harbor Symposia on Quantitative Biology, vol. 30, pp. 207–215. Cold Spring Harbor Laboratory of Quantitative Biology, New York 1965.
139 Vodyanoy, V., and Murphy, R.B., Single-channel fluctuations in bimolecular lipid membranes induced by rat olfactory epithelial homogenates. Science *220* (1983) 717–719.
140 Wang, E.A., and Koshland, D.E. Jr, Receptor structure in the bacterial sensing system. Proc. natn. Acad. Sci. USA *77* (1980) 7157–7161.
141 Wood, P., Shirley, S., and Dodd, G., Effect of concanavalin A on frog olfactory receptors. Biochem. Soc. Trans. *11* (1984) 781–782.
142 Wysocki, C.J., Whitney, G., and Tucker, D., Specific anosmia in the laboratory mouse. Behav. Genet. *7* (1977) 171–188.
143 Yamamoto, M., Comparative morphology of the peripheral olfactory organ in teleosts, in: Chemoreception in fishes, pp. 39–59. Ed. T.J. Hara. Elsevier, Amsterdam 1982.
144 Yoshii, K., and Kurihara, K., Role of cations in olfactory reception. Brain Res. *274* (1983) 239–248.

0014-4754/86/030241-10$1.50 + 0.20/0

Neural correlates of odor-guided behaviors

by J. Pager

Laboratoire d'Electrophysiologie, Université Claude Bernard, F-69622 Villeurbanne Cedex (France)

Key words. Olfaction; behaving subjects; chronical electrophysiology; multiunit/unit recording; organization level.

Introduction

To the same extent as audition, the olfactory processes contribute to extract information from distal sources. Close to gustation, they are also affected by proximal cues. Thus odorous signals can subserve any decision to start, maintain or interrupt behavioral sequences. Besides that, the olfactory pathways are extensively open to electrical self-stimulation[43]. Odors in themselves may be reinforcing[25], and are able to modulate the electrical reinforcement[41]. Their hedonic value turns out to be a conspicuous factor in multidimentional evaluation[46]. Therefore the sense of smell is likely to subserve the identification of odors not so much as chemical species, but even more as indices of possible reward.

In man, the hedonic dimension of food odors, but not that of control odors, is biased by the metabolic status of the subject, in an adaptative manner, which gave rise to the general definition of alliesthesia[5]. In most examples, finalism is satisfied with the statement that external stimuli are pleasant, as far as they signal a goal useful to individual or specific survival. In such models, the pleasantness of odors is their proper behavior-guiding dimension. Which does not exclude that odors in themselves are gratuitous goals for cognitive behavioral sequences, such as exploration. In any case, optimalized sensory mechanisms do require that the internal state of the subject should control the olfactory processing[24]. As a consequence, the definition of behavioral events has to be extended to internal events subserving behavioral sequences.

In fact the internal processes could interfere with the treatment of olfactory information at various functional levels, from the nasal cavity to the central integrative areas, through metabolic, endocrine, autonomic and central nervous processes. Various chapters in this issue illustrate it from the organizational, functional and psychophysical points of view. But although we know much