Sensory Perception and Its Mechanisms

(A visit to the Monell Chemical Senses Center, January 1988)

By Dr. Juan-Ramon Mor, Group Research Coordinator, Givaudan Research Co., Dübendorf, Switzerland;
Gabriel Sinki, VP-Technical, Flavors, Givaudan, Clifton, NJ; and
Stanley Allured, Publisher, *Perfumer & Flavorist,* Wheaton, Illinois

The Monell Chemical Sciences Center was founded in 1968 at the University of Pennsylvania in Philadelphia, based on a gift from the Monell Foundation. While very generous, this gift required a substantial additional investment in order to develop the facilities required for serious research into the senses of smell and taste. A strong appeal was directed at industry in general, and particularly the flavor and fragrance industry, to make up the necessary funds to build and furnish the building, and provide the start-up budget for this pioneering research effort.

Industry saw the necessity, and more important, the value, of this investment. It is a great credit to the leaders of the flavor and fragrance industry that they have continued funding this center in a major way. Industry supports the center by providing an important part of its annual budget, and by contributing ideas and suggestions to the research workers.

From the outset, Monell was unique in a number of ways. It was committed to a multidisciplinary approach to research, a concept which has become more widely promoted in the intervening twenty years; and it maintained a key relationship with industry, a policy which was criticized in the early seventies, but is now widely accepted, applauded and imitated.

Today, the Monell staff works on problems of immunology, chemistry, endocrinology, biochemistry, nutrition, behavior, genetics, physiology and other specialties as they relate to taste and smell.

Critical to the success of Monell has been its training program that prepares talented young scientists from a broad range of scientific disciplines for investigation of the mechanisms and functions of the chemical senses. Interdisciplinary training is emphasized.

Approximately 200 graduate students, postdoctoral fellows and visiting scientists have had research training at Monell. Monell is now the world's principal source of supply of such scientists trained in the chemical senses. Requests for these scientists have been numerous and are increasing. Former trainees now are located in government, various industries and universities.

Morley R. Kare, PhD, director of the Monell Chemical Senses Center since its inception says, "We have grown to be the largest single source of scientists and research publications on the chemical senses. The Monell Chemical Senses Center is an experiment that worked."

Monell is supported by nearly all of the major flavor and fragrance houses of the world. The industry participates in the budget at a healthy level of about 16%. The remainder is covered by grants from government and foundations for specific re-

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search projects.

However, it is the relatively small contribution of industry that is not earmarked for specific purposes, that allows the Center to add staff and initiate projects that it feels will bring important advances in scientific knowledge without waiting for the long process of special grant development for that project.

This report is the result of a visit to Monell aimed at a general review of our present knowledge of the science of taste and olfaction. This in-depth review was gleaned from conversations with the extensive scientific staff.

Physiology of Olfaction and Taste

The fragrance and flavor chemicals, either alone or as part of an essential oil or flavor or fragrance composition, first meet the olfactory and taste system at a receptor cell. Here begins the mystery of chemoreception. The details of the nature and process of this key element are beginning to be clarified thanks to decades of intensive research.

Theories on how these cells function have been proposed year after year, and today we are closer to answers than we were twenty years ago. We know that the shape of the odorant or taste molecule alone, proposed as one of the earliest ideas on specificity, is certainly not the whole answer, but it may well be a part of the answer.

Other physical and chemical characteristics of the odorant molecule have been proposed as the sole, or partial, answer to this question. None have proved to be the complete answer, but none, of course, have been ruled out entirely as a potential part of the answer.

The receptor cells are deceptively simple. They are there in plain sight at the surface of the tongue and the olfactory epithelium. They can be collected in quantity, separated into reasonable purity, and looked at biochemically and physically. We do know that some taste and olfactory stimuli bind to the receptor cell, but how this highly specialized cell differentiates between the almost infinite variety of molecules that momentarily touch it, leaving a very specific taste or smell impression, is not understood entirely.

There is a "holy grail" in the research field of the chemical senses, known as the "Olfactory code," which when discovered will explain the fundamental laws of smell as they relate the world of chemistry with that of biology. It is presumed to exist, presumed to be knowable, and when discovered, will certainly confer upon its discoverer one of the world's major scientific prices.

In order to review our present knowledge, let us start with a discussion of the anatomy and physiology of the sensory processes.

1. *The Olfactory Cell*

The anatomy of the sense of smell is relatively simple in its beginning, but becomes highly complex once the olfactory messages enter the central nervous system (CNS) and spread out to a number of brain regions.

Olfactory receptors responsible for the sense of smell are found in the patch of olfactory epithelium that covers the center and side walls of the roof of the nasal cavity. In addition to the receptor cells, this epithelium contains cells which produce the mucous secretion covering the epithelium, and cells that also act as support for the olfactory receptor cells. The olfactory cells project a dendrite which carries at its apex a group of 5 to 20 cilia that are bathed in the mucous coating of the olfactory epithelium.

It has been suggested that the function of the mucous surface of the olfactory mucosa may correspond to that of a gas cromatograph, separating molecules as they flow past the olfactory surface. Depending on the degree of solubilization a process of separation and concentration of molecules may take place on the mucous surface.

At its other end, the receptor cell narrows to a fine (.2 to .3 microns) axon. A large number of these axons bundle together within a Schwann cell sheath, penetrate the cribriform plate on the roof of the nose, and travel to the olfactory bulb of the brain, where synapses occur. The human olfactory nerve contains about 100,000,000 axons. 100 to 1000 primary olfactory neurons contact a single postsynaptic mitral cell to form complex synaptic structures called glomeruli. In this manner, information is processed by a complex neuronal circuitry in the olfactory bulb before it is relayed to the cerebral cortex.

The olfactory receptor cell forms a direct, unbroken, single pathway from the cilia through the dendrit, past the cell nucleus and along the axon to the olfactory bulb within the brain. This is the shortest and most direct contact with the brain of any of the senses, with a length of 2 cm to 3 cm.

2. *The Act of Olfaction*

The hypothesis that the cilia at the dendritic tips of the olfactory cells are the sites where the initial chemosensory recognition and transduction events are initiated is supported by increasing evidence. In 1980, Rhein and Cagan isolated olfactory cilia for biochemical studies from the rainbow trout. Subsequently, techniques were developed enabling cilia

isolation from other species. Biochemical studies of these organelles that contain the molecular components for olfactory reception have increased dramatically both in numbers and sophistication.

It is difficult to imagine that there is a specific receptor molecule for each of the thousands of odorants we can describe. On the other hand, our ability to distinguish some pairs of steroisomers does suggest the existence of specific odorant-receptor mechanisms. Although none of the hypothetical olfactory receptors has been isolated up to now, the existence of several hundred different specific receptor cell types is postulated.

It is now rather well demonstrated that odorants, which interact with yet unidentified olfactory receptor proteins in the membrane of olfactory cilia, activate enzyme cascades that lead both to the production of cyclic AMP (cAMP) and to inositol triphosphate (IP_3). A GTP-binding protein (G-protein) of the stimulatory type (Gs) appears to mediate coupling between receptor molecules and the enzyme adenylate cyclase, the latter producing cAMP from ATP. cAMP is then believed to interact, directly or indirectly, with the ion channels responsible for membrane depolarization. IP_3 may release calcium ion from internal stores, particularly from microsomes.

Dose-response and time course studies by Richard Bruch indicate that significant enhancement of cyclic AMP levels was obtained only at high odorant concentration or following prolonged exposure to stimulus. The combined results suggest that cAMP may participate in olfactory transduction during stimulus-induced adaptation perhaps by regulating ion channel proteins in the cilia.

Such mechanisms would be homologous to those found in visual transduction, where light activates the photoreceptor protein *rhodopsin*. Similarly, many hormones and neurotransmitters exert their action by modulating the activity of cyclic nucleotide-processing enzymes. Thus, a rewarding aspect of current olfactory research is the notion that chemoreception shares molecular details, beyond sterospecific receptors, with other cellular mechanisms involved in transmembrane signaling.

3. *Taste Buds and Receptors*

The detectors or receptor structures of taste are embedded in the epithelium of the oral and pharyngeal cavities. The dorsal or upper surface of the tongue is covered with numerous taste *papillae* of four types, which are named after their individual structure: The taste-bud bearing fungiform papillae, which are mushroom-shaped, are located mostly on the tip of the tongue. *Foliate* taste papillae, re-

sembling ridges, are located mostly on the sides of the tongue. *Vallate* taste papillae, which look like enclosed circles, are found on the back of the tongue. Finally, *filiform* papillae, highly keratinized pointed structures, are interspersed among the other types over the anterior two thirds of the surface of the tongue. These latter papillae do not contain taste buds.

The taste papillae are provided with taste buds which consist of a number of elongated neuroepithelial cells (75 to 100) that are somewhat curved, tapering at their ends. Taste buds are very dynamic structures with a rapid (7 to 10 day) turnover of cells.

The vallate papillae have many taste buds on their sides (each papilla bears 100 to 150 buds), while the other types have considerably fewer buds. Adults may have a total of about 2,000 buds while children have an even greater number. Each bud contains from 20 to 30 gustatory cells. The taste cells end with microvilli, where it is probable that the process of taste detection and reception actually takes place analogous to the cilia in olfaction.

Just below the apical ends, the cells are joined by desmosomes—thickenings of the plasma membrane—which seal off the intercellular spaces from the taste pore. Taste buds are innervated by both large and small nerves which enter the bud at its base. They end just below the taste bud in a synaptic connection that continues onto the brain. Clearly, this synaptic arrangement constitutes a major difference compared to the continuous nerves in the olfactory system.

4. *The Act of Taste Perception*

A major portion of taste research at Monell uses the catfish as a model system, since its taste buds are numerous, very sensitive, and easy to collect for study. So far, there is evidence that on the taste cell at least two different types of receptors can be identified. One shows specificity for L-arginine and the other for L-alanine and perhaps also for certain other short chain neutral amino acids (L-threonine, L-serine and glycine). The significance of this finding is easy to note from the dependence of the catfish on insects and other animal matter for food.

In humans, the sensation of taste is presently divided into four or five primary qualities, each of which can be elicited by a specific test compound. These are: sweet (sucrose), sour (hydrochloric acid), salty (sodium chloride), bitter (quinine) and a meaty or savory taste elicited by glutamate which the Japanese call "umami." The tip of the tongue is sensitive to all stimuli but especially sensitive to sweet and salty compounds; the sides of the tongue to sour and the posterior to bitter compounds. Sensitivity to heat, touch and chemical irritation decreases along the axis from the tip of the tongue to the larynx, whereas sensitivity to cold seems to be evenly distributed. While a strong relation between olfaction and trigeminal system sensation seems to exist, only a weak, if any, interaction between trigeminus and taste could be observed. Bitterness still remains the least understood basic taste.

Non-ionic substances evoking taste sensations are thought to act by binding to specific receptor sites on the apical parts (microvilli) of the taste cells. By a mechanism that is not yet fully understood, this binding causes an increase in the ionic conductance of the taste cell membranes, which then gives rise to a depolarization of the taste cell. This depolarization, in turn, leads to an increase of discharge of "firing" of the innervating sensory fibers.

A single taste cell often responds to more than one of the four primary taste stimuli, but not equally to each. Similar multiple responses are observed in taste fibers, each of which synapes with several cells. A considerable number of fibers have been observed to respond well, for instance, to both salty and acid stimuli, whereas fibers responding strongly to both salty and sweet stimuli are rare (salt-"best" and sugar-"best" axons). Within a given responding group, the relative sensitivity to different stimuli can vary widely. The gustatory system may not produce a single taste sensation from a single stimulus, but instead, the neural message for taste quality may result from the integration of a pattern composed of relative amounts of activity across a population of many nerve fibers. A major neurophysiological challenge will be to discover how these patterns are analyzed, compared and interpreted by the central nervous system.

Receptor Cells to Behavior

The initial event in both olfaction and taste is an interaction between chemical stimuli and specialized regions of the receptor cell membrane. This process triggers a sequence of actions in the cells (transduction) that ultimately leads to discharge of nerve impulses in olfactory receptor neurons and to secretion of neurotransmitter in taste receptor cells. This common pathway for activation of both olfactory and taste receptors involves changes in the movement of ions through selective macromolecular pores (ionic channels) in the cell membrane. Ionic channels are the fundamental excitable elements in the membranes of virtually all cells and modulation of these channels mediates the electrical activity of nerve, muscle, secretory and sensory

receptor cells. Over 50 different types of ionic channels have been characterized today, but probably over 100 do exist.

Substantial progress has been made in the past decade in understanding the biochemistry and biophysics of the receptor, and of transduction properties in taste and olfaction. We now possess a better understanding of the receptor processes in both taste and olfaction, and of the ionic channel types underlying the response of taste and olfactory cells. At the receptor level, a strong species-specificity is evident. Yet mechanisms at the transduction level seem to be more or less similar for different species (rat, mouse, catfish). The fact that different classes of receptors can be characterized and mapped allows us to selectively stimulate specific receptors in order to ask questions of receptor specificity and of transduction (second messenger and channel activation).

1. *Patch Clamp–Contemporary Research*

Recent studies seem to favor the theory that a stimulus is recognized by membrane-bound receptor sites, some of which are more specific than others. However, the common denominator of all these theories is that the process of recognition occurs in a single cell. Thus, it is important for experimental work on recognition to test the single receptor and channels within that single cell.

Single channel recording obviously eliminates the variables encountered in measuring the total conductance of many channels that open simultaneously. A recently developed technique, known as "patch clamping," allows accurate single channel recording.

Using a microscope, a micropipette is pushed against a single cell. After a high resistance seal between the pipette and cell membrane is established, several configurations can be generated. Single channel current can be measured in the "cell-attached" configuration. If the pipette is pulled away from the cell, a small "inside-out" patch of membrane can be obtained. With the pipette attached to the cell, the membrane under the pipette can be replaced by applying suction. In this "whole-cell" configuration, currents across the entire cell membrane can be recorded. If the pipette is pulled away from the cell, an "outside-out" patch can be obtained.

2. *Channels, Carriers and Pumps*

Specialized research directed toward measurements of action potential in single cells have led to the observation that there could be three different

types of transport systems. These transport systems, allowing the passage of ions and small molecules through the membrane, are termed "channels," "carriers" and "pumps."

Channel proteins seem to form continuous pores through the membrane. Some are specific to ions such as Ca^{++} or Cl^{-}, but some are non-specific. Open channels allow ions to flow at a relatively high flux, allowing measurement of current in a single channel.

In contrast, carriers have lower flux rates and can be studied by measuring and comparing data from several of these carriers. In contrast to channels, they allow one or a few ions through at a time, filling at one end and releasing at the other.

While both channels and carriers mediate passive flux down an electrochemical gradient, pumps can work against a gradient. They pump ions upward in an uphill transport fashion.

As the understanding of the receptor mechanisms increases, our ability to predict the activity of novel stimuli will also increase. Eventual isolation and detailed characterization of the receptors and their transductive components will permit rational design of new stimuli and inhibitors.

3. *Mapping Transduction*

Cranial nerve mapping is well advanced where locations of motor and sensory fibers are correlated to specific brain areas. We know where the nerves of the hands, feet, face and other parts of the body register in the brain. Now the same information for the sensory taste nerves is being performed by undertaking the task of precise cytoarchitectural localization of taste-elicited cortical responses. By visualizing the complex pathways of both olfaction and taste that have to be monitored in such experiments, one can appreciate the magnitude of this undertaking.

4. *Heat and Cold Effects*

Measurement of the sensitivity to heat of the various oral-facial regions on human subjects shows that the responsiveness to warming varies substantially across oral sites. It seems that responsiveness to cooling is more homogenous. Other researchers pursue the same subject, but with different stimuli, such as capsaicin. They report intense sensations from the tip-side of the tongue and posterior palate, and less intense sensations from the cheek and anterior palate.

5. *Genetics, Olfaction and Behavior*

Research on individual smell characteristics indicates that everyone has at least some individual idiosyncrasies in their sense of smell. Major differences are some well-known anosmias. However, small differences exist in everyone, particularly in the threshold levels of odorants.

An extensive study of twins in the Philadelphia area has shown that differences in some anosmias are genetically determined. In the case of identical twins, 100% of all who were anosmic to androsterone had an identical twin who was also anosmic to the same material. In the case of fraternal twins, only about half of them were anosmic, which is what one would expect. Further research will determine whether this is a dominant gene effect. In animals, anosmias to e.g. isovaleric acid are widespread and found to have a genetic cause.

Humans and other animals are thought to produce genetically-determined, individually-distinct body odors. Consequently, body odor, like a fingerprint, may provide a unique code to each individual of a species. Studies have focused on the role of a subset of genes—the Major Histocompatibility Complex (MHC)—in specifying individuality of odor.

The MHC is a cluster of about fifty genes found in all vertebrates. These genes exhibit the greatest diversity of any known set of loci and are thereby capable of labeling every individual in a population. No two humans, save identical twins, are

identical at the MHC. The MHC is also crucial in controlling *immune function.* The fate of tissue and organ transplants depends on the extent of MHC similarity between donor and recipient. Speculation that immune recognition may share analogies and homologies with olfaction consequently makes the study of this set of genes particularly attractive.

The Monell Center's experimental animal is the inbred mouse, strains of which can be bred to be identical at all other loci while maintaining diversity of the MHC. Previous studies had already shown that such strains of mice are distinguishable by scent as determined by other mice, rats or humans. New work has now identified a specific, single MHC gene as being involved in chemosensory individuality. With the availability of a single gene model system, studies can now be designed to investigate the pathway from gene to odor. Parallel investigations with humans are planned for the near future.

It can hardly be doubted that a genetically-based chemosensory communication system with such potent influence on reproductive behavior has far-reaching implications for those species that possess it. Whether there is any similar unconscious communication among the human population, in addition to communication of the myriads of the other genetically-variable visible attributes which we take for granted, is a fascinating question.

Outlook

The next few years of chemosensory research should lead to a comprehensive molecular analysis of the mechanisms of olfactory recognition and possibly also of taste transduction. This may open the way to a better description of the selectivity and diversity attributes of the chemosensory processes. An understanding of the molecular transduction machinery could allow one to control molecular amplification parameters, hence leading to the development of products that diminish or enhance olfactory and taste sensitivity.

Finally, a better understanding of the molecules that define the individuality of olfactory sensory neurons may help us to understand the cell-recognition processes that allow chemosensory axons to find their appropriate synaptic targets during development and regeneration.

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