Spring 1996

The Monell Connection

The Monell Connection… from the Monell Chemical Senses Center, a nonprofit scientific institute devoted to research on taste, smell, and chemosensory irritation.

O l f a c t i o n

Genes • Gender • Generation

Why are certain odors pleasurable to some and unpleasant to others?

Experience, heredity, gender, age. These, along with other factors, contribute to an individual's olfactory world.

Every person has a unique history, which helps determine how he or she will react to specific chemosensory events. What smells good to most — a rose, perhaps — might be offensive to some.

What could make a sweet-scented rose smell bad? Suppose, as a young child, a person had a pleasant walk through the garden, smelling roses for the first time while in a mother's loving arms. A fond memory, likely. But suppose roses were first smelled by the child while attending a mother's funeral?

In each case, a powerful association has formed between the initial encounter with roses and an emotional event. While these two scenarios represent extremes, in each person's life their own significant odor experiences will influence how that person responds to chemosensory stimulation.

Experience, however, does not explain all variation. Genetic differences also influence individual variation in odor perception. At present there are believed to be as many as 1000 olfactory receptor genes. With so many genes responsible for olfaction, it is likely that one or more could be defective, resulting in a faulty or missing receptor. If a specific odorant molecule normally interacts with only this receptor, and the receptor is not working, then that odorant would not be perceived.

Inability to smell a specific odorant in an individual with otherwise normal ability to smell is known as *specific anosmia*. Many already have been described, and more are likely to be identified in the future. Although the causes of specific anosmias are not yet fully understood, genetic defects in receptor or other mechanisms are most likely involved.

An interesting example of specific anosmia involves the compound androstenone. About half of all adults can detect its odor — typically described as musky or "urine-like"— while half cannot. Studies of twins indicate that capacity to smell androstenone is related to genetic differences. However, some "non-smellers" can acquire the ability to detect androstenone after repeated exposure to the compound.

Interestingly, if an odorant molecule is one that reacts with two molecular receptors, and one receptor is defective, only partial odor information would be available. In such a situation, odor quality most likely would be perceived differently from the way it would be by an individual who received information from two fully

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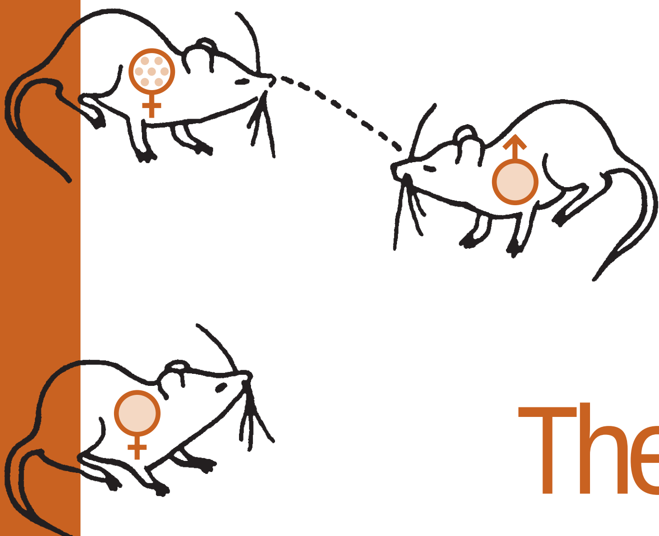
The olfactory system can recognize genetic differences in the immune system.

How does this happen, and what purpose does it serve?



The Scent of Diversity

Visitors from Japan coming to Monell — whether corporate representatives, visiting scientists, or students in the early stages of research training — can always be certain of a warm and helpful welcome from Dr. Kunio Yamazaki, whose own arrival in the United States more than two decades ago reminds him of what it is like to be in a new country.

Yamazaki's move involved more than a change of location. In the early part of his career, he was a microbiologist specializing in radioactive technology. Today, his research explores relationships among immunology, genetics, and olfaction.

A fondness for animals and the prospect of becoming a veterinarian led Yamazaki to a college degree in biology. Animals did not figure prominently in his work after college, however, when he took a research position at the government-owned Tokyo Metropolitan Isotope Research Institute. During his fourteen years there, he earned a Ph.D. in radiation microbiology from Tokyo University.

Research at the institute was engrossing, but as Yamazaki's seniority increased he had to attend "so many administrative meetings about projects and planning that I couldn't keep my experiments going."

He shared this frustration with a friend who had just returned from several years in New York and knew about a fellowship at Memorial Sloan-Kettering Cancer Center. The only catch was that it was in an immunogenetics laboratory — something quite unrelated to what Yamazaki was doing. What was appealing, however, was the opportunity to carry out research without administrative distractions.

The radiomicrobiologist quickly took a course to learn as much as possible about fundamentals of immunology and genetics, while also reviewing related techniques for using animals. When he arrived at Sloan-Kettering, he was given two projects. One was in the immunogenetics laboratory of Dr. Edward A. Boyse, now at the University of Arizona. In the evenings he worked on a second project, inspired by the late Dr. Lewis Thomas, a former chairman of Monell who at the time was president of Sloan-Kettering. Together, Thomas and Boyse were to become Yamazaki's mentors and longtime colleagues.

Thomas, whose interest in olfaction began during his medical residency in neurology, had observed a similarity between the olfactory and immune systems: both have the ability to identify some aspect of individuality. He noted that hounds track the scent of a specific human across a field containing numerous other human odors. This suggested that each individual has a

Kunio Yamazaki, Ph.D.

distinctive odor and that the olfactory system is capable of discriminating these odors from one another.

Comparably, the immune system can distinguish self from non-self, which is a fundamental property of individual recognition. This capacity is determined in large part by a linked set of more than 50 genes known as the Major Histocompatibility Complex (MHC). Genes in the MHC are critical to many aspects of immune function. Among other things, they provide the blueprint for proteins that stud the surface of most cells in the body. These protein markers enable the immune system to recognize cells that need to be destroyed, such as foreign cells — "invaders"— that do not belong in the body. In addition, because bits of protein from invaders are inserted into the markers after the invaders have entered the body's own cells, infected cells can also be identified and destroyed.

Virtually every individual — with the exception of identical twins — has a unique MHC, due to the extreme diversity of MHC genes. Because the immune system attacks cells that differ at the MHC, successful tissue and organ transplants depend on MHC similarity between donor and recipient.

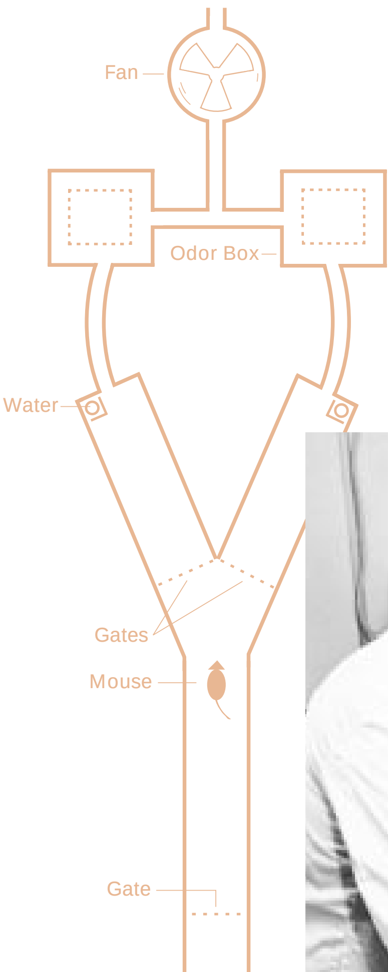
Thomas was intrigued that two systems served the same function: identification of individuality. He proposed the apparently outlandish idea that these two systems might be related to one another, and suggested that the immune-regulating MHC genes also control olfactory individuality.

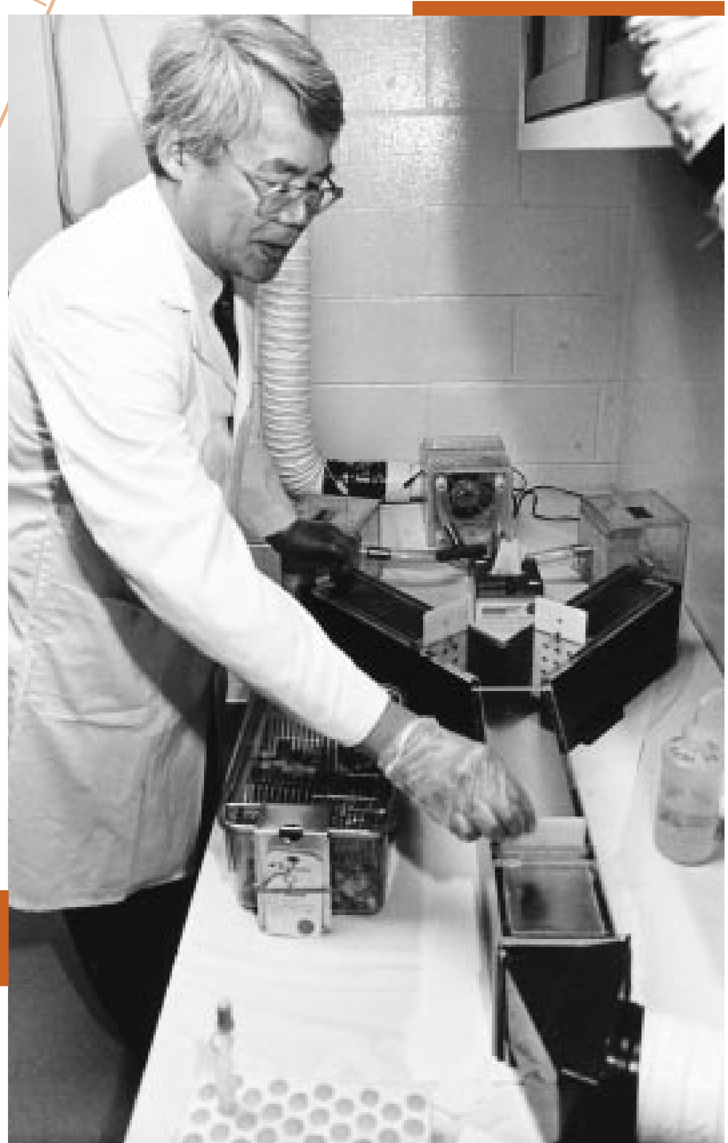
This was the puzzle waiting for Yamazaki. Thomas had discussed his ideas with Boyse, who used many different strains of inbred mice in his laboratory. Among the mice were two strains which were genetically identical *except* for MHC genes. After he had finished his day's work, Yamazaki would remain in the laboratory to study mating preferences in the inbred mouse colonies. He observed more than 2000 mating trios. Each trio consisted of two female mice, identical except for their MHC genes, and one male who had the same MHC genes as one of the females.

Yamazaki found that males preferred to mate with females possessing MHC genes different from their own. This was the first observation of mating preference in mice that could be traced to variation of a specific gene or gene complex. This mating bias is believed to reduce inbreeding and maintain genetic diversity in immune systems of offspring, enabling them to better resist bacterial and viral attack.

The next step was to determine whether the mating preference might be determined by olfactory cues. But in the midst of this exciting work, Yamazaki's fellowship came to an end and the institute in Tokyo wanted the capable and experienced scientist to return. But Thomas, Boyse, and Yamazaki himself had other ideas. Letters were sent to the institute's president asking if Yamazaki could stay another year. The response was negative. Thomas even asked the mayor of New York to write, hoping that this high-level intercession might work. The answer was still no.

"I couldn't figure out why they wanted me back so much," Yamazaki muses. Even though it was difficult for him to leave his work in New York, he returned to Japan.





▲ Using a Y-maze that he designed, Yamazaki trains mice to distinguish which arm contains urine from mice differing at the MHC.

Once there, he found that his thoughts kept returning to the exciting experiments he had left behind in New York.

Scientists at Sloan-Kettering missed Yamazaki's input as much as he missed the work there, and in 1976 Thomas offered him a permanent position. After three months of deliberation, Yamazaki returned to New York to resume the research that had so intrigued him.

With increased focus on the role of olfactory cues, Yamazaki and his colleagues established that mice could be trained to distinguish between the odor of urine of mice differing only at the MHC. This showed that the MHC is involved in individual odor production and that the identifying odorants are contained in urine.

As busy as Thomas was, he stopped in Yamazaki's lab early every morning and then returned late in the evening to discuss how the work was going. "He was so interested," Yamazaki recalls, "that every day he found time to look at our data."

The fascinating results of the team at Sloan-Kettering attracted the attention of Dr. Gary K. Beauchamp, now Monell's director, who also had been studying individual recognition. Yamazaki gave a talk at Monell, and soon afterward was invited to transfer his work to Philadelphia. "It seemed appropriate," he says, "because what I was doing at Sloan-Kettering was not typical research for a cancer center. At Monell, which is a mecca for olfaction research, my work fit in well."

Over the years, Yamazaki has maintained his collaboration with Boyse and with Dr. Judith Bard, also at the University of Arizona. Thomas continued to be involved until his death in 1993. Together with Beauchamp and other Monell scientists, they went on to show that MHC-determined odors (now termed "odortypes") can be altered by varying a single gene within the MHC.

Pamela L. Goodman

A relevant question is whether odortypes play a role in human communication and behavior. While urine is most likely not an appropriate source of information for humans, Yamazaki's research suggests that odor cues regarding identity may also be conveyed through more suitable mediums, such as sweat, saliva, or mother's milk.

A recent study indicates that human fetuses have distinctive odortypes. It is possible that mothers and infants may use smell as an early form of identification, and perhaps as an aid to bonding.

The link between olfactory communication and immunogenetics has fascinated Yamazaki since his arrival in America. "What Dr. Thomas suggested more than 22 years ago," Yamazaki notes, "we now find is correct." Pursuing the extent of this will most likely consume many years, while Yamazaki continues to help visitors from abroad feel welcome at Monell. ■

Genes•Gender•Generation

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functional odor receptors.

It might seem that not being able to smell just one compound is a minor matter. However, if that single compound is the final top-note of a fine fragrance or wine, then a person who cannot smell the compound is missing out on the ability to fully appreciate the total experience.

In the real-world complexities of smell, one is rarely confronted with a single compound. Typically, what we smell is a mixture of stimuli.

On the flip side, if the compound is the single malodor in spoiled food, then most diners would reject a meal that the person with the specific anosmia considers quite all right to eat.

Gender also influences how individuals perceive odor. Research studies show that females are better at identifying odors. When men and women are tested, females find pleasant odors to be more pleasant and unpleasant odors to be more unpleasant. Females also have lower thresholds for many odorants, and they generally rate the intensity of an odor higher than do men.

In the not-so-distant past, it was thought that greater involvement of females with meal preparation was the driving force behind olfactory-based gender differences. However, differences in responsiveness to odors have been noted in newborns — before infants of either sex have done any cooking — thus suggesting a biological basis for at least some gender-related olfactory differences.

Age is yet another factor. Studies show that ability to detect odors declines later in life. Whether a decline occurs for every odor is not yet certain. For the aging, loss and diminution of sensitivity might result in a situation where olfactory experiences are distorted.

Are age-related decreases in olfactory sensitivity a direct result of the aging process? Or do other factors associated with age — such as long-term exposure to environmental toxins and increased likelihood of disease — also contribute to olfactory changes described in the elderly?

Every individual is unique. The way information about smell is received and processed is influenced by heredity, sex, age, and interaction with the surrounding world. Additional influences on olfaction include frequency of exposure, early flavor experience, flavor aversions associated with illness, and environmental factors such as chemical pollutants. Further, numerous disease states, including some not directly related to chemosensation, such as diabetes, also influence how odors are perceived.

The result is a sensory world that is different for each and every one of us. ■

Another Scientist's Perspective...

Beverly J. Cowart, Ph.D.
Director
Monell-Jefferson
Taste & Smell Clinic

Basic research often leads to development of techniques, instruments and insights that have many important implications for clinical care. Conversely, the study of disease and dysfunction adds substantially to understanding fundamental processes that underlie normal function.

Although Monell is a basic research institution, its scientists have long recognized the value of clinical interactions. For that reason, they frequently engage in clinical studies of chemosensory perception and preferences.

Involvement of basic scientists in clinical studies is greatly facilitated by close ties with a medical institution. Ten years ago, Monell established a formal collaboration with The Jefferson Medical College, primarily through its Department of Otolaryngology, Head & Neck Surgery, when the National Institutes of Health provided funds to establish a Chemosensory Clinical Research Center (CCRC).

Our core facility is the Monell-Jefferson Taste & Smell Clinic, located within Jefferson's Department of Otolaryngology. Through the application of measurement techniques developed in basic studies of human smell and taste function, clinicians are able to obtain unique information about chemosensory function in their patients. This is valuable for both diagnostic and prognostic purposes. At the same time, assessment of patients with primary complaints of smell or taste dysfunction adds substantially to scientific understanding of the forms these dysfunctions can take, their causes, and the ability of these sensory systems to recover from various insults — all of which have implications for basic mechanisms.

The clinic provides a point of contact and coordination for clinicians and basic scientists. Collaborative interactions spring naturally from this foundation and frequently build upon themselves. Ongoing studies of olfaction at the cellular and molecular levels are a prime example. Such studies had previously been limited to animal models, but given the accessibility of human olfactory epithelium for biopsy, biophysicists at Monell wondered whether human biopsies might provide suitable material for extending their cellular studies.

Otolaryngologists at Jefferson suggested that initially this question might be addressed through studies of tissue from patients who were already undergoing tissue removal in the course of elective sinus surgery. When it was demonstrated that viable human olfactory neurons could be isolated from this biopsied tissue, and that the neurons responded to odor stimulation with changes in intracellular calcium, a study plan was developed in which collaborating physicians obtained volunteers for use in studies by research scientists at Monell. This work has now revealed that although human olfactory neurons share many of the response characteristics observed in rats, they also possess their own unique properties.

The success of these basic research studies has in turn led to application of similar techniques to clinical populations, and also to additional collaborations between Monell scientists and physicians in Jefferson's Departments of Radiology and Geriatric Psychiatry.

In the first instance, we were able to take advantage of the fortuitous identification of two anosmic individuals with Kallmann syndrome at the Taste & Smell Clinic. In Radiology, examination by magnetic resonance imaging confirmed the existence of central olfactory system pathology (i.e., incomplete or missing olfactory bulbs) consistent with this syndrome. Significantly, however, assessment by Monell scientists of the responses of isolated olfactory neurons from these patients demonstrated that some neurons were able to respond selectively to odors, indicating that they were functionally mature. This suggests that contact with the olfactory bulbs is not necessary for maturation of human olfactory neurons.

In another instance of collaboration, this time with the Department of Geriatric Psychiatry, we are able to assess calcium regulation in olfactory neurons obtained through biopsies in patients who are suffering from Alzheimer's disease. The ability to study these cells is important because it has been hypothesized that irregularities in neuronal calcium regulation provide the final common pathway for the neuropathological changes associated with Alzheimer's disease.

Clearly, the work in Monell's CCRC epitomizes the mutual benefits, and scientific and clinical value, of cooperative efforts between research scientists and clinicians. Clinical studies are a major facet of Monell's overall research program. With completion of our new human testing facility, which includes a room designated for clinical assessments, this important aspect of science at Monell will continue to grow. ■

We Smell Like Garlic, Mom!

Exposure to characteristics of the mother's diet during early development may influence how a young child later responds to flavors and foods, and might also play a role in determining future food preferences. Previous research from Dr. Mennella's laboratory has begun to describe how flavors of foods consumed by the mother are conveyed through breast milk. The current study extends those findings to demonstrate that at least some characteristics of the mother's diet also are transmitted to amniotic fluid.

Forty-five minutes prior to routine amniocentesis, five pregnant women swallowed capsules containing garlic extract. Another five women swallowed placebo capsules. Later, a sensory panel of adults was asked to smell and compare pairs of amniotic fluid samples — one from a woman who had swallowed garlic and the other from a woman given the placebo. In four of the five pairs, the amniotic fluid from the woman who had consumed garlic was judged to smell stronger or more like garlic.

Whether *in utero* exposure to garlic alters the response of the postpartum human infant to garlic-flavored foods is not yet known. Earlier experiments by Dr. Mennella and Dr. Beauchamp have shown that infants are exposed to, and perhaps learn about, flavors including garlic soon after birth. The present study suggests that humans might start learning about characteristic flavors of their mothers' diets even earlier, while still in the womb.

Garlic ingestion by pregnant women alters the odor of amniotic fluid. Julie A. Mennella, Anthony Johnson (Thomas Jefferson University), and Gary K. Beauchamp. *Chemical Senses, 1995, 20, 207-209.*

Axillary Odors Tell All

Chemical communication in humans is not well understood, but might involve axillary (underarm) secretions and their odors. Humans can identify their own axillary odors and can distinguish odors of their own spouses and babies. Further, extracts from human axillary secretions can alter the length and timing of the female menstrual cycle, implying that certain axillary compounds may act as primer pheromones, which are chemical signals that have long-term effects on the recipient.

Previous research by the authors suggests that (E)-3-methyl-2-hexenoic acid (E-3M2H) and other volatile acids are responsible for characteristic human axillary odor. The current studies identified two proteins — Apocrine Secretion Odor-Binding Protein 1 and 2 (ASOB1 and 2) — in axillary secretions. One function of these proteins is to bind and carry 3M2H from axillary apocrine glands to the skin's surface.

Both this study and others using different mammals have shown that volatile odor molecules can be transported from the site of secretion by specialized carrier proteins. Thus, there are interesting chemical parallels between human axillary secretions and non-human mammalian secretions known to be involved in chemical communication. Future research will continue to characterize the chemistry of axillary secretions and explore the mechanism by which they alter menstrual cycles. The chemistry of additional body fluids (such as tears, nasal secretions, saliva) that also contain an ASOB1-type protein is also being explored.

Proteinaceous precursors of human axillary odor: isolation of two novel odor-binding proteins.
A.I. Spielman, X.-N. Zeng, J.J. Leyden (University of Pennsylvania), and G. Preti. *Experientia, 1995, 51, 40-47.*