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THE AROMA-CHOLOGY REVIEW℠

FOCUSING ON OLFACTORY DEVELOPMENTS AROUND THE WORLD

THE INFLUENCE OF HUMAN BODY ODORS ON MOOD AND MEMORY

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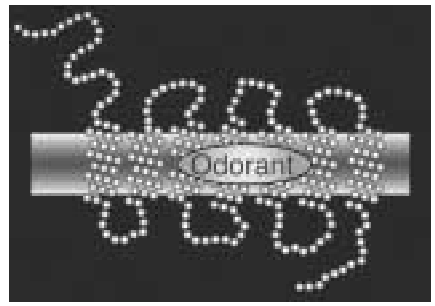
I stumbled upon olfactory research by accident about four years ago. I was doing an online literature search on a social psychology topic and noticed instead studies on the possible role of odors in regulating reproductive cycles in women. However, further search revealed that only a small fraction of the olfactory literature had to do with humans, and even less had to do with psychological, behavioral, or developmental research in humans. Fascinated by the topic, and encouraged by an enthusiastic and inspiring advisor, I decided to do my dissertation on human olfactory communication. I would like to thank the Olfactory Research Fund for the 1998 Tova Fellowship which helped to support my dissertation work.

Scents have been posited to influence people's mood (e.g., Rotton, Barry, Frey & Soler, 1978; Schiffman, Sallely-Miller, Suggs & Graham, 1995), memory (e.g., Ehrlichman & Halpern, 1988; Herz & Cupchik, 1995), perception of other people (e.g., Baron, 1981; McBurney, Levine & Cavanaugh, 1977), and behavior (e.g., Baron, 1980). Much of the evidence supporting these observations has been obtained from studies using synthetic odors, such as fragrances and chemical irritants. Much of that research supports the "hedonic congruency" explanation (e.g., Bower, 1981) which posits that pleasant odors tend to have a positive effect on people, and that odors perceived to be unpleasant tend to have a negative effect. Laboratory evidence concerning possible effects of natural body odors on mood, memory, and the perception of other people is lacking. Available studies on natural body odors have been largely limited to people's ability to identify, through olfactory cues, themselves (e.g., McBurney, Levine, & Cavanaugh, 1977), kin (e.g., MacFarlane, 1975; Russell, 1976), and gender of young adults (e.g., Doty, Orndorff, Leyden & Kligman, 1978; Russell, 1976). A recent report suggests that human-derived biochemicals can influence the neuroendocrine and behavioral systems of women independent of a perceived odor (e.g., Stern & McClintock, 1998).

In the study summarized herein, I explored the effects of underarm odors on the mood and autobiographical memory of young adult men and women in an effort to examine: (1) whether the personality, age, and gender attributes about the odors made by the observers would accurately reflect the actual age and gender of the donors; (2) whether underarm odors obtained from different developmental stages would differentially impact on *continued on page 6*



MOLECULAR MATCHMAKING

Randall R. Reed, Ph.D.
John Hopkins University

The past decade has produced an explosion in our understanding of the fundamental biological mechanisms underlying the detection of olfactory stimuli. Organisms as diverse as humans, mice, fish, flies and worms utilize a similar paradigm to convert an *continued on page 14*

ALSO IN THIS ISSUE

HIGHLIGHTS FROM ACHEMS XXI

Craig B. Warren, Ph.D.
Consultant to the Fragrance Industry

Each spring approximately 500 scientists from 19 countries migrate to Sarasota, Florida to attend the Association for Chemoreception Sciences (AChemS) annual meeting. This year's meeting (April 14 –18), featured over 350 presentations of research findings, special symposia and workshops. The detailed program for the meeting can be found on the web at: http://neuro.fsu.edu/achems/prog99.htm.

The majority of attendees at AChemS are researchers interested in how olfaction works in mammals, fish and insects. The commercial world, on the other hand, is interested in the effect of odor on human behavior (Aroma-Chology), and the relationship between an odorant's molecular structure and its performance in commercial application. This difference in interests is most likely responsible for the poor representation of the commercial community at this meeting. Perhaps it is time to think again about this difference. I feel that our understanding of how olfaction works has reached the point where some commercial applications can be envisioned. The small sampling of slide and poster presentations described below is intended to illustrate this point. Additional information on these presentations and the addresses of the authors can be found on the web site listed above.

Cells That Smell: Functional Cloning of Odorant Receptors

In 1991, two Columbia University researchers, Drs. Richard Axel and Linda Buck discovered around 1000 genes that code for odor receptors. However, for many years it was never possible to show that that the proteins identified by Axel and Buck were in fact odorant receptors as there was no way to show that they would respond to odorants. Two groups now report the functional cloning of olfactory receptors from single olfactory neurons. Dr. Randy Reed, Howard Hughes Medical Institute investigator at Johns Hopkins, presents his findings in this issue of The Aroma-Chology Review. Dr. Kazhuhige Touhara and co-workers at Tokyo University reported similar findings at this year's AChemS meeting. Their study, "Functional Cloning And Reconstitution Of An Odorant Receptor In Single Olfactory Neurons," (Paper No. 7) describes the development of both a cell containing a functional olfactory receptor that releases calcium upon odorant stimulation, and a technique to visualize this calcium release. Using this system, Touhara, et. al. found that one odor molecule was recognized by multiple odorant receptors while, on the other hand, one receptor recognized structurally related odor molecules. They postulate that olfactory receptors recognize structural sub-units in odor molecules and that different combinations of receptors recognize various odorants in a concentration-dependent manner.

Adult Computer Games: Molecular Models of Aldehyde Interactions in the I7 Olfactory Receptor

(Paper No. 263) Using the methods described above, the structure of two olfactory receptors has now been elucidated. Rat olfactory receptor "I7" was found to preferentially bind the odorant, n-octyl aldehyde. Mouse receptor "I7," was found to preferentially bind n-heptyl aldehyde. Yale University researchers Dr. Michael Singer and Dr. Gordon Shepherd have built 3D models of these receptors. The models revealed a pocket that contained lysine as the most prominent residue with hydrophobic residues lining the rest of the pocket. They postulate that the aldehyde portion of the odorant interacts with the lysine part of the pocket while the tail portion of the odorant contacts the hydrophobic residues lining the pocket of the receptor.

Adaptation, Now You Smell It, Now You Don't

This year's meeting featured a symposium entitled *Adaptation in Vision and Olfaction*. "Sensory adaptation allows organisms to reach behavioral equilibrium with the ambient environment and respond primarily to changes in stimulation." So says Monell Chemical Senses Center researcher Dr. Pam Dalton in her presentation entitled "Psychophysical and Behavioral Characteristics of Olfactory Adaptation," (Paper No. 11). Fragrance ingredients are no exception and humans, for example, readily adapt to ionones and musks. This adaptation manifests itself in the perceived loss of sparkle of a fragrance topnote containing an ionone or the perceived loss in intensity of a basenote containing musk. This area is ripe for investigation. For those interested I draw your attention to Dr. Dalton's paper and to University of Maryland researcher, Dr. Frank Zufall's presentation entitled, "Cellular and Molecular Basis of Odorant Adaptation," (Paper No. 12).

> **Our understanding of how olfaction works has reached the point where some commercial applications can be envisioned.**

Artificial Nose, Odor Evaluators Need Not Apply For Unemployment

Artificial noses while intriguing have not been widely accepted by the fragrance industry. This is due in part to reliability problems. After studying biological olfactory systems for years, Dr. John Kauer and coworkers at Tufts University have developed a new approach to the design of artificial olfactory systems.

In two posters: "A Portable Artificial Nose Based on Multiple Olfactory Principles," (Paper No. 138) and "Techniques for Quantifying Information in Olfactory Sensor Arrays," (Paper No. 139) Kauer and coworkers describe the use of photodiode and fluorescent detector arrays to detect odorants. They claim that after training, their portable system is capable of discriminating and identifying a number of odorant substances within seconds.

Understanding How The Inner Brain Processes Odor: Brain Imaging Of Olfactory Function

This AChemS meeting featured a satellite symposia entitled: "Advances in Brain Imaging and Electrophysiological Measurement of Olfactory Function in Health and Disease," chaired by Dr. Dick Doty (Smell and Taste Center, University of Pennsylvania), and a slide session entitled: "Assessment of Olfactory Function in Humans," chaired by Dr. Donald Leopold (Johns Hopkins University).

The visualization techniques covered were positron emission topography (PET), and functional magnetic resonance imaging (fMRI).

Dr. David Zald, University of Minnesota, in a paper entitled: "Positron Emission Studies of Olfaction and Olfactory Hedonics," (Doty Symposium), showed that the amygdala is stimulated by negative (hydrogen sulfide) but not by positive (vanillin) odorants. Zald also found that the left orbital frontal cortex was activated by both positive and negative odorants. This makes one wonder if negative smelling odorants are processed differently by the brain than positive smelling ones.

Dr. Noam Sobel, Stanford University, presented a paper entitled: "Odorants Increase the Variance but not the Amplitude of fMRI Activation in the Ventral Temporal Lobe of the Human," (Paper No. 66). To date, fMRI has failed to reveal increases in odorant induced activation in the primary olfactory (ventral temporal) regions of the brain. Dr. Sobel and his group at Stanford University painstakingly explored this problem and found that odorants did not induce a significant change in amplitude of fMRI activation rather they induced a small but highly significant increase in the variance of activation. According to Dr. Sobel, it is tempting to relate this significant increase in variance of activation to a possible odorant induced reduction in synchronization of neural activity in the ventral temporal region. If these results hold up it may be feasible to use fMRI to explore the deep regions of the brain where olfactory signals are processed.

Dr. B. Kettenmann, University of Erlangen-Nuremberg, of Dr. Gerd Kobal's group presented a paper entitled: "Olfactory Activity in the Human Cingulate Cortex Identified by fMRI," (Paper No 67). The cingulate cortex is involved in the processing of the emotional aspects of pain. Since emotions are also considered crucial for odor perception, Kobal's group hypothesized that the cingulate cortex is also activated by olfactory stimuli. Using vanillin and hydrogen sulfide, Kobal's group found that the cingulate cortex was indeed activated by both of these odorants. Future studies are planned to investigate the correlation between hedonic differences and functional topography of this part of the brain.

Human Olfaction

Have you ever had trouble identifying the components of a simple fragrance accord by smell alone? Dr. David Laing of the University of Western Sydney, Australia tells us that this is to be expected. In a presentation entitled: "Odor Identification in Mixtures; Is Olfactory Working Memory the Ultimate Limitation?" (Paper No. 223), Laing shows that humans have difficulty identifying the components of a mixture containing more than two components. Furthermore, this difficulty is not overcome by training, by using different odorants or by using different test paradigms. Laing and coworkers showed that the addition of neural information about a third component entering memory in less than 600 to 900 milliseconds after that of the initial two components wipes out the identity of the original two components. Dr. Laing attributes this to an olfactory memory overload.

Olfactory communication of fear has been demonstrated in rats. In the study: "Olfactory Communication of Emotion in Humans," (Paper No. 283), J. Haviland, Rutgers University, and Denise Chen, Monell Chemical Senses Center, show that humans can communicate happy and fearful emotions through their axillary odors. Female and male subjects correctly identified fearful odors as fearful, female subjects identified happy odors as happy while male subjects identified both blank and happy odors as happy.

End Note

So this is what they said at the 21st annual AChemS meeting. Twenty-one years ago we only had a sketch of how olfaction worked. This sketch was developed by chemists, biologists and psychophysicists without knowledge of the molecular biology of olfaction. If there is a story to take home from this year's meeting, it is that the molecular biologists and neuroscientists have done their job. What was a sketch is now a picture with considerable detail. This picture describes how odorants interact with receptors to generate signals and how these signals flow to the first olfactory processing station in the brain, the olfactory bulb. For the first time in the history of olfactory science, the necessary technology is in place to decipher the olfactory code, the Holy Grail of olfaction. To decipher the olfactory code is to determine which odorants interact with which receptors to give rise to a particular odor sensation. To deal with the issues of odorant molecular structures and odor sensations will require input from chemists and psychophysicists and so we will have come full circle. ■

InSites on the Web

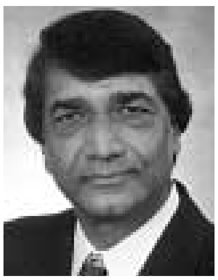
Avery N. Gilbert, Ph.D.

The commercial website from Leffingwell & Associates features promotional information about the company's products, but also "Olfaction A Review in Progress." This is a very informative summary of the biology and chemistry of odor perception at the molecular level. It's well referenced (often with links to other sites) and generously illustrated with anatomical sketches and chemical structures — not to mention fab fanzine pix of leading researchers. Find it at **http://www.leffingwell.com/olfaction.htm**

File under "animal oddities": a study at the Dallas zoo was looking for odors that might attract or repel ocelots. After presenting all sorts of natural odors and getting no response from the big cats, the researchers tried Calvin Klein's Obsession. The ocelots loved it and rolled around in the scent boxes. Details at **http://www.exn.ca/html/templates/mastertop.cfm?ID=19990315-54** ■

OLFACTORY RESEARCH IN THE NEW MILLENNIUM

Dr. H. Rahman Ansari
Quest International
& Chairman, Olfactory Research Fund
Industry Advisory Committee



"Odors are becoming more and more important in the world of scientific experiments and in medicine, and the need of more knowledge of odors will bring more knowledge, as surely as the sun shines." — Alexander Graham Bell

Dr. Jack Mausner, Chairman Olfactory Research Fund, stated back in 1995; "The scientific investigation of the last two decades of the 20th Century explored the positive effects of fragrances on human behavior and have opened new vistas of olfactory research for scientists to explore and conquer in the 21st Century." Almost five years since then, we now stand at the doorstep of the 21st Century. We find ourselves in the midst of a sensory revolution. Getting in touch with our senses has led to the realization of the importance of the sense of smell in our daily lives. This realization has seized the attention of scientific as well as business communities. The scientific research in olfaction during the past 20 years has already helped to unfold some of the deepest mysteries of the sense of smell. But a lot more still remains to be done!

Aroma-chology has proven to be more than a fad or a shooting star on the horizon of the now passing 20th Century. As we approach the new millennium, we witness an increasing interest in the sense of smell both from scientists and consumers. While researchers are exploring the fundamentals and better understanding of olfaction, the visionary consumer product developing companies are translating these findings and their associated benefits into a myriad of innovative new products. Aroma-chology has already started to contribute to our overall well being and enrichment of our daily lives; it has proved its functional value. But we have only scratched the surface!

The developments referred to are not taking place in a sterile vacuum. A multidimensional revolution is in the making; we are amidst a big change explicitly driven by emerging technologies. To quote Tom Ford, Creative Director of Gucci: "It is all about technology, computers, video conferencing. We are going to live in a more graphic world. We will be surrounded by flat screen television that will allow us, at a whim, to recolor and redecorate our surroundings, possibly even ourselves." Take this concept further and visualize buildings in tune with inhabitants. Intelligent glass will darken or lighten in response to sunlight. Polymers in the color of paint will change with room temperature or lighting. Computerized scent release systems will fragrance the home environment to create a customized ambiance. The graphic world will be a product of the appropriate interaction of all our heightened senses — sight, sound, touch and smell — in harmony to create balance around us and to enrich our sense of well being. It is imperative that the changes brought about through high technology are appropriately balanced with spirituality and sense of well being.

"When our mind is peaceful, inner energies wake up and work miracles for us — without any conscious efforts on our part." — Deepak Chopra

It is our belief that "emotional intelligence" is more important than IQ. This realization is now gaining ground and general acceptability even in the corporate world. The world of fragrance is a world of emotions and human psyche. We know that fragrance is pure emotional communication. It can stimulate, re-energize, rebalance or it can relax, calm and soothe. Consequently, a better understanding of the sense of smell and its psychological implications have the potential to provide the necessary pathway to the exploration of emotional intelligence.

Some other promising new areas of research in the new millennium may very well be the areas of personal and social well being and education. For example, the use of fragrances in pain management, physiotherapy and psychiatry/psychology. Maybe even as a therapy in smoking cessation programs and identification of specific odors with color, so that visually impaired can "see" color as stated by a colleague of mine.

Concurrent to the above, accelerated developments in basic research on olfaction would target transduction mechanism, human olfactory genomes, sophistication in brain imaging in relation to odor and understanding olfactory memory and cognition.

The Olfactory Research Fund has supported innovative work on the sense of smell and human response to odors since 1982. During this period, several leading national and international scientists, psychologists and anthropologists have researched and published their funded work in leading scientific journals. The body of this work has shown that fragrances have effects on social interaction, memory connection, alertness, stress reduction, relaxation, quality of sleep, aging and quality of life and moods to mention a few. This pioneering work has laid a solid foundation for the scientist to build further on these results.

With the above mentioned olfactory research initiatives, the challenge to the consumer product industry is how to translate today's science into tomorrow's new consumer products with readily perceived consumer benefits. Applied research in the industry must pay attention and capitalize on trends of spirituality, harmony, balance and well being. Focus on natural alternatives for health involving fragrances would lead to good science and profitable business.

As a parting comment, I had the pleasure of attending this year's Association for Chemoreception Sciences Symposium and was impressed with the research activity on smell and taste (366 papers/posters). Even more inspirational was to see a large number of young and dedicated researchers pursuing the science of olfaction. The future of the exploration of sense of smell has been passed on to the next generation belonging to the new millennium. ■

MILLENNIUM

As the 20th Century draws to a close, the editors of *The Aroma-Chology Review* asked the Chairmen of the Olfactory Research Fund's Industry and Scientific Advisory Committees, along with the President of the Fund, to share their insights on the status of olfactory research and sensory trends we can expect to see in the future.

THINKING OUT LOUD

Annette Green
President, Olfactory Research Fund

Blame it on the Internet, changing public awareness or the demands of the over-saturated marketplace, but a trend is emerging which will determine the success or failure of products and services in the new millennium. It is focused on a surge of public interest in the senses in general and most particularly the sense of smell.

The Olfactory Research Fund must be credited with slowly and surely changing perceptions about how important the sense of smell is to each of us.

When I think of the subliminal impact the Olfactory Research Fund has had since its inception in 1981, I cannot help but remember an overall indifference. The very subject of the sense of smell was rarely addressed. In fact, even before the Fund was founded, we often presented sensory experts to the industry at informative symposia, and I can assure you there were those in the audience who thought the subject irrelevant. Not that recognizing the importance of the sense of smell was an easy sell. It wasn't then — and it isn't now — this, despite the consumers' increasingly obvious desire for fragrance experiences that are more holistic in nature rather then self-indulgent.

It is becoming increasingly obvious that much of the success in new product development — whether in the bath and body category or fragrance concepts — results from a wide range of ingredient choices to enhance the quality of life, with extra special benefits. Consumers have shown their enthusiasm, and will continue to do so, in my opinion, for fragrances which reduce stress, increase alertness, and energize.

If the Olfactory Research Fund has done nothing more than educate the public about the psychological roles of different fragrance ingredients, it will have provided an indispensable service to the industry. Be assured, however, the Fund does so much more. It is committed to cutting edge research which holds enormous potential for fragrance in the 21st century.

This is not a time for the faint of heart. The information age in which we find ourselves has placed new demands on the how, why, when and if women and men include fragrance in their fast-paced lives.

Fortunately, the fragrance industry has the vehicle in the Olfactory Research Fund to develop and substantiate an extraordinary new array of fragrance experiences. The Fund deserves the continuing support of the members of the international fragrance industry to trail-blaze the paths through research and public education to olfactory successes unimagined in the past. ■

FUTURE DIRECTIONS FOR USE OF ODORS IN OVERALL HEALTH

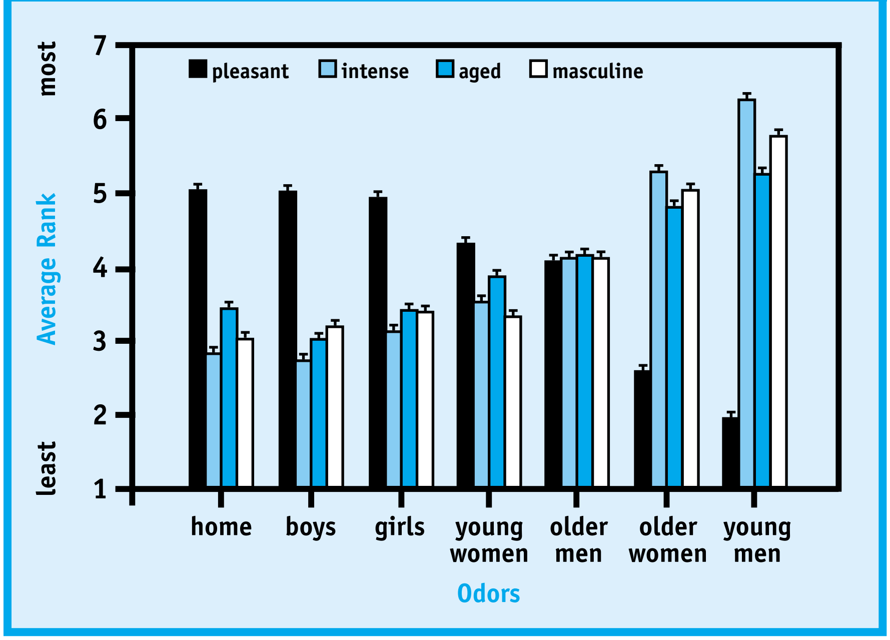
Susan S. Schiffman, Ph.D.
Duke University Medical School
& Chairman, Olfactory Research Fund Scientific Advisory Committee

Ten years ago there was little interest in Western Societies in use of aromas as a means of improving mood and overall health. Today, aromas are being utilized with greater frequency as an adjunct to conventional treatments for a variety of health conditions. Odorous compounds, especially volatile essential plant oils, are administered through inhalation or topical application to the skin (with or without relaxation therapies) to deal with the emotional aspects of illness. Inhalation of fragrant plant extracts has been used since ancient times to cope with a variety of health conditions. The desire for aromatic oils from medicinal plants was one of the reasons for the opening of trade routes by the Romans to East India and Arabia. Traditional Indian medicine known as ayurveda historically incorporated aromatic oils in its practice. The invasions of South America by the conquistadors led to the discovery of more plants that are aromatic and oils. North American Indians also used aromatic oils and produced their own herbal remedies.

Many claims are currently being made for use of aromas to cope with health conditions but more scientific data and improved methodology are necessary to assess these claims. Odors are gaining more frequent use in health care settings to provide an atmosphere of relaxation and comfort. An objective study of patients admitted to an intensive care unit found that patients who were exposed to the odor of the essential oil lavender reported significantly greater improvements in mood and perceived levels of anxiety than those who received massage therapy or a period of rest (Dunn et al., 1995). Six sessions of exposure to aromas produced a significant improvement in 58 cancer patients as measured by the Hospital Anxiety and Depression Scale (HADS) (Kite et al., 1998). Mean anxiety, depression, and combined scores dropped from 8.9 to 6.2, 6.1 to 4.0, and 15.0 to 10.2, respectively, $P < 0.001$, and at least half of the patients reported a significant improvement in the eight most commonly assessed symptoms. Odors have also been used to complement traditional medical treatments for other conditions including behavior problems and stress. However, these efforts require far more scientific documentation.

In the future, I think that more attention will be given to the potential of odors to improve the outcome of health problems by impacting mood, brain activity, ***continued on page 11***

FIGURE 1. Average Rank of Perceived Pleasantness, Intensity, Donor Age, and Donor Masculinity



THE INFLUENCE OF HUMAN BODY ODORS ON MOOD AND MEMORY

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observers' mood and memories; and (3) whether the olfactory effect on mood and memory, if any, would be contingent upon how the odors were judged or evaluated.

EXPERIMENTAL METHOD

Underarm odors were collected from 30 donors coming from six age by gender groups: (1) pre-pubertal boys and (2) girls, (3) college-aged young adult men and (4) women, and (5) older adult men and (6) post-menopausal women, respectively. Odors collected from the homes of each donor were pooled as the control. Donors were instructed not to use any fragrances / antiperspirant, not to shave their underarms 4 days prior to odor collection, and not to eat any odorous food such as garlic and onion. They took a shower the evening before each odor collection with fragrance-free soap and shampoo. The next morning, they taped a clean 4x4 inch gauze pad around each armpit, which was kept in position for 8 to 10 hours. Donors wore a new T-shirt to avoid contamination from laundry detergent. Once collected, odor samples were frozen in enclosed glass jars at -80 ° C until testing.

Three hundred and eight undergraduates participated as odor observers. They were randomly assigned to one of 7 odor groups: odors of boys, girls, young men, young women, older men, older women, and home odors, in a double-blind design. Each group of odors was contained in a large glass container labeled with a number on the bottom. Odor observers rated their mood before and after they smelled the odor, recalled a dream and a significant event that had occurred to them after they smelled the same odor, and performed 7 odor-ranking tasks in which they ranked all 7 odors by their perceived intensity, pleasantness, age, gender, and other qualities. A Principal Components Analysis which represents the mood questions into smaller categories showed that subjects' moods fell into 3 categories: depressive, hostile, and positive moods. Dream and event recollections were coded for the number of references to various types of characters (e.g., close acquaintances, family members, strangers) and the number of references to positive and negative emotion words. Changes in moods as a result of an odor was examined by hierarchical multiple regression analyses that statistically controlled for the effect of perceived odor intensity. Frequencies of a type of character mentioned, as a result of an odor, were then examined by the same method, which controlled, in addition to odor intensity, the length of the recall. Similarly, the number of positive and negative emotion words were examined by the same method, which controlled, in addition to odor intensity and the length of the recall, previous mood of the subjects. Analyses of covariance that yielded the same results as the regression analyses were also performed to yield the mean scores. How people ranked the odors relative to each other on odor-ranking tasks was analyzed by repeated measures ANOVAs.

RESULTS

Results showed that (1) observers were not able to identify the age or gender per se, but were able to discriminate between underarm odors based on their perceived intensity and/or pleasantness (Chen & Haviland-Jones, 1998), (2) underarm odors from different developmental stages differentially impacted on mood and memory (Chen & Haviland-Jones, 1998, 1999), and (3) such impact was consistent with the underlying characteristics of the donors but were independent of how the odors were judged by the observers.

More specifically, odors of homes and of children were perceived to be most pleasant, least intense, youngest in age, and most feminine, whereas odors of older women and young men were perceived to be most unpleasant, intense, oldest, and masculine (Fig.1). Odors of young women and older men were in the middle.

Although both odors of older women and young men were perceived most intense, the former produced a significant decrease in ratings of depression whereas the latter did not; odors of older women produced lower ratings of depression than odors of young men (Fig.2). Odors of older adults produced lower ratings of depression than did odors of young adults, and odors of women produced lower ratings of depression than did odors of men. All the comparisons remained significantly different after controlling for the observers' perceived odor intensity, pleasantness, perceived donor age, gender, and other qualities.

The effects of odors on the types of characters and emotions mentioned in memory recollections were mainly

Body odors carrying social biological information differentially bias odor recipients' moods and selectively guide the content of their memory recollections.

observed in female subjects. For example, even after the odor intensity was controlled for, odors of donors similar in age produced more references to close acquaintances than the control odor. Odors of donors older in age produced more references to both family members and strangers than did odors of donors similar in age. After controlling for odor intensity and observers' previous moods, odors of young adults produced significantly more negative emotion-words in event recalls than did odors of children or older adults.

The finding that underarm odors of older women were perceived similar to odors of young men appeared to challenge the existing assumption that apocrine gland activities (responsible for producing the underarm secretions) stop functioning in old age. The finding that underarm odors of older women were perceived as more similar to the odors of young men than to the odors of young women, and that odors of older men were perceived more similar to odors of young women than to odors of young men, may reflect changes in the hormonal status of individuals in old age.

The findings in this work also suggest that odors may be processed on different cognitive levels depending on the nature of the task. When people are asked to consciously discriminate between odors, their decision is largely guided by the more apparent and immediate olfactory qualities such as intensity and pleasantness. When not directly focused on the odors, people's moods, the types of relationship mentioned in the memory recollections and their emotional content, reflect the age and gender information in the odors.

Van Toller and Kendal-Reed (1995) made the distinction between an olfactory experience that lends itself to linguistic description (e.g., naming or labeling an odor) versus an olfactory experience that is intuitive and non-linguistic (e.g., emotional relationship with one's grandmother evoked by the smell of lavender water). Perhaps the mood and autobiographical memory tasks used in this work provide such a social linguistic environment for people to describe something that may be social but may not be intrinsically linguistic.

In conclusion, the findings of the present work replicate and extend past research on olfactory identification of gender. This work examines olfactory identification of gender in conjunction with age from a developmental perspective, and examines the effects of age and gender on people's mood and memories. It demonstrates that body odors carrying social biological information differentially bias odor recipients' moods and selectively guide the content of their memory recollections.

FIGURE 2. Changes in Depressive Mood by Odor Conditions

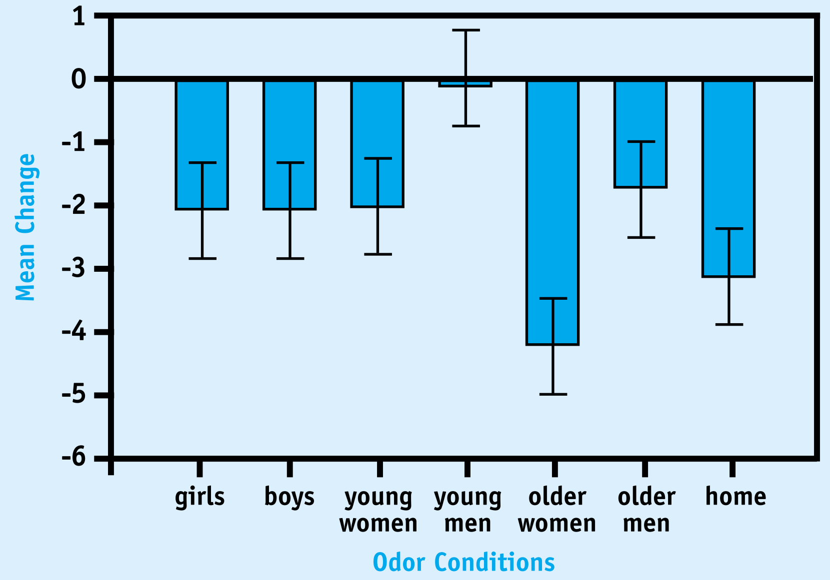

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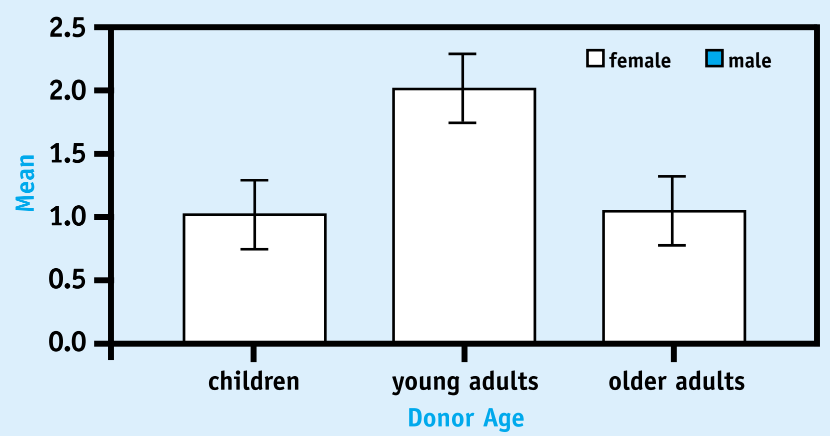
FIGURE 3. Mean Number of Negative Emotion Words in Events by Donor Age in Female Subjects



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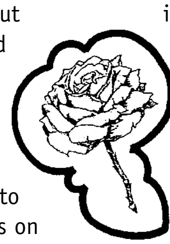
IMAGINING ODORS

A paper published recently in the *Journal of Mental Imagery* has shed light on people's ability to form mental images of odor. The study used a newly developed questionnaire to quantify the experience of olfactory mental imagery. The results show that perfumers and other fragrance experts have more vivid and realistic mental images of odor than non-experts. The investigators suggest that imagining an odor may activate the same brain processes used during actual odor perception.

A team of three sensory psychologists led by Dr. Avery N. Gilbert of Synesthetics, Inc., examined 27 fragrance experts and a control group of 95 non-experts. Study participants filled out the Vividness of Olfactory Imagery Questionnaire (or VOIQ), a sixteen-item inventory that asks a person to imagine specific odorous scenes and to rate the vividness and realism of the imagined scents on a five-point scale. Participants also completed the VVIQ, a widely-used questionnaire that measures the vividness of visual mental imagery.

Says Gilbert, "Other experiments have examined odor imagery but until now there was no method to directly assess a person's ability to create mental images of odor. The VOIQ fills that gap."

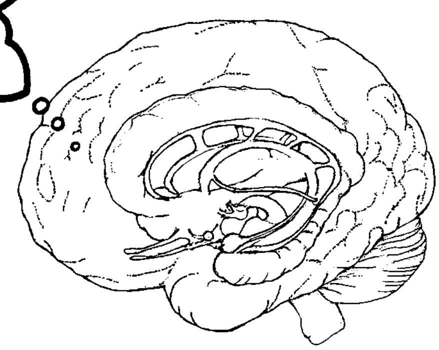


Fragrance experts proved to have significantly better scores on the VOIQ, indicating that their olfactory imagery ability was better than that of non-experts. In contrast, the two groups did not differ on visual imagery ability. Gilbert attributes the olfactory differences to the fact that perfumers are trained in the sensory evaluation of scents, and have substantial and ongoing experience in olfactory judgments, both real and imagined.

"The ability to anticipate the perceptual character of a novel odor mixture is a key asset for a professional fragrance expert," says Gilbert. "We find that perfumers do better on olfactory imagination in the same way that people with specialized visual and motor skills typically score better on the visual test."

In the field of visual neuroscience it is generally believed that the parts of the brain involved in visual perception are also active when a person imagines a visual scene. Gilbert suggests that the same may be true of olfaction. Recent studies show that mental representations of odor share many properties with actual odor perception. For example, when people rate the similarity of imagined odors the results are the same as when they rate the similarity of perceived odors. Also, imagined odors interact with one another as do perceived odors: the sensory phenomenon known as "mixture suppression" is found in both actual and imagined odors.

Gilbert believes the existence of brain mechanisms common to odor perception and odor imagination has profound implications for consumer behavior. "The very act of remembering a brand's fragrance activates an entire range of psychophysical responses in the consumer," he says. "These include emotions and correlated sensory impressions such as texture, color, and tactile feel. When fragrance is fully integrated into the brand image, even imagining the scent is enough to set off a cascade of sensory impressions." ■



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FOCUS ON THE FUND

NEWS AND EVENTS AT THE OLFACTORY RESEARCH FUND

FUND CREATES INTERNATIONAL "BLUE RIBBON" ADVISORY COUNCIL

As we approach the 21st century with its increasing focus on alternative health care, well-being and the important role of the senses in our lives, it seems a most propitious time for the Olfactory Research Fund to create an **International "Blue Ribbon" Advisory Council**. The Council will be composed of cutting edge visionaries, with an interest in all the senses and their role in improving quality of life.

Members of the Council are being drawn from diverse disciplines including the arts, the sciences, public affairs, sports & entertainment and the business community. The Council's primary mission is to establish an international exchange based on the growing importance of all the senses, and most particularly the sense of smell.

To date the following individuals have agreed to serve on the Council:

- **Dr. Robert Batcha**, President, Museum of Television & Radio
- **Francois Berthoud**, Managing Director, Group ISIPCA (Versailles, France)
- **Dr. Linda Buck**, Asst. Professor of Neurobiology, Harvard Medical School
- **Fabrizio Ferri**, Photographer, Universita dell' Imagine (Milan, Italy)
- **Dr. Alan Gelperin**, Research Scientist, Lucent Technologies
- **E. Edward Kavanaugh**, President, CTFA
- **Dr. Donald Leopold**, Director of Rhinology, Johns Hopkins University
- **Dr. Nathan Lewis**, Professor of Chemistry, California Institute of Technology
- **Beverly Sills**, Chairman, Lincoln Center for the Performing Arts
- **Steven Sunshine**, President & CEO, Cyrano Sciences, Inc.
- **Dr. Lionel Tiger**, Darwin Professor of Anthropology, Rutgers University
- **Edith Weiner**, President, Weiner Edrich Brown, Inc.

Twice a year, the Fund will send a questionnaire to Council members regarding sensory trends they may have observed or become aware of within their respective disciplines and travels. A report will then be generated based on responses received and sent to the Fund's corporate sponsors. ■

THE SIXTH ANNUAL "NIGHT OF HONORS" GALA

The **6th Annual Night of Honors Gala** is scheduled for Tuesday, December 7, 1999 at The Pierre Hotel in New York. The Fund is pleased to announce that **Stephanie George**, Executive Vice President, Group Publisher, Fairchild Publications will serve as the Honorary Chairperson of the gala which is themed "Sensations for the Millennium." Honorees include:

Corporate Vision Award – Avon Products Inc. Award to be accepted by Andrea Jung, President

Retail Sense of Smell Award – Bloomingdale's. Award to be accepted by Michael Gould, Chairman & CEO

Scientific Sense of Smell Award – Dr. Steven Van Toller, University of Warwick

Richard B. Salomon Award – Annette Green & Linda Dyett, authors of *Secrets of Aromatic Jewelry*

In addition, **Medals of Honor** will be presented in recognition of corporate contributions to the Olfactory Research Fund. ■

SENSORY SOLUTIONS: SPORTS REHAB PROGRAM UNDER DEVELOPMENT

The ever-growing interest in fitness and sports has led to one of the most interesting arenas for the potential application of Aroma-Chology research and the development of the Fund's newest public service educational program: **"Sensory Solutions: Sports Rehab."** This unique pilot project will study the effect of aroma on sport rehabilitation particularly during physical therapy. Findings will be used to develop and disseminate a self-help kit that will address the emotional aspects of the healing process for patients during the final recovery stage of sports-related injuries through rehabilitation programs in hospitals, sports medicine facilities and general health care settings.

This year the Olfactory Research Fund is supporting two research studies, which are the first in a series that will lay the scientific groundwork necessary to fully develop the "Sensory Solutions: Sports Rehab" program. A description of the studies can be found in the "Research Update" column on page 10. ■

NEW BOARD MEMBERS APPOINTED

The Board of Directors has unanimously elected and welcomes the following new members to the Board:

- **Julian Boyden**, Chairman, President & CEO – Bush Boake Allen, Inc.
- **Cosimo Policastro**, Sr. Vice President, Fine Fragrance – Givaudan-Roure
- **Roger Schmid**, President Fragrances & Cosmetics Worldwide – Dragoco
- **Judy Zaunbrecher**, Director Worldwide Home Care Product Development – S.C. Johnson & Son

In addition, the Board has appointed **Brian Schmalz**, President, Haarmann & Reimer Fragrance Division, North America to the position of Vice Chairman on the Executive Committee of the Board. Mr. Schmalz has served as a member of the Board since March 1998. ■

SECOND ANNUAL GOLF INVITATIONAL PLANNED

The Fund will hold its 2nd Annual Golf Invitational on Thursday, September 23, 1999 at the Fiddler's Elbow Country Club in Bedminster Township, NJ. The top "Hole-In-One" Prize will be a 2000 Volvo C70 Convertible sponsored by Volvo Cars of North America. Invitations were mailed to industry members in May. ■

For more news and updates about the Olfactory Research Fund, visit our website at www.olfactory.org

RESEARCH UPDATE

FOUR NEW GRANTS AWARDED FOR 1999

The Olfactory Research Fund is pleased to announce that it has awarded four grants for the 1999 grant period. Out of a field of fourteen proposals submitted in response to the Fund's annual call for research studies, three studies were selected for funding. In addition, the Fund has contracted with the Nicholas Institute of Sports Medicine and Athletic Trauma (NISMAT) to investigate the effect of odors on perceived exertion. These new projects and the questions they seek to answer are described below. As the work progresses, update reports will be included in future issues of The Aroma-Chology Review.

CAN AMBIENT ODORS AFFECT OUR PERCEIVED LEVEL OF EXERTION AND INFLUENCE HOW WE FEEL WHEN WE'RE EXERCISING?

Two separate studies will investigate this question.

The Olfactory Research Fund has contracted with the **Nicholas Institute of Sports Medicine and Athletic Trauma (NISMAT)** at Lenox Hill Hospital in New York to conduct an investigation of **"The effect of ambient scents on perceived exertion during sub-maximal exercise." Dr. Gilbert Gleim**, Director of Research at NISMAT, is heading up the study.

In describing the project, Dr. Gleim notes, "Exercising human beings innately work at an intensity level which corresponds to a comfortable level of perceived exertion. Respiratory rate, muscle fatigue, body temperature and perhaps even heart rate all give feedback to our cognitive perception of exercise intensity. If perceived exertion becomes too great, most individuals will decrease their intensity or stop unless they are training for some specific purpose."

Previous research has shown that psychological states and traits can affect the perception of muscular work. Likewise, recent studies have demonstrated that aromas can positively impact emotion and mood states so it is possible that scents may influence perceived exertion and influence how we feel when exercising. Thus a scented environment could conceivably lead to greater compliance with physical therapy and sports training programs as well as more effective workouts. The NISMAT project will clearly demonstrate the validity of this hypothesis.

A second study to be conducted by **Dr. Bryan Raudenbush** at **Wheeling Jesuit University**, will investigate **"The Effects of Odors on Objective and Subjective Measures of Physical Performance."** Dr. Raudenbush's hypothesis is that odors which improve mood, attention and motivation will result in reduced levels of physiological stress during exercise, and will also lower the perceived level of physical effort. An opposite set of results is expected for unpleasant odors.

The subjects in the study will be physically fit athletes recruited from college track and soccer teams. They will take part in a standard treadmill stress test. During the exercise they will wear a vital signs monitor that records their heart rate, blood pressure, and oxygen saturation. At the end of the test, subjects will fill out a questionnaire about subjective ratings of workload and perceived physical exertion. Each subject will take part in four tests during which one of three odorants or unscented air, is delivered through a nasal cannula.

A positive set of results from the two research projects described above, would open the door to a range of new fragrance benefits that are more physical than psychological. Finally they can easily lead to new applications in sports training, fitness and physical therapy.

HOW DO COGNITIVE PROCESSING STYLES INFLUENCE THE PERCEPTION OF ODORS AND THEIR MIXTURES?

Drs. Andrew Livermore and **Rachel Dryer** of **Charles Sturt University** in Bathurst Australia will conduct a research study to resolve the longstanding debate about the nature of odor perception and whether we perceive odors analytically or synthetically.

This Fund-sponsored project, will take a new look at the question by taking advantage of well-known differences in cognitive processing between the two sides of the brain. The left hemisphere tends to process information in a sequential (analytical) manner, while the right hemisphere tends to process in a global (synthetic) manner. In other words, the left hemisphere sees the trees; the right hemisphere sees the forest.

The investigators will use priming techniques to see whether they can bias the analytic vs. synthetic processing of odor mixtures. Will people primed with a global processing visual task be more inclined to perceive a mixture as a unified whole? If odor evaluation can be altered by cognitive processing style, the researchers will ask whether this is due to a change in perception or merely a change in task demand. They will also look to see whether a person's natural cognitive processing "style" determines how that person perceives odors. Finally, they will use brain activity and imaging techniques to trace the olfactory perception differences to a specific cerebral hemisphere.

This research will tell us how the different ways of "thinking about" odors can alter our perception of them. The potential applications of this information include better ways of assessing and evaluating smells, whether they are foods, perfumes or pollutants.

HOW DO CHANGES IN ENDOCRINE HORMONE STATUS (SEX STEROIDS) AFFECT OLFACTORY DISCRIMINATION DURING CHILDHOOD AND ADOLESCENCE?

It is a well-established fact that the ability of children to recognize and discriminate among odors increases with age. **Dr. Robert A. Richman** will lead an investigation at **SUNY Health Science Center** at Syracuse to determine the **"Changes in olfactory discrimination**

with age and steroids during childhood and adolescence." The studies will compare normal pre- and post-pubertal girls and normal pre-pubertal boys, with three very interesting patient populations of: girls with precocious puberty, pre-puberty girls with treated congenital hypothyroidism and girls with a mild virilizing disorder. Dr. Richman, a pediatric endocrinologist, has assembled the clinical population.

In the first study, the researchers will use a set of test odorants that produce age-dependent differences in a discrimination task. They predict that endocrine maturation will be associated with better olfactory discrimination. Specifically, they expect the older, normally post-pubertal girls and the younger girls with precocious puberty, to outperform all other groups.

The second study will examine a non-clinical population from five different age groups, between two and 18 years old. The experimental task involves sorting a set of odorants by odor similarity. The investigators hypothesize that older children will produce more odor groupings than younger children; classification of the children by pubertal status should produce an even sharper difference in group performance.

These studies will help clarify the contribution of physical (hormonal) maturity to olfactory performance increases during childhood. They may very well help us understand the biological source of the well-known sex differences in odor perception seen in adults. ■

SENSORY DIRECTIONS FOR THE NEW MILLENNIUM: FUTURE DIRECTIONS FOR USE OF ODORS IN OVERALL HEALTH

continued from page 5

immunity, and other biological functions. There are already reliable data that pleasant odors including fragrances and chamomile oil can impact mood and even reduce the doses of antidepressants necessary for the treatment of depression (see Komori et al., 1995). There are also reliable data that odors differing in hedonic properties have differential effects on electrical brain activity as measured by electro-olfactograms, electroencephalograms, and neuroimaging techniques (e.g. Zald and Pardo, 1997).

An emerging area of research is the role of odors in immune status. In animal models, odors are used to condition enhanced immune responses in a manner analogous to classical Pavlovian experiments. In several conditioning paradigms in animals, an odor has been paired with a physiologically active agent, i.e. poly-inosinic:poly-cytidylic acid (Poly I:C). Poly I:C generates interferon production which induces natural killer (NK) cell activity. For example, Spector et al. (1994) found significant increases in spleen cell NK activity using the odor of camphor as the conditioned stimulus (CS) and the injection of Poly I:C as the unconditioned stimulus (US). Conditioned immune responses to odor appeared to delay tumor growth (Ghanta et al., 1990), elevate cytotoxic T-lymphocyte (CTL) responses (Ghanta et al., 1995), and increase survival in tumor-bearing mice (Ghanta et al., 1988).

Odors may also enhance immunity in humans through conditioning (Ader and Cohen, 1991). For example, pairing odor with relaxation therapy may in part contribute to enhanced immunity through conditioning. Furthermore, odor may be conditioned to certain biological effects of essential oils. Many essential oils have been reported to have anti-inflammatory and antimicrobial activity in vitro, but it is not known if the concentrations of oils inhaled during aromatherapy are high enough to affect the immune system directly in vivo.

As we approach the 21st century, scientific researchers will continue to explore the effect and mechanisms by which odor may impact immune parameters, quality of life, and functional status in persons with a range of health conditions. As the global population ages, the need for cost-effective complementary strategies to improve immune status, quality of life, and functional status in older individuals will become more crucial. Use of aromas has the potential to be an adjunctive therapy in numerous instances.

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THEOPHYLLINE TREATMENT OF SMELL LOSS SHOWN BY fMRI

Robert I. Henkin, M.D.
The Taste and Smell Clinic, Washington, D.C.

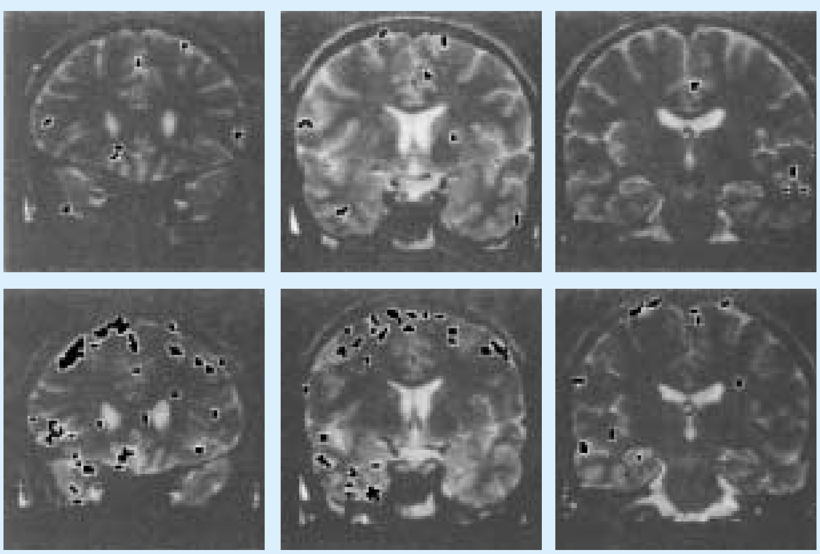
Among the most frequent inquiries received at the Olfactory Research Fund, are those from individuals who have lost their sense of smell who are looking for a cure. Because of this interest, we have decided to include this report even though its subject matter does not fall under the banner of Aroma-Chology. We feel, however, that the results reported by Dr. Henkin should be considered preliminary rather than definitive since the study utilized a small patient sample size. — ed.

THE PROBLEM

Smell loss (hyposmia) affects over 16 million people in the U.S. (1). It affects people of all ages, gender, races and ethnic groups and once it occurs it can last for the remainder of life. It occurs following a variety of causes including influenza, head injury, allergies, metabolic disorders and therapeutic drug use. In spite of its frequency, there has been no accepted method to either diagnose or treat it mainly due to lack of verifiable methods by which it can be documented and quantitated.

With realization of these difficulties, we developed a technique that provided an objective measurement of smell function with negligible patient participation. Through use of functional magnetic resonance imaging (fMRI) with olfactory stimulation, we obtained measurements of brain activation in response to olfactory stimulation in normal subjects (2) and patients with smell loss (3). Results provided both quantitative brain measurements of activation and brain maps of olfactory stimulation in patients with hyposmia demonstrating significantly less activation than in normal subjects [(3), Fig 1].

FIGURE 1. fMRI Brain Scan with Pyridine Stimulation in a Patient with Hyposmia After a Head Injury.



Before treatment, when the patient could not smell, is shown in upper three panels, after treatment with theophylline for four months when the patient had his smell return no normal, in lower three panels. Each panel is a representative brain section beginning anteriorly (left section), toward the middle portion (middle section) and posteriorly at the level of the red nucleus (right section). Changes are demostrated by activated pixels in brain in specific regions. Increased activity is demonstrated in all brain regions after theophylline treatment consistent with return of normal smell function.

To extend this concept further, we performed fMRI studies in a specific group of four men with hyposmia before and after theophylline treatment using each patient as his own control (4).

METHODS NEEDED TO SOLVE THIS PROBLEM

MRI brain scans were performed on each patient before and after 4-6 months of theophylline treatment; one patient developed hyposmia after severe head injury, 3, associated with allergic rhinitis.

MRI brain scans were obtained with 1.5 T clinical scanner with a quadrature head coil in 3 patients with a FLASH technique and in one patient using echo planar imaging (EPI) fMRI. Stimulation was obtaind by placement of an absolute solution of either acetic acid amyl ester (amyl acetate), L-menthone or pyridine approximately 1-2 cm above the patient's nose.

RESULTS

Prior to treatment each patient reported he could not smell and none recognized the character of any vapor. Using the FLASH technique, mean brain activation was less than that obtained previously in normal volunteers with responses significantly lower for menthone ($p<0.005$, t test) and for pyridine ($p<0.001$, t test) [(Table I) (Fig 1)]. Using EPI, activation in each section for each vapor was lower than normal (Table II).

After treatment 3 patients responded to theophylline with improvement in smell function (responders) and one did not (non-responder). In responders using FLASH fMRI, mean activation to each odor increased significantly over pre treatment [(Table I) (Fig 1)]. Responses to menthone and amyl acetate increased over five fold and to pyridine over 20 fold. Mean activation also increased significantly to each vapor studied with EPI MRI (Table II) with activation to menthone and amyl acetate increasing over two fold and to pyridine over three fold. Brain maps of responders (Fig 1) showed markedly increased enhancement of brain activation. In the non-responder there was no change in either brain activation or brain maps of olfactory response.

After treatment, responders showed changes in activation in specific brain regions. The greatest increase occurred in regions of the frontal lobes (Fig 1). Increases varied from three to five fold in cingulate gyrus region to three to four fold in the region of orbitofrontal cortex. Increases also occurred in temporal lobes (Fig 1), but relatively quantitatively less change occurred in these regions after treatment compared with changes in regions of frontal lobes. Post-treatment, activation in all regions identified in frontal lobes combined was significantly greater than that in all regions of temporal lobes combined ($X^2 = 18.0$, $p<0.01$, median comparison test).

MEANING OF THE RESULTS

Results demonstrate that fMRI with olfactory stimuli can be used to document smell loss in patients with hyposmia and then improvement in smell function following medical therapy using each patient as his own control (4). These changes have been shown both by quantitative measurement of increased brain activation (Tables I, II), by increased brain activation visualized on topographical maps (Fig 1) in responders and by lack of change in brain activation or in topographical brain maps in the non-responder. Thus, topographical brain maps are useful to follow treatment in patients with hyposmia who are undergoing therapy to correct their hyposmia just as x-rays are useful to diagnose bone fractures and to follow healing after treatment.

Results demonstrate that theophylline is effective therapy to improve smell function in patients with hyposmia secondary to both head injury and allergic rhinitis. This is important since it is commonly held that, following a sever head injury, as occurred in one patient studied, the hyposmia is due to destruction of the olfactory bulbs or to a severing of the fila olfactoria as they traverse the cribriform plate; this has led to the dictum that there is no accepted form of treatment for post traumatic loss of smell. Data from this patient, as well as prior data (1), cast doubt upon this dictum and should offer hope to the many patients who suffer hyposmia following a severe head injury (4).

The mechanism(s) underlying the theophylline effect observed in this study may be multiple. Theophylline enhances cyclic adenosine monophosphate (cAMP) activity through its action as an inhibitor of phosphodiesterase and this increased cAMP activity may enhance smell function. cAMP serves as an excitatory second messenger in olfactory transduction acting through a GTP-dependent (type III) adenylyl cyclase, G protein (g^{olf}) and putative olfactory receptor proteins. It may also play a role in neurite outgrowth and with nerve growth factor (NGF), a role in neurite extension. It may act as a type of growth factor influencing olfactory receptor growth and development.

continued on page 14

TABLE I: Brain Activation in Two Patients with Hyposmia to Three Olfactory Stimuli Before and After Effective Theophylline Treatment Using Flash fMRI.

AMYL ACETATE

Patient	Anterior		Middle		Posterior		Mean±SEM	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
C.P.	0.56	3.22	0.43	3.27	0.25	1.61	0.41±0.11	2.70±0.67[d]
J.L.	0.68	3.03	0.67	2.29	0.33	2.40	0.56±0.14	2.57±0.28[b]
Mean	0.62	3.12	0.55	2.78	0.29	2.00	0.49±0.12	2.63±0.26[a]
± SEM	0.08	0.06	0.17	0.31	0.06	0.25		

MENTHONE

Patient	Anterior		Middle		Posterior		Mean±SEM	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
C.P.	0.63	3.81	0.45	3.07	0.90	2.18	0.66±0.16	3.02±0.58[c]
J.L.	0.43	2.51	0.25	1.94	0.25	2.01	0.30±0.07	2.15±0.22[a]
Mean	0.53	3.16	0.35	2.50	0.58	2.10	0.49±0.05	2.59±0.24[a]
± SEM	0.14	0.92	0.14	0.80	0.46	0.08		

PYRIDINE

Patient	Anterior		Middle		Posterior		Mean±SEM	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
C.P.	0.30	3.78	0.32	2.50	0.22	3.55	0.28±0.04	3.38±0.54[b]
J.L.	0.12	4.54	0.07	4.06	0.04	4.73	0.08±0.03	4.44±0.24[a]
Mean	0.49	3.48	0.33	2.85	0.33	2.62	0.18±0.02[c]	3.86±0.22[ac]
± SEM	0.12	0.26	0.05	0.18	0.10	0.60		

Pre, pre treatment; **Post,** post treatment
a, p<0.001, t test, post vs. pre amyl acetate mean
post vs. pre methone mean
post vs. pre pyridine mean
b, p<0.005, t test, post vs. pre amyl acetate mean
post vs. pre pyridine mean
c, p<0.01, t test, post vs. pre amyl acetate mean
pre pyridine mean vs. pre menthone mean
pre pyridine mean vs. pre amyl acetate mean
post pyridine mean vs. post amyl acetate mean
post pyridine mean vs. post menthone mean
d, p<0.05, t test, post vs. pre amyl acetate mean

TABLE II: Brain Activation in Patient JC with Hyposmia to Three Olfactory Stimuli Before and After Effective Theophylline Treatment Using EPI fMRI.

	Anterior		Middle		Posterior		Mean±SEM	
Stimulus	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Amyl Acetate	0.98	2.05	0.81	1.39	0.74	1.69	0.84±0.09	1.71±0.23[e]
Menthone	0.43	1.18	0.37	1.56	0.27	1.08	0.36±0.06[c]	1.27±0.18[b]
Pyridine	1.68	8.09	1.57	4.59	0.91	1.18	1.39±0.29	4.62±2.44
Mean	1.03	3.77	0.92	2.51	0.64	1.32		
± SEM	0.44	2.66	0.43	1.27	0.23	0.23		

Pre, pre treatment; **Post,** post treatment
b, p<0.005, t test, post vs. pre menthone mean
c, p<0.01, t test, pre menthone mean vs. pre pyridine mean
e, p<0.025, t test, post vs. pre amyl acetate mean

THEOPHYLLINE TREATMENT OF SMELL LOST SHOWN BY fMRI

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The locus of action of this drug effect could be either in brain, olfactory nerves, olfactory receptors (where initial responses to odors occur) or at all three loci. Since no patient had CNS pathology there was no clinical reason to presume that either olfactory nerves or brain itself were injured; indeed, olfactory tracts and nerves were demonstrated to be present and of normal size in all patients in this study, even in the patient with head injury. Thus, brain activation observed in this study could be considered the result of primary changes of theophylline at olfactory receptors.

These results confirm the usefulness of fMRI with olfactory stimuli in evaluation and treatment of patients with hyposmia. This technique offers an opportunity to measure smell function with negligible patient cooperation. FMRI provides brain maps of CNS activation by which changes in olfactory acuity in response to theophylline or other medical therapy that can improve smell function can be followed clinically. This technique has many applications in studies of smell function since it is possible to define degree of smell loss and brain regions in which this loss may be most apparent.

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MOLECULAR MATCHMAKING

continued from page 1

environmental stimulus (odorant) into electrical signals sent by the olfactory nerve cells to processing centers in the brain. The common biological mechanism of olfaction is initiated by a set of structurally similar proteins, located on the surface of the nerve cells that serve as odorant receptors.

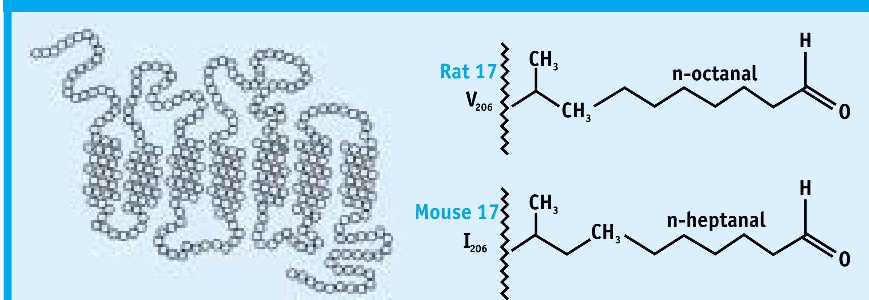
In humans and in mice, the best studied organisms at the genetic level, there appear to be approximately 1000 genes responsible for the production of these receptor proteins. The genes are scattered across the 40 or so chromosomes that are the reservoir for hereditary information. The great variety of receptor types they produce provides the molecular basis for our ability to perceive many thousands of odorous compounds. It has been hypothesized that each receptor protein is able to detect a limited number of odorants, and that there may be some overlap in the odorants detected by different receptors. Knowledge of the range of receptors that interact with each odorant, and of the contribution that each receptor makes to the overall pattern of odor quality recognized by the brain, could be a powerful tool. It might allow one to rationally modify odorant structures to optimize desirable sensory properties. However, since these initial studies almost a decade ago, significant technical and theoretical hurdles have delayed the realization of a complete molecular description of olfactory recognition.

To begin unraveling the basis of olfactory coding, we need to determine how the thousand different receptor molecules interact with an even greater number of odorant structures. This is a molecular matchmaking challenge of the highest order. Even the analogy of a needle in the haystack understates the immensity of the problem. A better image is a mountain of locks lying next to an even larger pile of keys. The task is straightforward but daunting: figure out which keys fit which locks. This problem had stymied investigators for almost a decade, but in the past 18 months four separate laboratories have described different but very promising ways to tackle the problem.

Haiqing Zhao, working in Stuart Firestein's laboratory at Columbia University, took an elegant approach to demonstrate specificity of receptor function. Zhao and Firestein reasoned that the introduction of a single, known olfactory receptor into the olfactory tissue of a rat would produce measurable alterations in the responsiveness of the animal to odorants that activated that receptor. They used a common cold virus (adenovirus) as the means of delivering a specific odorant receptor gene (called I7) into the sensory cells of a rat's nose. (Similar viruses introduced into other tissues are currently being tested for genetic therapy of human diseases.) When infected by the adenovirus, the rat cells incorporated the I7 gene and caused it to produce the I7 odorant receptor. The response of both normal and infected sensory cells to different odorants was assessed by measuring the cells' electrical activity (known as an electroolfactogram). Among the more than 50 odorants initially tested, only a single compound, n-octanal, produced strongly elevated responses in recordings from the infected tissue. Smaller responses were also observed, but only for structurally-related compounds, namely straight chain aldehydes containing one fewer and up to two additional methylene groups. Interestingly, octanoic acid and octanol failed to produce a specific response in the I7 receptor-expressing tissue.

In two independent efforts to identify olfactory receptors that bind specific odorant molecules (referred to generally as "ligands"), investigators in Linda Buck's laboratory at Harvard and T. Sato's laboratory at the University of Tokyo

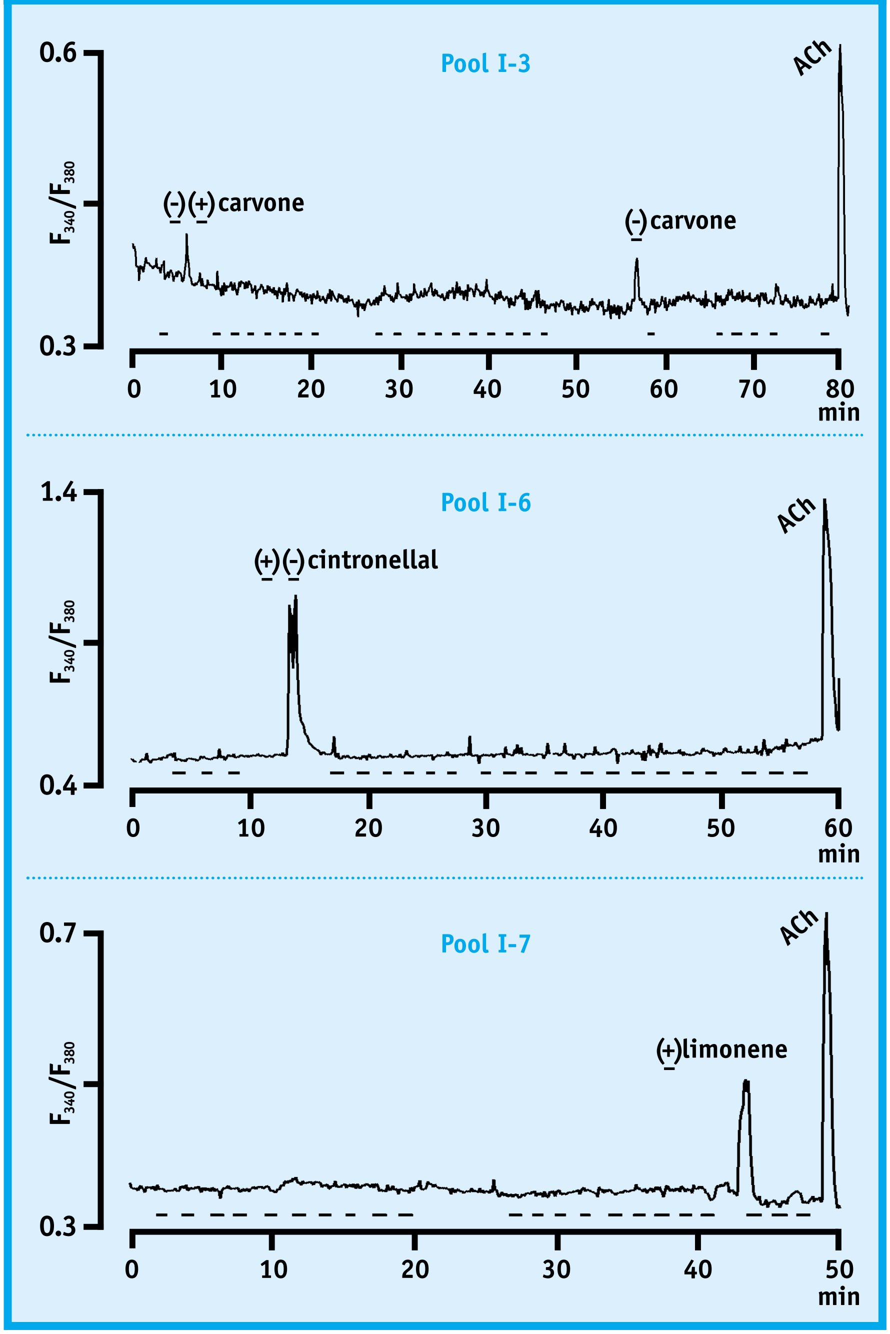
FIGURE 1. Defining the Binding Site in Odorant Receptors



decided to directly examine the native olfactory receptor cells for odorant activation. These laboratories began their experiments by obtaining a sample of mouse olfactory tissue and separating the individual sensory cells for examination. A collection of a hundred or so cells was examined simultaneously; changes in cellular activity arising from odor exposure were assessed under a microscope. The researchers were able to identify a few cells in each collection that responded to the test compounds. By utilizing extremely powerful DNA cloning methods, the olfactory receptor proteins expressed in the responding cells could be isolated and studied. Moreover, since the test odorants used in these experiments were structurally related, the investigators could demonstrate that each receptor protein produced a characteristic profile: it would respond to some stimuli but not to others. These observations have led the investigators to suggest that the ability to distinguish odorants derives from a combinatorial code in which unique patterns of receptor activations define the odor identity.

A fourth group led by Dietmar Krautwurst in our laboratory has successfully tackled the ligand-receptor specificity question from a completely different direction. In these experiments, DNA cloning methods were used to generate a collection containing hundreds of different olfactory receptor gene sequences. Five hundred individual clones were transferred to a grid-like array; their DNA sequence was determined and they were prepared for assessment of their ligand binding specificity. By screening groups of 8 receptors, a relatively large number of different receptor proteins could be examined. In their initial analysis, using only 80 receptors and 26 different odorant compounds, Krautwurst reported the identification of three new receptor-ligand interactions. One of these interactions displayed stereoisomer selectivity and relatively high specificity upon challenge with structurally related molecules. Interestingly, for one receptor the change of a single carbon atom at a critical position in the receptor (out of the 1500 or so in the receptor protein) produced a dramatic shift in selectivity from n-octanal to n-heptanal. This observation suggests that subtle changes in the amino acid sequence of the receptor protein may produce profound changes in the selectivity of that receptor. The screening approach described by Krautwurst could be employed in a high throughput method that would allow the screening of hundreds of receptors and thousands of odorant compounds.

FIGURE 2. Functional Assay of Receptor Pools



All of these research groups performed their experiments in rodents. This reflects the simpler and greater reliability of tissue isolation and the ability to perform subsequent, more elaborate experiments including genetic manipulation of the receptors in the animal. Nonetheless, extensive characterization of the receptor repertoire in rodents and in humans suggests that closely related receptors exist in both species and that the methodologies used in these recent studies are directly applicable to the analysis of the human olfactory system.

The mammalian olfactory system represents one of the best models in which to examine structure/function relationships between odorant ligands and their receptors. The potential to understand the relationship between odorant structure, the genes that underlie its detection, and the quality of the perception that it generates may not be too far in the future. In particular, the rational identification of agents that might block particular odorants and thus change their intensity or odor quality may represent a new paradigm for product development in the fragrance industry. ■

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