THE BIOLOGICAL SENSE OF SMELL:

OLFACTORY SEARCH BEHAVIOR AND

A METABOLIC VIEW FOR OLFACTORY PERCEPTION

Thesis by

Christine W.J. Chee-Ruiter

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"Solitude is the friend of thought, and the enemy of reason." --unknown sage

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ABSTRACT

Part I of the thesis describes the olfactory searching and scanning behaviors of rats in a wind tunnel, and a detailed movement analysis of terrestrial arthropod olfactory scanning behavior. Olfactory scanning behaviors in rats may be a behavioral correlate to hippocampal place cell activity.

Part II focuses on the organization of olfactory perception, what it suggests about a natural order for chemicals in the environment, and what this in turn suggests about the organization of the olfactory system. A model of odor quality space (analogous to the "color wheel") is presented. This model defines relationships between odor qualities perceived by human subjects based on a quantitative similarity measure. Compounds containing Carbon, Nitrogen, or Sulfur elicit odors that are contiguous in this odor representation, which thus allows one to predict the broad class of odor qualities a compound is likely to elicit. Based on these findings, a natural organization for olfactory stimuli is hypothesized: the order provided by the metabolic process. This hypothesis is tested by comparing compounds that are structurally similar, perceptually similar, and metabolically similar in a psychophysical cross-adaptation paradigm. Metabolically similar compounds consistently evoked shifts in odor quality and intensity under cross-adaptation, while compounds that were structurally similar or perceptually similar did

not. This suggests that the olfactory system may process metabolically similar compounds using the same neural pathways, and that metabolic similarity may be the fundamental metric about which olfactory processing is organized. In other words, the olfactory system may be organized around a biological basis.

The idea of a biological basis for olfactory perception represents a shift in how olfaction is understood. The biological view has predictive power while the current chemical view does not, and the biological view provides explanations for some of the most basic questions in olfaction, that are unanswered in the chemical view. Existing data do not disprove a biological view, and are consistent with basic hypotheses that arise from this viewpoint.

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Introduction to the Thesis

The computational task the olfactory system performs involves the recognition of objects based on the patterns of chemicals they release into the environment. In the natural world, such objects are almost exclusively of biological origin [1].

Solving this task requires handling tremendous chemical complexity. In general, natural olfactory stimuli display high chemical, spatial, and temporal diversity. For example, compounds of biological origin (natural compounds) are rarely found in a monomolecular state, and are generally part of a complex chemical mixture; mixtures released by different species may contain compounds in common, but will differ in the proportions of these compounds; a single source can release a mixture whose composition can change in time and on multiple timescales. However, much work in olfaction appears to be performed without a general understanding of the natural conditions under which the sense operates. The approach of probing the olfactory system with monomolecular stimuli is common, though interpretation of results rarely considers the experimental stimulus as an element of a more complex chemical pattern, with a fine spatial and temporal structure.

The nature of the sense of smell underlies these issues. Unlike visual or auditory stimuli, the organization of olfactory stimuli has been elusive. This complicates research

efforts, because no obvious order has been apparent in the organization of olfactory perception, either. For example, there is no clear series or sequence to the odors "Apple," "Green Bean," or "Fried Chicken." Consequently, the organizational concepts used in chemistry have been used as metrics for olfactory studies, and this has inadvertently shaped the interpretation of results.

For many years, research in olfaction has assumed that olfactory sensors (receptors) are organized along chemical families of the sort familiar to chemists. Homologous series are series of compounds related by a systematic structural change from one compound to the next. Members of homologous series are thus structurally similar, and have similar chemical properties. Thus, homologous series of compounds are almost universally used by olfactory researchers working from the level of single cells [3, 4] to perception [5].

However, evidence from single cells has consistently suggested that this organization may not reflect the organization of olfactory processing. Malnic, et al. (1999)[4] showed that single receptor neurons respond to a range of compounds that cross "series" lines. Within a series, cells responded differentially and only to a subset of compounds. Other studies show that single receptor neurons respond to specific compounds and not to other, structurally similar compounds [6, 7]. (Since Malnic, et al. (1999)[4] recently showed that vertebrate receptor neurons express only one receptor type, the responses of single receptor neurons may be taken to reflect the specificity of

their olfactory receptor proteins.) At the level of olfactory perception, compounds with highly different structures can elicit similar odors, while small changes in chemical structure can render a highly odorous compound completely odorless [8]. Thus, systematic changes in structure that generate homologous series only partly describe the order in olfactory stimuli. More generally, it has been impossible to predict the odor quality, intensity, or hedonic value that a compound will evoke based on its chemical composition, nor whether a compound will even elicit an odor.

Accordingly, it is likely that there is some other order existent in the chemical environment, and that this order is reflected in the organization of the olfactory system as it classifies objects based on the molecules they release. The primary objective of the work described in this thesis is to explore this hypothesis.

There are two parts to the thesis. Part I (Chapters 1-3) addresses how animals acquire an olfactory signal. Part II (Chapters 4-6) addresses the output of the olfactory process, perception, and what it implies about the natural order of chemicals in the environment.

What information is used by animals when responding to an attractive olfactory stimulus? Chapter 1 introduces concepts in olfactory search behavior, and describes for the first time the olfactory searching and scanning behaviors of a vertebrate animal in a *wind tunnel*. Rats appear to use non-olfactory directional information (e.g., wind direction) as well as olfactory information in locating the source of an odor. Rats display

an invariant behavioral sequence in olfactory search, consisting of four main phases. Within these phases, rats performed distinctive olfactory scanning behaviors, scanning in three spatial dimensions. These scanning behaviors occurred at regular positions in the wind tunnel, which were unique for each rat. These positions form landmarks on a spatial map of the environment, identifying the location of important activities performed during olfactory search. The spatial organization of these maps appears similar to the spatial organization of place fields described in hippocampal literature. Thus, the scanning seen during olfactory search may be a behavioral correlate to hippocampal place cell activity. In general, olfactory search behavior is a good paradigm in which to explore the relationship between nonspatial activities (e.g., odor recognition) and spatial activities (e.g., search).

Complex behaviors (for example, grooming in the rat) are often based on sequences of behavioral "building blocks" called fixed action patterns. Fixed action patterns have largely been studied in the context of motor (i.e., output) behavior, but could also function in the acquisition of sensory information. Olfactory scanning behaviors may be thought of as odorant-released fixed action patterns that may aid in odor detection, identification, or search. However, specific scanning behaviors are likely to be different in animals with different nose configurations. Comparing scanning behaviors between different animals may reveal both the function of scanning behavior and the common problems posed by natural olfactory stimuli. Chapter 2 describes for the

first time a detailed movement analysis of terrestrial arthropod olfactory scanning behavior. Arthropods have different morphological nose structures from vertebrates, allowing the dissociation of respiration from olfaction. Stimulation with an attractive odor elicits a striking change in antennal movements as well as postural and locomotory activity. Antennal movements comprise a three-dimensional scanning pattern.

Chapter 3 addresses the spatial and temporal nature of the physical olfactory stimulus, compares how bacteria, insects, and rats interact with odorant stimuli, and describes how these animals perform olfactory navigation and search. Biological principles of olfactory search may be applied to engineering designs for artificial chemical sensing devices.

The next three chapters focus on the organization of olfactory perception, what it suggests about a natural order of chemicals in the environment, and what this in turn suggests about the organization of the olfactory system. Chapter 4 discusses odor classification and describes the creation of a model of odor quality space (analogous to the "color wheel"). This model defines relationships between odor qualities perceived by human subjects based on a quantitative similarity measure. It is demonstrated that compounds containing the elements Carbon, Nitrogen, or Sulfur elicit odors that are contiguous in this odor representation, which thus allows one to now predict the broad class of odor qualities a compound is likely to elicit. Based on these findings, a natural organization for the chemical environment is suggested: the order provided by the

metabolic process. In this conception, an odorant precursor is subjected to spontaneous or enzymatic reactions producing a patterned mixture of volatile compounds. These reactions may take place endogenously within an organism, or exogenously by a consortium of organisms. The resultant odorant mixture is determined by the genetic makeup of the organism.

Chapter 5 tests this hypothesis by comparing compounds that are structurally similar, perceptually similar, and metabolically similar in a psychophysical cross-adaptation paradigm. In perceptual cross-adaptation, continuous exposure to one odorant results in a decrease in odor intensity of a second odorant. The results are thought to reflect a mechanism where both odorants share common neural processing pathways, so the more a compound can influence the perception of another compound, the more similar they are thought to be. Chapter 5 describes a modification to this paradigm by measuring changes in odor quality perception as well as odor intensity perception. Results show significant shifts in odor quality and odor intensity, with metabolically similar compounds consistently evoking shifts. Compounds that were structurally similar or perceptually similar did not show consistent results. Two conclusions can be drawn from this experiment. First, odor quality is not a fixed characteristic of the olfactory system in response to a stimulus, but may vary in a context that may be as simple as the presence of another monomolecular stimulus. Secondly, the defining characteristic of that context may be metabolic similarity to the test compound. This suggests that the

olfactory system may process metabolically similar compounds using the same neural pathways, and that metabolic similarity may be the fundamental metric about which olfactory processing is organized. In other words, the olfactory system may be organized around a biological basis.

The idea of a biological basis for olfactory perception represents a shift in how olfaction is understood. Chapter 6 discusses the implications of a biological basis for olfactory perception, and contrasts it with the common "general chemical classifier" view of olfaction. There are two basic differences between these views: they have different metrics by which to organize olfactory stimuli, and while the biological view places olfaction in an evolutionary context, the chemical view does not provide a reasonable context for olfaction. Thus, the biological view has predictive power while the chemical view does not, and the biological view provides explanations for some of the most basic questions in olfaction, that are unanswered in the chemical view. Existing data do not disprove a biological view, and are consistent with basic hypotheses that arise from this viewpoint.

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Chapter 1. Olfactory Search Behavior of Rats

Introduction

Animals search successfully in different environments: land, underwater, air. Animals search to find food, mates, shelter, and other resources. These searches involve visual, olfactory, and other cues. In certain cases, particularly at night, the search process is driven primarily by olfactory stimuli. Success in search is necessary for survival and reproduction, so we can expect search behaviors to be highly developed.

While a number of studies have focused on the olfactory search behavior of invertebrates (moths [1], lobsters [2], and crabs [3]), there have been no controlled studies of the olfactory search behavior of vertebrates. It is reasonable to expect that the search behavior of vertebrates might be very different from that seen in invertebrates. An obvious difference is that many of these studies have been done in marine arthropods, while we are interested in terrestrial mammals. Another potential difference is that arthropods have a different nose structure than mammals: their "noses" are antennae -- long, moveable arrays of olfactory sensory neurons--that can move through the olfactory stimulus. Vertebrates have stationary (relative to the body) noses, sheets of olfactory

receptors embedded in the respiratory mucosa. Olfactory stimuli are carried to the receptor sheet when the animal "sniffs." Olfaction is thus linked to respiration in vertebrates, unlike in arthropods.

We seek a general understanding of the principles underlying the search behavior of mammals. We are interested in observable, as well as non-observable, aspects of search. Observable aspects of the search process include the spatial and temporal patterns of movement displayed by the animal, as well as the physical structure of the animal's sensory organs. Non-observable aspects include details of the chemical stimulus, and the dynamics of the distribution of the stimulus. Because we cannot observe what the animal actually senses, or how the animal processes this sensory input, it may be possible that non-olfactory stimuli play an important role in olfactory search behavior. This is of interest because there are some problems, such as direction-finding, that are more easily solved using senses other than olfaction.

Another question of interest is if there is a general search strategy, or a basis set of search strategies, that can explain the different searching behaviors seen in animals. It seems plausible that the task facing animals is the same: find the source of a chemical distribution in a constrained environment. If we interpret an animal's searching behavior

as an *implementation* of a search strategy, it is possible that the different searching behaviors observed are all examples of the same search strategy.

In this paper, we will describe the searching behavior of rats in a wind tunnel. Rats are nocturnal scavengers, and thus depend on their olfactory sense to find food and other resources. Rats are also mammals, and so in addition to a different kind of olfactory organ they have a much more complex neural architecture than arthropods. For these reasons, we may expect to find that rats show different searching behavior than observed in other animals studied. By comparing rat behavior with other animals, we hope to identify some common principles of search.

Methods

Wind Tunnel

A 4' X 10' wind tunnel was constructed[1]. The top and one side were Lexan; the floor and the remaining side were wood. A 1m X 3m grid was applied to the wooden floor and side of the wind tunnel with tape, with each grid measuring $10cm^2$. The wood surface was then sealed in Teflon film. The inlet and outlet of the wind tunnel was

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[1] Design and construction by David Kewley of the Bower Lab, at the California Institute of Technology

formed by multiple metal honeycomb layers that served as "flow straighteners." Air was drawn through the tunnel by a suction fan attached to the outlet of the tunnel; flow was approximately 4 linear feet/second. Flow visualization tests showed near-laminar flow. Odorant was released from one of three sites at the upwind end of the tunnel (Figures 1 and 2).

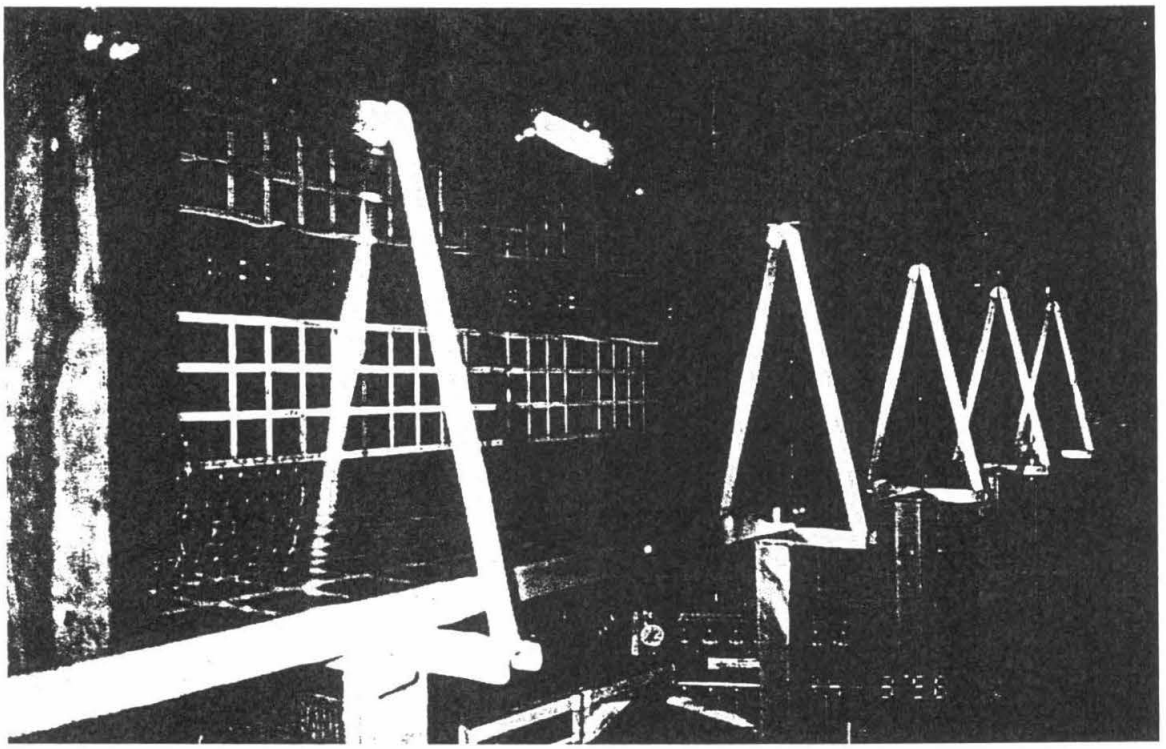

Figure 1. Side view of the wind tunnel.

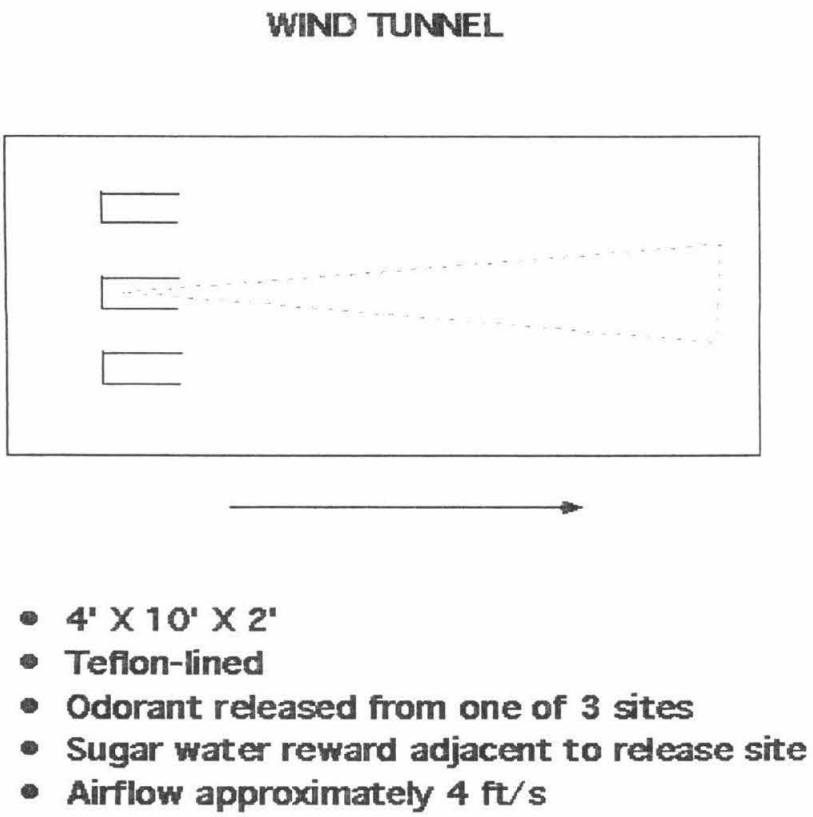

Figure 2. Schematic diagram of wind tunnel. Air flows from left to right. The dotted lines represent the approximate shape of odor plume as visualized using flow visualization techniques. Rats were released on the downwind side of the tunnel. The rectangular figures represent the possible reward sites.

Flow visualization tests of the odor delivery apparatus showed jet plume dispersion. The other two sites released non-odorized humidified air at the same flow rate as the stimulus. The odorant position was switched from trial to trial. Each release site was enclosed in a three-sided fence; entry within the fenced region was the criterion determining when the rat had made its choice. Each release site held a plastic cup containing either a sucrose solution or water.

Odorants

Two odorants of different chemical structure were used as stimuli. Early experiments used citral ($C_{10}H_{16}O$) as an odor stimulus, while later experiments were conducted using Toluene (C_7H_6). Citral evokes a Lemon odor in humans, while Toluene has a chemical odor. Both odorants were novel to these animals at the start of training. A citral emulsion was prepared with water at a ratio of 5 ml/25 ml respectively, and the emulsion was applied to absorbent paper to be used as stimuli (0.9 ml). Air drawn over the stimulus was directed to the release site. Toluene was delivered using an air dilution olfactometer, at a concentration of 2.084 parts per thousand at the point of delivery.

Odor Delivery System

Compressed air was filtered and dried before entering the low-flow (odorant) and a high-flow (background diluent) pathways (Figures 3 and 4). Both airflow pathways were controlled by separate flowmeter valve systems. Air was bubbled through pure liquid Toluene, producing saturated vapor. This vapor stream was then diluted by humidified background air to the desired concentration.

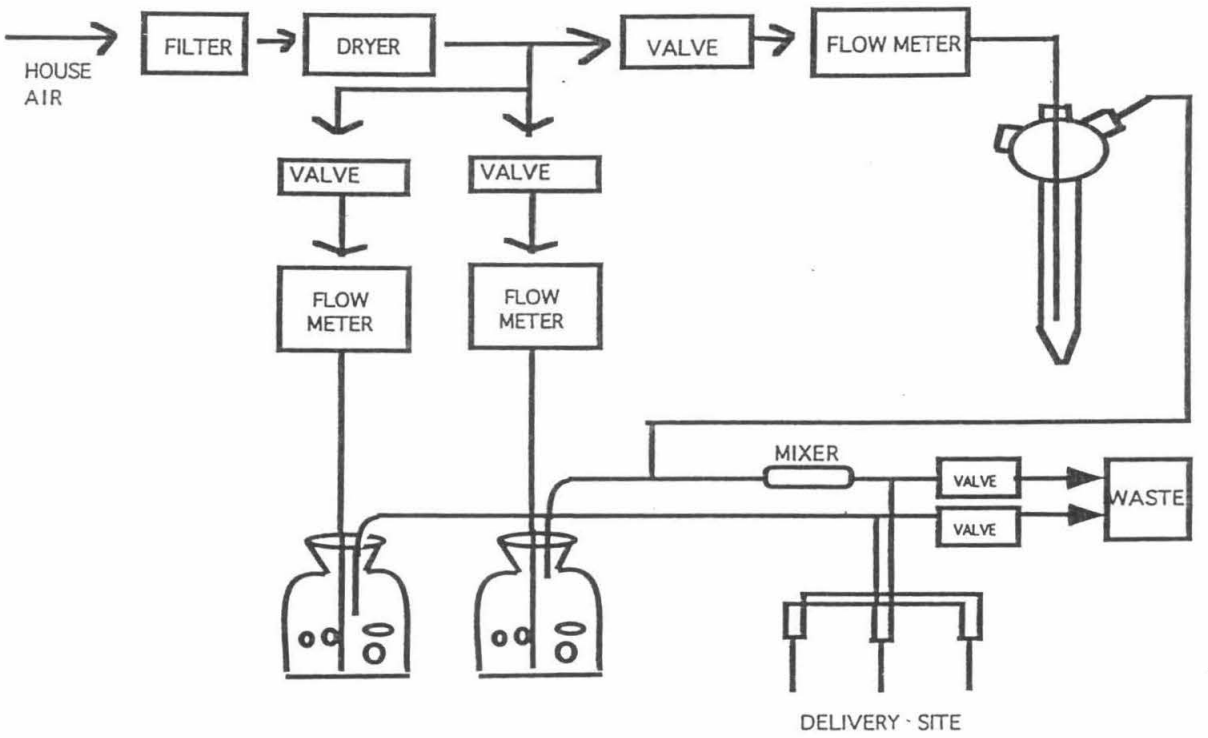

Figure 3. Schematic of the odor delivery system.

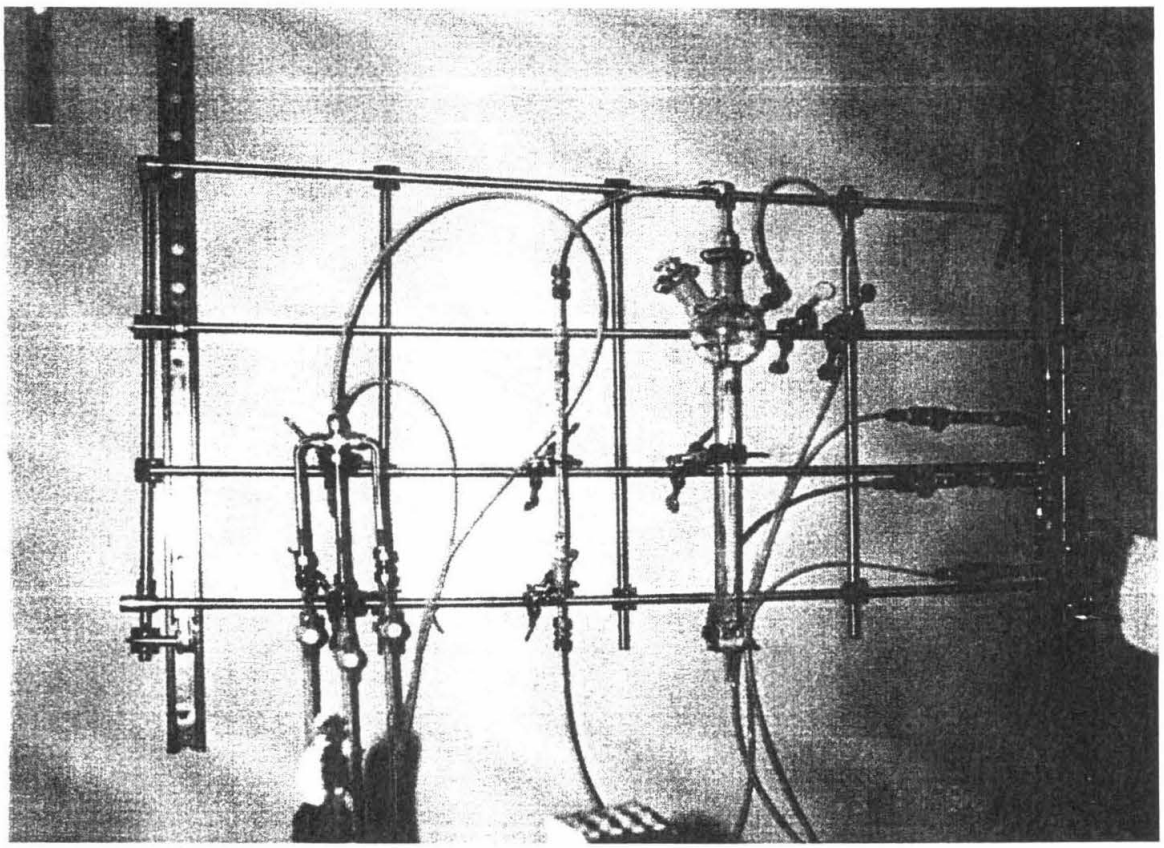

Figure 4. Photo of odor delivery system.

Initial Training

Six rats were used (4 female, 2 male). For each stimulus, three rats were trained using operant conditioning to associate the stimulus with a reward. Rats were trained to associate the odorant with a sucrose solution by simultaneously presenting the sucrose solution with one syringe, and puffing the odorant near the rat's nose with another syringe. Rats were then trained to follow the odorant to a sugar water reward using a "T" shaped maze (Figure 5). Odorized and non-odorized air streams were each drawn through an arm of the T-maze. Plastic cups containing either sucrose solution or water were placed in the arms of the T-maze. Flow visualization tests indicated that there was no mixing of the two air streams in the arms of the T-maze. Once rats were trained to follow an odorant to a reward in the T-maze, they were moved to the wind tunnel for training. Rats were allowed to explore the wind tunnel, to familiarize them with the reward sites (during training there was no odorant present, no airflow, and no fence around the reward site). Sucrose solution was placed in all three reward positions. Prior to experimentation, rats were water-restricted.

Experimental procedure

Trials were conducted between 3:00 PM and 6:00 PM. The rat was placed in a small cage, then placed near the downwind side of the tunnel. While the cage was being

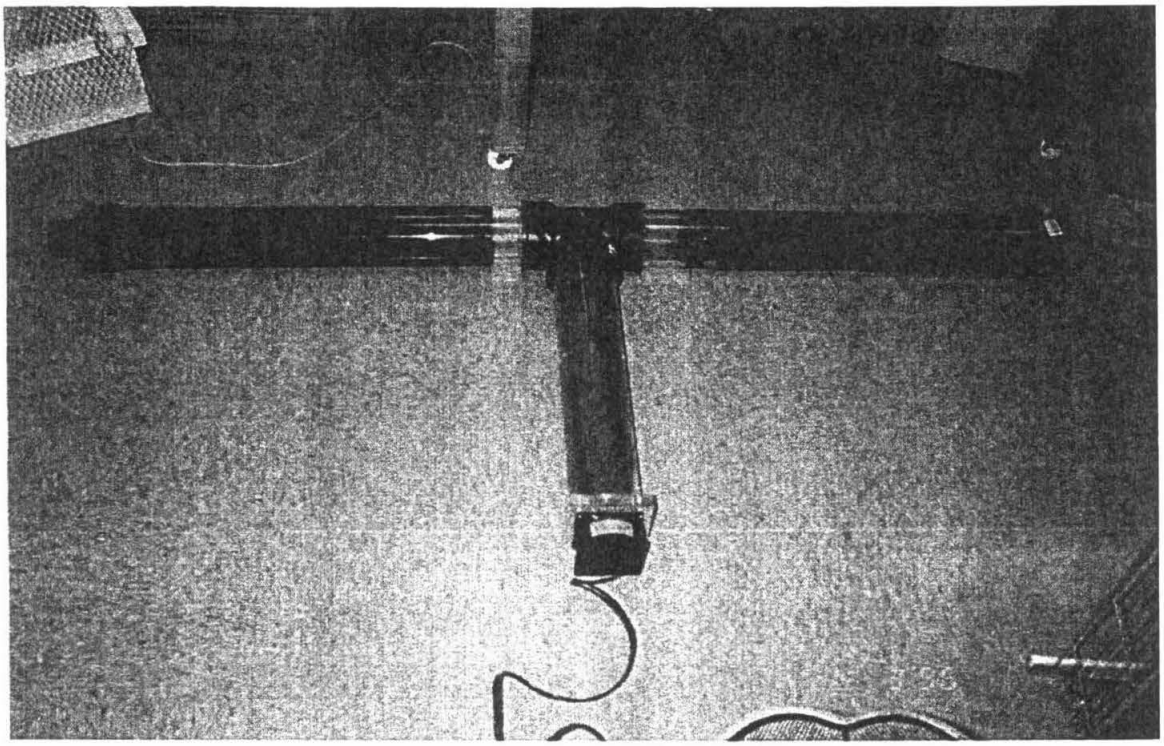

Figure 5. Photo of T-Maze. The tubes are covered with gel filters to darken the interior. Odorized air is drawn in through one arm of the maze, while humidified non-odorized air enters the other arm. Also in the arms of the "T" were plastic cups that held either plain water or sugar water. A vacuum fan attached to the base of the "T" pulls air through the maze.

installed in the wind tunnel the air was flowing in the tunnel, but no odorant was released. After the cage was installed, the odorant was introduced, and the rat released from the cage. After the rat selected its target, the odorant supply was turned off and the rat removed from the tunnel. In preliminary experiments, rats had been observed to follow the paths of other rats from previous trials. This behavior was interpreted to be mediated by pheromone signals. To prevent pheromone-following behavior, it was sufficient to mop the floor of the wind tunnel with 90% ethanol solution after each trial, then allow it to dry.

Video Analysis

Search movements were videotaped in a darkened room with a black-and-white video camera using infrared illumination. 50 trials, using three female rats and with Citral as the stimulus, were videotaped and analyzed. Of these, a representative 16 trials were analyzed in detail. Path configurations were recorded by hand, and temporal sequences were then classified and grouped. 169 trials, using three different rats (1 female, 2 male), were conducted and videotaped using Toluene as the stimulus. Of these, a representative 24 trials were analyzed in detail. Data were taken in the same way as the citral trials. Further, detailed behaviors were noted, classified, and grouped.

Analysis of search movements

Search movements made by rats were first broken down into the shortest possible path segments and common features between these were then noted. For example, for a particular trial one rat may circle along the edges of the wind tunnel in one direction, while another may circle in a different direction. The common feature is that both rats circle the tunnel near the tunnel edge. Once path segments were grouped into "phases," they were examined to see if temporal sequences existed. For example, all rats ran several times around the edge of the tunnel immediately after being introduced to the tunnel (Phase I), then all rats showed a perpendicular "dash" across the tunnel (Phase II); Phase I always preceded Phase II.

Results

Rats performed search movements

Rats were observed to perform search movements, with active sniffing and visibly directed locomotion. Each trial was approximately 106 +/- 92 seconds in duration. The normal speed of searching was approximately 50 cm/s. In 44 analyzed trials, the overall success rate was 61%.

Four main phases of olfactory search

16 of 50 Citral trials were analyzed for the overall path taken by searching animals, and the results were verified in 169 toluene-based trials using a different set of animals. There were four phases to rat olfactory search paths in the wind tunnel (Figure 6). The first and the last phases were relatively fixed, while the second and third were more variable. The sequence is invariant.

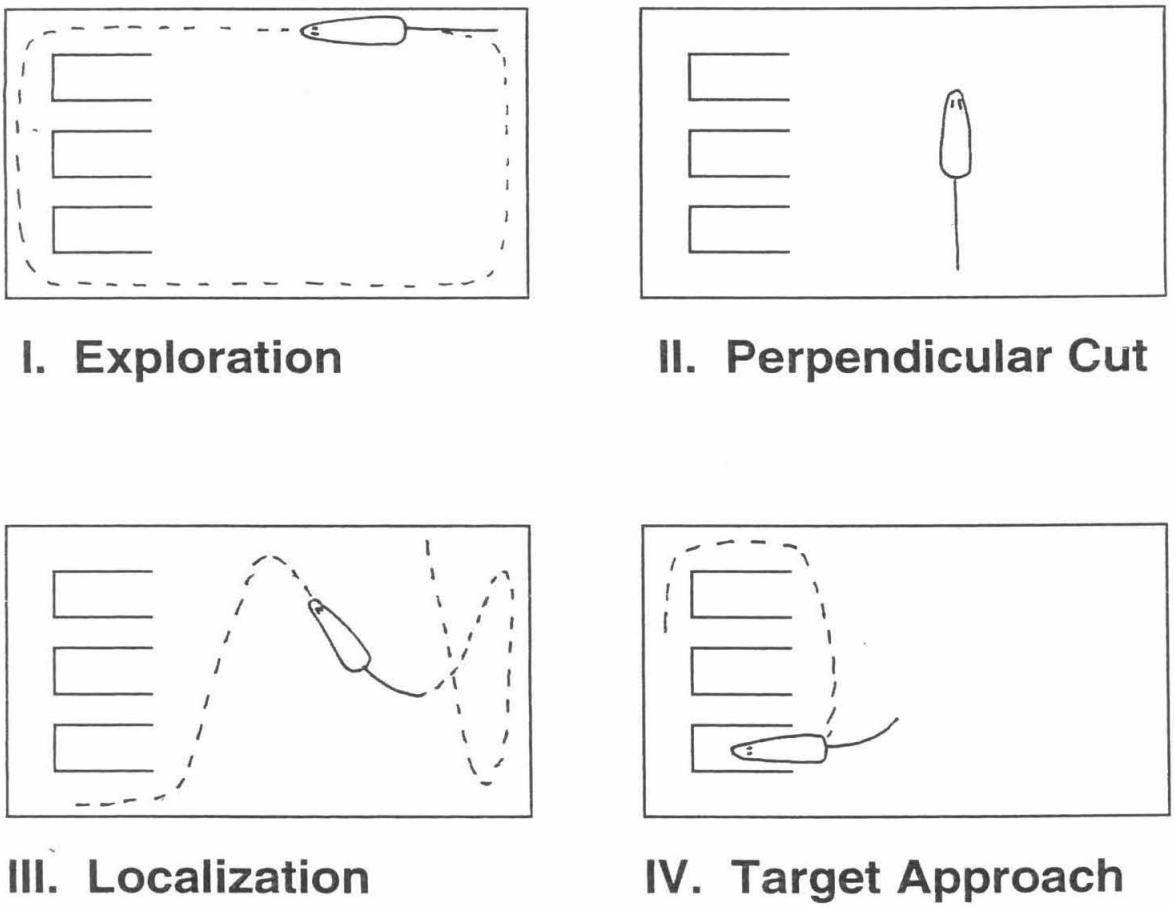

Figure 6. Four main phases of rat olfactory search behavior.

PHASE I: Exploration.

This phase is characterized by movements along the edges of the tunnel after release. Rats spent 58.1 +/- 67.4 seconds during this phase. This phase was present in all trials.

PHASE II: Movement Perpendicular to Wind Direction.

Rats spent 3.5 +/- 3.3 seconds during this phase. This phase was present in all trials. This phase can be divided into two subgroups. The first part of this phase is characterized by a movement perpendicular to wind direction. Starting from variable positions along the tunnel, rats dash across the tunnel floor perpendicular to the airflow. This part of Phase II was seen in all rats, though in 17% of the Toluene trials, some variation is seen. In these cases, a rat either does not complete the dash, turning back to the original edge less than halfway across, or crosses the tunnel completely but not strictly perpendicular to the wind direction. In contrast, the second half of this phase is more variable: some rats may make more dashes along the edges of the tunnel, while other rats may proceed directly to the third phase.

PHASE III: Increased Spatial Resolution.

This phase is characterized by many different crossing behaviors and much activity away from the edges of the tunnel. This phase is highly variable, and even missing in some rats. Rats spent 35.9 +/- 49.6 seconds during this phase.

PHASE IV: Target Approach.

Rats spent 8.8 +/- 7.4 seconds during this phase. This phase was present in all trials. Three types of approach were noted:

1. Rats move upwind and cross behind the delivery sites, continuing to the other edge of the tunnel. This is followed by a downwind movement, a turn toward the target, and then a turn directly into the target.
2. Rats move upwind and cross behind the delivery sites. Unlike (1), rats circle downwind between two of the fences and then make their target choice.
3. Rats move upwind, but do not pass the stimulus. They cross in front of the delivery sites before finally making a choice.

Classification of behaviors performed during olfactory search

24 of the 169 Toluene-based trials were analyzed in further detail, and several distinctive sensory movements were observed. There were two types of movements in the context of search: locomotory movements (e.g., walking), which position the animal in the environment, and sensory movements (e.g., positioning the nose, sniffing), which allow the animal to actively acquire sensory information. These sensory movements were called "scanning" behaviors. The animal was likely to perform both locomotory

movements and sensory movements at the same time. Additionally, grooming movements were also observed, which have no apparent connection to search.

Saltatory locomotion

Rats did not show continuous movement during search, but instead display a "saltatory" behavior characterized by frequent stops and starts. While the rat showed a variety of behaviors while moving, we focused on behaviors occurring while the animal was relatively still. A "pause" was defined as any speed less than 13.51 cm/s (i.e., rat stays within 5 cm distance in 11 frames, at 30 Hz frame rate).

Eight behaviors observed during pauses

All rats displayed eight distinctive behaviors while paused or stopped. During a stop, a rat may perform one or more of the individual behaviors. These behaviors may also occur while the rat is moving, but behaviors during movement were not included in this analysis. Throughout all the trials, the total number of behaviors observed for the three rats is 455 (Figure 7).

Sniffing with head lowered to the floor

After stopping, rats put their noses on the floor while hunching their backs. The nose is pointed directly at a specific spot on the ground and the head remains stationary.

This behavior is usually observed when the rat is along the edges of the tunnel. Sniffing with head lowered to the floor constitutes 24.4% of all the behaviors seen.

Sniffing with head lowered to the floor (short)

This behavior differs from the previous one in that the rat travels significantly slower from the normal traveling speed, but not slow enough to be considered a stop. This behavior can be seen when rats travel along the edges of the tunnel. Most of the time, this behavior occurs right before the rat changes to Phase III when it travels back-and-forth across the flow of the wind many times. This behavior is seen 9.9% of the time.

Sniffing with head up

After coming to a stop, the rat tilts its head back and points its nose to the air. This behavior always occurs in the company of other behaviors during a stop: it either precedes or follows another behavior within a single stop. This behavior occurs 14% of the time.

Sniffing with head moving up and down

This behavior combines the "head up" and "head down" postures previously mentioned. The starting movement can vary: a rat can move its head up first or down first. Movements up and down can be rapid or slow, and varies throughout the trials.

Unlike either the "head up" or "head down" behavior, the rat stops only momentarily when its head moves up and down. This behavior occurs 6.6% of the time.

Rearing

Coming to a complete stop, the rat stands with two rear paws on the ground while the front paws are held up in the air or against the wall of the tunnel. In this position, the rat then points its head straight up and sniffs its surroundings. This behavior occurs mostly near the ends of the tunnel, although sometimes a rat may stop in the middle of a perpendicular dash, and rear. This behavior occurs 4.8% of the times.

Sniffing with head moving in a semicircle

In this behavior, the head of the rat is first positioned straight in front of the body. While sniffing in this position, the rat moves its head from one side of the body to the other. This movement traces a semicircle in space before the rat. Rats either sniffed while moving their heads smoothly along the path of the semicircle, or they selected three discrete points within the semicircle to pause and sniff: one on either side of the body and one straight in front. This behavior composes 27.3% of all behaviors identified.

Swinging head into the wind

This behavior always occurs along the edges of the tunnel. When coming to a complete stop, the rat will shift its head into the wind flow while keeping its body close

against the edge of the tunnel wall. Sometimes, the rat will continue on its path along the edge of the tunnel after completing this behavior, but frequently will make a dash across the tunnel floor. This behavior occurs 8.2% of the time.

Grooming

Grooming is a stereotyped behavior, most common in male rats. (This was also observed during these experiments.) The number of grooming bouts were significant when rats where first introduced into the tunnel during training. By the time training was complete, the number of grooming bouts had significantly decreased. Rats groomed only along the edges of the tunnel. Throughout all the trials, grooming behaviors make up 4.8% of the observed behaviors.

Axially-directed scanning behavior

In addition to the eight behaviors described above, which occurred while the rat was nearly stationary, the rats also displayed a dynamic behavior. The rats were observed to perform a series of quick pauses that occurred within a specified timeframe (each pause lasted 270 msec), usually along the edge of the tunnel (i.e., along the direction of airflow). Pauses were approximately 10cm apart. All scanning behaviors were observed within the series of pauses. Isolated pauses described earlier occurred in addition to the axial scanning behaviors.

Consistency in spatial positions of "stops"

While moving in search of the odorant, rats paused 22 +/- 17 times, at approximately five locations on average. A pause (or stop) is noted whenever a rat slows to a speed at or below 13.51 cm/s. (The series of pauses composing axially directed scanning were not included in the analysis of where the rats stopped.) These pauses occur at about the same spatial position along the edges of the wind tunnel. The set of locations that a rat pauses at is unique for each rat and is consistent across all trials (Figure 8).

Discussion

Summary of local search behavior in the rat

Searching behavior can be broken down into a hierarchy of behavioral units, which occur in relation to the spatial distribution of resources [4]. In this framework, resource patches are found in habitats; search across resource patches is termed "ranging," and movements across habitats is termed "migration." For this study we focused on local search behavior, which normally occurs within a resource "patch."

Local search behavior combines locomotory movements, sensory movements, and other behaviors. Locomotory movements were examined both spatially (i.e., by

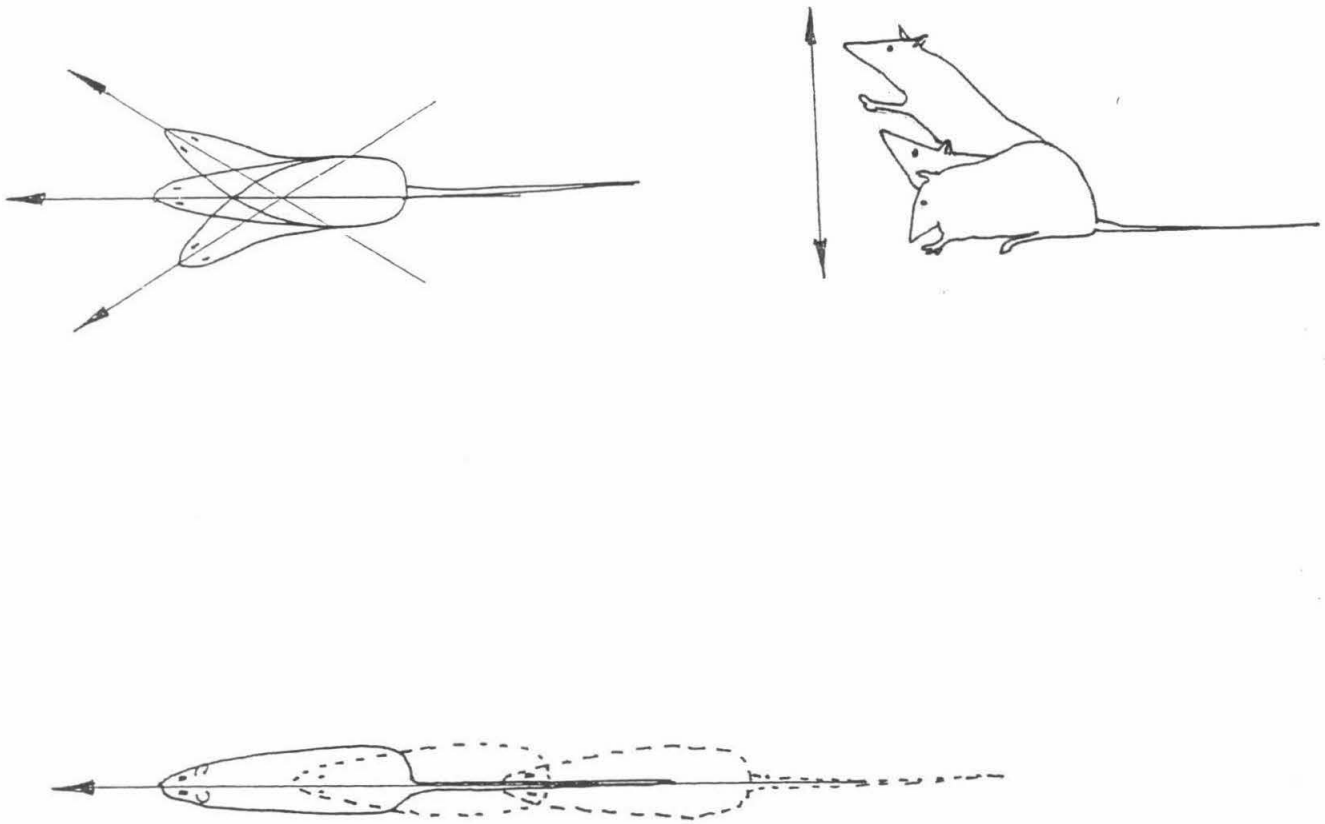

Figure 7. Summary of rat olfactory scanning behaviors. Eight behavior modules were observed, which when viewed with respect to their scanning planes, revealed three spatial dimensions of rat olfactory scanning behaviors.

identifying *paths taken* during search) and temporally (examining the pattern of stops and starts taken during search).

Rats display an invariant path sequence in olfactory search, consisting of four phases.

A four-phase spatial search pattern was seen in all six rats, regardless of the odor stimulus. The four phases are in the same order for every trial. The first and the last phases of the search showed the least variability while the second and third phases were highly variable. The first phase is probably for exploration, allowing the rat to investigate its environment. This is suggested because the rat shows no observable response to the odor, and the behavior is similar to the rat's naive behavior in the wind tunnel. This first phase is also a time when rats may create a spatial "image" of the wind tunnel. This "map" may provide spatial information that rats use to locate the source of the odor.

The second phase is marked by a dash perpendicular to the airflow. This perpendicular dash is present in every trial. Possibly, this is the point where a rat gathers most of the information about the odorant, and its position relative to the wind direction. By moving perpendicular to the airflow, a rat can sample all the information carried by the air. Because the dash is always perpendicular to the flow direction, wind is possibly an important cue used by rats in their search. Alternatively, the direction of the dash may

be an artifact of the rectangular shape of the wind tunnel. Since the shape of the wind tunnel contributes to the characteristics of the airflow, more experiments are necessary to tease apart the spatial characteristics of the search pattern from the spatial characteristics of the environment.

The third phase is likely for increased resolution of spatial information. This phase is the most variable of all phases. Rapid movement across the airflow, away from the edges of the tunnel, is the characteristic most representative of this phase. This phase often includes several dashes of varying lengths, suggesting that the rat is actively gathering information about the stimulus and that the crosswise paths are a way for them to gather the maximum amount of information.

The fact that the sequence of paths is invariant, and that the third phase is missing in some rats, suggest that in each phase the rat is using the information gathered from the previous phase. Even when the third phase is missing, the rats go directly to the fourth phase and are able to locate the odor with the same success rate as rats that perform the third phase. If the rat was not able to use (i.e., recall from memory) information from the first and second phase, it seems less likely that the rat would locate the odor successfully. In between the third and fourth phases, the rat increases its speed. This suggests that it has found the odor, and will then approach the site.

The *fourth* phase is the approach to the odor source. There are only three different patterns of approach. This suggests that the rat has already decided where the target is, and contact with the odorant is no longer of primary importance.

Within these phases, rats performed distinctive olfactory scanning behaviors, scanning in three spatial dimensions.

We next examined the overall four-phase search behavior in detail to determine if there were behavioral subcomponents within the general search pattern. Nine behaviors were identified. With the exception of grooming, all of the behaviors observed were sniffing strategies (scanning behaviors). The scanning behaviors can be grouped into three categories: vertical scanning, horizontal scanning, and axial scanning (Figure 7).

Rats appeared to use memorized spatial information during search

More interestingly, these scanning behaviors occurred at positions within the search environment that were unique for each animal. This behavior recalls the exploration and subsequent "polling" behavior of animals in the field [4]. This in turn suggests that a rat might be using memorized spatial information during search. In other words, rats may not be strictly stimulus-driven, but may integrate their own spatial position with sensory information in order to locate a target.

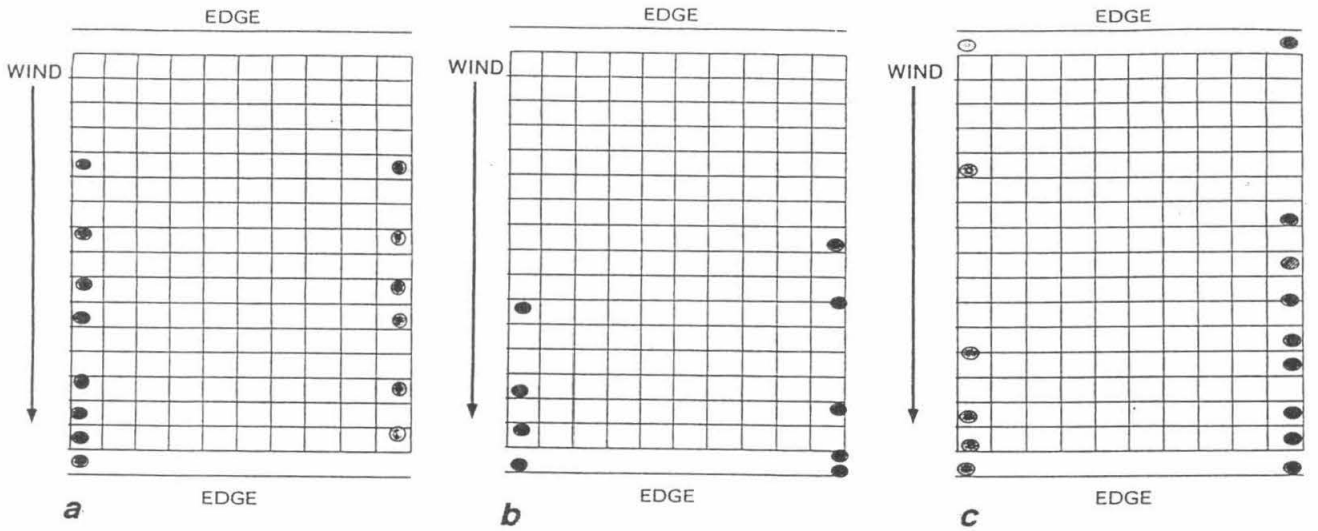

Figure 8. Map of spatial locations of positions where individual rats consistently "paused" during olfactory searching behavior.

The spatial organization of these maps is similar to the spatial organization of place fields described in hippocampal literature.

The spatial organization of the positions where rats paused during search shows a remarkable resemblance to the spatial organization of "place fields" described in the hippocampal literature. Place fields are spatially discrete locations which, when the rat is physically present in that location, evoke neuronal activity in populations of "place cells" in the hippocampus. The spatial organization of place fields shows a tendency towards clustering along the edges of the experimental arena. While the hippocampus has long been studied in the context of olfactory behavior [5], this is the first time that a link has been observed between the natural behavior of animals and the physiological properties of hippocampal neurons.

A likely role for the hippocampus in olfactory searching behavior

Current theories of hippocampal function suggest not only that the hippocampus plays a central role in olfactory searching behavior, but also provide a framework for understanding the olfactory search strategy used by rats.

The scanning seen during olfactory search may be a behavioral correlate to hippocampal place cell activity.

One theory of hippocampal function is that it integrates non-spatial information and spatial information [5]. Olfactory search behavior is a good example of a task that requires the integration of non-spatial (i.e., odor perception information, as well as temporal information about the chemical stimulus) and spatial (i.e., current location of animal; predicted location of odor source) information. The hippocampus might provide a reasonable locus for integration of this information.

Another idea is that the hippocampus coordinates sequences of information [5]. This provides a mechanism for the hypothesis that the function of a specific search strategy may be to produce specific patterns of sensory stimulation, which in turn provides the necessary information in the proper "format" to the nervous system.

Rats appear to search differently from the known search behaviors of other animals, suggesting more than one general search strategy

The primary goal of the experiment was to determine what type of search strategy is used by rats while searching for an odor stimulus. An animal's search behavior is a reflection of its search strategy, since different behaviors may accomplish the same search task. For example, if the search task were to determine the height above ground of

an odor source, *possible* behaviors might be to rear on its hind legs or climb on a rock while sniffing. A series of search tasks would then comprise a search strategy.

It was found that rats displayed the same spatial search pattern for different olfactory stimuli. This suggests that this level of search might be innate. To test this hypothesis, a careful analysis of the behavioral components of rat behavior while localizing the odor source was performed. All behaviors that occurred consistently throughout the trials were noted. While many of these behaviors were observed while rats were moving, a detailed study was performed only of behaviors that occurred when the rats paused during search. Eight searching behaviors were identified. These eight behaviors strongly suggest that there is indeed an innate search strategy and that during a search, multi-modal non-olfactory sensory information (e.g., wind direction) is gathered. This is supported by sniffing behavior with movements perpendicular to the axis of the odor plume as well as parallel with the plume axis. Furthermore, the behaviors are seen in all rats, and there was no "preferred" behavior seen in one rat over another.

Perpendicular "dash" not seen in arthropods

Earlier studies of arthropod search behavior did not report a crosswind "dash" behavior as was seen in this study. The dash behavior suggests that the rat may use the direction of the airflow as an orienting cue during olfactory search behavior. One interpretation of the significance of this behavior is that it provides the most information

interpretation of the significance of this behavior is that it provides the most information about the potential location of the odor source for the least energy (and possible risk of predation). Preliminary experiments showed that the rat may select its target after this phase, so that if the location of the odor source is changed after it has completed Phase II but before progressing to Phase III, the rat often selects the original position of the odor source as its target. This suggests that the rat may base its decision after this phase, disregarding information it may gather in subsequent movements, further suggesting that the rat may orient itself crosswise to the wind for this purpose. However, the dash may be an artifact of the configuration of the wind tunnel; a wind tunnel that was shaped differently might yield different orienting behaviors.

Spatial map not seen in arthropod search

The use of a spatial memory map was not indicated in studies of arthropod olfactory search behavior, even though marine animals do appear to use spatial information in other contexts (i.e., returning to a "home" crevice, etc.).

The map seen might be an artifact of the configuration of the wind tunnel, and the fact that the rats were familiar with the search arena. Thus rats in a novel environment, or rats in a differently shaped environment, may show different spatial patterns of search. However, that the spatial map emerged at all suggests that the behavior of the rats is quite

complex, and perhaps the experiment could be made more difficult to determine the limitations of the rat's searching abilities.

In conclusion, rats appear to search differently from the known search behaviors of other animals, which suggests that more than one general search strategy is used across different species. It may be that the search strategy used depends on the fluid regime the animal is searching in. If so, a single animal may make use of several search strategies. This has numerous implications about how animals process sequences of sensory information, and how sensory behaviors may be patterned in the brain to respond to the appropriate environmental cues while providing dynamic sensory information.

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Chapter 2. Olfactory Scanning Behaviors*

Introduction

Insects are favored models for studying sensory processing because their anatomy allows ready access to the nervous system, and because it is easy to combine electrophysiology and behavioral approaches. The premier sensory apparatus in insects are the antennae: the long articulated appendages attached to the head. The antennae bear receptors for touch, taste, temperature, and humidity, though the predominant receptor modality is olfaction. Consequently, the antennae show different movement patterns that are linked with one or more sensory modalities. The antennae produce both reflexive movements in response to tactile stimuli, and behaviors that are more complex associated with chemical stimuli and exploratory search. Although movements of the antenna are controlled by local circuits in the cerebral ganglia, its sensory input strongly influences the global behavior of the animal. Our approach is to view the antenna as a "chemo-sensorimotor" system, and to examine patterns of movement that may have functional significance to the animal.

Extensive work has been done on the insect olfactory system, especially in the last 50 years, characterizing the anatomy and physiology of the system. However, little work has described antennal behavior, especially as regards to olfactory responses. In this

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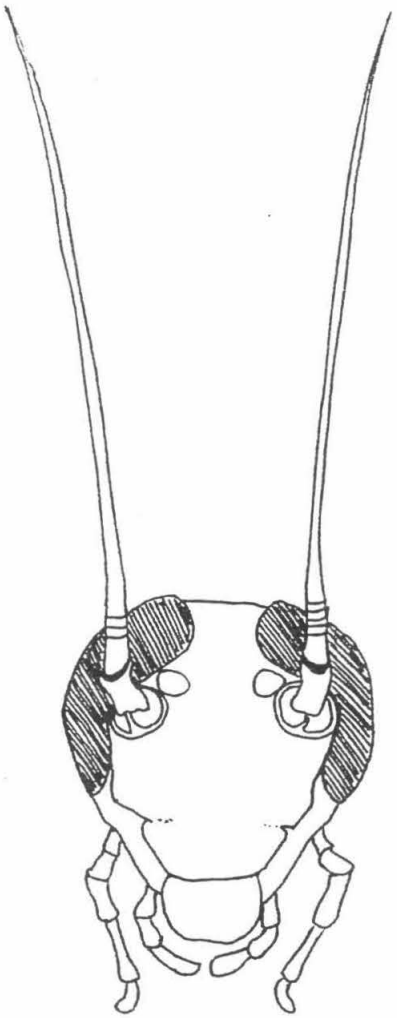
* This work performed in the laboratory of Gilles Laurent, at the California Institute of Technology

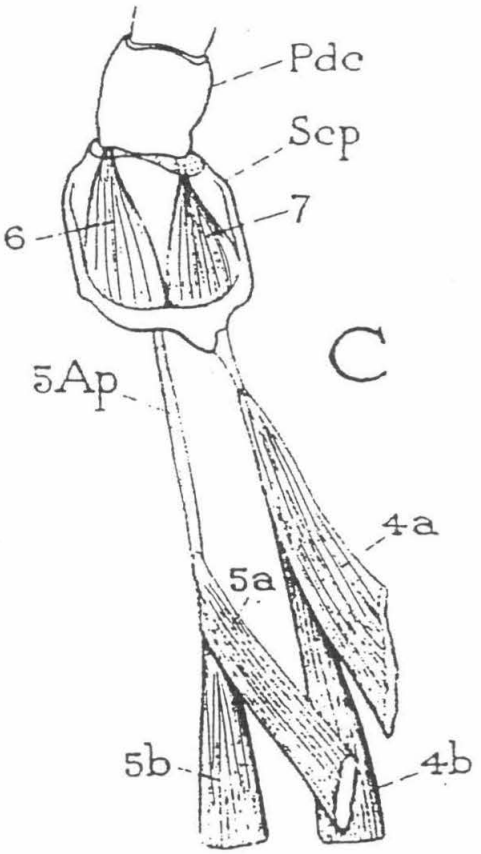
study, we examined the antennal responses of the American cockroach, *Periplaneta americana*, to food odors presented to the animal from different directions. The cockroach is a nocturnal scavenger that depends heavily on its antennae for sensing and exploring its environment, and it was likely that antennal movements might play a role in olfactory-evoked behavior.

We entered this study with several questions in mind: do the antennae show odor-evoked movement patterns? What may odor-evoked antennal movements tell us about olfactory processing? What does it tell us about olfactory perception in insects, and how is this information used in a complex behavior such as exploratory search? To explore these questions, we videotaped the movements of antennae at rest and in response to food odors; we also characterized antennal movements in response to varying degrees of air movements. These motions were then digitized, and recreated in two and three dimensions for analysis.

Background

In addition to olfactory receptor cells, the antennae bear sensory receptors of other modalities including taste, temperature, humidity, and touch. The sensory receptors are housed in sensilla, which are cuticular structures that cover the surface of the antennal segments. The sensory receptors within each sensillum project from the antenna via the





G.C. Crampton (1925)

Figure 1. Sketch of cockroach head and antennae. Abductor (7) and adductor (6) muscles attach to the scape (Scp) and insert onto the pedicel (Pdc), allowing movement of the pedicel and flagellum in the horizontal plane. Levator (5) and depressor (4) muscles insert onto the scape, providing for movement of the entire antenna in the vertical plane. Combined, these muscles allow the antenna three rotational degrees of freedom. (Diagram from [1])

antennal nerve to the antennal lobe, a glomerular structure in the deutocerebrum of the insect brain. Axons from a single sensillum are thought to project to up to several glomeruli in the antennal lobe [2]. From the antennal lobe, uni- and multiglomerular projection neurons carry odor information to the mushroom body [3] which, as a site of sensory integration, receives multi-modal sensory inputs as well as centrifugal inputs from other cerebral ganglia.

In addition to housing the vast array of sensory receptors, the antenna is also an articulated appendage (Figure 1). The cockroach antenna has three main segments, and is articulated only at the base. Abductors and adductors attach to the scape and insert onto the pedicel, allowing movement of the pedicel and flagellum in the horizontal plane. Levator and depressor muscles insert onto the scape, providing for movement of the entire antenna in the vertical plane. Combined, these muscles allow the antenna three rotational degrees of freedom [1].

The motorneurons controlling the antennal muscles lie in the deutocerebrum, the second of the three cerebral ganglia in the head of the animal. It is not known if there are direct connections from the antennal nerve to the antennal motorneurons.

Materials and Methods

All experiments were performed in a darkened room, under infrared illumination. All experimental animals were male, taken from the laboratory colony. Roaches were

fed *ad libitum* with Purina Rat Chow and water. All animals were introduced into the test chamber and allowed to dark-adapt for 30 minutes prior to filming. Two-dimensional (2D) experiments were performed on freely moving animals, while 3D experiments were performed on animals that were immobilized, with head and antennae free to move.

Test Chamber

The cylindrical test chamber had six ports through which odors could be introduced. This provided a spatially symmetric environment within which roaches were free to wander. The top of the chamber was sealed. The floor of the chamber was covered with a disposable filter paper liner, which was replaced after each trial to prevent unwanted food odors or pheromonal cues from confounding the experiments. Air was drawn into the chamber through the side ports, and exited the chamber through a screened vent in the center of the chamber floor, which was connected to a small fan which drew air outward. For 2-D odor experiments (N=15), all air ports were open, and odors were introduced at variable positions. For 2-D airspeed experiments (N=7, 49 trials), all six holes remained open, and the vacuum fan was turned off. A non-odorized air stream was directed through one of the ports using a variable-speed air pump. The airspeed was varied in a set sequence of 0-4-2-3-1, with airspeed settings corresponding to the maximum airspeed:

- air off
- 25% of maximum
- 50% of maximum
- 75% of maximum
- maximum airspeed

For 3-D odor experiments (N=9), animals were not filmed in the test chamber but were fixed such that only head and antennae were free to move.

Odor Stimuli

Each animal was stimulated while "alert" but stationary, meaning that it was not grooming or walking, but exhibited a characteristic low-frequency "beating" pattern with its antennae. Odors were introduced to the chamber by expelling air from a syringe containing small amounts of whole food items (ripe banana or cheese) immediately outside the odor port. Thus, odors were drawn into the chamber without the mechanical stimulus associated with an air "puff." For 3-D experiments, the syringe was used to gently "puff" odors at different positions near the antennae.

Motion Analysis

Because cockroaches are most active at night, we videotaped cockroaches in a darkened chamber. All videotaping was performed under infrared illumination using black-and-white CCD cameras using a frame rate of 60 Hz. Stimulus delivery was recorded simultaneously on each videotape using an optical signal. The Peak

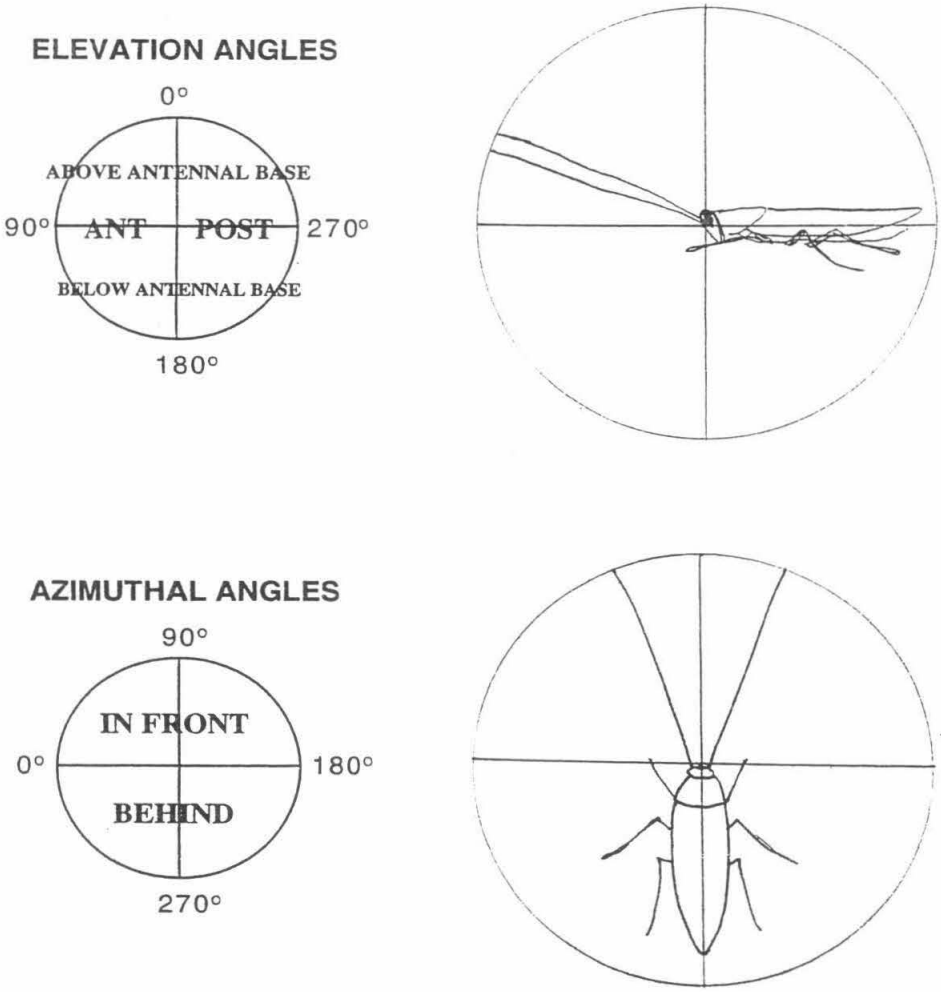


Figure 2. Reference frames used for angular measurement.

Performance Motion Analysis system [4] was used for both 2D and 3D motion analysis. To analyze videotaped movements, a spatial model was developed which defined the specific points of interest (base and tip of antennae, and fixed points on the head). The videotaped trial could then be digitized at any frequency up to or equal to the frame rate of 60 Hz, to capture the points specified by the spatial model. 2D odor and airspeed experiments required a single camera, and were digitized at 20 Hz. 3D odor experiments required two cameras, and were digitized at 60 Hz. Each camera view was calibrated for object size and orientation before each experiment using a calibration frame. Digitized points from the two simultaneous camera views were transformed in software to recreate the three-dimensional movement of the antennae.

Reference Frame for Angular Measurement

Antennal angles were determined according to Figure 2. Elevation angles measured movement in the vertical plane, while azimuthal angles measured movement in the horizontal plane.

Results

Summary of Antennal Behavior

At rest, "alert" animals exhibit a slow (0.5 Hz), low-amplitude "beating" pattern of antennal movement (Figure 3c). This movement is likely to be a response to low-

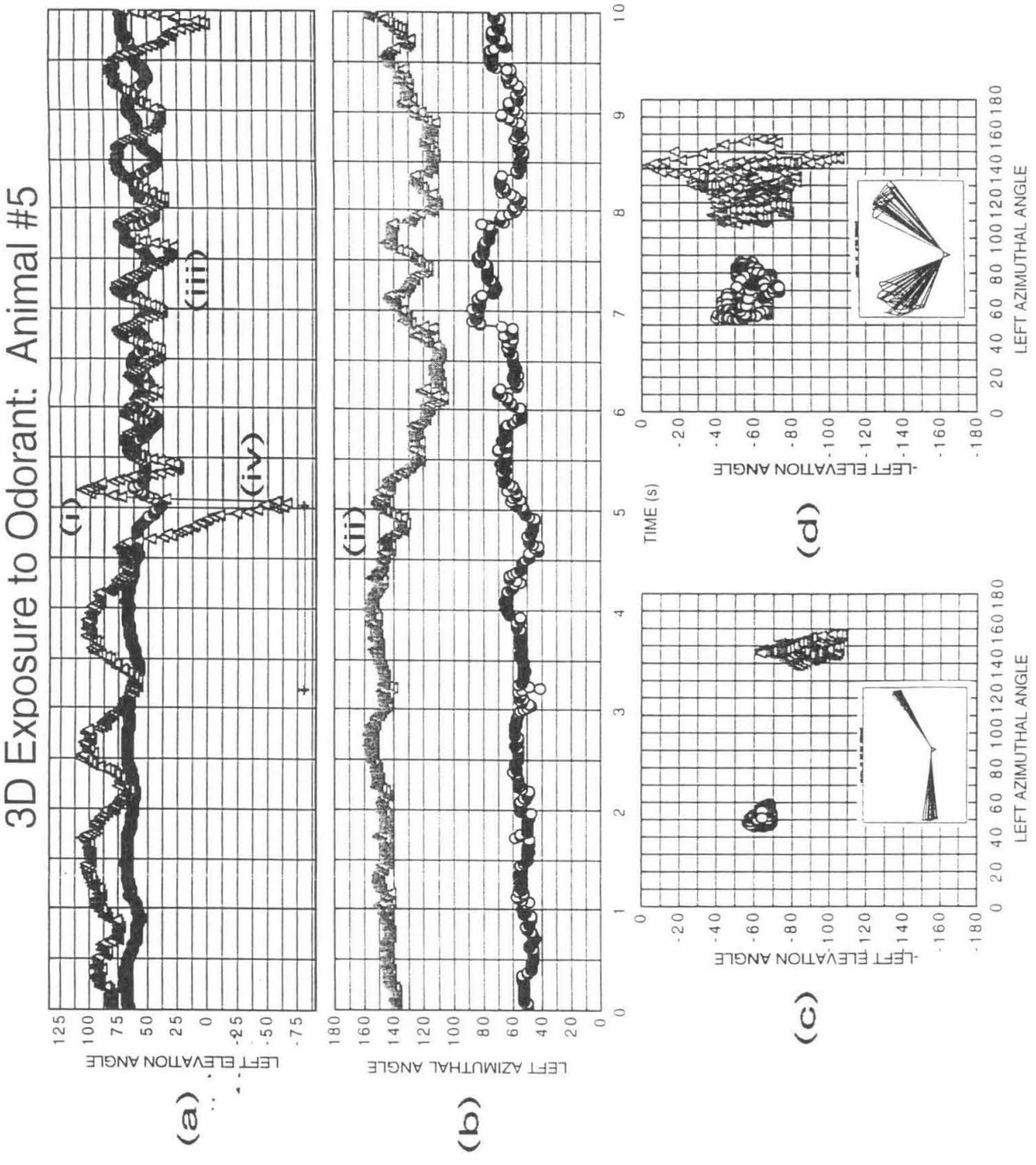


Figure 3. 3D antennal response to odorant stimulation.

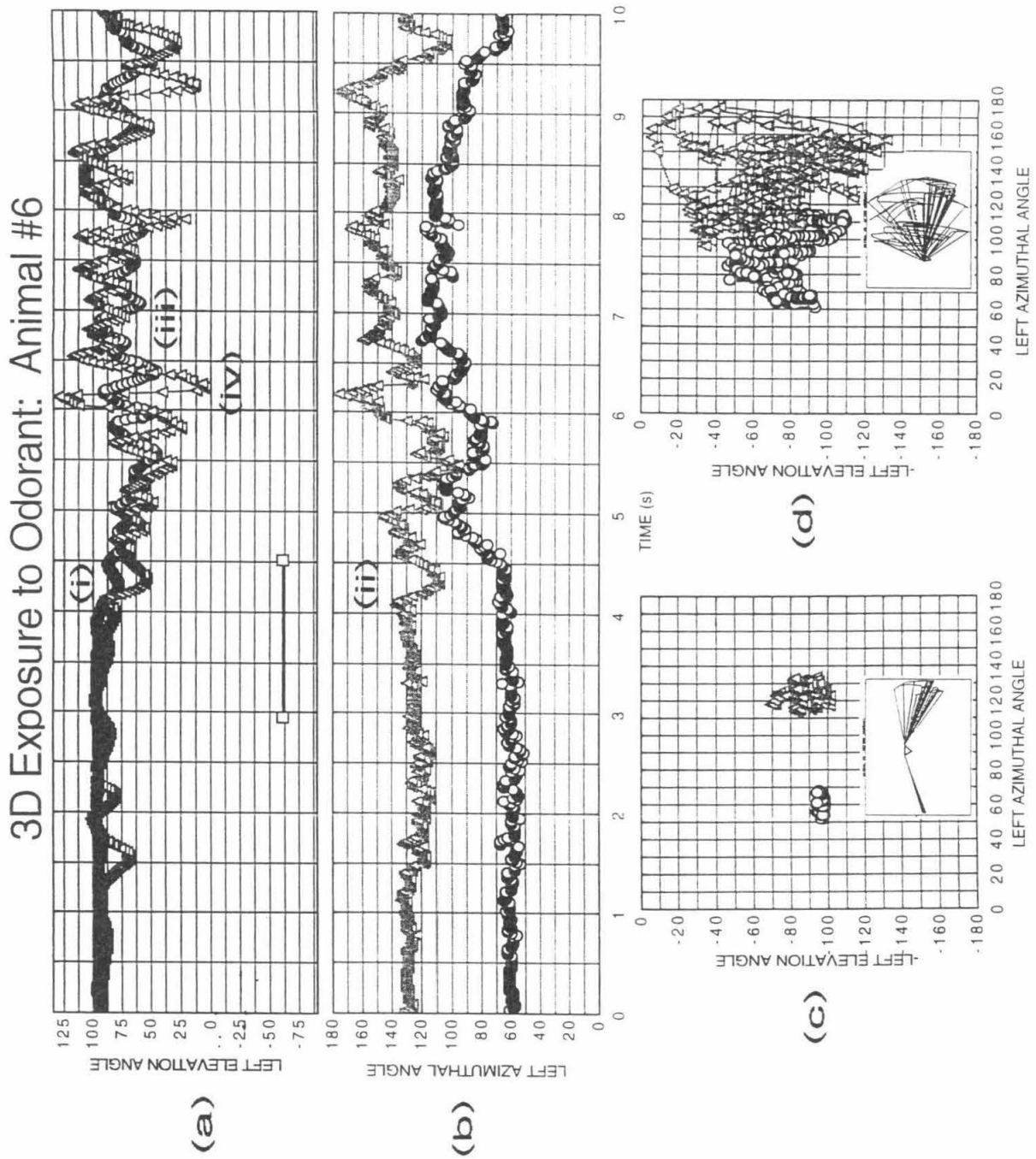


Figure 4. 3D antennal response to odorant stimulation.

Figures 3-4. 3D antennal response to odorant stimulation. Antennal movement patterns before and after odorant stimulation. Before stimulation, the right and left antennae move asymmetrically and scan a small volume. After odorant stimulation, the antennal waving frequency increases, though the pattern of movement is still asymmetric. Upon presentation of the odor, antennae were raised simultaneously (i) and were drawn together in the horizontal plane (ii) upon odor stimulation. Antennal waving frequency increased, primarily due to movements in the vertical plane (iii). The amplitude of antennal patterns increased (iv). The antennal patterns of motion were "arcs" or "circles." A shift in the average antennal angle corresponds to antennal "pointing" towards the odorant source (v). Antennal patterns differ between left and right antenna both spatially and temporally. (a) Left (Δ) and right (□) antennal elevation angles. (This graph may be viewed as if we were viewing the animal from the side, and the tips of the antennae were tracing out a path in time.) The bar near the bottom shows the duration of the stimulus event. Left axis shows angle measurement in degrees (as defined in Figure 3). Bottom axis shows time in seconds. (b) Left (□) and right (Δ) antennal azimuthal angles. (This graph may be viewed as if the tips of the antennae were dipped in ink, and the plot was a chart recorder scrolling towards the left.) (c) The small figure shows the digitized form of the animal (head is the triangle), and the stick-figure representation of antennal movement before stimulus presentation. The pattern of motion can be described as "arcs" or "circles" through space. The plot in the background illustrates the 2D projection of the 3D path traced by the tip of the antenna in the time period before stimulation; left axis is the inverse of the elevation angle, bottom axis shows azimuthal angle. (d) Change in movement after odor stimulation. The head has oriented towards the right of the drawing, and the antennae are now "pointing" towards the odorant source. Antennal amplitude and frequency have increased.

velocity air currents, as discussed below. Introducing food odors caused a visibly striking response. Immediately after food odors were introduced into the chamber, the antennae responded with a very specific pattern: both antennae are elevated simultaneously, the azimuthal angle between left and right antenna decreases, then antennae immediately started to wave rapidly (up to 3 Hz). The tips of the antennae traced out large circular or arcing paths, spanning the space around the animal. Furthermore, the antennal tips seemed to "point" towards the source of the odor (Figure 3d). In experiments with freely moving animals, the animals continued to wave their antennae in this manner as they walked rapidly towards the odor source, demonstrating a

clear ability to locate the source of the odor. These patterns were similar in the nine animals tested. No difference in responses to different odors was observed.

Frequency Analysis

The frequency of antennal movements increases after odor stimulation. Visual inspection of 2D digitized data suggested three major frequency components: ~0.1 Hz, ~1 Hz and ~10+ Hz. Plotting antennal angle vs. time, the appearance of the pattern is a noisy sinusoid with a slowly varying sinusoid baseline (Figure 5). However, Fourier analysis of 3D antennal displacement patterns reveals a flat spectrum at frequencies higher than 10 Hz (Figure 6). There is a very large peak at frequencies < 0.5 Hz; this is likely to be aliasing from noise. Several peaks appear in the 1-5 Hz range depending on the presence or absence of odor.

For both azimuthal and elevation angles before odor presentation, the frequency spectra show a broad peak centered on 0. After odor presentation, strong peaks appear in the 1-3 Hz range. Fourier analysis of azimuthal motion before and after stimulus suggests that frequencies in the 1.5-2.5 Hz range emerge as a result of odor presentation. Fourier analysis of levator motion before and after stimulus suggests that frequencies in the 1-3 Hz range emerge as a result of odor presentation. The increase in antennal waving frequency is primarily due to levator activity.

Figures 5a-c. 2D antennal response before, during, and after odorant stimulation. These are 2D projections of 3D movement (as if one were looking at shadows of moving figures on a wall). "Interantennal angle" is the angle between antennae.

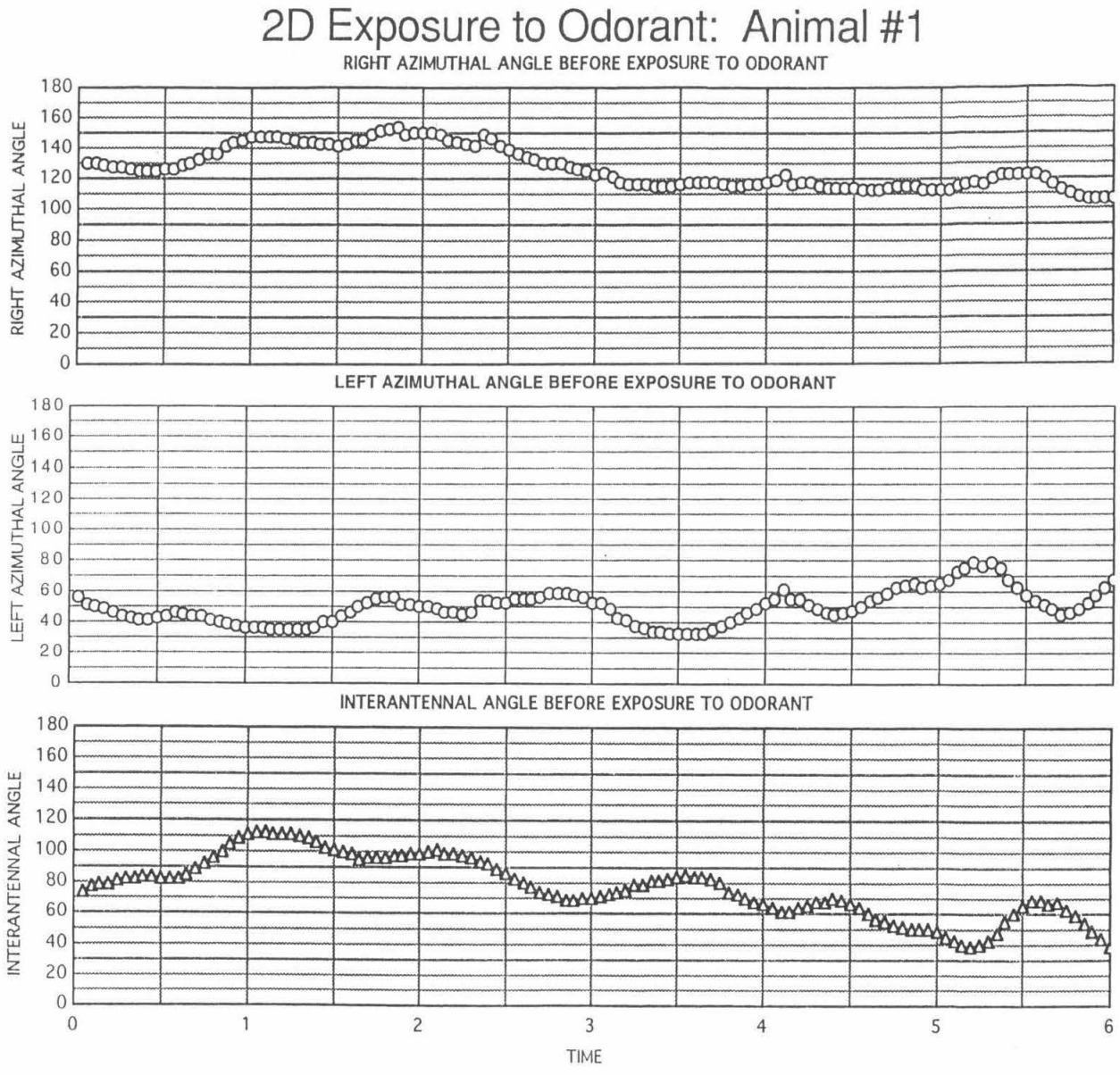


Figure 5a. 2D antennal response before odorant stimulation.

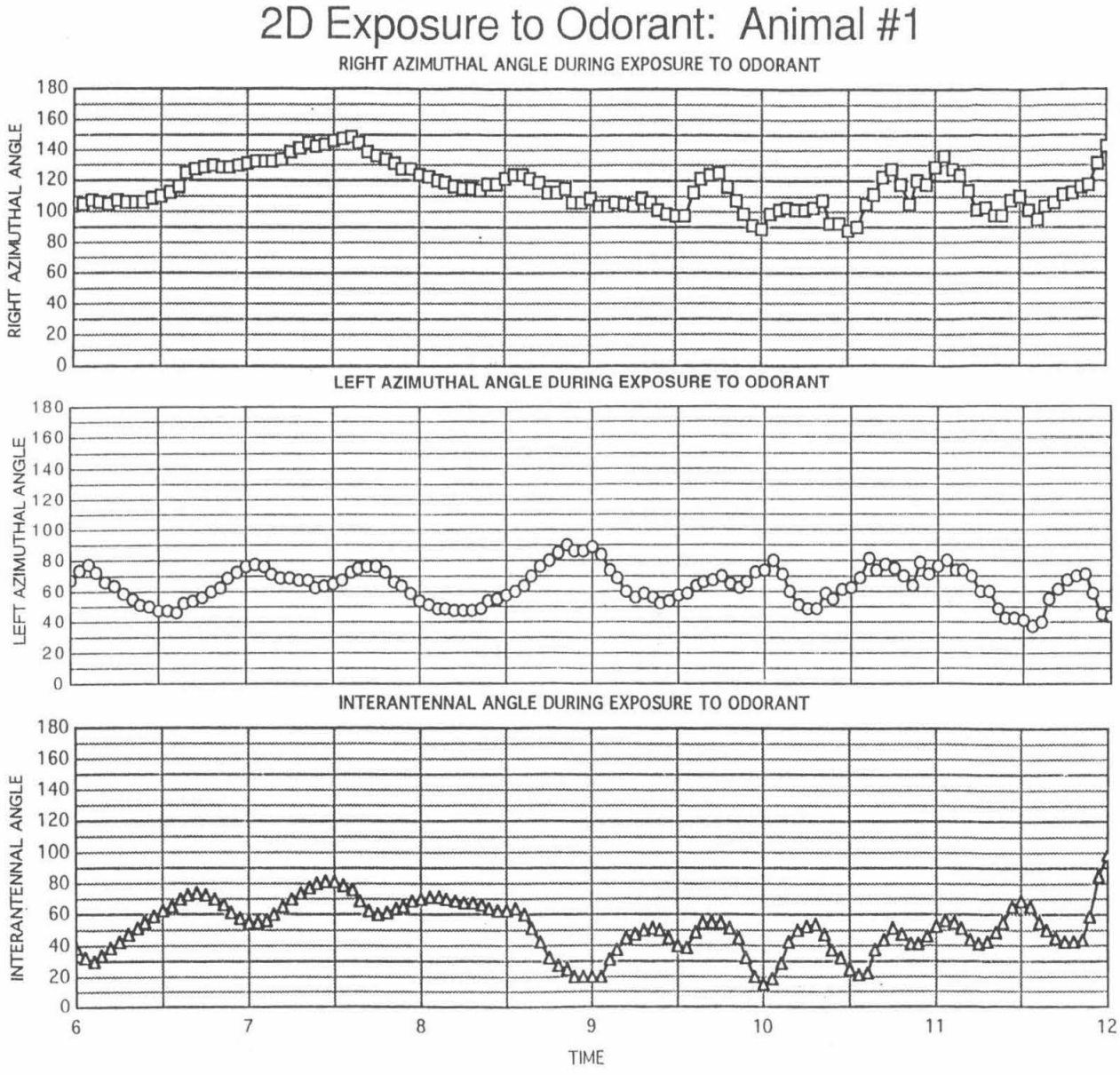


Figure 5b. 2D antennal response during odorant stimulation.

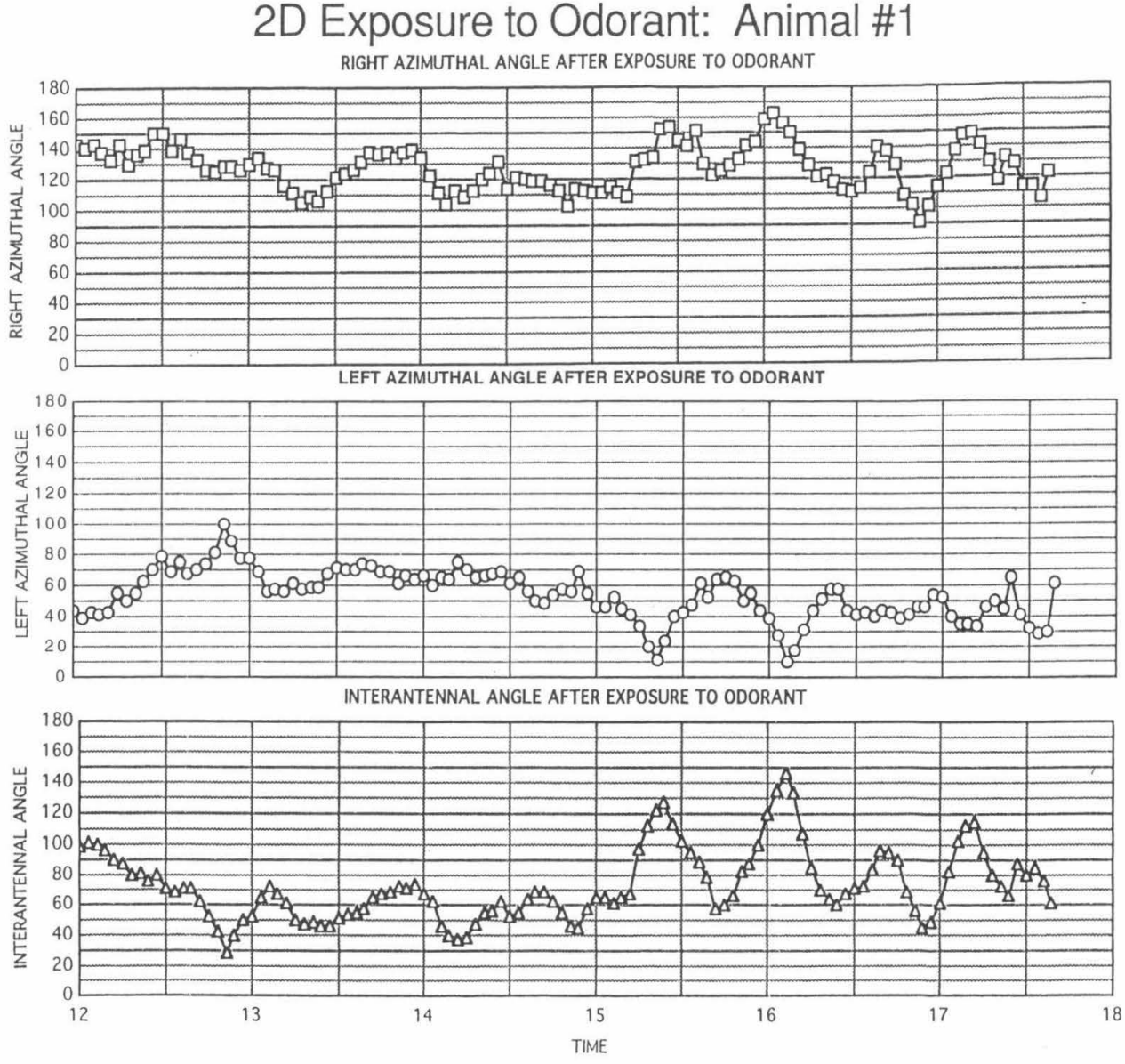


Figure 5c. 2D antennal response after odorant stimulation.

Spectral Analysis of 3D Odor Trials Before and After Stimulus

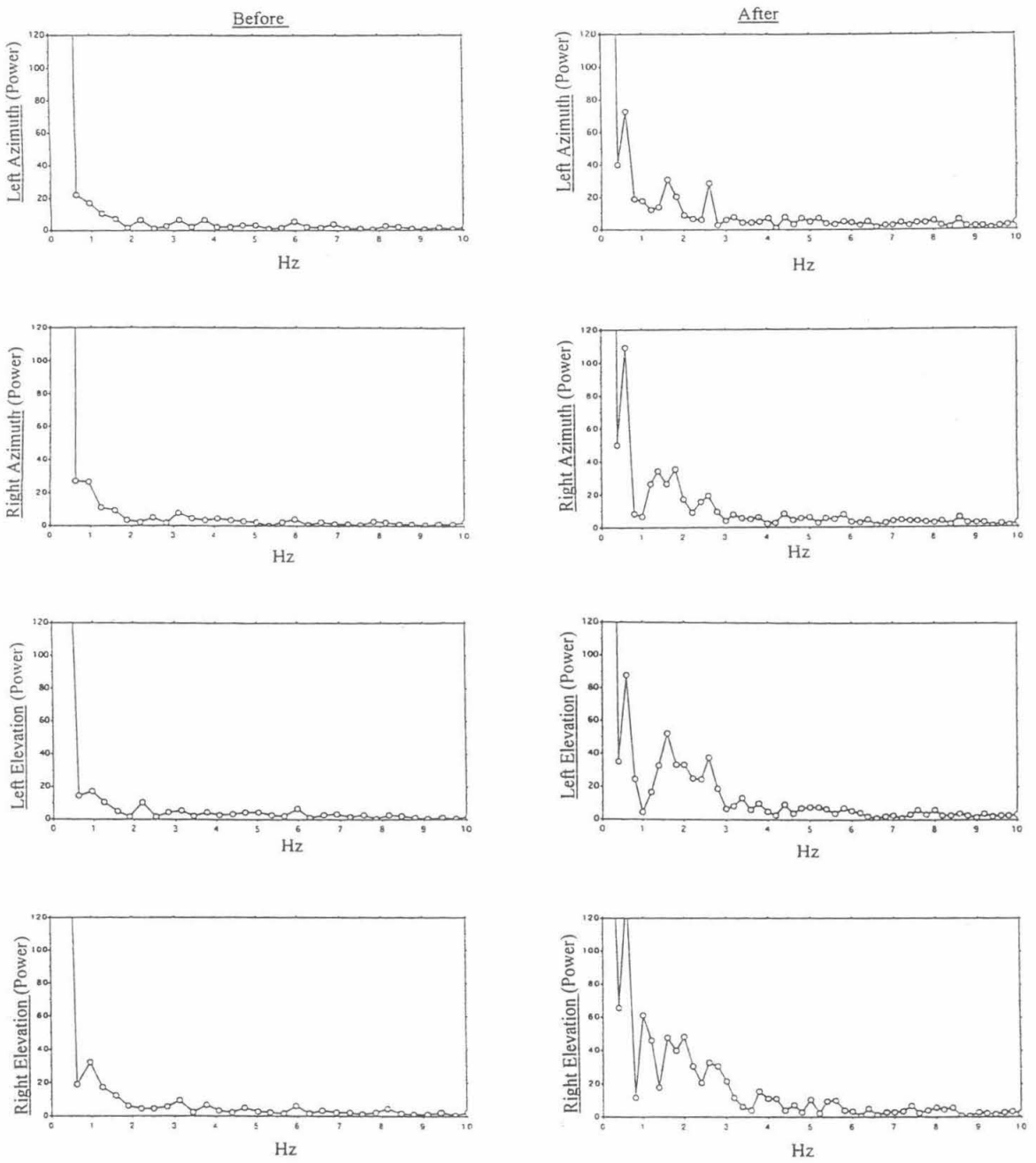


Figure 6. Fourier spectra of 3D odor trials before and after odorant stimulation. Spectral analysis of antennal waving frequencies shows that there are no significant frequency components higher than approximately 5 Hz. These eight panels show frequency spectra for both horizontal and vertical planes for both left and right antenna, before (a) and after (b) odorant stimulation. Increased power is seen in the 1-3 Hz range after stimulation.

Phase Analysis

How coordinated is the activity between left and right antennae? Immediately after odor stimulation, antennae are elevated simultaneously ((i) in Figure 3a), and move towards each other in the horizontal plane ((ii) in Figure 3b). However, this short period of coordinated movement appears to be the only time in which left and right antenna show coupled activity. 2D results suggested that prior to stimulus, antennae "scan" in anti-phase (laterally), and upon odor presentation the scanning behavior changed to a coordinated phase-matched sweeping. However, the 3D results did not verify the "scanning" behavior seen previously, although this might be a result of the animals being immobilized. Cross-correlations of antennal movement patterns after odor stimulation reveal no phase relationship between antennae. Visual inspection of Fourier spectra verify that movements of individual antennae show different frequency peaks.

Spatial Pattern Analysis

In general, the motion of the antennae appears to be a series of dorsal-ventral arcs or circles, sweeping laterally.

The amplitude of antennal motion changed after odor stimulation. Prior to stimulus, horizontal angle amplitude was approximately 5-10 degrees (maximum 20 degrees), while after stimulus, amplitude increased to about 30-40 degrees (maximum 50

degrees). In the vertical plane, elevation amplitude changed from about 5-10 degrees before (maximum 30 degrees) to about 40-60 degrees after stimulation.

The mean direction angle in the azimuthal plane also changed after stimulus presentation. In Figure 4b, this represented a shift towards the centerline of the animal. In Figure 3b, this represented a shift slightly to the right of centerline of the animal. This "DC shift" corresponded to a "pointing" of antennae towards the odorant source. The duration of "pointing" was approximately 2 seconds. In the vertical plane, the mean direction angle also changed after stimulus presentation. In both examples, this represented a shift slightly up, but other trials suggest this is not a general rule.

The musculature of the cockroach antenna allows for rotation about the longitudinal axis of the antenna during normal motion. Axial rotation of antenna increases surface area of antenna contacted by odorant molecules. However, video analysis indicated that rotational movement was not significant at the spatial and temporal resolution provided by our equipment.

Volumetric Analysis

Calculating the volume defined by the limits of the range of antennal movements includes the space scanned by the antenna, as well as the "sweep" towards the source of the odorant. The maximum antennal ranges observed were 180 degrees in elevation, and 200 degrees in azimuth. For an antennal length of 5 cm, the volume defined by the limits

of the range of antennal movements before odor stimulation is approximately 8.4cm^3 per antenna; both antennae sweep out a volume of 16.9cm^3. After odor stimulation, this volume increases to 85.1cm^3 per antenna. As there is a large degree of overlap in the area scanned by both antennae, the combined volume scanned is approximately 93.5cm^3. In general, after odor stimulation each antenna increases the volume of scanned space by an order of magnitude.

Influence of Air Speed on Antennal Movements in Absence of Odor

In unscented, still air, the cockroach moved its antennae up and down only slightly (about 0.5 Hz). When unscented air was pumped into the chamber at different speeds, at low velocity the antennae would "beat" in a slow (0.5 - 1.0 Hz) up-and-down motion (Figure 7). The largest change in the rate of beating was between the still air condition and when the air was moving, so it is likely that the antennae were responding to the general motion of the air, and not to the specific air speed.

Changes in spatial patterns were not observed. There is a trend towards increasing antennal "beat" frequency with increasing airspeed. The greatest variation in frequency occurs between Airspeed 0 and Airspeed 1. A paired-sample t-test shows the only significant difference is between antennal frequencies at Airspeed 0 and Airspeed 4. The increased antennal frequency after odorant stimulation is significantly higher than variation shown in non-odorized air.

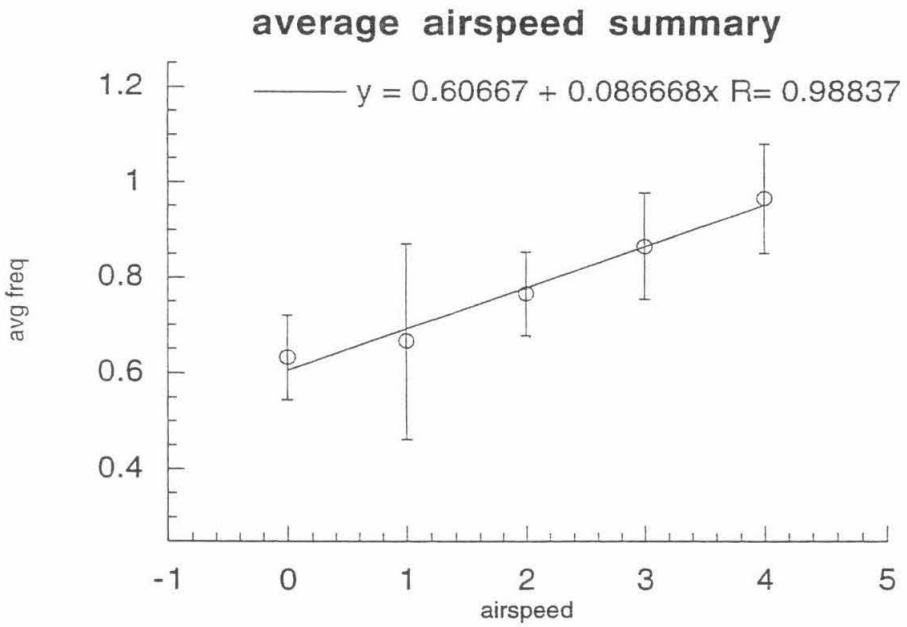


Figure 7. Influence of air speed on antennal movements in absence of odor. There is a trend towards increasing antennal "beat" frequency with increasing airspeed. The increased antennal frequency after odorant stimulation is significantly higher than variation shown in clean air. Error bars show standard error.

Search Behavior in Air Currents vs. Still Air

In the absence of air currents, cockroaches did not respond to an odor source puffed into the chamber. When whole mashed fruit was placed on substrate (odorant diffusing from source), cockroaches found the stimulus after several minutes through normal tactile search movements (locomotion across substrate with antennal "tapping" of surface). Cockroaches did not respond to the stimulus until antennae were directly over the sample. Upon locating source, cockroaches proceeded to eat. Antennal waving was not observed either before or after cockroach encountered stimulus.

With low-velocity air currents, cockroaches were observed to show a marked behavioral response to odor stimuli within one or two seconds of stimulation, including:

- Increased antennal waving frequency and amplitude
- Postural changes (e.g., rearing up and raising body on front legs, effectively raising base of antennae higher above substrate)
- Locomotion towards perceived source of odor

Sources of Noise.

Observer error.

Digitization of all points was done manually. Because the antennae are very thin, and the movements fast compared to the shutter speed of the camera, the contrast between the antennae and the background is low. Marking the antennae was not feasible, as even the smallest touch of marking fluid would cause the antenna to bend under the

extra weight. Thus, there could be errors in digitizing a moving point that is captured by two different cameras due to the observer's selection of the point to digitize. Nevertheless, repeated digitization of the same trial at 60Hz showed qualitatively equivalent patterns of movement.

Digitization error.

Digitization error is distinguished from observer error as the error in digitizing the same stationary point ("hand-eye-coordination"). This error proved to be approximately 4 degrees at 60 Hz, for the working volume in these experiments (volume enclosing head and antennae).

System error.

The computer-controlled VCR uses a time signal encoded on the videotape to grab the appropriate frame for subsequent digitization. While rare, it is not uncommon for the system to grab the wrong frame for digitization if the time code signal is not encoded adequately (perhaps due to noise in the cabling). This could result in a single-frame error in the trajectory of a point, at a digitization frequency of approximately 60 Hz.

Discussion

In this study, we characterized the three-dimensional antennal behavior to non-pheromonal (food) odors, to establish the operational regime of this chemosensory-motor

system. It is readily apparent that olfactory antennal behaviors are qualitatively different from other observed antennal behaviors based on their rapid movement and aerial nature. In olfaction, active movements may enhance the interaction between the stimulus and olfactory receptors by either controlling the flow of odor-carrying media past the olfactory receptor sheet (as vertebrates do), or by controlling the position of the olfactory receptors within the flow of the media, as arthropods do. The antennal movements observed here suggest that they may be linked to "sniffing" in vertebrates, and that discrete sampling of olfactory stimuli appears to be a common feature of olfactory systems.

Movement of the antennae may serve a quantitative function in olfactory sampling. Studies of the fluid flow characteristics around arthropod antennae suggest that antenna structure and movements may be optimized for odorant transport. Specifically, antennae were found to operate such that the fluid flow around sensilla structures (aesthetacs in crustaceans) was maintained at a Reynolds number that optimized the transport of odorant molecules into the sensory array. These movements were found to change as the animals increased in size, suggesting that animals altered their sampling strategy as they mature [5]. Similarly, transport properties of odorant-laden air may be a driving constraint on terrestrial insect antennal behavior.

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Chapter 3.

BIOLOGICAL SEARCH STRATEGIES FOR CHEMICAL PLUME TRACING*

Christine W. J. Chee-Ruiter, Sharlene Wu and James M. Bower

Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

Abstract

Following a chemical trace to its source poses a difficult engineering task, yet is a problem commonly solved by foraging animals. This paper describes the search strategies used by three different kinds of animals--bacteria, insects and mammals--in an effort to identify common behavioral properties that could guide further development of autonomous chemical-seeking devices. The results indicate the importance of behavioral sampling strategies, as well as the likely involvement of non-olfactory information in the chemical searching behavior of these animals. Analysis of their behavior also indicates the importance of computations involving space during olfactory search. Several recommendations are made based on this data for engineering applications.

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Introduction

Much research effort has been focused on the development of sensors to detect and identify chemical compounds and biological pathogens. The potential applications of such devices span military, industrial, environmental, manufacturing, and home uses[1]. However, considerably less attention has been paid to determining how such sensors could be used to actually locate the source of a released material. Terrorist attacks using biological or chemical agents, buried land mines, and chemical leaks are all examples of problems in which the actual localization of a source in space is at least as important as detecting the chemical released from the source.

It is our view that both understanding the problem and identifying possible solutions for source localization can benefit from a closer examination of the ways in which foraging animals find odorant sources. It is already clear that animals such as insects[2], crustaceans[3], and vertebrate species such as dogs[4] use highly developed olfactory search behaviors in tracking their prey. However, even single-celled animals such as bacteria demonstrate oriented movement in the presence of chemical compounds in their environment[5]. Given that the problem these animals solve is similar to the problem confronting human searchers using artificial chemical sensors, we believe that examining successful biological search strategies may yield insights into engineering design solutions for automated searching devices.

In this paper we consider the chemically-driven searching behavior of three very different types of animals: bacteria, the American cockroach, and the albino laboratory rat. In each case, the animal has been placed in a natural environmental situation, in which a chemical is released into a medium with the simplest possible flow conditions normally encountered by the animal. Under these conditions, odorant distributions can be expected to form a concentration gradient which decreases in some regular way, on the average,

with distance from the source. The farther away from the odorant source, the lower the average odorant concentration becomes. For this reason one could imagine that each animal might execute a relatively simple behavior to find the source (e.g., move in direction of increasing stimulus concentration). However, while this seems to be true in bacteria, it is clearly not true in the insect and rat. Further, the behavior of even simple bacteria implies a more complex computation is taking place than would be possible if the bacterial model was simulated with a single chemical sensor mounted on a wheeled robot. When the behavior of each of these animals is examined in more detail, it is clear that the complexity of the localization problem requires a more sophisticated computational and behavioral solution than initially seems necessary. In particular, it is clear that detecting the direction of a chemical source requires some understanding of the distribution of the chemical in space and time. For even simple environments, success is likely to require the use of multiple receptor surfaces, multiple sensor modalities, and complex behavioral strategies.

Chemotaxis in bacteria

Perhaps the best studied animal model for chemical-seeking behavior is bacterial chemotaxis. Bacterial locomotion involves the use of flagella which propel the animal within an aqueous medium. "Runs" are interrupted by brief pauses during which the animal "tumbles", and subsequent movement is resumed in a different, random direction[6]. Control of overall direction is effected by controlling how often the animal tumbles. A considerable amount is already known about the behavior of bacteria seeking chemical sources[7]. In addition there is a growing understanding of the biochemical networks that, in effect, "compute" the most favorable direction to move relative to a chemical source[8]. The reason for this level of understanding, of course, is that these animals are simple, and the environments in which they live are also relatively simple. For the most part chemical distributions from a source form a smoothly-varying gradient governed by the forces of diffusion. Accordingly, the strategy that bacteria have adopted (and implemented in their

biochemical machinery) appears to involve sampling the chemical concentration across multiple receptors (E. coli are reported to possess 30 chemoreceptors[9]) at various points in space. This information is integrated within the chemotactic network and the animal responds with a higher or lower rate of tumbling[10]. Thus, even in this simple animal in a simple environment, localizing odorant sources involves sampling at multiple spatial positions, and then, in effect performing a calculation that involves a "memory" of the environmental space[11].

Scanning behavior in insects

Perhaps not surprisingly, terrestrial animals have developed even more sophisticated behavioral strategies and sensor arrays for localizing chemical sources. The size of these animals relative to their natural environment subject them to airflows varying from still-air conditions, through laminar-flow conditions, to turbulence. Under most natural conditions, the chemical environment is highly complex[12] as wind, for example, can enormously change the spatial and temporal distribution of chemicals even at a short distance from the source.

As we describe here, however, even when placed in near-laminar flow conditions, terrestrial animals use complex behavioral sampling strategies to pursue the source of chemical plumes. In particular, several years ago we examined the response of the antennae of the American cockroach, Periplaneta americana, to chemical sources (in this case food odors) puffed at the animal from different directions. These insects are particularly good subjects for the study of olfactory search behavior because they are nocturnal scavengers who depend heavily on their olfactory sense to localize food and other resources. As in all insects, the "noses" of this animal are the long mobile antennae attached to the head. These antennae are covered at high density with multiple olfactory receptors[13].

In our experiments, these animals were placed in a fixed position in a dark chamber while odorants were puffed at the head of the animal from different directions. Infrared video was used to record the movements of each animal's antennae at rest and in response to this stimulus. These motions were then digitized and recreated in 3-D using Peak Performance Technologies motion analysis system[14].

The square in Figure 1 represents the head of the animal, and the bars and arrows show how the antennae move with time. Before the odorant is presented, the antennae move up and down slowly (each bar is separated by a uniform time period) in what we think of as a baseline scanning mode. Under these conditions, the antennae move asymmetrically in time and space. When food odor is puffed into the chamber, however, the animal "points" both antennae in the direction of the odorant source, increasing both the velocity and the amplitude of its antennal movements. In general, after odorant stimulation each antenna increases the volume of scanned space by an order of magnitude (from approximately 8.4 cm^3 prior to stimulus presentation, to 85.1 cm^3 after, for 5-cm long antennae).

As in the case of bacteria, it is apparent that the strategy used by this insect for chemical source localization also requires sampling environmental space. In our experiments, the animal accomplishes this spatial sampling by explicitly controlling where its antennae were pointed. However, it is important to also note that the insect does not merely point its antennae, but instead rapidly and repeatedly sweeps its antennae through space. In this way, we suspect that the insect is, in effect, imposing a spatial pattern onto the stimulus response by virtue of the movement of the sensory surface. Under these conditions, explicit memory-based comparisons are likely necessary to determine the direction of the odorant source.

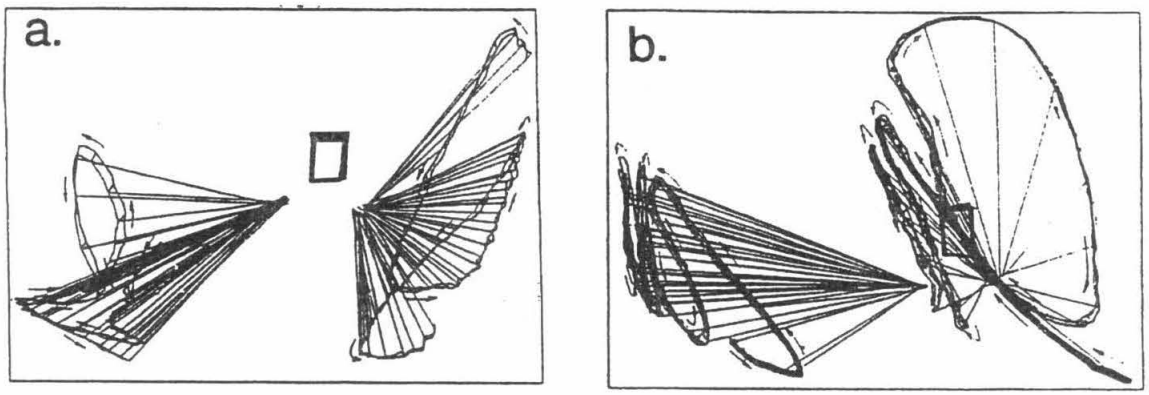

Figure 1. Insect olfactory scanning behavior. Digitized image of antennal response to food odor puffed from left side of figure. Square denotes head of animal. Each bar represents a uniform time period. Arrows denote direction of movement. a) before odorant stimulation. b) after odorant stimulation.

The scanning behavior of rats

Finally, most animals also use whole body motions to localize odorants. Unlike the whole body movements of bacteria, however, the searching movements of terrestrial animals do not appear random, but instead seem patterned. We have examined this aspect of odorant seeking behavior using albino laboratory rats. In these experiments, rats were initially trained in a light-shielded T-maze to follow a commercially-available odorant (citral) to a sugar-water reward. The odorant is released into the near-laminar airstream, forming a "plume" distribution. Subsequently, olfactory search behavior was examined in a 4' X 10' wind tunnel, in a darkened room. By their previous conditioning, the rats were motivated to find the source of the citral "plume", which could have been one of three possible release sites. When they found the correct site, they were given the usual sugar-water reward. As with the insects, the behavior of the animals was videotaped using infrared cameras.

In principle, given this experimental set up, one could imagine that the rats might simply adopt the strategy of following the chemical plume to the odorant source. When placed in the wind tunnel they are highly motivated by thirst to get to the odorant source quickly. However, rats cannot assume, as can bacteria, that the chemical sources they seek emit chemicals in a smoothly-varying distribution. In turbulent conditions, odorants can form discrete "patches" which would not provide an animal with a direct path to the odorant source. Furthermore, rats even in the dark are cautious, and prefer to stay close to the walls of whatever space they are in. Perhaps for both reasons, these animals exhibited much more complex source seeking behavior than seemed necessary given the experimental conditions.

Each rat placed in the wind tunnel went through four distinctly different phases in its search regardless of how many previous times the animal had performed the task. As shown in Figure 2 these phases consisted of: (1) an "exploratory" phase in which the animal ran around the outside wall of the wind tunnel. This phase might have been non-olfactory as there was never any overt indication that the animal was aware of the olfactory stimulus. However, as already stated, the animal was highly motivated to find the source of the odorant; (2) the exploratory phase was always terminated by a perpendicular run from one wall to the other, across the airflow and away from the end wall. All animals executed this perpendicular run which was then followed by behavior clearly olfactory in nature. Accordingly, we refer to the perpendicular run as the "detection" phase; (3) the perpendicular run was almost always followed by a highly variable phase characterized by high activity levels and multiple forays away from the edges of the tunnel. Based on our analysis, this phase almost certainly was directly related to determining the actual source of the odorant and is therefore referred to as the "localization" phase; (4) finally, each animal eventually returned to the wall of the tunnel and took the most direct course possible via the walls to the source of the odorant. We refer to this phase as "target approach and acquisition."

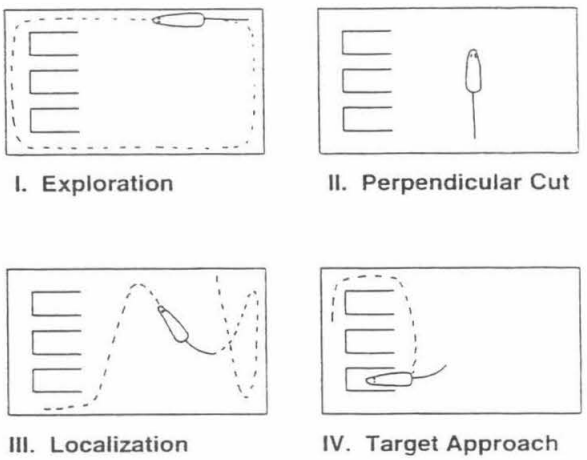

Figure 2. Rat olfactory searching behavior classified into four main phases.

In addition to these four phases of overall movement, rats were observed to periodically pause and execute one of several stereotyped head and body movements. (This overall pattern of "runs" and "stops" recalls the chemotaxis behavior of bacteria.) These behaviors, which we refer to as scanning behaviors, most often occurred in the localization phase (Phase 3) of the overall behavior. These are shown in Figure 3 and can be classified into three distinct groups: vertical scanning behaviors, horizontal scanning behaviors, and axially-directed scanning behaviors. In each case the rat appeared to take multiple sniffs in a small volume, in effect sampling the space around itself prior to another

bout of movement. Interestingly, individual rats were found to pause and execute these scanning behaviors at characteristic locations along the walls in trials occurring over multiple weeks. It is unlikely that these positions were related to some olfactory cue in the wind tunnel because each animal had its own characteristic stopping points, and the wind tunnel was de-odorized after each trial. The data strongly suggests that the rats have a spatial reference "map" of the wind tunnel and use it to determine the likely location of the odorant source. A similar use of space has also been reported in foraging behavior of animals in the wild[15].

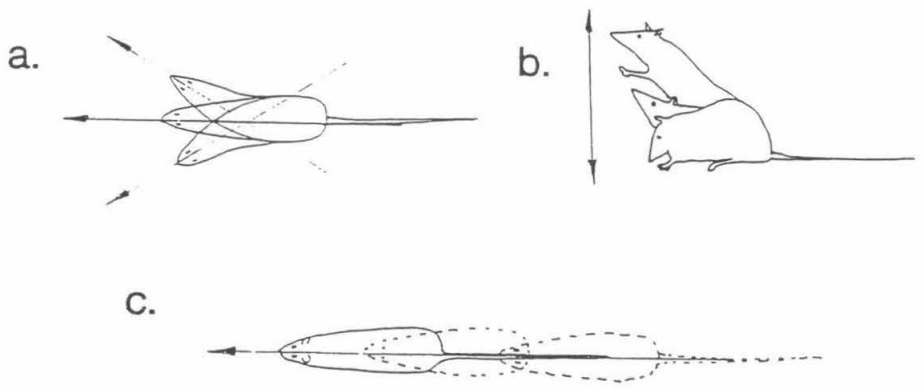

Figure 3. Rat olfactory scanning behaviors. a) Horizontal scanning. b) Vertical scanning. c) Axial scanning (along axis of stimulus plume).

Lessons learned from animals

Taken together it is clear that all three animals share several basic strategies in searching for the sources of odorants. In each case, chemical seeking behavior involves an explicit search of physical space. In the case of the rat, the first thing the animal does is run around the perimeter of the space, even though it is highly motivated to find the odorant source. Rats always start with Phase 1, even after they have been placed in the tunnel twice a day for several months. The subsequent (Phase 3) selection of specific locations for olfactory scanning also strongly implies that the animal is making computational decisions based on its understanding of the space it is operating in. Second, all three species exhibit scanning behavior during the process of odorant source localization. In the case of the bacterium, this scanning involves integrating odorant information across receptor sites during whole body movements through its environment. The cockroach scans its antennae through the surrounding space in a manner that is directly related to the presence and direction (though not identity) of the odorant source. In the case of the rat, the animal stops moving periodically to scan its receptors (i.e., its nose) through a small volume of air. Of course it uses its legs to move the entire receptor array around the space in a regular and repeatable pattern. This analysis makes it clear that chemoreceptors by themselves are insufficient to localize chemical sources in space (that is, a chemosensory *system* is required). From an engineering point of view, there are several specific recommendations that we can make as a result of our observations with respect to the efforts to engineer devices to find chemical sources:

Applications to engineering

The value of multiple chemosensors

From our analysis of the insect, it is clear that a multiple-sensor array can be used to provide critical spatial information. For example, if multiple chemosensors are arranged in a line (the simplest case), the axis of this linear array provides an inherent directional reference relative to the position of the detector base (Figure 4a). Placing the chemosensors in a distinct spatial arrangement is thus one method by which the detector can impose a spatial pattern onto the stimulus. Now if the array is moved to fixed positions within a plane (Figure 4b), one can then make a map of the two-dimensional space that the array covers, and overlay this map of space ("spatial map") with a map of sensor responses ("chemo-temporal map"). When these two maps are aligned, it is possible to calculate a reasonable estimate for the direction of highest average chemical concentration. If the array is large relative to the "patch" size of the odorant in the stimulus plume, and if the array moves fast relative to the speed of the plume, this provides sufficient information for orientation.

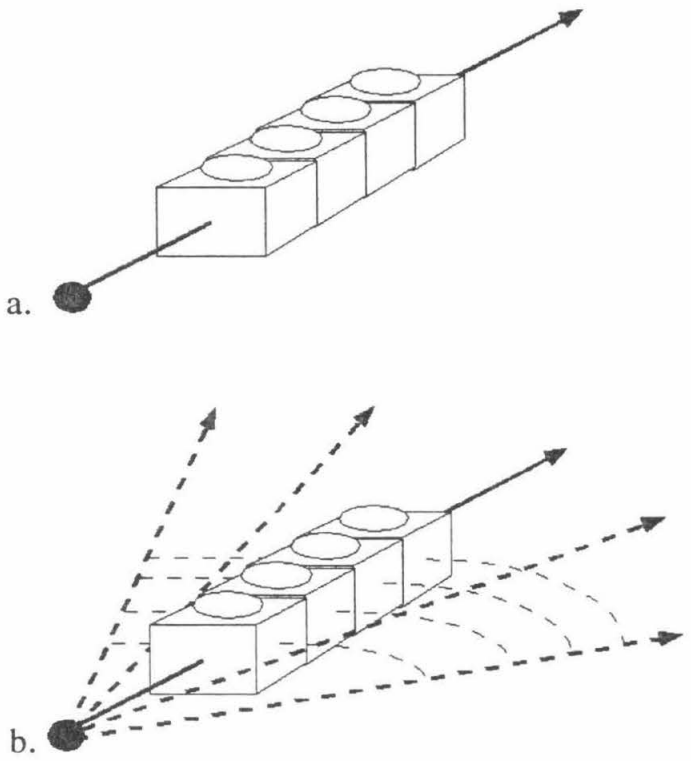

Figure 4. a) Linear sensor array provides inherent directional reference. b) Linear array moved to fixed positions allows detector to impose a spatial pattern onto the response. Simple movement required.

Spatial sampling strategies are critical

One can also extrapolate from the animal results presented above that, depending on the limitations presented by sensor sensitivity and/or spatial distribution, patterns of sensor movement can complement the detector hardware. The resulting spatial information can be extracted with appropriate computational effort. Comparing the case of the linear sensor array with that of a single sensor, it can be seen that to obtain the same two-dimensional spatial information, the pattern of movement of the linear array can itself be linear; if only a single sensor is available, it must move in a two-dimensional pattern. In other words, a detector with a linear array of sensors needs a much less elaborate scanning pattern than a detector with a single sensor to get the same information. Of course, the more sensors, the

more potentially complicated the task of deciphering their data. For this reason, sensor number and sensitivity, sensory acquisition behavior, and computational resources should be considered together in analyzing animal behavior as well as in designing tracking devices.

Data from other sensory systems may be equally important

In the real world, neither animals nor tracking devices need to operate based on chemosensation alone. Animals appear to use many different types of sensory data to localize odorants. While it is in principle possible, as just described, to obtain spatial information by interpreting data from chemosensors swept through the environment, these receptors cannot provide potentially vital information about the spatial surroundings which might be obtained from wind or visual cues. We know from our rat data that animals take into account information about wind direction as well as other environmental cues when localizing odorants. In fact, even insects appear to integrate multiple modes of sensory information to perform olfactory search and navigation[16]. In an engineering context, multi-modal sensory information is likely to make olfactory search more accurate and more efficient. Under natural conditions, multimodal sensory data is likely to be essential to find sources of chemical emissions.

Source localization intrinsically involves the need to interpret data in a spatial context

Finally, an important result of this analysis is that different species of animals all appear to use an understanding of the space they occupy to find the source of an odorant. This is most clearly seen in the rat, whose first response when put in the wind tunnel is always to run around its outside walls. As already mentioned, each animal consistently does this even after they have been placed in the tunnel many times and even though they are highly motivated to find the odorant source. Further, as noted above, each individual rat performs olfactory scanning behaviors at characteristic positions along the tunnel

walls. A particular rat stops in the same positions over multiple trials, over multiple days and even over multiple weeks. This strongly implies that the animal has adopted a particular search strategy linked to specific spatial locations in the tunnel in order to calculate the location of the odorant source. Future behavioral experiments with rats will examine the effects of wind speed and odorant concentration on these fixed stopping points. It is also interesting to note that the olfactory system in mammals is intimately related to the hippocampal formation, which is believed to be responsible for generating and maintaining an internal map of external space[17]. Thus, both behavioral and neurobiological data strongly suggest that olfactory searching involves both detecting the presence of a chemical and calculating its location using spatial information about the environment. In more complex (non-laminar, inhomogeneous) environments, we anticipate that extra-olfactory information will become even more important in localizing odorants.

Conclusions

At present there is considerable interest in identifying more efficient methods of locating the source and mapping the distribution of chemical leaks and contamination in many military, industrial, and environmental applications. Most current methods of locating chemical sources are based on humans carrying around hand-held detectors. This approach is labor-intensive, inefficient and, depending on the circumstances, dangerous. In principle, it should be possible to construct autonomous sniffing devices to perform these functions. However, to date most of the research on such devices has focused on the problem of detecting the appropriate chemicals, rather than on the larger problem of how the device finds the source of the chemicals once detected. Based on our beginning analyses of the search strategies used by three very different types of animals, it is clear that chemical detection alone will not solve the overall problem. We would like to suggest that

further studies of animal localization strategies, especially in more complex and realistic environments, could play an important role in the development of this technology.

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Chapter 4. A Map of Odor Quality

Introduction

Olfactory researchers commonly face difficulties in selecting appropriate stimuli for experiments, and often select odorants on the basis of their prior use or description in research, or on the basis of their chemical properties. There is logic to these methods, especially given that we cannot reliably predict whether a novel compound will elicit an odor, and often must sniff it ourselves for verification. However, it is fair to say that humans in general have a lack of intuitive understanding about the stimuli that evoke odors, and this lack of intuition exacerbates the problem.

Olfactory stimuli are chemical compounds, but not all chemical compounds elicit odors. The physical and biological characteristics of a compound must be experimentally determined, though general predictions may be made about its chemical activity. In particular, its ability to elicit odors must be verified empirically, and can be characterized by several parameters. *Intensity* describes the strength of the odor, and is related to the concentration of the stimulus. *Quality* refers to the "character" of an odor, and is described using *descriptors* such as "Apple" or "Orange." *Hedonic value* is the pleasantness or unpleasantness of the odor.

Structure-function studies of monomolecular stimuli and the odors they evoke have made much progress in the understanding of possible relationships between

chemical structure and how it contributes to odor quality perception. However, these studies have shown that small changes in structure can render a highly odorous molecule odorless [1]. The organization of stimuli appears highly fractured; an understanding of the chemical organization relevant to olfaction continues to be elusive.

While the relationship between one compound and the next is not fully understood, it does not prevent monomolecular compounds from being used as olfactory stimuli. However, because the organization of stimuli is unclear, the relationship of the odor responses to each other (odor qualities) is also unclear.

Although we do not yet understand how the olfactory system transforms a chemical stimulus into a specific odor, there is some regularity to the process. In a mental exercise, for example, a particular apple will evoke an Apple odor quality; if we were to sniff it several times during an hour, we might agree that each time we sniffed it, the odor had the same quality as the previous time. If we were to recruit ten of our friends and repeat the procedure, we expect to get the same results. From our experience, the mapping from stimulus to odor quality has some consistency. If we were to use an orange in the same experiment, we might find the same consistency in results. However, we would not be able to determine a relationship between the Orange odor quality and the Apple odor quality, nor would we between Apple and Banana. For example, if we were challenged to arrange the Apple, Orange, and Banana qualities, we could not logically place one "first," "second," or so on. This exercise illustrates that there is no

readily definable "order" in the space of odors (i.e., the collection of all odors), and that if there is order, it is not readily apparent.

The lack of "order" between stimuli makes conducting research difficult. A simple example is that a set of olfactory stimuli selected according to ad hoc methods may not span "odor space" appropriately (akin to testing color perception with only Long wavelengths of light).

For this and other reasons, a "map" of odor quality would be valuable to researchers. Such a map could be used much like any city map: it would describe the boundaries of the odor "space" and major odor features within the space. Odor "neighborhoods" could be visualized. Coupled with a searchable database of odorants, one could select stimuli according to the odor perceptions they evoke, and perhaps gauge a qualitative measure of "distance" between odor responses. Furthermore, if one had such a map, one might be able to discern patterns within the space that may not be readily appraised otherwise. If such patterns could be identified in a map of odor qualities, they might provide valuable clues to the organization of the stimulus space.

This idea of patterns in an odor map is analogous to the use of a "color wheel" in studying color perception. In the color wheel, light of opposing colors mix to form the color white, while adjacent colors mix to form intermediate colors, etc. The structure of the color wheel codifies relationships between the colors. While odors are not expected to have the same relationships as colored light in vision, the underlying idea is that

relationships between odor perceptions might be found that would suggest relationships between the stimuli that evoked those odors. Alternatively, relationships between odor perceptions might suggest how the olfactory system processes chemical information.

For a map to be created, quantitative measures must be found to relate the different odor qualities together. This chapter describes the methods we have used to obtain these measures, the creation of an odor quality map, and a description of general patterns found in the map.

Methods and Results

Odor Quality Pairings

We examined olfactory perceptual data to see if quantifiable relationships could be found between odor qualities. We sought to reduce the complexity of the problem by only considering odors evoked by stimulation with monomolecular compounds. To find a quantitative measure to relate odor qualities, we used the phenomenon that a monomolecular stimulus frequently elicits multiple odor qualities at a given concentration. (Because the odor quality evoked by a compound can be complex, each individual quality is termed an odor "note.") For example, Isoamyl hexanoate ($C_{11}H_{22}O_2$) elicits the odor notes of apple, pineapple, fruity, and green, simultaneously [2]. The specific odor notes may be linked by virtue of their being elicited simultaneously.

Data were obtained from two sources, which combined included approximately 1000 chemicals that were described using 327 odor descriptors. Data from Dravnieks (1985) [3] included 142 chemicals, evaluated by a panel of subjects using a standardized set of 146 odor descriptors. The other data set used was from Aldrich Chemical Company [4], which compiled 278 odor descriptors for 851 chemicals, from primary published sources ([2], [5]).

Because it was possible that the descriptions of the same compound could differ between the two data sources, we first compared evaluations from the two data sets using odorants that were described by both. A few odorants in common were found. It was determined that the evaluations from both data sources corresponded fairly well if, for the Dravnieks data, those descriptors that exceeded the 20% applicability [1] across subjects, as listed in the source, were used. This threshold was therefore used to "calibrate" the two data sets.

An example of this is Citral. In the Aldrich database, Citral is described by the odor descriptor Lemon. In the Dravnieks database, Citral is described in the raw data with the descriptors (at all percent applicabilities):

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[1] The percent applicability is a metric calculated from the pooled data. It is the geometric mean of the percentage of the subjects who used the descriptor and the ratio of the sum of their total scores to the maximum possible score.

- Fruity, Citrus
- Lemon
- Grapefruit
- Orange
- Fruity, Other than citrus
- Pineapple
- Floral
- Rose
- Cologne
- Perfumery
- Fragrant
- Aromatic
- Spicy
- Woody
- Minty
- Eucalyptus
- Sweet
- Turpentine
- Herbal, Green, Cut grass
- Soapy
- Chemical
- Sharp, Pungent, Acid
- Sour, Vinegar
- Oily, Fatty
- Light
- Heavy
- Cool, Cooling
- Warm

However, the odor descriptors that exceed the 20% applicability only include Fruity (citrus), Grapefruit, Lemon, and Orange. Thus, using this threshold for the data allowed the two databases to be compared.

The data from each source was treated separately. The descriptors for each data source are listed separately in Appendix A. Each chemical and individual odor note

within a data source was assigned a unique number. A matrix of chemical vs. evoked odor notes was then created for each data source.

	Odor Quality 1	**2**	**3**
Chemical 1	1	0	1
2	0	1	0
3	1	1	0
4	1	1	1

Figure 1. Example matrix A. A "1" denotes that the chemical evokes that odor; a "0" means that that odor quality is not elicited.

For each data matrix A, an odor cross-correlation matrix was created:

$C = A^{T} A = M \times M$ where M = number of odor notes

	Odor Quality 1	2	3
Odor Quality 1	3	2	2
2	2	3	1
3	2	1	2

Figure 2. Example matrix C. Each element of C describes how often those two odor notes were elicited together across all chemicals in our database, while the diagonal tells the frequency of occurrence of each odor note across all chemicals.

Each element of C describes how often two odor notes were elicited together across all chemicals in our database, while the diagonal tells the frequency of occurrence of each odor note across all chemicals. From the conditional probabilities (i.e., $P(X|Y) = C_{xy} / C_{yy}$) we determined the likelihood that one note will appear given that another is present. Note that these conditional probabilities may be asymmetric (i.e., P(B|A) != P(A|B)). For example, odor A may sometimes be elicited with odor B, and other times appears with

odors C and/or D, while odor B may only ever appear in combination with odor A. Thus, to capture the asymmetry of the link between two notes, we use the metric $I = P(B|A) * P(A|B)$. This metric is an approximation to the cross-entropy information measure [6], which is used as a goodness-of-fit measure between two distributions (e.g., the occurrence of odor A and the occurrence of odor B) [7].

The following figures show the odor quality maps for each data set. To be included on the map, an odor note must have been evoked separately by at least two different chemicals. Each eligible odor note is shown. Each arrow denotes the strongest (i.e., highest numerical value of the metric I) connection from one odor note to another. (Appendix A lists the ten highest values of I for each eligible odor descriptor for the Aldrich database.) This allows a series of odors to be linked: the strongest connection from A will be B, the strongest from B will be C, and so forth. Generally speaking, A, B, and C will all have lower level connections to each other, indicating that they are often evoked together simultaneously. In some instances, the strongest connections from two or more odors will be to each other, forming odor "islands." Odor islands are denoted by boxes in Figure 3. In this case, secondary connections are shown, to allow odor "islands" to be linked with larger groupings of odors. In other cases, a single odor may show multiple links because these links are of the same strength.

The position of odors relative to one another as described by these map configurations is somewhat flexible. Comparison of Figures 3 and 4 show that, while

odor "groups" may have flexible positions, in general the set of odors that are linked closely to each other are the same for the different data sets. Where there were no primary links to guide placement, odor notes were positioned near other odor notes with which they shared less significant connections.

Odors appear to "cluster"

While there is no *a priori* reason for simultaneously-elicited odor notes to smell similarly (e.g., garlic and onion are quantitatively linked as well has having similar odor qualities), there does seem to be a high degree of similarity between nearby odors on the map. Odors appear to "cluster" into familiar, though qualitative, groups. For example, citrus fruit notes are closely linked. In addition to neighborhoods of similar odors, it appears to the naïve viewer that different neighborhoods seem to relate to familiar food groups (e.g., "fruits," "herbs," and "snack foods"). Also, there are distinct groupings of "unpleasant" odors like those clustered around "Putrid," "Fatty," and "Sulfur."

Figures 3 & 4 (next). Each figure illustrates relationships between odor qualities for a separate data source. Figure 3 shows odor quality profiles from Dravnieks, while Figure 4 shows odor quality relationships obtained from Aldrich. In each diagram, an arrow denotes the strongest (i.e., highest numerical value of the metric "I") connection from one odor note to another. Each figure is just one configuration of many possible arrangements. In similar arrangements, odors near the top of the page have secondary links to odors placed near the bottom, and likewise between the left and right sides of the page. In other words, this map may be thought of as a 2D (Mercator) projection of a 3D "surface."

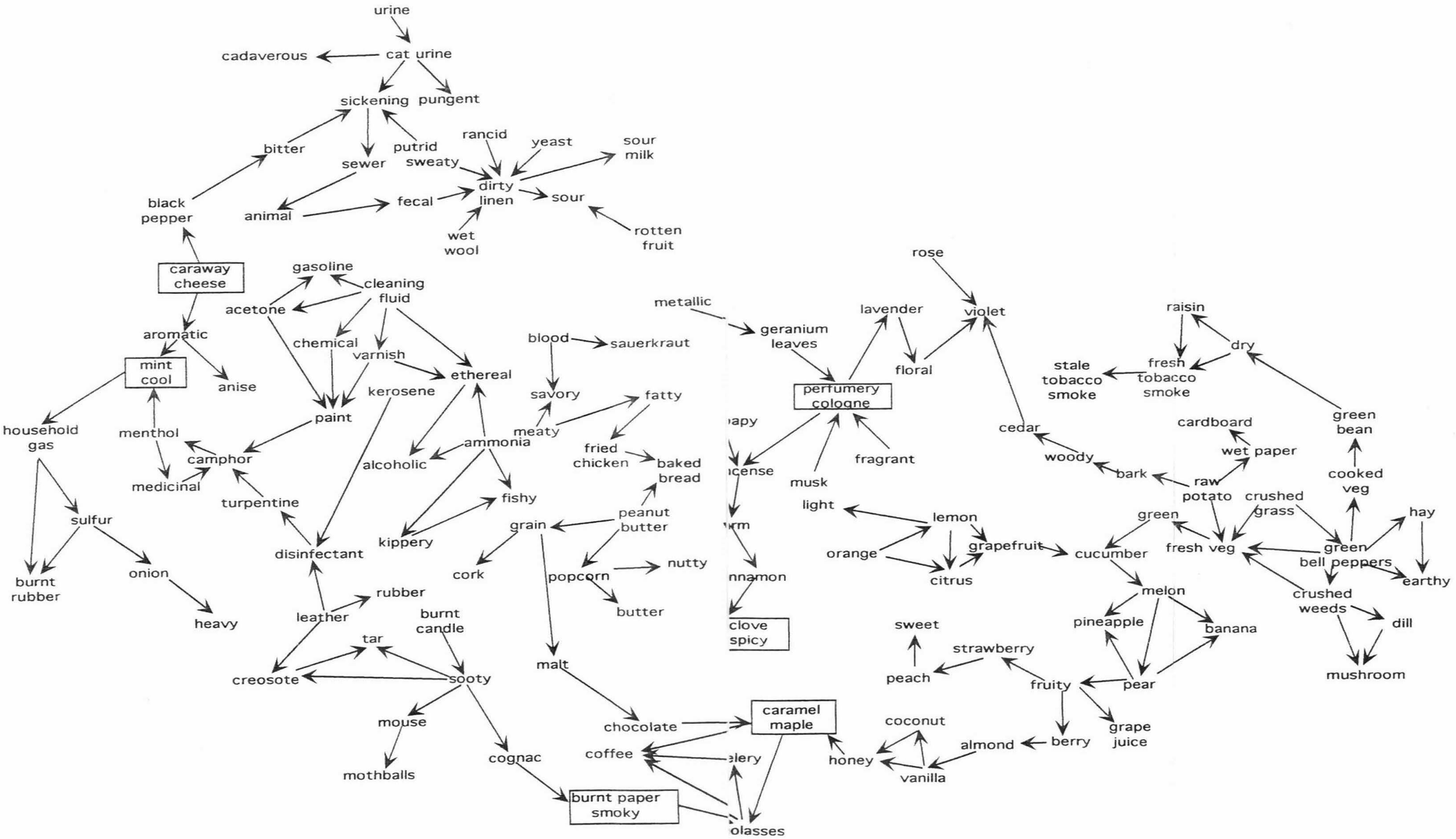

Figure 3. Odor Quality Map for Dravnieks data.

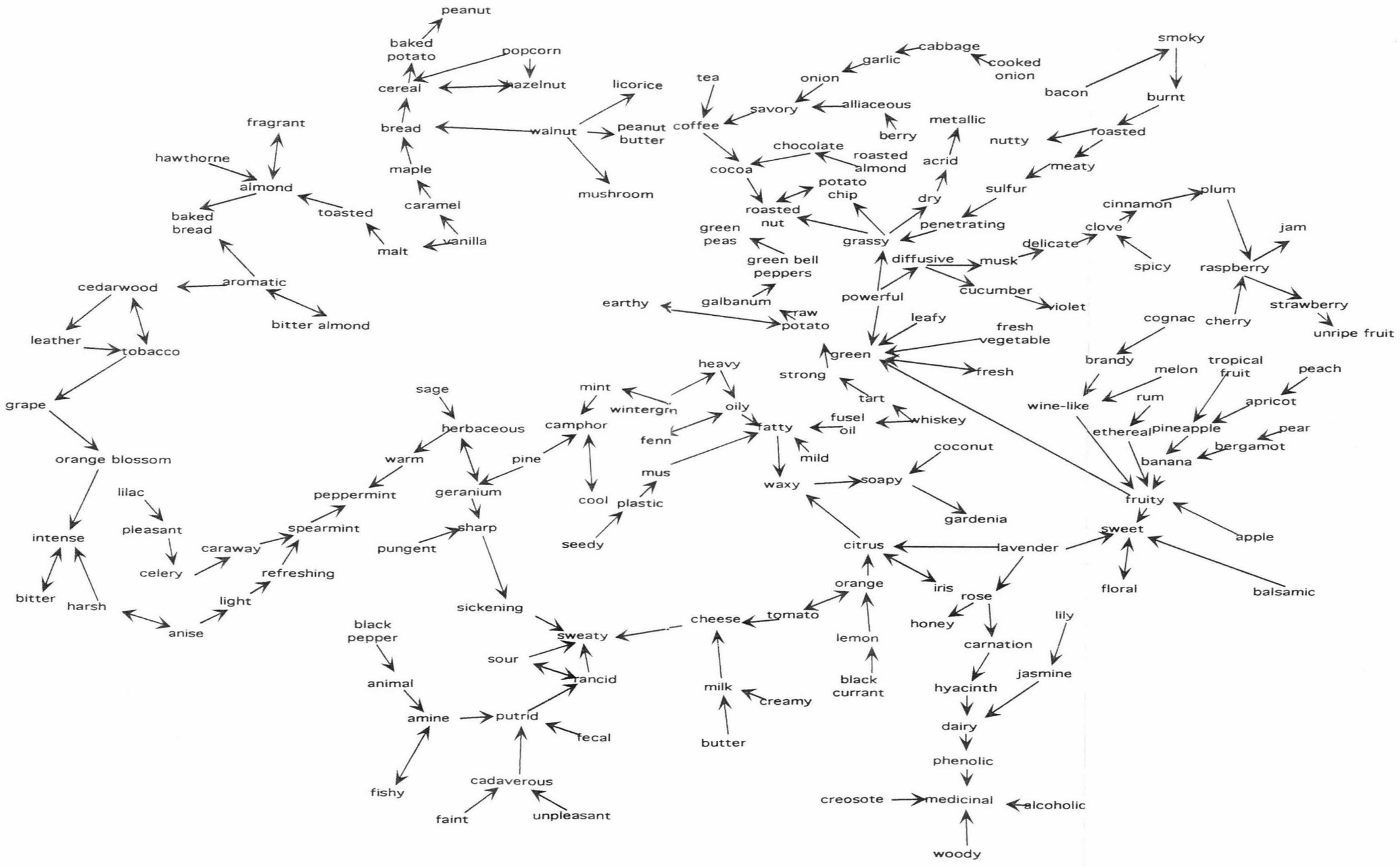

Figure 4. Odor Quality Map for Aldrich data.

More formal structure sought

While we quantified pair-wise relationships between odor notes, we wanted to quantify relationships between general groups of odors that would provide a structure for an odor map. An example of "structure" in the color wheel is the "axes" formed by the color opponents of green/red and blue/yellow. Similarly, it would be highly desirable to determine if any "structure" exists in maps of odor quality space. Such structure could allow us to gain insight into the olfactory processing mediating perception, and may provide an intuitive framework for understanding how odor mixtures might be perceived.

To obtain an additional quantitative means for linking general groups of odors, we used another well known perceptual phenomenon. For many monomolecular odorants, the odor quality elicited can vary as a function of concentration. These odor notes may be linked because they are elicited by the same compound, although they are not elicited simultaneously. Most commonly the variation in odor with concentration is slight; however, a small class of monomolecular stimuli can elicit not only vastly different odor

notes, but notes of opposing hedonic quality (i.e., pleasant to unpleasant), with changes in concentration. For example, Indole (C_8H_7N) elicits a jasmine note at low concentrations, while at high concentrations elicits a tarry-repulsive odor quality [2].

What we sought, then, were pairs of odor qualities that were evoked by a common compound, yet had opposing hedonic value. This property would exclude all odor qualities that did not have an opposite hedonic partner, so any pair of odor qualities that did have this property could be linked. To link these qualities, an "axis" could be drawn between them. For all odorants considered, surprisingly only three odor pairs were identified, which were each elicited by several odorants:

- Fruity - Sulfur
- Floral - Putrid
- Green - Fatty

Pleasant-unpleasant odor relationships suggest a metabolic link between compounds

What is the meaning of unpleasantness in odors? Where are unpleasant odors found? One answer is decaying organic matter. The process of decay (that is, the biological degradation of organic matter) complements the process of biosynthesis, and together these compose part of the general cyclic process of nature. Decomposition, as we are most familiar with it, is usually mediated by the metabolism of microorganisms.

In environmental microbiology, the metabolism of microorganisms is usually considered with respect to the Carbon cycle, the Nitrogen cycle, and the Sulfur cycle.

Interestingly, each of the axes identified above corresponds to one of these cycles: The fruity-sulfur axis corresponds to compounds containing Sulfur; the floral-putrid axis corresponds to compounds containing Nitrogen, and the green-fatty axis corresponds to Carbon compounds. Thus, the idea of a metabolic relationship between chemical compounds is consistent with the quantitative links we identified between specific odor qualities.

Compounds containing Carbon, Nitrogen, and Sulfur map to contiguous regions on an odor quality map

The following figure shows the odor qualities elicited by compounds containing Carbon, Nitrogen, and Sulfur, as they are located on the Aldrich map. In general, regions appear contiguously. A notable exception is the "islands" representing "foul" odors. As most compounds in the database are organic compounds, Carbon-elicited odors appear almost ubiquitously[2]. This means that it is possible for Carbon compounds not containing the key atom (i.e., Nitrogen or Sulfur) to still elicit odors in these regions; however, compounds containing Nitrogen or Sulfur are not likely to fall in the Carbon-only odor region. Nitrogen and Sulfur regions overlap near their boundaries.

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[2] An example of an inorganic compound is ammonia.

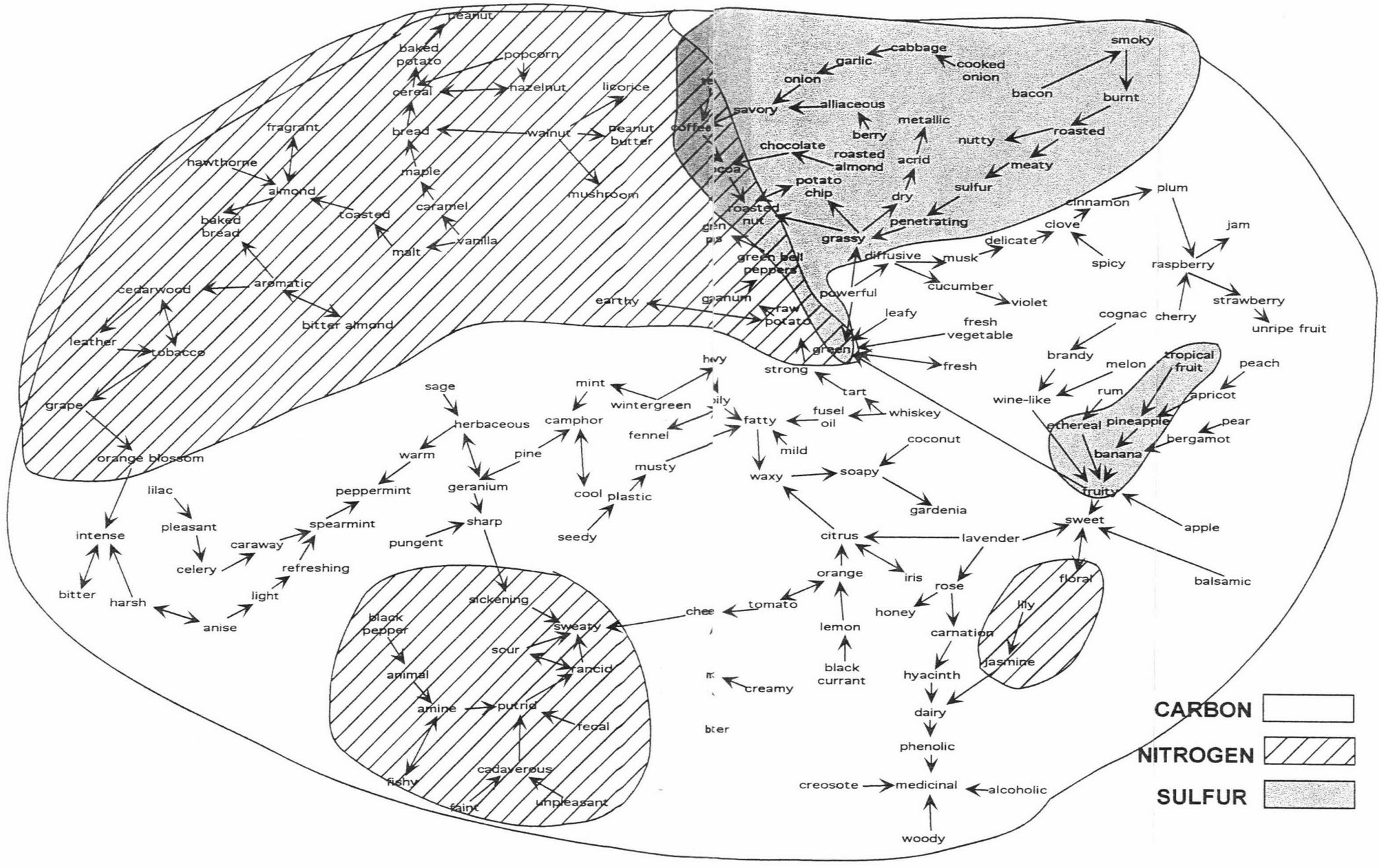

Figure 5. Carbon, Nitrogen, and Sulfur map to contiguous regions on the Aldrich map.

Discussion

Hypothesis: a metabolic basis to odor perception

The construction of an odor quality map allowed us to visualize "structure" in odor quality space in the form of pleasant-unpleasant odor pairs. This structure led us to qualitatively identify the location of the odor qualities elicited by compounds containing different key atoms, which were found to lie in contiguous regions on the map. Since, if there was no relationship between the atomic element and odor quality, we might expect the evoked odors to fall in random locations on the map, the fact that the odors did lie in contiguous regions suggested that there was such a relationship. These results in turn suggest a hypothesis that olfactory perception may be based on metabolic relationships between compounds. Specifically, compounds that are metabolically "close" (i.e., within the same metabolic pathway, or perhaps separated by only a few enzymatic reactions) may map to odor qualities that are "close" together as represented on a map of odor quality. It remains for further work, to test if metabolic similarity might be an appropriate distance metric for olfactory stimuli.

It may be possible to predict the general class of odor elicited by a compound

Our last result suggests that may be possible to predict the general class of odor that a compound may evoke. There has been no quantifiable way to predict what odor a

molecule will evoke based on its chemical properties. However, the biological properties of a molecule (i.e., what metabolic cycle it belongs to) may provide a context for mapping chemical compounds to odor perception. We suggest that the identity of key atoms (e.g., Carbon, Nitrogen, or Sulfur) may determine the general odor class elicited by a compound. These odor classes are large and overlapping, yet nevertheless form contiguous regions in a map of perceptual odor space.

Notes on the construction of the odor quality maps

In the maps shown, some odors were often connected weakly to odors which are placed distantly on the map, and often to odors on the opposite edge; in other words, these map configurations could be viewed as "Mercator Projections" of the surface of a sphere. This strongly suggests that the odor quality space has three or more dimensions, and that a more sophisticated graphing technique, and much larger database of chemicals, might yield fruitful results.

There is a possibility that the databases used to create these maps may be inadvertently skewed in selecting chemicals that evoked odors considered "pleasant" by the general population. However, inspection of the maps shows a healthy representation of "unpleasant" odors. It is hoped that this model will continue to serve as a repository for information on odor quality, and that time will serve to reconcile such bias if it exists.

Acknowledgements

Mitra Hartmann contributed equally in the formulation of the distance metric used in the maps. I thank Luke Wang for assistance in research and in initial preparation of the odorant database, and Lusine Danakian for preparation of the odorant database.

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Chapter 5. Perceptual Shifts in Odor Quality

Introduction

One of the most well known olfactory phenomena is perceptual self-adaptation, where sensitivity to an odorant decreases after continuous exposure. A related phenomenon is cross-adaptation, which occurs when sensitivity to an odorant decreases after continuous exposure to a different odorant.

Self-adaptation and cross-adaptation have been studied at the level of single olfactory receptors [1], as well as at the level of perception (e.g., [2], [3]). Cross-adaptation in particular has been used as a paradigm to measure the similarity between odorants. The reasoning is that an odorant which can influence the perception of another odorant is more similar to it than one that does not ([3], [4]).

While it is reasonable that adaptation and cross-adaptation may affect all dimensions of olfactory perception (i.e., odor quality and hedonic value as well as intensity), the perceptual data have almost exclusively focused on intensity responses. What happens to odor quality under adaptation? If the results from previous studies examining odor intensity are any indication, one might predict that the odor quality elicited by a test compound after adaptation should change from the odor quality elicited by that compound before adaptation. Following a period of disadaptation, the odor

quality elicited by the test compound should return to the quality elicited before adaptation. Thus, we may hypothesize that adaptation may induce variability in the perception of odor quality; that is, the odor quality elicited by a stimulus may shift depending on the context.

Few studies have so far addressed the concept of variability in the realm of odor quality ([5], [6]). Additionally, these studies reported results that were conflicting. O'Connell, et al. (1994) [6] reported that, except for quality shifts to the no-odor category, adaptation did not significantly alter odor quality reports. Lawless, et al. (1991) [5] reported mixed results: a significant shift was seen in the odor quality of a test odorant after adaptation to one set of compounds, but not the other.

Review of these studies suggests that cross-adaptation may still be a useful approach towards quantifying the similarity between olfactory stimuli, but that the choice of appropriate stimuli is crucial. Cross-adaptation studies have selected stimuli based on their structural similarity ([7], [8]) as well as their perceptual similarity ([5], [9]).

We aim to explore potential odor quality shifts, comparing the effectiveness of stimuli which are structurally similar and perceptually similar against stimuli which are metabolically (that is, biologically) related. We also include compounds that are dissimilar in all categories.

Methods

Subjects

Thirty volunteers (20 male, 10 female) were recruited and participated in the training phase for these experiments. They were not paid for their participation. Subjects were between the ages of 18-56, had no known respiratory or allergy problems, and had a self-reported normal sense of smell. In all subjects, informed consent was obtained. Of *these, five* subjects participated in the preliminary experiments described in the Methods, and ten subjects participated in the final experiment.

Odorant Selection

Odorants were selected using a searchable database developed in the course of this thesis work. Applicable data for the evaluation of the compounds selected were originally published in Fenaroli (1975) [10], Arctander (1969) [11], and Guenther (1948) [12].

The test stimulus was chosen based the following criteria: that it be a naturally occurring compound, that it occur in a number of natural sources, and that the natural sources have different characteristic odors. We reasoned that these criteria would allow the selection of compounds that were most likely to be influenced by cross-adaptation, since they naturally appeared in mixtures that evoked different characteristic odors. That

is, no matter what the odor evoked by the test stimulus in isolation, the context in which it was found would determine the resultant odor. The compound cis-Jasmone was selected from a set of compounds meeting the above criteria.

Adapting stimuli were chosen based on their perceptual similarity, structural similarity, and metabolic similarity to cis-Jasmone. Compounds found in a single organism (e.g., a plant oil) are most likely created by the metabolic processes of that organism, and were assumed to be metabolically related. In the absence of detailed descriptions of the complete metabolic pathways for individual organisms, this seems a reasonable approach. Adapting stimuli were further screened for overt trigeminal effects; those that were known to stimulate the trigeminal system (e.g., menthol, which elicits a "cooling" sensation that is mediated by the trigeminal system) were excluded. Table 1 shows relevant data for the adapting stimuli chosen. Table 2 describes how these stimuli were classified.

Table 1. Structural, Biological, and Perceptual Characteristics of Stimuli

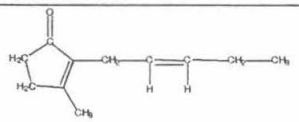
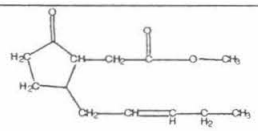
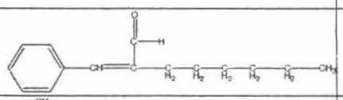
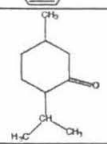
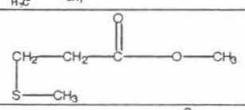
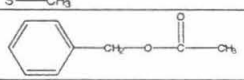
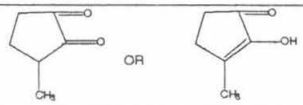
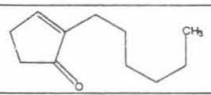
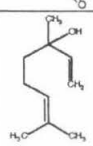
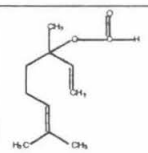
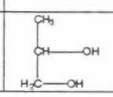
Name	Structure	Natural source	Odor description
cis-Jasmone		Jasmine, Jonquil, *Pittosporum glabrum*, Neroli, Peppermint, and Bergamot	Jasmine
Methyl jasmonate		Jasmine and Tunisian Rosemary	powerful, floral-herbaceous, sweet-persistent odor similar to Jasmine
α-Hexyl cinnamaldehyde		Not found in nature	Jasmine
l-Menthone		Russian and American Peppermint	a characteristic odor similar to menthol (i.e., Peppermint odor)
Methyl 3-(methylthio)propionate		Pineapple fruit	Onion-like
Benzyl acetate		Jasmine	Jasmine
Pyrrole		coal tar, bone oil, lemon and orange tree oil, and in vervain, lemon and bergamot leaves	sweet, warm-ethereal odor reminiscent of chloroform
Methylcyclo-pentenolone		tar oil, and in fenugreek	Nutty, burnt, coffee, maple syrup, caramel odor
Isojasmone		Not found in nature	green odor reminiscent of jasmine
Linalool		Jasmine, Clary Sage, Lavandin, Jonquil, California Lemon, Basil, Gardenia, many others	"typical floral odor"
Linalyl formate		Lavender, Clary Sage, Peach, Lime, Petitgrain, *Prunus persica, Prunus armeniaca*	fruity, floral (rose) odor reminiscent of bergamot and vervain
Propylene Glycol		Not found in nature	Virtually odorless

Table 2. Classification of Adapting Stimuli (Similarity to cis-Jasmone)

Structural Similarity	Perceptual Similarity	Metabolic Similarity	Stimulus Set
Yes	No	No	Methylcyclopentenolone
No	Yes	No	α-Hexylcinnamaldehyde
No	No	Yes	Menthone, Linalool
Yes	Yes	No	Isojasmone
Yes	No	Yes	Pyrrole
No	Yes	Yes	Benzyl acetate
Yes	Yes	Yes	Methyl jasmonate
No	No	No	Methyl-3-(methylthio)propionate, Linalyl formate

Odor Descriptor Selection

The aim was to select an optimal set of odor descriptors which best characterized the breadth of the complex odor elicited by the test stimulus. A number of factors needed to be optimized, so the selection process was involved, though systematic.

We were interested in being able to characterize the direction of unknown shifts. We reasoned that the characteristic odors of the natural sources in which the test stimulus occurred were good candidate descriptors: Jasmine, Jonquil, Pittosporum glabrum, Neroli, Peppermint, and Bergamot are all natural sources of cis-Jasmone.

Next, Laing and Francis (1989) [13] showed that subjects could only distinguish three to four odor notes in a complex odor, and rarely indicated that more than three components of odor were present. This suggested that we should limit the set of odor descriptors to a maximum of four.

Another issue was a problem encountered in preliminary testing, where it was discovered that subjects were not familiar with "common" odor descriptors. Subjects reported familiarity with the name of the odor, and some were familiar with the odor itself, but in interviews many stated that they would not be able to name the odor based on their personal experience, if they were presented with it. To address this problem, odor "standards" were required to train subjects to associate a name with an odor quality, and to consistently identify a fixed set of odor notes by name. Since odor descriptors are derived by analogy with a known odor quality, whole essential oils (i.e., naturally occurring mixtures of plant compounds) were used to represent "standard" odor qualities. This meant that the set of odor descriptors selected should be able to be represented by commercially available essential oils.

Towards what hidden odor quality "directions" might cis-Jasmone shift? We might find a clue if we could identify a compound that was related to cis-Jasmone, but also found in sources that cis-Jasmone was not found in--sources that have different characteristic odors. A compound that met these criteria was Linalyl acetate. Cis-Jasmone and Linalyl acetate both occur naturally in the essential oils of Bergamot,

Jasmine, and Neroli. However, Linalyl acetate occurs in Lavender, while cis-Jasmone does not; Cis-Jasmone occurs in Peppermint, while Linalyl acetate does not. By these facts it seemed plausible that the odor notes of Jasmine and Bergamot might be used to describe the normal character of cis-Jasmone, and that Lavender and Peppermint might be "boundary" odors for cis-Jasmone. These "boundary" odors were educated guesses for the directions of unknown shifts that might occur under cross-adaptation.

All of these factors combined suggested the selection of Jasmine, Bergamot, Peppermint, and Lavender as the set of descriptors to be used to characterize the odor quality of the test stimulus.

Once selected and procured, all stimuli to be used were sniffed by the experimenter to verify that the identified descriptors corresponded to the experimenter's perception. It was possible that the descriptors may have been inappropriate, since individual chemical lots may vary in odor quality (due to differences in the source material, environmental conditions, processing, and packaging). In addition, a procedural problem could arise in the case where there were missing descriptors, in which case subjects would not be able to report a significant odor note. These concerns were therefore checked empirically.

Test Procedure

The test session was divided into two parts, separated by a break that varied from 10 minutes to several days. Each part used the identical set of trials, except that the trial sequence was rearranged.

A computer-controlled system was used for training and stimulus odor delivery, and for data collection.

Subjects were asked to sniff an adapting stimulus from a jar continuously for 30 seconds. Immediately after, subjects were presented with three one-second puffs of the test stimulus, each separated by a 5-second interval. After evaluation of the test stimulus, there was a 1-minute delay. The total time for each part-session was approximately 24 minutes.

Subjects were asked to evaluate the complex odor quality of the test stimulus, using the descriptors Jasmine, Lavender, Bergamot, and Peppermint. Subjects were also asked to evaluate the intensity of both the adapting stimulus and the test stimulus. In all evaluations, subjects were instructed to provide a numerical answer ranging from 0 (did not detect) to 9 (maximum value). When evaluating odor quality, subjects were instructed to distinguish between the overall intensity of the odor and the "appropriateness" of a specific descriptor. For example, in a multi-component odor, the overall intensity might be very high (9), but the Lavender component may be moderate

(4) and it may have a small Jasmine component as well (2). Combinations of (0, 0, 0, 0) as well as (9, 9, 9, 9) were possible, as each component was to be treated independently.

Interspersed through the experiment were several control trials, in which the test stimulus was evaluated after adaptation to the diluent compound Propylene Glycol. All measurements of odor quality shifts were taken relative to the control values.

Subject Training

All subjects performed standard odor identification training, and repeated training in a short "familiarization" session before each experiment session. Because this experiment used a new experimental design, preliminary experiments and training were performed on a small number of subjects to ascertain the difficulty of the different aspects of the experimental task, and to verify that human subjects could indeed perform the task. Five subjects were additionally trained to perform these other training tasks.

"Aspects of Odor Perception" Instruction

When encountering a novel odor, subjects perceive a multi-faceted sensation. Odor may be decomposed into several components, including intensity, quality, and hedonic value. Some odors may contain a tactile component, such as the "cooling" effect of menthol. In the case of odorant mixtures, the resultant odor perception may have a

temporal component that can be used to discriminate one mixture from another [14]. Untrained subjects may be unaware of the different dimensions of odor, yet may unconsciously use differences in these perceptual components to discriminate between, and even identify, odors. Subjects were therefore given verbal instruction, to increase their awareness of the dimensions of odor perception pertinent to this experiment, and to enable them to focus their attention on the odor quality component.

Standard odor identification and testing

An issue identified in preliminary tests was that some subjects were naive to the standard odors (e.g., some subjects claimed they had never smelled Lavender before, and could not identify the odor as Lavender). It was important that the subject be able to correctly identify the standard odors, and to associate them with the appropriate descriptors. In this way, we could distinguish changes in perception from errors in reporting. It was noted that some subjects could learn odors and their descriptors quite rapidly, while others had difficulty in learning this task. Subects retained a working memory of the standard odors, so that if they repeated the experiment many days later, they would take minimal time to remember the odors. However, most subjects required retraining before each test session.

The odor descriptors used in the experiment were selected according to the criteria outlined above. The odor standards used were essential oils of Lavender,

Bergamot, Jasmine, and Peppermint and prepared as described below. Subjects were first allowed to sample each odorant *ad libitum*, and were allowed to view a poster which displayed the name of the plant as well as a photo of the plant from which the oil was extracted. After this familiarization session, subjects were trained to correctly identify and label the standard odors using a flashcard-style (interactive) method. A computer-controlled system was used for training and data collection.

Subjects were presented three puffs of a randomly selected odorant, and then were *required* to key in their answer. If subjects answered incorrectly, they were corrected, and were presented with the next randomly selected odor. Subjects were considered trained when they could identify ten consecutive samples without error.

Category Scaling Training (Color)

The task in the experiment is to evaluate a multi-dimensional odor quality on a multi-dimensional scale. This is a difficult task, and it was possible that some subjects may perform a scaling task much better than others. Further, even people with training in psychophysical procedures may be unfamiliar with the multidimensional character of odors. Thus, initial training using color stimuli instead of odors served a dual purpose: to evaluate variability between subjects performing a category scaling task, and to convey complex information about the sense of smell by using the analogy of color.

Subjects were verbally introduced to the concept that color is a multidimensional perception, and that they were being asked to consider just one characteristic (saturation level) of color. Subjects were first introduced to color samples that varied systematically in saturation level, but not in hue or in luminance. Color samples were prepared using Canvas on a Macintosh computer, and were presented using Microsoft Power Point. Subjects learned to identify 11 different samples of a Red hue according to saturation level. 100% saturation was assigned a value of 10, 90% saturation was assigned a value of 9, and so forth until 0% saturation was reached with a corresponding value of 0. The background was a neutral gray of 50% luminance such that at saturation level 0, the screen appeared completely gray. 0 was chosen as a lower limit to the scale rather than 1, because it is important that the subject is able to report the absence of the stimulus.

Subjects were then tested on their ability to categorize color samples according to saturation value. Four different hues were tested individually (Red, Orange, Green, and Blue) for a total of four tests. Prior to each test, subjects were shown representative samples of the test hue at 100% saturation ("10" on the value scale), as well as the 0% sample for comparison. Next, 50 samples of random saturation level were presented individually. Subjects were allowed 7 seconds to evaluate each sample.

Following the individual color tests, subjects were then tested on their ability to evaluate multiple color samples presented simultaneously. Each panel was composed of four color samples (one each of Red, Orange, Green, and Blue) of random saturation

level. 24 panels were presented. Subjects were allowed 30 seconds to evaluate each panel, followed by a 10-second blank.

In this manner, subjects were introduced to the concept that a single stimulus (4-color panel) could comprise multiple, independently varying objects, each of which required evaluation. Further, the skill required to perform this task is likely to be useful in analyzing odors with multiple odor notes.

Subjects were evaluated on their ability to respond consistently to variations in saturation level for each color, and in the 4-color panel task. Because they were trained in only one hue, their ability to perform consistently when tested with other hues was sufficient indication of their ability to generalize the task. Further, their ability to perform consistently in the 4-color panel task indicated that they understood and could perform this multidimensional evaluation in the modality of color vision.

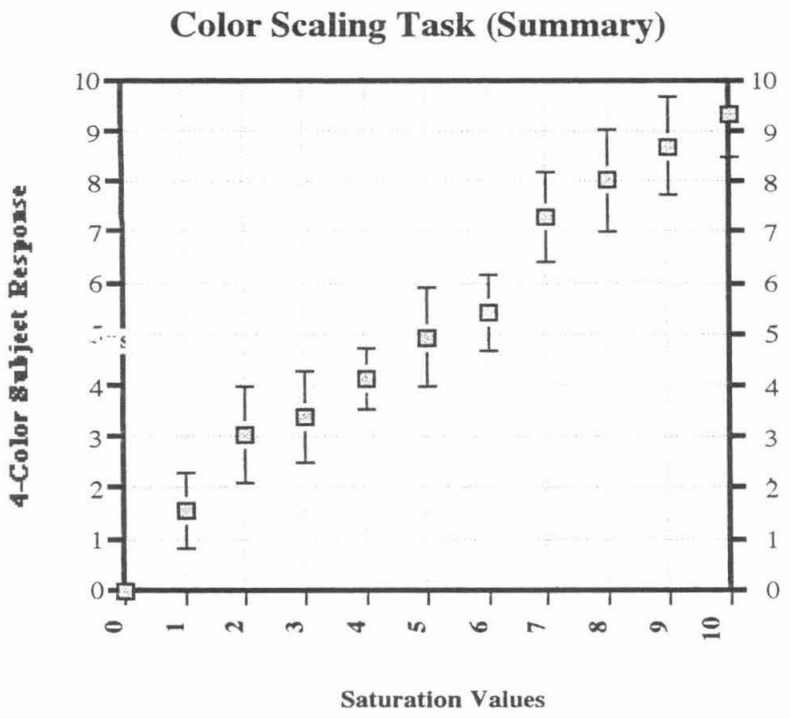


Figure 1. Subject responses to 4-color panel task. For each trial, subjects were required to evaluate the saturation level of each of four color swatches, on a scale from 0-10. This figure summarizes the responses for all colors. Error bars show standard error.

Category Scaling Training (Odor)

Subjects were verbally introduced to the concept that odor is a multidimensional perception, and that they were being asked to consider just one characteristic (odor quality) of odor. Further, they were introduced to the concept that odor quality is itself multidimensional, and may consist of several odor notes. Subjects were then presented with odorant samples from one of two sample series that varied systematically in the relative intensity of a specific odor note. When evaluating the samples, subjects were

instructed to distinguish between the overall intensity of the odor, individual notes in the complex quality of the odor, and the "appropriateness" of the descriptor (either Lavender or Jasmine) to describe that odor note.

Odor samples were prepared as described below. The objective was to keep the intensity of the sample approximately the same, but allow the specific intensity of an odor note to vary. Subjects learned to identify six different samples of a Lavender note according to the level of its specific intensity. Samples were labeled 0, 2, 4, 6, 8, and 10. Subjects were then tested on their ability to categorize the same series according to the specific intensity of Lavender, then another series according to the specific intensity of Jasmine.

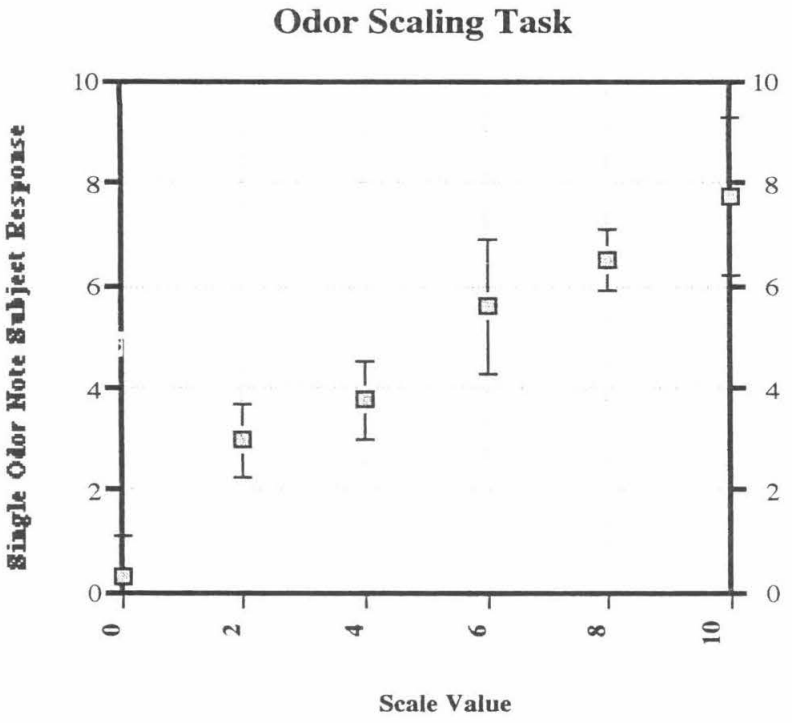


Figure 2. Subject responses to an odor scaling task. Subjects were required to identify the specific intensity of a particular odor note elicited by a stimulus evoking a complex odor quality, on a scale from 0-10. Error bars show standard error.

Stimulus Preparation

Standard "Odor" Training

For each descriptor, corresponding commercially available essential oils were obtained (i.e., Lavandula essential oil was selected to represent the descriptor "Lavender"). The Lavender, and Peppermint oils were from Aura Cacia; the Bergamot

oil was from Oshadhi, and the Jasmine Oil was from Aveda. 5 ml of each oil was applied to a sorbent pad and placed in a stoppered flask.

Odor scaling training

Two "linear" series of odor standards were prepared. Lavender oil, Ethanol, and Propylene Glycol were mixed in the proportions shown in Table 3. Similarly, a second series of odor standards were prepared using Jasmine oil, Ethanol, and Propylene Glycol. 5ml of each mixture was applied to a sorbent pad and placed in a wash bottle. Each bottle was assigned a value (0, 2, 4, 6, 8, and 10).

Table 3. Odor Scaling Stimulus Preparation

Value	Lavender oil (ml)	Jasmine oil (ml)	Propylene Glycol (ml)	Ethanol (ml)
0	**0**	**0**	**10**	**0**
2	**0.2**	**0.2**	**10**	**1.8**
4	**0.4**	**0.4**	**10**	**1.4**
6	**0.6**	**0.6**	**10**	**1.0**
8	**0.8**	**0.8**	**10**	**0.6**
10	**1.0**	**1.0**	**10**	**0.2**

Adaptation and Test Stimuli for Experiment

Adaptation and test stimuli were prepared as follows. All compounds except Isojasmone were obtained from Aldrich. Isojasmone was supplied by Bedoukian. With the exception of α-Hexylcinnamaldehyde and Methylcyclopentenolone, Propylene Glycol was used as a diluent.

Table 4. Stimulus Preparation for Adaptation and Test Stimuli

	Vol. (ml)	Propylene Glycol (ml)
Cis-Jasmone (adapting stimulus)	**0.60**	**15**
Cis-Jasmone (test stimulus)	**1.20**	**15**
Methyl jasmonate	**0.80**	**10**
α-Hexyl cinnamaldehyde	**0.40**	**2 ml Ethanol + 15 ml Diethyl Phthalate**
l-Menthone	**0.30**	**15**
Methyl 3-(methylthio)propionate	**0.25**	**10**
Benzyl acetate	**0.16**	**15**
Pyrrole	**1.90**	**15**
Methylcyclopentenolone	**0.006 g**	**15 ml H_2O**
Isojasmone	**0.30**	**15**
Linalyl acetate	**0.40**	**10**

5 ml of each odorant mixture was applied to a sorbent pad. Pads used for adapting stimuli were placed in wide-mouthed glass jars. Pads used for test stimulus were placed in a stoppered glass flask. The test stimulus was delivered using a computer-controlled system, so though the stimulus mixture was prepared at a higher concentration, at the point of delivery the concentration was reduced.

Results

Subjects were asked to treat specific odor quality notes, as well as overall odor intensity, as individual psychophysical variables. Henceforth, we will use the term *redolence* to define the specific intensity of an odor note when the odor note is one of several composing a complex odor quality. The redolence of an odor note is distinguished from the overall intensity of the complex odor.

Significant odor shifts seen

Significant shifts in odor quality as well as odor intensity were observed after a cross-adaptation procedure. Twelve adapting stimuli were used (including the test compound cis-Jasmone). Individual adaptation to four compounds resulted in large shifts in the odor quality of the test compound. Three compounds were effective at significantly reducing the intensity of the test compound after adaptation.

Table 5. Average Relative Shift In Redolence Of Each Descriptor (+ = increase)

Compound	Jasmine	Mint	Bergamot	Lavender	Intensity
Menthone	0.43	1.28	-0.98	-1.70	0.08
Isojasmone	0.08	0.88	-1.23	0.40	0.28
Methyl jasmonate	0.93	-0.98	-0.68	-1.00	-1.23
Methyl-3-(methylthio)-propionate	0.63	-0.93	0.33	-1.90	-0.58
α-Hexylcinnamaldehyde	0.88	-0.43	-0.68	-1.25	-0.83
Cis-Jasmone	0.68	-0.18	-1.13	-0.90	-1.03
Methylcyclopentenolone	-0.35	0.45	0.30	0.05	-0.60
Pyrrole	0.48	-0.88	-1.13	0.05	-0.08
Benzyl acetate	1.28	-0.63	-0.13	-0.40	-0.28
Linalool	-0.68	0.48	-2.13	0.85	0.58
Linalyl formate	0.73	-0.83	-1.33	0.65	-0.23

Table 5. Table shows the average of the relative shifts in redolence (i.e., the specific intensity of an odor note), experienced by individual subjects for each of the odor notes in the top bar, evaluated for the test stimulus cis-Jasmone after adaptation. The adapting odorants are shown in the left column. A negative value shows suppression of the note, while a positive value shows enhancement. The right column shows the average of the relative change in overall intensity of cis-Jasmone after adaptation.
Significant shifts ($P < 0.01$) are shown boxed in dark gray; ($P < 0.02$) in light gray; and ($P < 0.05$) boxed in white.

Structurally Similar Compounds

The compounds that were classified as structurally similar to cis-Jasmone were Methyl jasmonate, Isojasmone, Methylcyclopentenolone, and Pyrrole. Cis-Jasmone was also included in this category, to allow comparison of results to self-adaptation. Each of these contains a 5-membered ring with one or more side chains. None (including cis-Jasmone) produced a shift in odor quality of the test stimulus. Cis-Jasmone and Methyl jasmonate were significantly effective at reducing the perceived intensity of the test stimulus.

Perceptually Similar Compounds

The compounds classified as perceptually similar (i.e., odor quality-wise) to cis-Jasmone were Methyl jasmonate, Benzyl acetate, α-Hexylcinnamaldehyde, and Isojasmone. Cis-Jasmone was also included in this category, to allow comparison of results to self-adaptation. All compounds elicited a pronounced Jasmine note that was remarkably similar. Benzyl acetate was effective at producing a change in odor quality of the test compound. Cis-jasmone (under self-adaptation), Methyl jasmonate, and α-Hexylcinnamaldehyde were effective at reducing the perceived intensity of the test stimulus.

Metabolically Similar Compounds

The compounds classified as metabolically related compounds were Methyl jasmonate, l-Menthone, Linalool, Pyrrole, and Benzyl acetate. Cis-Jasmone was also included in this category, to allow comparison of results to self-adaptation. Of these, l-Menthone, Linalool, and Benzyl acetate produced shifts in odor quality of the test stimulus. Methyl jasmonate and cis-Jasmone (under self-adaptation) effected a reduced perceived intensity of the test stimulus.

Unrelated Compounds

Compounds that were not classified as structurally, perceptually, or metabolically related to cis-Jasmone included Methyl-3-(methylthio)propionate and Linalyl formate. Methyl-3-(methylthio)propionate produced a change in odor quality, while no effect was seen for Linalyl formate.

Shifts appeared in specific odor quality dimensions, and in intensity

As seen in Table 5, individuals reported changes in the redolence of specific odor quality notes elicited by cis-Jasmone after adaptation to a set of adapting stimuli. Each of these odor notes can be thought of as "dimensions" of a complex odor quality. Individuals also reported changes in intensity perception; odor intensity can be represented as an additional dimension to the odor perception. Thus, changes in odor

perception were observed after cross-adaptation, but they were not uniform across odor quality and odor intensity dimensions.

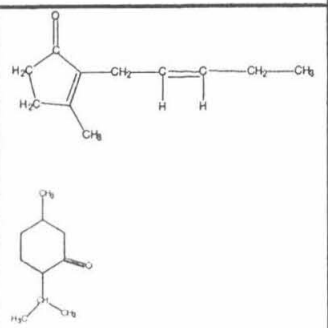
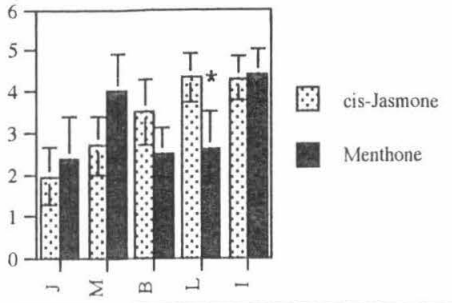
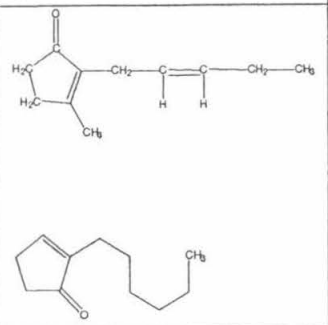
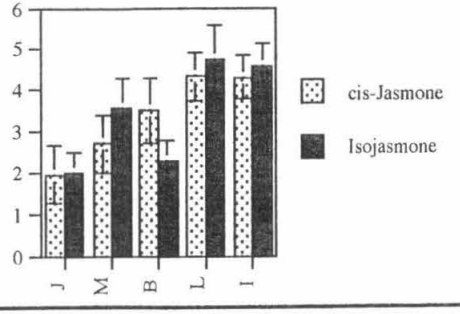
Odor Quality Shifts show both suppression and enhancement

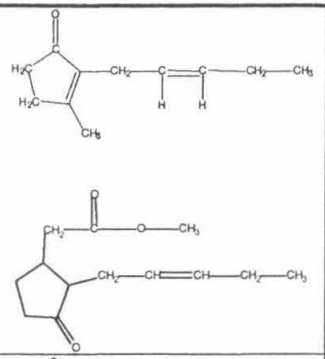
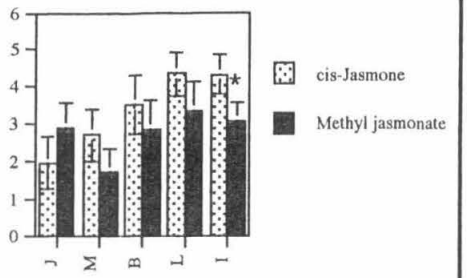
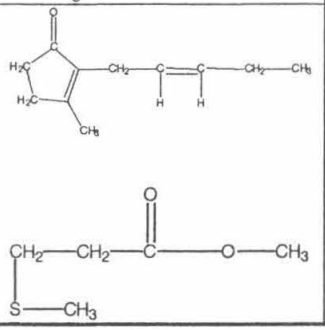
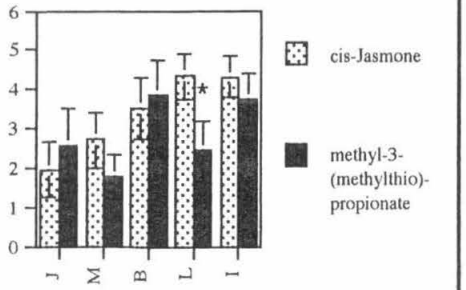
In general, the shifts in redolence indicate both suppression (denoted by negative values) and enhancement of specific odor quality dimensions. Suppression and enhancement of different odor notes may result after adaptation to the same stimulus; for example, the redolence of Jasmine showed an increase, while the redolence of Mint, Bergamot, and Lavender showed a decrease, under adaptation to Benzyl acetate. The significant shifts in odor intensity all show suppression, similar to that seen in other cross-adaptation studies.

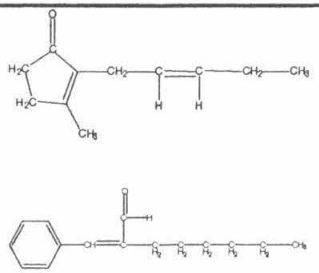
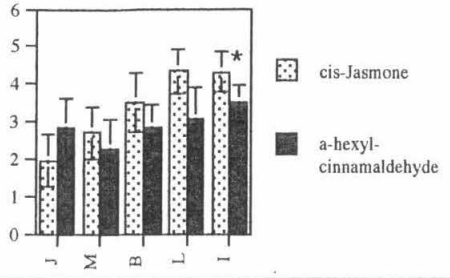
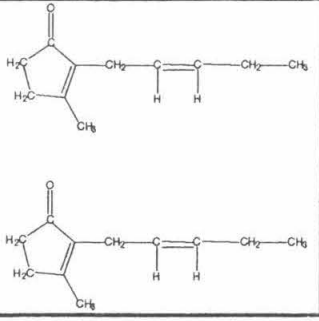
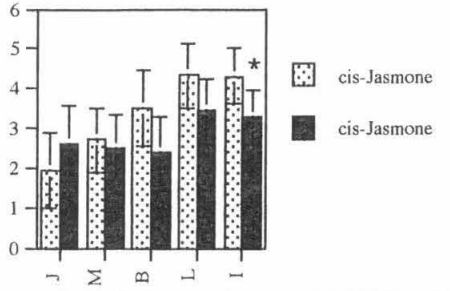
Odor Quality Shifts and Intensity shifts did not occur at the same time

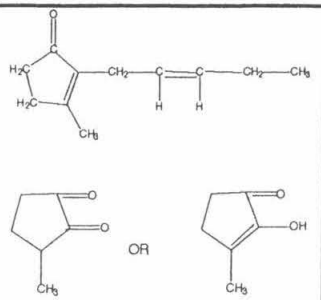
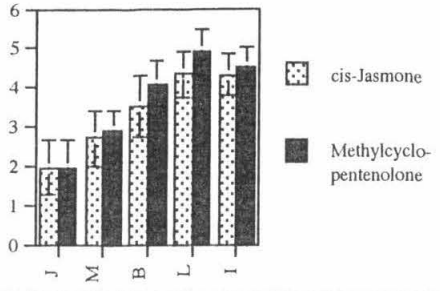
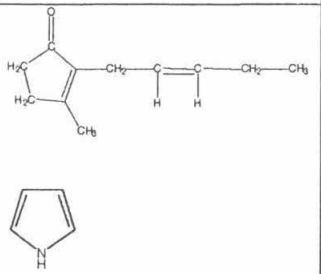
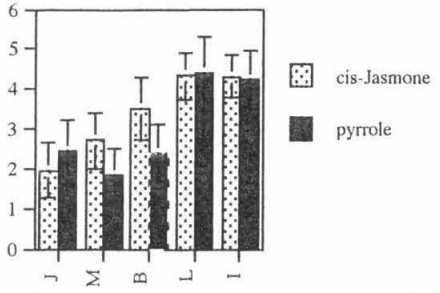
Redolence shifts and intensity shifts did not seem to occur at the same time. If a compound produced a change in intensity of the test stimulus after adaptation, it can be seen that shifts occurred in the odor quality realm as well, though these were not shown to be significant. However, if a compound produced a significant redolence shift, changes in the intensity realm were seen to be minimal. Further, while all intensity shifts were negative, indicating suppression, odor quality notes were seen to increase as well as decrease in redolence (i.e., Jasmine, for Benzyl acetate) after adaptation. These results

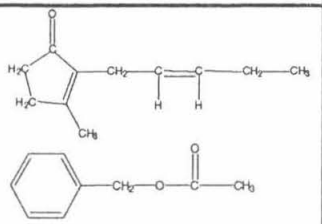
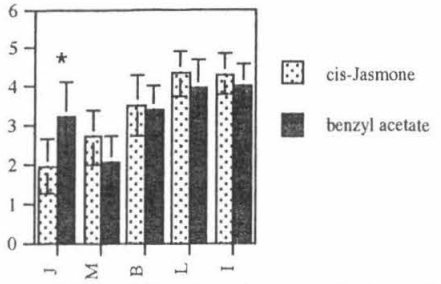
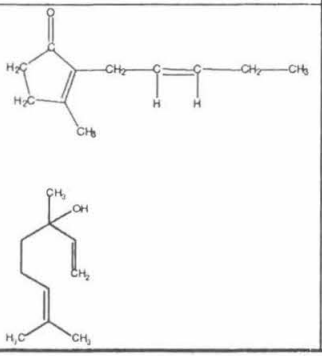
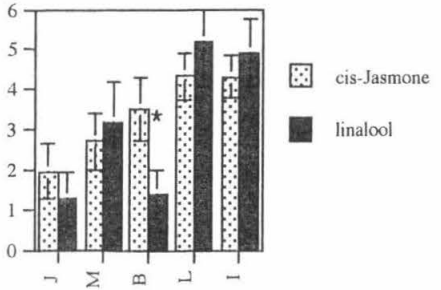
suggest the possibility that the redolence of a specific odor note in a complex odor quality may be processed in a different way from the overall intensity of the odor.

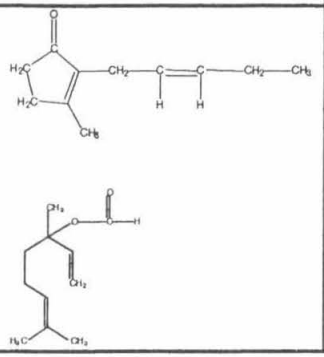
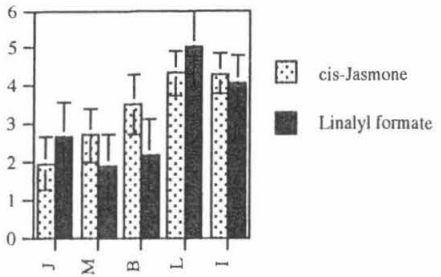
Adapting Compound	Structural Similarity	Perceptual Similarity	Metabolic Similarity	Nat. Orig	Effect of X-Adapt	Structures	Perceptual Effects of Cross-Adaptation
Menthone	No	No	Yes	Yes	Quality Shift		
Isojasmone	Yes	Yes	No	No	None		

Adapting Compound	Structural Similarity	Perceptual Similarity	Metabolic Similarity	Nat. Orig	Effect of X-Adapt	Structures	Perceptual Effects of Cross-Adaptation
Methyl Jasmonate	Yes	Yes	Yes	Yes	Intensity Decrease		
Methyl-3-(methylthio)-propionate	No	No	No	Yes	Quality Shift		

Adapting Compound	Structural Similarity	Perceptual Similarity	Metabolic Similarity	Nat. Orig	Effect of X-Adapt	Structures	Perceptual Effects of Cross-Adaptation
α-Hexylcinna-maldehyde	No	Yes	No	No	Intensity Decrease		
Cis-jasmone (self-adaptation)	Yes	Yes	Yes	Yes	Intensity Decrease		

Adapting Compound	Structural Similarity	Perceptual Similarity	Metabolic Similarity	Nat. Orig	Effect of X-Adapt	Structures	Perceptual Effects of Cross-Adaptation
Methylcylco-pentenolone	Yes	No	No	Yes	None		
Pyrrole	Yes	No	Maybe	Yes	None		

Adapting Compound	Structural Similarity	Perceptual Similarity	Metabolic Similarity	Nat. Orig	Effect of X-Adapt	Structures	Perceptual Effects of Cross-Adaptation
Benzyl Acetate	No	Yes	Yes	Yes	Quality Shift		
Linalool	No	No	Yes	Yes	Quality Shift		

Adapting Compound	Structural Similarity	Perceptual Similarity	Metabolic Similarity	Nat. Orig	Effect of X-Adapt	Structures	Perceptual Effects of Cross-Adaptation
Linalyl formate	No	No	No	Yes	None		

Discussion

Odor quality may vary

The perceived odor quality of a test compound was seen to vary after adaptation, depending on the identity of the adapting compound. These results suggest that odor quality is not a "fixed" or static property, but rather a relative measure of chemical information. This phenomenon is similar to the Bezold-Brücke effect in the study of color vision, where a change in hue is associated with a change in field luminance [15]. Boynton and Gordon (1965) [15] noted that the hue shifts observed were relatively small, but well established. Similarly, the odor quality shifts seen in this study were not large. The range of responses seen suggests that the degree of odor quality variability is likely to be bounded both in the number of odor notes that a compound may elicit, as well as in the redolence of individual notes.

Odor quality and odor intensity appear to be linked in a complex way

Studies of monomolecular compounds suggest that some perceptual characteristics, such as the threshold of detection for a compound, can be well defined in a simple manner. However, descriptions that are more complex might be needed for characteristics like odor quality and odor intensity, which are already known to vary with stimulus concentration. These characteristics surely depend on the identity of the compound that elicits them, though this and other studies suggest that odor quality and

intensity may depend on other factors as well (e.g., [14]). These factors include the chemical mixture within which the compound is presented as a stimulus, as well as the state of the olfactory system.

The odor quality and odor intensity of a stimulus may also be related to each other. The results from this work showed that if the odor was suppressed in intensity, the quality did not change significantly. Likewise, if the odor quality changed, it was not accompanied by a change in odor intensity. Thus, under cross-adaptation conditions, odor quality and odor intensity shifts appear to be somewhat mutually exclusive.

There could be several explanations for mutual exclusivity in shifts of odor intensity and shifts of odor quality. We can illustrate the kinds of potential interactions between odor quality and odor intensity that might lead to mutual exclusivity, under the effects of cross-adaptation, with the following hypotheses.

1. Adapting compounds may have varying levels of efficacy in cross-adaptation with respect to a target compound. Some compounds may not cause adaptation, resulting in no perceptual changes. Other compounds may cause only partial adaptation, resulting in a shift in odor quality but not in odor intensity. If a compound is effective in causing cross-adaptation, then it may succeed in suppressing any odor quality shifts that may otherwise have been perceived under partial adaptation. This hypothesis suggests that odor quality and odor intensity are linked, and that odor

intensity suppression may lie on one extreme of a continuum of interactions between odor quality and odor intensity parameters.

2. Adapting compounds may have varying levels of efficacy in cross-adaptation with respect to a target compound. Depending on the compound, cross-adaptation results in either odor intensity shifts, or odor quality shifts, but not both. If an adapting compound causes odor intensity to shift, then the odor profile (i.e., the proportional relationship between redolences of all notes in the complex odor quality) remains the same, but is suppressed (or enhanced) uniformly. If the adapting compound causes odor quality to shift, then the baseline odor profile is lost because individual redolences vary. This hypothesis suggests that odor quality and odor intensity changes are effected by two different pathways, and that odor quality shifts and odor intensity shifts are linked in an exclusive manner—an adapting stimulus either shifts odor quality, or odor intensity, but not both.

To our knowledge, there are no data to strongly support one of these hypotheses over the other. However, these hypotheses illustrate psychophysical concepts that can be useful in exploring related questions at different levels of olfactory investigation.

Structural similarity not sufficient to produce quality or intensity shifts

An important question addressed in this work is the nature of the measure of "distance" between monomolecular olfactory stimuli. The undefined neural mechanisms

that underlie psychophysical cross-adaptation were utilized to probe this "distance." It would be useful if we could correlate similarity between compounds to a particular psychophysical phenomenon, perhaps strongly enough to predict from a perceptual response the distance between two compounds, and vice versa. To approach this problem we selected compounds according to three different categories which are potential measures of distance between compounds: structural similarity, perceptual similarity, and metabolic (biological) similarity.

Structural similarity is often gauged by homology. Homologous series of compounds (a series of chemically similar compounds, where each differs from the next by a systematic change) have been used as olfactory stimuli by researchers at all levels of study in the olfactory system ([16], [17], [18]). The choice of homologous compounds as stimuli implicitly suggests the hypothesis that the olfactory system may be organized along similar lines; that is, that the olfactory system may discriminate and organize compounds based on their structural homology. To test this idea, we selected several compounds that were structurally similar to the test compound to see if they might be effective at inducing cross-adaptation.

- Compounds that were related to the test stimuli only by structural similarity (i.e., Methylcyclopentenolone and Pyrrole) were found to be ineffective at producing shifts in either odor quality or odor intensity.
- Across all compounds in this category, two compounds were effective in producing changes in the perception of the test compound after adaptation. The two compounds that were effective, cis-Jasmone (the test compound itself) and Methyl jasmonate,

were also the most similar to the test compound when compared to all other adapting compounds, and both produced odor intensity suppression.

- With these exceptions, across all responses to cross-adaptation, perceptual shifts in either quality or intensity were produced by compounds that were not structurally similar to cis-Jasmone.

These findings are consistent with the idea (for which there is ample evidence) that the olfactory system may use a different fundamental metric than structural homology in its organization. Results from single olfactory receptor neurons [16], mitral cells in the olfactory bulb [19], and at the perceptual level [20] show that olfactory response is discontinuous along homologous series of compounds. Only a range of several consecutive compounds of a series elicit similar olfactory responses. Studies have shown that the same olfactory receptor responds to compounds with varying structure [16], and that a small structural change can render a highly odorous molecule completely odorless [21]. These findings suggest that olfactory selectivity may be highly specific as well as diverse in terms of chemical structure.

Odor quality similarity linked to shifts (but is not a primary metric of "distance" between stimuli)

Pierce, et al. (1993) [9] showed that cross-adaptation, resulting in a decrease in perceived intensity, was achieved with an adapting compound which was perceptually (i.e., odor quality-wise) similar, but structurally dissimilar, to the test compound. The idea behind experiments of this type is that the olfactory system may map compounds,

which have an otherwise undefined relationship to each other, to a common odor quality perception. These compounds are thus "similar" by virtue of their common olfactory processing, and it is assumed that further work may define the metric used by the olfactory system that links the compounds together. Cross-adaptation resulting in suppressed odor intensity [9] supports the hypothesis that common neural pathways are used to process these compounds.

To test this idea, we selected several compounds which were odor quality analogs of the test compound to see if they might be effective at cross-adaptation. Some caution is required in interpreting results for compounds in this category, because the "similarity" classification used (odor quality) is not a property of the stimulus itself, but is a result of processing by the olfactory system. Odor quality similarity may be correlated with an undetermined metric of similarity between stimulus compounds, but cannot be the fundamental metric of similarity we seek. Thus, odor quality similarity is used here as an indicator of an unknown similarity metric.

- Compounds that were related to the test stimuli only by odor quality similarity showed mixed results. α-Hexylcinnamaldehyde produced odor intensity suppression, while Isojasmone was ineffective at producing perceptual shifts.
- Across all compounds in this category, all but one (Isojasmone) were effective at producing significant odor quality or odor intensity shifts.

- Across the set of responses to cross-adaptation, there was no clear relationship seen between effectiveness at producing an odor quality shift and odor quality similarity. That is, some compounds that produced odor quality shifts were not similar in odor quality to the test stimulus. However, all compounds that were effective at producing odor intensity suppression were also similar in odor quality (among these are the two structurally similar compounds we encountered earlier, cis-Jasmone and Methyl jasmonate).

This last result is complicated by findings that perceptually dissimilar (with respect to odor quality) compounds can cause odor intensity suppression after cross-adaptation [7]. Thus, odor intensity suppression may not strictly predict odor quality similarity. However, it suggests that a common neural pathway may link odor intensity suppression and odor quality similarity to the same distance metric between stimuli.

Metabolic similarity produces shifts in quality and intensity

A third category of similarity tested was metabolic similarity. Since naturally-occurring compounds are commonly produced as elements of a complex mixture by a biological source, it is reasonable that products from the same biological source may be "similar" by virtue of their common origin. Thus, to test the third category of similarity

we selected several compounds which have the same natural source as the test compound to see if they might be effective at cross-adaptation.

- Compounds that were related to the test stimuli only by metabolic similarity (l-Menthone and Linalool) were found to be effective at producing odor quality shifts after cross-adaptation.
- Across all compounds in this category, compounds that were classified as "metabolically similar" were seen to be effective at producing shifts in either odor quality or odor intensity of the test compound, with the exception of Pyrrole. However, Pyrrole was included in the group because it is found in Bergamot leaves, while cis-Jasmone is found in Bergamot fruit oil. It is possible that, while both compounds are found in the same plant, that they may not be closely related; essential oils from different parts of the same plant often contain different compounds [12]. Among these metabolically similar compounds are cis-Jasmone and Methyl jasmonate, the two structurally similar compounds which were effective at producing perceptual shifts.
- Across the set of responses to cross-adaptation, all but two instances of perceptual shifts were correlated with metabolic similarity. The exceptions were α-Hexylcinnamaldehyde, which is an odor quality analog discussed previously, and Methyl-3-(methylthio)propionate, which has no categorical similarity in this experiment. These two results may be understood in the context of the following discussion.

Our results suggest that metabolic similarity may be relevant to olfactory processing. Metabolic processes link compounds through a series of enzyme-catalyzed reactions. Thus, the "distance" between compounds may be as fine-grained as "one

hydrolytic cleavage apart" or more coarse-grained, such as "one metabolic pathway apart, sharing a common precursor." It remains to be seen if metabolic "distance" can be related to the "molecular receptive range" [19] of an olfactory receptor neuron. However, results from Zhang, et al. (1997) [22] show that olfactory receptor proteins may be tuned to metabolically related compounds, and are capable of discriminating compounds that are structurally similar but not metabolically related.

We can now formally hypothesize that metabolic similarity produces changes in odor intensity shifts or odor quality shifts in the perception of a test compound under cross-adaptation. Furthermore, we can propose that metabolic similarity may be the fundamental metric by which olfactory processing is organized. However, how can we explain perceptual shifts that are produced by compounds that are not metabolically similar? The specific open questions from the data that need to be answered are:

- From the perceptually similar group, how can we explain why α-Hexylcinnamaldehyde elicits perceptual shift, while Isojasmone does not?
- Why do Methyl-3-(methylthio)propionate and α-Hexylcinnamaldehyde produce effects when they are not metabolically similar to cis-Jasmone?

We return now to the question of what fundamental similarity metric odor quality might reflect. These results might possibly be understood in the context of the natural origin of compounds.

Naturally-occurring compounds

An idea we investigated was that the olfactory system might be "tuned" to naturally occurring compounds. Natural compounds were not tested categorically, but in the context of their structural, perceptual (odor quality), and metabolic (biological) similarity to the test stimulus. The process of stimulus selection informally emphasized the link between natural origin, odor quality, and metabolic similarity. For example, if a compound was of natural origin, and had similar odor quality to the test compound, then it was usually metabolically similar as well. This empirical observation suggested that odor quality similarity might be an indicator of a biological metric of similarity, if the compounds were both of natural origin. In the perceptually similar group, both Isojasmone and α-Hexylcinnamaldehyde are artificial compounds, not found in nature. Thus, the mixed experimental results from these compounds may reflect their artificial origin, and an arbitrary similarity to cis-Jasmone.

Unrelated compounds

The second question above concerns the last category of compounds that remains to be discussed: the Dissimilar group of compounds. Interestingly, of the two compounds that were dissimilar in all categories to the test compound, one was very effective in eliciting an odor quality shift: Methyl-3-(methylthio)propionate, which is a sulfur compound eliciting an onion-like odor. While so far an inconclusive observation,

it does suggest we rethink the assumption that only "similar" compounds might affect the odor perception of a test compound. It is known that in color vision, "induction effects" occur in the perception of a hue when juxtaposed with another color field [15]. Such effects may serve to heighten the contrast between visual objects, in effect forming a colored "edge." In olfaction, no spatial "edges" occur around an olfactory object; however, if an olfactory stimulus was presented against a chemically dissimilar background, a "contrast" mechanism might produce such an effect as seen here. This issue remains to be explored.

Summary

To summarize, we investigated whether cross-adaptation might produce shifts in odor quality as well as intensity in a test compound. We reasoned that adapting compounds which were "similar" to a test compound might produce shifts in odor perception after cross-adaptation. Adapting compounds were categorized according to one of several similarity measures: structural similarity, odor quality similarity, or metabolic (biological) similarity. These similarity metrics are candidate metrics to describe the similarity or "distance" between olfactory stimulus compounds. Compounds that were metabolically similar to the test compound were found to produce significant shifts in odor quality or intensity in the perception of the test compound. Metabolic similarity could account for effects produced by compounds that also had structural or

perceptual similarity to the test compound. Thus, these results suggest that metabolic similarity may underlie changes in odor intensity or odor quality in the perception of a test compound under cross-adaptation. Furthermore, metabolic similarity may be the fundamental metric by which olfactory processing is organized.

Because the olfactory system can also process compounds which are not found in nature (and therefore are not strictly "metabolically related" to natural compounds), some of these produced perceptual shifts under cross-adaptation. We propose that an arbitrary (i.e., cannot be categorized) similarity to the test compound is responsible for these effects.

Finally, we observed that compounds which are extremely dissimilar from the test compound could also produce perceptual shifts under cross-adaptation, and interpret this as an "induction" phenomenon which may underlie olfactory "contrast" between odor objects.

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Chapter 6. Olfaction--the biological sense

Our results raise the possibility that the olfactory system may be organized around a metabolic (i.e., a biological) order

In the last chapter, we found that metabolic similarity between compounds could produce shifts in odor perception in a cross-adaptation paradigm. This suggested that metabolically similar compounds might share the same neural processing pathways in the olfactory system, leading to the idea that metabolic similarity may be the fundamental metric by which olfactory processing is organized--that is, the olfactory system may be organized around a biological basis.

This idea represents a shift from the classical view that the olfactory system is a general chemical detector and classifier. In this view, the olfactory system is organized around chemical principles of "similarity," and chemical similarity (measured by molecular size, weight, structure, solubility, or other characteristic) determines whether compounds may share the same neural processing pathways in the olfactory system. This theory is attractive for several reasons. First, it makes no presuppositions about the "meaning" of chemical compounds: if a compound possesses definite molecular properties (surface activity, low polarity, some water solubility, a high vapor pressure, and a high lipophilicity), these are sufficient to elicit olfactory activity [1]. Second, it

provides a nice framework within which to select olfactory stimuli for experiments--homologous series are easy to identify, and it is fairly straightforward to define the difference ("distance") between two stimuli. Third, the olfactory system has been found to respond to most compounds meeting the general criteria above.

However, this theory has failed to explain some of the most basic questions in olfaction. For example, why do some compounds elicit odors, while others (that meet the criteria listed) do not [1]? Why do only a few compounds of a homologous series elicit activity in a given neuron, and why do those same neurons also respond to compounds which are not of that series [2]? Why do "small" structural changes (in terms of chemical metrics) render some highly odorous molecules odorless, and cause other molecules to elicit a different odor quality [1]? Issues that are more philosophical include understanding the role of a general chemical classifier in behavior and perception. Of what use is a general chemical classifier? Most important of these issues are that a chemistry-based view lacks predictive power. This shortcoming is resolved with a shift to a biological view of olfactory processing.

The olfactory system seems perfectly suited to sensing biological information. Odor perception can directly reveal the identity of the biological source, its age and health, and its relevance to the perceiver in terms of attractiveness or repulsiveness. Such information is often inaccessible using other sensory systems (e.g., vision or hearing). An animal that can perceive biological information has a clear advantage over a

competitor that could not. For example, a decayed fish and a fresh fish often look the same, but the consequences of ingesting decayed food can be serious. Similarly, the nutrient content of foods may be ascertained quickly through olfaction by foraging animals; this may explain the high selectivity in feeding shown by animals when many food sources are available [3].

In this view, the function of the olfactory system is to represent features in the biological environment, with timing, accuracy, and detail enough to enable the animal to respond appropriately. Thus, it is reasonable to think that there may be a correlation between features in the environment, represented by biologically based (i.e., life-based) chemical patterns, and patterns in olfactory perception.

The basic differences between a biological view and a chemical view

There are two basic differences between a biological view of olfactory perception, and a chemistry-based view.

There is a different order to chemical stimuli

First is the issue of "order" between chemical stimuli. In other sensory systems, the receptive field of a primary sensory neuron usually spans a range of ordered inputs. That is, whether wavelength, frequency or distance, they can be arranged in a sequence

that has a uniform "distance" and sequence between elements. However, no such natural, chemistry-based order has been demonstrated for olfactory stimuli.

Most studies use homologous series of compounds to probe the response properties of olfactory neurons. In homologous series, chemically similar compounds are related by a systematic structural change, as in an alcohol series where a single Carbon is successively added. However, olfactory neurons so far have only been shown to respond to a few compounds of any single series, while responding to compounds of other series as well [2]. Similarly, in perceptual studies it is known that a small change in the structure of a highly odorous compound can render it odorless [1]. Thus, while a group of compounds can define a "molecular receptive range" for an olfactory neuron [4], a chemistry-based principle underlying the relationship between those compounds is not clear.

In contrast, metabolic processes link compounds through a series of enzyme-catalyzed reactions. Thus, the "distance" between compounds may be as fine-grained as "one hydrolytic cleavage apart" or more coarse-grained, such as "one metabolic pathway apart, sharing a common precursor." Further, there is a distinct sequence to the process, so compounds may be arranged in order of their synthesis within a particular pathway.

Thus, a convincing chemical order or "chemotopy" does not exist in the chemical-classifier view of olfactory processing, but one does exist in the biological view.

The biological view places olfaction in an evolutionary context, while no reasonable context is provided by a chemistry-based view

Secondly, a chemistry based view of olfactory perception does not explain the significance of the set of chemical properties that have been shown to be important in enabling a molecule to elicit an odor (e.g., surface activity, low polarity, some water solubility, a high vapor pressure, and a high lipophilicity). It is clear that the properties of olfactory receptors are "matched" to complement these properties (since binding of ligands to olfactory receptor proteins is thought to underlie olfactory transduction). It has been recently suggested that the configuration of particular binding sites on olfactory receptor proteins may allow them to rapidly evolve to accommodate the recognition of new odorants that arise during the formation of new species [5]. It seems reasonable that the properties of a chemical stimulus that enable it to elicit odors may reflect a biological origin, and in fact may be characteristic of biologically relevant olfactory stimuli. This is consistent with a view that the olfactory system and the biological environment within which it functions are seen as co-evolving, reflecting a process by which species undergo reciprocal evolutionary change through natural selection [6].

A logical extension of this reasoning is that the olfactory system may be "tuned" for biologically relevant compounds (i.e., compounds whose presence negatively impacts survival, and whose presence may enhance survival), and ignores compounds that are not

biologically relevant. Thus, biological relevance provides a general context for classification of chemical compounds, that is harder to establish on a chemical basis.

Is there anything in the existing data that disproves a biological view?

Generalization across structurally similar compounds is consistent with a biological view

"Small changes" in chemical structure may be correlated to metabolic similarity. Many studies probe the ability of the olfactory system to respond to compounds that differ by a "small change" in chemical structure. For example, homologous series of compounds are considered structurally similar. Structurally similar compounds are encountered by the olfactory system in one of two settings: in the laboratory (produced by chemists), or in nature (produced by enzyme catalysis).

Enzyme-catalyzed reactions, as found in metabolism, are generally limited in kind; there are only six major classes of enzymes, and these are based on the type of reaction they catalyze [7]. These reaction types are:

1. Oxidation-reduction
2. Chemical group transfer
3. Hydrolytic cleavage of specific bonds
4. Non-hydrolytic cleavage, with the effect of leaving double bonds or of adding groups to a double bond
5. Change of geometrical (spatial) arrangement of a molecule
6. Joining together of two molecules

Some of the changes in molecular structure mediated by these reactions might be classified as "small changes," while others might not. However, because natural origin was not a controlled factor, study results demonstrating that olfactory neurons respond to several members of a homologous series (e.g., [2], [8]), as well as similar studies in the perception of homologous compounds [9], may be consistent with a metabolism-based chemotopy.

Discrimination between structurally similar compounds is also consistent with a biological view

Similarly, where it is found that the olfactory system discriminates between structurally similar compounds (e.g., [2], [8], [9]), it may also be consistent with the hypothesis that the olfactory system discriminates between:

1. biologically relevant and non-biologically-relevant compounds
2. compounds of different metabolic origins

"Broad tuning" vs. specificity paradox at the level of single cells and at the perceptual level is resolved with the idea that the olfactory system is biologically specific

From a chemical point of view, the specificity of the olfactory system seems both "broad" and "narrow." For example, a specific receptor protein is shown to respond to

compounds of varying chemical structure, suggesting "broad molecular tuning." However, because receptors respond to only a few compounds of a particular chemical class, this suggests "narrow" tuning, or higher specificity. As mentioned in earlier chapters, a metabolic-based chemotopy may explain the receptor specificity of single receptors in *C. elegans* [10], and also may resolve this paradox.

Since the neural organization of the olfactory system appears based on patterns of receptor specificity, its principles of organization seem consistent with a biological view

The neural organization of the olfactory system appears highly organized ([5], [11], [12]) and, potentially, chemotopically ordered around the specificity of olfactory receptors. A biological view of olfactory receptor specificity is consistent with the methods used to trace anatomical and functional pathways in the olfactory system.

The relationship between chemical structure and odor perception is consistent with the idea that biological information is encoded in chemical stimuli

Existing structure-function studies exploring the relationship between molecular properties and odor quality are consistent with the idea that biological information may be encoded at the level of monomolecular compounds. That is, information about the biological source of a monomolecular stimulus may be conveyed by the odor evoked. An

example of this is Menthol, a bioactive compound in Peppermint Oil that evokes the characteristic odor of Peppermint, as well as evoking the "cooling" sensation of Peppermint.

If a biological view were appropriate, what would it predict?

In considering that a biological view may be an appropriate framework from which to view olfactory perception and olfactory processing, a number of hypotheses present themselves. There is already much data that is consistent with these hypotheses.

The olfactory system should discriminate between biologically relevant compounds, and other compounds

Biology-based discrimination

For the olfactory system to represent features of the biological environment, it is reasonable to expect that it be able to discriminate biologically relevant compounds from a background of other chemical compounds. To evaluate the efficacy of the olfactory system in this task, it is helpful to understand the differences between naturally occurring biological compounds, and other compounds.

Quantifiable differences exist between naturally occurring compounds of biological origin, and other compounds

There are several general differences between naturally occurring biological and non-biological compounds. These differences are summarized in Figure 1.

1. Limited atomic composition - About 26 elements are known to be essential to life, and of these, only six are major components of living tissue. These are Carbon, Hydrogen, Oxygen, Nitrogen, Phosphorus, and Sulfur [13].
2. Physical distribution in the environment - compounds of biological origin (as well as those of geothermal origin, which also elicit odors) occur in atmospheric concentrations that are much higher than the amounts predicted from equilibrium geochemical analysis. However, other natural compounds tend to occur at equilibrium concentrations in the atmosphere [13].
3. Rate and relevance of change - Compared to most non-biological compounds in the environment, biological compounds change rapidly, and in biologically relevant ways. There are specific patterns of how biological compounds change, and these may be classified according to the primary element of the compound. One example is the biogeochemical Sulfur cycle, which comprises compounds containing that common atomic element, and describes how that atomic element is transformed into different compounds as it moves through the environment [14].
4. Bioactivity - Many compounds of biological origin elicit non-olfactory activity (e.g., toxicity, endocrine function) in target organisms.

There are also differences between naturally occurring compounds, and those not found in nature. For example, many compounds that are important to life are chiral; that

is, they have a left-handed and right-handed configuration. However, there are many examples where only a specific enantiomer of a chiral compound is found in nature (i.e., only one "hand" is of natural origin). One example is L- and D-alanine, the left-handed and right-handed enantiomers of the amino acid alanine. Both forms are found on earth (the D- form is of extraterrestrial origin), but only the L- form is incorporated in living tissue [15]. Most of the naturally occurring amino acids (the building blocks of proteins) are chiral, and these occur naturally only in the left-handed configuration.

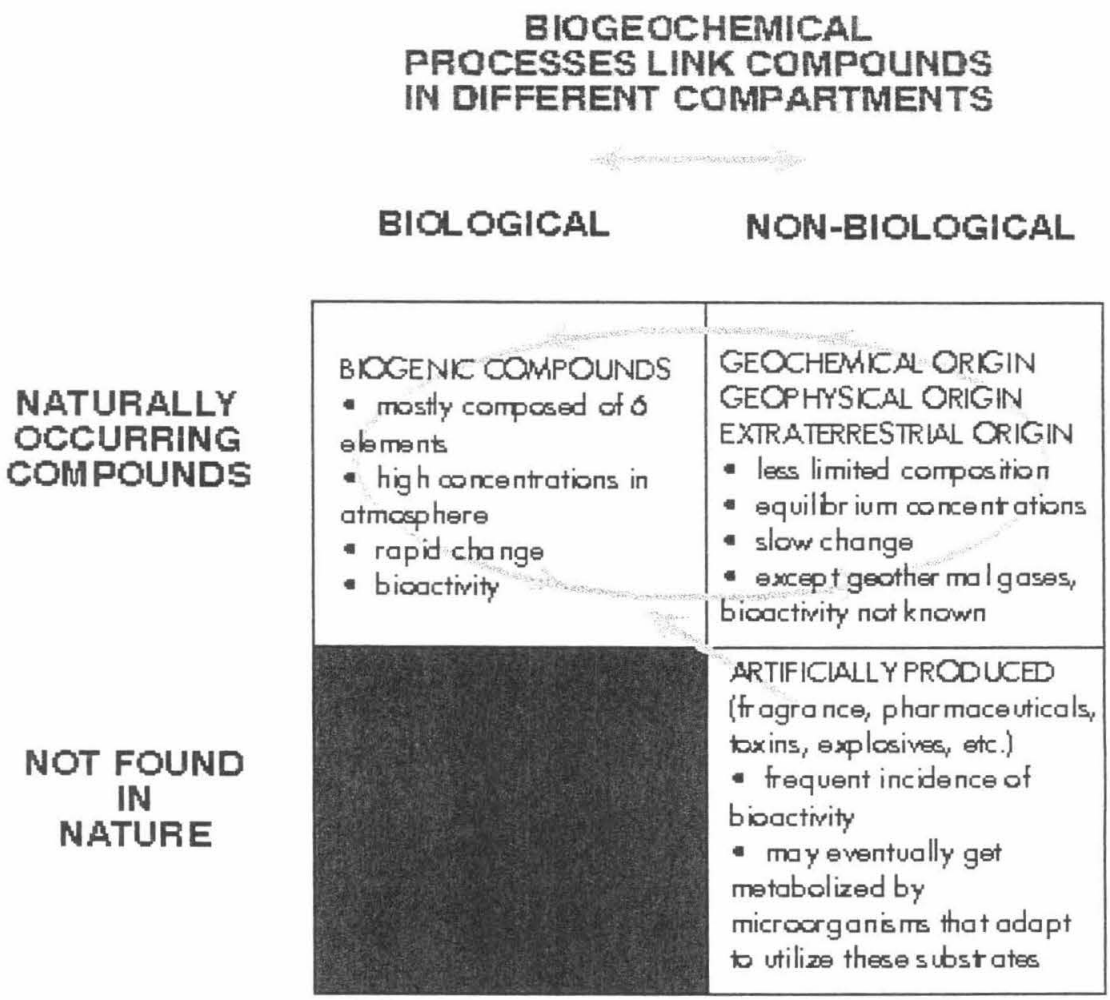

Figure 1. A biologically based classification of chemical compounds in the environment. This figure summarizes a classification of compounds, and dynamics of their change from compartment to compartment, especially between the naturally occurring compounds. Metabolic processes control how compounds change in the upper left compartment. Geochemical processes such as evaporation, and geophysical processes such as weathering, control how compounds change in the upper right compartment. Compounds in the lower right compartment are man-made; there is evidence that microorganism *metabolism* may adapt to utilize substrates which are not found in nature [16], which may introduce these compounds into the biogeochemical dynamic.

These examples demonstrate that quantifiable differences exist between naturally occurring biological compounds, and other chemicals. Insofar as biologically relevant compounds are to a first order likely to be of biological origin, it is reasonable to hypothesize that the olfactory system might be able to detect and discriminate natural biological compounds on this basis.

The olfactory system should discriminate between biologically dissimilar compounds

It makes sense that the chemical "background" to an olfactory stimulus might include other biological compounds that may have relevance in other scenarios, but is not of current interest to the animal. Discrimination between biologically relevant but dissimilar compounds is more conceptually complex. There is first the question of whether two compounds can be discriminated by their evoked odors (i.e., same, or different?). Then there is the question of how different two odors might be. The following discussion refers to compounds that elicit very different odor qualities, and we suggest that this is due to a large metabolic "distance" between compounds.

It is known that enantiomeric compounds often elicit very different odor qualities. For example, (-)-(S)-limonene (found in pine oils) evokes the odor of Turpentine, while (+)-(R)-limonene (found in citrus oils) evokes the odor of Orange [1]. The ability of the olfactory system to discriminate by perception is not limited to enantiomeric pairs.

Stereoisomers (cis- and trans-, or (Z)- and (E)- configurations) often elicit very different odors. Similarly, change in the functional group of a molecule can cause changes in the perception of the stimulus from odorous to odorless (or perhaps to a very different odor quality) [1]. The compounds used in our psychophysics experiments in Chapter 5 demonstrate that compounds that are arbitrarily similar in structure may elicit very different odor qualities. These observations might be explained by the olfactory system discriminating between compounds based on their different biological sources.

To support the idea that the olfactory system may be discriminating on the basis of biological information, as opposed to discrimination based on strictly chemical classification, there are numerous examples of how enantiomeric and diastereomeric specificity of the olfactory system is used to advantage in chemical communication between animal species. For instance, the bark beetle species *Ips pini* is attracted by (-)-(R)-ipsdienol, while the (+)-(S)-isomer intercepts the effect [17]. In some cases, separate enantiomers are used as pheromones by different species [18]. The (6R,7S)-configuration of a moth pheromone is used by *Colotois pennaria*, a winter-flying geometrid moth, while the (6S,7R)-configuration is used by a similar species *Erannis defoliaria* [18].

These and other studies suggest that chirality, and perhaps other specific features of biological molecules, may convey (and in fact, may be produced in order to convey) important biological information, and that the olfactory system may differentiate between

compounds based on the different biological information they carry. Revisiting previous studies in this area, as well as further research, may yield results consistent with a biologically based view of odor perception.

The olfactory system should be tuned to biologically patterned stimuli

Biological olfactory stimuli are complex mixtures of compounds. Because the olfactory stimulus is composed of discrete elements, there is no physical "boundary" around the compounds forming the stimulus. Since there is no limit to the number of odor-evoking compounds that might happen to be present (due to other odor sources), any hypothetical volume of space may contain compounds from overlapping chemical patterns, so the olfactory stimulus may be "noisy." The olfactory system must be able to extract the stimulus from its background. This is somewhat analogous to fitting together the pieces of a jigsaw puzzle, when the pieces are mixed together with pieces from other puzzles.

As the jigsaw puzzle example illustrates, there are two levels to this problem. The first problem is separating the different puzzles from each other. Hopfield (1999) [19] proposed that this signal-from-noise problem might be solved by recognizing the temporal relationship between stimulus elements. The idea is that compounds released from a common source will show the same temporal structure, while compounds from another source will show a different temporal relationship. This idea is supported by

results from Moore and Atema (1988) [20], who showed that distance information might be calculated from the dynamic structure of a chemical concentration profile. However, there is still a second problem to be solved--that of putting the pieces together correctly (as in the jigsaw puzzle example), or in this case, identifying the biological information conveyed by the odor. As anyone who has assembled a jigsaw puzzle knows, there are clues both in the shape of the piece, as well as the part of the puzzle image that one can see pictured on the piece.

If the olfactory system used metabolic relationships between compounds to link compounds within a stimulus together, then each compound in a stimulus could presumably carry enough biological information to link it to other compounds with which it belonged. It would be analogous to the part of the puzzle image that appears on a jigsaw puzzle piece. Our data (described in Chapter 5) suggests that the olfactory system may process metabolically similar compounds using the same neural pathways, and these may serve to modify (i.e., cause a shift in odor perception) the relevant signal. Thus, the metabolic relationships between compounds could serve to segregate relevant collections of biological compounds from each other, enhancing the ability of the olfactory system to discriminate between dissimilar biological patterns. The olfactory system may further employ a "contrast" mechanism to further differentiate between dissimilar patterns (described in Chapter 5).

Another issue in discriminating between biologically relevant and non-relevant compounds may occur once the pertinent pattern elements are segregated and discriminated from the chemical background. The compounds that comprise a biological mixture of chemicals can number in the hundreds [21]; yet, psychophysical results suggest that subjects can identify at most three or four odor notes in a complex odor quality [22]. Not surprisingly, some compounds found in these biological mixtures do not elicit odors when presented to the olfactory system monomolecularly [1]. It is likely that some of these odorants may not be processed by the olfactory system at all (i.e., are odorless because they do not bind to receptors). Why would the olfactory system ignore a natural, biological molecule that is part of an information-carrying mixture?

One answer is that these compounds probably do not carry specific biological information, because their presence does not allow the prediction of other compounds in the metabolic pathway. Some biological compounds seem to be ubiquitous in animal metabolism. One example is oxaloacetate, which is produced as part of the Krebs cycle and occurs in many bacteriological fermentation pathways [23]. Other compounds are likely indicators of specific metabolic pathways, for example the combination of citrate, pyruvate, and diacetyl [23]. In anaerobic conditions, citrate may be produced in large amounts (up to 1.5 g/l) while diacetyl only occurs in small amounts (2 ml/l). Pyruvate is an intermediate compound in the formation of diacetyl from citrate, as is oxaloacetate. However, the combination of citrate, pyruvate, and diacetyl specify (a) an anaerobic environment, in which (b) specific (usually lactic acid) bacteria are active, and where (c) citrate is the substrate from which this fermentation process is based. Thus, a worm (i.e.,

C. elegans) that can detect citrate, pyruvate, and diacetyl receives biological information about the presence of specific bacteria that are its food source. In fact, Zhang, et al. (1997) [10] have shown that a *C. elegans* olfactory receptor protein, ODR-10, specifically responds to citrate, pyruvate, and diacetyl, the markers of anaerobic citrate-based diacetyl formation. Additionally, ODR-10 did not respond to oxaloacetate, or a host of other compounds that were structurally similar to citrate, pyruvate, or diacetyl. This demonstrates that olfactory receptor proteins can be highly specific, and that their specificity might be explained by a biological basis for odor perception.

Thus, the specificity of the olfactory system seems appropriate for segregating biologically relevant patterns from a chemical background, as well as for discriminating between compounds that carry biological information and those that do not.

The olfactory system extracts chemically encoded biological information and re-encodes it as odor

In the biologically based view of olfactory perception, one of the most important tasks of the olfactory system is to convey biological information that is encoded in the chemical stimulus. In other words, specific odors may carry specific biological information. How is this information encoded in the chemical stimulus, and how is it decoded by the olfactory system and mapped to an odor perception? These questions may be best addressed by considering the evolutionary origins of the olfactory system.

The process of natural selection is what determines the genetic machinery that ultimately produces these chemical patterns, and therefore is what determines the biological information that is encoded. To say that biological information is encoded in chemical patterns suggests that even if we had an artificial means of sensing and processing these compounds, the biological information could be decoded--it is objective and quantifiable.

The encoding of biological information

The identity of unique molecules, or the biological patterns of more common molecules released by organisms, serve as biological "markers," or a "signature" for the organism. An example of a how metabolism may produce an array of volatile compounds (i.e., olfactory stimuli) is microbial fermentation. Organisms such as bacteria or fungi eat a food source and excrete a number of by-products. These compounds rapidly accumulate and eventually reach a steady state such that the proportions of individual compounds remain the same over time: e.g., a "fermentation balance." The identity of the by-products, as well as their proportions with respect to the steady-state balance, depends on the type of organism, its food source, and the environment (aerobic or anaerobic). Because the genetic programming of the organism determines what enzymes and metabolic pathways are used in the fermentation process, it is possible to identify the active organism in a culture by analyzing the fermentation balance of its

products. The fermentation balance acts as a "chemical signature" of the identity of the organism.

Decoding biological signals: role of evolution

The olfactory system and the biological environment within which it functions appear to complement each other. The origins of this complementary relationship may be explained by co-evolution, a process by which species undergo reciprocal evolutionary change through natural selection [6]. The opposing means by which species act on each other, and which would logically undergo evolutionary change, are biologically active compounds produced by organisms, and the resultant behavior of other organisms, which is mediated by olfactory sensitivity to these compounds. Thus, it is conceived that biologically active compounds evolved because their effects on target organisms provided advantage to their releasers, and in turn olfactory sensitivity towards these compounds evolved as either protection from, or as an opportunistic tool to take advantage of, the source of those compounds.

The need to decode information contained at the physical level of biological stimulus patterns may explain common structural features of olfactory system anatomy

Understanding that biological chemical patterns contain two levels of information (i.e., the jigsaw puzzle analogy where information is contained both in the shape and the image imprinted on a puzzle piece) permits us to consider that olfactory systems may

have evolved at two different levels, to allow decoding the information at these two levels. These levels are that of the physical distribution of the stimulus, and biological information about the source of the stimulus.

Four parameters may be used to characterize biological chemical patterns:

1. the total set of compounds that constitute the mixture (the whole stimulus)
2. the identity of each component compound
3. the relative concentration of each component with respect to the total concentration
4. spatial and temporal distribution of all the compounds in the mixture

It can be argued that these parameters of complex chemical patterns are, to a first order, common to all biological olfactory stimuli. Since the particle nature of the stimulus introduces unavoidable challenges to the olfactory system, it seems reasonable that biological principles of patterning might severely constrain successful olfactory systems. Visible evidence of such constraint is extremely clear.

Animal "noses" vary considerably in appearance from animal to animal. However, a striking phenomenon in olfactory systems is the similarity in structure of the second stage of olfactory processing: the olfactory bulb in mammals, and related structures in insects and other animals. The olfactory bulb is characterized by the appearance of structures called glomeruli, which are striking in appearance, as well as numerous (approximately 1000 in mammals). The glomerular organization of the

olfactory system has existed for 500 million years [24], and is present in both general and pheromonal systems, which suggests that its basic functions are similar (examples: insect, fish, crustacean, bird, rat). However, the function of the glomeruli is not yet known. It is believed that complex processing of chemically encoded information is performed at these sites due to the high density of neural processes and synaptic junctions contained within each glomerulus. Each glomerulus is therefore viewed as a complex processing structure, perhaps with a fine level of substructure that may carry out compartmentalized computations.

The similarity of structure of the olfactory bulbs across the animal kingdom strongly suggests that the structure is constrained by a common element in the tasks solved by the olfactory system. This element must be similar from animal to animal, regardless of marine or terrestrial environment. If we consider that fundamental principles may govern the creation and distribution of all biological olfactory stimuli, then this situation comes as no surprise. Metabolism is a process common to all life forms on earth, and so the characteristics of its products are similar, and ubiquitous.

Decoding information about the source involves the association between odorants and other relevant biological information, and the evolution of olfactory receptors to complement signal-carrying compounds

Evidence for evolution at the level of biological information is more problematic, yet within grasp. Studies of the biosynthesis of terpenes (which represent many of the compounds that elicit odors, and which are precursors to the steroids) suggest that they are formed by common processes, utilizing a limited set of enzymatic reactions [25]. Terpene biosynthesis may occur in parallel with alkaloid biosynthesis (whose products include many well known drugs and poisons). In certain classes of organisms, links between these two categories of compounds may be robust enough that animals may have "learned" (through natural selection) to associate specific odorous compounds with specific alkaloids or other bioactive products, resulting in particular food selection strategies. (Many odor-evoking compounds are themselves bioactive, as well.)

The responses of organisms to bioactive compounds are varied and can be categorized in view of the likely effect of these compounds on intended targets. Primary responses are those related to immune, endocrine, and communication functions. These include physical responses, physiological responses, and some behavioral responses including pheromone-evoked behavior. Secondary responses are those mediated by olfaction: odor-evoked behaviors, odor-evoked memories, and so forth.

There is a distinct link between the receptive properties of pheromone receptors, and the pheromones produced within an animal species. In the more general situation of allomones (signaling compounds, with release beneficial to the host), there may be a clear relationship between the evolution of plant odor and olfaction. This linkage may be particularly obvious in the case of primates, birds, and insects. The idea is that a foraging animal using olfaction may be guided to a food source by its particular chemical pattern, and then in turn may act as an agent for seed dispersal (adapted from [26]).

With kairomones (signaling compounds, with release beneficial to the receiver), the evolutionary relationship is less clear, though the utility is obvious in the case of predators and their prey (and vice versa). This is most likely because it illustrates a situation where an olfactory system has evolved to detect a chemical stimulus for which the organism is not the primary target. The compounds that the olfactory system detects are likely to be those compounds that are necessary to the survival of the host organism (e.g., primary metabolites).

Because the dispersal of a chemical pattern depends on air or water to carry it, the information contained in the pattern is effectively "broadcast" to the environment. Organisms other than the intended target may detect the pattern. Bioactivity may or may not be elicited in these unintended targets due to wayward chemical patterns. Likewise, responses of these organisms to these chemical stimuli may not affect the source of the compounds. Nevertheless, evolution of olfactory systems in response to an olfactory

stimulus may occur in networks of organisms whose members may not be the intended targets for a number of reasons. In addition to using compounds to identify prey (or predator) in the example above, another example may be using natural compounds released by stationary sources as a navigational marker [27].

The broadcast nature of olfactory stimuli, coupled with evidence that the olfactory system has evolved with broad specificity, suggests a trend for the olfactory system to evolve in response to its general, and not merely specific, biological environment. It seems reasonable that the receptor specificities of a given species of animal might show some correlation to the chemical environment it inhabits.

The olfactory system extracts biological information from a single compound, and this information is conveyed by its odor quality

The olfactory system senses biological patterns of compounds, and conveys the biological information encoded in them as an odor perception. As shown in Chapter 4, compounds containing the elements Carbon, Nitrogen, and Sulfur elicit odor notes that fall in a contiguous region on a graph of odor quality. Thus, it may be possible to predict the general odor family that a compound may elicit, just based on its chemical formula. Specific compounds particular to a biological source commonly elicit the characteristic odor of the biological source, or similar odors; this was seen in the range of odor notes elicited from a monomolecular compound, described in Chapter 4. These observations

suggest that the olfactory system can extract biological information about its source from a single compound, and that this information is conveyed in its odor quality.

Psychophysical data suggest that metabolically related compounds may cause the odor perception of a characteristic compound to shift (described in Chapter 5). Thus, as the metabolic processes (e.g., in the case of a ripening fruit) progress in time, the proportions of different compounds in the mixture will change, and the resultant odor may represent a gradual shift through a "neighborhood" of the odor of the original sample; this reflects the changing nature of the biological information in the sample. Studies have shown that the changing proportions of different compounds corresponds to the degree of ripeness in a fruit, and also to a corresponding change in the assessment of "ripeness" as conveyed by odor [28].

In this manner, a particular odor may convey particular biological information about the source of the odor stimulus.

Summary

Viewing odor perception as organized around a biologically based odor environment--as opposed to a chemically based odor environment--changes how we understand the olfactory detection process and its behavioral significance.

We propose that olfactory perception may be organized around a biological basis, as opposed to a purely chemical basis. While there is much direct, as well as

circumstantial, evidence that biologically based chemical patterns in the environment may be correlated with patterns of olfactory specificity, neural activity, and perception, this has not yet been critically investigated. However, a biological framework provides answers to many unanswered questions: why do some compounds elicit odors, while others do not? Why do only a few compounds of a homologous series elicit activity in a given neuron? Why do those same neurons also respond to compounds which are not of that homologous series? Why do "small" structural changes (in terms of chemical metrics) render some highly odorous molecules odorless, and cause other molecules to elicit different odor qualities? Why do enantiomeric (i.e., left-handed and right-handed) pairs elicit different odors? These questions can be addressed by a few broad principles of biological based olfactory sensing:

1. The olfactory system may be "tuned" for biological compounds
2. Metabolic similarity may be the fundamental metric by which olfactory processing is organized
3. Odor quality may carry specific biological meaning (e.g., the identity of the biological source of the compound that elicits the odor)
4. Change in odor quality may reflect a change in metabolic distance

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APPENDIX A

The following pages show additional information for Chapter 4, A Map of Odor Quality. Lists 1 and 2 contain the odor descriptors used for the Aldrich and the Dravnieks database, respectively.

1 putrid	24 wine-like	47 lemon
2 roasted	25 coffee	48 lime
3 meaty	26 smoky	49 orange
4 burnt	27 chemical	50 ethereal
5 rancid	28 fruity	51 nutty
6 pungent	29 apple	52 almond
7 fatty	30 apricot	53 hazelnut
8 butter	31 banana	54 peanut
9 cheese	32 berry	55 walnut
10 creamy	33 cherry	56 spicy
11 oily	34 coconut	57 pepper
12 sour	35 grape	58 medicinal
13 balsamic	36 grapefruit	59 mint
14 anise	37 jam	60 floral
15 (balsam)	38 melon	61 blossom
16 caramel	39 peach	62 carnation
17 chocolate	40 pear	63 gardenia
18 cinnamon	41 pineapple	64 geranium
19 honey	42 plum	65 hawthorne
20 sweet	43 quince	66 hyacinth
21 vanilla	44 raspberry	67 iris
22 soapy	45 strawberry	68 jasmine
23 waxy	46 citrus	69 jonquil

List 1. Odor descriptors for Aldrich data.

70 lilac
71 lily
72 marigold
73 narcissus
74 rose
75 violet
76 woody
77 green
78 mossy
79 vegetable
80 herbaceous
81 caraway
82 sage
83 earthy
84 musty
85 camphoraceous
86 sulfurous
87 egg
88 cabbage
89 metallic
90 alliaceous
91 onion
92 garlic
93 animal
94 (pungent)
95 tart
96 leafy
97 strong
98 powerful
99 fragrant
100 aromatic
101 faint
102 popcorn
103 potato chip
104 toasted grain
105 bread crust
106 heavy
107 cocoa
108 cereal
109 bread
110 odorless
111 (anise)
112 phenolic
113 harsh
114 bacon
115 savory
116 horseradish
117 amber
118 dry
119 elegant
120 incense
121 oriental
122 eggyolk
123 hard-boiled egg
124 penetrating
125 fennel
126 mushroom
127 cadaverous
128 gasoline
129 pleasant
130 mild
131 bitter almond
132 repulsive
133 urine
134 quinoline
135 rubbery
136 fresh
137 fishy
138 peppermint
139 cresylic
140 milk
141 rum

List 1. Odor descriptors for Aldrich data.

142 warm
143 sharp
144 sweaty
145 spearmint
146 refreshing
147 terpene
148 cool
149 clove
150 cassia
151 lemon peel
152 intense
153 acid
154 raisin
155 prune
156 musk
157 weak
158 unpleasant
159 baked potato
160 sautéed garlic
161 clams
162 orange blossom
163 very strong
164 fenugreek
165 licorice
166 diffusive
167 butyric
168 roasted crude sugar
169 mildew
170 moldy
171 whiskey
172 peanut butter
173 new leather
174 roasted nut
175 grassy
176 grilled chicken
177 tea
178 roasted barley
179 boiled poultry
180 delicate
181 magnolia
182 plastic
183 seedy
184 light
185 brandy
186 (sour)
187 burnt almond
188 chamomile
189 passion fruit
190 dried fruit
191 maple
192 butterscotch
193 tobacco
194 leather
195 rhubarb
196 skunk
197 candy
198 raw potato
199 wintergreen
200 cognac
201 mustard
202 baked bread
203 ripe
204 lavender
205 smoked sausage
206 toasted
207 sickening
208 alcoholic
209 (leafy)
210 acrid
211 bitter
212 tropical fruit

List 1. Odor descriptors for Aldrich data.

213 unripe fruit
214 hot sugar
215 fecal
216 fusel oil
217 mango
218 pine
219 turpentine
220 celery
221 grape skin
222 green bell peppers
223 green peas
224 tomato leaves
225 ammonia
226 cedarwood
227 blueberry
228 rooty
229 creosote
230 clean
231 bergamot
232 malt
233 black currant
234 mercaptan
235 galbanum
236 roasted almond
237 roasted peanut
238 (gardenia)
239 candy circus peanuts
240 dairy
241 buttermilk
242 stinging
243 cucumber
244 watermelon
245 acrylic
246 (bread)
247 roasted corn
248 boiled cabbage
249 fried
250 cooked onion
251 cooked meat
252 crackers
253 wild
254 menthol
255 rich
256 brown
257 tomato
258 parmesan cheese
259 romano cheese
260 ricotta cheese
261 green bean
262 sherry
263 amine
264 acetic
265 saffron
266 mothballs
267 decayed
268 bland
269 petroleum
270 cauliflower
271 fermented soybean
272 lard
273 burnt caramel
274 roasted coffee
275 wet
276 orange peel
277 mandarin
278 flat

List 1. Odor descriptors for Aldrich data.

1	fragrant	24	malt	49	animal
2	sweaty	25	cinnamon	50	vanilla
3	almond	26	popcorn	51	fecal
4	burnt, smoky	27	incense	52	floral
5	green, herb, grassy	28	melon	53	yeasty
6	ethereal	29	tar	54	cheese
7	sour, acid	30	menthol,	55	honey
8	blood, raw meat		eucalyptus	56	anise, licorice
9	dry	31	fatty	57	turpentine
10	ammonia	32	mothballs	58	vegetable, fresh
11	disinfectant,	33	gasoline	59	medicinal
	carbolic	34	cooked vegetables	60	orange
12	aromatic	35	sweet	61	butter
13	meaty, cooked	36	fishy	62	burnt paper
	meat	37	spicy	63	cologne
14	sickening	38	paint-like	64	caraway
15	earthy, musty,	39	rancid	65	bark, birch bark
	moldy	40	mint, peppermint	66	rose
16	pungent, sharp	41	sulfur	67	celery
17	camphor	42	citrus	68	burnt candle
18	light	43	fruity	69	mushroom
19	heavy	44	putrid, decayed	70	wet wool, wet dog
20	cool	45	woody, resin	71	chalk
21	warm	46	musk	72	leather
22	metallic	47	soapy	73	pear
23	perfumery	48	onion, garlic		

List 2. Odor descriptors for Dravnieks data.

74	stale tobacco smoke	96	crushed grass	121	burnt milk
		97	chocolate	122	sewer odor
75	cucumber	98	molasses	123	sooty
76	raw potato	99	alcoholic	124	crushed weeds
77	mouse	100	dill	125	rubbery
78	black pepper	101	chemical	126	baked bread
79	green bean	102	creosote	127	cognac
80	banana	103	green bell peppers	128	grapefruit
81	burnt rubber	104	household gas	129	grape juice
82	geranium leaves	105	peanut butter	130	egg, fresh
83	urine	106	violet	131	bitter
84	beer	107	tea	132	cadaverous
85	cedarwood	108	strawberry	133	maple
86	coconut	109	stale	134	savory
87	rope	110	cork	135	apple
88	seminal, sperm-like	111	lavender	136	soupy
		112	cat urine	137	grain
89	carbona cleaning fluid	113	pineapple	138	clove
		114	fresh tobacco smoke	139	raisin
90	cardboard			140	hay
91	lemon	115	nutty	141	kerosene
92	dirty linen	116	fried chicken	142	acetone
93	kippery (smoked fish)	117	wet paper	143	rotten fruit
		118	coffee	144	berry, cherry
94	caramel	119	peach	145	varnish
95	sauerkraut	120	laurel leaves	146	sour milk

List 2. Odor descriptors for Dravnieks data.