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# TOWARD A PHARMACOLOGY OF ODOR RECEPTORS AND THE PROCESSING OF ODOR IMAGES

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**Summary**—Odor molecules may be considered as molecular ligands which bind to receptors in the olfactory sensory neurons to give rise to the sensory response. Binding studies in whole sensory epithelia suggest that the receptors also bind muscarinic cholinergic antagonists. Preliminary electrophysiological evidence indicates that muscarinic and beta adrenergic antagonists block odor-elicited membrane currents in single isolated salamander sensory neurons. These results support the idea that models developed for analyzing ligand binding by members of the 7 transmembrane domain family of membrane receptors may apply rather closely to olfactory transduction. We suggest that sensory neurons express single receptor types with differing degrees of affinity for different ligands. We further suggest that glomeruli in the olfactory bulb function as labeled lines for particular sets of odor ligand determinants, and that interglomerular circuits bind together similar glomeruli and enhance contrast between dissimilar glomeruli. The odor image laid down in the sensory neuron population is thus subjected to abstracting and enhancement at the glomerular stage, prior to being transmitted for further processing in the deeper layers of the olfactory bulb and in the olfactory cortex.

## INTRODUCTION

A general framework for the organization of vertebrate olfactory circuits has been available for some time [cf. 1–4], but an understanding of the functional operations of these circuits has been limited by lack of knowledge about the form of the sensory information transmitted from the olfactory receptor neurons. In view of the fact that odor stimuli do not convey information about external space, neural space in the olfactory pathway is available for processing information contained in the stimulating molecules. Evidence that odor stimulation elicits spatiotemporal gradients of mitral cell activity in the olfactory bulb [5] gave rise to the idea that the brain constructs "odor images" [cf. 6, 7]. The 2-deoxyglucose mapping technique showed further that spatial activity patterns at the level of the glomerular layer are extremely detailed and complex [cf. 8]. This has given rise to the more precise postulate that neural space is used to map molecular properties as expressed in the binding of ligands to olfactory receptors, and that these maps of molecular properties in effect constitute "molecular images" in the neural domain [8, 9].

The mechanisms of ligand binding to olfactory receptors are obviously critical to an understanding of molecular images and their processing in the olfactory pathway. In this paper we focus on the potential of pharmacological methods for elucidating the nature of transduction of odor molecules in vertebrates. We first describe preliminary studies involving binding studies and electrophysiological analysis. We then indicate how computer-generated molecular models may be useful. We compare these results with recent evidence from the cloning of a candidate family of olfactory receptor proteins. Finally, we discuss the mechanisms by which the transduced molecular information is subjected to processing by central olfactory pathways. Where appropriate, we cite evidence regarding mechanisms relating to pheromones as well as to more general types of odor molecules.

## OVERVIEW OF STUDIES ESTABLISHING THE ODOR TRANSDUCTION PATHWAY

Our present understanding of vertebrate olfactory transduction owes much to the pioneering studies of Lancet and his colleagues. Building on previous suggestive evidence, these

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workers showed that cilia-enriched membrane preparations of frog olfactory epithelium have very high levels of adenylate cyclase activity [10]. They demonstrated that this activity satisfies the usual pharmacological criteria for G-protein mediation, and that exposure to odor compounds elicits a GTP-dependent enhancement of the activity in a dose-dependent manner. By analogy with photoreception, it was predicted [cf. 11] that the cAMP produced by the adenylate cyclase activity might act directly on a channel protein, and this was borne out by the critical study of Nakamura and Gold [12], who showed that both cAMP and cGMP directly modulate a membrane conductance in patches pulled from the cilia or dendrite of freshly dissociated bullfrog receptor neurons. Evidence that the cyclic nucleotide-gated channel is indeed identical to the channel that mediates the odor-induced current in the sensory membrane has been presented recently [13–15].

Based on the early evidence of a cAMP-mediated transduction pathway, and in parallel with corresponding studies of the equivalent elements in other tissues, the molecular characterization of the entire cascade has been obtained within the space of only a couple of years. This includes the Gs protein specific for olfactory tissue (Golf [16]), the adenylate cyclase [17] and the cyclic nucleotide-gated channel [18, 19]. Very recently, the cloning and sequencing of genes encoding putative olfactory receptor proteins has been reported [20]. In addition, ancillary proteins have been characterized, including odor-binding protein (OBP [21]) and the cytochrome enzyme *P*450 system [22–24]. Here we wish to focus only on those aspects of these studies that bear directly on the problem of specificity of the odor receptor molecules for their odor ligands.

## PHARMACOLOGICAL BINDING STUDIES

For purposes of pharmacological analysis, we take as a working hypothesis that olfactory reception shares properties with membrane receptor mechanisms at neuronal synapses [cf. 25, 26]. The general model for the synapse involves the binding of a ligand to a receptor, the ligand being the particular species of neurotransmitter molecule, and the receptor being an integral membrane protein linked directly or indirectly to a membrane conductance channel. By analogy, we assume that an odor molecule functions as a ligand to activate an integral membrane receptor protein located in the sensory region of the olfactory receptor neuron, though we do not rule out other actions of odor molecules as well. Dodd and Persaud [25] were among the first to characterize an "odorant" as a "ligand" [cf. 27, 28]. We therefore refer to the odor molecule as an "odor ligand", or simply "ligand".

Beginning in the late 1970s, several authors began to carry out biochemical and pharmacological studies of homogenates of the olfactory epithelium in order to identify fractions that bind labeled ligands. Of particular interest to us was the study of Hirsch and Margolis [29], who screened a number of radiolabeled drugs known to bind to different types of neurotransmitter receptors at brain synapses. It was found that homogenates of mouse olfactory epithelium bind: [$^3$H]quinuclidinyl benzilate (QNB), a competitive antagonist at cholinergic muscarinic receptors; [$^3$H]clonidine, WB-4101 and dihydroergocryptine, all $\alpha$-adrenergic antagonists; and [$^3$H]dihydroalprenolol, a $\beta$-adrenergic antagonist. The binding characteristics in each case were similar to those obtained from brain membrane fractions. Olfactory bulb ablation experiments suggested that the QNB binding was associated with olfactory neurons, not the supporting cells, in the epithelium.

We confirmed that the basic findings of Hirsch and Margolis regarding QNB binding apply to the salamander as well. We found further that QNB binds to a protein in the epithelium with a $K_d$ of 0.8 nM, and that this protein has the pharmacological profile of a cholinergic muscarinic receptor [30]. By themselves, these results could imply that acetylcholine modulates odor reception, or modulates some other function of the receptor neuron.

We addressed the question of whether the odor receptor is specifically related to the muscarinic receptor by testing the ability of odor stimulation to compete with the binding of the muscarinic antagonist [31; B. Hedlund, E. Perdahl, J. R. Cooper and G. M. Shepherd, unpublished observations]. The experiments were carried out on cilia-enriched fractions of salamander olfactory epithelium. A typical result is illustrated in Fig. 1. As can be seen, over a concentration range 0.01–0.1 mM, the odorous substance camphor caused a reduction in the specific binding of [$^3$H]QNB, suggesting a close relation between muscarinic receptor binding and odor receptor binding. Tests of several other odor ligands (amyl acetate, butanol,

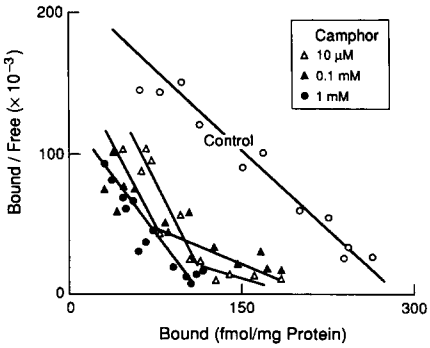


Fig. 1. Scatchard plot of the binding of [$^3$H]QNB, a muscarinic antagonist, in the salamander olfactory epithelium, in the absence and presence of 0.01, 0.1 or 1 mM camphor. Data from 1 representative experiment of 3 are shown. The epithelium was subfractionated by transferring 15 epithelia to 10 ml of a deciliation medium (0.1 NaCl, 2 mM EDTA, 30 mM Tris, 10% ethanol, pH = 8.0), to which $CaCl_2$ was added to a final concentration of 10 mM. After stirring for 5 min at room temperature and centrifugation at 1000 *g* for 5 min the supernatant obtained was diluted 10 to 15-fold in Krebs–Ringer buffer (104.4 mM NaCl, 1.8 mM KCl, 3.6 mM $CaCl_2$, 0.7 mM $MgCl_2$, 5 mM Hepes, 1 g/l glucose, pH = 7.4) and used without further treatment. Approx. 0.15 mg protein was added to each tube and was incubated with [$^3$H]QNB and appropriate drugs for 60 min at room temperature. The highest concentration of [$^3$H]QNB used was 5 nM. Nonspecific binding was determined in the presence of 10 $\mu$M atropine. The reaction was stopped by addition of 10 ml ice-cold normal saline and filtration through Whatman GF/F filters. The radioactivity on the filters was measured in a Beckmann liquid scintillation counter at an efficiency of 30%. (B. Hedlund, E. Perdahl, J. R. Cooper and G. M. Shepherd, unpublished observations.)

dimethyl disulfide) showed similar results, with inhibition constants, $K_i$, in the range of 4–9 $\mu$M. By contrast, two non-odorous substances, quinoline and sucrose, had no effect on [$^3$H]QNB binding. The results were specific for olfactory epithelium, because the odorous substances had no effect on [$^3$H]QNB binding to homogenates of salamander brain tissue. Phospholipase treatment had no effect on inhibition at lower amyl acetate concentrations, indicating that at these concentrations the interaction was mainly with membrane proteins and not with the lipid moiety of the membrane.

These early results indicated some of the potential of pharmacological binding studies for characterizing the nature of the odor receptor, but they also showed some of its limitations as well. Pace and Lancet [28] summarized these problems as follows:

"1. Olfactory receptors most probably have low equilibrium binding constants and high dissociation rate constants, conditions which hinder the application of most receptor binding techniques." [32, 33]

"2. Odorants are lipophilic and they easily bind to nonreceptor proteins and to the lipids of the membrane. This may result in a high degree of nonspecific binding."

"3. Olfactory receptors are ... likely to be heterogeneous: a biochemical preparation from entire epithelia will contain many receptor types. Odorants may bind to more than one receptor type, leading to complex saturation curves."

The relatively high dissociation rate constants, high ligand concentrations, complex inhibition curves, and effects of [$^3$H]QNB binding on all odor ligands tested, indicated that these limitations applied to the interpretation of our experiments. Although the preliminary results were promising in suggesting a close relation between the odor receptor and one member of the neurotransmitter receptor family, we abandoned further experiments of this type pending more precise information about the molecular nature of the odor receptor.

With regard to pheromones, mention here will be limited to experiments carried out by Persaud *et al.* [34] on binding of 5$\alpha$-androstan-3-one to olfactory epithelial membranes. These studies gave evidence of saturable binding to a supernatant fraction from sheep olfactory epithelium with an affinity constant of approx. 10 nM. The large amount of nonspecific binding, due to the hydrophobicity of the pheromone molecule, limited further analysis of binding activity. The elution profile from gel chromatography showed radioactively labeled pheromone bound to a sharp peak at 148 kDa, suggesting a relatively high affinity site, and a low broad peak at 40 kD, suggesting lower affinity sites with fast dissociation rates. Competitive binding studies gave evidence that several compounds with similar molecular shapes and similar "urinous" odors could displace 5$\alpha$-androstan-3-one, whereas a related compound with no odor showed only weak displacement.

## ELECTROPHYSIOLOGICAL STUDIES OF ODOR RESPONSES AFFECTED BY NEUROTRANSMITTER ANTAGONISTS

An inherent limitation of binding studies is that they assess receptor binding of ligands in the steady state, and therefore have uncertain applicability to natural events taking place over brief periods of time. Electrophysiological analysis of the physiological odor response in real time combined with pharmacological

manipulations is therefore the method of choice. We have used these methods to obtain evidence for each of the steps in the cAMP second messenger cascade [13–15]. Recently, we have broadened these studies to the receptor mechanisms.

Our point of departure has been the fact that in all second messenger systems involving GTP-binding proteins, the GTP-binding protein is coupled to a receptor that belongs to the family of 7 transmembrane domain (7TD) proteins, which includes rhodopsin and a number of neurotransmitter receptors defined by their specific ligand (cholinergic muscarinic, $\beta$-adrenergic, dopaminergic, serotonergic; see Ross [35] for a review). We have therefore hypothesized that the odor receptor might share sufficient homology with the other members of this family that some neurotransmitter antagonists might also affect odor receptors. In view of our previous evidence from binding studies (see above), we first tested the muscarinic antagonists QNB, atropine and scopolamine, and found that they reduced odor-elicited membrane currents in voltage-clamped salamander olfactory neurons by up to 50% [36; S. Firestein and G. M. Shepherd, in preparation]. The inhibitory $EC_{50}$ was in the range 70–100 $\mu$M. We then tested the $\beta$-adrenergic blocker alprenolol, and found that it was somewhat more effective, with an $EC_{50}$ of approx. 20 $\mu$M (see Fig. 2). Although the concentrations of these antagonists were relatively high, no nonspecific effects on the membrane properties of these cells were observed.

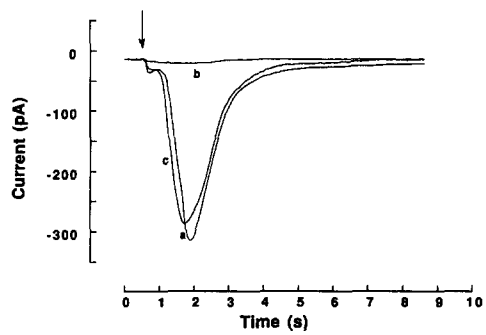


Fig. 2. Alprenolol, a $\beta$-adrenergic antagonist, blocks the odor-induced membrane current in an isolated salamander olfactory neuron under whole cell voltage clamp. A 50 ms odor pulse of amyl acetate was delivered at the arrow and (trace a) elicited a current response of >300 pA. Alprenolol (100 $\mu$M) was perfused into the bath, and almost totally blocked the odor current (trace b). This effect was almost completely reversed by returning to normal Ringer (trace c). The holding potential was −55 mV. (S. Firestein and G. M. Shepherd, unpublished observations.)

These preliminary results have several interesting implications. First, they give added evidence that the odor receptor belongs to the family of seven transmembrane domain, G-protein coupled, receptors. Second, they indicate that the shared properties are not limited to a single member of that family, but are shared more broadly with that family. Third, since there are no known odor antagonists, neurotransmitter antagonists may have a role to play in analyzing odor receptor mechanisms and perhaps in classifying odor receptor families. Finally, to the extent that they are consistent with the results on muscarinic antagonist binding, the results lend some credence to the binding studies as at least an adjunct in obtaining clues to olfactory receptor mechanisms.

## MOLECULAR MODELS RELEVANT TO ODOR LIGAND–ODOR RECEPTOR INTERACTIONS

An ultimate aim of studies of odor transduction is to provide a complete description of the molecular properties of the receptor that determine the specificity of binding to different types of odor ligands. As in other ligand–receptor systems, we anticipate that molecular models will have an important role to play in correlating this information.

Of particular interest in this regard are the studies of Strader and her colleagues of a model for ligand binding by the $\beta$-adrenergic receptor ($\beta$AR) [37, 38]. As already mentioned, the $\beta$AR belongs to the 7TD, G-protein linked, receptor family. Using oligonucleotide-directed mutagenesis, regions of the $\beta$AR gene were deleted, the mutated genes expressed, and the ligand binding properties of the gene products analyzed. These and other experiments have shown that the regions critical for ligand binding are located within the hydrophobic domain of the receptor protein, corresponding in this respect with the position of retinal within rhodopsin, another member of the 7TD family. An overview of the tertiary structure of the $\beta$AR, and the position of a $\beta$-adrenergic ligand, carazolol, within the core "pocket" formed by the transmembrane segments, is shown in Fig. 3.

For higher resolution analysis, effects of single amino acid substitutions on ligand binding were examined. These studies pointed to the model illustrated in Fig. 4. for the binding of the $\beta$-adrenergic agonist isoproterenol. In this model, specific binding of isoproterenol was particularly dependent on interactions with

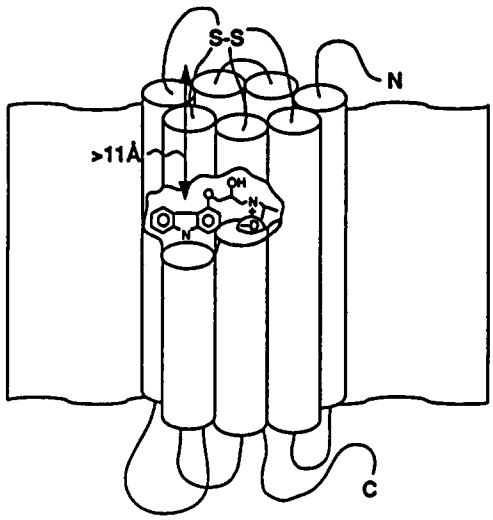


Fig. 3. A model for the $\beta$AR. Studies employing fluorescence spectroscopy have indicated that the $\beta$-adrenergic antagonist carazolol binds deeply within the hydrophobic pocket within the plane of the plasma membrane formed by the 7TD segments of the receptor protein. The approximate position of the residue Asp 113 in trasmembrane helix III is indicated by the letter D. From Ref. [38].

aspartate residue 113 in segment III, two serine residues, 204 and 207, on successive turns of the alpha helix of segment V, and the phenylalanine residue 290 in segment VI. There was a further suggestion that aspartate residue 113 is involved in both agonist and antagonist binding. The isoproterenol molecule interacts with these residues in a position within the plane of the membrane similar to that indicated in Fig. 3.

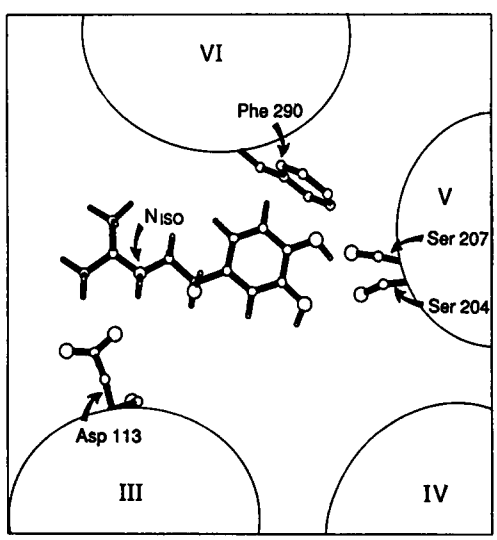


Fig. 4. A model for the ligand binding site of the $\beta$AR. Four transmembrane segments (III–VI) are shown viewed from the extracellular side of the membrane. A molecule of the $\beta$-adrenergic agonist isoproterenol is shown within the pocket formed by these segments; it interacts with specific residues as indicated. From Ref. [37].

It appears that the specificity of this model is adequate to account for the kind of selectivity that must be required for odor receptor molecules to distinguish between the determinants of odor ligands [cf. 9]. Assessment of this hypothesis requires detailed consideration of both odor receptors and odor ligands. With regard to the receptors, Lancet [33] and Lancet and Pace [26] after noting the similarities with neurotransmitter receptors, made several remarkable postulates: that there are "different odorant binding sites in a 'variable region' of the molecule"; that "olfactory receptor molecules constitute a repertoire of many different receptor types, each with the usual narrow agonist range"; that "olfactory receptor proteins constitute products of a multigene family"; and that "the upper limit for the olfactory (gene) repertoire may be $10^2$–$10^4$". The recent landmark study of Buck and Axel [20] provides dramatic confirmation of all of these predictions. They report that the odor receptor is a member of the superfamily of 7TD proteins; that it shares extensive sequence homology with other members of that family; that it also shows marked sequence diversity within transmembrane segments III, IV and V; and that this diversity indicates a gene family estimated to be at least 100 and possibly up to 1000–2000. Buck and Axel [20] suggest that the blocks of non-conserved residues "could reflect the sites of direct contact with odorous ligands"; each member of the receptor family may recognize "only one or a small number of odorants", which could provide "a plausible mechanism to accommodate the diversity of odor perception".

With regard to the odor ligands, a first question is whether there is sufficient similarity between them and neurotransmitter ligands to support the possibility that odor ligands interact with their receptors in the manner depicted by Strader *et al.* [37, 38] for neurotransmitter binding. In order to assess this question, Fig. 5 provides a simple comparison between a set of commonly used odor ligands and the set of neurotransmitter ligands that bind to 7TD proteins. It is immediately evident that both sets consist of relatively small molecules that have a general similarity in size and the presence of a partial or complete ring structure with short lengths of carbon chains attached. It is not difficult to imagine, for example, that any of these odor ligands could fit rather closely in the environment of residues within the transmembrane pocket of helices pictured in Fig. 4. This

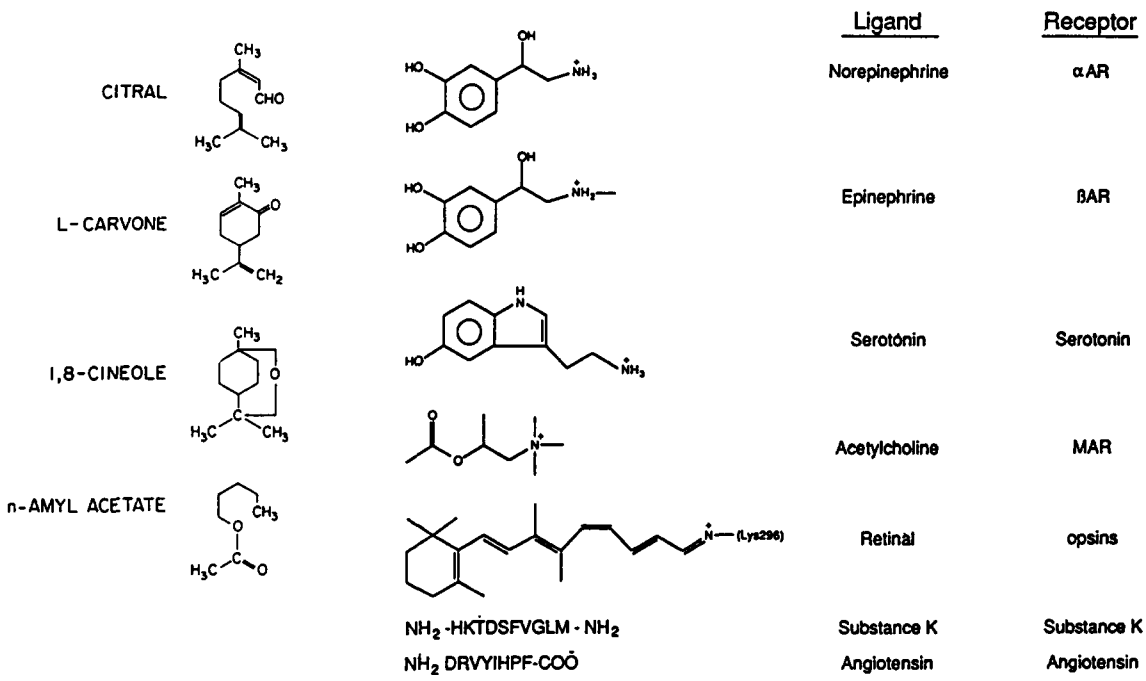


Fig. 5. Comparison of the molecular structures of some typical odor ligands (left) with the neutrotransmitters and neuropeptides (right) that act at specific members of the family of the 7TD, G-protein linked, receptors. Left, from Ref. [28]; right, from Ref. [37].

direct comparison of neurotransmitter and odor ligands suggests rather strongly that the specificity of the 7TD receptor for its ligands provides a model of selectivity that is quite adequate for distinguishing between the determinants of odor ligands [cf. 9].

With regard to pheromones, these molecules are mostly medium size. For example, bombykol (a sex attractant of moths) is a 16-carbon chain, and 5α-androstan-3-one (a sex pheromone of boar) is a 20-carbon steroid molecule. These molecules also have their counterparts in size among ligands for other members of the 7TD family. Retinal, for example, is a 21-carbon molecule, which fits within the hydrophobic pocket of rhodopsin. Peptides interacting with 7TD proteins, such as substance K and angiotensin, are of intermediate size (see Fig. 5). These comparisons suggest that pheromone molecules are not precluded on the basis of size from interacting with amino acid residues within the hydrophobic pocket of 7TD odor receptors, although of course one does not exclude the possibility that they, like other odor ligands, may also act at other sites on the molecule, or on other types of receptor molecules.

An interesting possibility to consider for the receptor for mammalian steroid pheromones is suggested by recent studies indicating that the rat type I phosphoinositol-specific phospholipase C isozyme contains a 44 residue segment that is similar to an estrogen-binding region of the estrogen receptor [cf. 39, 40]. Although the estrogen receptor shows little recognition of androgen steroids (the group into which many mammalian pheromones fall), estrogen and androgen molecules are similar enough to raise the possibility that phospholipase C could function as a pheromone receptor in mammals.

## CENTRAL MECHANISMS FOR PROCESSING ODOR IMAGES

From these considerations, it may be suggested that the irreducible "primitives" of the olfactory sense are the individual "determinants", such as chain length, charged groups, and other properties that determine the interaction of an odor ligand with its receptor site(s) on the odor receptor molecule. A given odor ligand is discriminated on the basis of its set of determinants which, though overlapping with the sets of other ligands, is uniquely different from the others.

How is the detection of a given set of molecular determinants signalled to the central nervous system? Several authors have discussed the organization of connections relevant to this question [1–3, 41]. Here, we will focus on the

results of application of the 2-deoxyglucose (2DG) mapping technique to the olfactory bulb. Exposure of an awake behaving rat to a given odor, such as amyl acetate, elicits a distinct pattern of uptake on the glomerular layer of the bulb (see Fig. 6). The pattern is complex: there are intense punctate foci localized to some glomeruli, broader glomerular regions of moderate 2DG uptake, and glomeruli showing very low, perhaps suppressed, levels of uptake [42–45]. These results have been confirmed and extended in several important ways: by careful mapping of foci over the bulbar surface for different odor ligands [46–48]; the finding of foci over identifiable glomerular groups ([49]; see Fig. 6); the induction of specific glomeruli related to behavioral plasticity ([50]; see Fig. 6); the finding of foci in response to putative mammalian pheromones [44]; and the consistency of patterns during development [47, 51] and across species [52]. Recently, a new type of probe has become available with the development of hybridization to c-fos mRNA [53]. Preliminary results show a close and complementary correspondence between c-fos and 2DG foci.

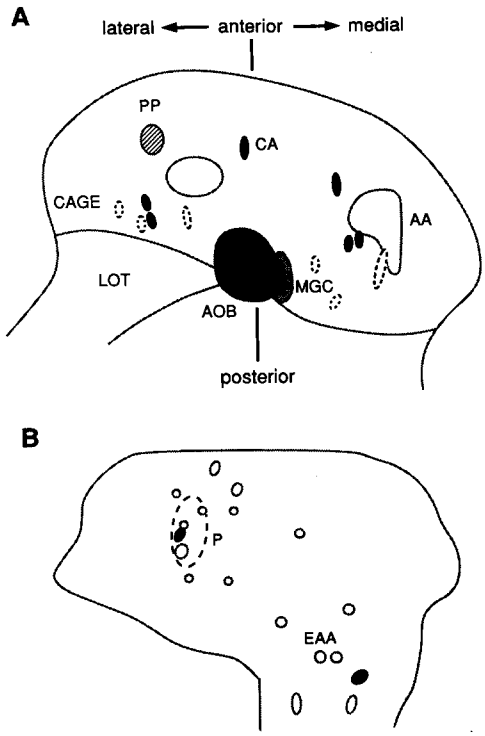


Fig. 6. Comparison of patterns of dense 2DG foci in the glomerular layer of the rat olfactory bulb elicited by olfactory stimulation with different odor ligands. Results of typical individual experiments are summarized by superimposing outlines of dense 2DG foci on reference maps. A: open profiles—amyl acetate [45]; filled profiles—camphor [45]; dashed profiles—cage air [45]; hatched (lines) profiles—peppermint, in the learning paradigm of Wu *et al.* [50]; shaded (dots) profiles—modified glomerulus activity in suckling rat pups [49]. B: open profiles—moderate intensity foci for ethyl ethanoacetate [47]; filled profiles—same experiment showing intense foci for ethyl ethanoacetate; dashed profile—propionic acid [48]. For most odor ligands the sites of foci vary somewhat in different animals, defining an overall "domain" for that ligand; for peppermint and suckling, however, the foci are associated with specific glomeruli identifiable from animal to animal. The foci shown are for the strongest odor concentrations; with weaker concentrations, the foci are, in general, less extensive. In addition to these foci, the odor ligands elicit complex patterns of lower peaks and valleys of activity through most of the glomerular layer, indicating parallel processing through distributed glomeruli.

Several properties of these patterns are critical. First, using the domains of highest uptake as a shorthand way of representing the entire pattern, the domain for a given odor is reproducible from animal to animal, and characteristically overlaps those for other odors, but is distinct from them. This finding suggests that a particular set of activated glomeruli represents the particular set of determinants characteristic of a given odor ligand. This set of glomeruli thus represents the molecule in neural space, and constitutes at the first synaptic relay in the olfactory pathway an abstracted "molecular image", equivalent to a visual image at the earliest stages of visual processing.

Second, the extent of activity varies with odor concentration; at low levels (near threshold for perception by the experimenter) there are only a few foci scattered within a domain; as concentration increases, the number of foci increases and regions of increased 2DG uptake expand to define the entire "domain" for that odor. This finding indicates that the effect of increasing odor concentration is not limited to causing a given set of receptor cells to fire impulses more intensely, as indeed they do, but that there is also recruitment of additional cells, presumably with higher thresholds to (i.e. lower affinity for) that ligand, with a wider distribution in the sensory epithelium.

Third, at high resolution, it is possible to see that neighboring glomeruli often differ distinctly in their intensity of 2DG labeling, but, for a given glomerulus, the level is relatively uniform throughout the glomerular interior. This finding has provided direct experimental evidence for the idea of the glomerulus as a "functional unit" in the processing of odor information. Since multiple widely distributed foci are the general rule for activation by a given odor, these results also provide direct evidence for parallel processing of odor information. To the extent that each "functional unit" is specific for a given set of

inputs from physiologically-defined receptor cells, a glomerulus may be considered as a "labeled line" for transmitting and processing that information. One therefore has the idea that a glomerulus may be labeled for a given set of odor ligand determinants; that there are multiple parallel labeled lines; and that glomeruli may share labels for ligands with overlapping sets of determinants.

We have described thus far the broad pattern of activity associated with a given odor ligand. Within this pattern are several levels of organization that involve synaptic interconnections between particular sets of mitral, tufted and periglomerular cell dendrites inside a glomerulus. These "microcircuits" apparently have several functions. If a glomerulus is a convergence site for receptor cells with shared ligand specificities, the *intra*glomerular microcircuits may function to spread the excitatory input in order to mediate the "unitary" function of the glomerulus in processing this input. In addition, intraglomerular inhibitory interactions may serve to sharpen the discrimination of particular inputs (such as those representing closely similar sets of determinants) that are closely similar. By contrast, *inter*glomerular excitatory interactions may serve to bind together the activity of glomeruli receiving similar inputs, whereas inhibitory interactions may serve to sharpen contrast between dissimilar inputs.

## CONCLUDING REMARKS

The evidence that odor molecules function as ligands for receptor proteins places the problem of olfactory transduction firmly within the mainstream of current research on membrane signaling mechanisms. This in turn implies that the range of techniques available for analysis of ligand–receptor interactions is appropriate and should have a high priority for application to the problem of olfactory reception. For this work, pharmacological methods can play an important role. The work reviewed here suggests that traditional neurotransmitter antagonists may be useful tools in at least characterizing families of olfactory receptors. It is not impossible to envisage that this could lead to a pharmacological classification of olfactory receptor types and subtypes similar to that for classical neurotransmitters and neuromodulators. This work may also give clues to the nature of ligand-binding domains and structures, through the use of computer modeling techniques, as indicated above. The results from analysis of receptor mechanisms must then be correlated with results from pharmacological analysis of the steps in the second messenger pathways linking the receptors to the ion channels generating the sensory receptor potential and impulse discharge. Here one has a real advantage in the olfactory system in being able to move from traditional ligand-binding methods to analysis of physiological responses of identified neurons in real time.

The complex, sharply defined activation patterns of glomeruli indicate that the glomeruli are critical for assembling the information that needs to be abstracted in order to identify the odor ligands. Glomeruli are constant features of the olfactory pathways of most invertebrates and vertebrate species, implying that grouping or "modularization" of connections of receptor cells into "functional units" at the first level of synaptic integration is essential to this process. The distinctiveness of the patterns of glomerular activity gives hope that application of pharmacological agents that selectively block specific types of ligand–receptor interactions or specific steps in the second messenger pathways can produce specific and detectable effects at the glomerular level. It seems a useful working hypothesis that odor receptor types and subtypes, identified pharmacologically at the receptor level, may be reflected in patterns of activation of "functional units" at the glomerular level. Tests of this hypothesis should provide a clearer idea of the construction of molecular images in neural space. They should also provide a better understanding of the nature of the output from the olfactory bulb to higher levels of odor processing underlying perception and the control of olfactory behavior.

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