

COMMENTARY

Interactions of Odorants with Olfactory Receptors and Other Preprocessing Mechanisms: How Complex and Difficult to Predict?

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Abstract

In this issue of *Chemical Senses*, Münch et al. present a thorough analysis of how mixtures of odorants interact with olfactory receptors (ORs) borne by olfactory receptor neurons (ORNs). Using fruit fly ORNs expressing the receptor OR22a, they provide a clear example of mixture interaction and confirm that the response of an ORN to a binary mixture can be sometimes predicted quantitatively knowing the ORN responses to its components as shown previously in rat ORNs. The prediction is based on a nonlinear model that assumes a classical 2-step activation of the OR and competition of the 2 odorants in the mixture for the same binding site. Can this success be generalized to all odorant–receptor pairs? This would be an encouraging perspective, especially for the fragrance and flavor industries, as it would permit the prediction of all mixtures. To address this question, I outline its conceptual framework and discuss the variety of mixture interactions found so far. In accordance with the effects described in the study of other receptors, several kinds of mixture interactions have been found that are not easily predictable. The relative importance of the predictable and less predictable effects thus appears as a major issue for future developments.

Key words: allostery, insect olfaction, mixture interaction, olfactory sensilla, pharmacology, syntopy

Many ideas are deeply attractive because they seem obvious, or simple or merely widely accepted. Such deeply rooted ideas, infrequently made fully explicit and thus rarely questioned, act as powerful obstacles that bar the way to clarification of concepts and slow down the pace of research and discovery. A case in point in the field of olfaction is the idea that olfactory receptor neurons (ORNs) encode passively their stimuli so that the central nervous circuits alone are endowed with the special property of processing sensory information. Another is that the natural reference to which to compare the response of an ORN to a mixture of 2 or more odorants must be, one way or other, the sum of its responses to the odorants applied separately. In recent years, several converging studies gave evidence that these preconceived ideas are wrong. In this issue of *Chemical Senses*, Münch et al. (2012) present a well-designed set of experiments based on calcium imaging of ORNs in *Drosophila melanogaster* that provides a timely confirmation that the peripheral olfactory

system must not be considered as a passive linear encoder but as a complex system with preprocessing and nonlinear properties. This complexity arises in large part from the deep connections of olfaction with pharmacology and a full understanding of these experiments requires some familiarity with this discipline. Pharmacology is not so much the science of drugs and medicines as the science of receptors in general (Kenakin 2004; Rang 2006), of which olfactory receptors (ORs) are only an example among many others.

Single odorants

The whole edifice of olfactory perception is based on an initial event that is a weak and reversible interaction of odorant molecules to ORs located at the membrane surface of ORNs. This interaction can be analyzed as a sequence of 2 reversible reactions: first the binding of the odorant to the OR (and its release from it) and then the activation (and

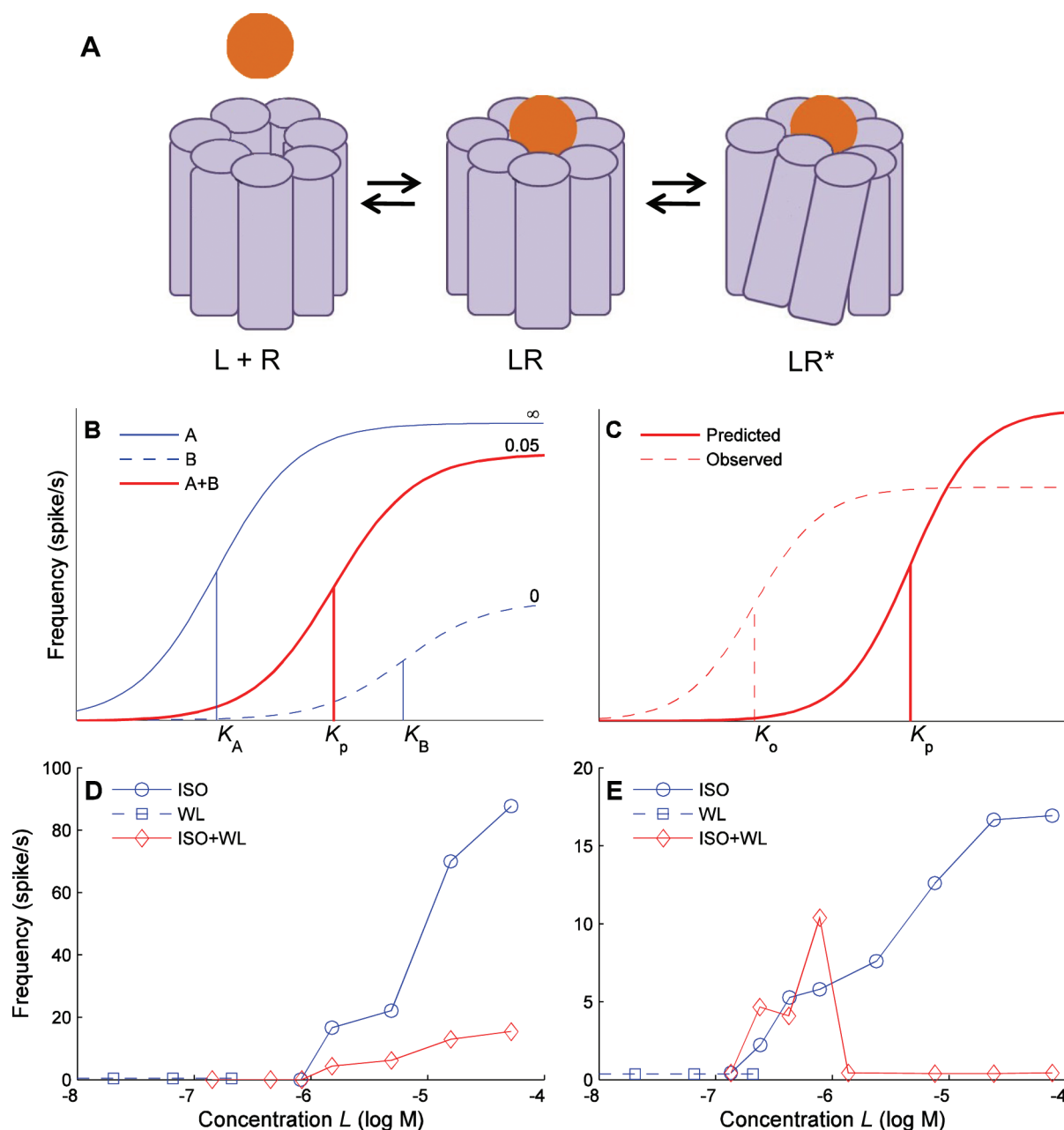


Figure 1 Variety of odor–receptor interactions. **(A)** The 2 reversible reactions model of odorant–OR interaction can predict neuron responses to single odorants A and B and in some cases to their mixture A + B. **(B)** Predictable responses for a constant ratio of concentrations A/B in the syntopic model: the mixture curve (here with $A/B = 0.05$) is intermediate between the curves for A ($A/B = \infty$) and B ($A/B = 0$). The apparent affinity (K_p) and maximum response (R_{MP}) for A + B can be calculated knowing the corresponding properties for A (K_A , R_{MA}) and B (K_B , R_{MB}). **(C)** Example of unpredictable response: the observed mixture curve is shifted both horizontally (amplification by increase of affinity) and vertically (suppression by decrease of efficiency) with respect to the curve predicted by the syntopic model used as reference (Rospars et al. 2008). This type of response is compatible with allosteric interactions. **(D)** Example of inhibition of isoamyl acetate (ISO, agonist) by whiskey lactone (WL, antagonist) (from Duchamp-Viret and Rospars 2012). **(E)** Example of complex action with synergy at low concentration ($<10^{-6}$ M) and antagonism at higher concentration (from Chaput et al. 2012).

deactivation) of the OR ascribable to a change of its conformation (Figure 1A). In pharmacological terms, an odorant is a ligand because it can bind to the OR and an agonist because it can activate it. This model with binding followed by activation was first applied by Del Castillo and Katz (1957) to the acetylcholine receptors of muscles. Although

subsequent research showed that many more reversible steps have to be considered to account for experimental results, 2 steps are sufficient to explain the most obvious ones. The usefulness of this model stems from the possibility to calculate the number of activated ORs (denoted LR^* or, for the sake of brevity, R^*) as a function of odorant concentration

(denoted A). Mathematical analysis of the model based on elementary chemical kinetics (e.g., Kaissling 1987; Rospars et al. 1996) reveals 3 remarkable properties. First, R^* follows a hyperbolic curve when A increases or, equivalently, a sigmoid logistic curve of $\log A$ (Figure 1B, blue curves). Second, the position of the curve along the concentration axis reflects the affinity of the odorant for the OR and depends only on the rate constants of the 4 reversible reactions (binding/release and activation/deactivation), not of the total number of receptors R_T . Third, the maximum number of activated receptors at high odorant concentration depends on R_T and the rate constants of the activation–deactivation reaction (but not of the binding–release reaction).

The conformational change triggers a cascade of events ultimately resulting in the measurable ORN responses: calcium entry, membrane depolarization, or firing of action potentials, depending on the technique utilized. It can be shown experimentally that the same ORN (and therefore OR) stimulated with 2 different odorants will respond with sigmoid curves that differ by their positions along the concentration axis and by their maximum responses. In pharmacological terms, odorants have different apparent affinities and different efficiencies (maximum responses). The 2-reaction model accounts well for these 2 facts. The main difference between the OR activation curve and the ORN response curve is their slopes: it can be shown empirically that the latter curve can be derived from the former by adding another parameter, the so-called Hill coefficient n that transforms the logistic curve for R^* (with $n = 1$) into a Hill function ($n \neq 1$). This change of slope is the only easily visible effect of the transduction cascade interposed between the OR and the measured response, as the other changes are not directly visible (they increase both the sensitivity and the power of the response by moving the neuron response curve to the left of the OR curve and by increasing the number of molecules participating in the response; Rospars et al. 1996, 2003; Gu et al. 2009).

Odorant mixtures: syntopic model

What happens when a mixture of 2 odorants is applied to the ORN preparation? The question is biologically relevant because almost all natural odors are mixtures of several odorants in specific proportions. Therefore, it is not appropriate to use classical protocols in which the concentration of a single component is varied but that of the other is kept constant, as this would change the quality of the odor. Keeping the quality of the odor the same while changing its concentration can be achieved only if the ratio of concentrations of the 2 odorants remains constant. Using this assumption, the number of receptors activated by any concentration of the mixture can be deduced from the 2-reaction model above knowing the affinity and the efficiency of its components applied alone, at least in the simple case where the 2 odorants compete for the same binding site; for this reason, it is called as syntopic

(Neubig et al. 2003). Then it can be shown that the number of ORs activated by the mixture also follows a logistic curve as a function of concentration $A + B$ and that it is always intermediate between the numbers R_A activated by odorant A at concentration A and R_B activated by odorant B at concentration B (Rospars et al. 2008). R_{AB} may be closer to R_A or to R_B depending on the affinities of A and B and the ratio A/B (Figure 1B, red curve). This curve can be considered as a reference because it is based on the simplest interaction (competition only) and the mere application of reaction kinetics with no added changes in the receptor–ligand system and no a priori reasoning. At least, chemistry gives no reason to expect that the OR response to the mixture could be linearly derived from the OR responses to its components.

Now, how does the transduction cascade modify the logistic equation for ORs? By analogy with the case of single odorants, the Hill coefficient n is introduced in the same way (note that this transformation is not a straightforward mathematical derivation but was done by analogy with the single odorant case). The theoretical solution obtained is logically consistent as it tends to the Hill equation describing the responses to single odorants, either A alone (if A/B is large) or B alone (if A/B is small, see Figure 1B). But is it also in agreement with experimentally recorded neuron responses to mixtures?

To check this prediction, the spiking responses of rat ORNs in vivo were recorded in response to odorants applied singly and in binary mixtures. The main condition to fulfill is that all 3 response curves to A, B, and A + B are complete, that is, with thresholds and maximum responses visible within the range of applied concentrations. It was found that about half of the complete mixture curves obtained were statistically undistinguishable from those predicted by the syntopic model based on the measured responses to their components given alone (Rospars et al. 2008). A more systematic approach has now been followed by Münch et al. (2012) taking full advantage of the possibilities offered by *D. melanogaster*. Instead of recording at random from single ORNs, the authors performed Ca^{2+} imaging of many ORNs expressing a single type of OR (OR22a) whose activation triggers Ca^{2+} increase that is revealed by a fluorescent calcium indicator. The main advantage of this preparation is that experiments can be repeated on the same OR in different animals (this is presently not possible with the rat). In the experiments where full dose–response curves were obtained for both the mixture and its components, Hill curves were found and the mixture curves were well predicted by the syntopic model. This is an elegant proof that the interaction of odorants with ORs can obey simple rules based on elementary reaction kinetics.

Beyond the syntopic model

Is it the end of the story? Not exactly. Other experimental results give evidence that the full series of classical

pharmacological effects are present in odorant–ORs interactions, like competition, antagonism, and allostery to name the most common.

The simplest of them is competitive antagonistic interaction. In this case, the antagonist odorant binds to the same site as the agonist but is unable to activate the receptor. Consequently, the response to the mixture agonist + antagonist is lower than the response to the agonist alone. Such effects have been described in heterologous systems (Oka et al. 2004; Sanz et al. 2005) and in vivo (Duchamp-Viret et al. 2003; Chaput et al. 2012, see Figure 1D).

Other effects may exist that can be collectively termed non-competitive interactions because they involve interactions between 2 different binding sites. Noncompetitive antagonism has been recently described in insects: the antagonist blocks the ion channel (coreceptor) associated with ORs (Jones et al. 2012), both forming an heteromeric complex.

Another example involving 2 binding sites is allostery. The main binding site permits OR activation by agonists as described above, whereas occupation of the second site does not permit activation but can modify the binding or activating properties of agonists at the main site. If odorants act on certain ORs as allosteric ligands, the curve predicted by the syntopic model will no longer be a good description of the response to the mixture, because the observed curve will be shifted with respect to the syntopic curve, along the concentration axis if affinity is modified, along the response axis if efficiency is modified (via a change of the activation reaction), or both (Figure 1C). In our experiments with rat ORNs, half of the mixture curves displayed such shifts, with the same number of affinity shifts to the left and to the right and of efficiency shifts upward and downward (Rospars et al. 2008). An important consequence here is that amplification, also called synergy, can result from at least 2 different mechanisms that must be carefully distinguished, depending on whether affinity (shift to the left) or efficiency (shift upward) or both are modified. The same is true for suppression, also called inhibition, which corresponds to the 2 other shifts. Therefore, an adequate description must specify whether amplification (or suppression) results from affinity or efficiency changes, and to this end full dose–response curves must be available. Although these phenomenological effects can be interpreted as the result of allosteric interactions, direct proofs are still missing. Interestingly, Münch et al. reported no examples of noncompetitive behavior in *Drosophila*. This could mean that no agonist odorant with this property was investigated or that the OR22a (or its transduction machinery) is immune to allosteric (or allosteric-like) effects.

This short list does not cover all mixture interactions known because other dose-dependent effects have been described. For example, in a systematic search for various ORs/ORNs response to 2 odorants, isoamyl acetate (ISO) and whiskey lactone (WL), in rats, Chaput et al. (2012) found a few ORNs not responding to WL alone but displaying a synergic effect at low concentration (the

response to ISO + WL is higher than to ISO alone) and an antagonistic effect at higher concentration (no response to ISO + WL and strong response to ISO alone; Figure 1E). This suggests that odorant–OR interactions can be complex, in full accordance with the teachings of present-day pharmacology.

Predictable and not so predictable effects

One of the major aims of science is to predict phenomena. In olfaction, this means inter alia to be able to predict the response of the population of ORNs to a mixture of odorants (in known proportions at any concentration) assuming that the responses to the individual odorants are known. Is this objective reachable? If all interactions with ORs were of the competitive syntopic kind, the answer would be positive. However, the available evidence is not so comforting as it suggests the existence of several other types of interaction that cannot be predicted from the knowledge of the responses to single odorants. To predict such mixture interactions, modelling would have to go 1 step further and include the prediction of interactions based on a detailed knowledge of the molecules involved, both ligands and receptors (Lai and Crasto 2012). This is a difficult problem that exceeds the scope of this commentary. A more immediately solvable problem is to know the relative importance of nonsyntopic interactions and whether they can be neglected in some practical applications.

Other effects make the prediction of response to mixtures difficult. Odorants could interfere with other molecules than ORs, in perireception (odorant binding proteins, enzymes) and postreception. Both excitatory and inhibitory signalling pathways have been reported in the same ORN (Ukhanov et al. 2011). Moreover, inhibitory interactions between adjacent ORNs bearing different ORs, inferred from the electrical circuit of insect olfactory sensilla and their known properties (Vermeulen and Rospars 2004), have been experimentally confirmed (Su et al. 2012) and may well apply also to the vertebrate olfactory epithelium. They provide another mechanism by which mixture interactions can take place, not on the same OR but on different ORs borne by adjacent neurons. This purely passive effect should be up to a point predictable. As a last word of caution, it must be kept in mind that all effects summarized above assume steady-state conditions and that ignoring time may be an unwarranted simplification in some cases.

Whether or not it proves predictable, the preprocessing of odorants by ORNs resulting from their competition for the same site, from binding to different sites of the same OR (e.g., in allosteric interaction and noncompetitive antagonism) or to ORs of different ORNs in electrical contact or other mechanisms still to be discovered, can no longer be ignored. It introduces a new level of complexity, rich of potentially interesting effects, in the pharmacological mapping of the

entire OR repertoire (Olender and Lancet 2012). At the present time, the relative importance and frequency of unpredictable (or difficult to predict) interactions can be only a matter of conjecture. It is possible that, as knowledge of pre-processing increases, simplifying features will appear leading to a better predictability than now envisioned, but this is not certain. Time will tell.

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