

Laying a controversial smell theory to rest

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In the 50 y before the cloning of the odorant receptors (ORs) by Linda Buck and Richard Axel in 1991 (1), two competing mechanisms for odor detection were discussed, one chemical and one spectral. The chemical theory posited that “detectors” respond to physicochemical attributes of the odorant, including molecular size, molecular shape, and functional groups (2, 3). The alternative spectral theory hypothesized that the olfactory system detects molecular vibrations of the odor molecules (4, 5). This vibration theory was revived in 1996, along with a proposed mechanism of inelastic electron tunneling spectroscopy activated by ligand–receptor interactions (6). These early theories of how ORs detect odorants were developed using psychophysical experiments, in which human volunteers sniffed odor molecules and described what they perceived. In PNAS, Block et al. (7) shift the “shape vs. vibration” debate from olfactory psychophysics to the biophysics of the ORs themselves. The authors mount a sophisticated multidisciplinary attack on the central tenets of the vibration theory using synthetic organic chemistry, heterologous expression of ORs, and theoretical considerations to find no evidence to support the vibration theory of smell.

What are the predictions of the vibration theory of smell and how would one test it? Proponents of the theory argue that the answer lies in chemical stimuli that are considered “identical” in structure but are fully deuterated analogs in which hydrogen atoms are exchanged with deuterium atoms. The thinking is that such molecules, known as isotopomers, would have identical molecular shapes and functionality but very different vibrational spectra. Sniff-testing of isotopomers has yielded mixed results. Although acetophenone and fully deuterated acetophenone were said in the original 1996 theoretical paper to be readily discriminable by humans (6), this observation was not reproduced in a later study (8). These same isotopomers were tested in insects and shown to be discriminable by flies (9) and honey bees (10). Gane et al. (11) returned to the acetophenone question in 2013 and now

confirmed that humans could not discriminate the acetophenone isotopomers after all, but offered new musk isotopomers that could be discriminated. Aside from being poorly reproducible, these psychophysical experiments are fraught with confounding variables. One cannot rule out that minute quantities of impurities in the isotopomers account for their discriminability or that the molecules are enzymatically transformed to novel odorants by nasal mucus long before they interact

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with their cognate OR, and subsequently yield a smell percept in the brain (12). Finally, it seems unlikely that fundamental subatomic mechanisms of OR ligand–receptor interactions can be inferred from human perceptual experiments alone.

No Evidence for Vibration-Sensing Odorant Receptors. Block et al. (7) solve these problems by taking the theory to where it should have been all along: at the interface between odorant molecules and the receptor. They performed clean chemical deuterations of the three major classes of isotopomers used in previous work, acetophenone, benzaldehyde, and musks, for a total of over 15 different stimulus pairs. Taking advantage of recent advances in functional expression and deorphanization of ORs, they systematically screened 330 human receptors to identify a single receptor, OR5AN1, strongly activated by cyclopentadecanone and related musks. In these assays, OR5AN1 responded equally to all musk isotopomers tested. They went on to test another nine ORs with known

ligand profiles and challenged them with many deuterated and nondeuterated isotopomer pairs. Again, no receptor was found that discriminates between isotopomers. The paper closes with a reexamination of the theoretical grounds supporting the vibration theory of olfaction (13), and finds them unrealistic in a biological milieu.

Precisely because this elegant and systematic study finds no evidence for the spectral theory, it suffers from the inevitable problem of being a series of persuasive negative results. Even having shown that the ORs profiled here are not the theoretical ones that can discriminate isotopomers, the authors cannot exclude the possibility that such an OR would eventually be found. Moreover, all of the work was carried out in tissue culture cells, not in native olfactory sensory neurons. Although such experiments are the gold standard for studying ligand–receptor interactions of all other G protein-coupled receptors, it is possible that heterologous expression does not reproduce the exact conditions in which the OR finds itself in the nasal epithelium. Perhaps the proposed inelastic electron tunneling mechanism fails to operate outside the nose. The ball is now in the court of the believers of the spectral theory, who need to produce experimental and not theoretical evidence that such problems are actually occurring in these experiments.

Why Should Odorant Receptors Be Exceptional? The paper by Block et al. (7) brings the field to an interesting juncture. Is this negative evidence sufficiently persuasive to lay the theory to rest, or should the screening of ORs and isotopomers continue until the absence of evidence passes the standard of reasonable doubt that would condemn the spectral theory? One interesting feature of the vibration theory is its exceptionalism. Vertebrate ORs are structurally and functionally related to the G protein-coupled receptor superfamily that senses hormones, neurotransmitters,

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tastants, and light (1, 14, 15), yet no one has proposed that these evolutionarily related proteins signal via vibrational mechanisms. Recent advances in membrane protein structural biology have enabled detailed high-resolution studies of non-OR G protein-coupled receptors (16). To date, no one has succeeded in solving the high-resolution crystal structure of an OR with its odor ligand. Once this technical barrier is broken, it will be fascinating to look into the insides of the ORs to see if there is any evidence of protein mechanisms that set the ORs apart from their close nonolfactory receptor relatives.

The Curious Persistence of a Radical Theory. The year 2016 marks the 25th anniversary of the cloning of the ORs (1) and the 20th anniversary of the revival of the vibration theory of olfaction (6). In the intervening years, the chemosensory field has largely ignored the vibration theory, yet it persists. The current paper seeks to determine its fate once and for all, but is this the end of the story? It is unlikely to go away for a number of reasons, sociological and scientific. The theory has sociological appeal because people are sympathetic to the underdog, as vividly brought to life in a popular book on the topic (17). The minority opinion is also critically important for the progress of science. Some “fringe” ideas like those questioning that HIV causes AIDS were swept away by evidence, and are now largely forgotten. Other provocative ideas were ultimately shown to be correct by lonely scientists who persisted in the face of great skepticism from colleagues. An important recent example is the Nobel prize-winning work of Barry J. Marshall and J. Robin Warren showing that ulcers are caused by infection of the stomach with the

bacterium *Helicobacter pylori* (18). After Marshall bravely drank the causative agent and developed an ulcerative condition, this fringe hypothesis was broadly accepted. As Max Planck said, “Truth never triumphs — its opponents just die out.” (19), and it is important for controversial ideas to be tested with empirical data and not reflexively repressed. This is how science progresses, but sometimes a fringe theory is just a fringe theory.

The vibration theory has been touted to solve one of the central problems in olfaction, and this promise accounts, in part, for its continued place in the spotlight. After centuries of conjecture on how a molecule leads to a smell percept, we still lack a convincing framework to predict the smell of a molecule from its chemical structure. If it were possible to predict the percept of a molecule reliably simply from spectroscopic analysis, our

understanding of the biology of olfaction would advance dramatically. If molecular vibration were in fact predictive of an odor percept, the application of the theory would dramatically accelerate the development of raw ingredients by the fragrance industry, which is currently guessing what new molecules will smell like rather than engineering them. However, none of these promises have been delivered by proponents of the vibration theory. Some have pointed out that it is a waste of time to expend effort to refute a controversial theory that has few advocates, and that attention should be turned instead toward how smell works (20, 21). Motivated by “disproving” the vibration theory, Block et al. (7) bring us important insights into structure–activity relationships in olfaction, one of the most important problems facing the field.

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