



Status of the Vibrational Theory of Olfaction

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The vibrational theory of olfaction is an attempt to describe a possible mechanism for olfaction which is explanatory and provides researchers with a set of principles which permit predictions allowing for structure-odor relations. Similar theories have occurred several times throughout olfactory science; this theory has again recently come to prominence by Luca Turin, who suggested that inelastic electron tunneling is the method by which vibrations are detected by the olfactory receptors within the nose. This work is intended to convey to the reader an up-to-date account of the vibrational theory of olfaction, both the historical iterations as well as the present iteration. This text is designed to give a chronological account of both theoretical and experimental studies on the topic, while providing context, comments, and background where they were found to be needed.

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1. INTRODUCTION

Olfaction is the oldest and most fundamental chemical sense by which lifeforms interpret their surroundings. This sense has always fascinated the sciences, delivering a great number of unique theories each divining the mechanism of this process. Richard Axel and Linda B. Buck published a landmark paper reporting their the discovery and cloning of the genetic code for several mammalian olfactory receptors (ORs) within a larger gene family [1]. Soon thereafter the first insect olfactory receptors were independently discovered by three research teams, all working on *Drosophila melanogaster* [2–4]. Olfaction within vertebrates and insects differs in the types of receptors used; in humans olfaction is performed via G-protein coupled receptors (GPCRs), whereas olfaction in insects primarily uses insect olfactory receptors (insect ORs). At present, the most accepted theory by which olfaction operates is based on the electrostatics and van der Waals surface of the odorant permitting binding to the receptor, after which the receptor undergoes a conformation change from its inactive state to its active state. The interplay between active and inactive conformations was validated as a likely description of activation in central nervous system (CNS) GPCRs through the analysis of the dynamics of the histidine and adenosine receptors [5–10].

Quantum effects in biology are a growing field of interest; this includes coherent energy transfer within photosynthetic bacteria proteins, mechanism of the avian magnetic compass and the possibility of inelastic electronic transfer (IET) occurring in olfactory receptors [11–15]. Considering the possible importance of quantum mechanics in biology, each suspected case of non-trivial quantum effects in biology should be examined skeptically [16]. Our intention in compiling this review is to present the vital findings and models used during the validation and examination of the contemporary Vibrational Theory of Olfaction (VTO), and to address an important question suggested by Barwich [17]: What, if anything, is so special about smell?

2. HISTORICAL VIBRATIONAL THEORIES

The present vibrational theory is an iteration in a historical movement within olfactory science to propose a relationship between molecular vibration and osmic properties. The proposition of vibrational modes controlling the osmic mechanism originates from the works of Malcolm Dyson [18, 19]. Coming off the contemporaneous development of Raman spectroscopy, Dyson believed that probing molecular vibrations of a molecule would elucidate correlations between these vibrations and the osmic properties of the odorant. The proposition was that the thermalized vibrations inherent to a molecule at a given (physiological) temperature would activate the receptor protein. Within his works he made it clear that the considerations of the vibrational components were independent of other properties necessary for odorant detections, such as the molecular volatility. Dyson continued to suggest that vibrational modes within the range of 1,400–3,500 cm^{-1} were responsible for the activation of olfactory receptor sites. Due to the contemporaneous nature of the application of Maxwell-Boltzmann statistics within his work, the ability of vibrational modes to be thermally excited at physiological temperatures was not addressed; a consideration later taken up by proponents of vibrational theories.

The VTO was revisited by Wright [20] who examined Dyson's model, noting that in higher energy regions vibrations correlate specifically with functional groups; therefore odor could equally be correlated to either vibrational frequency or functional groups. Additionally, the high energy ranges proposed by Dyson were found to have so small a population that it was unlikely that they could be responsible for a reliable olfactory sense. By considering this, Wright proposed that it was vibrations of $\sim 700 \text{ cm}^{-1}$ or less that were responsible for any activation process. Additionally, Wright espoused that the modes below 700 cm^{-1} were both "more characteristic of the molecule as a whole" and less dependent on the particulars of specific functional groups. In a second work Lloyd [21] analyzed the low energy Raman spectral region of several almond odorants, finding that the collection of almond-odorants all possessed a similarly strong band within the 150–200 cm^{-1} range. They emphasized that the nose need not act as a Raman spectrographic device, but that the underlying molecular motion is detected through a mechanical—not spectroscopic—means by the receptor.

Contemporaneously, Amoore and Moncrieff independently published the basic concepts and arguments that formed a stereochemical/steric theory governing olfaction; an important theory whose aspects have remained with us to the present [22–26]. Later Wright and Michels completed a survey of 50 test odorants. First, using trained human subjects within two counter-ordered groups to place the odorants into one of nine training classes and then to use these data through a standard similarity matrix method to compare with the Raman spectra. Although the raw Raman data had a low correlation score, the rotationally oriented data (along several olfactory factors) were found to have a high significance factor, implying strong correlation [27].

Doolittle et al. completed a study of the olfactory responses of melon flies (*Bactrocera cucurbitae*) with deuterated variants of a

known attractant. They concluded that there was no relationship between the low-energy infrared frequencies and the perceived effect on the behavior of the exposed melon flies [28, 29]. Blum et al. conducted a similar deuteration study with the Florida harvester ant (*Pogonomyrmex badius*). Ants were exposed to several ketones to determine which are capable of mimicking the natural alarm pheromone—4-methyl-3-heptanone—and the successful candidates were then deuterated to test the VTO. They found no evidence supporting a molecular vibration related mechanism for olfaction. A brief report was published by Barker et al. [30] providing highly suggestive evidence that *Apis mellifera* L. are also incapable of discerning between deuterated variants of nitrobenzene. This paper prompted a response from Wright as the negative conclusion drawn did not include information concerning the infrared spectrum, explicitly lacking information concerning the low energy region. In 1977, Wright completed the basis of his—and several facets of the modern iteration VTO, asserting the feasibility of the theory within a biological context [31]. Subsequent studies were conducted on both the German and the American cockroaches with mixed results. Sugawara et al. [32] and Havens and Meloan [33] Hara contextualized his results as altered molecular motion—vis-à-vis Wright's theory—in his report finding that whitefish (*Coregonus clupeaformis*) are capable of discriminating between glycine and d_5 -glycine in a behavioral study [34].

In 1978, Wright completed an information theory analysis that maintained the plausibility of the VTO in the context of a limiting number of receptor sites, and that the osmic spectrum could be feasibly detectable under his theory [35]. Wright attempted to justify the inclusion of his VTO within the context of enantiomeric osmic differences, by noting that the stereochemical aspect alone is insufficient to account for differences [36]. Eriksson et al. completed a study comparing the predictive nature of the contemporary olfactory theories on substituted pyridine and the perseverance of the pure pyridine odor; it was found that a particular out-of-plane collective vibration was present in all the osmically pyridine odorants. It was determined that vibrations were no more predictive than the molecular shape and space filling nature of the electron cloud; additionally, both examined properties were found to be necessary, but not essential properties [37]. Wright further attempted to provide a vibrational explanation for human perception of odor blending [38]. It was not long after these final works that it was clear that the VTO—as proposed by Wright—had no more predictive ability than considering facets of the odorant's structure alone [37, 39, 40].

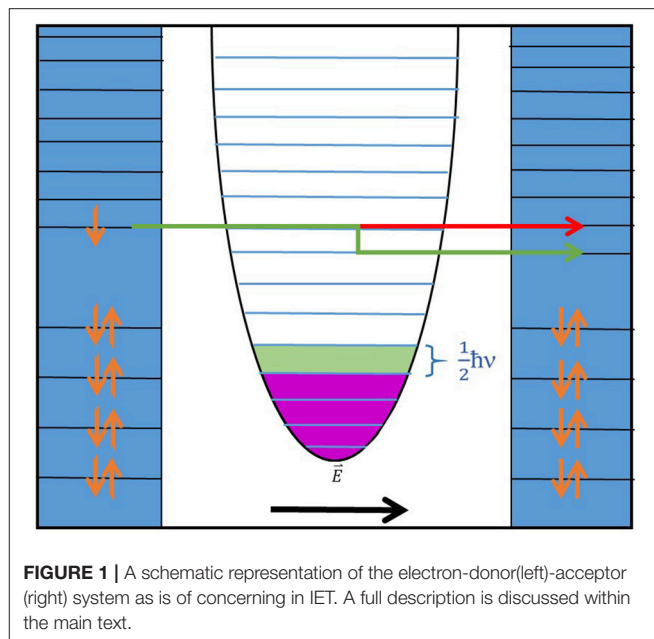
3. REINCARNATION

A contemporary iteration of the VTO was introduced in an effort to generate a systematic approach to the olfactory process, as well as to forward better odorant classification, prediction, and molecular design [41, 42]. Here we will provide a brief description of aspects associated with the VTO as Turin presents it.

Beginning with the works of Dyson and Wright, Turin appends the general theory with a mechanism physically

capable of detecting vibrational modes feasibly within biological environments: IET. Many of the important early theoretical methods were devised by Lambe and Jaklevic [43], Kirtley et al. [44], Lambe and McCarthy [45], Khanna and Lambe [46], and Phillips and Adkins [47], while an excellent introductory overview of this spectroscopic method is available from Reed [48]. Speaking generally, this is a method capable of detecting the vibrational modes of a molecule. Importantly, as no light-matter interaction is involved, both IR and Raman active modes appear in IET spectra with roughly equal weight as no invocation of the dipole rule is required. Furthermore, this non-photon driven process would permit a larger range ($0\text{--}4,000\text{ cm}^{-1}$) of possible olfactory vibrations to be examined, rather than the highly truncated range ($\sim 0\text{--}500\text{ cm}^{-1}$) attributable to a thermally driven process [40].

Specifically, within a laboratory environment, an analyte molecule is deposited upon a metal surface in close proximity to another metal surface, while a tunable electrostatic potential is generated across the insulating gap between the plates. This potential will drive electrons from one side of the gap to the other via evanescent tunneling. During the elastic tunneling processes, electrons are driven from the donor (D) to the acceptor (A) while obeying strict energy conservation; if this is the only process, the junction is Ohmic. This elastic process is shown in **Figure 1** as the red arrow; as electrons traversing the gap from the left (donor) are deposited in an energy level on the right (acceptor) with no change in energy. As we have deposited an analyte molecule on one surface, other processes may take place. The tunneling electron will be capable of donating a quantum of energy to the analyte before moving on to the acceptor; this process being termed first-order inelastic tunneling, shown as the green arrow within **Figure 1**. The electron leaves the donor energy level, and traverses the gap, falling into an energy level at the acceptor with less energy than initially, due to the electron having donated a quantum of energy to the “molecule” within the figure. Within the figure, the “molecule” is represented by a one-dimensional quantum harmonic oscillator, while in reality the molecule is comprised of anharmonic collective oscillations. Yet this distinction makes no difference in the physics as the important facets remain: the molecule retains the ability to resonantly absorb specific quanta of energy, absorption of off-resonant energy will result in emission, and highly local absorption/interaction can result in energy redistribution throughout the normal coordinates of a specific phase-consistent motion. Higher energy levels of a real molecule may undergo energetic shifts due to the incorporation of more anharmonic character, yet a system evolved to detect molecules in this fashion would likely (entirely hypothetically) have evolved with an “understanding” of the typical energy required to provide a quantum of energy to the molecule (on average) while it sits in a typical (most populated, average) vibrational energy distribution. Subsequently, the vibrational state population of the oscillator will increase from merely thermally activated (magenta shading) to a new distribution incorporating the additional excitation originating from the electron (green shading). This new pathway for electron transfer presents itself as a minute increase in the measured tunneling



current across the gap: non-Ohmic behavior. These increases are typically small enough that plotting the second derivative of the IR curve with respect to V , $\frac{\delta^2 IR}{\delta V^2}$, is beneficial for their visualization. There are other processes involved, such as second-order elastic processes (where the electron donates and accepts quanta from the molecule), but such higher-order processes (not pictured in **Figure 1**) typically present diminishing contributions to the over-all current with an increase in order.

Within the context of Turin’s theory the odorant binding site of the OR acts as the tunneling junction. The electron donor and acceptor sites within the biological system have been suggested to be charged amino acid sidechains or, possibly, a redox ready metal. Odorants, in general, are not redox ready or charged; both of these traits would hinder the volatility of the odorant, detrimentally affecting the ability of the molecule to act as an odorant. As there is no continuum of unoccupied states within the likely biological acceptor, this biological tunneling gap is capable of electron transfer at a finite number of specific energy values; that carries two important implications: (1) The probability of a biological Ohmic junction is very low (and would be entirely undesirable for a spectroscopic junction, as non-Ohmic junctions would prevent constant misfiring of the receptor in the absence of an appropriately bound ligand). (2) Contrary to laboratory IET spectroscopy, the biological case is likely to be many single apparatuses (unique ORs) each operating at a single difference frequency. Also, the interaction between the electron and dipole is very close in proximity, thereby preventing the application of the dipole approximation, forcing one to employ the exact form of the interaction. This leads to an IETS intensity $\propto \sum_i x_i^2 q_i^2$, where x_i^2 and q_i^2 describe the displacement of the partial charge, q_i^2 , along a single coordinate, x_i^2 . This implies the sensitivity of the method to both partial charge and to displacement (from equilibrium position); it is for this reason

that the effects of large atom isotopic replacement would be smaller than that of hydrogen, as hydrogen displacements within normal mode coordinates are typically much larger.

Within the receptor environment, an electron will likely originate from an electron donor site—either the metal cofactor or an electron rich amino acid sidechain—and travel through or near the bound ligand to an electron acceptor site's energy level. Within Turin's original proposal, no concern was given to electron spin; this is likely due to his nearly direct application (and thereby close mirroring) of standard IETS theory, where electrons would originate from the electron sea of the donor and traverse toward a vacant conduction band at the acceptor. Under this situation, spin is able to be treated as an ergodic property in the system, as it lacks a dance partner. That stated, there is little present understanding of the exact (hypothetical) origin or destination of the ballistic electron; that is, there is no observation that the metal undergoes oxidation and no evidence of a charged side-chain (both suggested scenarios require a direct excitation as either oxidation or a charge side-chain would lose energy through donating its electron). Complicating the problem even further, Turin's proposal is an activation method for the protein, not a method of describing the totality of the events determining perception. This is more noteworthy as Turin's theory cannot describe any perireceptor events, aspects of intermolecular interactions during the docking of the ligand, nor consider any degree of complexity in the olfactory coding.

Finally, the standard laboratory procedure typically requires the IET spectroscopic junction to be cooled to cryogenic or near-cryogenic temperatures to reduce the effects of thermal broadening as the rotational states couple with the vibrations, or vibrations coupling with electronic states, where one performs IETS to activate electronic states. Thermal broadening cannot be eliminated in the biological environment, as the homeostatic temperature of the body (or near epidermal temperature for nasal olfactory receptors) is much higher than cryogenic temperatures. Yet, within the confines of the orthosteric binding site the ability of the ligand to freely rotate or translate is severely hindered, likely narrowing the Gaussian profile contribution for these motions. Competing with the aforementioned reduction, thermally active vibronic coupling between the ligand and protein environment will remain, and will increase the overall number of degrees of freedom. These effects were considered in a recent theoretical work to be discussed later [49].

The adaptation of the standard metal-electrode model mechanism of an IETS to an olfactory receptor is *ad hoc*; yet within his works, Turin provides a number of tertiary supporting facts. Foremost, electron transfer and tunneling mechanisms are prevalent throughout biological systems [50–53], whose early models were hypothesized by Marcus [54] and Siddarth and Marcus [55]. Secondly, he hypothesizes that there should be a redox-ready metal near the activation site to donate the electron necessary to the process. The possibility of a metal cofactor playing an important role in either binding or activation at GPCRs and other non-GPCR chemokine receptors is largely substantiated. It is known that divalent cations such as Mg(II) and Zn(II) are vital for both binding and the formation of activated complexes for opiate,

MC1, MC4, and CXCR4 receptors [56–60]. Na⁺ has been shown to allosterically modulate several GPCRs, including opiate and dopamine receptors via a specific binding site between helices 1, 2, and 7 [61]. Zn⁰ nanoparticles, specific to oxidation and size, have been shown to enhance olfaction in rodents and canines [62–66] and have been shown to inhibit certain rodent ORs through competitive binding [67]; circumstantially, it should be noted that large concentrations of zinc have been found in specific areas of the brain [68–71]. Ionic copper has been shown to be vital for the robust activation of thiol-detecting olfactory receptors [72–74]. Ethylene hormone receptors of the *Arabidopsis* genus require a copper cofactor and specific mercury complexes have been known to inhibit the olfaction of *Heliothis virescens* [75, 76]. Finally, an extensive number studies (especially in rodents) have shown both the presence and the importance of metals/metal ions in olfaction [77–81].

4. EXPERIMENTS

The prior two decades have provided several experimental works concerning Turin's proposed mechanism. The works have taken place in vacillating waves of pro and con stances, and have utilized—for purpose of experimentation—both human and insect test subjects. We will now delineate these studies, addressing each article in chronological order of publication and illuminating the article both within the contest of the conflicting theories.

The earliest experimental work evaluating Turin's theory was performed by Haffenden et al. [82]. Those authors obtained several highly pure, commercially available labeled benzaldehydes; three isotope-substituted variants, as well as the natural abundance compound. Panelists were assigned to complete a series of three forced judgement, duo-trio tests [83, 84]. Unadjusted *P*-values suggested that the ¹³C analogs were not discerned at statistically relevant rates, whereas the deuterated variant was. After further correcting the values for perceived sequence bias, it was found that after considering the 50% chance of guessing there existed a 47 ± 32% “actual true” detection rate of the deuterated compound, while neither ¹³C-labeled compound was discernable. These findings were in line with the initial suggestions made by Turin that the difference in vibration-mode displacement of the carbon atoms is too small to perhaps be perceived, whereas deuteration causes a more appreciable response. Haffenden et al. argue that their findings—coupled with infrared spectrum studies—indicate that the 3,000–2,500 cm⁻¹ range could play host to one of the bitter-almond bands. Although their finds provide some support for this region playing host to the cause of the bitter-almond scent, it should be further noted that this spectral region is characteristic of such a plethora of functional groups (including the saturated C-H, S-H, N-H salt, aldehyde and carboxylic acid stretching bands) that it is an entirely non-descript descriptor; especially, as any structural aspect (whichever in the list) causing this band would become the primary correlation between odorant and odor.

A series of experiments were conducted to test several claims put forth by Keller and Vosshall [85]. The first test examined the claim of odor blending originating from Wright [38]. The claim of odor blending appears twice in Turin's original paper [41], most notably the addition of guaiacol and ethylbenzaldehyde to replicate a vanilla note through the addition of spectral features. Keller and Vosshall determined that at either of the concentrations presented of a 1:1 mixture of guaiacol:benzaldehyde panelists discerned no additional vanilla odor when compared to a respective concentration of either individual component. This is sensible as even under Turin's proposal, this was the least feasible assertion: that two molecules—which clearly would not occupy the same receptor—adding their individual spectra together (in uncorrelated and unrelated environments) to generate a third “ghost” odor, and without any foreknowledge of the structural olfactory code. Secondly, within Turin's original paper, it was claimed that for aldehydes of chain length C_8 through C_{12} , aldehydes with an even number of carbons are perceived to be fruity (citrusy) whereas those with an odd number of carbons are found to be floral (or waxy). Pairs of aldehydes—from a selection of unbranched aldehydes spanning butanal to dodecanal (L.B. Vosshall, personal communication April 16-18, 2017)—with the same parity of carbons did not present with more dissimilar odors than those with different parity; in fact, the difference in perceived odor increased generally with an increase in carbon chain length. It should be noted that several of the aldehyde molecules examined by Keller and Vosshall fall outside the range of aldehyde chain lengths discussed in Turin's initial claim and thus may very well bias the statistics concerning the C_8 - C_{10} range through out-of-scope sampling. Though, Turin himself likely determined the root cause of this odor difference, yet ascribed it to a IET-scattering mechanism rather than simple conformation space. It was found that the rotation of the aldehyde group was more hindered in the odd numbered chains; this fact would present any receptors able to encapsulate (in whole or par) the aldehyde to perceive a different free energy pathway toward the ground state of the complex, this additional hindrance to rotation (larger energy barrier to rotation) could negatively affect the receptor's ability to reach the active conformation. Finally, the claimed difference in odor between acetophenone and d_6 -acetophenone was examined; the human panelists employed perceived no difference in odor between the compounds when they were presented via a triangle test. Secondary testing of those who perceived the odor differences returned statically average findings.

To test the VTO, Franco et al. [86]. employed several strains of (*Drosophila melanogaster*, which is commonly used in biological research. Untrained *Drosophila* were examined and found able to both recognize and react (attraction/aversion) to various levels of deuterated ACP during a simple T-maze test [87], and the aversion response roughly scaled with the level of deuteration. These flies were also trained to react to specific levels of deuterated vs. natural abundance species through application of negative electronic stimulus in a similar T-maze. A generalization experiment was also performed showing that *Drosophila* trained to avoid 1-octanol vs. d_{17} -1-octanol were able to generalize some characteristics and showed selective avoidance for ACP vs.

d_5 -ACP, thereby suggesting that there is a salient odor feature provided by deuteration that is generalizable across molecules. A group of mutated anosmic flies no longer displayed untrained avoidance of either d_8 -ACP or d_{17} -1-octanol. The evidence provided by the anosmic flies is chiefly that the flies discerned these compounds through olfaction. The scaling of aversion response and trainability could likely have been due to (larger degrees of deuteration requiring) larger concentrations of a reactant/contaminant within the samples, where anosmic flies would detect neither the analyte nor any contaminant present.

Gane et al. [88, 89] examined the VTO through two tests employing both trained and untrained human subjects. Furthermore, great effort was expended to address the concern of impurities; assuring GC-level purities of all odorants, even those purchased at high purity. In a forced-choice same-difference test 5 panelists (3 perfumers, 2 untrained) were presented with GC purified, stored samples of commercially available 99% ACP and d_8 -ACP. Agreement was reached with the findings of Keller and Vosshall [85]; explicitly, humans are not able to distinguish between these two isotopologues of ACP. The second test examined the human ability to discriminate deuterated variants of musk-class odorants, including: cyclopentadecanone, cyclopentadecanolide, 1,4-dioxo cyclohepta decane-5,17-dione, and 1-(3,5,5,6,8,8 - hexamethyl-6,7-dihydronaphthalen-2-yl) ethanone. This test was conceptualized by considering the larger ratio between carbons and hydrogens in the (typically saturated) musk compounds, and that this may generate a system where the possible isotopic characteristics may present the largest differences. Upon analysis of the data from 11 subjects, the p -values of the individual subjects ranged from 0.109 to 7.62×10^6 ; the statistical chance of the aggregate trials (119 correct identifications during the aggregated 132 total trials) to have occurred accidentally was found to be 5.9×10^{-23} . In brief, deuterated musk odorants were determined discernible from their non-deuterated counterparts by the study subjects. Gane et al. attribute the musk odor perception to be symptomatic of groups responsible for a strong absorption within the $1,380$ - $1,550 \text{ cm}^{-1}$ IR region. Yet a broader examination of the IR and Raman absorptions of musks, including popular nitro-musks, structurally similar non-musks, and recently examined silio-musks (see [90]), show no clean relationship between musk odor and any single spectral feature.

Gronenberg et al. examined the ability of the western honey bee (*Apis mellifera*) to discern between isotopomers [91]. Bees were tested with three chromatographically pure compounds ACP, BNZ, and OCT against their fully deuterated counterparts. Although the learning curve response was flatter than expected by the authors, it was clear that the bees were able to discriminate between these two related molecules. Discrimination testing over the deuterated and un-deuterated pairs revealed that the bees were able to differentiate between the two isotopomers; this effect was truly independent of whether the deuterated or natural analog was the “trained” stimulus. The authors did find it noteworthy that the deuterated odorants tended to confuse the bees more than the h-BNZ vs. h-ACP test—possibly alluding to a greater degree of difficulty in isotope discrimination compared to chemical discrimination. This confusion could also be a product

of unintended contamination of samples during the deuteration, minute amounts of solvent or reactant causing confusion as they present as accidentally introduced, untrained stimuli. A control experiment was also undertaken, generating mixtures (95:5) and pure samples of h-ACP and h-BZN. The addition of a minor component did not affect the bees' response compared to the pure training test. Gronenberg believes this shows that the bee's ability to respond to minor impurities within the samples is minimal. It should be noted that although the 95:5 mixture of h-ACP:h-BZN did not elicit a novel response, neither of the components were deuterated, and could have possibly lacked a systemic contaminant which elicited the trainable responses seen in the previous experiments.

Block et al. [92]—instead of providing another psychophysical experiment with either insects or humans—examined the theory at the receptor level [92]. Within the main paper, Block conducted three unique series of experiments: (i) Examination of receptor activation by isotopologues of Exaltone; (ii) Activation by isotopomers of acetophenone and benzaldehyde; and (iii) Testing isotopomers of ten receptors and ten h/d ligand pairs. In the first series of experiments, a collection of 330 human ORs were screened with h-cyclopentadecanone (Exaltone, a musk odorant) and four deuterated variants (d_4 , d_{24} , and d_{28}). A single OR, OR5AN1, was found to be a “bona fide” receptor for this musk. They reported no receptor responded to only one of the four compounds (a claim refuted by [93]), which would contest the possibility that different ORs respond to different isotopomers. EC_{50} 's and dose-response curves were found to be very similar for each of the 4 isotope-specific compounds. Secondly, Block and colleagues tested acetophenone and benzaldehyde at various mouse ORs, and repeated this with isotopologues of these compounds. No significant differences were found between the activations of the receptors by the isotope-specific ligands. Finally, several ligands—most importantly MTMT and bis(methylthiomethyl) disulfide—were examined with their isotopomers. In none of the cases was a significant difference in activation found. MTMT and bis(methylthiomethyl) disulfide are known to require a copper cofactor in their overall activation scheme, most likely during binding; for this reason, Block et al. repeated the above series of tests while also introducing $30 \mu\text{M}$ of Cu^{+2} . Introduction of copper—to provoke a differential response due to increased cofactor concentration—provided no results divergent from the original conclusion.

Acknowledged by Block et al., differentiation of a pair of isotopomers does not necessarily prove an IET-esque mechanism of OR activation; nor does a collection of negative results absolutely disprove such a theory. (I speak here of proper evidence of absence, not absence of evidence!) A possible reason that previous behavioral studies (explicitly [41, 82]) that determined various abilities at discerning isotopologues—whereas the present study did not—are those events that occur before the activation of the receptor itself, perireceptor events. Three pertinent perireceptor events for behavioral studies conducted above would be (i) Enzymatic activity within the nasal mucosa converting chemical species or exchanging hydrogen [94, 95]. (ii) The action of odorant binding proteins within

the nasal mucosa in transporting specific species through the hydrophobic environment. (iii) The ability of the nasal mucosa itself to act as a separatory media through differential diffusion or solubility [96, 97]. Alteration of protium to deuterium has other important effects on systems resembling the prescient solvated ligand-receptor system, e.g., isotopic alteration changes the zero point energy of the bond and will have an effect on intermolecular forces, for H/D this effect is manifest in hydrogen bonds. A possible IET-esque mechanism would be extremely sensitive to both geometry and orientation. The geometry of the bound ligand will differ greater from the gas phase geometry, affecting the vibrational modes. Also, orientation has been described to be so important in IETS that it is likened to a selection rule, and electron tunneling is known to be a very local process. We do not know the true crystal structure of an OR, its ligand-bound structure, nor the possible donor or acceptor sites, thus we cannot possibly predict the appropriate IETS of any such system. It should also be noted that Block reported that d_4 -muscone lacked activity in the IR region ($1,380\text{--}1,550 \text{ cm}^{-1}$) that Gane et al. [88] had claimed should have an “intense” absorption to realize a musk odorant. One should recall that any electron tunneling mechanism would be evident in both Raman and IR spectra [44, 47], therefore solely examining the IR spectra will not elucidate the totality of possible vibrational modes.

To better address the conflicting accounts of odorant isotopologues sensitivity, two independent works revealed that insects can differentiate isotopologues of odorants. First, Paoli et al. [98] examined the local activity of specific odorants at the glomeruli of the honey bee. Four odorants (1-octanol, benzaldehyde, acetophenone, and isoamyl acetate) and a single deuterated variant of each (d_{17} , d_5 , d_8 , and d_3 , respectively) were examined at 19 *Apis* glomeruli. Initially calcium flux was used to determine the activity of the glomeruli, and that was followed up by employing two-photon functional microscopy to assist in visualizing the local activity on the topography of the sensory organ. It was noted that several of the glomeruli displayed differential flux responses with respect to exposure to isotopologues of an odorant. When regional activity of the glomeruli were examined it was found that—although there was differential detection of isotopes—the ability of *Apis* to differentiate isotopologues is smaller than the ability to differentiate unique chemical species. Drimyli et al. [99] examined the ability of *Drosophila* to differentiate isotopologues. Both behavioral (T-maze) and electroantennography (EAG) tests were conducted; the behavior tests were performed to corroborate that any differences found in the EAG were salient and perceived by the insects themselves. EAG revealed a differential response between deuterated and non-deuterated ligands. With forethought to possible criticisms and non-VTO explanations, Drimyli et al. conducted a preemptory discussion concerning purity, perireceptor events, and the extent to which size or number of deuteriums may affect the EAG. As there was no variation in the EAG rise times (the time at which a $2/3$'s maximal response was recorded [100]), Drimyli et al. argue that the effect of polarity difference between ^1H and ^2H has a minimal effect on the totality of perireceptor events [101], as well as the binding/activation steps. In a cross comparison of maximal

EAG response between each of the odorants, prodeutero-analogs and several non-prodeuterated versions it was found that the differential response to any deuteration was similar; implying that the detection ability is less sensitive to the extent of deuteration than the difference between deuterated and non-deuterated, implying that the insect detects deuterium or it does not. That does suggest—though not conclusively—that the extent of isotopic exchange is unimportant, in opposition to the commentaries: [93, 102]. This dichotomy of detection also supports the hypothesis that the effect is caused by the presence of impurities, as unique impurities may likely be present in deuterated samples compared to the natural abundance sample.

Voshall [103] made two poignant observations: (1) Generally, psychological experiments are both poorly reproducible and have many (both obvious and obfuscating) variables that complicate the interpretation of results, and (2) The unlikelihood that a molecular-level mechanism could be clearly discerned through perception-based, *in vivo* psychological studies on humans. Therefore, the finding of humanity's ability to discern isotopologues of musk compounds is important; yet, it does not prove that an IET-esque mechanism—or any activation mechanism—is dependent on vibrations. Human subject examination—such as the Gane [88] or Haffenden [82] studies—are entirely *in vivo*; for this reason the examiner is provided no ability to attribute this isotope-sensitivity to events that occur in the nasal mucosa [94], with odorant binding proteins [104–106], at the antechamber of the receptor [10], during docking [107], or during activation itself.

Block et al. [92] provided an extensive discussion over their concerns about impurities in previous works, and various isotope effects within biology and the nasal passage. In a reexamination of an early experimental work, Turin determined that the ability of *D. melanogaster* to discriminate between acetophenone and d_5 -acetophenone was very likely due to minute impurities within the commercially “pure” isotopologue samples; this finding does not invalidate the VTO, yet does remove one of the most important experimental findings in support of the theory, as well as calling into question several other studies where GC-levels of purity were not observed [108]. As an example, throughout Franco's experiments the deuterated compounds used were commercially available at highly pure levels ($\sim 99\%$); this was done in reaction to one of the many counter-points to a previous experimental evaluation of the VTO. Though, commercially pure extremely minute concentrations of any impurity involved in the synthesis of either isotopomer—which could not be removed through the performed GC purification—would be present. In the light provided by the complexity provided by olfactory coding, the spatial mapping of olfactory lobes, the building of olfaction spaces and the developing picture of dynamic protein activation (coupling dynamics with VDW/ ΔG /volume sensitive receptor and low reorganization energies), the question pertaining to the VTO which must be immediately addressed is: Is it needed?

5. THEORY

Before the present iteration of the VTO gained attention, Quantitative Structure-Activity Relations (QSARs) were developed to determine novel drug molecules from known

batches of molecules and their known experimental activities by developing statistical relationships and machine learning algorithms to find correlation between particular protein activations by ligands and specific characteristics of those ligands. A particular descriptor used within QSAR is the EigenValue (EVA), developed at Shell Research Ltd., [109, 110] which attempts to correlate activities to vibrational eigenvalues as opposed to characteristics that more logically allude to binding (e.g., size, polarity, functional groups, hydrogen bond forming groups, etc.). An early implementation of the EVA descriptor to predict odor relationships was performed by Takane and Mitchell [111]. Within this work, 47 structurally diverse molecules were sampled and dendrograms were formed by considering the region of the IR spectra where each molecule possessed vibrational modes. They found that the EVA descriptor was an excellent zero-order approximation, and determined into which of 7 categories (ambergriis, bitter almond, camphoraceous, jasmine, rose, muguet, and musk) an odorant fits. The authors of this work note that although the vibrational frequencies of the molecules naturally placed the odorants into their appropriate categories, this was merely likely due to the vibrations reflecting structural aspects of the molecules; making the EVA a “once-removed” structural analysis tool. This conclusion is in direct relation to why Wright's theory originally failed (and the clear problem with the Haffenden et al. experimental conclusions); a common cause likely exists between the particularities of odorant perception and the existence of particular absorption bands, yet that does not mean the bands cause the perception.

The first attempt at building a model to determine the viability of Turin's VTO re-constructed a Marcus-type expression for the time-scales of electron transfer in a biological environment and determined parameter ranges reasonable to physiological conditions [112]. Within the context of the Marcus theory formulation, a Coulombic interaction was introduced between the tunneling electron and an introduced intermediate set of oscillating partial charges representing the odorant molecule. Important biological factors determined by Brookes et al. include: the Huang-Rhys factors, the importance of limiting $D \rightarrow A$ electron transfers in the absence of an odorant (likely due to either distance between D and A or a non-Ohmic junction), and the possibility that the electron/hole source may likely be a biological oxidizing agent. It was determined that the charge transfer rate was adequately feasible for use in neurological detection and signaling. Additionally, bounds for a reasonable reorganization energy were formulated by considering a hydrated system to display an upper bound ($\sim 1\text{eV}$) and resonance condition to define the lower bound. They determined that if the reorganization energy was only slightly larger than $k_B T$ a large signal to noise ratio was obtainable. Brookes et al. conclude that the overall non-mechanical mechanism suggested by Turin was feasible within the system, and incorporate it as a possible mechanism in a general Swipe Card model of olfaction (where selective docking plays a role, but activation of the OR protein is based on a non-mechanical actuation) [113].

The question as to the relative rates between the elastic and inelastic processes with respect to both the donor-acceptor energy difference and the reorganization energies associated with real molecules was addressed by Solov'yov [114]. They

devised a model Hamiltonian coupling the olfactory receptor and odorant via a harmonic approximation, simulated an environment through a collection of harmonic oscillators and a tunneling term built around the hopping integral. The odorant molecule was optimized and vibrational frequencies and IR activities were determined through a B3LYP-DFT approach. They approximate the electric field of the receptor to be equal to a Zn^{+2} dication at a distance of 15.4 Å, as histidine and zinc ions have been previously discussed to be a probable acceptor/donor pair [41]. Several protio and deuterio variants of acetophenone, citronellyl nitrile, and octanol were examined; it was determined that energy regions of highly IR active vibrations were also parametric regions where there were large enhancements to the inelastic/elastic tunneling ratio; additionally, some less active modes were able to make contributions. Additionally, Solov'yov et al. propose a scenario by which deuterated analogs of similarly-sized (or functionalized) molecules may accidentally activate novel (non-endogenous, non-intended) receptors. The authors conclude that it is possible to determine molecules based on tunneling rate ratio enhancement because they correspond to characteristic vibrations under physiological conditions (as the analogous non-physiological statement has generally been known for decades).

Turin then provides several insights as to the molecular and physiological factors concerning his theory, and revises the underlying theory [115]. The insights concerning his theory are threefold: (1) The discriminatory factor of ORs on odorants cannot be merely electronic as isotopomers are detected (even only sometimes) as distinct odors. (2) The basis for discrimination cannot be mass alone, as a fully deuterated acetophenone differs only 3% from the protio isotopomer. (3) Under physiological conditions, the C-H/C-D modes do not drastically alter the heat capacity. A revision of the VTO is undertaken as it is argued that the standard IET spectroscopy theory is not valid because all orbitals within the biophysical system are localized; this lack of direct overlap requires some slight alteration in the theory. A new simulation model is developed based on a donor-bridge-acceptor model Hamiltonian. This new approach predicts the probable discrimination between acetophenone and d_8 -acetophenone, while predicting that deuterated isotopomers of ethylene cannot be discriminated. The paper continues by presenting the results of a T-maze experiment testing and validating the predicted hypothesis concerning acetophenone and d_8 -acetophenone, yet no evidence was provided to support the claims concerning ethylene.

A spin-boson model was developed to describe the importance of dissipation in electron transfer processes under a polar master equation by Chęcińska [116]. Within the model, the environment is again described by a collection of harmonic oscillators, the physical parameters being determined by physiological arguments similar to previous works [112, 114], and the odorant molecule is modeled as a single oscillator in an expandable manner. They determined that under resonant conditions reverse transfer ($A \rightarrow D$) is a likely event without preventative mediation from the surrounding protein environment, likely in the form of reorganization. They also

found that reduced dissipation limits the frequency detection and discrimination of the system. Operating under a similar model, Tirandaz et al. [117] examined the system's ability to discriminate enantiomers based on an asymmetric double well potential. They found that such a system was able to discriminate enantiomers based on the ratio of the tunneling frequency to the localization frequency, used as an overall measure of chiral interactions.

Block et al. [92] had previously made three claims to be answered by those supporting the VTO. These claims are: (1) The suggested mechanism by which electrons are delivered to the system is too unreliable to support such a vital system as olfaction. (2) Electron transfer mechanisms are highly sensitive to both bonding and average environmental fields that are not adequately considered in present models. (3) Assumptions concerning environmental fluctuations are not realistic. To answer claims 2 and 3, Solov'yov et al. determined acetophenone to be a viable odorant molecule to examine, selected based on experimental utility and docking into a homology model of an OR based on rhodopsin apoprotein [49]. The vibrational properties of the odorant were calculated under the influence of the OR while the newly perturbed Huang-Rhys factors were determined. Finally, alterations in binding due to post-ET reorganization are considered and comments on this are provided. Within the homology model, particular candidate amino acid residues—favorable to the binding of acetophenone—were selected as putative donor and acceptor sites; interestingly, methionine was selected as a possibly active residue as it was previously suggested to be involved in electron relays [118]. It was determined that the electron transfer has only a minor impact on the potential energy of the OR, and that the changes in vibrational character of the odorant will likely not affect electron transfer in the system. Furthermore, chemical fluctuations (including proton and methyl radical transfers) and dynamic fluctuations were considered and shown to have no appreciable impact on the average IR spectra of the odorant. Finally, it was reiterated that the reorganization energies of membrane proteins can be so low as to be negligible, e.g., a reorganization energy of 0.03 eV is observed for the photosynthetic protein of *Rhodobacter capulatus* at room temperature [119].

Apart from directly modeling the electrostatics and dynamics of the system, there have been several studies attempting to divine other testable aspects of the odorant-receptor system to validate/invalidate the VTO. A possible test for the VTO was designed based on the use of a ^{13}C isotope instead of the more commonly employed ^2H isotope [120]. Therein, it was suggested that committing a number of $^{12}\text{C} \rightarrow ^{13}\text{C}$ exchanges to match the mass shift in a deuterated isotopomer of the same molecule you are capable of generating odorants with (roughly) the same molecule, differing vibrational spectra, and roughly the same Gibbs free energy. That allows one to probe the on/off rate of—let's say $^{13}\text{C}_8$ -acetophenone and d_8 -acetophenone—and determine whether any change in this quantity is due to a free energy difference or possibly due to a difference in vibrational spectra. It should be here noted that no perceived effect was seen in the behavioral study by Haffenden et al. that included ^{13}C isotopic exchange, possibly giving credence to the VTO within

the context of this test [82]. Additionally, Maia et al. completed MM/MD calculations to obtain geometries of 23 molecules (16 musks and the remainder being structurally similar to the musks, yet without musk odor character) and employed B3LYP-DFT calculations to obtain the vibrational spectra. Considering central frequency bands centered at intense peaks $\pm 200\text{ cm}^{-1}$ at 700 cm^{-1} , $1,000\text{ cm}^{-1}$, $1,500\text{ cm}^{-1}$ and $1,750\text{ cm}^{-1}$; it was determined that no single frequency or band could be responsible for the activation of a musk receptor (otherwise musks would not activate or non-musks would activate). The response was likely—if at all related—due to a collection of modes sitting roughly between 700 and $1,700\text{ cm}^{-1}$; the authors further disputed Turin's assessment that a carbonyl stretch at $2,000$ – $2,400\text{ cm}^{-1}$ was responsible for the musk odor because this band appears at $1,680$ – $1,810\text{ cm}^{-1}$ in the present work and is not present in several of the studied musk odorants.

Saberi and Seyed-allaei [121] examined the molecular volume (considering both size and flexibility of the binding pocket, characteristics featured in other works, such as [122, 123]) and attempted to correlate it to olfactory sensitivity for the olfactory receptors of *D. melanogaster*. They cite neural measurements that allude to the use of a combinatorial coding in olfaction, yet wished to determine the vital discriminatory properties of the olfactory system. They, logically, assumed that any mismatch in the volume/receptor relationship will affect the physiological response. An initial assumption was that the total activity was a separable function of volume effects and all remaining considerations; where molecular volumes are determined by a Gaussian distribution built on tuning curves constructed by neural responses of individual neurons. It was thought that if all receptors had the same preference for molecular volume/flexibility, then changing the volume of an odorant would affect the intensity of odor and not the character. Molecular volume was found to be a factor, but not the sole one involved in olfactory discrimination; yet there did seem to be a non-linear volume dependence that may be capable of masking other factors involved in olfaction. This study was explicitly concerned with *D. melanogaster*, yet could be repeated for other species.

Finally, it is prescient to consider the more overt ways by which isotopic exchange may affect enzymatic processes. Krzan et al. [107] examined the effects of ^2H isotope exchange via saturation in deuterated media. They—of course—found contributions to the enzymatic rates and binding affinities due to the Ubbelohde effect as $^1\text{H}\rightarrow^2\text{H}$ exchange within the protein effects intramolecular bonds and thereby alters the energy balance between the active and inactive states of the protein. This is—the authors claim—the first attempt at examining isotopic effects within the context of the protein's atoms; yet there are decades of work examining the effects of isotopic specific endogenous and synthetic ligands. Within these previous studies the binding isotope effect, alterations in zero-point energy, and changes in vibrations effecting hydrogen bonding have all been discussed [124–126].

Recently, Tirandaz et al. [127] reexamined their chiral-odorant polaron Hamiltonian model—expanding it to determine a non-planar vibrational state—and determined several aspects

of the model that could provide new tests of the vibrational theory, while also displaying the versatility of a system behaving within the model. Therein they constructed a rate expression describing the IET frequency based on an odorant capable of undergoing a tunneling interconversion between two chiral states, similar to the classical ammonia tunneling problem. Using this model, the authors determined a lower thermal limit to the possible detection of an odorant within the VTO context, and limiting relationships and trends with respect to odorant concentration (thermodynamic pressure) and isotopic mass substitutions. Importantly, they determined that the substitution of higher isotopic masses increases the system's sensitivity under the VTO. Finally, though very narrowly, they find that the VTO is capable of chiral recognition under biological conditions. In a series of papers, Kraft et al. examined—through both *in vivo* calcium ion imaging and computational methods—the validity of the “shape-based” theory of olfaction by examining the response to several Group 14-for-carbon substitution of the t-butyl functional group in the linal and Bourgeonal odorants [128–130]. As these authors state, the benefit of this substitution series is that it maintains the molecular geometry while increasing the hydrophobic bulk of the molecule. Within the series of papers, their focus was to examine the molecular mechanism responsible for odor detection and perception through a three pronged approach: (1) conduct *in vivo* detection threshold experiments, (2) *in vitro* calcium ion imaging of activation, and (3) computational support. They determined that the human OR17-4 receptor was activated—and likely solely activated—by the odorants above through both structure-odor-relationships and *in vivo* analysis. Afterward, a homology model was constructed for the receptor based on the rhodopsin protein and docking simulations of the various substituted odorants were made, noting their change in free energy. They then compared the requirements for breaking of the disulfide bond, which binds the intracellular G-protein to the transmembrane GPCR. They take that the energy required to directly break the disulfide bond to be a bound for the energy harvested from the vibrational quantum; using this bound they find that the energy ranges defined by these criteria do not match the activation patterns seen in their (both *in vivo* and *in vitro*) works. The free energy changes seen in the docking calculations with respect to the increasing hydrophobic bulk do match those results. It was finally determined that olfaction was largely based on van der Waals (VDW) surface and electrostatic surface properties, finding no evidence that the VTO played any role in olfaction [130].

6. GPCR/EVOLUTION-BIOLOGICAL-GENETIC

Dating back to the unicellular stages of evolution, chemoreception was the earliest form of communication between cells and their environments (including other cells) [131]. Similar chemoreception systems solve similar problems between those early unicellular life forms and the individual cells of today's multicellular life. It should not be surprising

that many metazoan signaling systems are constructed from components already found in their unicellular ancestors [132], while other systems were coopted entirely from pre-existent systems [133–135]. Note that in the case of GPCRs, the unicellular precursors of metazoans already possessed most of the cytoplasmic components of GPCRs, including all of the G_α subunits. Nature seemed to grab the right (or closest-to-right) tool for a job and set it to a new task.

One of these novel receptor classes developed by metazoans was the GPCR system, where most GPCRs families have their ancient origins in the last common eukaryotic ancestor [136]. Generally speaking, GPCRs' transmembrane signaling proteins are comprised of seven helical domains crossing from the extra- to the intra- cellular environments. Each receptor possesses a ligand binding pocket near the extracellular surface and an intracellular heterotrimeric G-protein complex, which dissociates after the GPCR is activated. Members of the family mediate extracellular chemical signals and share homologous structure [137]. Speaking generally once more, GPCRs are divided into 5 major categories by the most used classification system: Glutamate, Rhodopsin, Adhesion, Secretin, and Frizzled. Although this system is designed specifically for GPCRs, it has been suggested to extend a similar system to include non-GPCRs, including insect ORs [138].

GPCRs populate batteries of receptors in the mammalian olfactory system (including: ORs, vomeronasal receptors, olfactory TAAR, Formyl peptide receptors, GC-D receptors) and neurotransmitter receptors (NRs) in many species, e.g., vertebrates and terrestrial varieties of both *Nemertea* and *Annelida*, use GPCRs as ORs [139, 140]. At a fundamental system level these jobs are similar, as both accept a chemical species traveling through space to interact with a binding protein whose signal is eventually detected by an ionotropic receptor (IR) after having undergone several intermediate processes [141]. NRs typically have lower sensitivity (nano- to mili- molar range) and deal with a smaller, controlled number of chemical compounds, many of which are highly specific ligand/receptor relationships [141]. By contrast, in olfaction, individual odorants activate subsets of receptors instead of single receptors and activation/inhibition/antagonism all play a role and possibly generate temporal dynamics that are important to the overall odorant code and possibly novel odorant perceptions [142]. Slight changes in either structure or concentration of an odorant are capable of affecting the activation pattern, leading to a different odorant code [139]. This odorant code permits mammals, including humans, to perceive a larger variety of odors than ORs expressed, as humans are capable of perceiving over 10,000 odorants with a trillion being an extremely liberal estimate under contention [131, 143–145].

The architecture of terrestrial vertebrate olfactory systems is fairly conserved. Air is up-taken through a stereo arrangement of nostrils, an arrangement important to odor tracking. This air, laden with odorant molecules, passes along the epidermis of the nasal cavity where receptor lined neurons project into the cavity; each olfactory receptor neuron (ORN) expresses one receptor type [139]. Within this span of receptors and neurons, some pairs are considered generalists (those that are typically involved in the

activation of odors) and some pairs are specialists (those involved in the detection of pheromones) [139]. After the receptor is activated, its neuron conducts the signal to a glomerulus, an organ that acts as an encoding pre-process site before the signal reaches the olfactory lobe of the brain [146–148].

Similar to the terrestrial vertebrate system, the overall system-level olfactory procedure is well conserved in insects. Air passes through a porous cuticular wall—typically along the antennae—where the sensillum contains dendrites for ~5 ORNs. The axons of these ORNs project into glomeruli within the antennal lobes of the insect brain. This physical pathway is utilized by neurons transferring mechanosensory, thermosensory, hygrosensory, and gustatory information as well [141, 149]. The zonal organization of the glomeruli is not precisely understood [149], but important electrophysiological studies are being done to understand these patterns and to build representations of odorant spaces [4, 98, 99, 150]. Importantly, these glomeruli patterns show similarity between individuals within a species [151]. Generally, different ORNs respond to different odors, and these responses are varied in type (excitatory or inhibitory) and dynamics [149]. The importance of this glomeruli pattern-based code is supported by cisgenic monoexpression of an OR in its non-wildtype neuron, which changes the overall innate behavioral response to an odorant [152].

Insect olfaction utilizes three types of chemosensory protein (CSP) receptors: insect ORs, IRs, and gustatory receptors (GRs) [139, 141]. An insect OR is a transmembrane protein of roughly 44 kilodaltons showing no homology to GPCRs, yet having similar topologies and functionalities. Of note, insect ORs possess inverted topology to GPCRs, as they have an extracellular C-terminus and an intracellular N-terminus [153, 154]. It is generally suggested that insect ORs act as ligand-gated ion channels [155], yet may also—through odorant sensing complexes—activate a G-protein pathway [156]. This dual channel mechanism is capable of providing both a rapid (ionotropic) and sensitive (metabotropic) detector [156]. This dual channel construction comes from a heteromultimer composition of one ligand-specific receptor acting as a ligand-gated ion channel coupled with a (highly conserved) ORco-type receptor [141, 157]. Whereas most ORs vary widely in sequence [158, 159], the ORco receptors show good sequence identity; these coreceptors have been identified in *Lepidoptera*, *Diptera*, *Coleoptera*, *Hymenoptera*, *Hemiptera* and *Orthoptera* [154, 157].

Insects typically express two types of olfactory protein on a single ORN (or sometimes four IRs, see [139]); this is different from their vertebrate counterparts. It is vital to note that the secondary protein expressed on the ORN is typically the ORco receptor for that species; this is to say, an ORN contains a single OR and the species' ORco [151, 158]. As ORs vary widely in their sequence identity—suggesting that species developed their present repertoire of ORs individually—the ORco receptors show amazing similarity across species [151, 158]; a study of several species have shown a 65–87% sequence similarity for their ORco analogs, including: *D. Melanogaster*, *A. Gambaie*, *H. Zea*, and *C. Capitata* [158]. It has been thought that the expression of multiple olfactory proteins per ORN was an adaptation to

expand the number of odorants perceived while maintaining a low number of neurons and ORs [151]; it is more likely that the glomeruli pattern holds the sensory diversity, and the OR-ORco pairing is designed to exploit multiple signaling pathways. That is evidenced by the fact that OR83b (the ORco analog of *D. Melanogaster*) is not directly involved in odorant recognition [139]. Suppression of the expression of the ORco analog has been shown to deplete the overall activity of the olfactory system [158, 160]. Additionally, these ORco analogs are capable of fulfilling the role of their interspecies counterparts; an anosmic mutant *D. Melanogaster*—without expressed OR83b—regains its electrophysiological response to odorant stimuli when transgenic Or83b orthologs were inserted and expressed in the fly's ORN [158].

7. FUTURE OUTLOOK/CONTEXTUALIZATION

Some studies have attempted to relate the vibrational theory to GPCRs within the CNS. Oh et al. [161] have completed studies attempting to find relationships between the molecular vibrations and activity of compounds at the histamine and adenosine receptors. A selection of several agonists for the candidate receptor were optimized at the BLYP-DFT level of theory and their molecular vibration frequencies were calculated. Analysis within a Corralled Intensity of Molecular Vibrational Frequency (CIMVF) framework suggested that the 0–5,000 cm^{-1} energy range of molecular vibrations is divided into 1,000 partitions of 5 cm^{-1} , and the value of each partition is the sum of the intensities within the partition. Upon completing a hierarchical clustering of the CIMFV data, it was determined that this method above others was capable of clustering the agonists of both the histidine and adenosine receptors within a tree. Dendrographic analysis of ligand activity correlated with vibrational frequencies [161–163]. Oh does not attribute that directly to the mechanism by which these CNS GPCRs are activated, yet describes it as a possible useful tool in divining novel ligands for these receptors.

In a similar vein to the discussion of Barwich [17], Hoehn et al. attempted to expand Turin's IET-based VTO by applying it to predict possible effects of deuterated ligands for non-olfactory GPCRs in the CNS [164]. They initially examined several well-known psychedelic 5-HT receptor agonists and algorithmically determined shared spectral features that could be responsible for their collective activation properties through a spectral indexing method. This putative active peak was similar in frequency to those previously reported for the histamine and adenosine receptors [161–163]; that is not entirely unreasonable as a steric/docking consideration providing additional discriminatory facilities to the receptor, as it is known that receptors interact with several ligands and a ligand is capable of interacting with several receptors [165]. After ascertaining a putative peak, their focus shifted to validation by comparing the peak volume—proportional to the tunneling probability associated with this vibrational mode—to the activities of known ligands at the receptors. The selected ligands were DOX and 2C-X (X = Br, I, trifluoromethyl, and an ethyl silyl ether). The tunneling

probability did roughly scale with the efficacies of the selected substances. Based on these findings, the relative efficacies of several deuterated analogs of the LSD molecule were predicted and proposed as a test of the vibrational theory for general GPCRs. In a follow-up paper, the authors performed the prescribed test on DAM-57, a less legally controlled variant of the LSD molecule [166]. The findings conclusively showed that the predicted pattern of efficacy for isotopologues of DAM-57 did not match the experimental findings. Thus the VTO is unlikely to participate in non-olfactory GPCRs; and as the lineage of GPCR maintains many similarities in both form and function, it makes it unlikely that the VTO participates in olfaction, as well.

A great deal of recent work has been directed toward determining the nature and the cipher of the odorant olfactory code [167, 168]. Olfactory lobe maps have begun to be made for both insect [169–172] and vertebrate species [146, 147, 173, 174]. The coding has proven to be sufficiently complex as to have evaded deciphering and provides an ample enough vector space to encode all perceivable odors [143, 144, 175]. It was suggested, though likely hyperbolic, that the total number of individual odors perceivable by the human olfactory system was upwards of 1 trillion [143], but that has been contested [144, 145]. The dimensionality of the odorant space has been examined to determine the minimal number of relevant olfactory classes for characterization [145, 176, 177]. Recent evidence has also shown—through inserted electrical probes in live mice—that the encoding of the olfactory lobe appears in the piriform cortex and posterolateral cortical amygdala, brain regions responsible for olfactory learning and innate behavioral response to odorants [178]. Interestingly, it was recently determined through exploring the perception of multi-component odorant mixtures that these mixtures are better understood as unique vectors within odor space than as averages between the two component odorant vectors [168]; this possibly explains early claims of odorant mixing in the vibrational theories of Wright and Turin [38, 41, 85]. As the complexity inherent in understanding structure-odor relationships led to the VTO, it should be noted that the unexplored complexity associated with olfactory coding may justify the lack of a simple structure-odor relationship and remove the impetus behind the VTO.

The topic of artificial olfaction is growing and has been adopting the more useful aspects of the discussion of organic olfaction, such as coding, metalloprotein-based detection and IETS [142, 179–182]. It is worth stating that even if all of this work does nothing to describe the biophysical and physiological process of olfaction, we are inadvertently proposing novel methods and tools for the development of artificial olfaction. Herein alone, electron transfer mechanics have been shown to be a possible means of artificial olfaction, while multiple sensors can provide a level of detection sensitivity and versatility that single detectors cannot. In general, many articles pertaining to the VTO have appeared within highly ranked journals; it is apparent that this conflict in vision—a conflict that neither side may win in the end—is one that the greater community would like to see resolved. Such a resolution will require far better communication between disciplines and a far more fair discussion between the entrenched camps on both sides of the debate.

AUTHOR CONTRIBUTIONS

All authors conducted background research for this article. RH completed most of the writing, while other authors heavily edited the text.

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