

# Testing odorant-receptor interaction theories in humans through discrimination of isotopomers

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## Abstract

Odour reception takes place on the olfactory receptor neuron membrane, where molecular receptors interact with volatile odorant molecules. This interaction is classically thought to rely on chemical and structural features of the odorant, *e.g.* size, shape, functional groups. However, this model does not allow formulating a correct prediction for the smell of an odorant, suggesting that other molecular properties may play a role in the odour transduction process. An alternative model of olfaction maintains that odorant receptors can probe not only the structural and chemical features, but also the molecular vibration spectrum of the odorants. This constitutes the so-called vibration model of olfaction. According to this model, two isotopomers of the same molecule,

*i.e.* two forms of the same molecule, one unaltered and one in which one or more hydrogen atoms are substituted with deuterium – which are therefore structurally and chemically identical, but with different molecular vibration spectra – would interact differently with an olfactory receptor, producing different olfactory perceptions in the brain. Here, we report on a duo-trio discrimination experiment conducted on human subjects, testing isotopomer pairs that have recently been shown to be differentially encoded in the honeybee brain.

## Introduction

Regarding the molecular basis of olfaction, there are still several unanswered questions, particularly concerning the interaction between odorant molecules and receptor proteins, which leads to olfactory signal transduction.<sup>1</sup> Traditional views on the topic<sup>2</sup> have been challenged by recent findings,<sup>3-5</sup> but conclusive evidence could not be provided yet, leaving the debate widely open.<sup>6-9</sup>

Odour perception initiates on the membrane of olfactory receptor neurons, where volatile odorants interact with olfactory receptor proteins triggering a signalling cascade. As suggested by comparison with other receptors from the G protein-coupled receptor (GPCR) family, to which human odour receptors belong,<sup>1</sup> such interaction might rely purely on chemical binding, probing the size, shape, and functional groups of an odorant. This model is predominant in textbooks, and constitutes the framework within which the majority of research on olfaction is conducted.

Although most of the scientific evidence on odour reception collected in different species is well in agreement with this model,<sup>10-13</sup> it does not provide satisfactory explanation for the existence of many molecules of completely different chemical properties, *i.e.* size, shape, and functional groups, resulting in identical odours.<sup>14</sup> At the same time, in large molecules, the substitution of a single inner atom, not contributing to chemical binding, can alter strongly the molecule's odour.<sup>15</sup> Because of these inconsistencies, an alternative interaction mechanism has been proposed, connecting odour character to the vibration spectra of the odorants.<sup>16,17</sup>

Luca Turin suggested a first concrete physical explanation for vibration-sensing detection, which is based on an inelastic electron tunnelling spectroscopy mechanism.<sup>18</sup> The theoretical feasibility of this ideas was supported by several quantum mechanical simulations.<sup>19-22</sup> An experimental test of the vibrational model was suggested in Turin's original work:<sup>18</sup> evaluating the odour discriminability of isotopomers, *i.e.* standard odorants and their deuterated counterparts, in which one or more hydrogen atoms were substituted for deuterium. These substitutions do not change chemical properties like shape, size, or functional groups of the molecules, but

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cause a substantial increase of the nuclear masses and therefore lead to major changes in the vibrational spectra. However, it has to be noted that some physical properties, such as the molecule's dispersion forces, are also slightly changed by the deuteration.<sup>23,24</sup> By now, various behavioural studies of isotopomer discrimination were performed in humans,<sup>24-26</sup> in *Drosophila*,<sup>20,27</sup> and in honeybees.<sup>28</sup> While outcomes of human tests were mixed, insect behavioural tests showed isotopomer discrimination. A first *in vivo* imaging experiment in the honeybee brain traced back the effect to the antennal lobe,<sup>4</sup> providing the first non-behavioural indication for different neuronal activation patterns corresponding to hydrogenated and deuterated odorants. In particular, response patterns to isotopomers of 1-octanol and benzaldehyde were clearly distinguishable. Recent electrophysiology experiments in *Drosophila* traced back differential responses to isotopomers to the odour receptor neurons of the antennae.<sup>3,5</sup> However, a recent imaging experiment in *Drosophila* showed that an observed differential response to benzaldehyde in one olfactory receptor could be fully explained by a minute impurity of 6 ppm within the odour sample.<sup>29</sup> Such impurity, undetectable by gas chromatography, had overshadowed the response to the nominal odorant.

Another study suggested limitations of theoretical models and reported a lack of isotopomer discriminations in an *in vitro* analysis of one human and several mouse olfactory receptors.<sup>7</sup> However, the heterologous odour receptor expression in a kidney-derived cell line raised doubts about their functionality.<sup>1</sup>

Altogether, a final explanation of the molecule-receptor interaction is not in sight, and further experimental tests of any model will support this important research goal.

In the present study, we challenged human subjects with an olfactory discrimination task regarding the same pairs of isotopomers discriminated by honeybees. Overall results did not show a significant discrimination of hydrogenated from deuterated odorants, but an interesting trend in benzaldehyde suggests how to improve the design of future studies.

## Materials and Methods

### Participants

Subjects ( $n=201$ , of which 108 females and 93 males) were recruited during a public event in Trento, Italy (*La notte dei ricercatori*) in September 2015. Their average age was 24.8 years (see Figure S6 for age distribution).

### Odorants

Samples of the odorants were prepared a few hours in advance in 15 mL Falcon tubes. The odorants used were: 1-octanol (OCT-h), purity >99%, and 1-octanol- $d_{17}$  (OCT- $d_{17}$ ),  $CD_3(CD_2)_7OH$ , purity 98.5%, isotopic enrichment 98.8%; benzaldehyde (BZA-h), purity >99%, and benzaldehyde-2,3,4,5,6- $d_5$  (BZA- $d_5$ ),  $C_6D_5CHO$ , purity 99.9%, isotopic enrichment 99.7%. They were diluted 1:50 in mineral oil up to a 200  $\mu$ L volume. Those concentrations were chosen such that healthy subjects could clearly perceive the odour character of each compound. To exclude potential anosmic subjects, volunteers were asked whether they sensed a difference between OCT and BZA, which was answered positively by all participants. Data of those who could not provide clear answers to the tests were discarded.

## Behavioural task

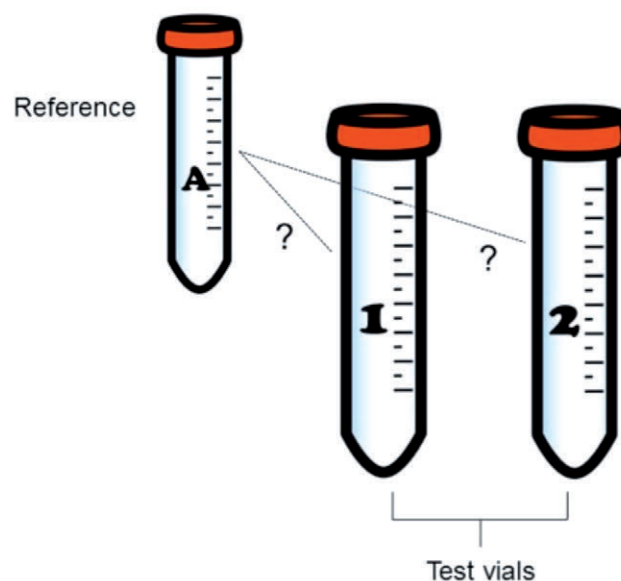
For the isotopomer discrimination tests, odorants were presented in three identical vials: one reference vial, and two test vials (Figure 1). One test vial contained the same isotopomer as in the reference vial, the other one a different isotopomer of the same odorant, forming a vial triplet for a so-called duo-trio test. In this duo-trio discrimination task, a participant is asked to compare the reference with the two test vials and report which one of the two test vials had the same odour as the reference. There were two vial triplets prepared for benzaldehyde (one with BZA-h and one with BZA- $d_5$  as reference) and two for 1-octanol (one with OCT-h and one with OCT- $d_{17}$  as reference). To perform a double-blind experiment, the four reference vials were marked with letters A-D and the test vials with numbers 1-8 by one experimenter. Each participant was randomly assigned by a second experimenter to two vial triplets, and then tested on both odorants, benzaldehyde and 1-octanol (50% of participants, randomly assigned, in this order, and the other 50% in the reverse order). All experiments were conducted indoors within 3h to guarantee stability of sample and ambient conditions.

## Statistics

Data processing and statistical analyses were conducted respectively in R and Matlab. Percentages of correct answers were compared against chance level through one-tailed binomial tests; P-values throughout the text were Bonferroni-corrected for family-wise errors (FWE).

## Results

Responses to the tests were analysed for both isotopomer pairs: 1-octanol-h/ $d_{17}$  and benzaldehyde-h/ $d_5$ . 98 out of 198 subjects



**Figure 1.** In the duo-trio test, participants are asked to first smell a reference vial and then confront it with two test vials. They are then asked to report which test vial contains the same odorant as the reference.

could perform a correct distinction in the 1-octanol test, whereas 108 out of 198 correctly responded to the benzaldehyde test (Figure 2). Binomial one-tailed tests provided probabilities for deviation from chance level of  $P=0.94$  for 1-octanol and  $P=0.23$  for benzaldehyde, respectively (Bonferroni corrected for the 2 tests). Although one might see a slight trend for discrimination of BZA, none of the two tasks was significantly solved.

To analyse the trend in BZA in more detail, we tested whether there was an effect of the order of presentation on the discrimination abilities of the subjects. From this analysis, a higher correct discrimination of the BZA isotopomers was found in those subjects performing first the BZA test: 63 out of 104 (Figure 3) with respect to those performing the BZA test after being tested on OCT: 45 out of 94 (Figure 3). A one-tailed binomial test on the subjects tested first on BZA provided a probability for deviation from chance of  $P=0.019$ . However, the Bonferroni correction for FWE of the 4 potential tests on 2 odours and 2 administration orders reduces this value to a non-significant trend ( $P=0.078$ ). In the case of 1-octanol, the presentation order was not affecting the proportion of correct answers (see Supplementary Figure S1).

Further exploratory analyses on potential effects of hydrogenated or deuterated odorants as reference (Supplementary Figures S2 and S3),<sup>25</sup> of sex (Supplementary Figures S4 and S5), and of age (Supplementary Figures S6-S8) did not show significant deviation from chance level.

## Discussion and conclusions

Discrimination of isotopomers has been suggested in various species, from insects<sup>20,27,28</sup> to humans.<sup>24,25</sup>

Here, we tested the possibility that naïve human subjects may be able to discriminate two of the isotopomer pairs used in a previous study in honeybee:<sup>4</sup> 1-octanol and benzaldehyde. For both odorants, we performed duo-trio tests, revealing that the analysed overall population failed to significantly discriminate hydrogenated from deuterated odorants. This indicates that these isotopomer pairs could not be distinguished by human subjects under the tested conditions or, alternatively, that the number of discriminators in the tested population was too low to cause a significant effect. However, we noted that the order of odour presentation and/or the time between single tests should be carefully chosen in future studies, since those subjects that had first been tested on BZA showed a trend to correctly discriminate the isotopomers, while subjects tested on BZA after the OCT tests failed to deviate from chance level. This might indicate that when BZA was tested second, receptors activated by both odours might have still been saturated from the OCT test, rendering a discrimination of potential subtle differences between BZA isotopomers impossible.

Interestingly, the ability of human subjects to discriminate benzaldehyde isotopomers was previously tested, although with a dif-

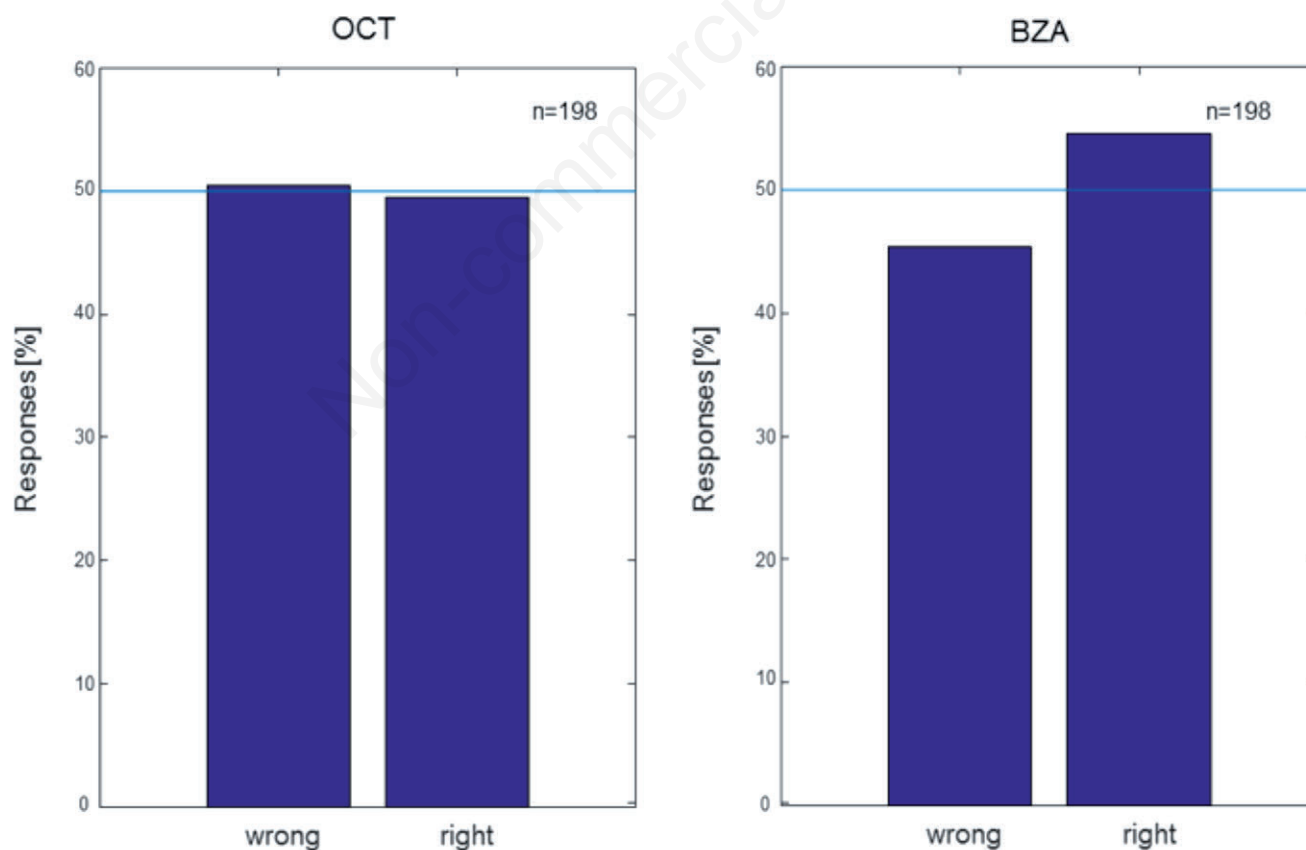
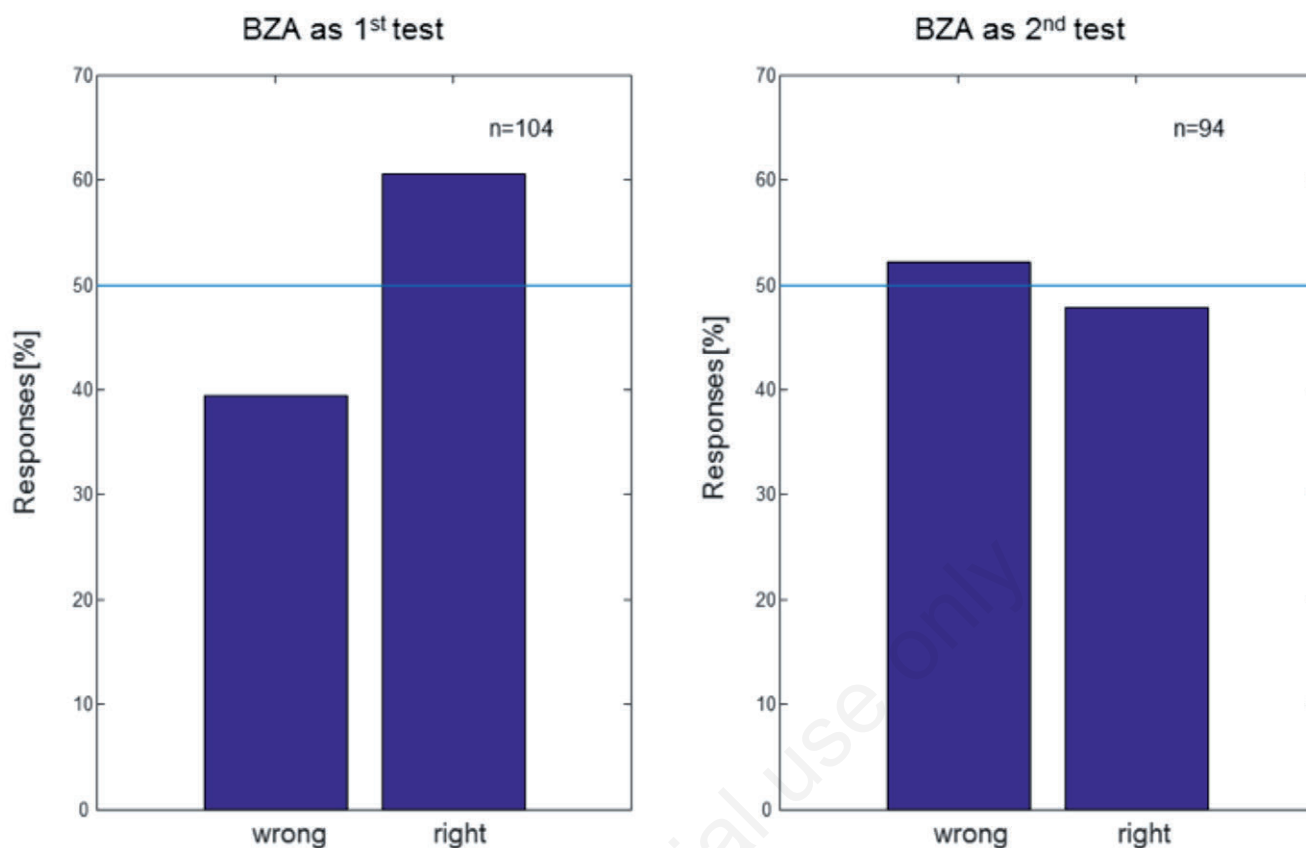


Figure 2. Percentage of wrong and right responses to the 1-octanol and benzaldehyde tests (absolute counts: wrong/right 100/98 and 90/108, respectively). The line at 50% represents chance level. None of the two proportions of correct answers was significantly different from chance level (binomial one-tailed tests, respectively  $P=0.95$  and  $P=0.23$ , Bonferroni corrected).



**Figure 3.** Percentage of wrong and right responses to the benzaldehyde (BZA) test, depending on the order of presentation. When the test on benzaldehyde was preceding that on 1-octanol (left plot, absolute counts: wrong/right 41/63), there was a higher number of correct answers than in the benzaldehyde tests following 1-octanol tests (right plot, absolute counts: wrong/right 49/45). When all 4 odour-combinations are tested, the FWE correction reduces the effect in the left plot below the significance level (binomial one-tailed test,  $P=0.078$ , Bonferroni corrected). The line at 50% represents chance level.

ferent protocol and a far smaller number of participants.<sup>25</sup> In that study, the subjects were previously trained, to enhance their discrimination abilities, and a significant differentiation between BZA-h and BZA-d<sub>6</sub> was reported.<sup>25</sup> This discrepancy with our results might be explained by two factors. First, Haffenden *et al.* used a higher level of deuteration exchanging all 6 hydrogen atoms of the benzene ring, while we worked with the slightly lower deuterated BZA-d<sub>5</sub>. The increase of isotopomer discrimination with the degree of deuteration was reported in several insect studies.<sup>4,20,27</sup> On the other hand, the two studies looked at distinct aspects of olfactory discrimination. While the current report investigates the spontaneous discrimination abilities within a large population, Haffenden *et al.* tested this capability in a few trained subjects. This suggests that benzaldehyde isotopomers exhibit at most minor differences to human subjects, but training may enhance subjects' perception of these differences.

However, from our study we conclude that an influence of molecular vibrations on odour discrimination between the two tested isotopomer pairs could not be resolved in a broad naïve population. Our study further suggests that when several odours are tested, increased recovery times should be allowed between single tests, in order to avoid receptor saturation. In addition, we suggest to consider for future human studies also the results of a recently

published insect experiment,<sup>29</sup> showing that odour stimuli need to be purified by gas chromatography to avoid biases of impurities.

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