A psychophysical test of the vibration theory of olfaction

Andreas Keller & Leslie B Vosshall

At present, no satisfactory theory exists to explain how a given molecule results in the perception of a particular smell. One theory is that olfactory sensory neurons detect intramolecular vibrations of the odorous molecule. We used psychophysical methods in humans to test this vibration theory of olfaction and found no evidence to support it.

A book about the physiologist Luca Turin[1], reviewed previously in *Nature Neuroscience*[2] and elsewhere[3,4], has generated new interest in the theory that the smell of a molecule is determined by intramolecular vibrations rather than by the molecule's shape. Vibration theory was introduced in the 1930s[5] and was later extended[6], but no biological mechanism to convert molecular vibrations into neuronal activation was proposed. As a result, the theory has been largely neglected in the research community. In the 1990s, Turin proposed a transduction mechanism involving inelastic electron tunneling[7]. Whether because of skepticism or 'scientific conspiracy' (as alleged in the book and echoed in most reviews), his predictions have failed to generate empirical tests by other researchers. In the present study, we tested vibration theory's key psychophysical predictions.

All subjects gave informed consent to participate in this study and were tested in a well-ventilated examination room of the Rockefeller University hospital. Procedures were approved by the university's Institutional Review Board. To minimize observer bias, we used a double-blind protocol such that neither the subjects nor the test administrator knew the identity of the odorant in a given vial (see **Supplementary Methods** online).

Turin predicts that the smell of a mixture of guaiacol and benzaldehyde has a vanilla character not found in its components because the combined molecular vibrations of benzaldehyde and guaiacol approximate the vibrations of vanillin[7]. To test this prediction, we asked subjects to rate the vanilla character of benzaldehyde, guaiacol and a 1:1 mixture of both. Subjects were first familiarized with the individual stimuli at two different concentrations under non-blind conditions. In a subsequent test, vanillin at both concentrations was identified with an accuracy of 84%. After being familiarized with the 13-point rating scale (1 = no vanilla, 13 = extremely vanilla), subjects rated the vanilla character of the individual components and the two- and three-component mixtures, presented in random order. This procedure was done at two concentrations: a higher concentration (1/100 dilution; **Fig. 1a**) and a lower concentration (1/10,000 dilution; **Supplementary Fig. 1** online, panel a). At neither concentration did the mixture of benzaldehyde and guaiacol have a stronger vanilla character than that of its individual components. A similar result was obtained when odor pairs were rated on an odor similarity rating scale (**Supplementary Fig. 1** online, panel b).

A second prediction of vibration theory as proposed by Turin is that aldehydes with an even number of carbon atoms have a different odor than those with an odd number[7]. Subjects rated pairs of aldehydes (1/10 dilution) that differed in chain length by up to six carbon atoms. Subjects rated the two aldehydes as smelling more dissimilar as the difference in carbon atom number increased (**Fig. 1b**). Similar results were obtained with pure aldehydes (**Supplementary Fig. 2** online). Contrary to Turin's prediction, pairs consisting of two odd or two even numbered aldehydes were not perceived as more similar than pairs consisting of an odd and an even numbered aldehyde (**Fig. 1c**). We found instead, as suggested in previous studies, that the carbon chain length of these molecules is the salient feature sensed by the olfactory system[8].

A third prediction of Turin's vibration theory is that acetophenone (AP) and completely deuterated acetophenone (AP-d8), which have the same shape but different molecular vibrations, should have distinguishable smells[9]. First, subjects rated paired odors for similarity using a 10-point scale (0 = same; 10 = very different). Similarity scores for the AP versus AP-d8 pairing were no different from those of the identical-odorant pairings (**Fig. 2a**). In addition, we used a triangle test in which subjects were asked to identify the odd stimulus from among three vials (two of which contained the same substance).

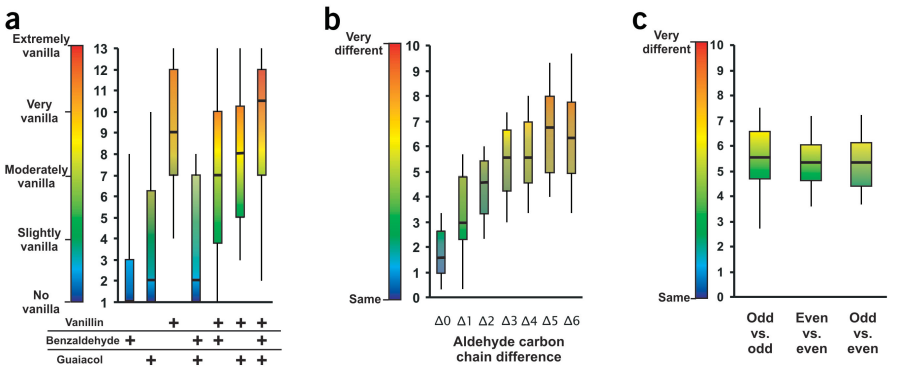

Figure 1 Additive synthesis and homologous series. (**a**) Subjects rated (on a 13-point scale[13]) the vanilla character of stimuli (1/100 dilutions) presented with an inter-trial interval of 30 s. The benzaldehyde/guaiacol mixture did not have a vanilla character stronger than either of its components (horizontal black line on each bar indicates median, boxed regions indicate 25–75% quantiles, whiskers indicate 10–90% quantiles; $n = 24$ subjects, 12 female; $P > 0.05$; Newman-Keuls test for multiple comparisons after Friedman's test). The olfactory sensation produced by vanillin is suppressed by trigeminal stimulation[14], but at the stimulus concentration used here there was no such interference, as is evident by the high score of the three-component mixture. Equivalent results with the same subjects were obtained at a 1/10,000 dilution (**Supplementary Fig. 1a** online). Purity of odors: benzaldehyde >99%, guaiacol 99.7%, vanillin 99.9%. (**b**) Odor dissimilarity of pairs of aldehydes was rated on a scale from 0 (same) to 10 (very different). Each subject ($n = 24$, 12 female) rated three randomly picked pairs from each of the seven groups (Δ0, Δ1, Δ2, Δ3, Δ4, Δ5 and Δ6). Odor solutions were 1/10 dilutions. (**c**) The data shown in **b** are replotted to compare the median similarity rating for pairs of aldehydes consisting of two odd, two even, or an odd and an even chain length. No difference between groups was found; see **Supplementary Methods** online for details.

Laboratory of Neurogenetics and Behavior, The Rockefeller University, 1230 York Avenue, Box 63, New York, New York 10021, USA. Correspondence should be addressed to A.K. (kellera@mail.rockefeller.edu).

Published online 21 March 2004; doi:10.1038/nn1215

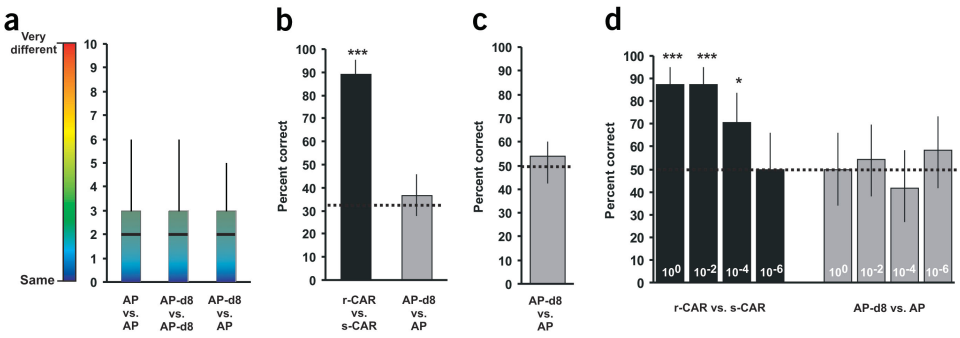

Figure 2 Isotope substitution. (**a**) The similarity between the smells of regular acetophenone (AP) and deuterated acetophenone (AP-d8) was rated on a scale from 0 (same) to 10 (very different) (horizontal black line on each bar indicates median, boxed regions indicate 25–75% quantiles, whiskers indicate 10–90% quantiles; $n = 108$, 36 and trials, respectively, for the 3 comparisons shown left to right; 36 subjects, 22 female). (**b**) Subjects easily distinguished r-carvone (r-CAR) from s-carvone (s-CAR) in triangle tests (one test per subject; $n = 36$ subjects, 22 female), but not AP from AP-d8 (two tests per subject; $n = 72$). (**c**) In duo-trio tests, two odors were presented and the subject was asked to identify the one identical to a third reference smell. Each of six subjects (1 female) took this test 30 times over the course of three days ($n = 180$ trials). (**d**) Duo-trio tests were performed with different dilutions of both r-carvone/s-carvone and AP/AP-d8 ($n = 24$ subjects, 12 female). (**b**–**d**) The percentage of correct choices and the 95% confidence intervals are shown. The dashed lines indicate chance performance. Chi-square tests were used to compare observed and expected frequencies. $^{***}P < 0.001$; $^{*}P < 0.05$. Purity of odors: AP 99.3%, AP-d8 99.9%, r-carvone/s-carvone >99%.

To verify that subjects understood the task, we included enantiomers (r-carvone and s-carvone) that are readily discriminable[10] and differ in shape but not vibration. Subjects easily distinguished the enantiomers but could not distinguish AP from AP-d8 (**Fig. 2b**). Finally, we used a duo-trio test in which two stimuli were presented and the subject was asked to identify the one identical to a third reference smell. In a separate session, we tested six subjects who had successfully distinguished AP from AP-d8 to determine whether their correct selections reflected chance performance or true discrimination of these two odorants. None of the six subjects was able to distinguish the two smells. The proportion of correct choices ranged from 43% to 67% (mean, 53%; standard error ± 14%; **Fig. 2c**).

To rule out interference of the trigeminal chemosensory system with olfactory perception seen at high stimulus concentrations[11], we used duo-trio tests to show that AP and AP-d8 were not distinguished at a wide range of concentrations (**Fig. 2d**). It has recently been reported that naive subjects perceive a difference between the odors of deuterated and regular benzaldehyde, but this previous study[12] was not run double-blind and used an anomalous version of the duo-trio test. Taken as a whole, our results provide no evidence that regular and deuterated acetophenone smell different to naive subjects. We cannot exclude the possibility, however, that olfactory training or experience could alter the outcome of the tests done here.

After testing a variety of psychophysical predictions of vibration theory, as formulated by Turin, we conclude that molecular vibrations alone cannot explain the perceived smell of an odorous molecule.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

We thank A. Gilbert for expert advice, members of the Vosshall laboratory for comments on the manuscript, and E. Gotschlich, B. Coller and the staff of the Rockefeller University Hospital. A.K. is an M.S. Stoffel Fellow in Mind, Brain and Behavior.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 18 December 2003; accepted 21 January 2004
Published online at http://www.nature.com/natureneuroscience/

1. Burr, C. *The Emperor of Scent* (Random House, New York, 2002).
2. Gilbert, A.N. *Nat. Neurosci.* **6**, 335 (2003).
3. Maslin, J. *The New York Times* February 6 (2003), p. E8.
4. Givhan, R. *The Washington Post* February 16 (2003), p. X–T8.
5. Dyson, G.M. *Chem. Ind.* 647–651 (1938).
6. Wright, R.H. *J. Theor. Biol.* **64**, 473–502 (1977).
7. Turin, L. *Chem. Senses* **21**, 773–791 (1996).
8. Laska, M. & Teubner, P. *Chem. Senses* **24**, 263–270 (1999).
9. Turin, L. & Yoshii, F. in *Handbook of Olfaction and Gustation* (ed. Doty, R.L.) 275–294 (Marcel Dekker, New York, 2003).
10. Laska, M. & Teubner, P. *Chem. Senses* **24**, 161–170 (1999).
11. Livermore, A. & Hummel, T. *Int. Arch. Occup. Environ. Health* **75**, 305–313 (2002).
12. Haffenden, L.J.W., Yaylayan, V.A. & Fortin, J. *Food Chem.* **73**, 67–72 (2001).
13. Watson, W.L., Laing, D.G., Hutchinson, I. & Jinks, A.L. *Dev. Psychobiol.* **39**, 137–145 (2001).
14. Kobal, G. & Hummel, C. *Electroencephalogr. Clin. Neurophysiol.* **71**, 241–250 (1988).