THE ROYAL SOCIETY

doi 10.1098/rspb.2000.1258

Olfactory coding in a simple system: adaptation in *Drosophila* larvae

Matthew Cobb[1*] **and Isabelle Domain**[2]

[1]*CNRS-UMR 7625, Bâtiment A 7e, 7 Quai St Bernard, 75005 Paris, France*
[2]*CNRS-UMR 8620, Université Paris-Sud, 91405 Orsay, France*

Drosophila melanogaster larvae were pre-stimulated with high concentrations of six homologous alcohols (C4–C9) and then tested for adaptation and cross-adaptation using these same alcohols, four aliphatic *n*-acetates and three acids. Pre-stimulation with hexanol effectively reduced to zero (abolished) test responses to all six alcohols, whereas test responses to hexanol were only affected by pre-stimulation with hexanol. This substance appears to play a fundamental role in the organization of the larval olfactory system. Test responses to butanol and pentanol, and the effect of pre-stimulation with butanol and pentanol, were not significantly different, indicating that they are sensory equivalents. Heptanol, octanol and nonanol induce a complex set of responses among one another. Cross-adaptation between functional groups was observed, in particular following pre-stimulation with hexanol, but there was also evidence that functional groups are coded separately. A model of olfactory processing in the fruitfly maggot is presented that explains the data and provides predictions for future anatomical, genetic and electrophysiological studies.

Keywords: *Drosophila melanogaster*; olfaction; alcohols; larvae; adaptation

1. INTRODUCTION

The olfactory responses of *Drosophila melanogaster* larvae represent a neurobiological enigma. Fruitfly maggots respond to most odours with which they are presented (51 out of 62 substances tested; Cobb 1999), and yet they possess only 21 olfactory receptor neurons (see Cobb (1999) for a review). Until recently, the *Drosophila* genome was thought to contain about 100 olfactory receptor genes (Clyne *et al.* 1999; Vosshall *et al.* 1999), but following the completion of the genomic sequence, this has now been reduced to approximately half this figure (Vosshall *et al.* 2000). We know nothing about (i) the ligand specificity of these receptors, (ii) how many genes are expressed at the larval stage, and (iii) how many types of receptor molecules are present on each larval receptor neuron. These are the key elements of a fundamental problem of olfaction: Can the system discriminate all detectable odours, or are some stimuli subject to common processing, i.e. what are the coding principles used by the olfactory system?

Most models of non-pheromonal olfactory processing assume that odour receptor ligands represent characteristic features of odour molecules ('odour primitives' (Shepherd 1991) or 'odotopes' (Hildebrand & Shepherd 1997)), which are shared by a number of odour molecules and are used to construct a central 'molecular image'. This implies that there are at least two levels to olfactory coding: the detection of 'odour primitives' and their integration.

In adult *D. melanogaster* our understanding of the principles underlying olfactory coding remains extremely limited, despite some recent electrophysiological work (de Bruyne *et al.* 1999). In adult *Drosophila*, as in vertebrates, it appears that each olfactory receptor neuron only expresses one type of olfactory receptor molecule. If the same is true at the larval stage, then at most 21 different 'odour primitives' will be detected and many odours will turn out to be sensory equivalents, i.e. sharing one or more 'odour primitives'. It is also possible that each larval olfactory receptor neuron carries more than one type of receptor molecule, as is the case in *Caenorhabditis elegans* (Troemel *et al.* 1995), each perhaps linked to a different second messenger system (Ache & Zhainazarov 1995). In this case, it is unlikely that many odours will be sensory equivalents, and the limiting factor on the detection capacity of the larval olfactory system would be the number of receptor molecule types that exist. Given the number of olfactory receptor genes in the fruitfly genome, there should thus be around 50 types of sensory equivalent. However, none of the known *Drosophila* odorant receptor genes has yet been shown to express in larvae (Vosshall *et al.* 2000), perhaps indicating that there is a separate, larva-specific family of olfactory receptors in this species.

In vertebrates and many invertebrates, olfactory integration takes place in cerebral glomeruli, which integrate signals from a large number of neurons and appear to play a major role in olfactory coding (Mori 1995). In *Drosophila* larvae, olfactory neurons show a regional arborization in the brain, reminiscent of a glomerular organization (Heimbeck *et al.* 1999). Molecular images may thus be constructed by the larval brain from the inputs of several receptor neurons, increasing the range of odours that can be discriminated.

The most rigorous way of determining receptor–ligand relationships and the nature of any integrative functions would be to carry out single-cell electrophysiological recordings in both the larval dorsal organ (where odours are detected) and in the antennal lobe of the larval brain. However, although an 'electro-antennogram' has been recorded from *Drosophila* larvae (Oppliger *et al.* 2000), no single-cell recordings from the dorsal organ have yet been reported.

Another approach is to use direct observation of larval behavioural responses to odours to reveal which odours

*Author for correspondence (mcobb@snv.jussieu.fr).

are sensory equivalents. This is more problematic than might first appear—the most reliable method is that of 'cross-adaptation' (Cobb 1999). Continual stimulation of the olfactory system with a given odour tends to result in 'adaptation' (also known as 'desensitization'), as shown by a change in the response to that odour, most often its attenuation or disappearance. If adaptation with odour A affects the response to odour B ('cross-adaptation'), it can be assumed that the two odours share some aspect of stimulus detection or processing pathways (either central or peripheral, or both). In the most extreme cases, the two odours can be taken to be 'sensory equivalents', i.e. they cannot be distinguished through detection, processing or responses. This technique has been used in systems such as bacterial chemotaxis (see Koshland (1980) for a review), isolated lobster olfactory cells (Borroni & Atema 1988, 1989; Daniel *et al.* 1994), *Drosophila* maxillary palps (de Bruyne *et al.* 1999), in *C. elegans*' behavioural responses (Colbert & Bargmann 1995) and in intact rats (Farley & Silver 1992). Although the genetic and physiological bases of adaptation in adult *Drosophila* have been studied (Störtkuhl *et al.* 1999), adaptation has never been reported in fruitfly larvae.

There appear to be two kinds of adaptation: (i) that occurring within a few seconds of stimulation, most frequently detected in individual cells by biochemical or electrophysiological methods, and (ii) that which can be characterized as psychophysiological, involving both the whole organism and much longer periods of stimulation (from several minutes to several weeks; Murphy 1987; Wang *et al.* 1993). A number of peripheral mechanisms have been invoked to explain short-term adaptation in vertebrates: a feedback loop involving Ca^{2+} channels (Kurahashi & Shibuya 1990), direct inhibition by odorants of a wide variety of ion channels (Kawai *et al.* 1997), and multiple kinetic forms of adaptation with different molecular bases (Leinders-Zufall *et al.* 1999). The cellular mechanisms involved in psychophysiological adaptation are unknown.

A range of odours with different functional groups (e.g. benzaldehyde, iso-amyl alcohol, butanone, diacetyl, pyrazine and thiazone (Colbert & Bargmann 1995); diethyl propionate, ethyl acetate, E2-hexanal and iso-amyl acetate (de Bruyne *et al.* 1999)) have been used in cross-adaptation tests and, partly for this reason, no precise conclusions have been reached as to the underlying coding principles. By using a narrower range of substances from closely related functional groups, and systematically studying odours of similar molecular sizes, it should be possible to identify the odour primitives being coded by the olfactory system, in particular if it is relatively simple, such as that possessed by the *Drosophila* larva. That was the aim of the study reported here.

2. MATERIAL AND METHODS

(a) *Preparation of larvae*

All larvae used were of the *D. melanogaster* Canton-S strain. All tests were carried out on larvae that had been fed on yeast paste for 24 h; olfactory responses in *Drosophila* larvae are maximal at this age (Cobb *et al.* 1992; Cobb & Dannet 1994). Before testing, larvae were washed from the yeast paste and starved for 1 h on a clean dish covered with agar.

(b) *Olfactory tests*

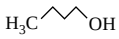
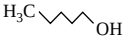
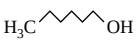
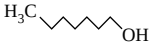
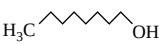
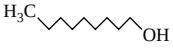
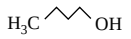
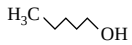
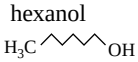
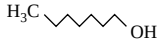
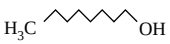
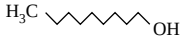
Olfactory responses were measured using the method of Cobb *et al.* (1992). A full discussion of this method and the behavioural, physicochemical and psychophysical factors involved appears in Cobb (1999). Briefly, between 20 and 70 larvae were placed at the centre of a 10 cm diameter Petri dish covered with 10 ml of 2.5% agar. The test odour (2.5 µl, in all cases) was placed on a small filter paper on one side of the dish, using a micro-syringe. All chemicals were Merck analysis grade. A control paper with no odour was placed on the other side of the dish. The lid of the dish was replaced; after 5 min the number of larvae on each side of the dish, plus the number of larvae that had not 'chosen', were noted. A larva was considered to have failed to choose if, at the end of the test, any part of its body was touching a 3 mm wide zone running across the middle of the dish. This category includes those larvae that were immobile and those that were apparently making their way towards the odour having initially gone in the other direction (Cobb 1999). The overall percentage of larvae defined as 'not responding' in the control data presented in table 1 was $15.90 \pm 1.40\%$. A response index (RI) was calculated such that $RI = ((n_{odour} - n_{control})/n_{total}) \times 100$. RI varies between -100 (total repulsion) and $+100$ (total attraction). This test does not measure gustation (Cobb *et al.* 1992) and the presence of relatively large numbers of larvae does not produce a 'stampede effect' (Monte *et al.* 1989). Each test was repeated between eight and 16 times. Odour and control sides were rotated from dish to dish. Statistical analysis was carried out using two-way ANOVA using Tukey's honestly significant difference test for differences between means, and *t*-tests where appropriate.

(c) *Adaptation*

To produce adaptation, larvae were pre-stimulated with an odour. A large piece of filter paper was placed in the lid of a Petri dish and an odorant was added to it. Larvae were placed on the agar surface of the Petri dish, the lid was then replaced, and pre-stimulation occurred. Larvae were then removed from the pre-stimulation dish with a wet brush, placed on dry filter paper for 10 s, then transferred onto a clean test dish, where responses to test odours were measured as described above. To define the appropriate conditions for adaptation, larvae were subject to pre-stimulation with six alcohols (butanol, pentanol, hexanol, heptanol, octanol and nonanol, C4–C9) separately, at varying doses and for varying times, and then tested with 2.5 µl of the same substance. The operational definition of adaptation was arbitrarily determined as a mean RI between -15 and $+15$; on this basis the durations and volumes of pre-stimulation were fixed at 1 µl h^{-1} except for pentanol (10 µl h^{-1}) and heptanol (2.5 µl 30 min^{-1}). The best mean auto-adaptation value found for heptanol (-15.14 ± 8.40) was slightly greater than the maximum allowed by the operational definition (-15). Nonanol produced no auto-adaptation at any dose, but at 100 µl 55% of larvae failed to move, presumably because of toxicity effects. Octanol induced no significant responses in control tests, but pre-stimulation induced significant repulsion to octanol at all pre-stimulation doses (1–50 µl). To control whether adaptation was permanent, adapted larvae were transferred to clean dishes for 60 min and then tested. RIs approached their control values (e.g. hexanol $= 58.43 \pm 4.26$, as against 72.94 ± 3.21), indicating that adaptation is reversible.

Table 1. *Effects of pre-stimulation with six alcohols (C4–C9) on olfactory responses to these alcohols*

(Responses are given mean response indices ($n > 7$), with standard errors in parentheses. The shaded row and column highlight the role of hexanol and aid inspection of the table. For full details of stimulus volumes, see § 2.)

	test					
	butanol	pentanol	hexanol	heptanol	octanol	nonanol
						
pre-stimulation	C4	C5	C6	C7	C8	C9
control	54.34 (8.44)	53.97 (5.80)	72.94 (3.21)	36.68 (2.48)	–3.78 (5.37)	–32.82 (3.62)
butanol C4 	9.27 (4.91)	12.74 (5.21)	36.49 (6.12)	–1.66 (4.99)	–1.80 (4.55)	–32.96 (6.05)
pentanol C5 	8.76 (2.38)	–5.50 (7.55)	49.88 (4.55)	9.42 (3.08)	–16.31 (5.20)	–20.19 (4.32)
hexanol C6 	5.21 (4.42)	–3.36 (6.14)	11.07 (3.14)	8.54 (6.60)	–1.41 (5.31)	–11.33 (7.20)
heptanol C7 	51.97 (4.29)	20.78 (5.77)	56.44 (4.32)	–15.14 (8.40)	–27.35 (8.64)	–30.05 (3.61)
octanol C8 	47.27 (4.80)	31.85 (8.08)	75.41 (5.04)	–2.26 (8.52)	–25.76 (6.32)	–46.08 (4.77)
nonanol C9 	43.71 (3.94)	21.29 (4.22)	77.63 (3.46)	–7.93 (7.83)	–21.27 (7.41)	–40.87 (7.07)

3. RESULTS

Adaptation was studied in six alcohols (butanol (C4)–nonanol (C9)) in 36 pre-stimulation/test combinations (table 1). As expected (see § 2), no adaptation occurred for nonanol, whereas the test response to octanol became repulsive following auto-adaptation. The other four alcohols all showed auto-adaptation.

Pre-stimulation with hexanol (shaded row on table 1) effectively reduced to zero (abolished) test responses to five of the six alcohols tested here (octanol induced no significant control response), with no significant differences between the responses ($F_{5,55} = 2.09$, $p =$ n.s.). Conversely, the test response to hexanol (shaded column on table 1) was abolished only in auto-adaptation, although pre-stimulation with the other five alcohols did significantly reduce the test response to hexanol ($F_{4,45} = 10.38$, $p < 0.001$).

Responses to test doses of butanol and pentanol following pre-stimulation with these two alcohols showed no significant differences (F (pre-stimulation) = 3.24, d.f. = 1,54; F(test) = 1.07, d.f. = 1,54), and no interaction ($F_{1,54} = 2.91$). This shows that reciprocal adaptation takes place between butanol and pentanol and implies that they are sensory equivalents. This is supported by the fact that they had identical effects on test responses to hexanol ($t_{20} = 1.60$, $p =$ n.s.), heptanol ($t_{14} = 1.89$, $p =$ n.s.), octanol ($t_{25} = 1.98$, $p =$ n.s.) and nonanol ($t_{26} = 1.77$, $p =$ n.s.).

Heptanol, octanol and nonanol tended to produce similar effects both as pre-stimulatory substances (rows in table 1) and as test substances (columns in table 1), yet do not appear to be sensory equivalents, in particular given the different responses induced by control tests (first row in table 1). Pre-stimulation with heptanol, octanol and nonanol did not lead to cross-adaptation to butanol, pentanol or hexanol, although there was a highly significant reduction in the test response to pentanol ($F_{3,49} = 7.60$, $p = 0.0003$). There were no significant differences between the three substances in their effect on each other ($F_{2,84} = 0.04$, $p =$ n.s.), nor was there any interaction ($F_{4,84} = 1.34$, $p =$ n.s.). Test responses to heptanol, octanol and nonanol following pre-stimulation with all six alcohols were significantly different ($F_{2,181} = 33.18$, $p < 0.001$), but there was no interaction ($F_{10,181} = 1.69$, $p =$ n.s.), indicating that test responses to all three substances vary in the same way as a function of pre-stimulation.

The effects of pre-stimulation with four alcohols (hexanol, heptanol, octanol and nonanol) on test responses to four acetates and three acids of similar chain lengths were studied (table 2). No systematic cross-adaptation was observed, indicating that odours with different functional groups are, at least in part, processed

Table 2. *Effects of pre-stimulation with four alcohols (C6–C9) on olfactory responses to four aliphatic acetates (C7–C10) and three acids (C7–C9)*

(Responses are given mean response indices ($n > 7$), with standard errors in parentheses. For full details of stimulus volumes, see § 2.)

	test						
	pentyl acetate	hexyl acetate	heptyl acetate	octyl acetate	heptanoic acid	octanoic acid	nonanoic acid
pre-stimulation	C7	C8	C9	C10	C7	C8	C9
control	75.62 (3.36)	−13.39 (5.75)	−31.34 (3.71)	−46.41 (6.12)	51.01 (2.72)	38.79 (4.75)	13.37 (7.24)
hexanol C6	17.48 (9.78)	−14.37 (3.65)	−14.45 (5.56)	−36.93 (3.46)	−13.56 (6.14)	−11.41 (2.99)	−0.61 (4.73)
heptanol C7	8.07 (4.42)	−35.49 (4.91)	−32.75 (4.15)	−55.11 (5.88)	13.33 (2.55)	−7.64 (4.23)	−8.66 (9.49)
octanol C8	29.82 (3.48)	−25.81 (2.58)	−12.66 (5.51)	−42.82 (2.52)	37.91 (4.56)	7.71 (4.26)	3.44 (3.57)
nonanol C9	20.17 (4.82)	−27.69 (3.95)	−20.60 (6.07)	−62.58 (7.02)	46.94 (3.85)	8.50 (5.16)	−4.78 (8.52)

separately. Pre-stimulation with hexanol severely affected test responses to two of the four acetates tested here. Pre-stimulation with the other three alcohols was not as effective: heptanol, octanol and nonanol had significantly different effects on test responses to the four acetates ($F_{2,83} = 11.22$, $p = 0.0001$). However, there was no interaction between pre-stimulation and test factors ($F_{6,83} = 1.30$, $p =$ n.s.), indicating that despite the different effects of each alcohol and the different responses induced by each acetate, there were no significant differences in the way test responses to these acetates varied as a function of alcohol pre-stimulation.

Pentyl acetate (C7) was the only acetate to be systematically and clearly affected by pre-stimulation with alcohols ($F_{4,43} = 13.81$, $p = 0.0001$), although only in one case (heptanol) was the test response to pentyl acetate abolished according to the operational definition adopted here. Test responses to hexyl acetate were significantly affected by pre-stimulation with alcohols ($F_{4,34} = 4.75$, $p < 0.004$): pre-stimulation with heptanol, octanol and nonanol all produced a significant repulsive response, although pre-stimulation with hexanol had no effect. The test response to octyl acetate was not abolished by pre-stimulation with any of the four alcohols, and even the largest reduction in test responses (pre-stimulation with hexanol) was not significant ($t_{14} = 1.35$, $p =$ n.s.).

Pre-stimulation with alcohols significantly reduced the test response to acids compared with control levels in all but two cases (octanol/heptanoic and nonanol/heptanoic, $F_{2,25} = 3.37$, $p =$ n.s.). The overall effects of hexanol and heptanol pre-stimulation on test responses to acids were not significantly different ($F_{1,42} = 2.79$, $p =$ n.s.), abolishing the differences in the test responses to the three acids ($F_{2,42} = 1.45$, $p =$ n.s.). Octanol and nonanol showed no significant differences in their effects ($F_{1,42} = 0.02$, $p =$ n.s.). Octanoic acid and nonanoic acid showed no differences in their responses to stimulation with alcohols ($F_{1,36} = 0.22$, $p =$ n.s.).

4. DISCUSSION

We have shown that *D. melanogaster* maggots show adaptation and cross-adaptation in their responses to olfactory stimuli, including those between functional groups. For the first time, we have a glimpse of olfactory coding in this important model organism. We draw the following three major conclusions—each merits further investigation, particularly to see whether they also apply to adult olfactory coding.

First, the processing pathway that underlies the response to hexanol plays a major role in olfactory response coding in *D. melanogaster* larvae. Pre-stimulation with hexanol abolished test responses to all alcohols, acids and acetates, with the only clear exceptions being the weak or non-existent responses to octanol and hexyl acetate and the strong repulsive response to octyl acetate (this also demonstrates that pre-stimulation with hexanol does not completely abolish the olfactory response). The apparent importance of hexanol is supported by the fact that hexanol induces the strongest electrophysiological responses in *Drosophila* larvae (Oppliger *et al.* 2000), and suggests this substance may play a particularly important

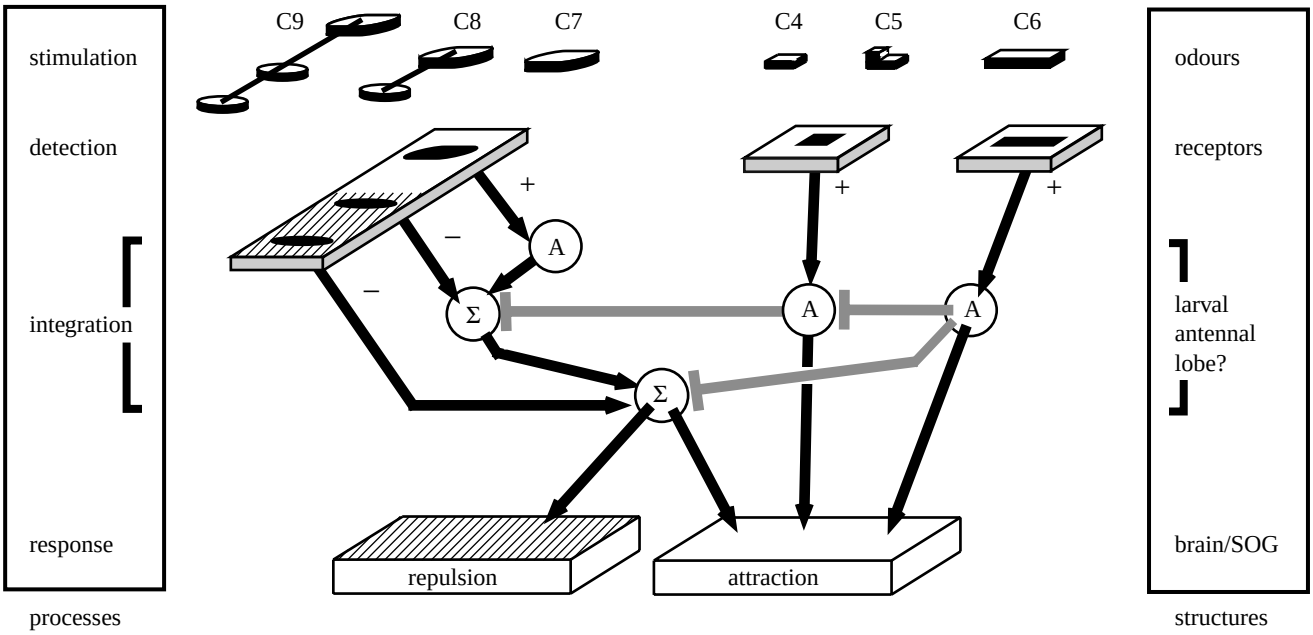


Figure 1. Model of olfactory processing of alcohols (C4–C9) in *Drosophila melanogaster* larvae, based on data in table 1. Odour molecules are represented at the top. 'Receptors' refers equally to receptor molecules and receptor neurons. SOG denotes sub-oesophageal ganglion. Round structures represent integration sites, involving either adaptation (A) or an integrative function (Σ) that sums positive (+) and negative (−) signals. Arrows indicate the direction of signal transmission; if adaptation occurs, there is no signal transmission beyond the A integration site. Grey, barred lines indicate lateral inhibition following adaptation. A site that receives lateral inhibition does not transmit its signal. Adaptation was operationally defined as a mean response index that was between −15 and +15. The only exception to this is heptanol auto-adaptation, which had a mean value slightly greater than the maximum operational definition (-15.14 ± 8.40; see § 2). This criterion means that some significant effects reported in table 1 (e.g. the reduction in hexanol test responses following pre-stimulation with butanol or pentanol) are not included in this model, which thus deals primarily with qualitative effects.

The simplest way of understanding the model is to follow the events it describes. C4 and C5 have different structures but this has no effect on detection by their shared receptor. C6 has a unique receptor. C7, C8 and C9 share a receptor that can give different outputs according to the zones that are bound. Stimulation of the C6 and C4/C5 receptors has three effects: (i) the behavioural response is attraction; (ii) excessive stimulation (i.e. pre-stimulation) leads to auto-adaptation (via the structures labelled A) and thus to no behavioural response; (iii) pre-stimulation leads to inhibition of some neighbouring integrative structures. The C4/C5 receptor only has one inhibitory effect (on C7–C8 integration); C6 affects both C4/C5 and C7/C8/C9 integration.

The C7/C8/C9 receptor has a more complex structure and can lead to either attraction or repulsion. The response is determined by two summing functions (Σ) that receive input from the three parts of the receptor, one of which can be inhibited by C4/C5, the other by C6. The part of the C7/C8/C9 receptor that is bound by all three odours produces a signal with a positive sign and can lead to auto-adaptation. The parts that are bound by C8 and C9 and by C9 alone produce a signal with a negative sign. The first summing function receives input from C7 and C8; if the positive and negative signals are equal (i.e. following a test presentation of C8), the output is zero and there is no behavioural response. If there is an inhibitory signal following adaptation with C4/C5, the output is again zero. If there is adaptation of the C7 pathway (i.e. no output from the C7 receptor following pre-stimulation with C7, C8 or C9) followed by test stimulation with C8, the output is negative. This integrative structure transmits its signal to a second-level integrative structure, which also receives negative signals from the part of the C7/C8/C9 receptor that is stimulated only by C9, and can be inhibited following pre-stimulation with C6.

role in the ecology of this species. Further behavioural, electrophysiological and ecological studies will be necessary to confirm this.

Second, the relatively simple larval olfactory system is able to generate a substantial degree of discrimination. Alcohols, acids and acetates can be discriminated, suggesting the separate detection of their functional groups, and only two of the six alcohols tested in cross-adaptation (butanol and pentanol) appear to be sensory equivalents. This high degree of discrimination may indicate that the choice of stimuli—all of which can be detected in fruit, which constitutes the natural medium of *Drosophila* larvae (e.g. in cherries; Mattheis *et al.* 1992)—was such that we have approached saturation of the olfactory system and that further investigation will reveal other substances to be sensory equivalents of the stimuli tested here. However, it seems intuitively more likely that the system is in fact capable of responding to a wide range of stimuli, which implies that the larval olfactory system possesses one or more of the following factors: a large number of specific receptors, differential ligand–receptor binding, or the integration of different 'odour primitives' to form a 'molecular image'.

Third, the existence of some examples of cross-adaptation between alcohols and acids and between some alcohols and pentyl acetate suggests that common coding principles are at work, perhaps related to chain-length detection. For example, test responses to C7 and C8 acids are profoundly affected by pre-stimulation with C6 and C7 alcohols. Furthermore, test responses to C7 acid are

unaffected by pre-stimulation with C8 and C9 alcohols, whereas test responses to C8 acid are profoundly affected. More extensive studies will be required to confirm and precisely define this apparent coding principle, and to investigate its cellular and molecular bases.

The conditions used here (30–60 min of pre-stimulation) were similar to those employed in the study of adaptation in the behavioural responses of *C. elegans* as measured in a similar paradigm (Colbert & Bargmann 1995). Nevertheless, the stimulus intensities and durations might appear extremely unnatural, and it could be argued that these data are an artefact produced by overloading the olfactory system. There are two responses to this criticism: first, inducing 'abnormal' responses from a system can nevertheless reveal aspects of its functioning; second, larvae spend their life burrowing in rotten fruit where the intensity and the duration of olfactory stimuli are probably extreme—the kind of processes described here, far from being aberrations, may be important in the ecology of this species.

In order to explore the coding principles that underlie the results presented here, the data contained in table 1 were interpreted in a graphical form (figure 1). A satisfactory model of olfactory coding should be able to explain the results and provide predictions for further studies, be they behavioural, molecular, electrophysiological or anatomical.

There are three classes of receptor in the model (each represented by a slot), which are activated by stimulus molecules. Following odour detection, signals with a positive or negative sign are transmitted to integrative structures that either lead to adaptation following pre-stimulation (A), or to a summing function. Adaptation blocks transmission of the signal to the response structures, and leads to lateral inhibition of some other integrative structures. For further explanation, refer to the figure legend.

Whereas the processes involved in the model are inferred from the data in table 1 and are thus empirically based, their equivalent anatomical structures are largely hypothetical. For example, the role given to 'receptors' in the model could be that of receptor molecules or receptor neurons, and there is no direct evidence to support the idea that the larval antennal lobe is involved in this aspect of olfactory coding. Furthermore, the kind of lateral inhibition suggested here, which is frequently encountered in studies of visual processing, is hypothetical in the case of olfaction (Laurent 1999), although lateral inhibition in the antennal lobe has been implicated in certain forms of olfactory learning in the honeybee (Linster & Smith 1997).

This model not only provides a simple explanation of the data in table 1, it also constitutes a framework for future anatomical, genetic and electrophysiological studies. Genetic variants already exist that can test some of the interpretations presented here. In particular, there are striking parallels between the genetic and psychophysiological data for C7, C8 and C9 processing. For example, there is a specific genetic anosmia to heptanol, which is located on chromosome II; larvae with this character are also anosmic to octanol, but their responses to nonanol are normal (Cobb *et al.* 1992). Furthermore, the *Indf* mutation (Cobb 1996) abolishes the response to nonanol and to octanoic acid, but not to heptanoic acid, just as pre-stimulation with nonanol abolished the response to octanoic acid, but not to heptanoic acid. *Indf* also abolishes the response to heptyl acetate, while pre-stimulation with nonanol significantly reduces the response to this substance. Finally, octanol becomes repulsive following pre-stimulation with nonanol, while one allele of *Indf* produces a strong repulsive response to octanol. These data suggest that the common C7–C9 processing network described in figure 1 is the focus of action of a number of genes.

Recent studies of olfactory coding in vertebrates and invertebrates tend to support the data presented here. For example, in isolated rat olfactory neurons, all of the receptors that responded to octanol and nonanol also responded to heptanoic, octanoic and nonanoic acid, whereas receptors that responded to hexanol only responded strongly to heptanol (Malnic *et al.* 1999). High correlations have been found in the glomerular responses of bees to heptanol, octanol and nonanol, and to pentanol, hexanol and heptanol (Sachse *et al.* 1999). Furthermore, there is a general tendency in both vertebrates and invertebrates for responses to aliphatic alcohols and acetates to show differences between C4–C7 substances and substances greater than C7 (Averill *et al.* 1988; Bargmann *et al.* 1993; Cobb & Dannet 1994; Tonosaki & Tucker 1982). These data tend to reinforce the idea that there are common coding principles at work across phyla (Hildebrand & Shepherd 1997). Part of the reason for this may be the physico-chemical properties of these substances, which would thus impose certain constraints on the evolution of the olfactory detection and processing system. The way in which the environment and its components have influenced the development of the olfactory system, in both its molecular detail and its overall sensitivity, will be an important feature of the study of olfaction.

Jean-François Ferveur is thanked for his comments and continual encouragement over the long gestation of this article. Two anonymous referees made valuable comments on a previous version.

REFERENCES

Ache, B. W. & Zhainazarov, A. 1995 Dual second-messenger pathways in olfactory transduction. *Curr. Opin. Neurobiol.* **5**, 461–466.

Averill, A. L., Reisig, W. H. & Roelofs, W. L. 1988 Specificity of olfactory responses in the tephritid fruit fly, *Rhagoletis pomonella*. *Entomologia Exp. Appl.* **47**, 211–222.

Bargmann, C. I., Hartwieg, E. & Horvitz, H. R. 1993 Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell* **74**, 515–527.

Borroni, P. F. & Atema, J. 1988 Adaptation in chemoreceptor cells. I. Self-adapting backgrounds determine threshold and cause parallel shift of response function. *J. Comp. Physiol.* A **164**, 67–74.

Borroni, P. F. & Atema, J. 1989 Adaptation in chemoreceptor cells. II. The effects of cross-adapting backgrounds depend on spectral tuning. *J. Comp. Physiol.* A **165**, 669–677.

Clyne, P. J., Warr, C. G., Freeman, M. R., Lessing, D., Kim, J. & Carlson, J. R. 1999 A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron* **22**, 327–338.

Cobb, M. 1996 Genotypic and phenotypic characterization of the *Drosophila melanogaster* olfactory mutation *Indifferent*. *Genetics* **144**, 1577–1587.

Cobb, M. 1999 What and how do maggots smell? *Biol. Rev.* **74**, 425–459.

Cobb, M. & Dannet, F. 1994 Multiple genetic control of acetate-induced olfactory responses in *Drosophila melanogaster* larvae. *Heredity* **73**, 444–455.

Cobb, M., Bruneau, S. & Jallon, J.-M. 1992 Genetic and developmental factors in the olfactory response of *Drosophila melanogaster* larvae to alcohols. *Proc. R. Soc. Lond.* B **248**, 103–109.

Colbert, H. A. & Bargmann, C. I. 1995 Odorant-specific adaptation pathways generate olfactory plasticity in *C. elegans*. *Neuron* **14**, 803–812.

Daniel, P. C., Fine, J. B., Derby, C. D., & Girardot, M.-N. 1994 Non-reciprocal cross-adaptation of spiking responses of individual olfactory receptor neurons of spiny lobsters: evidence for two excitatory transduction pathways. *Brain Res.* **643**, 136–149.

de Bruyne, M., Clyne, P. J. & Carlson, J. R. 1999 Odor coding in a model olfactory organ: the *Drosophila* maxillary palp. *J. Neurosci.* **19**, 4520–4532.

Farley, L. G. & Silver, W. L. 1992 Self- and cross-adaptation to chemical stimulation of the nasal trigeminal nerve in the rat. *Chem. Senses* **17**, 507–518.

Heimbeck, G., Bugnon, V., Gendre, N., Häberlin, C. & Stocker, R. F. 1999 Smell and taste perception in *D. melanogaster* larvae: toxin expression studies in chemosensory neurons. *J. Neurosci.* **19**, 6599–6609.

Hildebrand, J. G. & Shepherd, G. M. 1997 Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *A. Rev. Neurosci.* **20**, 595–631.

Kawai, F., Kurahashi, T. & Kaneko, A. 1997 Nonselective suppression of voltage-gated currents by odorants in the newt olfactory receptor cells. *J. Gen. Physiol.* **109**, 265–272.

Koshland, D. E. 1980 Bacterial chemotaxis in relation to neurobiology. *A. Rev. Neurosci.* **3**, 43–75.

Kurahashi, T. & Shibuya, T. 1990 Ca^{2+}-dependent adaptive properties in the solitary olfactory receptor cell of the newt. *Brain Res.* **515**, 261–268.

Laurent, G. 1999 A systems perspective on early olfactory coding. *Science* **286**, 723–728.

Leinders-Zufall, T., Ma, M. & Zufall, F. 1999 Impaired odor adaptation in olfactory receptor neurons after inhibition of Ca^{2+}/calmodulin kinase II. *J. Neurosci.* **19:RC19**, 1–6.

Linster, C. & Smith, B. H. 1997 A computational model of the response of the honey bee antennal lobe circuitry to odor mixtures: overshadowing, blocking and unblocking can arise from lateral inhibition. *Behav. Brain. Res.* **87**, 1–14.

Malnic, B., Hirono, J., Sato, T. & Buck, L. B. 1999 Combinatorial receptor codes for odors. *Cell* **96**, 713–723.

Mattheis, J. P., Buchanan, D. A. & Fellman, J. K. 1992 Volatile compounds emitted by sweet cherries (*Prunus avium* Cv. Bing) during fruit development and ripening. *J. Agric. Food Chem.* **40**, 471–474.

Monte, P., Woodard, C., Ayer, R., Lilly, M., Sun, H. & Carlson, J. 1989 Characterization of the larval olfactory response in *Drosophila* and its genetic basis. *Behav. Genet.* **19**, 267–283.

Mori, K. 1995 Relation of chemical structure to specificity of response in olfactory glomeruli. *Curr. Opin. Neurobiol.* **5**, 467–474.

Murphy, C. 1987 Olfactory psychophysics. In *Neurobiology of taste and smell* (ed. T. E. Finger & W. L. Silver), pp. 251–273. New York: Wiley.

Oppliger, F., Guerin, P. M. & Vlimant, M. 2000 Neurophysiological and behavioural evidence for an olfactory function for the dorsal organ and a gustatory one for the terminal organ in *Drosophila melanogaster* larvae. *J. Insect Physiol.* **46**, 135–144.

Sachse, S., Rappart, A. & Galizia, C. G. 1999 The spatial representation of chemical structures in the antennal lobe of honeybees: steps towards the olfactory code. *Eur. J. Neurosci.* **11**, 3970–3982.

Shepherd, G. 1991 Computational structure of the olfactory system. In *Olfaction: a model system for computational neuroscience* (ed. J. L. Davis & H. Eichenbaum), pp. 3–41. London: MIT Press.

Störtkuhl, K. F., Hovemann, B. T. & Carlson, J. R. 1999 Olfactory adaptation depends on the Trp Ca^{2+} channel in *Drosophila*. *J. Neurosci.* **19**, 4839–4846.

Tonosaki, K. & Tucker, D. 1982 Olfactory receptor cell responses of dog and box turtle to aliphatic *n*-acetates and aliphatic *n*-fatty acids. *Behav. Neural Biol.* **35**, 187–199.

Troemel, E. R., Chou, J. H., Dwyer, N. D., Colbert, H. A. & Bargmann, C. I. 1995 Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell* **83**, 207–218.

Vosshall, L. B., Amrein, H., Morozov, P. S., Rzhetsky, A. & Axel, R. 1999 A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96**, 725–736.

Vosshall, L. B., Wong, A. M. & Axel, R. 2000 An olfactory sensory map in the fly brain. *Cell* **102**, 147–160.

Wang, H. W., Wysocki, C. J. & Gold, G. H. 1993 Induction of olfactory receptor sensitivity in mice. *Science* **260**, 998–1000.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.