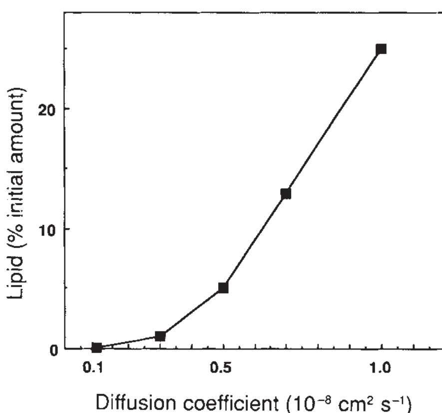
Lipid diffusion in neurons

SIR — Kobayashi *et al.*[1] suggest that the compositional differences between the axonal and somatodendritic domains of neuronal membranes are maintained by a diffusion barrier at the axon hillock. This novel suggestion is based on observations of the movement of fluorescently labelled lipids inserted exclusively into the axons of neurons infected by viruses. Although the fluorescent lipid is mobile in the axonal membrane, labelling is abruptly interrupted at the point where the axon emerges from the cell body; the cell body and dendrites do not appear to become labelled even after an hour of observation.

Using analysis similar to that developed for quantifying diffusion from a spherical cell surface to a tubular projection[2], we have calculated the amount of lipid that would diffuse from the axon to cell body after 1 h in the absence of a barrier, for lateral diffusion coefficients ranging from 10^{-9} to 10^{-8} cm^2 s^{-1} (see figure). For a lipid diffusion coefficient of 6×10^{-9} cm^2 s^{-1}, as measured in spinal cord neurons[3], only 9% of the lipid initially present in an axon of 100-μm length would diffuse into the cell body after 1 h. Under these conditions, labelling in the axonal initial segment would not disappear, as was observed[1]. Direct measurement of the diffusion coefficient of the fluorescent lipids in the virus-infected neurons would permit evaluation of the contribution of a diffusion barrier in restricting movement of axonal lipids into the cell body.



Amount of lipid in the cell body after 1 h (as a percentage of the initial amount in the axon) against diffusion coefficient. The diameters of the cell body and axon were taken as 10 and 1 μm, respectively, and the axon length as 100 μm. The amount of lipid in the cell body after 1 h does not vary significantly for longer axon lengths or larger cell body diameters (not shown). Note also that for low diffusion coefficients (10^{-9} cm^2 s^{-1}), lipid that diffuses from the axon to cell body is restricted to the area of the cell body proximal to the axon, but for higher diffusion coefficients (10^{-8} cm^2 s^{-1}), lipid is almost uniformly distributed over the whole cell body surface.

We propose that a physical barrier may not be necessary to maintain the polarized distribution of cell surface molecules in axons. Mature axons in the central nervous system are often several millimetres long and can be as long as a metre in the peripheral nervous system. Assuming typical diffusion coefficients of approximately 10^{-8} cm^2 s^{-1} for lipids and 10^{-9} cm^2 s^{-1} for proteins (although many proteins move more slowly), and using the equation $t = L^2/2D$ (where D is the lateral diffusion coefficient, L is length and t the time taken for the molecule to move L), a lipid would take approximately 6 days to move 1 mm from the distal to proximal end of an axon, and a protein 60 days. Even for a shorter axon of 100 μm, a lipid would take 80 min and a protein 14 h to diffuse this distance. It is generally assumed, at least in growing axons, that membranous material is added at the distal end of an axon. As approximately 50% of the membrane surface is internalized every hour (as measured in baby hamster kidney cells[4], although a direct measurement of the rate of membrane internalization has not been performed in neurons), the time a molecule spends in the axonal membrane before internalization will be much shorter than the time taken to diffuse long distances. Thus, the polarized distribution of a cell surface molecule could be obtained by targeting and inserting intracellular vesicles containing axonal molecules to a location in the axon that would prevent diffusion back to the cell body within a reasonable time. The significance of a physical barrier to lipid diffusion should be considered in the light of these geometrical considerations.

Anthony H. Futerman
Department of Membrane Research and Biophysics
Raya Khanin
Lee A. Segel
Department of Applied Mathematics and Computer Science,
Weizmann Institute of Science,
Rehovot 76100, Israel

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Odour detection in bees

SIR — Breed and Julian[1] argue that data they obtained from controlled agonistic interactions of worker honey-bees ". . . suggest a system of ordering of priority of cues by the bees. . ." in the context of nestmate discrimination. This interpretation builds on the "hierarchy of importance of cues" hypothesis proposed by Carlin and Hölldobler[2] to explain nestmate discrimination behaviour in carpenter ants. But Carlin and Hölldobler's data are also consistent with a concentration or, more correctly, proportion hypothesis in which the recognition signature of the queen has diminishing influence as the size of the colony increases[3,4].

Similarly, Breed and Julian's data are also consistent with a proportion hypothesis which predicts that as the proportion of a salient odorant component increases in a blend, the blend is perceived as being increasingly similar to this component (as has been demonstrated for the case of blends of two fatty acids and blends of two *n*-alkanes[5] and provided that the total concentration of the blend does not reach the point where the perception of quality is lost through saturation of the olfactory system[6]). Because the molecular mass, volatility, and receptor affinity varies among odorants, without the right sort of controls we cannot be sure that an odorant at one concentration dominates another at the same or a different concentration. Our understanding of salience of odorants as cues is complicated because salience may vary with the genotype and experience of individuals. Further, we could misinterpret our results if we do not identify the threshold concentrations below which the odorants are not detected[7]. This point is not considered by Breed and Julian who hypothesize that ". . . the presence or absence of each cue, rather than the relative concentrations of cues, seems to have the greatest importance [in mediating kin discrimination]."

To exclude the effect of relative concentration, Breed and Julian would have needed to vary the relative concentration of their odorants in binary mixtures of the two, ranging from the hexadecane component increasing from its detectability threshold concentration to methyl docosanoate decreasing to its detectability threshold concentration. A proportion hypothesis — assuming concentrations c_1 and c_2 provide equally salient stimuli of odorants 1 and 2, respectively — predicts that worker bees would increasingly favour introduced bees exposed to odorant 2 over those exposed to odorant 1, if for some increasing value of α ($0<\alpha<1$) the receiving bees themselves had been exposed to a

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$(1-\alpha)c_1{:}(1+\alpha)c_2$ blend of the two odorants. A complete switch in preference, however, might occur for a relatively small value of α. This, together with a possible greater salience for honey-bee workers of hexadecane over methyl docosanoate, could explain Breed and Julian's data under a proportion hypothesis.

A priority cue hypothesis requires that the components of a blend are individually perceived. In worker honeybees, however, olfactory receptors do not function under a "labelled-line" paradigm but rather respond to classes of compounds[8,9] to generate "across-fibre" patterns in the antennal lobe of their brains[4,10]. Thus it is unlikely, in general, that bees can identify individual components in a stable blend. This is not to say that they do not perceive a blend that has a dominant component (in terms of salience) as being similar to that component on its own, or that they cannot identify components in blends that vary across space or with time[11]. On the other hand, interaction effects[12] may cause the quality of a blend to be perceived as different from, rather than intermediate to, its component odorants, in which case the quality of the component odorants may be completely masked.

In making the statement ". . . the choice of [kin discrimination] cue compounds may be driven by the prioritization system. . . ", Breed and Julian cite a study[13] which actually supports a proportion rather than a priority hypothesis. In that study, aggression increases in a graded though asymmetrical way as differences in the discrimination signature are increased from purely genetic differences to both genetic and environmental differences. Further, data in a companion to that study[14] indicate that the level of an aggressive response among individuals in a species of ant, as in the honey-bee[15], is modulated in a graded fashion by both individualistic and *Gestalt* components of the nestmate recognition signature. These ant and honey-bee data are contrary to a priority cue hypothesis, which predicts that either the individualistic or *Gestalt* component should dominate so that no graded response is observed.

Wayne M. Getz
Department of Entomology,
University of California,
Berkeley,
California 94720, USA

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BREED REPLIES — Getz, of course, is quite wrong: his critique fails on both empirical and logical grounds. Empirically, he suggests that hexadecane might simply be overpowering methyl docosanoate at the concentrations used, and that had a series of ratios been tried we might have found a pair of concentrations giving the contrary result. There are three problems with this argument.

First, the two compounds are equally effective in changing the recognition status of individuals. If hexadecane were more overpowering than methyl docosanoate, we would expect it to have a stronger effect in the controls; it does not. Second, the effect of hexadecane remains the same over a broad range of concentrations; it operates as an off/on signal rather than a graded signal over an order of magnitude of concentration differences (M. D. B. and R. Bowden, unpublished observations). The experimentally imposed cues do not work in a vacuum but co-occur with the cues that the bees already possess: if ratios were important the ratios between existing and imposed cues would assert themselves. Third, I tested tetracosanoic acid (which is equivalent in this system to methyl docosanoate) and hexadecane in 1:3 and 3:1 ratios, and found that 27.5% (n = 51) of the bees receiving a 3:1 ratio of tetracosanoic acid to hexadecane were attacked by bees treated with a 1:3 ratio, while the attack rate was 18.2% (n = 39) in controls (in which bees all received a 1:3 ratio). This difference is not statistically significant (χ^2 = 1.86, d.f. = 1). This result is consistent with the data presented in our original paper.

The logical problem with Getz's ratio model is that as a bee flies to and from floral resources its surface is heated due to both internal heat production (from muscular activity) and to solar radiation. The thorax of a flying bee is usually several degrees warmer than the ambient temperature. Compounds that are known to be active in honey-bee recognition vary considerably in their volatility. As a flying bee heats up, concentrations of individual compounds will change differentially. Thus, the ratios of a bee arriving back at the colony may differ substantially from the ratios of departing bees. Getz's model cannot account for how bees accommodate these changes.

Getz favours the ratio model but has no empirical evidence in this behavioural context to support his argument. He attempts to undermine our position by suggesting an impossibly difficult experimental design, based on the idea that if one tries enough different ratios and enough concentrations, ultimately evidence for a ratio model will appear. I suggest that the data in our original paper, perhaps strengthened by the additional information mentioned here, establish the principle of cue prioritization firmly enough that it can only be dislodged by an empirical demonstration to the contrary.

Michael D. Breed
Department of Environmental, Population and Organismic Biology,
University of Colorado,
Boulder, Colorado 80309-0334, USA

Inside information

SIR — Gonnet and Benner[1] claim that words obtained from the one-letter code for amino acids in protein sequences "are candidates for the most unusable pieces of information". On the contrary, such words in a sequence can give valuable information about the protein. I have come across two such cases without even searching. The end of the sequence of the insulin-responsive glucose transporter (IRGT, Glut 4) from rat, mouse and man reads . . .END (. . .Glu Asn Asp)[2-6]. The second case, which may not be obvious to non-Swedes, is in the sequence of the pig lactate dehydrogenase: . . .SVIN. . . (. . .Ser Val Ile Asn. . ., at positions 302–305)[7], which in Swedish means 'pig', and which should leave no doubts about the protein origin. However, the chicken lactate dehydrogenase H-chain is also labelled SVIN (ref. 8), which calls into question the reliability of these built-in pieces of information.

Lars C. G. Andersson
Department of Biochemistry (N),
Biomedical Center,
University of Uppsala,
PO Box 576,
S-751 23 Uppsala, Sweden

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