

to ascertain what component of the psychopharmacological effects of chocolate, if any, is mediated by an indirect activation of the endogenous cannabinoid system.

Di Marzo and co-workers have tested the role of endogenous cannabinoids in chocolate craving by investigating the extent to which NAEs and anandamide reach the bloodstream and exert their pharmacological effects after oral administration. To test whether anandamide or oleamide (which is not an NAE, but inhibits the enzymatic hydrolysis of anandamide) elicit overt cannabis-like effects in mice, they gave anandamide or oleamide orally in quantities similar to those found in chocolate, and assessed their psychotropic effects by a standard behavioural procedure that is used in the screening of cannabinoid-receptor ligands. From their results, they conclude that the content of NAEs and other cannabinoid-related compounds in cocoa is insufficient to elicit cannabis-like effects.

These results lend themselves to two considerations. First, it seems illogical to presume that compounds present in chocolate should display a pharmacological profile similar to that of cannabis. This would be the same as assuming that cocoa and cannabis have comparable psychoactive effects, which is evidently not the case. It would have been more informative to compare anandamide and oleamide to cocoa in a standard drug discrimination test. Second, the effects of *N*-oleylethanolamine and *N*-linoleylethanolamine were not investigated: not only are these NAEs present in chocolate in greater amounts than anandamide, but they are also produced in neurons through an activity-dependent mechanism that is similar to the one implicated in anandamide formation⁶. The possibility that these compounds act synergistically to prevent anandamide degradation *in vivo* therefore remains to be investigated.

In conclusion, although the results of Di Marzo *et al.*'s study will reassure manufacturing companies that the risks of chocolate consumption do not include cannabis-like intoxication, they provide little new information on the intriguing psychopharmacology of cocoa. This substance remains, in R. J. Huxtable's apt words, "more than a food but less than a drug"⁷.

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- Desarnaud, F., Cadas, H. & Piomelli, D. *J. Biol. Chem.* **270**, 6030–6035 (1995).
- di Tomaso, E., Beltramo, M. & Piomelli, D. *Nature* **382**, 677–678 (1996).
- Arnone, M. *et al. Psychopharmacol.* **132**, 104–106 (1997).
- Colombo, G. *et al. Life Sci.* **63**, PL113–PL117 (1998).
- McGregor, I. *et al. Proc. Int. Cannabinoid Res. Soc. Meet.* **76** (1997).
- Di Marzo V. *et al. Nature* **372**, 686–691 (1994).
- Huxtable, R. J. *Nature* **382**, 411–412 (1994).

Honeybees link sights to smells

It is common for a smell or a sound to trigger a vivid recollection of an associated event in the past, even if it involves a different sensory modality and the episode occurred a long time ago^{1,2}. The human brain displays impressive cross-modal associative recall. Here we investigate whether this capacity extends to insects. We find that trained honeybees can recall a specific colour when they encounter a particular scent.

To investigate whether recall of a colour can be triggered by exposure to a scent, the two kinds of stimulus should be presented sequentially. Furthermore, the colour should not be present when the scent is encountered, and vice versa. We met these requirements by using the apparatus shown in Fig. 1a. As the bees entered the first part of the apparatus, chamber A, they received an olfactory stimulus from a vial placed at the entrance. They then had to fly to chamber B, which has two exits, one marked with the colour blue and the other with yellow. The bees had to choose the yellow exit if they had encountered the scent of mango at the entrance to chamber A, and the blue exit if they had encountered the smell of lemon. The blue and yellow labels in chamber B were interchanged every 10 minutes, and the reward moved with the appropriate colour, to make sure the bees found the reward by associating each scent with the appropriate colour, rather than with a particular exit in chamber B (left or right).

After one day's training, the bees were tested in the apparatus one at a time. When the bees encountered the scent of mango at the entrance to chamber A, they showed a strong and significant preference for the

yellow exit. When the entrance was lemon scented, they preferred the blue exit (Fig. 1b, experiment 1). The scent of mango evidently evoked recall of yellow, and lemon triggered recall of blue. Bees could also be trained to make the opposite associations: yellow with lemon, and blue with mango (Fig. 1b, experiment 2).

Bees trained on this task sometimes hesitate to choose a colour in chamber B, then return to the entrance of chamber A and hover in front of the scented vial with extended antennae, as if to sample the scent once more, before returning to chamber B to make their choice. We also found the reverse phenomenon, that bees can be trained to recall a specific scent when they see a particular colour (data not shown).

'Associative learning' describes the process by which an organism learns to associate a particular sensory stimulus with a reward or a punishment^{3–9}. The associative recall that we describe here, however, is more elaborate than this. It involves both the grouping together of reward and signalling sensory stimuli of different modalities in memory, and the recall of one member of a grouped pair when the other member is encountered.

For a foraging honeybee, cross-modal associative recall can facilitate the search for a food source; for example, detecting the scent of lavender can initiate a search for purple flowers. Indeed, one might surmise that the nectar samples received by a potential foraging recruit from a dancing bee may stimulate the recruit into recalling many of the host flower's other attributes, such as its colour, shape and texture, and perhaps even the route to the flower patch if the recruit has previously visited it.

In nature, the smell and appearance of flowers are often (but not always) detected at the same time. In our experiments we presented the olfactory and visual stimuli

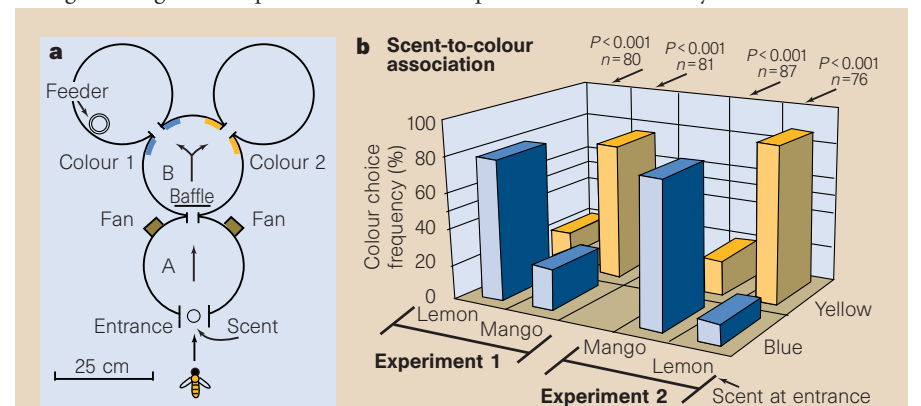


Figure 1 Cross-modal associative recall in honeybees. **a**, Experimental set-up. Bees had to choose the appropriate colour in chamber B (blue or yellow) according to the scent (lemon or mango) they experienced from a vial at the entrance to chamber A. The fans created a slight positive pressure in chamber A, ensuring that the bees encountered the scent only as they entered that chamber, and not in chamber B. The baffle ensured that the bees did not see the colours until they entered chamber B. **b**, Results of tests; *n* indicates the number of choices analysed and *P* represents the confidence level¹⁰ for choice frequencies being significantly different from random choice (red line).

sequentially, to ensure that we were eliciting associative recall. It remains to be seen whether bees that have experienced the colour and the scent of an object simultaneously can recall one of the object's attributes when the other is presented in isolation.

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1. Hintzman, D. L. *The Psychology of Learning and Memory* (Freeman, San Francisco, 1978).
2. Baddeley, A. *Your Memory: A User's Guide* 169–172 (Penguin, London, 1993).
3. Dickinson, A. *Contemporary Animal Learning Theory*. (Cambridge Univ. Press, 1980).
4. Mackintosh, N. J. *Conditioning and Associative Learning* (Oxford Univ. Press, 1983).
5. Menzel, R. in *Neurobiology of Comparative Cognition* (eds Kesner, R. P. & Olton, D. S.) 237–292 (Erlbaum, Hillsdale, NJ, 1990).
6. Gould, J. L. & Gould, C. G. *The Honey Bee* (Freeman, New York, 1988).
7. Bitterman, M. E. *Anim. Learn. Behav.* **24**, 123–141 (1996).
8. Wehner, R. in *Handbook of Sensory Physiology* Vol. 7/6C (ed. Autrum, H.) 287–616 (Springer, Berlin, 1981).
9. Couvillion, P. A. & Bitterman, M. E. *Anim. Learn. Behav.* **16**, 67–74 (1988).
10. van Hateren, J. H., Srinivasan, M. V. & Wait, P. B. *J. Comp. Physiol. A* **167**, 649–654 (1990).

Immobile plasticizer in flexible PVC

Plasticized poly(vinyl chloride) (PVC) is one of the most widely used polymeric materials in medical and related applications, and usually contains up to 40 per cent di-(2-ethylhexyl)phthalate (DEHP), which acts as the 'plasticizer' to impart flexibility to an otherwise rigid PVC¹. The plasticizer can migrate from PVC-based devices and storage bags into physiological fluids, however, and has been detected in storage media such as blood, plasma, serum, drug solutions and fatty foods^{2–4}, as well as in the bodies of patients undergoing haemodialysis and transfusion⁵. This is a concern because DEHP is a lipid-removing liver carcinogen⁶, and causes hepatic⁷ and reproductive toxicity⁸ in rodents, although opinion is divided on its toxicity in humans⁹.

Attempts to prevent this migration have included coating, grafting or blending PVC with other polymers, glow-discharge treatment of the surface of PVC, prolonged ultraviolet irradiation, and photocrosslinking of dithiocarbamated PVC¹⁰. We have developed a surface-modification technique to prevent this migration, and show that plasticized PVC becomes extremely resistant to migration when treated with sodium sulphide in the presence of a suitable phase-transfer catalyst in water. The treatment should benefit the medical and related applications of flexible PVC.

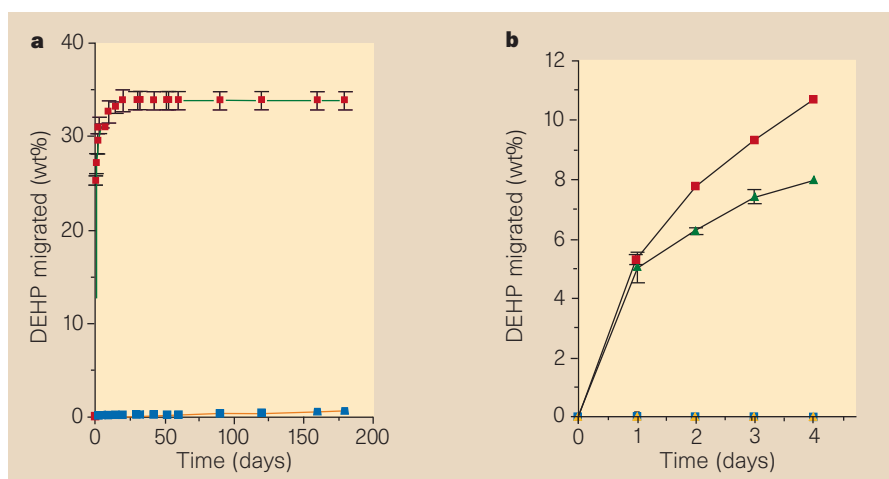


Figure 1 Surface modifications prevent migration of DEHP from PVC. **a**, Migration of DEHP from unmodified (red squares) and modified (blue squares) PVC tubes into petroleum ether at 30 °C. **b**, Migration of DEHP from unmodified (red and green symbols) and modified (blue and yellow symbols) PVC tubes into cotton-seed oil (squares) and poly(ethylene glycol)-400 (PEG400; triangles) at 70 °C.

The formation of dialkyl sulphide from an alkyl halide and sodium sulphide is well documented and proceeds readily in the presence of a suitable phase-transfer catalyst¹¹. The chlorine atoms on PVC are labile and can be substituted by various other nucleophiles¹². Our strategy was to substitute the chlorine on PVC using a dianion such as sulphide, which causes the polymer chains to crosslink. If crosslinking is confined mainly to the surface of PVC by reacting plasticized PVC in a solvent in which the polymer is insoluble, such as water, then such surface crosslinking should retard or prevent the diffusion of the plasticizer.

We therefore treated medical-grade PVC tubes (from Solmed, Denmark) with sodium sulphide (7.0 mol per litre) in the presence of tetrabutyl ammonium hydrogen sulphate (0.15 mol per litre) as the phase-transfer catalyst at 80 °C in water for 5 h, with occasional shaking. Specimens were then washed with plenty of water, sonicated for 1 min in a bath-type sonicator and dried to constant weight at 50 °C. X-ray photoelectron spectroscopy and elemental sulphur analysis showed that sulphur was present in the modified PVC specimens. The crosslinked network formed on the surface of PVC could be separated after treating it with tetrahydrofuran, causing only the uncrosslinked part to dissolve, along with other additives in flexible PVC.

The amount of DEHP that migrated into petroleum ether, a potential extractant for DEHP, over 6 months at 30 °C was determined spectrophotometrically from unmodified and surface-modified PVC tubes (Fig. 1a). Virtually no plasticizer migrated into this medium from surface-modified specimens, whereas unmodified PVC lost almost all its plasticizer in a day. Accelerated migration at 70 °C, followed by monitoring the change in weight gravimetrically, indicated that no migration

occurred from the modified specimens into media of different polarities, such as cotton-seed oil and PEG400 (Fig. 1b).

Surface modification imparted a slight yellow colour to the tubes, causing optical transmittance to decrease by about 20 per cent in the 400–500 nm region of the spectrum. At 500–700 nm, the optical transmittance of the modified tubes was similar to that of the unmodified tubes (about 80 per cent).

When the surfaces of flexible PVC sheets were similarly modified, we found that plasticizer migration could be prevented completely, as it was in PVC tubes. However, modification led to a decrease in the stress (about 8 per cent) and strain (about 28 per cent) at the breaking point for PVC sheets. There was also significantly reduced adhesion of platelets and bacteria to the surface-modified sheets.

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1. Blass, C. R., Jones, C. & Courtney, J. M. *Int. J. Artif. Organs* **15**, 200–203 (1992).
2. Jaeger, R. J. & Rubin, R. J. *Science* **170**, 460–462 (1970).
3. Waugh, N. W., Trissel, L. A. & Stella, V. J. *Am. J. Hosp. Pharm.* **48**, 1520–1524 (1991).
4. Castle, L., Mayo, A. & Gilbert, J. *Food Addit. Contam.* **7**, 29–36 (1990).
5. Plonait, S. L., Nau, H., Maier, R. F., Wittfoht, W. & Obladen, M. *Transfusion* **33**, 598–605 (1993).
6. Ledwith, B. J. *et al. Mol. Carcin.* **8**, 20–27 (1993).
7. Winberg, L. D. & Badr, M. Z. *Toxicol. Lett.* **76**, 63–69 (1995).
8. Davis, B. J., Maronpot, R. R. & Heindel, J. J. *Toxicol. Appl. Pharmacol.* **128**, 216–223 (1994).
9. Cadogan, D. F. *Proc. Inst. Materials Int. Conf. 'PVC 96'* (British Plastics Federation, London, 1996).
10. Lakshmi, S. & Jayakrishnan, A. *Polymer* **39**, 151–157 (1998).
11. Starks, C. M. & Liotta, C. *Phase Transfer Catalysis: Principles and Techniques* (Academic, New York, 1979).
12. Okawara, M. & Ochiai, Y. in *Modification of Polymers* (eds Carraher, C. E. & Tsuda, M.) 41–57 (American Chemical Society, Symp. ser. 121, Washington DC, 1980).