3. Casselman, J. M. in *Proc. 1980 North Am. Eel Conf.* (ed. Loftus, K. H.) 74–82 (Ontario Ministry of Natural Resources, Ontario, 1982).
4. Radtke, R. L. *Comp. Biochem. Physiol. A* **92,** 189–193 (1989).
5. Kalish, J. M. *J. Exp. Mar. Biol. Ecol.* **132,** 151–178 (1989).
6. Secor, D. H. *Fish. Bull. US* **90,** 798–806 (1992).
7. Tzeng, W. N., Severin, K. P. & Wickström, H. *Mar. Ecol. Prog. Ser.* **149,** 73–81 (1997).
8. Angino, E. E., Billings, G. K. & Anderson, N. *Chem. Geol.* **1,** 145–153 (1966).
9. Nakai, I., Iwata, R. & Tsukamoto, K. *Spectrochim. Acta B* (in the press).
10. Otake, T., Ishii, T., Nakahara, M. & Nakamura, R. *Mar. Ecol. Prog. Ser.* **112,** 189–193 (1994).

Trick or treat from food endocannabinoids?

The discovery of the endogenous cannabinoid *N*-arachidonoylethanolamine (anandamide)[1] and other *N*-acylethanolamines (NAEs) in chocolate[2] has led to speculation that the purported rewarding properties of cocoa are due to the presence of compounds "that could act as cannabinoid mimics"[2]. This observation raises some important questions. First, are NAEs and anandamide, or the 'endocannabinoid' 2-arachidonoylglycerol (2-AG)[3], present in widely consumed foods (such as milk) that are less 'rewarding' than chocolate? And second, to what extent do these compounds reach the bloodstream and exert pharmacological effects when consumed orally? We believe that the content of endocannabinoids in foods, and in cocoa in particular, is not sufficient to produce cannabis-like effects in mammals.

We purified NAEs and 2-AG from various foods (including mature human, bovine and goat milk, and cocoa at various stages of processing) and quantified them by gas chromatography and mass spectrometry. Milk was the only food analysed in which an endocannabinoid, 2-AG, was found in relatively high concentrations (0.33 ± 0.11 μg ml^{-1} in human milk, for example). The level of NAEs varied between 0.003 and 0.024 μg ml^{-1}, with anandamide being the least abundant compound. Oleamide, a sleep-inducing substance[4] that inhibits the hydrolysis of anandamide[5,6], was also present (0.055–1.27 μg ml^{-1}).

In cocoa-derived samples, we detected NAEs (0.01–5.8 μg per g) and oleamide (0.17–6.0 μg per g), but no or very little anandamide and no 2-AG. NAE levels are much lower in unfermented cocoa beans than in cocoa powder (which contained less than 0.003 μg per g anandamide). Tiny amounts of anandamide in cocoa could therefore be explained as artefacts of processing[2]. Like all higher plants, cocoa plants cannot synthesize arachidonic acid or its derivatives[7]. Notably, in their NAE and oleamide content, cocoa and dark chocolate are similar to other plant-derived foods (including soybean, hazelnuts, oatmeal and millet), in which we detected up to 2.3, 1.1 and 2.8 μg per g oleamide, *N*-oleoyl-ethanolamine and *N*-linoleoyl-ethanolamine, respectively.

To establish whether endocannabinoids survive their passage through the digestive system, we assayed anandamide and 2-AG after oral administration in a series of *in vivo* tests that are used widely to assess cannabimimetic activity[8] (Table 1). The two compounds were active in four of the five behavioural tests, but only at very high concentrations relative to those in foods. In contrast, a smaller dose of the psychoactive component of marijuana, (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC), was sufficient to give a strong effect in all tests.

Because intraperitoneal anandamide and 2-AG are active in the same tests at doses 20- to 60-fold lower[3,9], these results indicate that only 1.6–5% of the orally administered compounds enter the bloodstream, probably owing to the high levels in the gastrointestinal tract of the enzyme fatty acid amide hydrolase[10], which catalyses the hydrolysis of both compounds. Therefore, it is unlikely that the amounts of anandamide and 2-AG found in food are sufficient to produce observable psychotropic effects. We did not test other unsaturated NAEs that have been suggested to enhance endogenous anandamide activity by inhibiting its inactivation[2], but instead assayed oleamide — for which this kind of action is also supported by pharmacological data[5,6]. High oral doses are again necessary for activity to be observed *in vivo* (Table 1).

Our results show that the amounts of anandamide, 2-AG and oleamide in foods, including milk and cocoa, are several orders of magnitude below those required, if administered by mouth, to reach the blood and cause observable 'central' effects. The assays used here provide a gross evaluation of cannabimimetic activity, and tests monitoring more subtle behavioural changes that might be induced by low oral doses of NAEs/oleamide are needed before the relevance of these compounds to the purported mild rewarding and craving-inducing effects of cocoa can be dismissed. The presence in milk of 2-AG (which may be released from triglycerides during digestion) and oleamide also needs to be investigated.

Table 1 **Effects of test substances on performance of behavioural tests in mice**

	Olive oil (vehicle)	Anandamide (300 mg kg^{-1})	2-AG (400 mg kg^{-1})	Δ^9-THC (90 mg kg^{-1})	Oleamide (200 mg kg^{-1})
Ambulation	77 ± 11	51 ± 8*	13 ± 9**	34 ± 7**	39 ± 6**
Rearing	29 ± 5	17 ± 4*	3 ± 2**	3 ± 3**	12 ± 2**
Immobility	22 ± 7	70 ± 20*	157 ± 19**	146 ± 18**	85 ± 19*
Analgesia	12 ± 0.4	16 ± 3	10 ± 1	25 ± 2**	15 ± 3
Hypothermia	−0.2 ± 0.25	−2.30 ± 0.42**	−3.2 ± 0.5**	−4.1 ± 1.0**	−2.8 ± 0.56**

Effects of orally administered olive oil, anandamide, 2-AG, Δ^9-THC and oleamide on mouse ambulation (number of square crossings) and rearing (number of offspring reared) in an open field, immobility (time spent motionless on a ring, in seconds), analgesia (response latency to hind paw lick on a hot plate (54 °C), in seconds), and hypothermia (decrease in rectal temperature, in °C)[6,7,9,10]. The procedures are not harmful to mice and meet with NIH ethical standards. Substances were administered to Sabra female mice by mouth by gavaging. Doses lower than those shown did not produce any statistically significant effect. Results are mean ± s.e.m. from 4–9 mice. *, $P < 0.01$; **, $P < 0.005$; assessed by analysis of variance followed by Newman-Keuls post-hoc tests for group differences.

Vincenzo Di Marzo*, Nunzio Sepe*, Luciano De Petrocellis†, Alvin Berger‡, Gayle Crozier‡, Ester Fride§, Raphael Mechoulam§

**Istituto per la Chimica di Molecole di Interesse Biologico and †Istituto di Cibernetica, Consiglio Nazionale delle Ricerche, Via Toiano 6, 80072 Arco Felice, Naples, Italy*
e-mail: vdm@trinc.icmib.na.cnr.it
‡Nestlé Research Centre, Nestec Ltd, Vers-Chez-Les Blanc, 1000 Lausanne 26, Switzerland
§Department of Natural Products, David Bloom Centre for Pharmacy, Faculty of Medicine, Hebrew University, Jerusalem 91120, Israel

1. Devane, W. A. *et al. Science* **258,** 1946–1949 (1992).
2. di Tomaso, E., Beltramo, M. & Piomelli, D. *Nature* **382,** 677–678 (1996).
3. Mechoulam, R. *et al. Biochem. Pharmacol.* **50,** 83–90 (1995).
4. Cravatt, B.F. *et al. Science* **268,** 1506–1509 (1995).
5. Mechoulam, R. *et al. Nature* **389,** 25–26 (1997).
6. Bisogno, T. *et al. Eur. J. Biochem.* **254,** 634–642 (1998).
7. Cook, H. W. *Biochemistry of Lipids, Lipoproteins and Membranes* 141–169 (Elsevier, Amsterdam, 1991).
8. Martin, B. R. *et al. Pharmacol. Biochem. Behav.* **40,** 471–478 (1991).
9. Fride, E. & Mechoulam, R. *Eur. J. Pharmacol.* **231,** 313–314 (1993).
10. Katayama, K. *et al. Biochim. Biophys. Acta* **1347,** 212–218 (1997).

Beltramo and Piomelli reply — The two most abundant *N*-acylethanolamines (NAEs) found in chocolate (*N*-oleyl-ethanolamine and *N*-linoleylethanolamine) do not activate brain cannabinoid receptors but effectively inhibit anandamide degradation[1,2]. This observation suggested to us that these compounds might contribute to the hedonic properties of chocolate by causing non-metabolized anandamide to accumulate at its sites of action[2].

How can this hypothesis be tested? One possibility is to determine whether antagonists of cannabinoid receptors prevent or reduce chocolate craving. However, because cannabinoid antagonists also reduce the intake of other palatable foods and drinks[3–5], the results of this experiment would be difficult to interpret. Another approach would be to test whether *N*-oleyl-ethanolamine and *N*-linoleylethanolamine act as reinforcing stimuli in behavioural experiments. It is possible, for example, to determine in animals whether these compounds possess discriminative properties, a measure of their 'subjective' effects. In either case, the aim is

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 formation[6]. 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].

Massimiliano Beltramo, Daniele Piomelli
Department of Pharmacology, 360 Med Surge II, University of California at Irvine, Irvine, California 92697-4625, USA

1. Desarnaud, F., Cadas, H. & Piomelli, D. *J. Biol. Chem.* **270,** 6030–6035 (1995).
2. di Tomaso, E., Beltramo, M. & Piomelli, D. *Nature* **382,** 677–678 (1996).
3. Arnone, M. *et al. Psychopharmacol.* **132,** 104–106 (1997).
4. Colombo, G. *et al. Life Sci.* **63,** PL113–PL117 (1998).
5. McGregor, I. *et al. Proc. Int. Cannabinoid Res. Soc. Meet.* 76 (1997).
6. Di Marzo V. *et al. Nature* **372,** 686–691 (1994).
7. 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

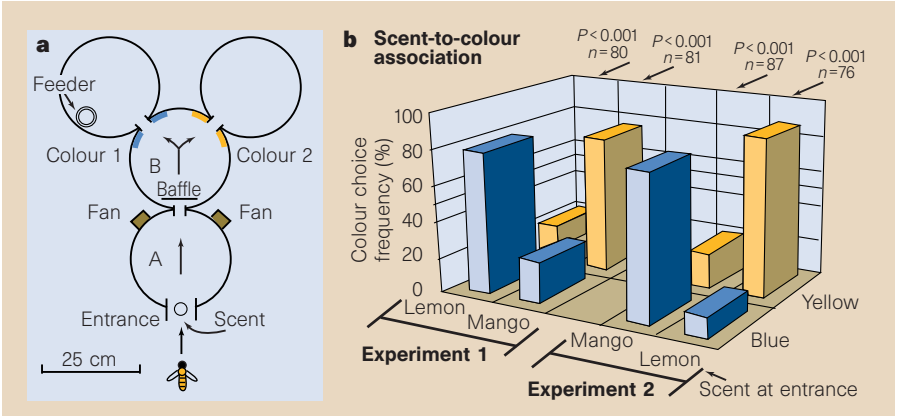

Figure 1 Cross-modal associative recall in honeybees. **a,** Experimental set-up. Bees had to choose the appropriate colour in chamber B (blue or yellow) according to the scent (lemon or mango) they experienced from a vial at the entrance to chamber A. The fans created a slight positive pressure in chamber 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 level[10] for choice frequencies being significantly different from random choice (red line).