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Food preferences in mice: an analysis using Gentiana lutea extract and enanthic acid

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Biologia. — *Food preferences in mice: an analysis using* Gentiana lutea *extract and enanthic acid* (*). Nota di MARISA MAINARDI, ANTONIO PASQUALI, BRUNO SCACCHETTI e ADRIANA DAMONI, presentata (**) dal Socio S. RANZI.

RIASSUNTO. — È stata saggiata la preferenza alimentare in topi adulti con diversa esperienza alimentare precoce. L'esperienza era fatta con cibo commerciale addizionato con estratto di genziana o con acido eptanoico oppure con cibo commerciale neutro. La preferenza è stata valutata mediante un test di scelta binaria tra cibo trattato e cibo neutro che fornisce una informazione quantizzabile. I risultati mostrano come l'esperienza precoce con l'estratto di genziana non cambi il comportamento in età adulta nei riguardi di questo cibo. Al contrario l'acido eptanoico si è rivelato sostanza efficace nel condizionare le preferenze alimentari in questa specie.

Animals have the capacity to recognize which substances they can take as food, and thus to choose between edible and non-edible matter. Moreover, in some cases it has been established that an animal can discriminate between two or more kinds of food on the basis of the physical and chemical stimuli associated with them. However, the studies on the determinism of feeding preferences are few, and cover a rather limited number of species.

An interesting question is whether the behavioural patterns of food choice are genetically determined, or whether they are the result of previous experience. In the mouse (*Mus musculus*), it has been demonstrated that the preference for saccharin can be traced to a single gene [1]. In some snakes (*Natrix* spp.), it has also been shown that certain preferences are purely genetic [2, 3, 4]. In other cases the choice is presumably mediated by early experiences. This is true of the turtle *Pseudemis scripta elegans* [5] of the bird *Taeniopigia castanotis* [6] of the dog (*Canis familiaris*) and the cat (*Felis catus*) [7]. In the rat, the via-milk experience of maternal diet may influence later food choices [8, 9, 10].

However, in all these cases the effect of feeding experiences on later choices is not permanent, and tends to disappear with the passage of time. In the ring

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dove (*Streptopelia risoria*) the preference for rice or wheat depends on early food experiences and seems to be independent of time [11]. In the garter snake (*Thamnophis sirtalis*) the preference is stable and it is directed at the food first ingested at the time of hatching [12, 13]. A similar phenomenon is found in two gull species, *Larus argentatus* and *L. delawarensis* [14], and in the turtle *Chelidra serpentina* [15]. In the chicken (*Gallus gallus*), the data recorded by Hess [16] and Burghardt [3] have shown the acquisition of permanent feeding preferences resulting from a specific experience during a sensitive period—a case of food imprinting.

In the mouse (*Mus musculus*), the subject of our research, it has been shown that there is a consistent preference for wheat in a choice between wheat and barley. However, this preference changes when the subjects are reared exclusively on barley or on wheat [17]. Subjects submitted to a binary test between rice and wheat showed a tendency to prefer the food different from that on which they had been reared. Hence, this species is capable of memorizing the stimuli associated with rice and wheat. When similar tests were carried out on mice reared on a commercial diet versus mice reared on the same diet mixed with chrysalis flour, it was revealed that chrysalis flour does not represent a stimulus capable of influencing later food choices [18].

Thus, in mice, the choice of food may depend, at least in part, on a learning mechanism. However, only some of the stimuli provided by the food are effective in determining the learning process.

In this research it was proposed to further clarify this learning phenomenon by using unpleasant foods, with a strongly distinctive smell and taste, not included in the natural diet of this species. Our experiments were designed in order to test the hypothesis that early experience with these foods may make them agreeable, or at least indifferent, to the adult individual.

Methods

The apparatus for testing food preferences consisted of a series of containers $70 \times 70 \times 50$ cm. In the centre of each container there was a plastic structure made up of two trays $6 \times 3 \times 3$ cm connected to a central tube. Incorporated into this structure (and in the middle of the trays) was a system of photoelectric cells connected via the central tube to two electromagnetic counters (hand reset). Each time a subject put its muzzle into the food tray the counter recorded the event. From the record made in this way it was possible to obtain the number of times each subject approached the food contained in one tray in a given period of time.

In our experiment the information concerning the food chosen was obtained by leaving each subject in one container for 24 hours. The container was supplied with water, nest-material, and with two different kinds of food (one per tray). The subjects had been fed normally. At each test, the two trays were exchanged so that 50 % of the subjects would experience one kind of food on the left and 50 % on the right. The subjects were assigned a score in terms of percent contacts made with one food tray over total number of contacts made with both trays in 24 hours.

We did two different experiments with gentian extract and with heptanoic acid respectively. The extract obtained from the roots of the yellow gentian (*Gentiana lutea*) contains bitter glycosides (gentiopicrin, gentsin, gentiomarine, and gentiocaulin), an alkaloid (gentianin), a yellow pigment (gentisin), and pectin, ethereal oil, sugars and tannin. The food pellets used in our tests were soaked in a 10 % solution of gentian extract in water, left to dry, and were then milled into flour and sieved. The same technique was used to obtain the control food but the pellets were soaked in water. Enanthic acid is a fatty acid with seven carbon atoms ($CH_3(CH_2)_5COOH$). Again, the commercial food pellets were soaked in a solution of fatty acid in methyl chloride, and the methyl chloride was then removed in a vacuum pan. The proportion of heptanoic acid was 1 cc per 100 grams food.

RESULTS

In the first set of experiments, 52 albino mice (Swiss strain), three-month-old males and females, were submitted to a binary-choice test (gentian treated pellets versus non-treated pellets). The feeding preference of these subjects was quantified in terms of percent contacts with treated pellets over the total number of contacts in 24 hours. The test was repeated one week later (51 subjects). Table 1 shows that the average score of the subjects decreased in the second test but the difference between test 1 and test 2 is not significant ($t = 1.88$; $P > 5\%$).

TABLE I

Comparison of average scores obtained by subjects in the parental generation in two tests (non-treated food versus food treated with gentian extract).

	TEST 1	TEST 2
Total individuals	52	51
Average score	41.17	34.77
Standard error	2.79	2.01
t	1.88	
D.o.f.	101	
P	> 5%	

The subjects were then interbred and the females isolated as they became pregnant. One group of females, starting from the 15th day of pregnancy, was fed only on commercial food treated with gentian extract. They and their offspring were kept on this diet until the latter were one month of age. A second group of females (with their offspring) was fed only on non-treated food. In this way, in the first generation, we produced 80 mice reared exclusively on treated food and 45 mice (controls) reared exlusively on non-treated commercial pellets.

TABLE II

Comparison of average scores obtained by subjects of the first generation reared on food treated with gentian extract and controls.

	Reared on treated food	Controls
Total individuals	80	45
Average score	33.19	31.96
Standard error	2.99	2.41
t	0.38	
D.o.f.	123	
P	> 5 %	

Both groups of offspring were then fed on non-treated food until they were two months of age, when they were tested for binary choice in our apparatus. Table 2 shows that the behaviour of the two groups of offspring toward the treated pellets did not differ markedly. As for their parents, all the individuals of the first generation refused the treated food, even when they had had an early and prolonged experience of it ($t = 0.38$; $P > 5\,\%$).

In the second set of experiments, 49 albino mice (Swiss strain), three-month-old males and females, reared on a commercial diet, were contrasted with a binary-choice situation (non-treated pellets versus pellets treated with enanthic acid). The subjects were re-tested one week later (48 individuals). Table 3 shows that there was a significant decrease in the aversion for the food treated with enanthic acid ($t = 2$; $P < 5\%$).

TABLE III

Comparison of average scores obtained by subjects in the parental generation in two tests (non-treated food versus food treated with enanthic acid).

	TEST 1	TEST 2
Total individuals	49	48
Average score	35.4	44.9
Standard error	3.3	3.3
t	2	
D.o.f.	95	
P	< 5%	

These subjects were then caged in groups, and the females isolated as they became pregnant. From the 15th day of pregnancy until the litters reached one month of age, one group of females was fed only on commercial pellets, while another group was fed only on pellets treated with enanthic acid. Then all the individuals of the first generation were fed only on commercial pellets until they were two months old. At this age they were exposed to a binary-choice situation (non-treated commercial pellets versus pellets treated with enanthic acid). In this way we obtained 36 mice reared on commercial food treated with enanthic acid and 38 mice reared on non-treated pellets. The results are given in Table 4 and show that the experience with food treated with enanthic acid had a significant influence on the behaviour of adult mice.

TABLE IV

Comparison of average scores obtained by subjects in the first generation reared on treated food and controls.

	Reared on fatty acid	Controls
Total individuals	36	38
Average score	55.6	39.9
Standard error	3.0	3.4
t	3.44	
D.o.f.	72	
P	> 0.2%	

Although the control group maintained their refusal of pellets treated with enanthic acid, as did the parents, the response of the other group was significantly different. This result suggests that early experience with enanthic acid can cause an acceptance of this kind of food.

In conclusion, it is clear that in mice feeding choices may depend, sometimes to a large extent, upon the subject's previous experience. Moreover, the effectiveness of the conditioning stimuli may change markedly from food to food. Previous experience with gentian extract did not influence their feeding behaviour. On the other hand, early experience with enanthic acid did condition their feeding preferences. So mice are capable of memorizing, without any reinforcement, experiences had at an early age, and will remember them at least until adult age.

References

[1] J. L. Fuller (1974) – *Single-locus control of saccharin preference in mice.* « J. Hered. », *65*, 33–36.

[2] G. M. Burghardt (1968) – *Chemical preference studies on newborn snakes of three sympatric species of Natrix.* « Copeia », *4*, 732–737.

[3] G. Burghardt (1969) – *Effects of early experience on food preference in chicks.* « Psychon Sci. », *14*, 7–8.

[4] G. M. Burghardt (1970) – *Intraspecific geographical variation in chemical, food cue preferences of newborn garter snakes* Thamnophis sirtalis. « Behaviour », *36*, 246–257.

[5] I. Y. Mahmoud and N. Lavenda (1969) – *Establishment and eradication of food preferences in Red-Eared Turtles.* « Copeia », *2*.

[6] V. Rabinowitch (1969) – *The rcle of experience in the development and retention of seed preferences in Zebra finches.* « Behaviour », *33*, 3–4.

[7] Z. Y. Kuo (1967) – *The dynamics of behaviour development: an epigenetic view.* New York Random House.

[8] B. G. Galef and P. W. Henderson (1972) – *Mother's milk: a determinant of the feeding preferences of weaning rat pupe.* « J. Comp. Physiol. Psychol. », *78* (2), 213–219.

[9] B. G. Galef and F. D. Sherry (1973) – *Mother's milk: a medium for transmission of cues reflecting the flavor of mother's diet.* « J. Comp. Physiol. Psychol. », *83* (3), 374–378.

[10] P. J. Capretta and H. L. Rowls (1974) – *Establishment of a flavor preference in rats: importance of nursing and weaning experience.* « J. Comp. Physiol. Psychol. », *33* (4), 670–673.

[11] D. Csermely (1980) – *Early food learning in the ring dove* (Streptopelia risoria). « Acc. Naz. Lincei », *69*.

[12] J. Fuchs and G. Burghardt (1971) – *Effects of early feeding experience on the responses of garter snakes to food chemicals.* « Learning and Motivation », *2*, 271–279.

[13] S. J. Arnold (1978) – *Some effects of early experience of feeding responses in the common garter snake* (Thamnophis sirtalis). « Animal Behav. », *26*, 455–462.

[14] V. Rabinowitch (1968) – *The role of experience in the development of food preferences in gull chicks.* « Anim. Behav. », *16*, 425–428.

[15] G. M. Burghardt and E. H. Hess (1966) – *Food imprinting in the snapping turtle,* Chelydra serpentina. « Science », *151*, 108–109.

[16] E. H. Hess (1964) – *Imprinting in birds.* « Science », *146*, 1128–1139.

[17] D. Mainardi, M. Mainardi, S. Parmigiani and A. Pasquali (1975) – *An analysis of seed preferences of the house mouse.* « Ateneo Parm. », *11*, 133–139.

[18] M. Mainardi, S. Parmigiani, D. Csermely and A. Pasquali (1976) – *Food preference in mice: an analysis using wheat, rice, chrysalis flour and commercial food pellets.* « Acc. Naz. Lincei », *61*, 635–640.