uted between 1800 and 3700 hertz. The low- and mid-frequency units probably derive from the amphibian papilla, whereas the high-frequency units originate in the basilar papilla (*11*). The histogram reveals a large population of fibers with BEF's in the range of the "Co" note, whereas there are few units with BEF's in the range of the "Qui" note. Neither the low-frequency nor the high-frequency populations in this histogram coincide with the energy in the male's call. Low-frequency sensitivity may play a role in detection of predators for some anuran species (*8*). The role played by the high-frequency fibers is not obvious. Occasionally the male *E. coqui* emits a call whose function is unknown and which contains energy as high as 2700 hertz (*12*). Perhaps the high-frequency fibers provide the basis for detection of this call. The mean BEF of the low-frequency units is not significantly different in males and females ($P > .05$); however, the differences between the mean BEF's of both the mid- and high-frequency units are significantly different for the two sexes ($P < .001$) (*13*).

The sharpness of tuning of an auditory nerve fiber is given by its Q value (the BEF divided by the bandwidth of its tuning curve at 10 db above threshold). The sharpness of tuning of the fibers sensitive to low and middle frequencies was not significantly different in males and females. However, the high-frequency units showed a distinct difference in tuning sharpness between the two sexes; for females, the average Q of the high-frequency fibers was 0.91 whereas in males it was 1.34 (*14*).

The fibers sensitive to high frequencies in females tend to have lower BEF's and broader tuning than those in males. This indicates that the basilar papilla in the inner ear is tuned differently in males and females. Such sexual selectivity in the response properties of the anuran's auditory organs is yet another adaptation for peripheral stimulus filtering. Partitioning the call so that it serves (at least) two functions, and differentially adjusting the peripheral sensitivities to enhance reception of the parameters of the call of interest to each sex, provides a novel mechanism whereby a male can simultaneously communicate with both sexes in a complex sound environment (*15*).

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9. The best excitatory frequency of an auditory nerve fiber is the tonal frequency to which the fiber is most sensitive (that is, has the lowest threshold).
10. A unit is inhibitable if its response to an excitatory tone within its tuning curve can be totally suppressed by the simultaneous addition of a second tone of higher frequency and appropriate intensity (up to 30 db above the excitatory tone) outside the unit's excitatory tuning curve.
11. In the bullfrog (*Rana catesbeiana*) the auditory nerve fibers which innervate the amphibian papilla are sensitive to low- and mid-frequency tones and the fibers which innervate the basilar papilla are sensitive to higher-frequency tones [A. S. Feng, P. M. Narins, R. R. Capranica, *J. Comp. Physiol.* **100**, 221 (1975)]. A similar organization is presumed to be the case for *E. coqui*.
12. G. E. Drewry, in *Puerto Rico Nuclear Center Rain Forest Project Annual Report 1970* (Puerto Rico Nuclear Center, San Juan, 1970), pp. 16–63.
13. The test used was the Kolmogorov-Smirnov two-sample test [S. Siegel, *Non-Parametric Statistics* (McGraw-Hill, New York, 1956)]. A measurement of nine morphological characters in 20 male and female *E. coqui* revealed a pronounced sexual dimorphism in size of all nine characters; for example, mean snout-to-vent length: male, 37.2 mm and female, 52.2 mm; mean longitudinal tympanum diameter: male, 2.0 mm and female, 2.9 mm [R. Thomas, *Q. J. Fla. Acad. Sci.* **28**, 375 (1966)]. The shift in frequency sensitivity between males and females might be related to sexual dimorphism, such as a size difference in the auditory organs of the two sexes. In addition, the characteristics of the mating call of male *E. coqui* vary with the elevation within the rain forest (G. E. Drewry, personal communication; P. M. Narins and R. R. Capranica, unpublished data). Furthermore, males at higher elevations are larger and produce lower-pitched calls than those at lower altitudes. This raises the interesting question of whether the frequency sensitivity of the inner ear in an animal of this species varies with its body size.
14. The Q values for the high-frequency fibers in males are significantly different from those in the females ($P < .001$, Kolmogorov-Smirnov two-sample test). This test also revealed that the Q values for the low- and mid-frequency units were not significantly different in males and females ($P > .05$).
15. At least eight sympatric species of eleutherodactylids contribute to the sonic background in the El Yunque rain forest [A. Schwartz, *Stud. Faun. Curacao Other Caribb. Is.* **91**, 1 (1967)].
16. E. Batschelet, *Animal Orientation and Navigation* (NASA SP-262, Government Printing Office, Washington, D.C., 1972), pp. 61–91.
17. The ++ response was a stronger indication of female preference than a + response. The linear distance from the release point to the midpoint of the ++ response area is 1.75 m; the distance to the midpoint of the + response area is 0.75 m. The ratio of these two distances is 2.33. Assuming responsiveness to be a linear function of distance traveled by the female, we assigned a weight of 2.33 to each ++ response and a weight of 1 to each + response.

18. We thank G. E. Drewry, J. Colón, and D. Narins for providing live *E. coqui*; P. Capranica for aid with the field experiments; the Puerto Rico Nuclear Center for the use of its field facilities; and the members of our laboratory for comments on the manuscript. Supported by NIH research grant NS-09244 to R.R.C. and a Sigma Xi grant-in-aid of research to P.M.N.

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Incentive Contrast in Honey Bees

Abstract. *Bees trained to come to the laboratory for a 20 percent sucrose solution accept it readily, but bees trained with a 40 percent sucrose solution and tested with the 20 percent solution show a pattern of interrupted feeding that may last for several minutes. Bees trained with 20 percent and tested with 40 percent sucrose are undisturbed. When the animals are offered two samples of the 20 percent solution simultaneously, they drink to repletion from whichever they first taste on each visit, but if both a 20 percent and a 40 percent drop are offered the 20 percent solution is rejected after a single experience of the 40 percent solution. Although these results are analogous in many respects to incentive contrast effects found in mammals, they can be understood in sensory terms and do not require the assumption of learning about reward.*

In diverse mammalian species, experience with a preferred food may reduce the acceptability of a less favored food. Monkeys will eat lettuce from a cup they have earlier seen baited with lettuce but not from one earlier seen baited with banana (*1*). Rats trained with bran mash in a maze or a problem box will not accept sunflower seeds substituted for the mash, although sunflower seeds are readily accepted by rats trained with them from the outset (*2*). Goats will normally eat grain, but not in a situation in which they have previously found both grain and bread (*3*). These results have been cited in support of the view that rewards do not serve simply to strengthen response tendencies as reinforcement theorists have proposed (*4*), but that rewards may be learned about, and anticipation of their specific properties may play an important role in instrumental behavior (*5*). Incentive contrast does not, however, necessarily imply learning about reward. In honey bees (*Apis mellifera*), I have found contrast effects, directly analogous to the mammalian effects, that can be explained in simple sensory terms.

Bees were trained individually to fly from the hive into the laboratory through an open door and to drink their fill from a large drop of sucrose solution on a square of paper at the center of a large gray turntable. The training method employed was a conventional one. A young bee was picked up in a matchbox from a feeding platform outside the laboratory (the platform was approximately 3 m from the door and supported a large jar of 12 percent sucrose solution inverted on a glass plate), carried into the laboratory, and set down at the drop of 20 percent or 40 percent solution offered there. As the animal drank, it was marked with a spot of colored lacquer, after which it was permitted to return to the hive. A regular pattern of shuttling between hive and laboratory was established in the animal within two or three placements of this sort. In the 4 to 6 minutes between the animal's successive visits to the laboratory the old lure was discarded and replaced with a fresh square of paper containing a fresh drop of sucrose solution. Recruitment of other animals by the trained ones was not a problem; no unmarked bee ever landed on the turntable in the course of the work (*6*).

Upon arriving in the laboratory, the bees drank uninterruptedly to repletion whether the concentration of sucrose used in training was 20 percent or 40 percent, but a bee trained with the higher concentration would show agitation when tested with the lower. After its first contact with the solution, it would fly upward, return after a time to the food, fly upward once more, and so forth; the bouts of drinking gradually became longer and the periods of abstention shorter until the animal finally settled down to a period of continuous drinking terminated only by return to the hive. The mean number of interruptions for five bees on their first encounter with the lowered concentration was 29 (range, 18 to 47). Interruption of drinking in bees has been observed before under conditions of low sucrose concentration and low influx velocity of the solution (*7*), but it occurred here with a freely available solution of relatively high concentration as a function of experience with a still higher concentration. The disruption was found primarily on the first visit to the less concentrated solution; there might be some transient disturbance on the second visit, but there was usually none. Four animals trained with the lower concentration and tested with the higher were entirely undisturbed; the marked difference in behavior ($P < .01$) indicates that disruption is not produced by change itself but by reduction in concentration (*8*). In this respect, too, the results are analogous to those for mammals (*1, 2*).

In a choice experiment patterned after the work with goats (*3*), I trained four bees with two squares of paper, 3 cm on a side and about 10 cm apart, each holding a large drop of sucrose solution. When the concentration of both drops was 20 percent, a bee would choose one square on each visit (sometimes the left, sometimes the right) and drink uninterruptedly from it alone. When, however, the concentration of one drop was 20 percent and the other 40 percent, with the positions of the two concentrations changed in random order, every bee refused the lower concentration after a single experience with the higher. If an animal chanced first to choose the higher, it remained there; if the lower, it broke off contact at once, went directly to the higher, and remained there. When the color of the paper square (yellow or blue, presented according to a balanced design) was used to signal the concentration of sucrose it held, four bees learned with no more than two errors to choose the higher concentration on each visit, although a fifth animal (previously trained with gray paper and a 20 percent sucrose solution) that encountered the 20 percent solution on the first choice trial continued to choose the associated color (20 percent color) on subsequent trials. Forcing the animal to the 40 percent solution on a single trial (by presenting only the 40 percent color) produced subsequent rejection of the 20 percent solution and rapid development of a preference for the 40 percent color.

I was led to this work by the informal observation of von Frisch that when flowers are scarce bees will accept a sucrose solution of low concentration that they would otherwise reject. "Bees, like men, become more fastidious under better conditions," he concluded; "the *threshold of perception* . . . is invariable under good and poor conditions alike" (*9*). Additional observations suggest, however, that, despite their similarity to the mammalian effects, the contrast effects shown by bees can be understood in sensory terms. The only assumption required is that adaptation during ingestion and regurgitation of the 40 percent solution reduces the apparent sweetness of the 20 percent solution to the point of unacceptability for a short period of time, during the course of which sensitivity gradually recovers, and the 20 percent solution becomes acceptable once more. The results for color discrimination are attributable to the fact that on visits subsequent to experience with the 40 percent solution, the 20 percent solution is not sweet enough initially to be reinforcing.

Rats given access to a 4 percent solution of sucrose after experience with a 32 percent solution drink it less rapidly than do control animals (given only the 4 percent solution) even with a 24-hour interval between the two sessions (*10*), but incentive contrast in bees occurs only in a briefer period, as the sensory interpretation suggests it should. Two bees trained with the 40 percent solution for 80 to 100 visits on one day accepted the 20 percent solution on the first visit of the next day with no sign of disturbance. In one experiment, I permitted a bee to make 25 visits to the 40 percent solution, after which I closed the laboratory door for 20 minutes and substituted the 20 percent solution. When the bee returned about 4 minutes after the door was opened, it accepted the 20 percent solution with no sign of disturbance. I repeated this experiment four times with the same animal over a 2-day period. With no delay between visits, samples of 20 percent sucrose subsequent to experience with 40 percent sucrose would produce the familiar pattern of interrupted feeding, but a delay between visits long enough to permit a foraging trip to the feeding station was enough to make the 20 percent solution immediately acceptable on the animal's next visit to the laboratory.

From additional observations, one can argue against the possibility that the memory of the 40 percent solution is responsible for choice of the correlated color and makes the 20 percent solution unacceptable in choice experiments. After a preference for the 40 percent color had been established and expressed in a long series of visits, I forced a bee to the 20 percent color in two subsequent visits by presenting that color alone. On the first occasion, disruption was marked, but the animal finally settled down and drank its fill of the 20 percent solution. On the second visit, there was little or no evidence of disturbance. On the third visit, I presented the animal with the 40 percent color but the 20 percent solution, from which it drank to repletion without interruption. On subsequent trials, it continued to choose the 40 percent color even though both colors were paired with the 20 percent solution. The same results were obtained with two more bees, for one of which a single interpolated visit to the feeding station was substituted for the two visits to the 20 percent color. The color preferences established in these experiments were themselves highly stable, persisting even after intervals of 2 to 3 days during which either no training was given or other experiments with gray papers were conducted.

Not all instances of incentive contrast in vertebrates require the assumption of learning about reward, although that capability has been clearly established in several mammalian species. Instrumental contrast experiments of the so-called simultaneous and successive types must be distinguished on the ground that simultaneous contrast permits an interpretation in sensory terms when training trials are massed (as typically they are), with large and small rewards following each other in close succession (*11*). It is not difficult from this perspective to understand why goldfish should show simultaneous but not successive contrast (*12*) or why under special circumstances simultaneous but not successive contrast may be found in rats (*13*). Nor do the present experiments rule out the possibility that bees learn about reward. Other results might well be obtained in experiments with qualitative rather than quantitative variation in reward or with quantitative variation in some different rewarding substance. It is chastening that results like those for sucrose found in one of the rat studies already cited were not obtained in companion work with saccharin (*10*). We can say now only that such evidence as we have of incentive contrast in bees does not argue against the sufficiency of the reinforcement principle. We should be wary, however, of easy anthropomorphic allusion; the results underscore the danger of mistaking what may be superficial similarities in the behavior of diverse species for commonalities of underlying mechanism.

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Hyperphagia and Obesity Following Serotonin Depletion by Intraventricular *p*-Chlorophenylalanine

Abstract. *Loss of brain serotonin was associated with overeating and increased body weight. Rats injected with* p-*chlorophenylalanine intraventricularly began overeating after 3 days and continued to display marked hyperphagia, primarily in the daytime, accompanied by increased body weight for 1 to 2 weeks. The effect was related to drug dose and to the degree and duration of serotonin depletion. Norepinephrine and dopamine levels were not significantly affected. It is concluded that* p-*chlorophenylalanine disinhibits feeding, as it does a number of other behaviors, by depleting serotonin. This suggests that hypothalamic lesions or dietary deficiencies which selectively and sufficiently deplete serotonin would lead to overeating.*

Attempts to define the neural substrate of satiety began with the clinical study of patients with hypothalamic tumors, and then focused on electrolytic destruction of localized tissue. Recent technological advances include experiments employing radio-frequency lesions: local subcortical anesthetization, and circumscribed knife cuts. All of these provide models of tumor damage and improved brain surgery techniques, but none mimic the neurochemical deficits that might follow genetic enzyme abnormalities, ingestion of neurotoxins, or dietary amino acid deficiencies.

Recent advances in neurochemistry

Table 1. Mean food intake and maximum body weight after intraventricular injection of PCPA or saline. Data for experiment 1 show a dose-related hyperphagia and obesity with PCPA in females; the top line corresponds to Fig. 1. Data for experiment 2 show hyperphagia in males as well as females and an increase in intake during both daytime and nighttime. Experiment 3 confirms the effect in ovariectomized females. The number of rats in each group is given in parentheses. The *P* values indicate a statistically significant change from the saline control.

			Food intake (g)			Body weight (g)		
Sex	Time	Group	5 days base-line	Days 3 to 7 after injection	Change (%)	5 days base-line	Maximum after injection	Difference (g)
			Experiment 1					
Female		PCPA* (7)	20.0	30.3	+52	296	345†	49
		PCPA‡ (6)	22.9	28.9	+26	310	336†	26
		NaCl (6)	20.8	20.8	0	310	325	15
			Experiment 2					
Female	Day	PCPA (5)	2.7	4.9§	+81	346	413†	67
		NaCl (6)	2.7	2.8	+4	338	362	24
	Night	PCPA (5)	14.1	16.1†	+14			
		NaCl (6)	14.9	12.9	−13			
Male	Day	PCPA (8)	2.0	5.7§	+185	392	426	34
		NaCl (7)	2.7	2.5	−7	413	438	25
	Night	PCPA (8)	15.5	13.1	−15			
		NaCl (7)	16.7	14.4	−14			
			Experiment 3					
Female	Day	PCPA (6)	4.4	14.2¶	+223			
		NaCl (7)	5.3	6.7	+26			
	Night	PCPA (6)	21.3	29.0†	+36			
		NaCl (7)	20.8	24.2	+16			

*Three milligrams. †$P < .05$. ‡Two milligrams. §$P < .01$. ¶$P < .001$.