in a few neurons increasing the information transmitted by a single response to 2 bits (that is, allowing discrimination of four categories). This suggests that each primary fiber had less effective excitatory action on second-order neurons in nucleus caudalis than in nucleus oralis; definition of stimulus intensity by a caudalis neuron without loss of information might then occur only when the stimulus parameters ensure engagement of a maximum number of convergent excitatory fibers.

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Cues: Their Relative Effectiveness as a Function of the Reinforcer

Abstract. *Two cues, either size or flavor of food pellet, were conditionally paired with either malaise induced by x-ray or pain induced by shock in four groups of rats. The combination of flavor and illness produced as conditioned decrement in consumption, but that of size and illness did not. Conversely, the combination of size and pain produced an inhibition of eating, but flavor and pain did not. Apparently, effective associative learning depends on central neural convergence of the paired afferent input.*

Pavlov (*1*) proposed that "any natural phenomena chosen at will may be converted into conditioned stimuli." For example, any discriminable cue, such as an audible tone or a visible light, which precedes a food reinforcer on several occasions can elicit responses associated with feeding in the absence of the reinforcer as confirmed by much experimental evidence.

However, consideration of the adaptive responses of rodents to poisoned foods may require qualification of Pavlov's notion. Animals that survive a poisoning attempt subsequently avoid the poisonous food but not the place where the food was consumed (*2*). In this situation, the visual, tactual, and other stimuli defining the place of the poison do not become conditional stimuli, perhaps because they are not as intimately associated with eating as the gustatory and olfactory stimuli are.

In our experiments, we attempted to discover whether nongustatory attributes of food (for example, size of pellet) could serve as conditional stimuli (CS) with illness as the unconditioned stimulus (US). We compared the relative effectiveness of both gustatory and nongustatory stimuli as cues when the consequence of eating was either a general internal malaise or a specific peripheral pain.

Four groups of eight young adult male rats (300 g, Sprague-Dawley) were trained with one form of food that was conditionally paired with the noxious stimulus and another form of the food that was not so paired (Table 1). Two size groups received food pellets of similar flavors but different sizes. The large size was a whole Purina Chow pellet (approximately 2.5 by 1.5 cm); this was cut into four equal parts for the small size. Two groups received pellets of the same size, but differing in flavor of the coating. Quartered pellets were rolled in flour or in powdered sugar so that their flavor differed but their appearance was similar. Some animals had small pellets associated with the noxious stimuli; others had the large pellets so associated. Flour and sugar were balanced in the same way.

Table 1. Stimulus combination used in conditioning four groups of animals.

Groups	Cue (CS)	Reinforcer (US)
1	Size of pellet	X-ray (illness)
2	Flavor of pellet	X-ray (illness)
3	Size of pellet	Shock (pain)
4	Flavor of pellet	Shock (pain)

The animals were habituated to eating the nonconditional form of the food for 1 hour each day. After a week, conditioning began. During each conditioning day the conditional form of the food was provided during the 1-hour feeding period, and the noxious stimulation was applied. Five conditioning days were carried out every 2 to 4 days. On the intervening days, the animals ate the nonconditional form of the food without the noxious stimulation. Two days after the final conditioning session the animals were tested with the conditional form of the food without noxious stimulation. The latency to begin eating and the total amount consumed in 1 hour were observed. Similar observations were recorded for consumption of the nonconditional food the day before and the day after the test.

One flavor group and one size group were conditioned with electric shock delivered to the paws by an electric shock generator with constant current through a grid floor of the eating compartment. Shocks (0.2-second pulses) were delivered immediately after the rat put the conditional form of the food pellet into its mouth. The intensity caused the rat to drop the pellet (approximately 2.0 ma). The animal received a shock when it placed a pellet in its mouth during the 60-minute conditioning session.

The other flavor group and the other size group were conditioned with x-ray. [Previous studies demonstrated that ionizing rays produce behavioral effects similar to those of toxins but without the peripheral pain of an injection (3).] An exposure to 50 r of 280 filtered x-ray (half-value layer, 1.4 mm of Cu) was delivered in 4.5 minutes immediately after each 60-minute conditioning session.

The flavor of the pellet was an adequate CS when combined with x-rays. Every animal in the flavor-x-ray group ate more of the nonconditional flavored food than of the conditional flavored food. The animals showed little hesitation in picking up either form of food and sampling it; thus the amount eaten was a more effective measure than latency to begin eating. However, the size of the pellet did not acquire this same conditional power although it was associated with identical x-ray treatment. By comparison, the size of the pellet was an excellent conditional stimulus when paired with shock to the

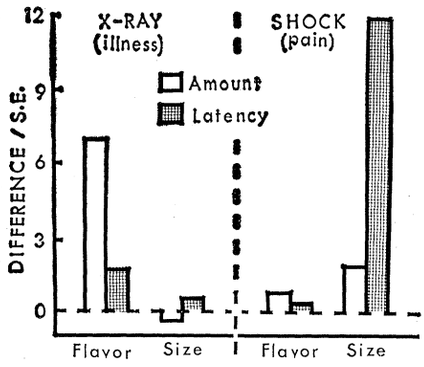

Fig. 1. Relative effectiveness of two attributes of food pellets (size or flavor) to act as cues after conditional pairing with two forms of noxious reinforcement (shock or x-ray) in four groups of rats. The mean difference between measures obtained with conditional and nonconditional forms of the food is scaled in terms of the standard error of that difference. (One S.E. is approximately 0.7 g or 8.2 seconds.) Amount reflects depressed consumption, and latency reflects hesitation before eating caused by the given cue on tests in absence of the reinforcer.

paws. Latency to begin eating was a more effective measure than amount eaten since every rat in the size-shock group hesitated much longer before eating the conditional size food; but once they commenced eating they continued to do so. However, the flavor of the pellet did not acquire significant CS properties when combined with the pain of shock. By contrast to the flavor-x-ray group, no animals in the flavor-shock group showed a decrease in preference for the shocked flavor even though the rats were shocked immediately after they began eating the flavored pellet (Fig. 1).

These data indicate: (i) that both the size cues and the taste cues were discriminable, (ii) that both x-ray and shock disrupted eating behavior, but (iii) that learning occurred only for certain stimulus combinations. Apparently, pairing a perceptible cue with an effective reinforcer does not insure effective associative learning; the cue must be "appropriate" for the consequences that ensue.

Since flavor is closely related to the chemical composition of food, natural selection would favor associative mechanisms relating flavor to the aftereffects of ingestion. The rat has such specialization in its anatomical structure, in gustatory receptors which sample food before it is incorporated by the internal viscera. Both the gustatory and visceral receptors send fibers that converge in the nucleus of the fasciculus solitarius (*4*). Other sensory systems do not send fibers directly to this nucleus; thus the neural organization reflects the propensity of the animal to associate flavor cues (but not size cues) with a subsequent malaise that is internally referred. When the consequences are beneficial (corrected vitamin deficit), the animal exhibits an increased preference for the flavor, and these shifts in preferences occur even though the subsequent reinforcement is delayed for hours (*5*).

Our evidence points toward a similar relation between the telereceptors (vision, audition) and cutaneous receptors. Externally referred sights and sounds are readily conditioned to the peripheral pain of shock. Although the mechanisms are considerably more complex, these systems also appear closely related both behaviorally and neurologically. When the consequence of eating is immediate peripheral pain, the animal exhibits fear responses (hesitation, jumping, squealing) to the nongustatory attributes of the food. But it does not display a reduced preference for either the gustatory or nongustatory attributes of the food. At higher intensities, both telereceptive and cutaneous cues produce similar orienting and startle reactions. These afferents may converge subcortically also probably at the level of the posterior thalamus (*6*). The probability of establishing associative learning depends in part on central integration of the particular afferent channels through which the conditionally paired stimuli are presented.

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Macrophage Spreading: Inhibition in Delayed Hypersensitivity

Abstract. *The capacity of peritoneal macrophages to spread was studied with cells of mice infected with* Listeria monocytogenes *and with cells of guinea pigs sensitized with BCG (bacille Calmette Guérin) vaccine or immunized with ovalbumin. In macrophages taken from animals having delayed hypersensitivity, this ability was markedly decreased by the presence of specific antigen for less than 1 hour. Such an effect was not observed in guinea pigs having only circulating antibodies.*

Rich and Lewis (*1*) showed that migration of cells from animals having delayed hypersensitivity to tuberculin could be inhibited by the presence of specific antigen. This basic fact has been extended to methods allowing in vitro quantitation of the inhibition of macrophage migration in systems specific for delayed hypersensitivity (*2*). Macrophages from animals treated with glucocorticoids lose their migratory capability, and, at the same time, such macrophages do not spread on glass surfaces (*3*). The absence of migration of such cells might reflect their inability to spread; the phenomenon of spreading inhibition might explain the macrophage-migration inhibition observed in delayed hypersensitivity. Experiments were therefore made to investigate the spreading ability of macrophages from animals exhibiting delayed hypersensitivities of the kind occurring in mice infected with *Listeria monocytogenes* (*4*) or in guinea pigs given BCG (bacille Calmette Guérin) vaccine. *Listeria monocytogenes* was grown in a medium of casein hydrolyzate (4 percent), NaCl (0.5 percent), and dextrose (0.2 percent) at *p*H 7.0; the culture supernatant was dialyzed and lyophilized as test material.

Female mice (N.C.S.) 4 to 5 weeks old (19 to 25 g) were injected intraperitoneally either with 7.5 to 9.5×10^4 living *Listeria monocytogenes* or with saline (controls). Peritoneal cells were collected 4 to 10 days later (*5*), the peritoneal cavities being washed with 5 ml of medium 199 containing 20 mg of bovine serum albumin (BSA) and 5 units of heparin per milliliter. The washings yielded 4.5 to 6.5×10^5 macrophages and 2.4 to 3×10^5 lymphocytes per milliliter. The cells were incubated in polyethylene tubes at 37°C for 30 minutes in the presence of