The potentiation of an auditory cue by taste illness mediation in rats

Catherine E. Whitmore

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THE POTENTIATION OF AN AUDITORY CUE BY TASTE ILLNESS MEDIATION IN RATS

A Thesis
Presented to the
Faculty of
California State College
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts
in
Psychology

by
Catherine E. Whitmore
Fall 1982
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Approved by:

Chairman

Date
ABSTRACT

It has been shown that animals rapidly associate gustatory cues with illness but have difficulty associating nongustatory cues with illness when the CS and US are paired directly. However, recent research has indicated that an association can be formed between olfactory cues and illness and visual cues and illness in rats when the nongustatory cue is presented in such a way as to be experienced contiguously with a distinct taste as part of the overall food stimulus. It has been suggested that the taste cue potentiates the association between the nongustatory cue and illness by indexing the nongustatory cue as a food cue in memory. If a distinct taste and contiguity are the only conditions necessary for an association to take place, then it may be that any arbitrarily selected nongustatory cue could be potentiated as well. The purpose of this experiment was to determine if rats can associate illness with a spatially and temporally contiguous auditory cue presented in a stimulus compound with a distinctive taste. Hungry rats were first presented with a choice between plain food and noisy-plain food. All food was powdered, and the noisy-plain food had a speaker in the food at the bottom of the food receptacle. When a rat's head entered the noisy-plain food receptacle a photo beam was broken causing a tone to be
presented through the food from the speaker. After demonstrating an equal preference for plain food and noisy-plain food the rats were presented with two treatments of noisy-sweet food followed by an injection of lithium chloride. The rats were then tested for a preference between plain food and noisy-plain food. It was found that the rats demonstrated a tendency to avoid the noisy-plain food.

A second group of rats was run as a control to determine if the auditory cue could be directly associated with illness or, was the tone illness associated potentiated by taste. The experimental procedures were exactly the same as in the first experiment except that during the aversion training wire screens were placed over the food receptacles which denied the rats access to the sweet food. It was found that a direct association between the auditory cue and illness does not take place without the mediation of a distinct taste.

A third group of rats was run to see if the tone must be spatially contiguous with the taste in the CS compound in order to be associated with illness. The experimental procedures were exactly the same as in the first experiment except instead of presenting the tone through the powdered food, the tone was produced by a speaker which was attached to the back wall of the apparatus. It was found that an association between a tone in a CS compound with taste does not take place unless the auditory cue is spatially as well as temporally contiguous with the distinct taste.
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INTRODUCTION

The feeding behavior of mammals can be divided into two phases: appetitive and consumatory. During the appetitive phase animals search out food; either approaching or avoiding potential food on the basis of distal cues (i.e., visual, auditory, olfactory). During the consumatory phase food is either accepted and ingested or rejected on the basis of proximal cues, such as taste. The ultimate internal effects of ingestion appears to regulate the perceived palatability of the food. For example, if illness follows ingestion, an animal will acquire an aversion for the taste of the food. Conversely, if recuperation from illness follows ingestion, the taste of the food will be favored. Conditioning to the homeostatic effects of food can occur in a single trial (particularly if the food is novel), even though the consequences of eating such foods are delayed for several hours (Garcia, Hankins, & Rusiniak, 1974). This phenomenon allows for the acquisition of taste preferences for new sources of food, while at the same time assuring a distaste for potentially toxic substances, and appears to be widespread throughout the animal kingdom (Garcia & Hankins, 1975).

While an association between taste and illness can readily be demonstrated, it has been much more difficult to establish
an association between the nongustatory cues of a food and illness (Domjan & Wilson, 1972; Garcia & Ervin, 1968; Garcia & Koelling, 1966; Holmstrom, & Wollman, 1974), even though the use of distal cues in guiding appetitive behavior has long been noted in naturalistic studies. Recently, however, researchers have shown that under certain experimental conditions a distinct taste can facilitate an association between nongustatory food cues and a subsequent illness (Clarke, Westbrook, & Irvin, 1979; Galef & Osborne, 1978; Rusiniak, Hankins, Garcia, & Brett, 1979). The purpose of this paper is to extend the investigation of this phenomenon.

**Conditioned Taste Aversion**

Although a clear demonstration of poison avoidance had been reported by Rzoska (1953) in his studies of rat eradication, Garcia and his colleagues were the first to consider conditioned taste aversion from the point of view of learning theory (Bolles, 1979). During the 1950's Garcia and associates (Garcia & Kimeldorf, 1957, 1958; Garcia, Kimeldorf, & Hunt, 1956, 1957; Garcia, Kimeldorf, & Koelling, 1955), carried out a series of radiation aversion studies which stimulated widespread interest in taste aversion learning. In one of the first experiments demonstrating conditioned taste aversion in a laboratory (Garcia et al., 1955) taste cues were separated from an illness producing agent by first presenting saccharin flavored water (a distinct taste) to rats, and then making them ill with X-radiation. A subsequent test showed an aversion
for the saccharin water which persisted through many weeks of post-testing. Since this early study, conditioned taste aversion has been produced in the laboratory in a variety of species, using diverse tastes and illness producing agents. Monkeys, Ceropithcus sabacus (Johnson, Beaton & Hall, 1975), cats, Felis domesticus (Braveman, 1974), chickens, Gallus gallus (Capretta, 1961), garter snakes, Thamnophis sirtalis (Burghardt, Wilcoxon, & Czaplicki, 1973), and cod fish, Gadus morhua L. (Mackey, 1974) form a partial list of the species which have demonstrated conditioned taste aversions.

Taste aversion conditioning has also been successfully replicated in the field and has been found to be effective in reducing coyote predation on domestic livestock. Coyotes formed an aversion to livestock after consuming a carcass or meat package of the potential prey which had been laced with lithium chloride (LiCl is an emetic agent) (Ellins & Catalano, 1980, Ellins, Catalano, & Schechinger, 1977). Field work on the application of taste aversion learning has also been done with wolves (Gustavson, 1979); bears (Colvin, 1975); raccoons (Nicolaus, Hoffman, & Gustavson, 1979); and hawks (Brett, Hankins, & Garcia, 1976).

Conditioned Taste Aversion vs. General Process Learning

One impetus for the interest that was generated by the early taste aversion studies was the fact that taste aversion learning appeared to contradict traditional principles of
basic learning processes (Rozin & Kalat, 1971; Seligman, 1970). Although conditioned taste aversion is assumed to fit the Pavlovian paradigm, it challenges several Pavlovian assumptions. For example, in most traditional animal learning experiments the association between the conditioned stimulus (CS) and unconditioned stimulus (US) has to occur many times in close spatial and temporal contiguity (ideally within seconds) for optimal learning to occur. However, in taste aversion learning studies, a single pairing of a food with illness is often sufficient to produce a strong aversion (Garcia et al., 1955). In addition, conditioned taste aversions can be demonstrated even though several hours have elapsed between the ingestion of food and the inception of illness (Garcia, Ervin, & Koelling, 1966; Garcia, et al., 1974; Revusky, 1968; Smith & Roll, 1967).

Another characteristic of taste aversion learning that appears to contradict traditional learning principles is the assumption of equivalent associability which states that all stimuli and all responses are equally associable. For example, in an early study by Garcia et al. (1957), it was noted that while rats quickly learned an aversion for taste cues paired with delayed radiation illness, it was much more difficult for rats to avoid a distinctive compartment in which they had received the radiation exposure. It appears that in certain learning situations some stimulus events may be more associable than other stimulus events. Garcia and Koelling (1966)
were the first to demonstrate this phenomenon in a classic experiment in which rats were hooked up to a circuit that produced a compound stimulus consisting of a click, a light, and a sweet taste each time the rat's tongue touched the nipple of a bottle containing saccharin flavored water. Two different types of consequences were given after consumption of the bright-noisy-tasty water; either foot shock or illness. Garcia and Koelling found that rats which were given foot shock after water consumption learned to inhibit drinking bright-noisy water, but continued to drink the water which had been flavored with saccharin. Conversely, rats that experienced illness after water consumption learned to avoid drinking the sweet water but continued to drink bright-noisy water. An attempt to train an association between bright-noisy water and illness, and between taste and shock failed.

Using the same paradigm as Garcia and Koelling (1966), similar results have been obtained in a number of other experiments (Domjan & Wilson, 1972; Garcia & Ervin, 1968; Green et al., 1974). From a compound CS consisting of taste cues and audio-visual cues, rats were far more likely to associate taste cues and illness than the nongustatory cues and illness. When the unconditioned stimulus was changed from illness to shock, however, the nongustatory cues became more associable than the taste cues. This apparent selectivity of cue to consequence has been found to be true for odors as well. Taste cues are more effective than odor cues when paired with illness (Hankins,
Garcia, & Rusiniak, 1973), while odor cues are more effective than taste cues when paired with shock (Hankins, Rusiniak, & Garcia, 1976).

Taste aversion studies further challenge a fourth assumption of the traditional principles of learning; the assumption of equivalent associability across species which states that all animals will associate the same cues equally. While it appears that most animals utilize taste cues as the primary mediating stimulus in the formation of a food aversion, there is evidence that not all species rely on taste cues to the same extent that the rat does. For example, Wilcoxon, Dragoin and Kral (1971) contrasted the behavior of the rat, a nocturnal feeder which has relatively poor visual acuity, to that of the quail, a diurnal feeder known to have high visual acuity (Walls, 1963), in response to blue sour water paired with illness. While the quail did show an aversion to the sour taste, when the sour taste was presented in a compound with blue color, the visual cues overshadowed or blocked conditioning of the taste. The rat, however, formed an aversion to the sour taste in the compound, which appeared to overshadow the blue color (Pavlov, 1927; Kamin, 1969). In addition, long delay learning has been demonstrated in quails with up to two hours between the presentation of visual cues and a subsequent illness (Wilcoxon, 1977). Various studies have indicated that chickens (Capretta & Moore, 1970), codfish (Mackay, 1971), monkeys (Gorry & Ober, 1970), and guinea pigs (Braveman, 1974) are also capable of using visual cues more
effectively than rats in forming poison based aversions.

Rozin and Kalat (1971) suggest that the critical dimension in poisoned based aversions is not taste versus other cues, but whatever cues a particular species uses in food selection. For example, Braveman (1974) has shown that guinea pigs, which like rats have poor visual acuity, use both taste and visual cues in forming an aversion to illness producing foods. Although the level of sophistication of the sensory system may influence to some extent the degree to which an animal uses that system in food selection, it is apparent that the ability to form poisoned based aversions to visual cues is not solely dependent on a sophisticated visual system. This would suggest that guinea pigs (like the quail) form aversions to the visual properties of food because the appearance of food is an important eating related cue (Braveman, 1974).

Preparedness

The facility with which rats (and numerous other animals) learn to associate gustatory qualities of food and subsequent illness has been viewed as reflecting an adaptive specialization of learning (Rozin & Kalat, 1971). Seligman (1970) suggests that through evolutionary processes an organism brings specialized neurological and associative equipment into the learning situation which prepares it to quickly learn the contingencies needed for the food selection problems it must
The special properties of taste aversion learning noted above which appear to contradict basic assumptions of traditional learning theory can be explained in terms of evolutionary adaptation. For example, in regards to one trial and delayed learning, an animal must be able to associate taste with the effects of ingested poison in very few trials (since every trial is potentially lethal). Also, because of the inherent delay between the ingestion of a poison and its metabolic consequences, the association must be made with long temporal delays between food consumption and a following illness. The concept of "preparedness" implies that the brain, through evolutionary specialization, is structured to facilitate this type of learning. In addition, the facility with which rats associate gustatory cues with illness as opposed to their apparent inability to associate nongustatory cues with illness makes sense in that certain kinds of associations allow an animal to survive in the environment while certain others are irrelevant. However, since different species live in different environmental niches, they therefore need to learn different things in order to survive. Thus rats are "prepared", through a biological predisposition to associate taste, their dominant food selection modality, with illness, and likewise, quail, a visual feeder, is "prepared" to associate the visual characteristics of food with illness.
The biological basis of the concept of preparedness has been studied with regard to taste as a prepotent cue for illness. According to Garcia and Ervin (1968), taste aversions to poison foods are mediated by the anatomical convergence of gustatory and viseral projections in the medulla and adjacent reticular formation of the brain. More recent research (Coil, Rogers, Garcia, & Novin, 1978; Kiefer, Rusiniak, Garcia, & Coil, 1981) suggests that a complex feedback system exists between the brain and abdominal structures via the vagus nerve which allows the animal to associate taste cues with a subsequent illness. The vagus further functions to mediate a conditioned emetic response when a taste stimulus is presented and apparently plays a critical role in the maintenance of a taste aversion.

The special neurological taste mechanisms proposed by Garcia and Ervin (1968), Coil et al. (1978), and Kiefer et al. (1981), to account for the robustness of taste aversion learning, however, does not explain how the quail and other species use visual cues and other nongustatory cues in mediating aversive conditioning, including long delay learning. It can only be postulated that some type of specialized mechanism exists which allows an organism to adapt to their specific feeding requirements (Nachman, Rauschenberger, & Ashe, 1977).

**Associability of Nongustatory Cues**

Learning appears to be greatly facilitated in a situa-
tion that takes advantage of an animal's natural disposition to learn a relationship between taste and illness; however, this does not necessarily mean that other associations are not possible. There is evidence, for example, that under certain conditions rats can learn to associate nongustatory cues with illness as well (Best, Best, & Mickley, 1973; Garcia, Kimeldorf, & Hunt, 1961; Mitchell, Kirschbaum, & Perry, 1975). However, conditioning to nongustatory cues was found to be considerably weaker than conditioned aversions to taste in that it appeared to resemble the learning found in more traditional conditioning studies requiring many trials and close spatial and temporal contiguity between the CS and US. When a learned aversion did occur to a distinctive nongustatory cue it was often dependent upon the total environmental and training situation, such as whether or not a distinct taste was also present (Best et al., 1973), and the amount of prior exposure to the training situation (Mitchall et al., 1975).

In addition, whenever a comparison was made between taste and a nongustatory cue, taste was always the most associable cue.

Conditioning to Compound Cues

In most conditioning situation, many CS's are presented simultaneously; that is, organisms are not presented with a unitary CS and a US. For example, in Garcia and Koelling's (1966) bright-noisy water study, three conditioned stimuli were used: a light, a click, and a sweet taste. When multiple
stimuli are presented simultaneously, they are considered a compound stimulus.

In work by Pavlov (1927) and Kamin (1969), one stimulus was made salient by associating it with an aversive US. If that CS were then placed in a compound with another CS and followed by the aversive US, the more salient CS would overshadow or block any association between the US and the other CS in the compound. That is, if the other CS in the compound were then presented alone there would be no conditioned response. The results of the early taste aversion studies might be interpreted as a case of overshadowing or blocking in that taste more readily associated with illness than did other nongustatory cues; that is, when presented in a compound the taste overshadowed the other cues (e.g., Domjan & Wilson, 1972; Garcia & Ervin, 1968; Garcia et al., 1957; Garcia & Koelling, 1966; Green et al., 1974). If these studies in fact represent examples of overshadowing, the other cues in the compound with taste, such as a light or a tone, would have also associated with illness if presented independently in training. Although studies in which nongustatory cues have been used alone or in which rats have been placed in a distinctive compartment have been somewhat effective in producing a learned aversion when paired with illness (Best et al., 1973; Garcia et al., 1956; Mitchell et al., 1975), such conditioning does not always occur and is not as strong as taste aversion learning (Galef & Osborne, 1978;
Palmerino, Rusiniak, & Garcia, 1980).

Such studies, however, do not explain how animals use nongustatory cues effectively in avoiding a food previously paired with illness. For example, predation studies have indicated that coyotes, after ingesting a meat bait or carcass of potential prey laced with LiCl, will learn to suppress attack behavior on live prey of that species after only one or two poisonings (Ellins & Catalano, 1980; Ellins et al., 1977); Gustavson, Garcia, Hankins, & Rusiniak, 1974; Gustavson, Kelly, Sweeney, & Garcia, 1976). In these studies, the coyote was presented with a compound CS (consisting of taste, odor, and visual cues of the meat bait or carcass), followed by a US (illness), resulting in a conditioned aversion to the taste. However, when the coyote was subsequently presented with live prey, it was often avoided based on odor and visual cues without killing and tasting the meat. According to the principle of overshadowing there should have been no aversion to the nongustatory cues of the live prey; the coyote should have still killed the prey, since the taste ought to have overshadowed the other cues in the CS compound. The fact that the live prey was not killed, indicates that the nongustatory cues as well as the taste were associated with illness.

Recently, additional studies have shown results that support the notion of aversive conditioning to nongustatory cues. That is, when a taste is paired with a nongustatory cue in a CS compound, instead of the nongustatory cue elicit-
ing a smaller conditioned response, it elicits a stronger response becoming much more effective than if it had been conditioned by itself. For example, this phenomenon has been observed in studies with rats in which almond scented water alone proved to be a very weak cue for illness, while sweet water proved to be a very effective cue. However, after the almond scented water and sweet taste were combined in a compound CS, followed by illness, the almond odor functioned as a very powerful cue for poisoned water as well (Palmerino et al., 1980; Rusiniak, Hankins, Garcia, & Brett, 1979). Rusiniak et al. (1979) found that instead of being overshadowed by the saliency of the taste, the odor appeared to become aversive in its own right, and in almost direct proportion to the saccharin concentration. In a similar experiment using pigeons (Clarke et al., 1979) blue water alone proved to be a weak cue for illness. However, when salt was mixed into the blue water, the pigeons acquired a strong visual avoidance of blue water in a single trial.

Although, Rusiniak's et al. (1979) and Palmerino's et al. (1980) studies indicated that a distinct taste could facilitate an association between olfactory cues and illness in rats, the results did not necessarily apply to other non-

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1Clarke, Westbrook, and Irwin (1979) acknowledge that these findings are the "opposite to those anticipated by an approach to food selection that relates the associabilities of cues with toxicosis to birds' structural development and ecological niche." Clarke et al. suggest that the results indicate "that anatomical development per se provides little ground for anticipating which species will readily develop what aversion" (p. 26).
gustatory cues. Olfaction probably plays an important role in the survival of the rat (a nocturnal animal) and it is likely that rats use such cues in their dietary regulation. On the other hand, rats have poor visual acuity which is assumed to be linked to their nocturnal feeding patterns (Walls, 1963). Galef and Osborne (1978) were interested in whether rats could learn an association between visual cues (a presumably weak nongustatory cue from an ecological point of view) and illness. If so, it may be possible that any nongustatory cue contiguous with taste in a CS compound might associate with illness as well. In the Galef and Osborne (1978) study, rats were subjected to illness after ingesting visually distinct gelatin food capsules with either a bitter or sweet taste. The results indicate that rats can form an association between visual cues and illness. However, subjects which lacked exposure to either the bitter or sweet taste during training failed to develop an aversion to the visually distinct food capsules.

According to Rusiniak, et al. (1979), in taste aversion conditioning the presence of a distinct taste in the compound during training synergistically potentiates the association between the nongustatory cue and illness. Evidence for this type of potentiation has been reported in several other species; for example, between odor cues and taste in coyotes (Ellins & Martin, 1981), and between visual cues and taste in hawks (Brett et al., 1976), and chickens (Capretta & Moore,
Statement of the Problem

Testa and Ternes (1977) have suggested that the spatial relationship between a cue and the food object is an important determinant in poison based aversions. They hypothesize that organisms will more readily form poison based aversions to cues that "covary in time and space", that is, to cues that are temporally and spatially contiguous with the ingested object. In each of the above studies (i.e., Clarke et al. 1979; Galef & Osborne, 1978; Palmerino et al., 1980; and Rusiniak et al., 1979) in which a strong association between a nongustatory cue and illness was found, the nongustatory cue was presented in such a way as to be experienced contiguously with taste as part of the overall food stimulus. A distinct taste has been found to be an integral part of the CS compound--potentiating the association of the nongustatory cue with illness. If a distinct taste and contiguity are the only conditions necessary for an association to take place between nongustatory cues and illness, then it may be that any arbitrarily selected nongustatory cue in a CS compound contiguous with a distinct taste could be potentiated as well.
EXPERIMENT I

The purpose of this experiment was to determine if rats can associate illness with an auditory cue (a cue not normally associated with appetitive feeding behavior in rats), given that it is spatially and temporally contiguous with a distinct taste in a CS compound.

Method

Subjects. The subjects were five female and 10 male adult Sprague-Dawley rats, weighing 270-555 g., reduced to 80% of their ad libitum weight. The rats were individually housed in (18 x 21 x 24 cm high) stainless steel cages when not participating in the experiment. All rats had free access to water throughout the experiment, both in their home cages and in the experimental box except where specified.

Apparatus. The apparatus consisted of a modular test cage, 21 x 31 x 33 cm high (model E10-01, Coulbourn Instruments). The end walls and top were constructed of aluminum sheeting and two side walls were made of clear Plexiglas. The box had a metal grid floor consisting of stainless steel rods paced 1.5 cm apart. A latched door, 29 x 20 cm high on one side provided access into the box. The interior walls, ceiling, and floor of the experimental box was lined with
.8 cm thick white polyfoam. The box was enclosed in an isolation chamber to attenuate extraneous light and noise.

The experimental chamber was constructed such that a tone could be selectively presented through food in either one or both of the receptacles. One end of the chamber was modified to hold two galvanized steel V-shaped food receptacles (Figure 1), each equipped with a speaker (model SC-628, 6-28 VDC, Soundalert), a photocell and photocell light (Figure 2). Two clear Plexiglas V-shaped envelopes held the food in each receptacle. Each food envelope had a 3.5 cm hole in the back, screened with cloth which sat against the speaker and a 3 cm hole on the top providing food access. The photocells were positioned in such a way that every time a rat's head entered the food receptacle to obtain food, a light beam was broken which caused a tone to be presented through the speaker at the base of the receptacle. The tone was maintained as long as the beam was broken. Each speaker was attached to a variable adjustment knob on a power supply board which allowed the intensity of the tone to be varied by the experimenter while a rat was feeding. The photocell of each food receptacle was linked through a timer to a digital stop clock which recorded the amount of time the light beam was broken.

Unadulterated powdered Purina Rat Chow and powdered Purina Rat Chow adulterated with 25% (by volume) brown sugar was used.
Figure 1. Experimental Chamber
Figure 2. Food receptacle for emitting a tone.
**Procedure.** The subjects were placed individually in the apparatus for 20 min. each day for 14 consecutive days of habituation. The first 10 days were run to habituate the subjects to eating in the apparatus. During this period plain food was presented in one food receptacle and the other receptacle was empty: The positions of the two receptacles were randomly alternated across days and subjects. On the 11th day, the subjects began the second phase of habituation. During this period the rats were presented an equal number of times with either noisy-plain food or plain food, with only one food available on each day. The subjects were slowly habituated to the noisy-plain food by gradually increasing the intensity of the tone to a predetermined level while they were feeding. The positions of the noisy-plain food and plain food were randomly alternated across days and subjects.

Pretesting began on the 15th day with each subject given a 50 min. choice between noisy-plain food and plain food which was presented simultaneously. This procedure was followed on the next day (day 16) by an additional 50 min. choice pretest in which the positions of the noisy-plain and plain food were counterbalanced for sides. The food was weighed in the food envelopes before and after each session. This weighing procedure was also followed in the pretest, training, and post-test sessions.

Aversion training began on the 17th day. All subjects were given a 20 min. access to noisy-sweet food (25% sucrose)
which was presented in both food receptacles simultaneously, and were immediately injected intraperitoneally with 1.2% of body weight solution of .15 M lithium chloride (Nachman & Ashe, 1973). They were then placed back in their home cages without food or water. On day 18, the subjects had access to plain food and water, and then were food deprived for 24 hr. On the 19th day an additional training session was run, using the exact procedures as above. The subjects were then returned to their home cages without food or water, and then were food deprived for 24 hr.

Posttesting began on the 21st day after 24 hr. of food deprivation. During the post preference test, the subjects were given a 50 min. choice between noisy-plain food and plain food. A second post preference test was run on the next day (day 22) in which the positions of the noisy-plain food and plain food were reversed.
RESULTS

In order to reduce variability and to clarify differences between groups and subjects, the data was transformed into preference ratios; the amount of noisy-plain food ingested over the total amount of food ingested (noisy-plain plus plain food) (see Appendix A for complete derivation).

The mean pretest and posttest preference ratios for Experiment I are presented in Figure 3. During the pretest an approximately equal amount of noisy-plain food and plain food was ingested, however during the posttest only 42% of the total amount of food ingested was noisy-plain food. This is a decrease of 18% in the amount of noisy-plain food ingested during the posttest when compared to the pretest. Individual preference ratios are presented in Figure 4. During the posttest 80% of the subjects consumed less noisy-plain food over the total amount of food when compared to the pretest. A t-test was run between the pre and post test ratios, which was found to be significant, $t(14)=3.45$, $p<.05$. The results of this experiment indicate that rats can associate an auditory cue with illness if the auditory cue is spatially (as well as temporally) contiguous with a distinct taste in a compound stimulus.

To determine if the large decrease in the amount of noisy food consumed between the pre and post test noted in rat #14
significantly weighted the results of Experiment I, a second t-test was run on the data with rat #14 removed. The results were again found to be significant, $t(13)=3.62$, $p<.05$. The data from rat #14 did not significantly change the results of Experiment I.
Figure 3. Mean pretest and posttest preference ratios.
Figure 4. Individual pretest and posttest preference ratios: Experiment I.
EXPERIMENT II

The results of Experiment I indicate that rats can associate an auditory cue (tone) with illness if the auditory cue is experienced contiguously with a distinct taste in a CS compound.

Rusiniak et al. (1979) suggests that the stronger taste cue in the CS compound synergistically potentiates the aversion for the weaker nongustatory cue. For example, in the Rusiniak et al. study it was demonstrated that while almond odor alone is a weak cue for illness, it becomes a very effective cue for illness after it is combined in a CS compound with a distinct taste. This apparent potentiation of a nongustatory cue by a distinct taste has been reported in a number of studies (Brett et al., 1976; Capretta & Moore, 1970; Clarke et al., 1979; Ellins & Martin, 1981; Galef & Osborne, 1978; Palmerino et al., 1980), including Experiment I in the present study.

If the auditory cue-illness association demonstrated in the first experiment was potentiated by the distinct taste in the CS compound, then it is reasonable to assume that in the absence of the taste the association between the nongustatory cue and illness would not occur. Experiment II was run as a control to determine if an auditory cue can be directly associated with illness.
METHOD

Subjects
The subjects were 10 adult female Sprague-Dawley rats, weighing 250-284 g., reduced to 80% of their ad libitum weight.

Apparatus
This experiment was conducted in the same apparatus used in Experiment I. However, during the aversion training sessions wire screens were placed over the food receptacles thereby denying the rats access to the sweet food.

Procedure
The procedure was exactly the same as in Experiment I with one exception. The amount of time that the light beam was broken (which triggered the tone) in the training sessions in the first experiment was averaged, and that duration of tone was presented to the rats in random 50 sec. intervals during the 20 min. training sessions in the present experiment.
RESULTS

The data analysis was exactly the same as in Experiment I. The mean pretest and posttest preference ratios are presented in Figure 3. In both the pretest and posttest, approximately equal amounts of both noisy-plain and plain food were consumed with less than 3% difference in the preference ratios between the two testing periods. Preference ratios for each subject are presented in Figure 5. A t-test was run on the data which indicates no significant difference between the pre and post test ratios, t(9) = .191, p > .05. The results of this experiment indicate that a direct association between the auditory cue and illness does not take place without the mediation of a novel taste.
Figure 5. Individual pretest and posttest preference ratios: Experiment II.
EXPERIMENT III

It has been shown that nongustatory cues are much more difficult to condition to illness than gustatory cues (i.e., Garcia et al., 1957; Garcia & Koelling, 1966; Green et al., 1974). There have been several recent studies, however (i.e., Clarke et al., 1979; Ellins et al., 1981; Galef & Osborne, 1978; Palmerino et al., 1980; Rusiniak et al., 1979), including Experiments I and II of the present study, which indicate that a distinct taste in a CS compound with a nongustatory cue potentiates the association between that cue and illness. In each of these studies, the nongustatory cue was presented as part of the overall food stimulus and not as an environmental cue. These findings support Testa and Ternes' (1977) hypothesis which states that animals will more readily form aversions to cues that "covary in time and space" with the ingested food object, than cues that do not. If the Testa and Ternes hypothesis is correct then a cue that is not spatially contiguous with taste will not associate with illness. Experiment III was run as a control to determine if an auditory cue can be associated with illness in a CS compound that is temporally but not spatially contiguous with a distinct taste.
METHOD

Subjects

The subjects were nine female and one male adult Sprague-Dawley rats weighing 232-284 g. All rats were reduced to 80% of their ad libitum weight.

Apparatus

The experiment was conducted in the same apparatus used in Experiment I with one exception. Instead of presenting the tone through the powdered food at the base of the food receptacle, the tone was produced by one of the same speakers used in Experiment I attached to the middle of the back wall of the apparatus (see Figure 1).

Procedure

The procedure was exactly the same as in Experiment I.
RESULTS

The data analysis was exactly the same as in Experiment I. The mean pretest and posttest preference ratios are presented in Figure 3. During the pretest 41% of the total amount of food consumed was noisy-plain food, while during the posttest 46% of the total amount of food consumed was noisy-plain food. This is an increase of 12% in the amount of noisy-plain food ingested during the posttest when compared to the pretest. Individual preference ratios are presented in Figure 6. A t-test was run between the pre and post test ratios, which was found not to be significant, t(9) = .109, p > .05. Although the auditory cue was temporally contiguous with the taste cue in this experiment, the results indicate that an association between a tone in a CS compound with taste does not take place unless the auditory cue is spatially (as well as temporally) contiguous with the distinct taste.
Figure 5. Individual pretest and posttest preference ratios: Experiment III.
DISCUSSION

The results of the present study indicate that a novel taste can synergistically potentiate the conditioning of an auditory cue with illness, which is consistent with the findings of Clarke et al. (1979), Palmerino, et al. (1980), Rusiniak, et al. (1979), and Galef and Osborne (1978). Several explanations for the potentiation phenomenon have been offered. According to Rusiniak et al. (1979) potentiation occurs as a result of a distinctive taste indexing the nongustatory cues as food cues in memory. Without taste identification, the capacity of an animal to form aversions for nongustatory cues after a poisoned meal would leave an animal vulnerable to the formation of supersitious aversions since innumerable experiences involving nongustatory cues can intervene between the ingestion of a poisoned food and a subsequent illness. In this way, taste, a cue that controls ingestion, mediates an aversion to a nongustatory cue used in food selection.

Durlach and Rescorla (1980) suggest that the joint occurrence of the two CS's (taste and a nongustatory cue) during conditioning results in an association between the two stimuli in the compound. The within-compound association adds to the aversion attributed to the nongustatory cue's
association with the US, producing a potentiation. Thus, a subsequent avoidance of a specific nongustatory food cue would reflect not only its association with illness, but also its association with taste. Durlach and Rescorla, (1980) demonstrated within-compound association in a study in which rats learned an aversion to an odor cue potentiated by the presence of a distinct taste during conditioning. It was then found that by extinguishing the aversion to the distinct taste the potentiation of the odor cue could be disrupted.

The present findings contradict the results of studies in which the more salient stimulus in a CS compound appeared to overshadow or block the association of the weaker stimulus, such as in the bright-noisy-water experiment (Garcia & Koelling, 1966). Overshadowing or blocking infers competition between cues in the CS compound, and in the potentiation studies where both the nongustatory and gustatory cues constitute different elements of the same food stimulus there is no competition. Both types of cues within the food stimulus have a specific function in the feeding behavior of animals. Non-gustatory cues are used to guide appetitive approach responses, whereas taste cues guide the ingestion of food through a process Garcia, Hankins, and Rusiniak (1974) refer to as hedonic palatability, whereby the quality of the taste is modified based on the ultimate internal afteraffects.

Although the present experiment is similar to the Garcia and Koelling (1966) study in which an auditory cue (and visual
cue) was paired with sweet water and an association with illness was not found, it is importantly different. In the Garcia et al. study, the rat activated an electrical circuit which produced a click in a box over the rat's head (as well as a flash of light) every time it's tongue licked a water bottle spout containing sweet water. Although the rat had three temporally contiguous cues (a click, a light, and distinct taste), only one cue (taste) was actually part of the water. The other two cues in the CS compound were environmental cues, being spatially divorced from the food stimulus. Therefore, the more salient taste cue competed with the non-gustatory cue for association with the US. In the present study, however, the food receptacle was designed in such a way that the auditory cue was also part of the food, thus there was no competition between cues in the CS compound.

Although studies have indicated that under certain conditions environmental cues could become associated directly with illness (i.e., Best et al., 1973; Garcia et al., 1961; Hankins et al., 1973), the conditioning was found to be considerably weaker than a conditioned aversion to taste—often depending upon numerous training trials and reduced delays between the CS and US. This appeared to be the case in Experiment II and indicates that it was in fact the taste that potentiated the auditory-illness association.

Testa and Ternes (1977) proposed that an important determinant of the associability of a specific cue with
illness is the degree of spatial and temporal contiguity between the cue and the food stimulus. They hypothesized that animals will more readily form poison based aversions to cues that are contiguous with the ingested food than cues that are not. The importance of spatial contiguity in learning a poison based aversion has been suggested in studies in which the potentiation of a nongustatory cue has been found. In each of these studies, the nongustatory cue was presented spatially as well as temporally contiguous with the taste during training. For example, in the Rusiniak et al. (1979) study, rats were presented with almond scent and sweet taste in a compound water solution. In the Palmerino et al. (1980) study, almond extract was placed on filter paper which surrounded the end of the water drinking tube. Blue food coloring was combined with salty flavored water in the Clarke et al. (1979) study. While in the Galef and Osborne (1978) experiment, sweet powdered food was presented in visually distinct gelatin capsules. In the present experiment a tone was presented through sweet powdered food which was provided by a speaker at the base of the food receptacle.

The results of Galef and Osborne's experiment (1978) as well as the present experiment indicate that even a distal cue that is presumably minor in the appetitive food behavior of rats (i.e., visual, auditory) can become associated with illness, if during consumption the nongustatory cue is spatially and temporally contiguous with a distinct taste as part of the
CS compound. Thus it appears that in addition to which cues a particular species may be biologically prepared to associate with illness (Seligman, 1970), the circumstances under which they will attend to cues associated with illness is also important.

The importance of the spatial distance between nongustatory cues and the food stimulus in associating those cues with illness was further supported in a recent study by Galef and Dalrymple (1981). Using a slightly different procedure than the above studies, rats were first pretrained to select palatable food using the visual properties of their food, food bins, and feeding chambers. Following the discrimination pretraining, the subjects were injected with LiCl after eating visually novel food, or after eating from a visually novel food bin or from a visually novel feeding chamber. In accordance with Testa and Ternes hypothesis (1977), Galef and Dalrymple's results indicated that the associability of the visual cue with toxicosis decreased as the visual cue became more diffuse and less spatially contiguous with the food object. That is, the rats formed the greatest illness-based aversion to the visual properties of the food stimulus. They formed a weaker aversion to the visual properties of the food bin, and almost no aversion to the food chamber. These findings suggest that if in the present study the temporal contiguity between the auditory cue and the other cues in the CS were maintained but the spatial contiguity between these
cues slightly changed, the strength of the association between the auditory cue and illness would decrease.
APPENDIX A

CONSUMPTION DATA:

EXPERIMENT I

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APPENDIX B

CONSUMPTION DATA:

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APPENDIX C

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REFERENCES


Colvin, T.R. Aversive conditioning black bear to honey utilizing lithium chloride. Proceedings of the 29th Annual Conference of the Southeastern Association of Game and Fish Commissioners.


Nachman, M. & Ashe, J.H. Learned taste aversions in rats as a function of dosage concentration, and route of administration of LiCl. Physiology and Behavior, 1973, 10, 73-78.


