TASTE AVERSION MOTIVATED BY

STOMACH DISTENTION

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BY

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ABSTRACT

Previous research has indicated two distinctive characteristics

of flavour-aversion learning in rats: (1) rats very readily associate

flavors with an internal malaise (toxicosis), as evidenced by their sub-

sequent aversion to the flavor, but they do not readily associate fla-

fors with peripherally-applied electric shock. In contrast, rats readily

associate auditory and visual stimuli with shock but not with toxicosis;

(2) rats associate flavors with a subsequent toxicosis even when the

gustatory stimulus is removed hours prior to onset of toxicosis. How-

ever, associations are formed between audio/visual cues and shock only

if the offset of the signal does not precede onset of shock by more than

one or two minutes.

It has been suggested that the unique features of flavour-aver-

sion learning result from the fact that toxicosis is primarily a vis-

ceral experience while shock is applied to somesthetic receptors. How-

ever, toxicosis differs from shock along a number of dimensions in addi-

tion to receptor site. Most notably, toxicosis typically rises to a peak

intensity over a period of many minutes and lasts for hours while shock

is usually applied with a rapid onset (milliseconds) and short duration

(seconds or milliseconds). Inasmuch as aversion learning experiments have

confounded the receptor site of the aversive stimulus with its distinctive

temporal features, it is not clear whether receptor site or temporal

features is the functionally important characteristic of toxicosis as an

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aversive stimulus in the taste-aversion learning preparation.

To determine the role played by the temporal features of the aversive stimulus in taste-aversion learning, rats were prepared with a stomach balloon and stomach balloon inflation was paired with ingestion of a flavored solution. In contrast to toxicosis, the onset/offset rate and duration of balloon inflation may be directly manipulated thus permitting application of a relatively discrete internal stimulus (in comparison to toxicosis) to visceral receptors.

Experiments presented here found: (a) rats associated a flavor with a stomach balloon inflation as indicated by an aversion to the flavor during a two-solution preference test. In contrast to toxicosis, the stomach balloon inflation had a rapid onset (seconds) and short duration (minutes). Control groups demonstrated that the rapid onset, short duration balloon inflation did not produce the long lasting malaise characteristic of toxicosis. (b) Rats associated a flavor with a rapid onset, short duration balloon inflation even when exposure to the flavor was terminated many minutes prior to onset of balloon inflation. (c) Rats readily associated a flavor with balloon inflation but not with shock, and an auditory stimulus with shock but not with balloon inflation, even though balloon inflation and shock were equated in terms of their temporal parameters.

These findings clearly indicate that the very slow onset and long duration characteristics of toxicosis are not the function-

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ally important features of toxicosis as the aversive stimulus in the taste-aversion learning preparation. Furthermore, the unique temporal features of toxicosis and shock do not appear responsible for the distinctive characteristics of flavor-aversion learning in rats.

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minutes.   Number of subjects per group is indicated in parenthesis.

CHAPTER 1: INTRODUCTION

Pavlovian Conditioning

Pavlovian conditioning is characterized by a set of operations in which a subject is exposed to the repeated presentation of two environmental events or stimuli. The presentation of these two stimuli is arranged so that (a) one stimulus reliably predicts the occurrence of the second and (b) the stimuli are presented without reference to any behavior emitted by the subject. In the traditional Pavlovian terminology, the second of the two stimuli is termed the unconditional stimulus (US) while the signal stimulus which reliably predicts the US is termed the conditional stimulus (CS).

The US is typically a biologically significant event that, without prior presentations, elicits a response termed the unconditional response (UR). For example, two widely used USs have been food presented to a hungry subject and nociceptive electric shock. Food elicits a variety of alimentary behaviors such as chewing, licking, salivating and gastric secretion. Nociceptive shock, on the other hand, may elicit responses such as leg flexion, eyelid closure and changes in heart rate.

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In contrast to the US, with its reflexively elicited behavior, the conditional stimulus is usually selected because it is a relatively neutral environmental event that elicits little behavior prior to its pairing with the US. As a function of (i.e., conditional upon) its repeated pairing with the US, however, the CS comes to elicit behaviors relevant to the US with which it is paired. The response elicited by the CS conditional upon CS-US pairings is termed the conditional response (CR). In Pavlov's original observations (1910), for example, environmental cues such as the sight of the person who normally fed the dogs and the smell of the food which inevitably preceded the presentation of food to dogs became capable of eliciting stomach acid secretion. Here the preceding cues (CS) were coincidentally paired a number of times with food (US) and these environmental cues by themselves became capable of eliciting gastric activity.

In Pavlov's later (and better known) conditioning research (1927) a variety of arbitrarily selected CSs (bells, light, tactile stimulation, etc.) were paired a number of times with food, each food presentation unconditionally eliciting salivation. After a number of such pairings salivation was noted to occur following presentation of the CS alone.

Arbitrariness and the Choice of CS and US

In the approximately 75 years that have followed Pavlov's pioneering investigations, Pavlovian conditioning procedures have been extensively used to investigate associative learning in man and other animals (Beecroft, 1966; Black & Prokasy, 1972; Prokasy, 1965). The emphasis of much of this research has been placed on the formulation of "laws of learning" that would be applicable in a wide variety of experimental situations and with a wide variety of subject species. Most of the experimentation, however, has been conducted within a limited number of species (e.g., rat, dog and pigeon) and experimental situations selected for reasons of convenience. It was assumed that once allowances were made for sensory and motor capacities of the subject, results from experimentation with different subject species and experimental situations would differ only quantitatively (Estes, 1959; Skinner, 1938). Thus, any one experimental situation was expected to be as satisfactory as any other situation in the investigation of associative learning:

> Pigeon, rat, monkey, which is which? It doesn't matter. ... once you have allowed for differences in the ways in which they make contact with the environment, what remains of their behavior shows astonishingly similar properties. (Skinner, 1959)

Pavlov, himself, had suggested that any CS and US could be selected for the investigation of the associative process:

It is obvious that the reflex activity of any effector
organ can be chosen for the purpose of investigation,
since signaling stimuli can get linked up with any
of the inborn reflexes. (Pavlov, 1927, p. 17)

Any natural phenomenon chosen at will may be converted
into a conditional stimulus. (Pavlov, 1928, p. 86)

It was hoped that the arbitrary selection of CS,

US and subject species would, in itself, assure a certain

generality with respect to the principles obtained from the

study of associative learning (Seligman, 1970). By the

1950s and early 1960s it appeared that the intensive

investigation of a limited number of experimental situations

had succeeded in establishing a number of general principles

regarding the associative process (e.g., Kimble, 1961).

Recently, however, several learning theorists have

suggested that, inasmuch as the neurological structure (i.e.,

associative mechanism) which underlies learning is a

biological characteristic of an organism, it should be

adapted through natural selection to the demands of the

organism's natural environment (Bolles, 1970; Garcia,

McGowan & Green, 1972; Rozin & Kalat, 1971; Seligman, 1970;

Seligman & Hager, 1972). Therefore, different species

could possess distinctively different associative mechanisms

and, as a result, qualitative features of data obtained from

learning experiments might well be determined by a

researcher's choices of organism or experimental situation.

Support for the argument that principles of

learning based on results from arbitrarily selected subject

species and experimental situations might themselves be

arbitrary and limited in generality has been derived from several different sources (Breland & Breland, 1961; Brown & Jenkins, 1968; Garcia & Ervin, 1968; Shettleworth, 1972). Nowhere, however, has the role played by an experimenter's choice of specific CS and specific US in the study of learning been more clearly illustrated than in the investigations of aversion learning in the rat (Garcia & Ervin, 1968).

Conditioned Taste Aversions

Rats readily acquire an aversion to a gustatory conditional stimulus if exposure to that stimulus is followed by a US which causes an internal disturbance. Domjan and Wilson (1972a), for example, exposed rats to the taste of saccharin flavored water (the CS) and then injected subjects with a sublethal dose of lithium chloride (the US), a drug known to produce visceral distress in man and other animals (Schou, 1957). Control subjects experienced either the taste of saccharin or the lithium toxicosis, but not both. When subsequently offered a choice between the saccharin solution and tap water, rats that had experienced the taste of saccharin followed by lithium toxicosis preferred to drink water while control rats preferred the saccharin solution. Thus, paired presentations of gustatory CS and toxic US produced a taste

aversion (the CR) while presentation of CS or US alone did not.

Not only are the operations required to produce a conditioned taste aversion equivalent to those of Pavlovian Conditioning but many results obtained from taste-aversion learning experiments are also consistent with results obtained from other Pavlovian conditioning preparations (Garcia, Hankins & Rusiniak, 1974; Mackintosh, 1975). For example, (a) the magnitude of a conditioned taste aversion is a direct function of the number of CS-US pairings experienced by the subject (e.g., Dragoin, 1971), (b) the strength of a learned taste aversion may be attenuated by interpolating a temporal delay between exposure to the gustatory CS and the subsequent administration of the US, with no taste-aversion learning occurring if the temporal delay between CS termination and US onset is sufficiently long (e.g., Domjan & Bowman, 1974; Kalat & Rozin, 1973) and (c) the magnitude of a conditioned taste aversion is a function of US aversiveness (Dragoin, 1971; Revusky, 1968).

Results from taste-aversion learning experiments are, however, quite unusual in two respects. First, rats associate gustatory CSs with toxic USs even when a very large temporal delay is interpolated between CS and US, a delay many times larger than would have been expected based on results from experiments with other CSs and USs. Second, rats do not readily associate gustatory CSs with nociceptive

USs applied to the surface of the rat's body even though these nociceptive events are quite effective USs when paired with CSs other than gustatory CSs. It is the rat's association of gustatory CS with toxic US over very long CS-US intervals and the rat's selective association of CS and US which are frequently cited as indicating the importance of an experimenter's choice of conditioning preparation to the conclusions reached about the associative process. Each of these findings is discussed in more detail below.

Aversion learning and the trace conditioning paradigm. It has been generally accepted that close temporal contiguity between CS and US is necessary for associative learning. Indeed, reviews of the relevant literature have provided extensive support for this principle (Kimble, 1961; Renner, 1964). Delaying onset of the US for even a few seconds after termination of the CS, a procedure known as trace conditioning, can dramatically attenuate the CS-US association. Optimal CS-US trace intervals have typically been described in terms of seconds or milliseconds (Ellison, 1964; Smith, Coleman & Gormezano, 1969). The longest trace intervals over which associative learning has been demonstrated with traditional Pavlovian conditioning preparations appears to be a few minutes. Kamin (1965) reported that rats could associate an auditory stimulus with subsequent electric shock even when shock

onset was delayed by two minutes following termination of the CS. Little evidence of associative learning was obtained when the CS-US interval was three minutes. There was until recently, therefore, little reason to question the assumption that close temporal contiguity between CS and US was necessary for associative learning.

To explain associative learning with relatively short trace intervals between CS and US, it was usually assumed that some central nervous system representation (a central trace) of the CS persisted for seconds or minutes at most to bridge the interval between CS and US (see Pavlov, 1927). Close temporal contiguity between CS and US was necessary for associative learning because the CS trace simply did not persist for a long period of time.

Close temporal contiguity between CS and US does not, however, seem to be a requirement for taste-aversion learning. A gustatory stimulus may be presented to a rat and then removed hours before the onset of toxicosis without eliminating associative learning. An experiment by Garcia, Ervin & Koelling (1966) illustrates this point. Rats were allowed to ingest a saccharin solution and, at different delays following removal of the gustatory stimulus, were injected with a toxin, apomorphine. Only when apomorphine administration was delayed by at least two hours after the gustatory stimulus was removed was aversion learning prevented. Numerous studies have since

demonstrated substantial taste-aversion learning with CS-US trace intervals of from 2 to 12 hours (e.g., Domjan & Bowman, 1974; Revusky, 1968; Smith & Roll, 1967).

Delaying administration of the US does not in itself rule out the possibility of CS-US contiguity. Rats might regurgitate the flavored solution during sickness, or the gustatory stimulus might persist in the rat's mouth for a long period following ingestion. In either case, physical remnants of the gustatory stimulus could occur contiguously with the US. However, rats do not regurgitate during sickness (Garcia & Ervin, 1968) and it seems unlikely that any physical trace of a gustatory stimulus could persist in the mouth over a twelve hour CS-US interval (Smith & Roll, 1967). In addition, it has been demonstrated that rats learn an aversion to a novel gustatory stimulus even when a second gustatory stimulus is interpolated between the initial stimulus and the toxicosis (Kalat & Rozin, 1970; Revusky & Bedarf, 1967). If physical remnants of the novel gustatory stimulus were responsible for CS-US contiguity, the interpolated stimulus should have altered these remnants and thereby prevented an acquisition of an aversion to the original gustatory CS. Finally, rats have learned to avoid ingesting slightly acidic water even though a litmus paper test demonstrated that acidity in the subject's mouth returned to normal well before the onset of illness (Garcia, Green & McGowan, 1969).

It appears, therefore, that physical remnants of the CS do not bridge the CS-US interval when CS offset precedes US onset by an hour or longer. This finding is in marked contrast to the close temporal contiguity required for association in other classical conditioning preparations.

Inasmuch as it has seemed unreasonable to assume that a central representation (trace) of the gustatory CS persists for hours, several investigators have suggested that new theories are required to account for taste-aversion learning in the trace conditioning paradigm (Revusky & Garcia, 1970; Rozin & Kalat, 1971; Seligman, 1970). The substantive issues dealt with in this thesis do not require an extensive treatment of the data or theories related to taste-aversion learning with a delayed US onset. [The interested reader will find detailed presentations of this information by Kalat and Rozin (1973), Revusky and Garcia (1970) and Rozin and Kalat (1971).]

Selective association of CS and US. It has been a prevalent view among learning theorists that, once allowances were made for the sensory and motor capacities of the subject, the choice of CS and US for the study of associative learning was arbitrary. That is, any one CS-US pair should lead to essentially the same conclusions

about the associative process as any other CS-US pair.
This view was supported by a substantial body of research
demonstrating that subjects did associate a wide variety
of visual, auditory, tactual, thermal and proprioceptive
stimuli with an equally wide variety of USs (see Hull,
1934; Kimble, 1961). Recent taste-aversion learning
research has shown, however, that different CS-US pairs
can lead to substantially different conclusions concerning
associative learning in the rat.

Although rats very readily learn an aversion to
gustatory stimuli paired with toxicosis, rats do not
easily learn a taste aversion when gustatory stimuli are
paired with electric shock (Domjan & Wilson, 1972b;
Garcia & Koelling, 1966; Garcia, McGowan, Ervin & Koelling,
1968). In contrast, rats readily learn to avoid auditory
or visual stimuli when these stimuli are paired with shock
but not when paired with toxicosis. A study by Domjan and
Wilson (1972b) clearly illustrates the rat's selective
association of CS and US. Two groups of rats were exposed
on four separate occasions to a solution of sodium
saccharin in tap water. Following each exposure to the
saccharin solution one group experienced a toxicosis
induced by a lithium chloride injection and the other
group experienced a nociceptive electric shock. The group
which experienced the saccharin solution followed by
toxicosis learned a taste aversion while the group which

experienced saccharin solution followed by electric shock did not learn a taste aversion. Two additional groups of rats were exposed on four separate occasions to an auditory stimulus consisting of an irregularly pulsed buzzer. One group of rats experienced a lithium induced toxicosis following each exposure to the buzzer while the second group experienced the electric shock. (Shock intensity and lithium chloride dose level were the same as used for the two groups exposed to the saccharin solution.) The group that experienced the buzzer followed by shock learned an aversion to the auditory stimulus while the group that experienced the auditory stimulus followed by toxicosis did not.

The selectivity of the rat's gustatory-toxicosis association in the Domjan and Wilson (1972b) study is unambiguous. Taste-aversion learning could not have been due to any extraordinary salience of the gustatory stimulus because the same stimulus was not avoided following its pairing with shock. Nor could it have been due to any extraordinary aversiveness of the toxicosis since the toxicosis was not able to motivate an aversion to the auditory stimulus. (A similar argument can be made for the selective association of the auditory stimulus with shock.) It seems, therefore, that the choice of specific CS and specific US can effect the outcome of a learning

experiment in a manner not anticipated by conventional views of associative learning.

Prepared Associations

To account for selective association--gustatory stimuli with toxicosis; audiovisual stimuli with shock-- several investigators have suggested that by virtue of their evolutionary history rats are "prepared", in the sense of being genetically predisposed, to associate certain types of stimuli with certain biologically important events (Garcia & Ervin, 1968; Rozin & Kalat, 1971; Seligman, 1970). For example, Garcia (Garcia & Ervin, 1968; Garcia, Hankins & Rusiniak, 1974) argues that rats are prepared to associate gustatory stimuli with visceral sensations and visual or auditory stimuli with nociceptive events applied to the surface of the rat's body. Garcia further suggests that the evolutionary basis for this selective association is easily understood when the rat is considered in its natural environment. Rats are omnivorous creatures dependent primarily upon olfaction and taste to detect and identify foods. Since the environment contains harmful as well a beneficial foods, it is advantageous for the rat to readily associate the internal consequences of harmful foods with the gustatory stimuli that identify the food. Similarly,

Garcia argues that it is advantageous for rats to associate auditory and visual stimuli with external aversive events because such associations would allow rats to readily acquire an avoidance response to predators detected at a distance. In support of this view Garcia and his colleagues (i.e., Garcia & Ervin, 1968; Garcia, Hankins & Rusiniak, 1974) cite neurological evidence suggesting gustatory and visceral afferents are integrated in a subcortical center separate from the center integrating visual and auditory afferents with somesthetic afferents. It is this neurological organization which, according to Garcia, provides the basis for selective association. Rats associate toxicosis with gustatory and not auditory stimuli because toxicosis stimulates visceral afferents and not somesthetic afferents, the reverse being true for shock.

At least one investigator (Revusky, 1971) has incorporated Garcia's selective association mechanism into an explanation of the rat's ability to associate gustatory CSs with delayed presentation of a toxic US. First, Revusky (1971) suggests that the temporal interval over which a CS and US may be associated depends upon the number of stimuli that occur in the CS-US interval and thereby compete for association with the US. In effect, stimuli that occur in the CS-US interval interfere with the subject's formation of an association between CS and US.

When the interference is great enough, that is, when a
large number of stimuli occur in the CS-US interval, the
association of CS with US can be prevented altogether.
Second, Revusky argues that only stimuli that subjects are
prepared to associate with a particular US actually
compete for association with that US. Thus, only
gustatory (and perhaps olfactory) stimuli compete for
association with toxicosis while auditory or visual
stimuli compete for association with shock. According to
Revusky (1971) then, rats are able to associate gustatory
CSs with toxic USs over long CS-US intervals because rats
experience relatively few competing stimuli between the
gustatory CS and toxicosis onset. In contrast, rats are
continually exposed to a variety of auditory and visual
stimuli which can compete for association with an external
US. Even a brief interval between auditory or visual CSs
and external USs would permit a large number of relevant
stimuli to compete for association with the US. Therefore
associative learning with auditory or visual CSs and an
exteroceptive US is attenuated by the interpolation of
even very short delays between CS termination and US onset.

Site of US Application vs. US Temporal Features

The mechanism suggested by Garcia (e.g., Garcia &
Ervin, 1968) as the basis for selective association assumes

the important functional difference between shock and
toxicosis is that the former is somesthetic while the
latter is visceral. However, shock and toxicosis differ
on several dimensions other than site to which the US is
applied. The shock administered in learning experiments
is typically a localized environmental event having a
rapid onset (i.e., rising quickly to a maximum intensity),
short duration and rapid offset. In contrast, toxicosis
is a pervasive internal experience having a slow onset,
long duration and a slow recovery.

Not only do shock and toxicosis differ on a
variety of dimensions, but published demonstrations of
selective association have clearly confounded a number of
these differences with US receptor site. Domjan and
Wilson (1972b), for example, demonstrated selective
association with a 500 millisecond shock and a lithium
toxicosis as external and internal USs, respectively.
The 500 millisecond shock is clearly a punctate US with a
rapid onset and short duration while the lithium toxicosis
is a diffuse experience which typically develops over a
period of five to fifteen minutes and lasts from one to
four hours (Barker & Smith, 1974; Nachman, 1970). Thus
subjects in the Domjan and Wilson (1972b) study could have
associated the lithium toxicosis with the gustatory CS and not
the auditory CS because the toxicosis had a slow onset and

long duration and not because the toxin acted upon visceral receptors.

A similar argument can be made with respect to other demonstrations of selective CS-US association, even when exposure to ionizing radiation, instead of a toxic chemical, was used as the "internal" US (Garcia & Koelling, 1966; Garcia, McGowan, Ervin & Koelling, 1968). Just as administration of lithium chloride leads to a visceral distress which has a slow onset and long duration, exposure to ionizing radiation also produces an internal malaise, "radiation sickness", which has a slow onset and long duration (at least at dose rate and level reported in these experiments) (Garcia & Ervin, 1968). Thus in each case, the selective association of gustatory CS with toxic US (either administration of a chemical or irradiation) and auditory or visual CS with shock could have been a function of US temporal characteristics rather than site of US application.

Perhaps the temporal characteristics of the US are particularly important for the acquisition of a conditioned taste aversion. If so, then an internal US having a rapid onset, short duration and limited site of application might not be (a) readily associated by rats with a gustatory CS, (b) readily associated with a gustatory CS over a relatively long CS-US trace interval, or

(c) selectively associated with gustatory stimuli.

Research Strategy

To investigate the possibility that the temporal characteristics of the internal US play an important role in the distinctive results obtained from taste-aversion learning experiments, it is necessary to control directly rate of US onset and duration of US application. Inasmuch as control over onset and duration of chemical or radiation induced toxicosis did not seem possible without manipulation of a confounding variable such as peak blood concentration of a toxin, another form of internal US was sought.

One form of internal US is distention of the stomach by means of a chronically implanted balloon.[1] Miller (1957) reported that distention of a rat's stomach by means of a chronically implanted balloon could disrupt bar pressing for a food reward and that rats also learned to avoid the arm of a T-maze associated with stomach

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[1] Another form of internal US which was investigated in a preliminary fashion was electric shock applied directly to the viscera. However, experiments in which an electric current was applied to the mucosa of the stomach through implanted electrodes encountered several obstacles. The electrodes rarely remained in place more than a day or two. A low voltage shock failed to motivate taste-aversion learning even when applied for a long period of time. At higher intensities the current caused contractions of thoracic and abdominal striate muscles, which are not usually classed as visceral.

distention, suggesting that stomach distention can act as an aversive stimulus. Furthermore, since rate of inflation, rate of deflation and duration of inflation are readily controlled, the temporal features of balloon inflation can be manipulated to produce an internal stimulus which is temporally similar to either shock or toxicosis while maintaining the same site of US application. Thus, stomach distention by means of a chronically implanted balloon would seem well suited for the exploration of the role played by the temporal features of the internal US in taste-aversion learning.

CHAPTER 2: DISTENTION PARAMETERS

Inasmuch as direct, mechanical distention of the stomach had not been previously used to motivate taste-aversion learning, the initial experiments were designed to (a) assess the efficacy of stomach distention in the taste-aversion preparation, and (b) to explore the relationship between basic balloon inflation parameters (volume and duration) and the magnitude of taste-aversion learning.

Experiment 1: Initial Demonstration

Experiment 1 was designed to determine if taste-aversion learning would be motivated by inflation of an intragastric balloon. Two groups of rats were prepared with stomach balloons. One group of rats drank a distinctively flavored solution prior to stomach balloon inflation; the second group drank the same solution but their stomach balloons were not inflated. Following this training, preference for the flavored solution was assessed by allowing subjects to choose between the flavored solution and tap water.

If stomach balloon inflation effectively motivated

taste-aversion learning, subjects' whose stomachs were distended following ingestion of the flavored solution would subsequently evidence lower preference for this solution in comparison to subjects who experienced the flavored solution but not the stomach distention. However, the distention subjects might evidence an aversion to the flavored solution for reasons other than because they associated the flavor with the gastric stimulation. For example, rats are generally reluctant to ingest large quantities of relatively novel solutions, and the experience of internal malaise has been reported to enhance this behavior (Rzoska, 1953; Rozin, 1969). Thus mere distention by itself, whether or not preceded by a gustatory CS, might cause subjects to subsequently avoid any flavored solution. To assess this possibility, and the possibility of other nonassociative consequences of the distention experience, a third group of rats was prepared with stomach balloons. This group did not drink the flavored solution until several minutes after balloon inflation was terminated. Subjects in the third group, therefore, experienced the same exposure to gustatory CS and balloon inflation US as did subjects in the first group, but the sequence of exposure eliminated the forward pairing of CS and US usually considered necessary for aversion learning. That is, rats cannot "learn" an

aversion to a stimulus which does not precede or at least occur contiguously with an aversive US (Mackintosh, 1975). Thus, an aversion resulting from the backward pairing of CS and US is typically attributed to some nonassociative consequence of the subjects experiencing these events. To unambiguously demonstrate taste-aversion learning, subjects that ingest the flavored solution before stomach distention must subsequently show a greater aversion to the flavored solution than subjects that ingest the flavored solution after termination of stomach distention.

Method

Subjects and pre-experimental preparation. Eighteen male rats, (Rattus norvegicus, Charles River strain, obtained from Canadian Breeding Farms, St. Constant, Quebec) weighing 275 to 300 grams, were individually housed and maintained on food (Purina Rat Pellets) and water ad libitum except as noted below.

Each subject was placed under general anesthesia and surgically prepared with a stomach balloon before it participated in the experiment. A detailed description of balloon construction and surgical procedure is presented in Appendix 1. In brief, a stomach balloon with cannula was prepared from a section of latex finger cot tied to a length of Clay-Adams "Intramedic" polyethylene tubing

(size, P.E. 160). The balloon was inserted through a small incision into the antral portion of the stomach and positioned along the greater curvature (see Figure 1a). The cannula portion of the balloon was passed through the muscle wall of the abdomen and threaded subcutaneously to the back of the neck where it was externalized. The stomach was sewn to the peritoneal cavity wall at the point where the cannula passed through the muscle (a point just posterior to the most caudal extension of the left rib cage) and the externalized portion of the cannula was held in place with a small plastic washer trapped by the flared end of the polyethelene tubing.

Subjects typically regained preoperative weight and appeared normal in activity and response to handling within two or three days after surgery.

Apparatus and stimuli. During daily experimental sessions a subject was placed in a Fisher Scientific small animal restrainer (chamber area, 18 centimeters long by 6.5 centimeters wide) with its tail taped to an extension at the back of the restraint (Figure 1b). An opening in the front wall of the restraint allowed insertion of a drinking tube and a second opening on top of the restraint allowed connection of an extension to the stomach-balloon cannula. Fluids, either water or a .15% (w/v) solution of sodium saccharin in water, were presented to a restrained

Figure 1

Experiment 1:   1a) Location of the balloon within the rat's stomach.   1b) Restraint.   1c) Drinking spout and reservoir.

Figure 1

1a)

1b)

1c)
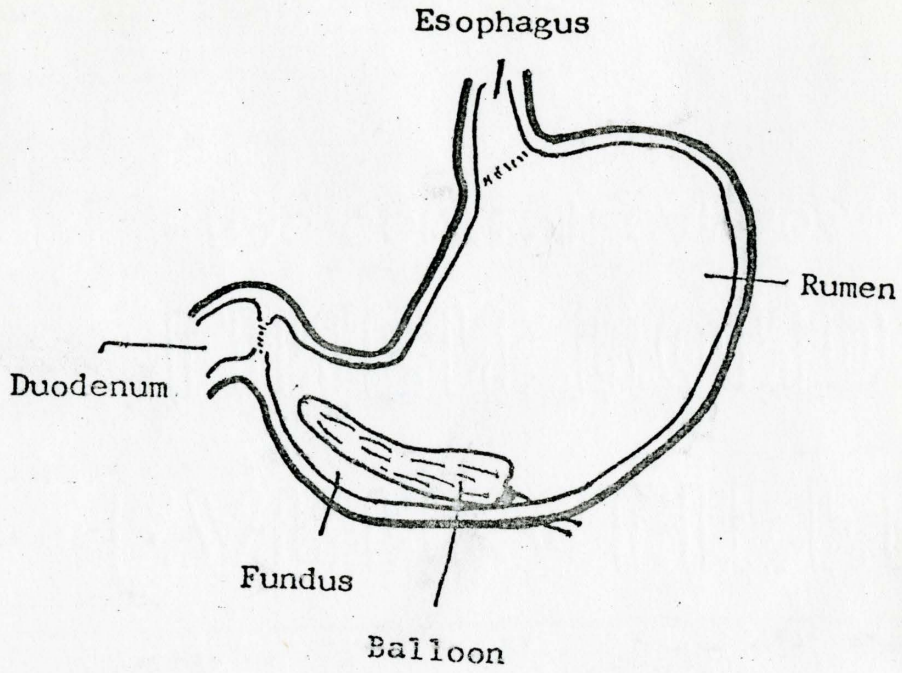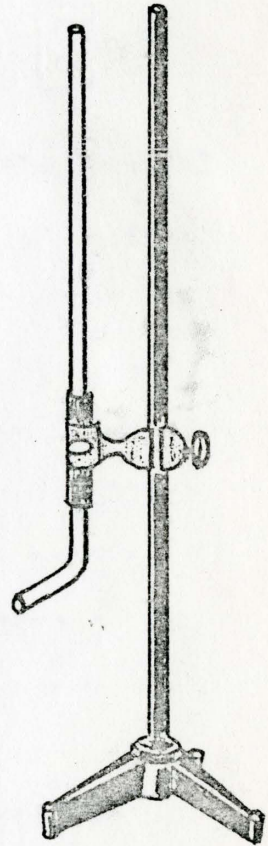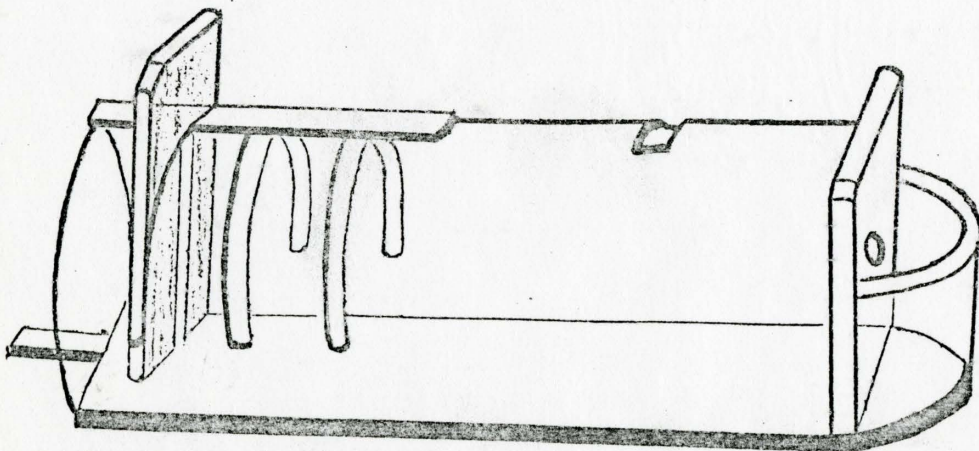
subject through a stainless steel drinking tube connected via a rubber collar to a fluid reservoir (a #8 Pyrex, thin-walled glass column). The drinking tube and reservoir were mounted on a ring stand to allow easy insertion into and withdrawal from the restraint apparatus (Figure 1c).

Inflation of the stomach balloon in the restrained subject was accomplished by manually infusing a premeasured volume of room temperature water into the stomach balloon through a cannula extension affixed to the externalized portion of the balloon cannula. The period of balloon distention was terminated by withdrawing the water from the balloon.

Adaptation. The experiment began with the removal of water from the rats' home cages five days after surgery. Each day thereafter each subject was removed from its home cage to an experimental room and placed in the restraint apparatus for one-half hour. During this restraint period each subject was allowed to drink water for three minutes from a spout inserted into the restraint. Following this one-half hour of restraint each subject was returned to its home cage where it received further access to water for one-half hour.

Treatment. Following five days of adaptation, subjects were assigned to one of three treatment conditions: operated, saccharin only control (Group Operated Control-- OpC, $\underline{n}=6$), forward pairing of CS and US (Group Forward

Conditioning--FCd, n=6), or backward pairing of CS and US, with exposure to the saccharin solution being delayed until five minutes after balloon deflation (Group Backward Pairing, 5 minutes--Bkd-5, n=5).[2] During each of two treatment sessions, one per day, OpC subjects were exposed to the CS solution and 10 minutes later were returned to home cages. FCd subjects were exposed to the saccharin solution and then immediately experienced 20 minutes of balloon inflation before being returned to home cages. Bkd-5 subjects experienced 20 minutes of balloon inflation and, five minutes after balloon deflation, were exposed to the saccharin solution before being returned to home cages.

Each exposure to the saccharin solution was limited to either (a) 180 seconds from the first lick, or (b) a total volume of 3.0 milliliters, whichever occurred first. Each balloon inflation consisted of infusing water into the balloon until resistance was met or to a limit of 20 ml. All subjects were given their daily one-half hour access to water immediately after each treatment session.

Testing. Twenty-four hours after the second treatment session, two calibrated cylinders, one containing tap water and the other the saccharin solution, were placed on each subject's home cage. Fifteen minutes later the cylinders were removed and the amount of each fluid

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[2] Six subjects were originally assigned to Group Bkd-5, but one died during the course of the experiment, and its data are excluded.

consumed recorded. For each subject, a saccharin preference ratio was calculated by dividing the volume of saccharin solution ingested by the total volume of fluid consumed (saccharin plus water). This ratio can range from a value of 0.0 (complete refusal of the saccharin solution) to 1.0 (complete preference for saccharin).

To summarize briefly, the experiment consisted of eight daily experimental sessions: five adaptation, two treatment and one test. Experimental treatments consisted of subjects (a) ingesting the saccharin solution (Group OpC), (b) ingesting the saccharin solution and then experiencing stomach distention (Group FCd), or (c) experiencing stomach distention and then ingesting the saccharin solution (Group Bkd-5). Finally, saccharin preference was assessed with a two-solution choice test conducted in the home cage.

Results

All two-group statistical comparisons described below are based on the nonparametric Mann-Whitney U test, and all reported probability levels are two-tailed.

Inflation volumes during treatment sessions. Median inflation volumes during Treatment Sessions 1 and 2 were, respectively, 18.3 ml and 13.0 ml for the FCd group, and 18.0 ml and 15.0 ml for the Bkd-5 group. The groups

Figure 2

Experiment 1: Median saccharin preference ratios
for groups that drank a saccharin solution either before
(FCd) or after (Bkd-5) balloon inflation during treatment
sessions. The saccharin only group (OpC) did not
experience stomach distention. Number of subjects per
group is indicated in parenthesis.

Figure 2
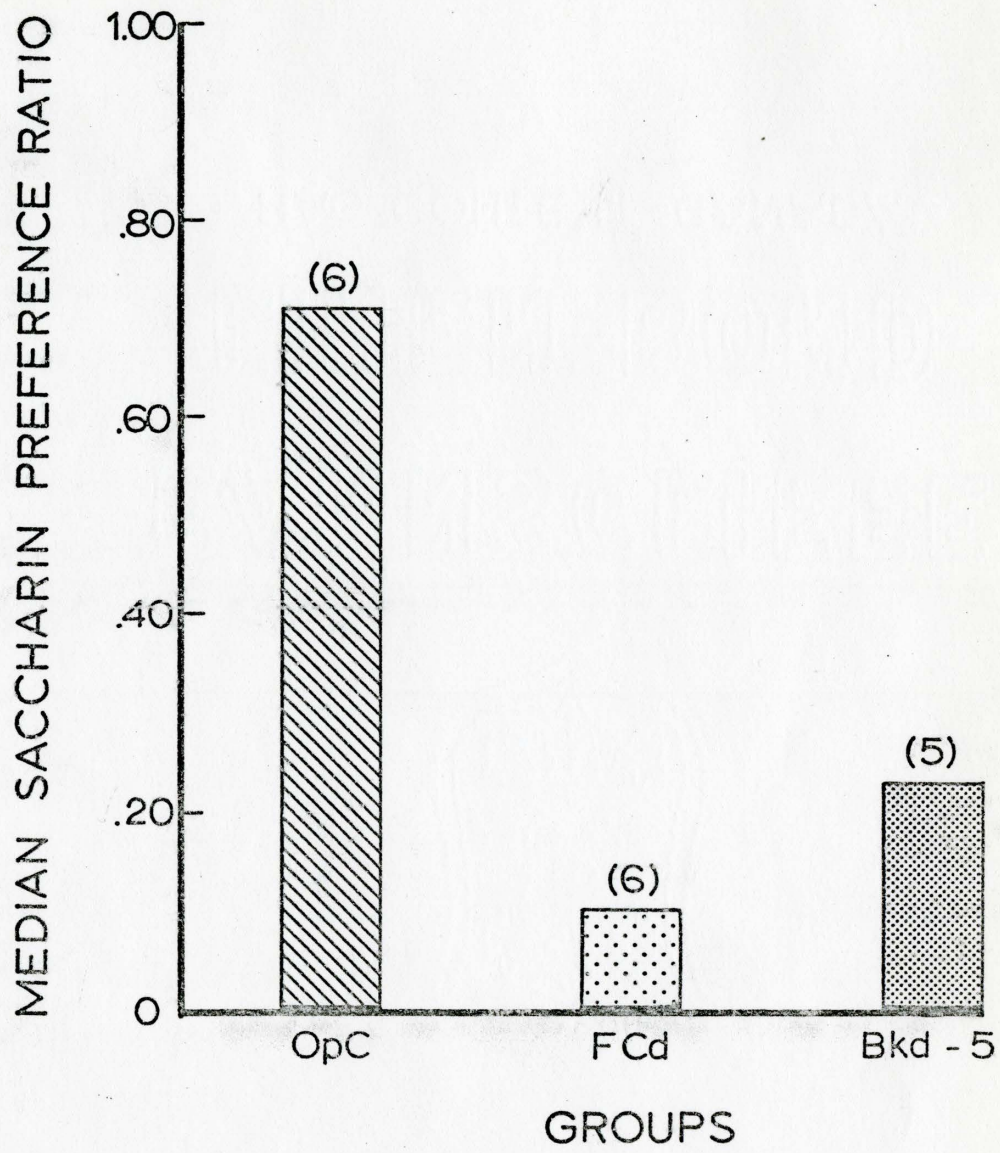
did not differ significantly in inflation volume during either treatment session.

Test session. The median saccharin preference ratio for each group is presented in Figure 2.[3] Subjects that experienced the 20 minute balloon inflation either before (Bkd-5) or after (FCd) ingestion of the saccharin solution during treatment sessions evidenced significantly lower saccharin preference ratios than did subjects who experienced only the saccharin solution on training trials (FCd vs. OpC, $U=1$, $p=.004$; Bkd-5 vs. OpC, $U=1$, $p=.008$). Although the FCd group appeared to have a stronger aversion to saccharin than the Bkd-5 group, the difference between groups was not statistically significant ($U=11$, $p>.20$).

Discussion

Because the backward pairing group evidenced a significant aversion to the saccharin solution, the aversion to saccharin exhibited by the forward pairing subjects does not provide an unambiguous demonstration of taste-aversion learning with stomach distention as the US. The aversions apparent in both groups could reflect a nonassociative consequence of saccharin ingestion and/or

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[3] Appendix 2 presents median milliliters of total fluid ingested by each group in each experiment during preference tests and discusses these data in relation to between group comparisons based on preference ratios.

the stomach distention experience.

In the introduction to this experiment it was indicated that rats could not learn an aversion to a stimulus unless that stimulus preceded or occurred contiguously with the US. The fact that backward pairing subjects were exposed to the gustatory stimulus five minutes after balloon deflation had occurred would therefore appear to preclude taste-aversion learning. If, however, the stomach distention procedures used in Experiment 1 induced an internal malaise which did not end with balloon deflation, then rats experiencing backward pairing could have had an internal distress both during and after exposure to the saccharin solution. Onset of the gustatory stimulus need not occur before the onset of internal malaise for the effective acquisition of a learned taste aversion: see, for example, Boland, 1973; Barker and Smith, 1974.

Indeed, observation of the rats indicated that subjects in both forward and backward pairing groups appeared to experience a malaise for many minutes following deflation of the stomach balloon. Subjects in both groups were lethargic, unresponsive to handling and appeared uninterested in food for at least an hour after balloon distention. In contrast, subjects in the control group, that is, subjects that did not experience balloon inflation

during the treatment session, ate and drank avidly when returned to home cages. It is possible, therefore, that Bkd-5 subjects experienced internal malaise following the taste stimulus and that the taste aversions apparent in both forward and backward pairing groups reflect an associative process rather than a nonassociative consequence of the distention experience.

Experiment 2:   Distention Volume

Experiment 1 did not provide an unambiguous demonstration of taste-aversion learning because subjects that experienced balloon inflation before ingesting the saccharin solution (a backward pairing of CS and US) subsequently evidenced a saccharin aversion during the preference test.   It was suggested, however, that subjects in this backward pairing group may have actually learned a saccharin aversion by associating the gustatory CS with an extended internal malaise produced by the distention volume and duration used as the US in Experiment 1.

Inasmuch as the distention procedures used in Experiment 1 appeared to be very aversive to rats, it seemed likely that distention volumes smaller than 15 to 18 ml could be effectively used as internal USs without causing the subject to experience a long lasting internal malaise.   If inflation volumes smaller than used in Experiment 1 are aversive to rats, than a clearer demonstration of taste-

aversion learning motivated by stomach balloon inflation might be possible using a smaller inflation volume as the US. Experiment 2 was designed to assess the relation of balloon inflation volume to subsequent preference for the CS solution.

Method

All unspecified detail of the method and apparatus remained as described in Experiment 1.

Twenty-four rats were prepared with stomach balloons, and, following a recovery period, adapted to handling, restraint, and the $23\frac{1}{2}$ hour water deprivation schedule. Originally, three subjects were assigned to each of six different distention volume groups (3, 6, 9, 12, 15 and 18 ml) and six subjects were assigned to a saccharin only group (0 ml). However, the balloon of one subject in each of the 15 ml and 18 ml groups ruptured during the experiment, and one subject in the 18 ml group died prior to testing. The data from these three subjects are excluded.

During each of the two treatment sessions, subjects drank the saccharin solution and then experienced stomach balloon inflation of the designated volume for twenty minutes. Twenty-four hours after the second treatment session saccharin preference was assessed with the two-

solution choice test.

Results and Discussion

The results of the saccharin preference test are presented in Figure 3 as group median preference ratios. Although there is no overlap between the 0 ml and the 15 or 18 ml groups in saccharin preference, the small sample size in the latter two groups precludes these differences from reaching statistical significance. The saccharin preference of the 9 and 12 ml groups, however, is significantly lower than the 0 ml group (both Us=1, both ps=.048). Although the median saccharin preference ratio in the 6 ml group is comparable to the median preference in the 9 ml group, the 6 ml group is not significantly different from the 0 ml group (U=3, p>.10). The saccharin preference of the 3 ml group is also not different from the 0 ml group (U=6, p>.20).

From the results of this experiment, we may conclude, (a) smaller balloon inflation volumes produce weaker taste aversions, and (b) inflation volumes substantially less than used in Experiment 1 are aversive to rats. The results suggest that balloon inflation volume may be analogous to shock intensity and drug and irradiation dose level in the direct manipulation of US aversiveness (Kamin & Brimer, 1963; Nachman, 1970;

Figure 3

Experiment 2: Median saccharin preference ratios for groups that ingested the saccharin solution and then experienced 0, 3, 6, 9, 12, 15 or 18 ml of balloon inflation for twenty minutes during each of two treatment sessions. Number of subjects per group is indicated in parenthesis.

MILLIMETERS OF DISTENSION

GROUPS
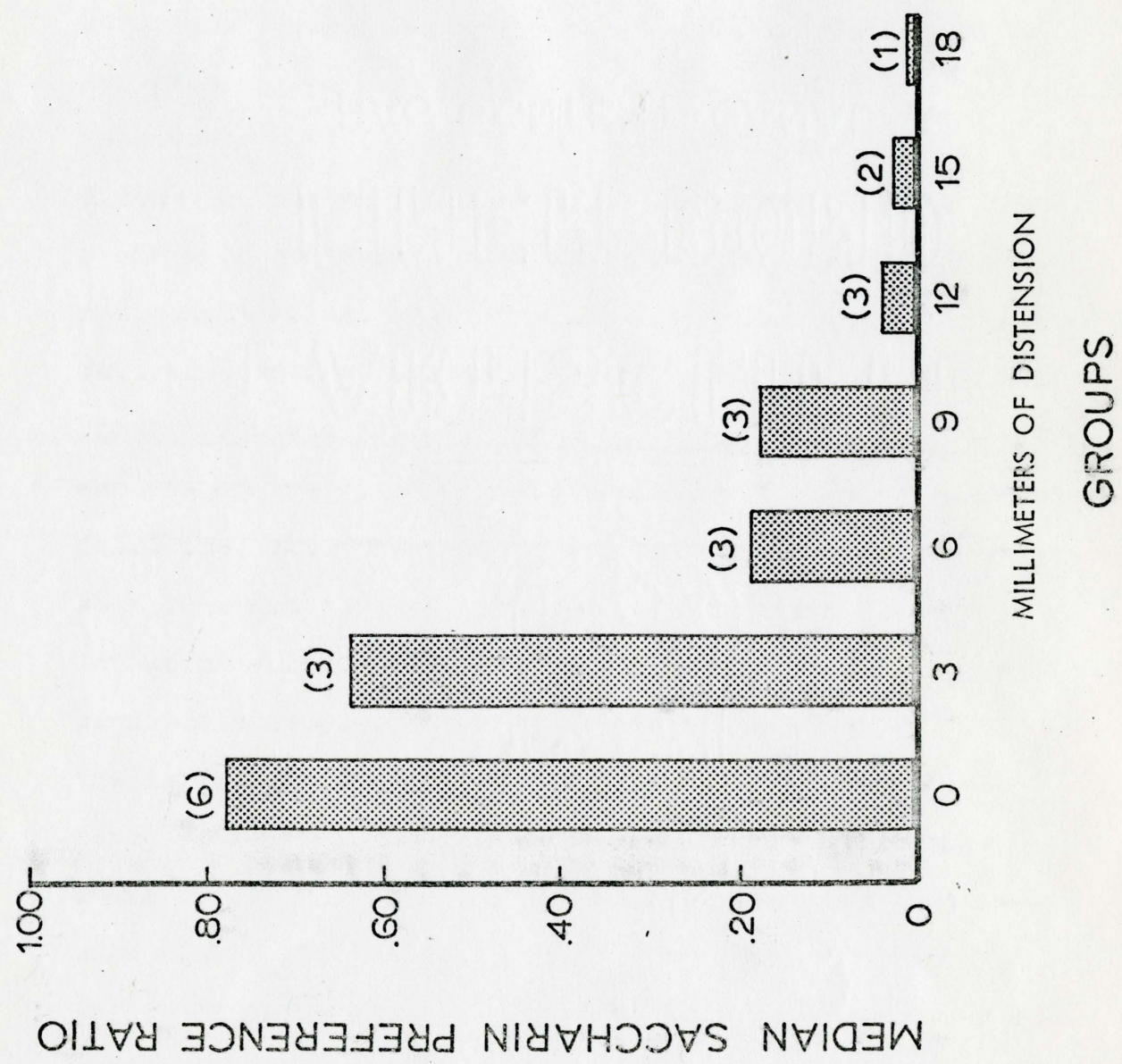
Figure 3

Revusky, 1968). Observation of the subjects indicated
that larger volumes of balloon inflation, in addition to
producing stronger taste aversion, also elicited more
behaviors suggesting an aversive experience. Balloon
inflation volumes of 3 or 6 ml produced almost no overt
reaction during distention while the 9 ml inflation volume
consistently elicited a mild response. During the
distention period subjects in the 9 ml group lay on their
right side and turned their head as if to look over the
left shoulder (recall that the stomach was anchored to the
left side of the peritoneal cavity just posterior to the
ribs). Subjects in all three of these groups were active
and responsive to handling immediately following balloon
deflation, and each subject ate and drank with alacrity
when returned to its home case. In contrast, balloon
inflation volumes of 12 ml or greater usually elicited
vigorous struggling and vocalization, particularly during
the first few minutes of distention. Furthermore, when
returned to their home cases subjects that experienced
these larger volumes of balloon inflation were relatively
unresponsive to handling and did not begin immediately
to eat or drink when returned to their home cases, as did
control subjects.

Experiment 3:   Distention Duration

(0, 8, 12 and 16 Minutes)

Inflation of a stomach balloon was selected as an internal US because it appeared to have the potential for temporally discrete stimulation of the viscera (when compared to toxicosis). The results of Experiment 1--an experiment designed to demonstrate taste-aversion learning motivated by balloon inflation--suggested, however, that the inflation parameters used in that experiment may have produced an internal malaise that lasted an hour or longer. It appeared necessary, therefore, to investigate the relation of balloon inflation parameters such as volume and duration to US aversiveness to determine more appropriate balloon inflation parameters for the study of taste-aversion learning with a discrete internal US.

Experiment 2 was designed to determine the relation of balloon inflation volume to US aversiveness. The aversiveness of the US was assessed by pairing the US with a flavor and then subsequently testing the subject's preference for that flavor. The results of Experiment 2 indicated that an inflation of 6 or 9 ml was aversive to the rat without producing behaviors suggesting a long lasting internal malaise.

The present experiment was designed to investigate the relation of inflation duration to subsequent preference for a CS solution. All distended subjects experienced the

same volume of balloon inflation and differed only with respect to the duration of this distention.

Method

The general methodology was similar to that described in Experiments 1 and 2. Subjects were prepared with a stomach balloon and allowed five days for recovery from surgery. Following recovery, subjects (a) were adapted to restraint, handling and a 23½ hour water deprivation schedule (one adaptation session per day for five days), (b) experienced two treatment sessions (one per day), and (c) were tested for their CS solution preference 24 hours after the second treatment session.

Details of the procedure are described below with all unspecified aspects remaining as previously described.

Feeding schedule. During Experiments 1 and 2 subjects were maintained on an ad libitum food schedule. In the present and subsequent experiments, subjects were deprived of food 4½ to 5 hours prior to each of the two treatment sessions, thereby minimizing differences between subjects in volume of food in the stomach at the time of balloon inflation. Food was returned to a subject's home cage immediately following each treatment session.

Conditional stimulus. In Experiments 1 and 2 subjects were allowed to ingest 3.0 ml of the CS solution or to drink for 180 seconds, whichever came first. In this and

all subsequent experiments exposure to the CS solution

(the .15% solution of sodium saccharin in tap water) was

limited to 110 seconds beginning from first lick. This

time limit was chosen because 110 seconds was the average

time required for a subject to ingest 3.0 ml of the CS

solution during the first treatment session of Experiments

1 and 2. Consequently, the change in procedures used to

present the CS did not substantially alter the amount of

CS solution consumed prior to distention.

Unconditional stimulus. A 9 ml volume of balloon

inflation was chosen for this experiment because it was the

smallest volume that reliably produced a taste aversion

(Experiment 2). In this and all experiments that follow,

the time required to accomplish complete infusion of 9 ml

of water into the stomach balloon was 7 to 10 seconds (rate,

approximately .1 ml/second) while balloon deflation required

18 to 25 seconds (rate, approximately .4 ml/second). A

specified duration of distention (e.g., 10 minutes) refers

to the period of complete balloon inflation.

Treatment sessions. Twenty-three subjects were

prepared with stomach balloon and assigned to one of three

distention conditions (US durations of 8, 12 or 16 minutes,

each $n=6$) or a CS only condition (Group OpC, $n=5$). Six

additional subjects that had not been prepared with

stomach balloons but had otherwise been treated as had

surgically prepared subjects were assigned to a second CS

only condition (Group Nonoperated Control--NopC). During each treatment session subjects from the three distention groups ingested the CS solution and then experienced 9 ml of balloon inflation for the specified duration (8, 12 or 16 minutes). Subjects in the CS only groups (OpC or NopC) ingested the CS solution, remained in the restraint for 10 minutes and then were returned to their home cages.

Twenty-four hours after the second treatment session preferences were assessed with the two-solution choice test.

Results and Discussion

The preference test results are presented in Figure 4. Operated (OpC) and nonoperated (NopC) CS only groups did not differ in their preference for the saccharin solution ($U=14$, $p>.10$) and their data were therefore combined for statistical comparisons with distention groups.

Both 12- and 16-minute distention durations effectively motivated a taste aversion. Each group was statistically different from the combined saccharin only control groups ($U=2$ and 6, for 12- and 16-minute groups, respectively, $p<.002$). The apparently greater aversion of the 12-minute group as compared to the 16--minute group did not achieve statistical significance ($U=6$, $p=.064$).

In contrast to the 12 and 16 minutes of stomach

Figure 4

Experiment 3: Median saccharin preference ratios
for groups experiencing 8, 12 or 16 minutes of stomach
distention. NopC and OpC refer to nonoperated and
operated, saccharin only control groups, respectively.
Number of subjects per group is indicated in parenthesis.

Figure 4
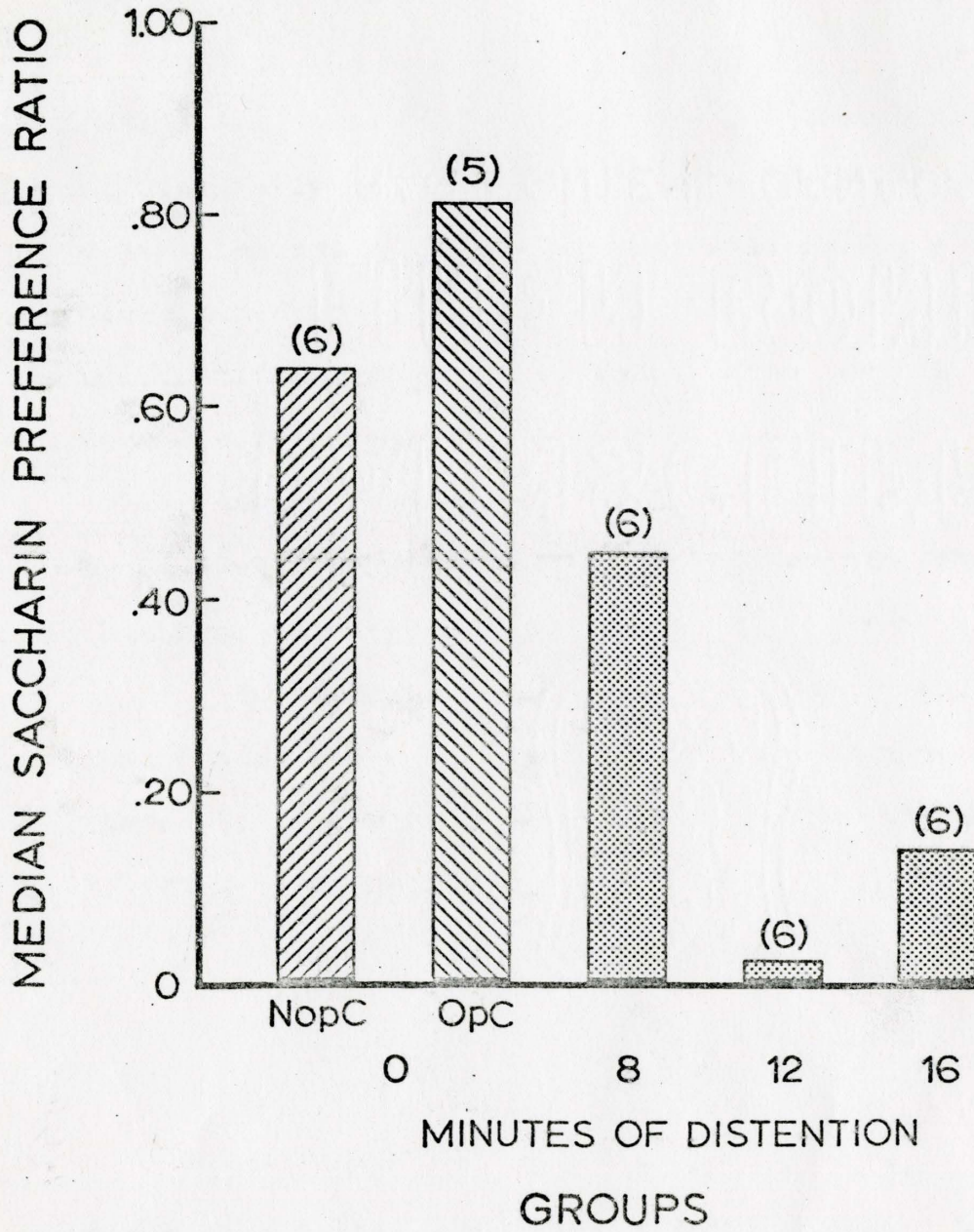
distention, 8 minutes of distention did not produce a reliable saccharin aversion. Subjects in this group did not differ statistically from those in the combined control groups ($\underline{U}=8$, $\underline{p}>.10$). On the other hand, because three subjects in the 8-minute group evidenced low preference ratios (less than 0.15), this group was also not statistically different from the 12- or 16-minute distention groups ($\underline{U}=8$ and 11, respectively, $\underline{p}s>.10$). Given two prior exposures to a .15% sodium saccharin solution, a saccharin preference ratio less than 0.15 is exceedingly unlikely (in a water vs. saccharin choice test) unless exposure to the saccharin solution has been followed by an aversive internal US. It is possible, therefore, that some rats found the 8-minute distention aversive.

In summary, the results of this experiment suggest that reducing distention duration from 12 minutes or longer to 8 minutes substantially reduces the aversiveness of the 9 ml stomach distention.

Experiment 4: Distention Duration
(0, ½, 4 and 10 Minutes)

Experiment 3 found that a 12-minute, 9 ml distention reliably produced a taste aversion while an 8-minute distention of the same volume did not. Experiment 4 continued the investigation of distention

duration with with distention periods of $\frac{1}{2}$, 4 and 10 minutes. Since the nonoperated, saccharin only control group did not behave differently from the operated, saccharin only control group in Experiment 3, only nonoperated control subjects were used in this experiment.

Method

All unspecified detail of the procedure remained as described in Experiment 3. Each of twenty subjects was prepared with a stomach balloon. Seven subjects were assigned to a 10-minute distention duration group, six to a 4-minute distention duration group and seven to a $\frac{1}{2}$-minute distention duration group. Thirteen nonoperated subjects were assigned to a saccharin only control group (NopC). During each of the two treatment sessions, subjects in distention groups drank the saccharin solution and then experienced 9.0 ml of balloon inflation for the appropriate duration. Subjects in Group NopC drank the saccharin solution, remained in the restraint for 10 minutes, and then were returned to their home cages.

Each subject was tested for saccharin preference 24 hours after the second treatment session. In contrast to previous experiments, an additional test of saccharin preference was conducted 24 hours after the first preference test.

Results and Discussion

Median saccharin preference ratios for Test Day 1 (left panel) and Test Day 2 (right panel) are presented in Figure 5. Results of the first preference test suggest that only the 10-minute distention was aversive to subjects. The 10-minute group is statistically different from both 1/2- and 4-minute groups (U=0 and 1, respectively, ps<.002) as well as the NopC group (U=0, p<.001). On this first test day neither the 1/2- nor the 4-minute group was significantly different from the NopC group (U=25 and 27, respectively, ps>.20). The second test, however, indicated an aversion to saccharin in the 4-minute group which was not apparent during the first test. The 4-minute group was statistically different from both the NopC and 1/2-minute groups (U=13 and 5, respectively, ps<.05), while the 10-minute group continued to show the strongest saccharin aversion, being significantly different from the 4-minute group (U=4, p=.014), as well as the NopC and 1/2-minute groups (U=0 and 3, respectively, ps<.01). The aversion to saccharin apparent in the 4-minute group seems to have resulted from an interaction between the prior stomach distention experience and exposure to the saccharin solution during the first preference test. Median saccharin preference ratios rose from .53 (Test 1) to .78 (Test 2) for the NopC group and from .38 to .93 for the 1/2-minute group while saccharin preference remained

Figure 5

Experiment 4:   Median saccharin preference ratios

for groups experiencing $\frac{1}{2}$, 4 or 10 minutes of stomach

distention.   Test Day 1 is shown in the left panel with

Test Day 2 shown in the right panel.   NopC refers to the

nonoperated, saccharin only control group.   Number of

subjects per group is indicated in parenthesis.
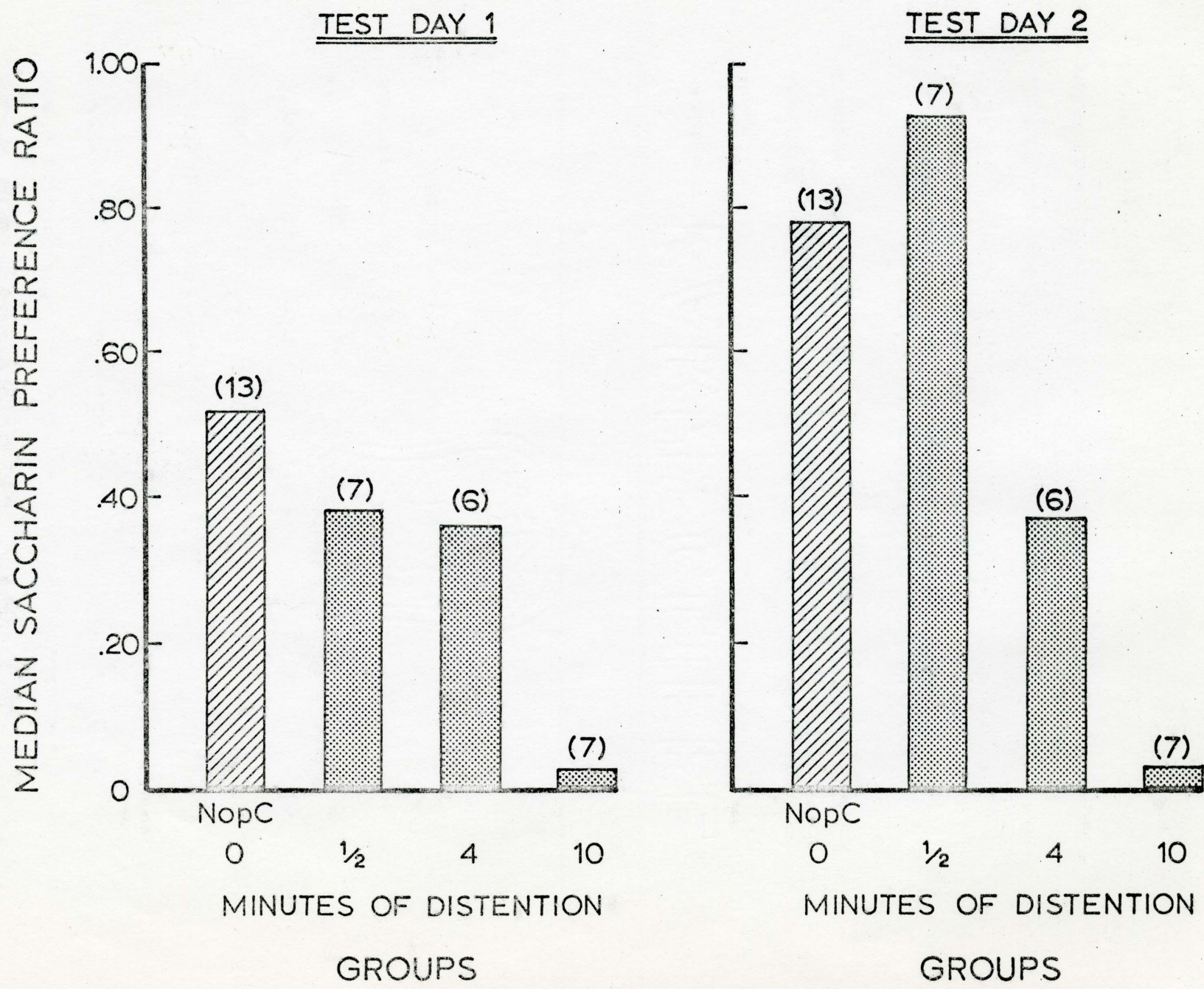
Figure 5

virtually unchanged in the 4- and 10-minute groups. This suggests the 4- and 10-minute distention durations were sufficiently aversive to block, via associative learning or some nonassociative process, the facilitation of saccharin preference due to the exposure of subjects to the saccharin solution during Test 1.

To summarize, 9 ml of distention lasting 4 or 10 minutes was sufficiently aversive to produce a taste aversion. However, there was no reliable evidence that subjects found aversive a distention of the same volume lasting only one-half minute.

Experiment 5: Backward Conditioning with Free Ingestion of the Saccharin Solution

Experiment 1 failed to provide an unequivocal demonstration of taste-aversion learning. Subjects that experienced two forward pairings of gustatory stimulus and stomach distention did not show a significantly stronger taste aversion than subjects that experienced two backward pairings of the gustatory CS and stomach distention. Thus, taste aversions evidenced by both groups could have been a function of some nonassociative consequence of the distention and/or saccharin exposure experiences. It was argued, however, that aversions apparent in both backward

and forward pairing groups might, in fact, have been learned. The behavior of the subjects following balloon deflation suggested that the inflation procedures used in Experiment 1 actually induced an internal malaise which extended well beyond the period of balloon inflation. As a result, subjects in the backward pairing group could have experienced an internal malaise following ingestion of the saccharin solution during treatment sessions and this could have served as the basis for taste-aversion learning even though US preceded CS. If this argument is correct, then a clearer demonstration of taste-aversion learning motivated by stomach balloon inflation should be possible with balloon inflation procedures which are aversive but do not cause a long lasting internal malaise.

Experiments 2 through 4 demonstrated that balloon inflation volumes and durations substantially smaller than used in Experiment 1 were aversive to rats. Furthermore, these smaller balloon inflations did not appear to produce effects which extended beyond the period of balloon inflation.

Experiment 5 was designed to demonstrate taste-aversion learning motivated by stomach balloon inflation using balloon inflation parameters (9 ml for 10 minutes) that reliably produced a taste aversion without appearing to produce a long lasting internal malaise. (The 10-minute

inflation duration was selected because it was the shortest duration which reliably produced taste aversions in the first preference test.) In addition, Experiment 5 was designed to directly assess whether the 9 ml, 10-minute balloon inflation induces an extended internal malaise and, if so, the duration of this malaise.

Method

Design. As in previous experiments five days were allowed for recovery from surgery and the recovery period was followed by five adaptation sessions, two treatment sessions and one test session.

During treatment sessions subjects were allowed to drink a saccharin solution either before (forward pairing) or after (backward pairing) their stomach balloon was inflated for 10 minutes. Subjects that drank the saccharin solution after distention were divided into three groups that differed only in the length of time from the end of distention (i.e., balloon deflation) to the beginning of saccharin drinking. One group ingested the saccharin solution immediately after ballon deflation while for the second and third groups delays of 10 and 30 minutes, respectively, were interpolated between balloon deflation and saccharin ingestion.

In addition to the four distention groups (one

forward and three backward pairing groups) a fifth group ingested the saccharin solution. This group, however, did not experience balloon inflation and thus constituted a CS-only control condition.

Associative learning would be most clearly demonstrated if subjects that experienced the forward pairing of gustatory stimulus and balloon inflation evidenced a taste aversion during the preference test while subjects that experienced the backward pairing of CS and US did not.

If one or more of the backward pairing groups does exhibit an aversion to the gustatory CS during the preference test, this may reflect either (a) some nonassociative consequence of the distention experience or (b) persistent aftereffects of distention being effectively forward paired with the flavor CS. The design of the present experiment permits an evaluation of the extent to which the effects of distention persist beyond balloon deflation, thus enabling an assessment of the contribution of persistent distention aftereffects to any apparent backward conditioning.

One means of estimating the duration of an internal malaise is through overt signs of visceral distress, especially the loss of thirst. Barker and Smith (1974), for example, estimated the onset and duration of lithium

toxicosis by recording the volume of water ingested during short drinking periods at different delays following a lithium injection. As long as water ingestion remained suppressed it was inferred that subjects continued to experience an internal malaise. During the treatment sessions of Experiment 5, different backward pairing groups received a short period of access to the saccharin solution at different temporal delays following balloon deflation. If the 9 ml, 10-minute balloon inflation produces an internal malaise which extends beyond the period of inflation, ingestion of the saccharin solution might be distrupted in one or more of the backward pairing groups. Recovery from the balloon inflation experience would be suggested by the absence of disruption of saccharin ingestion.

Procedure. Twenty-two subjects were prepared with a stomach balloon. Five of these were assigned to a forward conditioning group (Group FCd) in which subjects ingested the saccharin solution before they experienced balloon inflation. The remaining seventeen subjects were assigned to backward pairing groups in which the saccharin solution was ingested 1/2 minute (Group Backward-$\frac{1}{2}$, Bkd-$\frac{1}{2}$, $n=6$), 10 minutes (Group Bkd-10, $n=5$) or 30 minutes (Group Bkd-30, $n=6$) after ballon deflation. Finally, seven nonoperated subjects were assigned to a saccharin only

control condition (Group NopC).

The different temporal relationships between CS and US are schematized in Figure 6. During each of the two treatment sessions saccharin only (NopC) and forward conditioning (FCd) groups began by drinking the saccharin solution for 110 seconds. All three backward pairing groups (Bkd-$\frac{1}{2}$, Bkd-10 and Bkd-30) began by drinking water for the same period of time. This assured that backward pairing subjects experienced stomach distention following ingestion of a fluid volume equivalent to that drunk by the FCd group. Following distention, the forward conditioning (FCd) group drank water while the backward pairing groups drank the saccharin solution after the appropriate delay. Again the duration of the drinking period was 110 seconds. The saccharin only group (NopC) drank water for 110 seconds 10 minutes after drinking the saccharin solution. Thus all groups were balanced for number of water and saccharin solution drinking periods as well as volume of fluid in stomach during distention, differing only in the temporal relationship between ingestion of the saccharin solution and stomach distention.

All other aspects of the experimental procedures remained as previously described.

Figure 6

Experiment 5: Treatment procedures for saccharin only (NopC), forward conditioning (FCd) and backward pairing (Bkd-½, Bkd-10 and Bkd-30) groups. Each saccharin solution (Sacch.) or water (H_2O) drinking period lasted 110 seconds. The temporal features of distention onset and offset have been exaggerated to indicate the continuous change from deflated to inflated and back again.

GROUP | TREATMENT

NopC — SACCH. ... H₂O

FCd — SACCH. DISTENTION ... H₂O

Bkd−½ — H₂O ... SACCH.

Bkd−10 — H₂O ... SACCH.

Bkd−30 — H₂O ... SACCH.
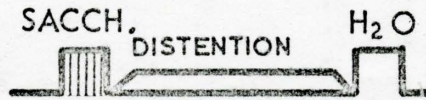
MINUTES ⊢ 5 ⊣
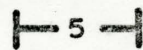
Figure 6

Results and Discussion

Preference test. Preference test results are presented in Figure 7. The forward pairing group (FCd) evidenced a significantly lower saccharin preference than did the saccharin only control group (NopC) ($U=0$, $p=.002$), while none of the Bkd groups provided evidence of taste-aversion learning. The difference between each of the Bkd groups and the saccharin only control group did not approach statistical significance (NopC vs. Bkd-½, $U=17$; NopC vs. Bkd-10, $U=11$; NopC vs. Bkd-30, $U=17$; all $ps>.20$).

The absence of aversion learning in the backward pairing groups clearly indicates an associative basis for the taste aversion obtained in the forward pairing (FCd) group. In addition, these data suggest that the aversive consequences of the distention procedures are substantially limited to the period of balloon inflation.

Post-distention ingestion of the saccharin solution. Figure 8 presents median milliliters of saccharin solution ingested by each group during each treatment session. During Treatment Session 1 (left panel) both Bkd-½ and Bkd-10 groups drank significantly less saccharin solution than combined FCd and NopC groups ($U=3$, $p<.002$ and $U=10$, $p<.02$). (FCd and NopC groups were combined for this comparison since neither group experienced stomach distention before saccharin ingestion. Also, these two

Figure 7

Experiment 5: Median saccharin preference ratios
for groups experiencing saccharin only (NopC), forward
(Group FCd) or backward pairing (Groups Bkd-$\frac{1}{2}$, Bkd-10
or Bkd-30) of CS and US during treatment sessions. Number
of subjects per group is indicated in parenthesis.

Figure 7
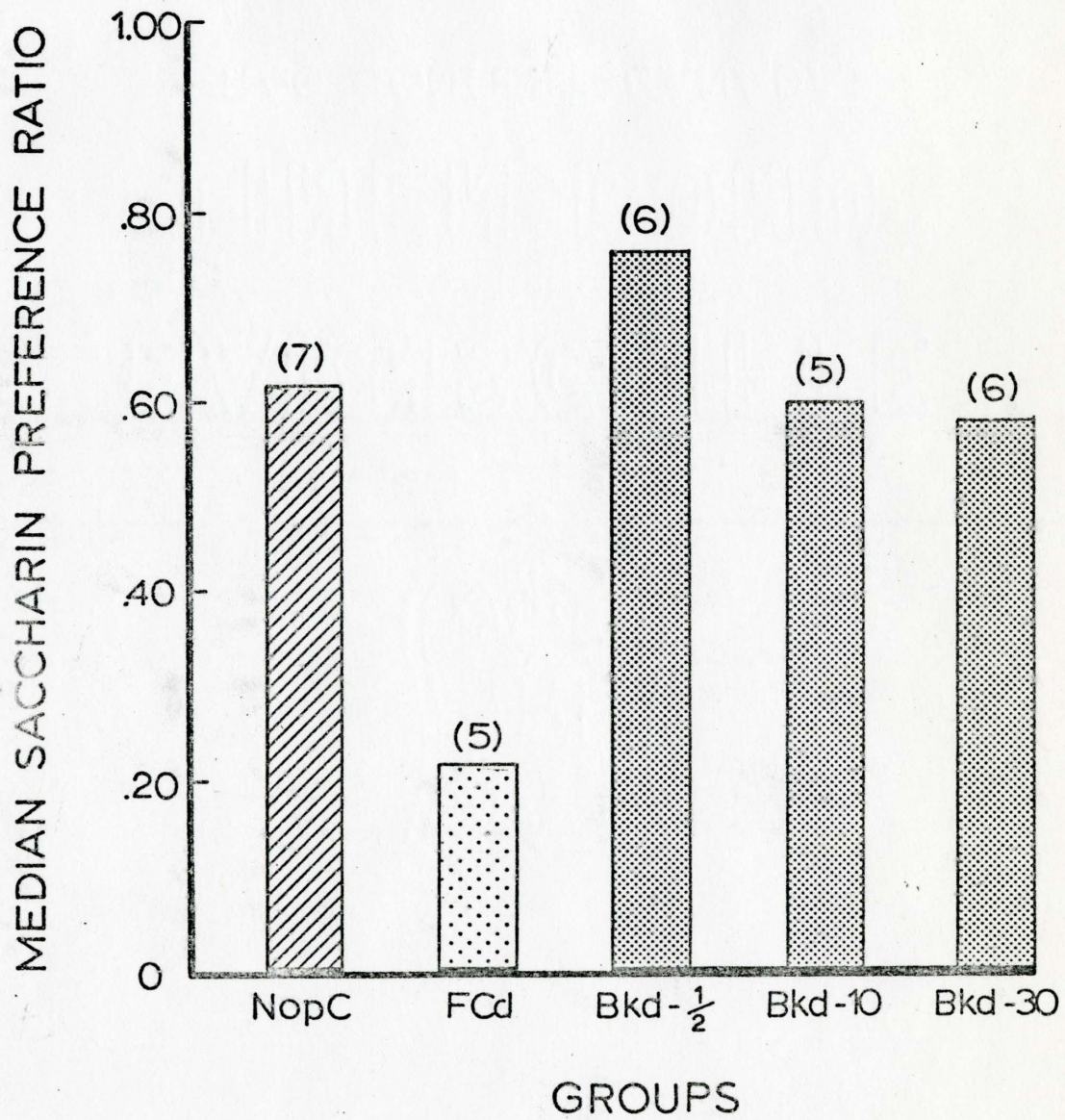
Figure 8

Experiment 5: Median milliliters of saccharin solution ingested during the 110 second access period during each Treatment session. The groups are forward pairing of CS and US (FCd), saccharin only control (NopC) and backward pairing of CS and US with CS delays of 1/2 (Bkd-$\frac{1}{2}$), 4 (Bkd-4) or 10 (Bkd-10) minutes. Number of subjects per group is indicated in parenthesis.
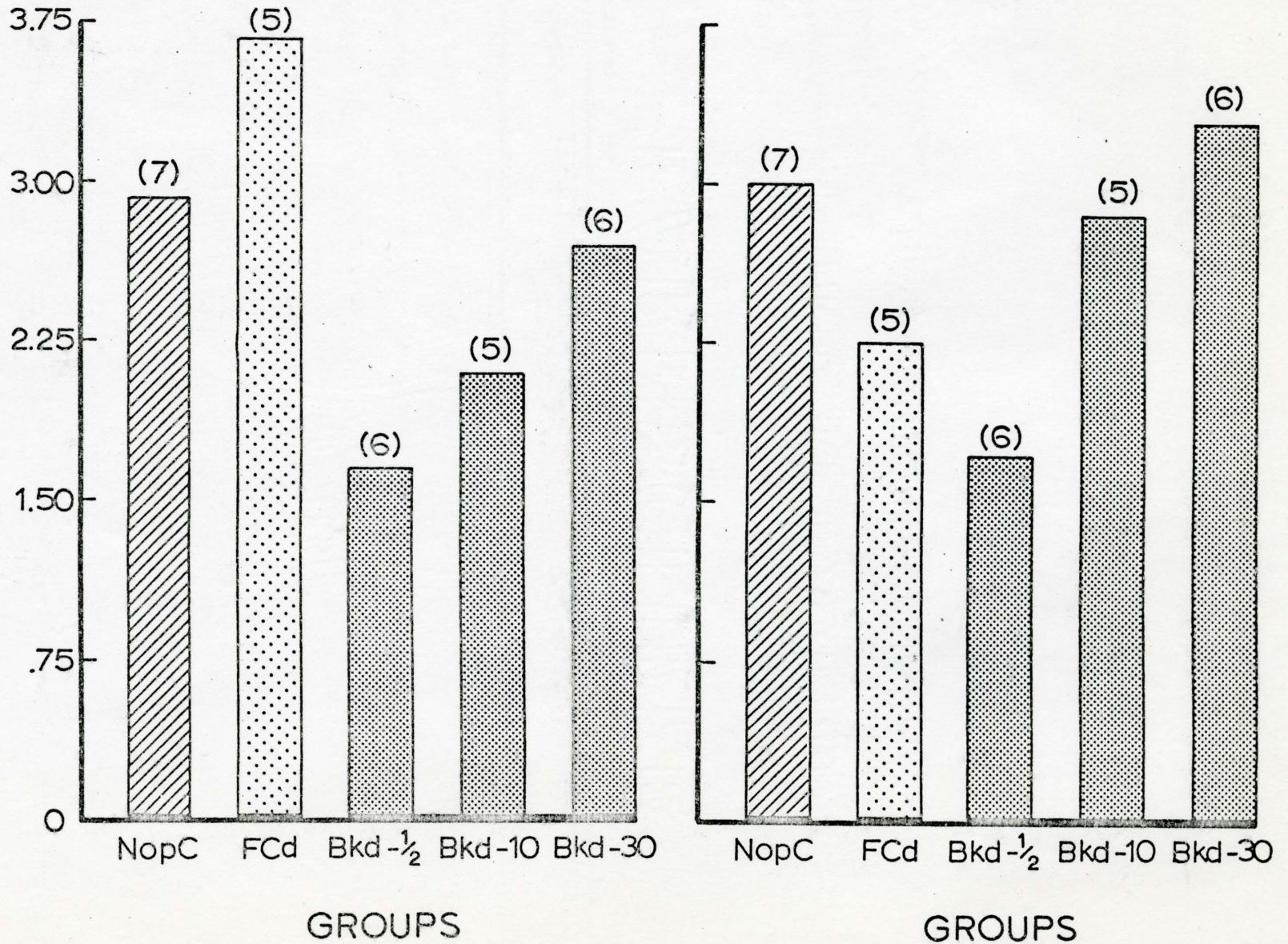
Figure 8

groups did not differ statistically in the volume of saccharin solution ingested.) During Treatment Session 2 (right panel) only the Bkd-½ group drank significantly less saccharin solution than the NopC group ($U=4$, $p<.014$). (It was not appropriate to combine FCd and NopC groups for the Treatment Session 2 comparisons since the FCd group's level of saccharin solution ingestion was suppressed as a function of the saccharin-distention pairing of Treatment Session 1.)

These results suggest the 9 ml, 10-minute balloon inflation may have produced an internal distress which extended briefly beyond balloon deflation. Certainly, however, this period of aversiveness did not extend to the ingestion of the saccharin solution in the Bkd-30 group. In fact, the period of aversiveness did not even extend to the ingestion of the saccharin solution in the Bkd-10 group during the second treatment session. Furthermore, the aversive consequences of the distention procedures did not extend sufficiently beyond the ingestion of saccharin in the Bkd-½ group to effectively motivate taste-aversion learning. Thus, Experiment 5 provides the first unambiguous demonstration of taste-aversion learning motivated by an internal US of relatively short duration which is applied directly to the viscera.

Experiment 6: Backward Conditioning with

Forced Exposure to the

Saccharin Solution

Experiment 5 demonstrated subjects exposed to a
CS solution after stomach distention did not learn to avoid
that solution while subjects that drank the saccharin
flavored water before distention did learn to avoid the
solution.   However, all groups did not receive equal
exposure to the taste of saccharin during the conditioning
trials.   In particular, the Bkd-$\frac{1}{2}$ group, the group most
likely to show effects of extended US aversiveness, drank
substantially less of the CS solution than the other
groups (see Figure 7).   This smaller volume reflects a
shorter time spent drinking and thus a shorter duration
of direct contact with the CS solution.   It is possible
that taste-aversion learning was in some way obscured or
prevented by this shorter period of contact with the CS
solution.

The procedures used in Experiment 6 were similar
to those of Experiment 5 except that subjects were exposed
to the CS solution by infusing it directly into the mouth
through a chronically implanted oral cannula.   Direct
infusion of the saccharin solution into the mouth, in
contrast with free ingestion, allowed control of duration
of direct contact with the CS solution, and thus all
groups could be equated for duration of exposure as well as

number of exposures to the flavored solution.

Method

Apparatus and subject preparation. A cannula consisting of a small diameter, polyethylene tube ("Intramedic", Clay-Adams, P.E. 205) was threaded subcutaneously from the back of the neck into the oral cavity just anterior to the right molar teeth. The cannula was held in place by flaring with heat each end of the cannula over small polyethylene washers. In addition, the cannula was secured with a single suture (5-0 silk) through both the cannula and the subject's cheek.

To infuse a fluid into the subject's mouth an extension was affixed to the oral cannula at the back of the neck which in turn was connected to a syringe mounted on an infusion pump (Harvard Apparatus, Model 941). Infusion rate and duration were controlled through the pump and associated electronic timing equipment with infusion rate, as measured at the connection to the oral cannula, being 2.1 ml/minute. Each oral infusion lasted 100 second and thus forced 3.5 milliliters of solution into the subject's mouth. During oral infusion a subject was allowed to move freely in a cylindrical compartment (1 foot diameter, 2 foot wall and a wire grill floor) mounted over a metabolism tray, permitting collection of

any fluid not ingested by the subject.

Procedure. Each of seventeen rats was prepared with a stomach balloon. One week later each subject was anesthetized again and an oral cannula implanted. Six subjects were assigned to each of two backward pairing conditions (Group Bkd-½ and Group Bkd-10) and five subjects were assigned to a forward pairing group (FCd). In addition, six subjects were prepared only with oral cannula and assigned to a saccharin only condition (Group NopC).

Four days after oral cannulation, subjects were placed on the 23½ hour water deprivation schedule and for the next five days were adapted to handling and oral infusion procedures. On each oral infusion adaptation day subjects were (a) placed in the cylindrical chamber and orally infused with 3.5 ml of water, (b) restrained for 10 minutes, (c) returned to the cylindrical chamber for a second infusion of water, and finally (d) returned to their respective home cages for their daily one-half hour of water.

Treatment procedures were similar to those described for Experiment 5 (see Figure 6) except that saccharin solution and tap water were presented by oral infusion. During each of two treatment sessions saccharin only subjects were infused with the saccharin solution, restrained for 10 minutes and then infused with tap water.

Forward conditioning subjects (FCd) were infused with the saccharin solution, distended and then infused with tap water. Backward pairing subjects were infused first with tap water, then experienced stomach distention and finally were infused with the saccharin solution. The delay between end of stomach distention and onset of saccharin solution was either 1/2 minute (Bkd-$\frac{1}{2}$) or 10 minutes (Bkd-10). Each oral infusion, whether of water or saccharin solution, was accomplished in the cylindrical chamber with each distention (9 ml and 10 minutes for all groups) being applied while a subject was restrained. Backward pairing groups spent the delay between stomach distention and oral infusion of the saccharin solution in the restraint, being transferred to the cylindrical chamber just before the infusion.

Twenty-four hours after the second treatment session all subjects were tested for saccharin preference.

Results

During adaptation sessions subjects readily learned to ingest all the fluid infused into their mouths. During treatment sessions the volume of saccharin solution collected by the metabolism tray was negligible for all groups, indicating that the oral infusion procedures successfully equated groups for both duration of exposure and volume ingested.

The results of the preference test are presented
in Figure 9. As in Experiment 5, backward pairing subjects
clearly did not acquire an aversion to the saccharin
solution. There were no significant differences among the
NopC, Bkd-½ and Bkd-10 groups (all $ps > .20$). In contrast
to Experiment 5, the FCd group was not reliably different
from all Bkd and NopC groups. The FCd group showed a
significantly lower preference for the saccharin solution
than the Bkd-10 group ($U=4$, $p=.05$) with the difference
between the FCd and NopC group approaching significance
($U=5$, $p=.08$). The difference between the Bkd-½ and FCd
groups was not statistically significant ($U=6$, $p=.13$).
The failure to obtain significant differences in the latter
two comparisons was not due to particularly low saccharin
preferences in the Bkd-½ or NopC groups but to the failure
of a single subject in the FCd group to acquire an aversion
to the saccharin solution. In fact, that single subject
was responsible for all overlap between the FCd group and
the other three groups.

Discussion

The results of this experiment are consistent with
those of the previous experiment in indicating that the
aversive consequences of the 9 ml, 10-minute stomach
distention do not extend sufficiently beyond the period of

Figure 9

Experiment 6: Median saccharin preference ratios for groups experiencing saccharin only (NopC), forward conditioning (FCd) and backward pairing (Bkd-½ or Bkd-10) during treatment sessions. Number of subjects per group is indicated in parenthesis.

Figure 9
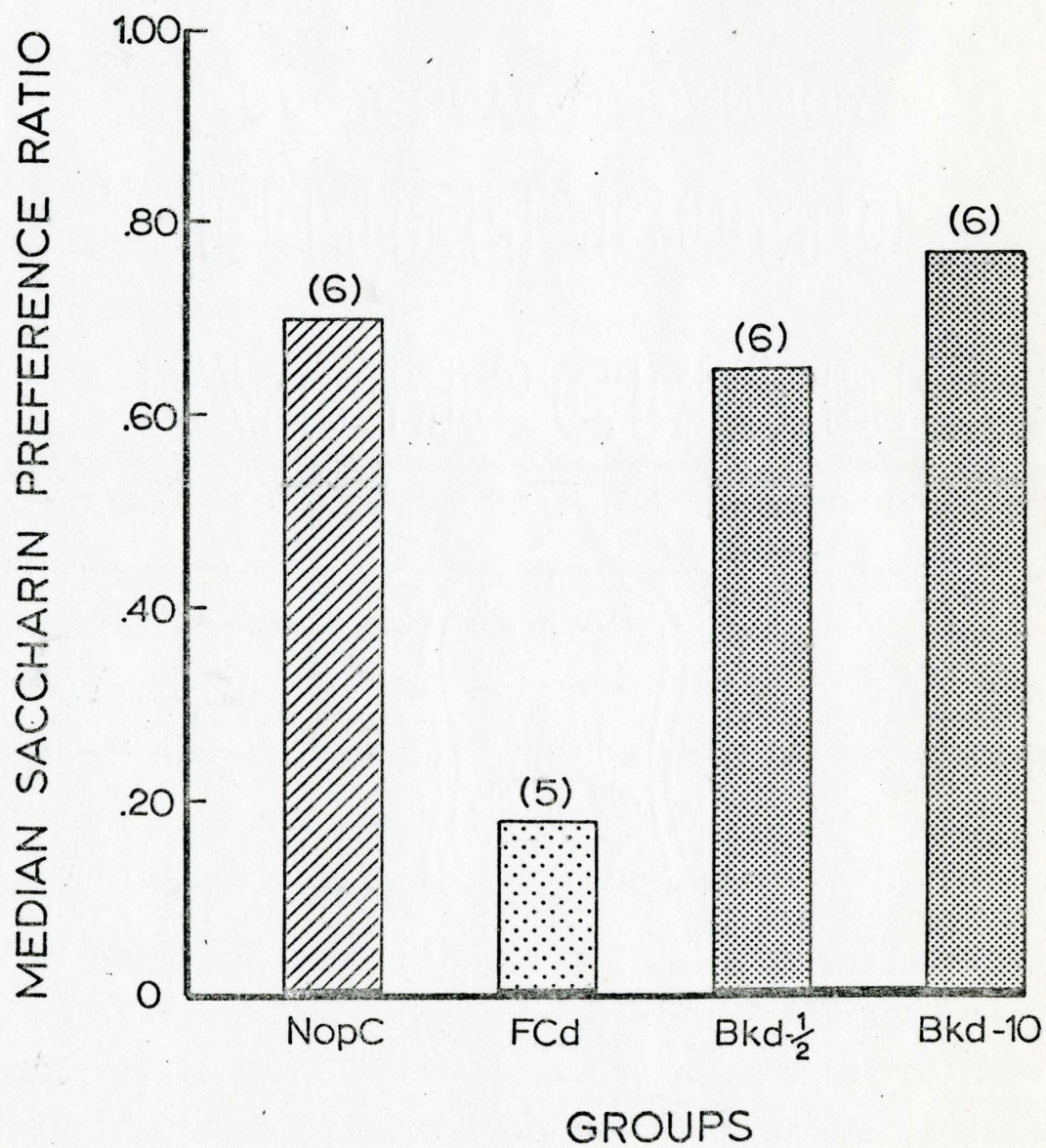
balloon inflation for an aversion to be formed to a novel gustatory stimulus presented immediately after the distention period. In addition, these findings suggest that the shorter duration of exposure to the CS solution in the Bkd-$\frac{1}{2}$ group of Experiment 5 was not responsible for the absence of aversion learning in that experiment.

One aspect of the design of Experiments 5 and 6 deserves further comment at this point. In both experiments each backward pairing group ingested tap water just prior to balloon inflation (see Figure 6). If this led backward pairing groups to associate water ingestion with stomach distention and thus to acquire a water aversion, a preference test based upon choice between water and the saccharin solution would not have been a very sensitive measure of saccharin-aversion learning in these groups.

In general, water aversions are not readily acquired (Garcia & Koelling, 1966; Garcia, McGowan & Green, 1972) and one important reason for this is the extensive exposure to water rats typically receive prior to its pairing with toxicosis (e.g., Nachman, 1970). In Experiments 5 and 6, for example, subjects had continuous access to water in their home cages from weaning (21 days of age) until they participated in the present experiments at approximately 100 days of age. Such extensive exposure to water in the absence of an aversive US would be expected

to substantially retard, if not prevent entirely, the subsequent association of water ingestion with aversive internal events (e.g., Revusky & Garcia, 1970). In fact, only one study has reported water-aversion learning when subjects had extensive prior exposure to water (Nachman, 1970, Experiment 3). Certain methodological features of Nachman's (1970) study appear, however, to limit the generality of his finding. Most notably, Nachman's rats received substantial exposure to a saccharin solution as part of the experimental procedures. The presentation of water as a CS within the context of repeated exposures to a saccharin solution may have increased the salience of water as a stimulus and thereby increased its associability. It seems then, that water-aversion learning is not a necessary outcome of pairing water ingestion with internal malaise, but may occur under certain conditions.

Two observations suggest that water-aversion learning did not occur in Experiments 5 and 6. First, all backward pairing subjects avidly drank water when returned to their home cages shortly after balloon deflation. This suggests that if a water aversion was being acquired during treatment sessions it certainly did not generalize to the home cage where saccharin preference was subsequently assessed. Second, seven of the fifteen backward pairing subjects in Experiment 5 actually drank more water prior to the second balloon inflation experience than prior

to the first.   (Similar data was not available from
Experiment 6 because both water and saccharin solution
was presented by the method of oral infusion.)   If
ingestion of water prior to the first distention produced
a water aversion this should have led to less water
consumption prior to the second distention.   (Subjects
ingesting the saccharin solution prior to the first
distention typically drink less of that solution prior to
the second distention.)

Although the possibility of water-aversion learning
was not directly assessed and therefore cannot be entirely
ruled out, the observations presented above certainly
suggest that its role, if any, in the results of
Experiments 5 and 6 was a minor one.

General Discussion: Experiments 1-6

Summary of results.   The stomach balloon
preparation was developed to obtain direct control over
physical and temporal characteristics of an internal US in
order to investigate the role of these characteristics in
taste-aversion learning.   Experiments 1 through 4
investigated balloon inflation parameters of volume and
duration and found: (a) a 20-minute, 20 ml balloon
inflation led to a taste aversion when it followed or
preceded saccharin ingestion (Experiment 1), (b) as

distention volume was decreased (Experiment 2) or duration shortened (Experiments 3 and 4) the resulting taste aversion became weaker, and (c) a 9 ml distention lasting only ½ minute did not motivate aversion learning. Experiments 5 and 6 demonstrated that aversions motivated by the 9 ml, 10-minute balloon inflation represented an associative rather than a nonassociative consequence of the distention experience by showing that this distention produced a taste aversion when it followed, but not when it preceded, ingestion of a saccharin solution. In addition, results from Experiments 5 and 6 indicated that the aversive consequences of the 9 ml, 10-minute stomach balloon inflation did not extend substantially beyond the period of balloon inflation.

Taste aversions as a function of US duration. Experiments 3 and 4 demonstrated that the magnitude of the CS aversion was a direct function of balloon inflation duration. Two interpretations are possible for this relation of aversion magnitude to US duration. Perhaps within the range of parameters used in Experiments 3 and 4, inflation duration was an important determinant of US aversiveness. Several studies have shown that all other conditions equal, more aversive USs produce stronger taste aversions (e.g., Dragoin, 1971; Revusky, 1968). Thus subjects that experienced the 10 minute balloon inflation

may have acquired a stronger aversion than subjects that

experienced 4 minutes of ballon inflation because the 10

minute distention was a more aversive US. The absence of

aversion learning in the group that received only 1/2

minute of balloon inflation could simply reflect insufficient

US aversiveness.

Alternatively, subjects in Experiment 4 might have

shown progressively less taste-aversion learning as the

duration of balloon inflation was decreased because rats

do not readily associate temporally discrete USs with

gustatory stimuli. Krane and Wagner (1975) have recently

suggested that one reason discrete USs, such as shock,

have not been readily associated with a gustatory stimulus

is because gustatory stimuli (or, more accurately, a central

trace of such stimuli) persist after removal of a drinking

tube. This persistence extends the gustatory stimulus into

the post-US safety or recovery period which, in turn,

interferes with aversion learning. In support of this

hypothesis Krane and Wagner (1975) replicated earlier

findings demonstrating that a brief shock cannot motivate

taste-aversion learning when presented immediately after

removal of a drinking tube but further demonstrated that

this brief shock did motivate taste-aversion learning when

delayed by thirty seconds. Perhaps then, the 1/2-minute

distention failed to motivate aversion learning, not because

it lacked sufficient aversiveness, but because the gustatory stimulus persisted into the post-US period. The 4- and 10-minute distentions would be more effective as USs since their durations could have been sufficiently long to avoid the situation in which the gustatory stimulus effectively persisted beyond US termination. At this point either interpretation offered above seems to adequately account for the relation of balloon inflation duration to aversion learning.

Experiments 4, 5 and 6 clearly suggest that USs with a slow onset and a very long duration are not necessary for the effective motivation of taste-aversion learning. With stomach distention, onset of aversiveness is quite rapid. Overt signs of discomfort usually appeared as balloon inflation reached 7 to 8 milliliters and were clearly present in subjects experiencing 9 ml of distention. In addition, the aversive consequences of the 9 ml distention are substantially limited to the period of balloon inflation: (a) overt signs of discomfort disappeared when the balloon was deflated, (b) subjects exposed to the stimulus after balloon inflation was terminated did not learn an aversion, and (c) the distention had only a limited effect upon ingestion of the saccharin solution immediately following the distention period. Thus, an exogeneous chemical US or exposure to

ionizing radiation is not necessary for taste-aversion

learning. Rather, such learning may be studied with

aversive USs having relatively rapid onset and short

duation, and with parameters which may be manipulated by

the experimenter.

CHAPTER 3:

TRACE CONDITIONING WITH BALLOON INFLATION US

The experiments presented in Chapter 2 demonstrate that an internal US need not have the slow onset and long duration characteristics of chemical or radiation induced toxicosis to effectively motivate taste-aversion learning. Rather, rats readily associate a flavor CS with a US of stomach distention when the onset of US aversiveness is relatively rapid and the duration of US application is relatively short in comparison to toxicosis. Experiments presented in the present chapter were designed to assess whether aversion learning with a long CS-US trace interval, another characteristic considered unique to taste-aversion learning motivated by toxic USs, may be found with the balloon inflation US. If an internal US having a rapid onset and short duration produces a conditioned taste aversion even when US onset is delayed for several minutes after CS offset, then aversion learning with long CS-US trace intervals would appear attributable to US locus rather than distinctive onset or duration characteristics.

Rats can associate a flavor with toxicosis even if flavor termination precedes the onset of illness by several

hours. With other, more traditional, conditioning preparations (e.g., when an auditory or visual CS is paired with a shock US), the maximum CS-US trace interval over which associative learning may be demonstrated is typically measured in seconds or, at most, minutes (Mackintosh, 1975, p.58ff.). If the 10 minute balloon inflation, despite its relatively rapid onset and short duration, is functionally equivalent to toxicosis, then rats should associate the ballon inflation US with a gustatory CS even when the CS-US trace interval is more than a minute or two.

Experiment 7: Delay of Unconditional Stimulus
Onset (0, 5, 12 or 25 Minutes)

Experiment 7 was designed to investigate taste-aversion learning when balloon inflation onset was delayed for 0, 5, 12 or 25 minutes after removal of the gustatory CS.

Method

The general methodology was essentially the same as used in previous experiments. Subjects were prepared with the stomach balloon and allowed five days for recovery from surgery. The recovery period was followed by five adaptation, two treatment and one test session(s). All

detail of the adaptation, treatment and test sessions not specified below remained as previously described.

Treatment sessions. Six operated (prepared with stomach balloon) subjects were assigned to each of three delay-of-US conditions (5, 12 or 25 minutes), with five operated subjects assigned to a 0-delay condition. Seven nonoperated subjects were assigned to a CS only condition (Group NopC).

Four and one-half hours prior to each of the two treatment sessions all food was removed from home cages. During each treatment session CS only subjects (Group NopC) received access to the .15% saccharin solution for 110 seconds from the first lick, remained in the restraint for fifteen minutes and were returned to their home cages. Subjects in the four delay-of-US groups were exposed to the saccharin solution and, after the specified delay, their stomach balloons were inflated (9 ml for 10 minutes). Subjects remained in the restraint during the interval between removal of the drinking tube and onset of balloon inflation. Delay-of-US subjects were returned to home cages immediately following balloon deflation where food and water were available. The water remained available for only one-half hour.

Assessment of Aversion Learning. As in Experiments 1 through 6, each subject's saccharin preference was

assessed with a two-solution (water vs. saccharin solution) choice test conducted in the subject's home cage 24 hours after the second treatment session. In addition to the results obtained from the two-solution choice test, results from a second measure of aversion learning are reported in this experiment.

The volume of fluid ingested during a fixed period of time is frequently used as an index of the aversiveness (or attractiveness) of that solution (e.g., Domjan & Bowman, 1974). Thus the 110 seconds of access to the CS solution during each of the treatment sessions may be treated as a brief assessment of CS solution palatability. Furthermore, when subjects in the balloon inflation groups were allowed 110 seconds of access to the CS solution during the second of the two treatment sessions, they had already experienced one CS-US pairing. As a result, when compared to the CS only control group, the volume of saccharin solution ingested by balloon inflation groups during the second treatment session provides a measure of taste-aversion learning as a consequence of the single CS-US pairing.[4]

[4] Data from this single solution test of aversion learning were not reported in Experiments 1 through 5 because such data did not provide any additional information beyond that available from the two-solution choice test.

Results

    Preference Test. Figure 10 presents the results
from the two-solution preference test conducted in home
cages after two treatment sessions. For the balloon
inflation groups, median saccharin preference ratios were
a direct function of CS-US trace interval; the lowest
saccharin preference was found in the O-delay group, and
progressively larger preferences were apparent as US
onset was delayed 5, 12 and 25 minutes. These data
suggest that the delay over which rats can associate the
gustatory CS with the balloon inflation US is measured in
many minutes rather than in seconds or a few minutes as is
typical for shock motivated aversion learning.
Statistically, both 12- and 25-minute delay groups were
not significantly different from the saccharin only control
group ($U=20$, $p > .20$ and $U=11$, $p=.18$, respectively). The
difference between the saccharin only control (NopC) group
and the 5-minute delay-of-US group was significant ($U=6$,
$p=.034$) but the difference in saccharin preference between
the O-delay group and the control group did not reach
statistical significance ($U=9$, $p=.10$).

    Ingestion of the saccharin solution during treatment
sessions. Figure 11 presents median milliliters of
saccharin solution ingested by groups during the 110 second
flavor exposure period during each treatment session.

Figure 10

Experiment 7: Median saccharin preference ratio
for groups that experienced saccharin only (NopC) or
saccharin followed by balloon inflation (9.0 ml for 10
minutes) during each treatment session. The CS-US interval
for the four balloon inflation groups was 0, 5, 12 or 25
minutes. Number of subjects per group is indicated in
parenthesis.

Figure 10
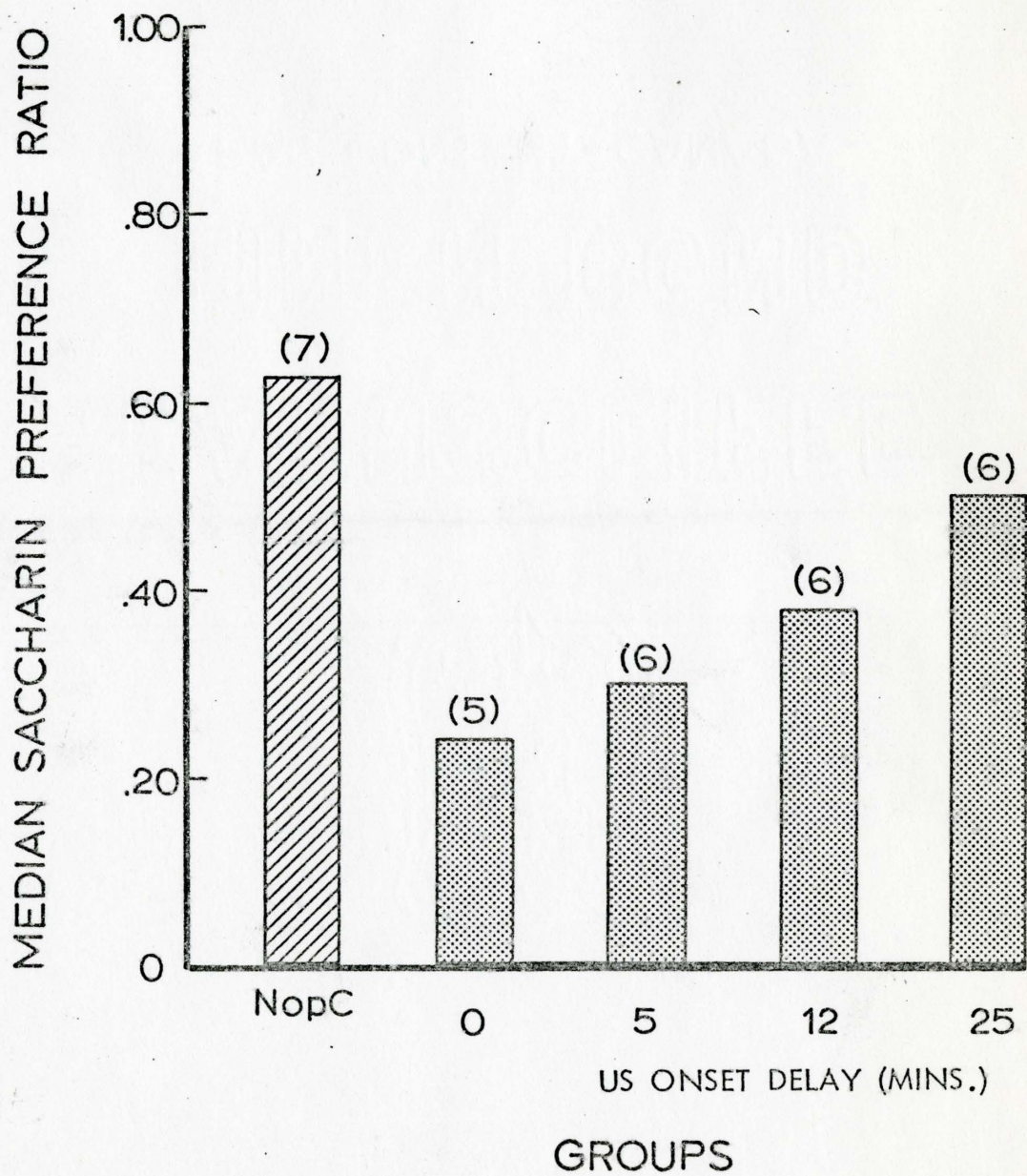
Figure 11

Experiment 7: Median milliliters of saccharin
solution ingested during the 110 second flavor exposure
period during each of the two treatment sessions. Number
of subjects is indicated in parenthesis.
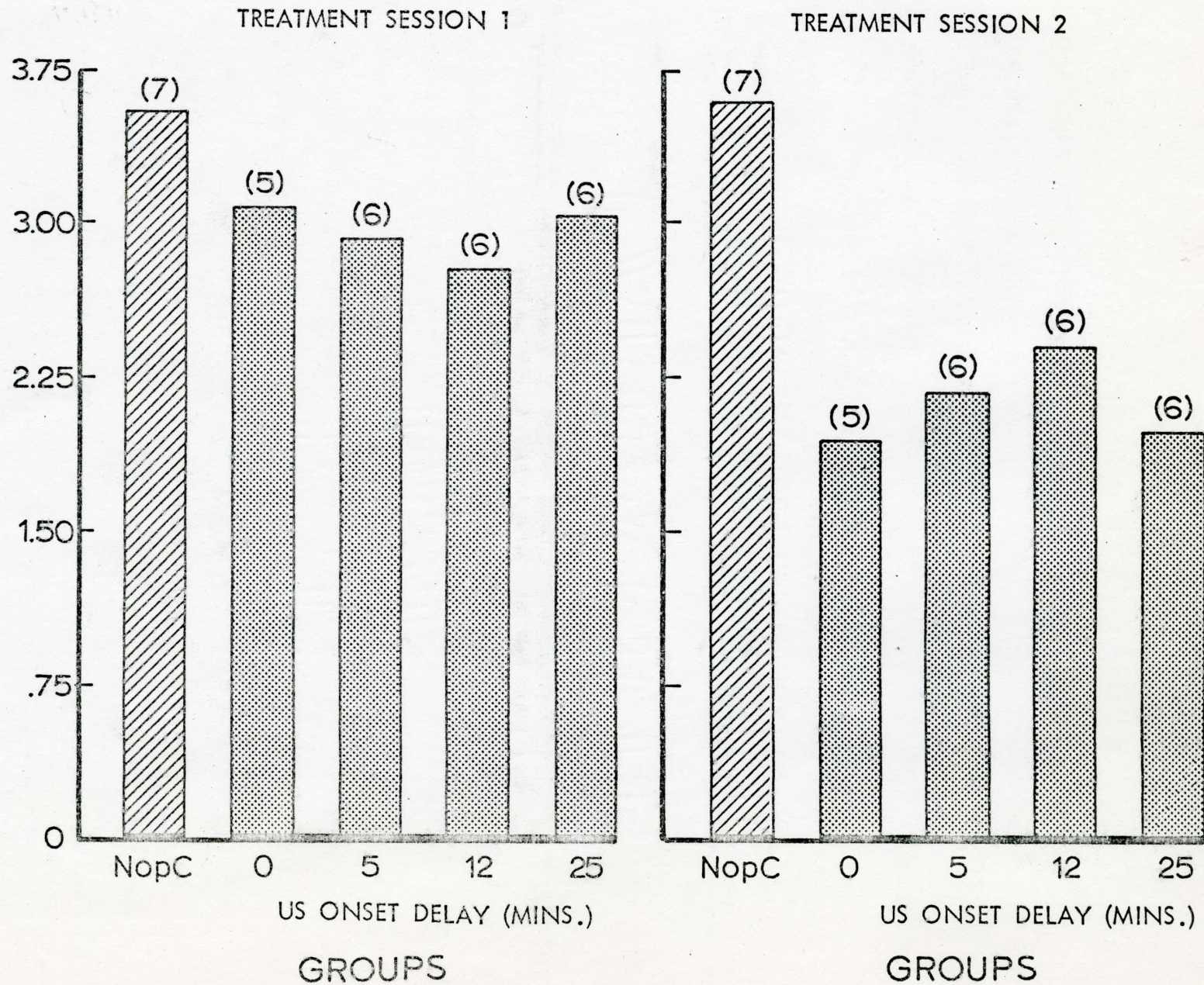
Figure 11

Treatment Session 1 (left panel) represents first contact

with the saccharin flavor and, as expected, there are no

significant differences among groups in the volume of

fluid consumed. During Treatment Session 2 (right panel),

however, all four distention groups drank substantially

less than the saccharin only control group (NopC vs.

0-delay, $U=6$, $p=.074$; NopC vs. 5-minute delay, $U=0$, $p=.002$;

NopC vs. 12-minute delay, $U=3$, $p=.008$; NopC vs. 25-minute

delay, $U=8$, $p=.074$). There were no significant differences

among the four delay groups.

Discussion

Previous demonstrations of Pavlovian conditioning

with CS-US intervals of more than a minute or two have all

employed exposure to ionizing radiation or administration

of chemical agents as USs. Each of these USs produces

internal consequences that have a slow onset and a long

duration. In the present experiment, the effect of

different CS-US delays on taste aversion learning was

assessed with another form of internal US, stomach balloon

inflation, which possesses onset and duration characteristics

very dissimilar from a radiation or chemically induced

malaise.

The volume of saccharin solution ingested during

the 110 second flavor exposure period of Treatment Session 2

clearly suggests that rats can associate a gustatory CS
with the balloon inflation US over a CS-US trace interval
as long as 25 minutes. It appears, therefore, that the
very slow onset and long duration characteristics of
toxicosis are not essential for taste-aversion learning
with CS-US trace intervals longer than a minute or two.
Indeed, with the single solution assessment, the magnitude
of the aversion was as strong in the longest delay condition
investigated (25 minutes) as in the 0-delay condition.

In contrast with the single solution test after the
first treatment session, the preference test (after the
second treatment session) indicated that if a delay of more
than five minutes was interpolated between the CS and US
there was little aversion learning. One especially
surprising feature of the preference test results was a
failure of the 0-delay group to show a statistically
significant aversion (although it does show the lowest
median saccharin preference ratio). This 0-delay group
was subjected to the same treatment as forward pairing
groups (FCd Groups) in Experiments 4, 5 and 6 in which
significant aversions were displayed with the two solution
preference test.

The following experiment was designed to provide
additional information concerning the effect of CS-US trace
interval on taste-aversion learning with stomach balloon

inflation as the US.

Experiment 8: Delay of US Onset

(0, 10 or 30 Minutes)

The delay-of-US groups in the previous experiment differed not only with respect to the time between removal of the gustatory stimulus and onset of balloon inflation but also with respect to the time from last ingestion of a fluid to US onset. Perhaps the effectiveness of balloon inflation as a US is in some way a function of time from last ingestion of a fluid. For example, each delay-of-US group in Experiment 7 drank approximately three milliliters of the saccharin solution prior to balloon inflation during the first treatment session. The ingestion of this solution would in itself produce, at least temporarily, a distention of the stomach. In the 0-delay condition, 9 ml of balloon inflation were immediately added to the volume of fluid in the stomach. In other delay conditions 5, 12 or 25 minutes intervened between ingestion of the saccharin solution and onset of balloon inflation. During this interval the ingested fluid could have been absorbed or passed through the stomach to the intestines. As a result, the 5, 12 or 25 minute delay groups may have experienced effectively less stomach distention than the 0-delay group. If the above argument is correct the relation of taste

aversion magnitude to CS-US trace interval observed in

Experiment 7 might reflect US aversiveness rather than the

CS-US trace interval. In the present experiment, delay

groups (with the exception of the 0-delay group) were

allowed to drink water for 110 seconds immediately prior

to balloon inflation. Thus, groups that experienced

several minutes between exposure to the CS and onset of

the US were closely matched to a 0-delay group in volume

of fluid ingested just prior to balloon inflation.

Finally, in order to reconfirm that nonoperated

control subjects do not differ from operated control

subjects in their preference for saccharin, both operated

and nonoperated control groups were included in this study.

Method

The general methodology remained as previously

described: (a) five days for recovery from surgery,

(b) five adaptation sessions, (c) two treatment sessions,

and (d) one test session. All detail of the procedure

not specified below also remained as previously described.

Twenty-four subjects were prepared with stomach

balloons with six subjects being assigned to each of three

delay-of-US groups (0, 10 and 30 minutes). The remaining

six operated subjects (Group OpC) and an additional eight

nonoperated subjects (Group NopC) were assigned to a

saccharin only control condition.

Experimental procedures during each of the two treatment sessions are schematized in Figure 12. Each subject drank the saccharin solution for one 110 second period and tap water for another 110 second period, the interval between drinking periods being spent in the restraint. The two control groups (OpC and NopC) and the 0-delay group drank water first and then eight minutes later drank the saccharin solution. The two remaining delay groups drank the saccharin solution first and then either 8 minutes later (Group 10-minute delay) or 28 minutes later (Group 30-minute delay) drank water. All delay-of-US groups experienced balloon inflation (9 ml, for 10 minutes) immediately after the second drinking period.

Twenty-four hours after the second treatment session saccharin preference was assessed in home cages. As in Experiment 7 results from both the two-solution choice test (conducted after the second treatment session) and the volume of CS solution ingested during each treatment session are presented.

Results

Preference test. The results of the two-solution preference test conducted 24 hours after the second treatment session are presented in Figure 13. The saccharin preferences shown by nonoperated control subjects (NopC)

Figure 12

Experiment 8: Treatment procedures for each of the five groups. In the treatment column, a vertical upward deflection of the time line indicates onset of the specified event (i.e., exposure to the saccharin solution, water, or balloon distention) while the vertical downward deflection indicates offset of the event. The onset and offset slopes of balloon inflation have been exaggerated to indicate the continuous change of the stomach balloon from deflated to inflated and back to deflated.
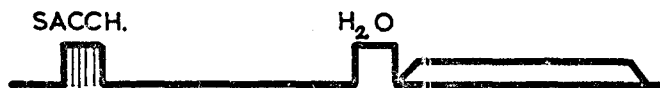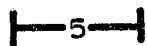
Figure 12

Figure 13

Experiment 8: Median saccharin preference ratios for groups that experienced saccharin only (NopC, nonoperated subjects; and OpC, operated subjects) or saccharin followed by balloon inflation during each treatment session. For groups that experienced balloon inflation, the interval between gustatory CS and balloon inflation US was 0, 10 or 30 minutes. Number of subjects per group is indicated in parenthesis.
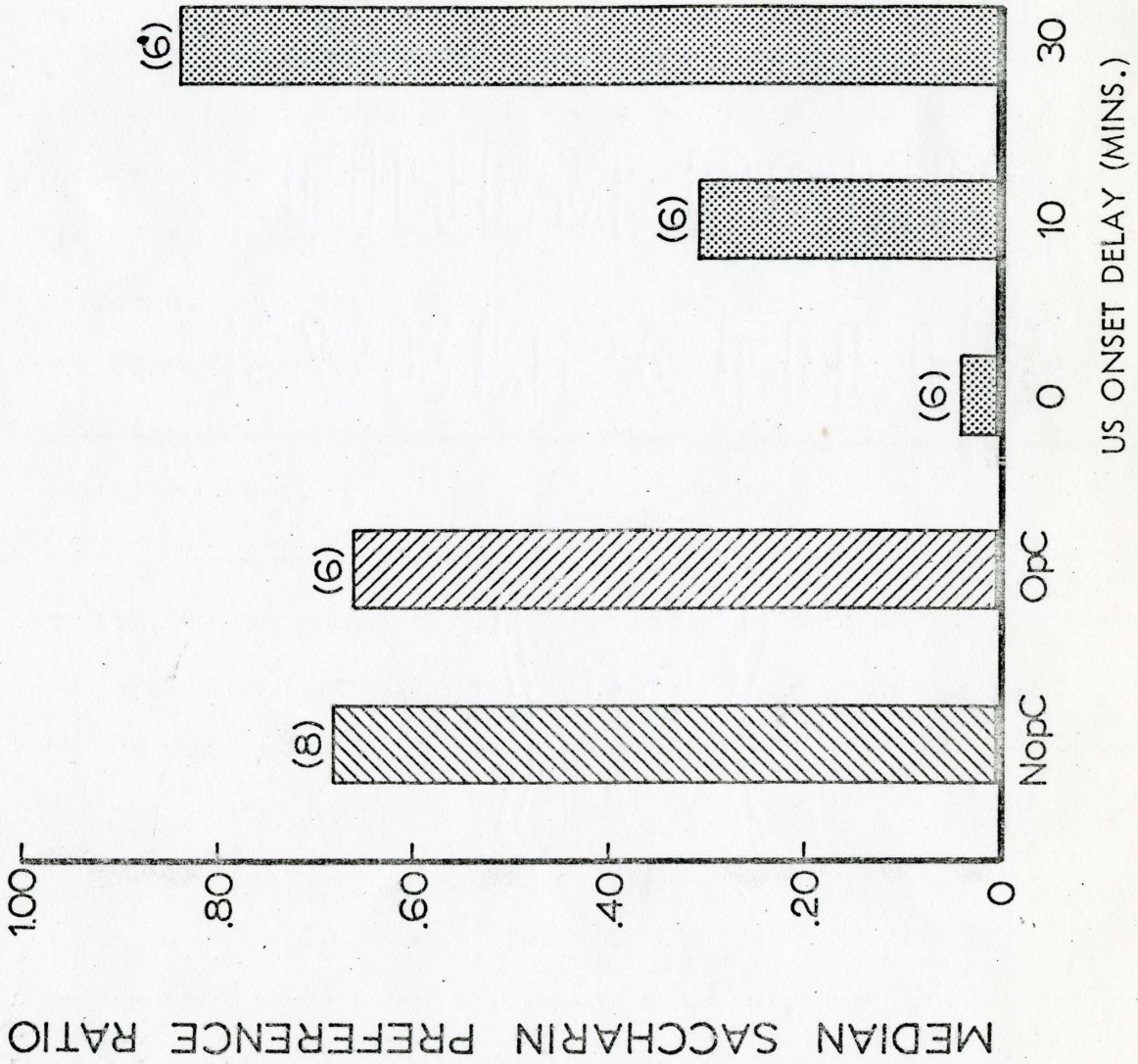
GROUPS

Figure 13

were not significantly different from the preferences
shown by operated control subjects (OpC) ($U=22$, $p>.20$),
and were therefore combined for statistical comparison
with delay-of-US groups.

The degree of saccharin preference in the different
delay-of-US groups was directly related to the length of
the CS-US interval during treatment sessions. The lowest
preference for the saccharin solution was shown by subjects
in the 0-delay condition. Subjects in this group
evidenced a significantly lower saccharin preference than
evidenced by subjects in the 10-minute delay group ($U=3$,
$p<.02$), the 30-minute delay group ($U=0$, $p=.002$) and the
combined control groups ($U=2$, $p<.002$).

The next lowest preference for the saccharin
solution was obtained from subjects in the 10-minute delay
group. The saccharin preferences of subjects in this
group were significantly lower than obtained from subjects
in either the 30-minute delay group ($U=0$, $p=.002$) or the
combined control groups ($U=11$, $p<.02$).

Finally, the preference for the saccharin solution
shown by subjects in the 30-minute delay group was not
significantly different from the preference shown by
combined control group subjects ($U=24$, $p>.20$), suggesting
that, on the basis of the preference test, subjects in the
30-minute delay group did not associate the gustatory CS

with the ballon inflation US.

Ingestion of the saccharin solution during treatment sessions. Figure 14 presents the median milliliters of saccharin solution ingested by each group during each of the treatment sessions. Since Treatment Session 1 (left panel) represented the first contact with the saccharin solution, no significant difference between groups in volume of saccharin solution ingested would be expected, nor was any obtained. During Treatment Session 2 (right panel) operated (OpC) and nonoperated (NopC) control groups did not differ significantly in volume of saccharin solution ingested and were therefore combined for statistical comparison with balloon inflation groups. All three delay--of-US groups drank significantly less than combined control groups during the second treatment session (combined control groups vs. 0-delay, $U=0$, $p<.002$; control vs. 10-minute delay, $U=1$, $p<.002$; control vs. 30-minute delay, $U=4$, $p<.002$). In addition, ingestion of the saccharin solution was suppressed to a greater extent in the 0-delay condition than in either the 10- or 30-minute delay conditions ($Us=2$ and 3, respectively, both $ps<.008$).

Discussion

The results of Experiment 8 provided additional evidence that rats can associate a gustatory CS with

Figure 14

Experiment 8:   Median milliliters of saccharin

solution ingested during the 110 second drinking period

within each of the treatment sessions. Groups are

nonoperated (NopC) and operated (OpC) saccharin only

control groups, and delay-of-US onset groups: 0, 10 and

30 minutes.   Number of subjects per group is indicated

in parenthesis.
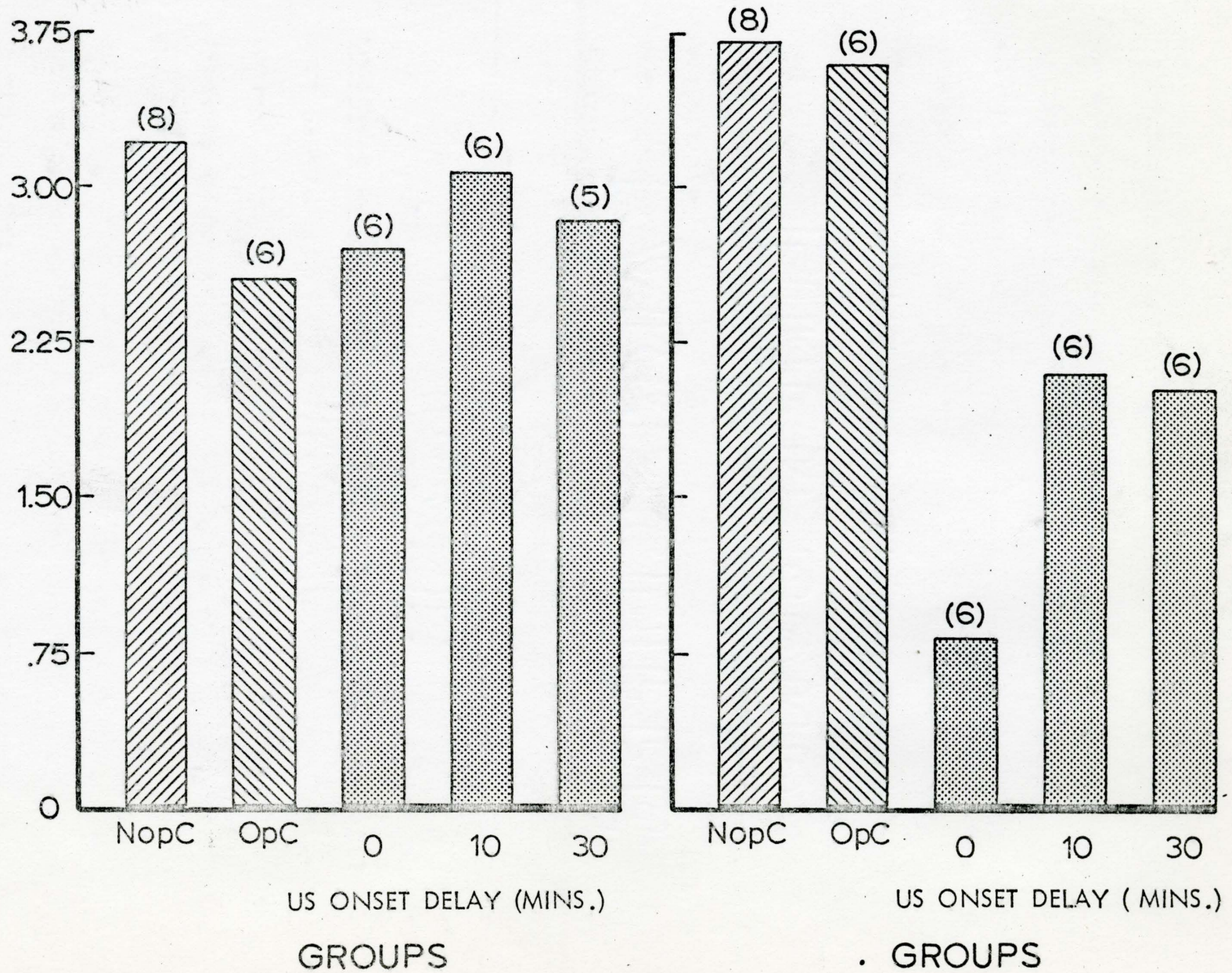
Figure 14

stomach balloon inflation even when US onset is delayed by more than a minute or two. Both measures of aversion learning, (a) the preference test conducted after two CS-US pairings and (b) the volume of saccharin solution ingested during the second treatment session, indicated that subjects associated the gustatory CS with the balloon inflation US over a CS-US interval of at least 10 minutes. In addition, the results from the volume of saccharin solution ingested during the second treatment session suggest that rats can associate a gustatory CS with balloon inflation US even when CS-US intervals are as long as 30 minutes.

General Discussion: Experiments 7 and 8

Ingestion of the saccharin solution during treatment sessions vs. the two-solution choice test. In both Experiments 7 and 8 the groups that experienced the longest interval between CS and US during treatment sessions evidenced an aversion to the CS solution following the first CS-US pairing (as assessed by the amount of CS solution ingested when it was the only fluid available) but not following the second CS-US pairing (as assessed by choice of CS solution compared with simultaneously presented water). In Experiment 7, for example, the 25-minute delay group drank significantly less saccharin

solution than the CS only control group during the 110 second CS exposure period of Treatment Session 2. In contrast, the saccharin preference evidenced by the 25-minute delay group during the two-solution choice test which followed the second CS-US pairing was not significantly different from the preference shown by the control group. This is a rather anomolous finding considering that assocations are usually strengthened, rather than weakened, by a second pairing of CS and US. In addition, it is usually suggested that a two-solution preference test is a more sensitive measure of taste-aversion learning than a single solution test (Dragoin, McCleary, 1971; Grote & Brown, 1971).

An adequate explanation of why a learned aversion was not observed in the choice test after having been demonstrated during the second treatment session would require experiments that are considered outside the scope of this thesis. Furthermore, the results of such research could not alter the basic conclusion of Experiments 7 and 8: with either assessment procedure, it is clear that rats can associate a gustatory CS with the balloon inflation US over a CS-US interval of several minutes.

Thus, Experiments 7 and 8 clearly demonstrate that Pavlovian conditioning with a CS-US interval longer than a minute or two is not limited to toxic USs which produce

internal consequences having a very slow onset (over a
period of several minutes) and a long duration (an hour
or longer). Rats readily associated a gustatory CS with a
10-minute distention of a stomach balloon even when 10,
25 or 30 minutes (depending upon assessment technique)
intervened between removal of the CS solution and onset of
balloon inflation.

Trace conditioning and US duration. Although the
experiments presented here clearly demonstrate that rats
can associate a flavor CS with a ballon distention US over
a substantial CS-US trace interval, the maximum CS-US
trace interval consistent with flavor-distention
association does not appear to be in the range of several
hours as is the case for flavor-toxicosis assocation. In
both Experiments 7 and 8 the two-solution preference test
indicated a maximum CS-US trace interval of less than 25
to 30 minutes. In addition, the single solution measure
indicated that even a few minutes between CS offset and US
onset attenuated aversion learning. These findings suggest
the maximum flavor-distention interval consistent with
aversion learning may be intermediate between the very
short CS-US intervals necessary for auditory/visual CSs
to be associated with shock, and the very long intervals
over which flavor cues can become associated with toxicosis.
Moreover, the US duration used in these experiments, 10

minutes, is also intermediate between the US duration in the experiments using a shock US (ranging from a fraction of a second to a few seconds) and the US duration in experiments using a toxicosis US (usually indeterminate but probably several hours). Perhaps gustatory CSs are readily associable with internally applied USs (e.g., mechanical distention of the stomach and toxicosis), and peripheral CSs with externally applied USs (e.g., electric shock), but given the favorable CS-US combination, it is US duration which determines the maximum CS-US interval over which associations may be formed.

Although a systematic investigation into the role of US duration in the CS-US interval function is beyond the scope of this thesis, two experiments were conducted to determine whether the short CS-US interval necessary for successful conditioning with external CSs and USs is due to the short shock duration typically used in these experiments. In both experiments conducted by this investigator subjects learned to avoid an auditory CS if a 12-minute shock was presented immediately after the CS but not if shock onset was delayed by 10 minutes. (Procedures used to present the auditory CS and shock and to assess aversion learning were identical to those described in Chapter 4, Experiment 9). There was no evidence that rats could associate an appropriate CS with a 12-minute shock

US when the CS-US interval was 10 minutes. Inasmuch as rats can associate a flavor CS with a 10-minute stomach distention US when the CS-US interval is 10 minutes, it appears that the long CS-US intervals conducive to taste-aversion learning are not attributable merely to the relatively long US duration used in the taste-aversion preparations.

Taste-aversion learning and US aversiveness. Chemical or radiation induced toxicosis readily motivates taste-aversion learning (as assessed by either single solution or two-solution choice test) over CS-US trace intervals of several hours. The 10-minute balloon inflation, on the other hand, did not motivate taste-aversion learning (as assessed by a two-solution choice test) over CS-US trace intervals of 25 to 30 minutes. One explanation for this apparent difference between toxicosis and balloon inflation as internal USs is in terms of US aversiveness. Revusky (1968), for example, has shown that the maximum CS-US trace interval over which rats will associate a gustatory CS with toxicosis is, in part, a function of US aversiveness, the less aversive the US the shorter the maximum CS-US trace interval. Although there have been no experiments in which the delay-of-US function has been investigated with a minimally aversive US (a US just capable of producing a learned taste aversion when

presented immediately after the flavor CS) it seems likely that a minimally aversive US might be readily associated with a flavor CS only over very short CS-US trace intervals.

The balloon inflation US used in these experiments appears to be far less aversive to rats than a typical radiation or chemical induced toxicosis. Certainly the duration of aversiveness with the 10-minute balloon inflation is less than for a typical toxicosis (Experiments 5 and 6). Also, a further reduction in balloon inflation volume or duration (Experiments 2 and 4) substantially attenuates the effectiveness of balloon inflation as a US even when presented immediately after the gustatory CS. The failure of the 10-minute balloon inflation to readily motivate taste-aversion learning (as assessed by the preference test) with a 30-minute CS-US interval may simply be another indication that the 10-minute inflation is only mildly aversive in comparison to the toxic USs used in previous studies of the delay-of-US effect in taste aversion learning.

CHAPTER 4:   SELECTIVE ASSOCIATION

Rats readily associate gustatory stimuli with a
chemical or radiation induced toxicosis but not with
nociceptive shock applied to the surface of the body.   In
contrast, rats readily associate auditory or visual stimuli
with shock but not with toxicosis.   Garcia and his
associates (e.g., Garcia & Ervin, 1968; Garcia, Hankins
and Rusiniak, 1974) have suggested that rats selectively
associate gustatory stimuli with toxicosis and auditory
or visual stimuli with shock because the two USs are applied
to different receptor systems.   Shock is applied to
somesthetic receptors while toxicosis is primarily a
visceral experience.   In Chapter 1, however, it was
suggested that the selective associability of shock and
toxicosis with different CSs may be a function of US
temporal characteristics (such as rate of onset and
duration), rather than the different receptor sites to
which the US is applied.   A shock US, for example, is
usually applied as a discrete stimulus with a rapid onset
and short duration.   Toxicosis, on the other hand, usually
develops over a period of minutes and lasts for hours.   It
was noted, in fact, that demonstrations of selective

association have confounded US receptor site (internal vs. external) with US temporal features (slow onset and long duration vs. rapid onset and short duration).

Although experiments presented in Chapter 2 clearly indicate that the slow onset and very long duration typical of chemically induced toxicosis is not necessary for the effective motivation of taste-aversion learning, those experiments do not preclude an important role for US temporal characteristics in the process of selective association. The experiments presented in this chapter were designed to assess whether rats selectively associated an internal US with a gustatory CS and an external US with an auditory CS when the temporal characteristics of US application were closely matched.

Experiment 9: Stomach Distention
vs. Electric Shock

Unlike toxicosis, stomach balloon inflation permits direct control over the temporal characteristics of stimulation, enabling the matching of groups of rats with respect to the onset, duration and offset of an internal (stomach distention) and external (electric shock) US. Experiment 9 was designed to investigate whether the selective association phenomenon exists when the different USs are temporally matched. A demonstration of selective

association with temporally matched USs would indicate that it is locus of US application that is relevant, rather than the unique temporal characteristics of the toxicosis US employed in previous demonstrations of the phenomenon.

Method

Design. Subjects were assigned to groups that experienced one of the following combinations of CS and US: (a) gustatory CS, shock US; (b) gustatory CS, distention US; (c) auditory CS, shock US; (d) auditory CS, distention US. These groups represent the four combinations of a 2 by 2 factorial design with two CSs (one gustatory, one auditory) and two USs (one stomach balloon distention, one shock). The USs (and CSs) were equated with respect to temporal characteristics, making this study unique among selective association experiments.

A fifth group of subjects experienced the auditory CS paired with a very brief shock. This group was included to determine if a long shock, one matched in temporal characteristics to stomach balloon inflation, would show a level of associability different from the level of associability shown by the more typically employed short duration shock.

Preexperimental preparations. Forty subjects were prepared with cheek cannula for oral infusion as described

in Experiment 6. One week later sixteen of these subjects were prepared with stomach balloon and the remaining 24 subjects prepared with a shock electrode consisting of a safety pin (2.7 cm in length) implanted just above the shoulder blades.

Gustatory CS. The gustatory stimulus, a 0.15% sodium saccharin solution, was orally infused at a rate of 2.1 ml/minute for 100 seconds. Since it was observed in Experiment 6 that subjects drank practically all of the orally infused fluid, no attempt was made to measure rejected solution, and oral infusion was accomplished while subjects were restrained.

Auditory CS. The auditory stimulus consisted of a click, generated 18 times per second (Scientific Prototype Click Generator, Model 4041) at an intensity of 70 decibels against a 55 decibel "white noise" background (General Radio Sound Level Meter, calibrated at 1000 Hz, Scale A), and presented for a period of 100 seconds. During each auditory stimulus presentation, subjects experienced oral infusion of tap water at the rate of 2.1 ml/minute. Oral infusion of water accompanied the auditory stimulus to insure that subjects experiencing distention following the auditory CS would ingest approximately 3.0 milliliters of fluid prior to balloon inflation (as had subjects that experienced the gustatory CS prior to balloon inflation).

Internal US. As in previous experiments, the
internal US consisted of a 9 ml stomach balloon inflation
of 10-minutes duration. Balloon inflation began at the
termination of the CS (either click presentation or
saccharin infusion) with complete balloon inflation
requiring 7 to 10 seconds. Ballon deflation required 18
to 25 seconds.

External USs. A 10-minute (long duration)
alternating current shock (Scientific Prototype A.C. Shock
Generator, Model 4007 J) was delivered through the implanted
safety pin and a spring loaded clip attached to the
midpoint of the rat's tail. To facilitate electrode
contact. Beckman EKG paste was applied to the surface of
the clip electrode. Onset, duration and offset of the
long shock were controlled manually through a variable
resister in series with the rat. Shock onset began as the
click presentation or saccharin infusion ended with
intensity rising from 0 to 90 volts (RMS, measured at
output poles on the shock generator) in a linear manner
over a 7- to 10-second period.[5] The duration of maximum

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[5] Subsequent to this experiment six of the subjects
prepared with electrodes were shocked as described above
with peak voltage and current across the rat recorded by
cathode ray oscilloscope. Peak voltage and currents were
found to range between 40 to 60 volts (RMS) and 0.6 to 1.0
milliamps, respectively.

voltage lasted for 10 minutes, when the shock was terminated by reducing voltage in a linear fashion from 90 to 0 volts over a period of 18 to 25 seconds.

The short duration shock US was presented at full intensity (90 volts, RMS) through closure of a relay for 500 milliseconds.

Procedure. Five days were allowed for recovery from stomach balloon surgery. Subjects were then placed on a 23½ hour water deprivation schedule and daily experimental sessions were begun. Each subject participated in the experiment for eight sessions: five adaptation, two treatment and one test session.

(1) Adaptation - For the first five experimental sessions, subjects were adapted to restraint, handling and oral infusion. During each adaptation session subjects were taken to an experimental room and restrained for fifteen minutes with all appropriate connections (stomach cannula extention, cheek cannula extention, tail electrode and safety pin electrode) fixed in place. During the restraint period each subject was orally infused with water for two minutes (rate approximately 1½ ml per minute). Following the restraint period each subject was returned to its home cage where it was allowed access to water for 1/2 hour.

(2) Treatment - Each of the two treatment sessions

began with the removal of all food from the home cages.
Four and one-half hours later each subject was removed
to the experimental room and restrained. During the
restraint period, two groups of subjects experienced the
oral infusion of the saccharin solution followed either
by 10 minutes of balloon distention (Group Saccharin
Distention, $n=8$) or the 10-minute shock (Group Saccharin
Long Shock, $n=8$). Two additional groups experienced the
combined oral infusion of water plus auditory CS, followed
by either 10 minutes of stomach balloon distention (Group
Click Distention, $n=8$) or 10 minutes of shock (Group
Click Long Shock, $n=8$). Finally, a fifth group also
experienced the combined auditory CS plus oral infusion
of water, but in this group the CS was followed by the 500
millisecond shock (Group Click Short Shock, $n=8$).

Following each treatment session subjects were
returned to their home cages and allowed access to water
for one-half hour. Food was also returned to the home
cages and remained available until 4 1/2 hours prior to
the next experimental session.

(3) Test - Twenty-four hours after the second
treatment session groups that had the saccharin CS (Groups
Saccharin Distention and Saccharin Long Shock) were tested
for saccharin preference and subjects that had the click
CS (Groups Click Distention, Click Long Shock, and Click

Short Shock) were tested for their click preference.
Testing procedures were slightly different from those used
in previous experiments. Each subject was removed to the
experimental room and placed in a cage identical to the
home cage. Saccharin preference was then assessed with
the usual two-solution, saccharin vs. water, choice test.
Click preference was assessed in the manner used in other
demonstrations of selective association (e.g., Domjan &
Wilson, 1972b): As in the saccharin preference test two
bottles were placed on the test cage. However, both
bottles contained tap water, with one bottle having
arbitrarily been predesignated as the click-water bottle.
When a subject drank from this bottle, the experimenter
activated the click generator and the auditory CS was
presented as long as the subject drank from that bottle.
When the subject drank from the alternate bottle, clicks
were not presented. Thus, subjects could choose between
click plus water or water alone. Both click and saccharin
preference tests were fifteen minutes in duration.
Saccharin preferences ratios were calculated as before,
and click preference ratios were calculated in an analogous
manner, that is, milliliters of water ingested from the
click plus water bottle divided by total milliliters of
fluid ingested (ml of click water plus ml of water). Thus,
a click preference ratio has the same range (0 to 1.0) and

interpretation as the saccharin preference ratio.

Results

Preference test results are presented in Figure 15 as median saccharin or click preference ratio for each group. Examination of this figure indicates that selective association occurred despite the fact that internal and external USs were matched in terms of their temporal characteristics. Subjects that experienced the auditory CS followed by either the long or short electric shocks drank significantly less water from the click bottle than did subjects that experienced the auditory CS followed by balloon inflation (Click Distention vs. Click Long Shock, $U=9$, $p<.014$; Click Distention vs. Click Short Shock, $U=0$, $p<.02$). In contrast, subjects that experienced the gustatory stimulus prior to the 10-minute balloon inflation subsequently showed less of a preference for the CS solution than subjects who experienced the gustatory CS prior to the long (10 minute) shock. This difference, however, did not reach statistical significance (Saccharin Distention vs. Saccharin Long Shock, $U=20$, $p>.20$).

Finally, subjects in the long shock group appeared to acquire a slightly stronger auditory aversion than subjects in the short shock group. The difference, however,

Figure 15

Experiment 9: Median saccharin preference ratios

for groups that experienced a gustatory stimulus

(saccharin) followed by either distention or long shock,

and click preference ratios for groups that experienced

an auditory stimulus (click) followed by distention, long

shock or short shock during treatment sessions.   Number

of subjects per group is indicated in parenthesis.

Figure 15

was not statistically significant (Click Long Shock vs. Click Short Shock, $\underline{U}$=27, $\underline{p}$>.20). (Of course both groups had such low click preference ratios that any difference between them may have been obscured by a floor effect.)

Discussion

Previous research has demonstrated a selective associability phenomenon using shock and toxicosis as USs. Rats readily acquire a gustatory aversion but not an auditory aversion when the US is an internal malaise having a slow onset and long duration. In contrast, rats readily acquire an auditory aversion but not a gustatory aversion when the US is a painful external stimulus having a rapid onset and a brief duration.

It is this reversal of CS associability with different USs which identifies the selective association process in rat aversion learning. Such a reversal of CS associability was obtained in Experiment 9 despite the fact that internal and external USs were applied with the same onset, duration and offset characteristics. Subjects that experienced a gustatory CS paired with a 10-minute internal US (stomach balloon inflation) subsequently evidenced a lower preference for the CS solution than did rats that experienced the gustatory CS paired with a 10-minute external US (shock) having the same onset/offset

characteristics as the internal US. On the other hand, subjects that experienced an auditory CS paired with the 10-minute external US subsequently evidenced a stronger aversion to the auditory CS than subjects who experienced the auditory CS paired with the 10-minute internal US. These results suggest that in previous demonstrations of selective association (e.g., Domjan & Wilson, 1972b), demonstrations in which US receptor site was confounded with distinctive US temporal characteristics, the important difference between USs was receptor site and not distinctive US temporal features.

In this experiment, the greater saccharin aversion acquired by the Saccharin Distention Group than by the Saccharin Long Shock Group is not statistically significant. This does not appear to have resulted from a lack of aversiveness on the part of the balloon inflation US. The median saccharin preference displayed by the Saccharin Distention Group (median=.17) is within the range of median saccharin preference ratios obtained from Saccharin Distention Groups in previous experiments (medians of .20 to .05). On the other hand, the saccharin preference ratio of the Saccharin Long Shock Group (median=.40) is substantially below the range of saccharin preference ratios observed in saccharin only control groups of Experiments 1 through 8 (medians=.55 to .70). Thus it is

possible that there was some association between the
flavor and the long shock. (Previous demonstrations of
the selective association phenomenon used brief shocks.)

The interaction in associability between temporally
matched CSs (gustatory vs. auditory) and USs (shock vs.
stomach distention) apparent in Figure 15 suggests that
the selective association phenomenon is dependent on locus
of US application, and not US temporal features.

Experiment 10:   Contiguous Presentation of

Auditory CS and Balloon

Inflation US

Experiment 9 demonstrated that rats readily
associated an auditory CS with shock, but not with visceral
stimulation, even when the temporal features of the two
USs were matched. However, observation of the rats during
Experiment 9 revealed a possible difference in the onset
latency of the affective qualities of shock and distention.
Shocked rats evidenced an overt reaction to the US
(struggling and vocalization) within two or three seconds
following shock onset. Distended rats, however, evidenced
an overt reaction to the US (rolling and head movement, as
described in General Discussion--Experiments 1-6) about
six to eight seconds following balloon inflation onset
(i.e., when balloon inflation volume reached about 7 ml).

Thus, although the duration and onset/offset characteristics of the internal and external USs were nominally equated, it is possible that the relatively low associability of the click US with the stomach distention US resulted from the relatively long time elapsing between CS offset and US aversiveness.

Experiment 10 was designed to assess whether the differential associability of shock and stomach distention with an auditory CS (as demonstrated in Experiment 9) would persist when the confounding of CS offset to US aversiveness interval and US locus of application was eliminated. In the present experiment, the duration of the CS was lengthened to 10 minutes, and it was presented simultaneously with a 10-minute US (either distention or shock). Thus, CS and US were completely overlapped and contiguity between CS and US aversiveness assured.

Method

Subjects and pre-experimental preparation. Twenty-eight subjects were surgically prepared for this experiment: eight with a cheek cannula for oral infusion, seven with cheek cannula and safety pin electrode, and thirteen with cheek cannula and stomach balloon. Stomach balloons and cheek cannulas were implanted in a single operation and therefore all subjects were allowed eight days to recover

from surgical procedures instead of the usual five.

Procedure. All details of the procedure not specified below were as described in Experiment 9.

Following the eight day recovery period all subjects were placed on the $23\frac{1}{2}$ hour water deprivation schedule, and adapted to restraint, handling, and oral infusion of water. Adaptation sessions were conducted once a day for five days.

During each of the two treatment sessions, two groups experienced a 100-second, oral infusion of water followed immediately by 10 minutes of the auditory CS (clicks at the rate of 18 per second). Throughout the 10-minute auditory CS one group experienced shock (Group Click Shock, $n=7$), while the second group experienced stomach balloon distention (Group Click Distention, $n=7$). As in Experiment 9 both USs were 10 minutes in duration and were matched in onset/offset characteristics. The onset of each US (shock or balloon inflation) began simultaneously with onset of the auditory CS. Thus, in both Click Shock and Click Distention Groups the overlap between the CS and US was complete.

A third group also experienced the 100-second, oral infusion of water followed immediately by the 10-minute auditory CS during each treatment session. This group, however, did not experience either US and therefore constituted a CS-only control group (Group Click Only, $n=8$).

Finally, a group was included that experienced a 100-second, oral infusion of the saccharin solution followed immediately by stomach balloon inflation (Group Saccharin Distention, $\underline{n}$=6) of the same temporal features as the balloon inflation experienced by subjects in the Click Distention Group. Such a group was necessary to determine if any failure of subjects to associate the auditory CS with the balloon inflation US was due to a lack of aversiveness on the part of the balloon inflation procedures.

Twenty-four hours after the second treatment session saccharin and click preferences were assessed as described in Experiment 9.

Results

Figure 16 presents median saccharin and click preference ratios for groups in Experiment 10.

Elimination of the temporal interval between auditory CS offset and onset of balloon inflation aversiveness does not appear to have caused rats to associate the auditory CS with the balloon inflation US. In fact, the Click Distention Group showed a higher preference for water from the bottle that produced the auditory CS than did the Click Only Control Group, although this difference was not statistically significant ($\underline{U}$=21,

Figure 16

Experiment 10: Median saccharin preference ratio
for the group experiencing a gustatory stimulus (saccharin)
followed by stomach distention, and median click
preference ratios for groups experiencing the auditory
stimulus (click) alone or paired with distention or shock.
Number of subjects per group is indicated in parenthesis.

Figure 16
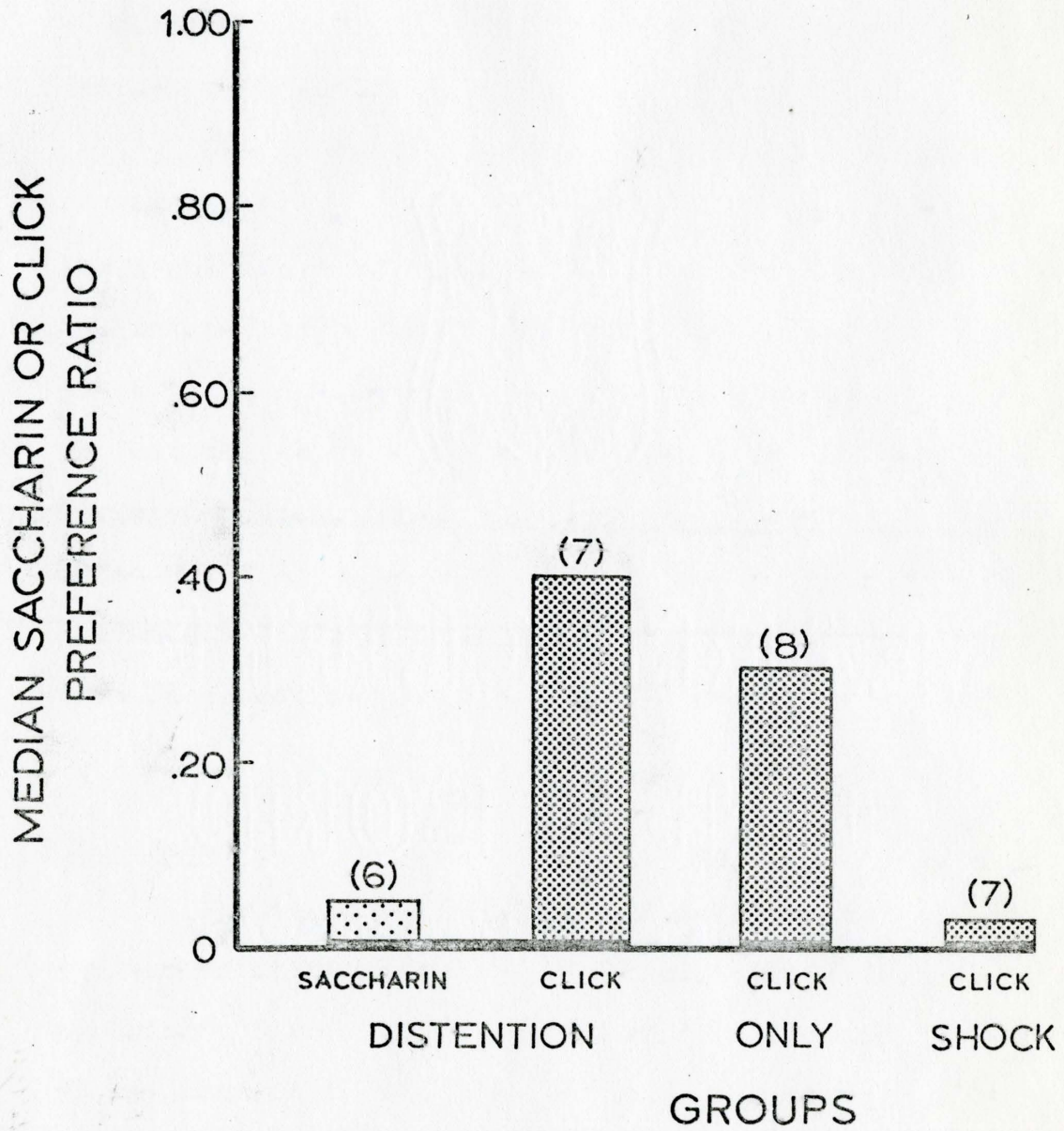
p>.20). Furthermore, the median click preference ratio
for the Click Distention Group of this experiment (median=
.40) is not significantly different from the click
preference of the Click Distention Group of Experiment 9
(median=.47) (U=18, p>.20).

The absence of click-aversion learning in the Click
Distention Group cannot be attributed to any general
ineffectiveness of the 10-minute auditory stimulus as a
CS, an auditory aversion was readily learned by the Click
Shock Group (Click Shock vs. Click Only, U=0, p<.001).
Nor can the absence of aversion learning in the Click
Distention Group be attributed to any ineffectiveness of
balloon inflation as a US. Subjects in the Saccharin
Distention Group clearly acquired a gustatory aversion
(median saccharin preference ratio=.07).

Discussion

The results of Experiment 10 indicate that the
failure of subjects in the Click Distention Group of
Experiment 9 to associate the auditory CS with the internal
US was not due to the separation of auditory CS offset from
onset of internal US aversiveness. Even when the auditory
CS was lengthened to 10 minutes and presented
simultaneously with the internal US (as was the case in
this experiment), subjects did not associate the auditory

CS with the internal US. Thus, Experiment 10 provided further evidence that US locus of application rather than US temporal features plays the predominant role in the selective association phenomenon.

General Discussion: Experiments 9 and 10

Experiments 9 and 10 were designed to determine if the rat's selective association of toxic USs with gustatory CSs and shock USs with auditory/visual CSs is attributable to the distinctive temporal characteristics of the USs rather than locus of US application. Previous demonstrations of selective association had confounded differences between the application sites of toxicosis and shock (internal vs. external) with differences between the USs in temporal characteristics (slow onset and long duration vs. rapid onset and short duration). In Experiments 9 and 10, the shock US was lengthened from its usual span of a few hundred milliseconds to ten minutes and applied with a slow onset and long duration. In comparison to chemical or radiation induced toxicosis, stomach balloon inflation applied with the same rate of onset, duration and rate of offset as the shock constituted a relatively discrete internal US. Experiment 9 demonstrated selective assocation of CS and US despite the use of temporally matched internal and external USs.

Rats clearly learned a taste aversion when the flavor CS was paired with the balloon inflation US but not when paired with the long shock. In contrast, rats learned an auditory aversion when the auditory CS was paired with the long (or short) shock but not when paired with the balloon inflation US. Furthermore, Experiment 10 demonstrated that the absence of aversion learning in the Click Distention Group was not the result of an artifactual temporal separation between auditory CS offset and onset of balloon inflation aversiveness. Rats did not learn a taste aversion even when auditory CS completely overlapped the balloon inflation US. These data are consistent with previous demonstrations of selective association and suggest that temporal properties of the US do not play a critical role in the selective association process.

CHAPTER 5: SUMMARY AND CONCLUSIONS

Previous research has shown that rats readily acquire a taste aversion when a gustatory stimulus is followed by toxicosis, but not when the same stimulus is followed by cutaneous shock. Also, taste aversions, in contrast to shock motivated auditory or visual aversions, are learned even when the gustatory CS precedes onset of toxicosis by several hours. Many investigators (e.g., Garcia & Ervin, 1968; Rozin & Kalat, 1971) attribute these distinctive features of taste-aversion learning to CS and US receptor sites with little consideration of the fact that taste and toxicosis differ from the more conventional stimuli, auditory or visual cues and electric shock, along a number of dimensions. For example, shock is usually applied with a rapid onset, short duration and rapid offset to a localized set of receptors (e.g., the paws or the tail of a rat). Toxicosis, on the other hand, is typically a diffuse experience which, in comparison to shock, has a very slow onset, long duration and slow offset. The present series of experiments was designed to investigate the role played by US temporal characteristics, such as rate of onset and duration, in the distinctive

features of taste-aversion learning.

In order to investigate the role of US temporal characteristics in taste-aversion learning, a conditioning preparation was developed in which an internal US that had readily controlled temporal features could be applied directly to visceral receptors. Inasmuch as this preparation was unique in the study of taste-aversion learning, the initial experiments (Chapter 2) were designed to (a) demonstrate taste-aversion learning motivated by stomach balloon inflation, and (b) investigate the relation of basic balloon inflation parameters such as volume and duration to aversion learning. It was demonstrated that a distention volume of 9 ml which lasted 4 or 10 minutes effectively motivated taste-aversion learning (Experiments 3 and 4). Furthermore, it was shown that a 9 ml, 10-minute balloon inflation did not produce an internal malaise that extended substantially beyond balloon deflation (Experiments 5 and 6). (Balloon inflation volumes larger than 9 ml and which lasted longer than 10 minutes produced taste aversions but also appeared to produce internal consequences that lasted far longer than the period of balloon distention (Experiments 1 and 2).) Thus, experiments presented in Chapter 2 provide the first unambiguous demonstration that the slow onset and long duration characteristic of chemical or radiation induced

toxicosis are not prerequisites for effective motivation of taste-aversion learning.

Experiments presented in Chapter 3 were designed to assess taste-aversion learning when CS termination preceded US onset (i.e., trace conditioning) by more than a minute or two. It was found that rats did associate the gustatory CS with stomach balloon inflation even when the trace interval was 10 to 30 minutes. This finding is in marked contrast to the close temporal contiguity necessary for associative learning when the CS is auditory or visual and the US is shock. Inasmuch as the balloon inflation used in these experiments constituted a relatively discrete internal US in comparison to toxicosis, the rat's association of gustatory CS with balloon inflation over CS-US trace intervals ten minutes (or longer) suggests the distinctive temporal characteristics of toxicosis are not necessary for associative learning with CS-US trace intervals longer than a minute or two.

Finally, experiments presented in Chapter 4 were designed to assess the relative associability of internal (stomach balloon inflation) and external (shock) USs when paired with an auditory or gustatory CS. These experiments differed from previous studies of selective association in the rat in that internal and external USs (as well as gustatory and auditory CSs) were closely matched with

respect to their temporal characteristics. Previous
investigations of selective association had, in fact,
confounded the site of US application (internal vs.
external) with US temporal characteristics (slow onset and
long duration vs. rapid onset and short duration). It was
found that rats selectively associated the internal US
(balloon inflation) with the gustatory CS and the external
US (shock) with the auditory CS even though shock and
balloon inflation were applied with the same rate of onset
and offset and for the same duration (Experiment 9). In
addition, it was demonstrated that failure of subjects to
associate the auditory CS with the balloon inflation US
was not attributable to any temporal separation between CS
offset and onset of US aversiveness. Rats did not
associate the auditory CS with stomach balloon inflation
even when CS presentation completely overlapped the US
(Experiment 10). Thus, the rat's selective association of
gustatory CSs with toxicosis and auditory or visual CSs
with shock does not appear to be based upon the distinctive
temporal features of the US but rather upon the receptor
sites to which the US is applied.

In summary, the results presented here suggest that
toxicosis is functionally distinct from cutaneous shock
because toxicosis has its effects internally rather than
externally. The distinctive temporal characteristics of

toxicosis, particularly its duration, may well play a role in determining the aversiveness of toxicosis but they are not necessary US features for (a) learning of a taste aversion, (b) aversion learning with CS-US trace intervals longer than a minute or two, or (c) selective association of gustatory CS with toxic US.

APPENDIX

A.   A detailed description of the surgical techniques

used to prepare subjects with stomach balloons.

B.   Preference ratios and total volume of fluid

ingested during preference tests.

APPENDIX A

Stomach Balloon Construction

Figure 17 indicates the construction of a stomach balloon. A length (15 cm) of flexible tubing (polyethylene 160 tubing, Intramedic, Clay-Adams, U.S.A.) was flared at one end by heating, and a short section (0.7-1.0 cm) of 18 guage steel tubing was inserted into the flared end of the flexible tube. (The steel tubing was obtained by clipping the hub and bevel from an 18 guage disposable needle and filing back each end of the shaft until the lumen has been reopened.) A latex rubber finger cot (reinforced finger cots, size--medium, Sterling Rubber Ltd., Guelph, Ontario, Canada) was cut to the length of 4 centimeters and tied as indicated in Figure 17 with 3-0 surgical silk to the flared end of the tubing.

Pre-surgical Preparation

Subjects weighing 275-300 grams were deprived of food and water the night prior to surgery. One half hour before surgery a subject was anesthesized with an intraperitoneal injection of phenobarbital sodium (64.8 mg/cc, Haver-Lochart Laboratories, Calgary, Alberta, Canada). All subjects were initially given 0.3 ml of the anesthetic,

Figure 17

Appendix A: Construction of stomach balloon plus
incision and exit sites.

Figure 17
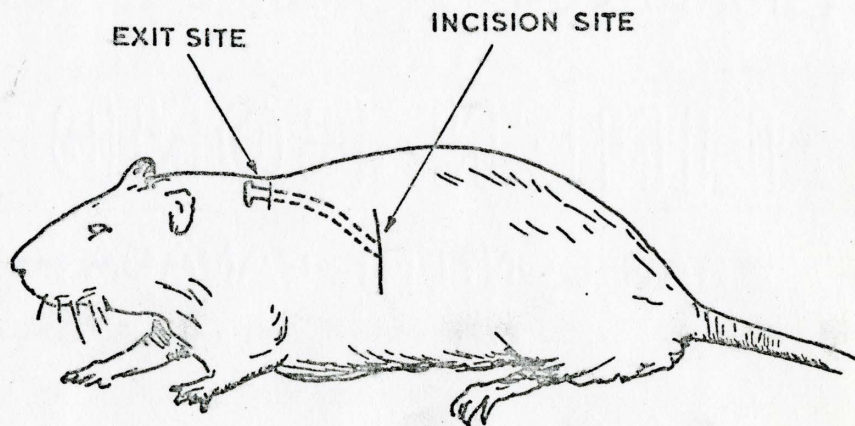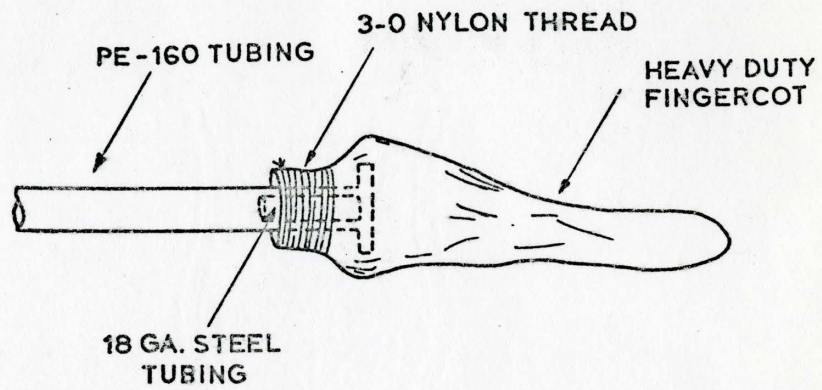
and, if a satisfactory depth of anesthesia was not obtained in 20 minutes, additional anesthetic was given in units of 0.10 ml.

Two surgical sites were shaved then swabbed with alcohol. One area (3.5 x 5.0 cm) was along the caudal curvature of the left rib cage and the other (2 x 2 cm) was immediately caudal to the shoulder blades.

Surgery

Surgery was begun with an incision, 2-3 cm in length parallel and just caudal to the left rib cage (see Figure 17 for approximate location of the incision). Next, the stomach was located and externalized. If necessary the spleen was separated from the stomach and replaced in the peritoneal cavity. Once externalized, the stomach and incision were packed with gauze pads soaked in sterile saline. The stomach was also periodically moistened with saline to prevent excessive drying. Following placement of the gauze pads, an oblong (1.5 x 0.7 cm) purse string suture (Markowitz, Archibald & Downie, 1964, pg. 44) was begun on the greater curvature at the intersection of the rumen and fundus and extended back into the fundus (suture material: 5-0 braided silk, Cardiovascular K-880 H, Ethicon Sutures Ltd., Peterborough, Ontario, Canada). Next, a small incision was made in the center of the purse-

string suture and the contents of the stomach removed by suctioning to reduce spillage during insertion of the balloon. Mild tension on the edges of the small incision was sufficient to stretch this small opening into a hole large enough for insertion of the balloon. The balloon was inserted into the stomach and positioned along the greater curvature, from the point of the incision to the pyloric sphincter, with the balloon cannula extending out through the incision. Following placement of the balloon, the stomach incision was closed by inverting the edges of the wound with thumb forceps and then gently drawing the purse string suture closed. When accomplished appropriately the cut edge of the stomach was buried and the serosa was in snug contact with the balloon cannula.

At this point the stomach was flushed with sterile saline and returned to the peritoneal cavity. Next the balloon cannula was passed through a small stab wound in the muscle anterior to the incision and threaded subcutaneously to the shoulder blades where it exited through a second small stab wound. The stomach was then positioned so that the closed stomach incision opposed the muscle stab wound. Thus, the balloon cannula passed immediately from the stomach through the muscle wall. The stomach was then anchored to the muscle wall with two 5-0 silk sutures, one on each side of the muscle stab wound.

Skin and muscle were closed separately with a continuous suture of 3-0 silk. Finally a small plastic collar was placed over the externalized portion of the balloon cannula. Excessive tube was then cut off and the end of the tube was flanged by heating. It proved to be quite important for the external portion of the balloon cannula to·be flush with the skin; otherwise, the rats were likely to chew off the end of the cannula and render the preparation useless.

Post-operative Care

Both incision sites were cleaned with alcohol immediately after surgery and each subject was given a 0.2 ml intramuscular injection of Strephanalean (M.T.C. Pharmaceuticals, Hamilton, Ontario, Canada), a combination of penicillin G and streptomycin. A second injection of Strephenalean (0.1 ml) was given 24 hours after the first. Food and water were returned to home cages immediately following surgery.

APPENDIX B

Transformation of the data into preference ratios
has the advantage of reducing within group variability
due to individual differences in general level of ingestion.
However, the validity of between group preference ratio
comparisons rests, in part, on the assumption that such
comparisons are not confounded by between group differences
in total volume of fluid ingested during a test. Table 1
presents median volume (in milliliters) of total fluid
ingested by each group in each experiment during preference
tests. Within each experiment all possible two-group
differences were tested for significance with the
nonparametric Mann-Whitney U at the 10% level (2-tail).
(The data from Experiment 2 are not presented because
sample size was insufficient for statistical analysis.)
However, a post hoc search for significant differences
between groups using multiple, pairwise comparisons
introduces another problem. Because many of these
comparisons are not independent, a statistical test is
likely to declare by chance alone more differences as
significant than specified by alpha. To maintain the type
I error probability at alpha or less, criterion levels
were adjusted according to the procedure suggested by

Ryan (1961).

Only two differences between groups in volume of fluid consumed during a preference test actually reached significance. In Experiment 8 the operated, saccharin only control group (Group OpC)· drank significantly more fluid than did the 10-minute delay group. There was no apparent reason for this difference. Furthermore, the important comparisons in Experiment 8 involved the combined OpC and NopC (nonoperated, saccharin only control) groups with distended groups. The median volume of fluid ingested by the combined control groups was 14.5 ml and this volume was not significantly different from the volume of fluid ingested by distention groups. In Experiment 10, the Click-Shock group drank significantly less fluid than did the Click-Only group. In addition, the Click Long Shock group of Experiment 9 also showed a tendency to drink less fluid during the preference test than did other groups, but none of these differences were statistically significant. The reason Click Long Shock subjects drank less fluid during the test was clear from their behavior. Once a Click Long Shock subject experienced the auditory stimulus while drinking from the designated drinking tube they were reluctant to drink from either of the available water filled reservoirs. Thus, the aversion to the auditory stimulus appeared to generalize from the drinking tube

associated with that stimulus to the tube not associated with the stimulus. Fortunately, however, the generalization was not sufficient to obscure the specific aversion to the auditory stimulus. Click Long Shock subjects of Experiment 10 showed a significantly lower preference for drinking from the tube associated with the auditory CS than did the Click Only group.

In summary, differences between groups in total fluid ingested does not appear to compromise any of the comparisons based upon preference ratios.

TABLE 1: MEDIAN VOLUME OF TOTAL FLUID INGESTED BY EACH GROUP IN EACH EXPERIMENT

Experiment 1

OpC	FCd	Bkd-5
15.0	14.8	15.5

Experiment 3

NopC	OpC	Duration 8	Duration 12	Duration 16
18.0	14.5	16.8	13.5	12.8

Experiment 4: first Preference Test

NopC	Duration 1/2	Duration 4	Duration 10
16.0	13.5	14.3	17.0

Experiment 4: second Preference Test

NopC	Duration 1/2	Duration 4	Duration 10
18.0	19.0	15.0	18.0

Experiment 5

NopC	FCd	Bkd-1/2	Bkd-10	Bkd-30
16.0	17.5	15.3	15.0	13.5

Experiment 6

NopC	FCd	Bkd-1/2	Bkd-10
13.3	10.5	12.3	13.0

Experiment 7

NopC	FCd	Delay 5	Delay 12	Delay 25
15.0	12.5	14.3	12.3	13.2

Experiment 8

NopC	OpC	FCd	Delay 10	Delay 30
14.0	15.5	13.0	12.0	12.3

Experiment 9

Saccharin Distention	Click Distention	Click Short Shock	Click Shock	Saccharin Shock
12.5	12.5	11.5	8.8	12.0

Experiment 10

FCd	Click Distention	Click	Click Shock
11.3	12.0	14.0	7.0

REFERENCES

Barker, L.M., & Smith, J.C. A comparison of taste aversions induced by radiation and lithium chloride in CS-US and US-CS paradigms. Journal of Comparative and Physiological Psychology, 1974, 87, 644-654.

Beecroft, R.S. Classical Conditioning. Goleta, California: Psychonomic Press, 1966.

Black, A.H. & Prokasy, W.F.(Eds.) Classical Conditioning II: Current Research and Theory. New York: Appleton-Century-Crofts, 1972.

Boland, F.J. Saccharin aversions induced by lithium chloride toxicosis in a backward conditioning paradigm. Animal Learning and Behavior, 1973, 1, 3-4.

Bolles, R.C. Species-specific defense reactions and avoidance learning. Psychological Review, 1970, 77, 32-48.

Breland, K. & Breland, M. The misbehavior of organisms. American Psychologist, 1961, 16, 661-664.

Brown, P.L. & Jenkins, H.M. Auto-shaping of the pigeon's key-peck response. Journal of the Experimental Analysis of Behavior, 1968, 11, 1-8.

Domjan, M., & Bowman, T.G. Learned safety and the CS-US delay gradient in taste-aversion learning. Learning and Motivation, 1974, 5, 409-423.

Domjan, M., & Wilson, N.E. Contribution of ingestive behaviors to taste-aversion learning in the rat. Journal of Comparative and Physiological Psychology, 1972a, 80, 403-412.

Domjan, M., & Wilson, N.E. Specificity of cue to consequence in aversion learning in the rat. Psychonomic Science, 1972b, 26, 143-145.

Dragoin, W.B. Conditioning and extinction of taste aversions with variation in intensity of the CS and UCS in two strains of rats. Psychonomic Science, 1971, 22, 303-304.

Dragoin, W., McCleary, G.E., & McCleary, P. A comparison of two methods of measuring conditioned taste aversions. Behavior Research Methods and Instrumentation. 1971, 3, 309-310.

Ellison, G.D. Differential salivary conditioning to traces. Journal of Comparative and Physiological Psychology, 1964, 57, 373-380.

Estes, W.K.   The statistical approach to learning theory.

  In S. Koch (Ed.), Psychology: A study of a science.

  Vol. 2. New York: McGraw, 1959.

Garcia, J., & Ervin, F.R.   Gustatory-visceral and

  telereceptor-cutaneous conditioning: Adaptation

  in internal and external milieus. Communications in

  Behavioral Biology, 1968, 1, 389-415.

Garcia, J., Ervin, F.R., & Koelling, R.A.   Learning with

  prolonged delay of reinforcement. Psychonomic

  Science, 1966, 5, 121-122.

Garcia, J., Green, K.F., & McGowan, B.K.   X-ray as an

  olfactory stimulus. In C. Pfaffman (Ed.),

  Olfaction and Taste: Proceedings of the Third

  International Symposium. New York: Rockefeller

  University Press, 1969.

Garcia, J., Hankins, W.G., & Rusiniak, K.W.   Behavioral

  regulation of the milieu interne in man and rat.

  Science, 1974, 185, 824-831.

Garcia, J., & Koelling, R.A.   Relation of cue to consequence

  in avoidance learning. Psychonomic Science, 1966,

  4, 123-124.

Garcia, J., McGowan, B.K., Ervin, F.R. & Koelling, R.A. Cues--Their relative effectiveness as a function of the reinforcer. Science, 1968, 160, 794-795.

Garcia, J., McGowan, B.K., & Green, K.F. Biological constraints on conditioning. In A.H. Black and W.F. Prokasy (Eds.), Classical Conditioning II: Current research and theory. New York: Appleton - Century - Crofts, 1972, 3-27.

Grote, F.W., Jr., & Brown, R.T. Conditioned taste aversions: Two-stimulus tests are more sensitive than one-stimulus tests. Behavior Research Methods and Instrumentation, 1971, 3, 311-312.

Hull, C.L. Learning: II. The factor of the conditioned reflex. In C. Murchinson (Ed.), A Handbook of General Experimental Psychology. Worcester, Mass.: Clark University Press, 1934.

Kalat, J.W., & Rozin, P. "Learned safety" as a mechanism in long-delay taste-aversion learning in rats. Journal of Comparative and Physiological Psychology, 1973, 83, 198-207.

Kalat, J.W., & Rozin, P. "Salience": A factor which can override temporal contiguity in taste-aversion learning. Journal of Comparative and Physiological

Psychology, 1970, 71, 192-197.

Kamin, L.J. Temporal and intensity characteristics of the conditioned stimulus. In W.F. Prokasy (Ed.), Classical Conditioning. New York: Appleton-Century - Crofts, 1965.

Kamin, L.J., & Brimer, C.J. The effect of intensity of conditioned and unconditioned stimuli on a conditioned emotional response. Canadian Journal of Psychology, 1963, 17, 194-198.

Kimble, G.A. Hilgard and Marquis' Conditioning and Learning. New York: Appleton - Century - Crofts, 1961.

Krane, R.V., & Wagner, A.R. Taste-aversion learning with a delayed-shock US: Implications for the "Generality of the Laws of Learning". Journal of Comparative and Physiological Psychology, 1975, 88, 882-889.

Mackintosh, N.J. The Psychology of Animal Learning. New York: Academic Press, 1975.

Markowitz, J., Archibald, J., & Downie, H.G. Experimental Surgery. Baltimore: Williams and Wilkens Co., Ed. 5, 1964.

Miller, N.E. Experiments on Motivation. <u>Science</u>, 1957,
   <u>126</u>, 1271-1277.

Nachman, M. Learned taste and temperature aversions due
   to lithium chloride sickness after temporal delays.
   <u>Journal of Comparative and Physiological Psychology</u>,
   1970, <u>73</u>, 22-30.

Pavlov, I.P. <u>Conditioned Reflexes</u>. New York: Dover, 1927.

Pavlov, I.P. <u>Lectures on Conditioned Reflexes</u>. New York:
   International Publishers, 1928.

Pavlov, I.P. <u>Lectures on the Work of the Digestive Glands</u>.
   Translated by W.H. Thompson, London: Charles
   Griffen and Company, 2nd Ed., 1910.

Prokasy, W.F. (Ed.). <u>Classical Conditioning: A Symposium</u>.
   New York: Appleton-Century-Crofts, 1965.

Renner, K.E. Delay of reinforcement: A historical review.
   <u>Psychological Bulletin</u>, 1964, <u>61</u>, 341-361.

Revusky, S.H. Aversion to sucrose produced by contingent
   X-irradiation: Temporal and dosage parameters.
   <u>Journal of Comparative and Physiological Psychology</u>,
   1968, <u>65</u>, 17-22.

Revusky, S.H. The role of interference in association over a delay. In W. Honig and H. James (Eds.), Animal Memory. New York: Academic Press, 1971.

Revusky, S.H., & Bedarf, E.W. Association of illness with ingestion of novel foods. Science, 1967, 155, 219-220.

Revusky, S.H., & Garcia, J. Learned associations over long delays. In G.H. Bower and J.T. Spence (Eds.), The Psychology of Learning and Motivation: Advances in research and theory, Volume IV. New York: Avademic Press, 1970, 1-84.

Rozin, P. Central or peripheral mediation of learning with long CS-US intervals in the feeding system. Journal of Comparative and Physiological Psychology, 1969, 67, 421-429.

Rozin, P., & Kalat, J.W. Specific hungers and poison avoidance as adaptive specializations of learning. Psychological Review, 1971, 78, 459-486.

Ryan, T.A. Significance tests for multiple comparison of proportions, variances, and other statistics. Psychological Bulletin, 1960, 27, 318-328.

Rzoska, J. Bait shyness, a study in rat behaviour. The British Journal of Animal Behaviour, 1953, 1, 128-135.

Schou, M. Biology and pharmacology of the lithium ion. Pharmacological Reviews, 1957, 9, 17-58.

Seligman, M.E.P. On the generality of the laws of learning. Psychological Review, 1970, 77, 406-418.

Seligman, M.E.P. & Hager, J.L. Biological Boundaries of Learning. New York: Appleton-Century-Crofts, 1972.

Shettleworth, S.J. Constraints on learning. In D.S. Lehrman, R.S. Hinde, and E. Shaw (Eds.), Advances in the Study of Behavior. Volume IV. New York: Academic Press, 1972, 1-68.

Skinner, B.F. A case history in scientific method. In S. Koch (Ed.) Psychology--A Study of a Science. New York: McGraw-Hill Book Company, 1959, 2.

Skinner, B.F. The Behavior of Organisms. New York: Appleton - Century - Crofts, 1938.

Smith, J.C., & Roll, D.L. Trace conditioning with X-rays as an aversive stimulus. Psychonomic Science, 1967, 9, 11-12.

Smith, M.C., Coleman, S.R., & Gormezano, I. Classical
conditioning of the rabbits' nictitating membrane
response at backward, simultaneous and forward CS-US
intervals. _Journal of Comparative and Physiological
Psychology_, 1969, _69_, 226-231.