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ABSTRACT

The perception of food involves input from different sensory modalities. In addition to taste input, other sensory cues like olfaction, texture, and temperature are involved with the flavor properties in ingestive behavior. Of these different types of sensory input, the effect of temperature on such feeding behavior is the least understood. While there has been a great deal of electrophysiological data at the level of sensory nerves to support an interaction between taste and temperature stimuli, there has been minimal evidence to support such an interaction at the behavioral level. To demonstrate such an interaction behaviorally, a number of criteria needed to be satisfied for temperature responses that were independent of taste cues. The first part of this research demonstrated that rats can respond to exclusively thermal input by displaying the following ingestive behaviors in different series of two-choice, short-term intake tests: thermal preferences, thermal aversions, discrimination between thermal stimuli, and generalization of a thermal response to other stimuli. Once these behaviors were characterized, the last part of this research examined any possible interactions between taste and temperature stimuli. This work established two features that are necessary properties of a taste and temperature interaction. First, the relative saliency of these cues was assessed to determine whether the expression a conditioned aversion to a mixture of taste and temperature cues was more influenced by either sensory input. This experiment demonstrated that although a taste and temperature interaction was not entirely clear, both sensory cues were readily associated with avoidance behavior. The second feature, the relative strengths of expressed aversions to a thermal cue, a taste cue, or a mixture of both cues was determined by measuring relative times of extinction for each condition. This experiment demonstrated a.) temperature and taste aversions extinguish differently under the conditions of the present experiment; and b.) the presence of both cues strengthen the expression of a conditioned aversion. Taken together, the present studies begin to behaviorally show that a taste and temperature interaction does in fact exist.
BACKGROUND AND SIGNIFICANCE

There are many different types of sensory input that influence ingestive behavior. Although the sense of taste, or gustation, is involved, there are numerous nongustatory factors that also may influence the preference or avoidance of different foods. Such things as olfaction, enzymatic components of saliva, blood chemistry within the tongue, prior history of food, early cephalic reflexes, texture, and temperature all may influence such behavior. Of these factors, orosensory temperature has been one of the least studied, as its influence on feeding behavior is not completely understood. In particular, the ability of rats to discriminate and learn about different temperatures and the influence of temperature on taste cues has not been characterized. While there has been a great deal of electrophysiological evidence that supports an interaction between taste and temperature, behavioral evidence showing any such influence is minimal at best. Such behavioral data need to be shown, for it would demonstrate that temperature, like other nongustatory influences, contributes to overall affects in the ingestion of foods. Thus, the goal of this research was to demonstrate that thermal input is not only independently involved with such behavior, but it interacts with other necessary forms of sensory input (i.e., gustatory input) at the level of the oral cavity.

Electrophysiological Evidence of a Temperature/Taste Interaction

There is electrophysiological evidence supporting a gustatory-temperature interaction at the peripheral level. More specifically, the effects of temperature on taste have been shown at the level of fibers that innervate the tongue (and presumably, taste receptor cells) of the rat. The earliest work to show this was conducted by Fishman (1957), who looked at effects of temperature on taste responses in the chorda tympani, a branch one of the primary taste nerves. His findings showed that water alone at a very low temperatures (3-5°C) would evoke an initial response similar to a 0.1 M salt solution at room temperature, but this response gradually decreased as the temperature of water was raised. He also demonstrated that responses to various concentrations of cold salt solutions gradually increased as the temperature was raised to room temperature. Thus, this work supports the claim that salt sensitivity for the chorda tympani
is affected by changes in temperature, such that maximal sensitivity occurs around room temperature (25°C).

Gustatory responses to temperature changes on the chorda tympani nerve have been investigated in the rat (Yamashita and Sato, 1965) and in the cat (Nagaki, Yamashita, & Sato, 1964; Yamashita, Yamada & Sato, 1964). In these studies, it was shown that maximum responses to different tastants were found at 30°C, and that deviations from this temperature resulted in decreased responses to the tastants, independent of preadaptation to the thermal input. Not only did these studies show that there is possibly a temperature effect in response rate to different taste stimuli, but it showed that this effect is not species-specific. Taken together, all of these studies lead to the following conclusions about responsiveness in the chorda tympani: a.) temperature input, by itself, can elicit an immediate response in single-cell units, at least at lower temperatures; b.) some single-fiber units in the chorda tympani can respond to both sensory and thermal input, even if thermal input is just modulating the gustatory response; and c.) temperature input does in fact have an effect on the response rate of various tastants.

There has also been evidence to support these temperature effects on peripheral taste responses at the level of geniculate ganglia. By recording from the rat geniculate ganglion, Lundy and Contreras (1999) supported previous work using single- and whole-nerve recordings of the chorda tympani (also, for review, see Contreras and Lundy, 2000). Furthermore, it was shown that with all of the classifications of neurons, the only cell type that specifically responded to discrete changes in temperature were those that also responded maximally to HCl, or a sour taste. In fact, responses to temperature were much greater in "acid-best" cells than in any other cell type. The relative rate of sensitivity for the respective tastants was also studied when temperature was reduced. Sensitivity was reduced in all neuron types for the various tastants except NaCl, where a group of cells, NaCl-specialist neurons, did not show any changes in response rate to NaCl when the temperatures decreased. Thus, it seems that the primary function of temperature sensitivity is to modulate quality of taste rather than to discriminate between thermal stimuli.

Lundy and Contreras (1994) also provided a unique perspective on the relationship between taste and temperature input on the rat lingual nerve, a branch of the trigeminal nerve that responds to somatosensory, not gustatory, input. Looking at individual nerve fibers, they showed there was some sensitivity to certain tastants. In this study, it was found that dilute
solutions of quinine, citric acid, and salt inhibited responses to warm thermal stimuli. This would imply that although taste input may not directly affect lingual nerve responses, it may have a modulating effect on temperature responses. This is the inverse of the role of temperature in the chorda tympani, where temperature modulates taste. However, this hypothesis remains to be supported by further evidence.

**Behavioral Evidence Supporting a Taste/Temperature Interaction**

Despite a great deal of electrophysiological data that supports a taste and temperature interaction, there is very little behavioral evidence that makes similar claims in the rat. However, there has been some human psychophysical work that supports a taste and temperature interaction. Early work by Hahn (cited in McBurney et al., 1973), suggested that when using an ascending series of the method of limits, temperature did have an effect on taste threshold, but this effect varied as a function of taste quality. However, Pangborn et al., (1970) specifically showed differences in responsiveness to sodium chloride at different temperatures, which yielded a “U-shaped” curve that showed a higher sensitivity to salt at temperatures between 22°C and 37°C rather than at extremes (0°C, and 55°C, respectively).

McBurney et al., (1973) expanded the idea of taste and temperature interactions by showing differences in sensitivity to tastants that covered the four basic taste qualities (sweet, sour, salty, bitter). In this study, where random presentations of tastants (at different concentrations) were compared to water on the tongue, detection thresholds were determined as a function of the stimuli’s temperature levels. Not only were differences in thresholds for each tastant found across a range of temperatures, but a minimum detectable concentration was found for each solution. As the temperature of a tastant deviated further from this “optimal” temperature, the sensitivity for that particular solution decreased (i.e., the minimal detectable concentration increased). Finally, Bartoshuk et al. (1982) showed that perceived responses to sweet substances is also affected by temperature, in which relatively low concentrations of sucrose are perceived as "sweeter" when their temperature is increased. Although these data may support the electrophysiological data that has shown taste and temperature interactions between tastants, it should be noted that these behavioral studies were done in humans, and most of the electrophysiological data were collected in the rat model. In order for a more direct relationship between electrophysiology and behavior to occur, it would be more appropriate to show behavioral evidence in the same animal model.
The only behavioral data in the rat that relate to temperatures of ingested substances involve the preference and avoidance of substances with no gustatory properties. The first of these studies was conducted by Nachman (1970), who demonstrated a learned aversion to water on the basis of a thermal cue. In this study, rats avoided water at 43°C after it was paired with a LiCl injection. Although this is evidence that a rat can discriminate between different thermal cues when they are ingested, there is a fundamental problem with the test stimulus. At this temperature (43°C), ingested water may be considered noxious and therefore would be avoided by the animal without any prior conditioning. Although the use of preferred a cold stimulus might have been more convincing, it was not tested in this study.

As far as any demonstrated thermal preferences in drinking behavior, Gold and Prowse (1974) showed that rats initially preferred cold water to warm water in a short-term comparison. Another study by Carlisle & Laudenslager (1976) did in fact show differences in short-term water intake when water temperature was more broadly manipulated. By comparing intake of water at different temperatures, it was shown that rats most prefer water at room temperature or slightly higher (up to 30°C) over water temperatures that fall above or below this range. Intake of water gradually decreased as water temperature decreased from room temperature, but intake of water warmer than room temperature sharply declined as water temperature increased. These studies suggest that rats can discriminate between different temperatures of water, but it also demonstrated a range of preference for water at different temperatures. However, the stimulus control in this study was somewhat questionable because distant circulating baths of warm or cold water maintained these temperatures. By using this method of temperature control, the experimenter would have to continually monitor the temperature of the bath so that the appropriate thermal input was transferred, making the experiment more open to human error.

**Thermal Stimulus Control**

An important limitation of these earlier behavioral studies was the method of stimulus control. In the electrophysiological literature, the delivery of a thermal stimulus was brief in duration, and the control of temperature was usually maintained by either a bath set to a certain temperature or by Peltier units that electrically produce a desired temperature. In a behavioral paradigm, this is not so easily obtained, as a stimulus must be maintained on the testing apparatus that also contains the animal being tested. This has been handled rather crudely in the past, as warm baths heat a substance (and cold baths cool a substance), but the transfer of such
energy ends as soon as the stimulus leaves the bath and is presented to the animal. To make matters worse, behavioral studies looking at temperature preferences have been previously measured under conditions where only one temperature was presented at a given time, and "preferences" were determined by a change in drinking over time (e.g., Gold and Prowse, 1974; Carlisle & Laudenslager, 1976). To determine clearer short-term temperature preferences, simultaneous access to more than one temperature may prove to be more effective.

To address these issues, we have developed a novel apparatus that allows precise control of fluid temperature. By using Peltier refrigerators attached to small CPU fans, fluids can be adjusted to a desired temperature to within 1°C. When the level of current is held constant, the temperature of a fluid is also held constant without continual manipulations to a warm or cold bath. This apparatus is therefore more analogous to that of an electrophysiological setup, so a higher level of stimulus control may be used in a behavioral paradigm. This will be discussed in more detail in a future section (see General Methods, Figure 1).

The goal of this research was to effectively measure any taste and temperature interactions in the rat. However, before a taste-temperature interaction could be behaviorally demonstrated, the effects of temperature alone on ingestive behavior (i.e., with minimal gustatory cues) needed to be tested. In other words, the response of a rat thermal input had to parallel responses found with taste input in an array of behavioral conditions. Therefore, the first part of this dissertation looked at the effects of temperature input on the following features typical of taste-guided behavior: preference behavior, avoidance behavior, discrimination, and generalization. Once these different responses were characterized when using thermal input, the second part of this dissertation examined whether taste and temperature cues influenced each other when both forms of orosensory input were initially present in one mixture. The relationship between these two forms of sensory input was assessed on the basis each cue’s relative saliency as well as an overall enhancement of a conditioned aversion when both cues were present. By conducting all of these experiments, this research attempted to show that thermal cues are not only effective as independent stimuli for ingestive behavior, but they influence (and thus interacted with) other forms of orosensory input (i.e., taste input) with more complex substances.
Preference for Thermal Stimuli

A great deal of evidence for preference in short-term intake comparisons has been collected with gustatory stimuli, in which rats show a greater intake for one tastant over another when both choices are simultaneously presented. It is assumed that with such a brief access period, a rat is responding to the orosensory properties of a food (presumably taste-guided) rather than any other forms of reinforcement (e.g., postingestional feedback). In such a test, the more preferred solution is consumed in higher quantities than the other solution. Although other methods of determining preference like licking analysis have been used to more precisely describe such preferences (e.g., Smith, Davis, & O’Keefe, 1992), intake comparisons provide an accurate understanding of what the rat prefers in a short-term period. To show the same behavior on the basis of temperature, preference for water at different temperatures was measured. In essence, these short-term preferences attempted to demonstrate similar preferences shown by others (e.g., Gold and Prowse, 1974; Carlisle & Laudenslager, 1976), but the present study provided access to two temperatures simultaneously.

Avoidance Behavior to a Thermal Cue (or Conditioned Temperature Aversion)

A well-studied phenomenon in regards to ingestive behavior has been conditioned taste aversion learning. When a rat is given access to a novel tastant (known as the conditioned stimulus, or CS) that is paired with an illness-inducing agent (the unconditioned stimulus, or US), the rat will subsequently avoid that tastant upon future encounters (for a review, see Riley & Tuck, 1985). It is assumed that an association forms between the taste cue and visceral effects from the illness. A similar avoidance response to a thermal cue was attempted, where a conditioned temperature aversion was acquired in rats paired with an illness-inducing agent (i.e., a LiCl injection). As mentioned previously, the work of Nachman (1970) has shown that rats avoid warm water when it is paired with a LiCl injection, but is unclear as to whether this aversion was innate or conditioned. Learned aversions are more demonstrable when the conditioned stimulus is initially highly preferred, such that conditioning dramatically reduces intake. Once the preferred range of temperatures was determined in the rat, then an appropriate temperature was chosen as a conditioned stimulus for this experiment. Then it was determined whether a novel (but generally preferred) thermal cue can be associated with visceral illness and be subsequently avoided as an expression of a conditioned temperature aversion.
Discrimination of Thermal Stimuli

Although responses to different water temperatures were demonstrated on the basis of preference, it was also important to determine how well a rat could discriminate between different thermal cues. Discrimination may be shown on the basis of preference, but such discrimination behavior is easier to observe by intake differences when the rat chooses between stimuli with a large difference in temperature. If the difference between these temperatures is decreased, then it is probable that a lack of preference between the two stimuli may not reflect the rat’s inability to discriminate between temperatures at this new range. It may be more effective to measure fine discrimination when one of the thermal cues is paired with illness-inducing effects (i.e., a LiCl injection). Thus, when given a two-bottle choice between the conditioned thermal cue and another thermal cue, rats would be more motivated to avoid the conditioned cue and consume the “safe” cue, thus demonstrating discrimination. A lack of avoidance under these conditions would more precisely define an inability to discriminate.

There is evidence that rats can discriminate between different concentrations of the same gustatory stimulus. Nowlis (1974) showed that the expression of an aversion to NaCl at different concentrations was dependent on the initial conditioning concentration. In other words, greater expression of a NaCl aversion was found when the concentration was closer to the original CS. Scott and Giza (1987) extended this idea even further by determining minimal ranges in concentration for an expressed taste aversion ($\pm 0.03$ M, for NaCl). The present study attempted to quantify the minimal discriminable range using thermal input in a conditioned aversion paradigm.

Generalization of a Thermal Aversion

It is also important to show that an aversion to a temperature cue can be generalized to other solutions having similar thermal properties. This form of generalization behavior can also be shown with a conditioned taste aversion paradigm. When a taste stimulus is paired with a LiCl injection, the rat demonstrates a generalized aversion to other mixtures containing the conditioned taste stimulus. A good example of this is shown by Smith and Theodore (1984), where rats were conditioned to avoid one of three tastants (sucrose, NaCl or HCl). After an aversion was conditioned, rats avoided all mixtures that contained the CS, but they did not avoid any of the solutions without the CS. Moreover, the expression of the conditioned aversion to
these mixtures was proportional to the concentration from the initial tastant paired with the illness-inducing agent.

In the present study, a similar type of generalization response was tested by pairing a thermal stimulus (having minimal thermal properties) with the effects of a LiCl injection. However, the generalization behavior was slightly different in regards to sensory input. Taste input can be classified on the basis of quantity (e.g., 0.025 M sucrose vs. 0.25 M sucrose) as well as by quality (e.g., sweet vs. salty, etc.). With temperature, however, differences can only be measured across quantity, or magnitude, of the thermal stimulus. Therefore, to measure generalization behavior using thermal input, the “generalized” mixtures require another form of sensory input. The most obvious choice for this is a mixture of thermal and taste stimuli. In this study, the ability to generalize was determined if an aversion to a presumably tasteless temperature cue generalized to tastants at the same temperature. If such a cue elicits this type of behavior, then rats should subsequently avoid any gustatory solution at the conditioned temperature.

Relative Saliency for Thermal and Gustatory Cues

The second part of this research focused on evidence that demonstrated an interaction between taste and temperature input. The first experiment to support this determined relative saliency for temperature and taste cues when both are presented in a mixture. If both thermal and gustatory cues are readily associated with LiCl-induced sickness, then a mixture containing both forms of input should be effective in the expression of a conditioned aversion to that mixture. However, it was unknown as to whether aversions to each sensory cues are comparable a function individually expressed aversions. Moreover, it was also unknown as to which cue, when simultaneously paired in a two-choice test, was avoided more in such a situation.

The process of conditioning an animal to avoid a solution having multiple components has been conducted by Smith et al. (2000). Rats were given access to a sucrose and corn oil emulsion and were then injected with LiCl to produce a conditioned aversion. After conditioning, a series of post-conditioning tests was conducted to determine the relative saliency of sucrose and corn oil components. The last of these tests showed that LiCl-injected rats displayed a more profound aversion to corn oil alone than to sucrose alone, suggesting that the corn oil component (possibly due to gustatory, olfactory, or textural properties) was the more salient cue from the mixture. In the present research, a similar post-conditioning procedure was
attempted, in which the conditioned stimulus possessing both thermal and gustatory features was employed. Aversions to these two types of sensory stimuli were compared in a series of short-term post-conditioning tests to determine a.) if rats avoided these orosensory components individually and b.) if one type of input was avoided more than the other when each cue was presented independently yet simultaneously.

Relative Extinction Times of Thermal and Gustatory Aversions

The last experiment of this present research examined the relative strengths of temperature aversions, taste aversions, and a combined taste and temperature aversion by measuring each aversion's relative time to extinguish. There is evidence that shows one form of orosensory cue can enhance the expression of an aversion to another sensory cue. This has been well studied with gustatory and olfactory cues in the rat. For example, Palmerino, Rusiniak, & Garcia (1979) showed that rats displayed a clear aversion to a weak odor when taste was also presented prior to a pairing with LiCl. This odor aversion was not shown when the odor was presented alone, which suggests that taste input actually influences the acquisition of a conditioned odor aversion. Similar to this finding, Batson and Batsell (2000) demonstrated that a taste aversion could also be enhanced in the presence of a preconditioned odor, suggesting that olfaction can also influence associations made with gustatory input. Instead of using olfactory cues, temperature was the second type of orosensory input used in the present study to determine whether a similar interaction occurred between taste and temperature.
GENERAL METHODS

**Subjects:** All subjects were naïve, male Sprague-Dawley rats that were maintained on a 12:12 light dark cycle, with the lights on at 0700 hours. Rats were housed in Plexiglas shoebox cages and were given ad libitum access to Purina standard rodent chow and distilled water. Ambient temperature of the room was recorded as approximately 25°C.

**Apparatus:** The apparatus that was used as the testing chamber for all of these experiments was a Plexiglas chamber with dimensions of 60 cm (length) by 60 cm (width) by 60 cm (height). The bottom of the chamber was a wire-meshed floor (2 cm² grids). To one side of the cage, there were two small openings where two fluid bottles could be placed onto the cage. In addition to passing through the Plexiglas cage, the sipper tubes of these bottles also passed snugly through aluminum blocks. Both blocks were attached to individual Peltier refrigerator units, which transferred thermal energy onto the aluminum blocks when electrical current was delivered to these units. Each Peltier unit, depending on the polarity and magnitude of the current, transferred one type of thermal energy (either warm or cold) to the attached aluminum block and the opposite type of thermal energy into a mounted CPU fan, which blew out the unwanted thermal energy. This system was controlled by a connection between the Peltier refrigerators and a central processor that determined the polarity and magnitude of the current. The relationship between current flow and the temperature of water was calibrated by using a temperature probe that was placed approximately 10 cm from the opening of each sipper tube. A schematic of this apparatus can be seen in Figure 1.

**Water Training:** Rats in each experiment were water deprived for 24 hours that started at approximately 0900 hours (i.e., 2 hours into the light cycle). After this initial deprivation period, all subjects received six days of water training. Each day of water training consisted of 10-minutes access to water in the testing apparatus, during which the temperature of the water was maintained at room temperature (25°C). During each session, water was made available in one of the two water positions. The position of
available water was alternated on each consecutive training day. After each daily training
session, rats were returned to their home cages, where food was always available. Rats
were then given a one-hour water supplement at approximately 1600 hours. After this
period of water training, rats were either used as subjects in a preference testing
experiment (Experiment 1) or in a conditioned aversion experiment (Experiments 2-6).

Preference Testing: Since Experiment 1 was the only experiment that entailed preference
testing, this procedure will be described later in Experiment 1.

Conditioned Aversion Testing: Prior to conditioning, rats were divided into groups based
on each animal’s mean water intake for the last two days of the training period. After
presentation of the appropriate CS, one group was given an injection of isotonic saline,
while the other group was given an injection of lithium chloride (LiCl). Lithium chloride
solution was obtained from Sigma Chemical Company and was prepared at 0.6 M, while
an isotonic saline solution was prepared at a concentration of 0.15 M. For each
experiment, rats were given 10 minutes access to a particular stimulus (CS), and each
animal was then given an intraperitoneal injection of either saline or LiCl five minutes
after the end of the intake period. The volume of this intraperitoneal injection was equal
to 0.5% of each animal’s bodyweight. This procedure was conducted in all conditioning
experiments except where noted otherwise.

The post-conditioning period of each experiment consisted of daily 10-minute,
two-choice intake tests between two fluid sources: the conditioned stimulus (CS) and
alternative substance from which intake comparisons were made. The positions of the
two sources on the cage were alternated on subsequent testing days. The specific
procedures will be described in detail for each experiment.
EXPERIMENT 1: SHORT-TERM PREFERENCE TO WATER AT DIFFERENT THERMAL CUES

Before any of the suggested experiments could be conducted, it was crucial to determine whether rats could show preference for water at different temperatures during short-term intake. There have been a couple of studies (Gold & Prowse, 1974; Carlisle and Laudenslager, 1976) that showed a rat's intake changed when the water temperature varied from room temperature in a single-bottle test. However, it is unknown as to whether rats can show a preference between two water sources at different temperatures in a two-bottle test. Thus, two-choice simultaneous access to different thermal cues was studied in this experiment by pairing very warm (40°C) water or very cold (10°C) water with room temperature (25°C) water and then by pairing these extremes with each other. Since the discrimination of thermal input in the oral cavity has not been conducted in the rat, the most conservative way to test for short-term preference was to use thermal cues that were relatively far apart from each other. By doing so, this experiment had two basic purposes. First, it was important to show that rats show differences in water intake in a two-bottle test when they are different in temperature. The second purpose of this experiment, although more subtle, was to determine a water temperature that was preferred in a two-bottle test. This was necessary to determine, for in subsequent experiments using a conditioned aversion paradigm, the thermal cue (CS) should be novel and preferred.

Methods

Subjects: Subjects were 20 naïve, male Sprague-Dawley rats that weighed between 300-400 grams.

Procedure: After the water training period, rats were given 10-minute access between room temperature (25°C) water and warm (40°C) water. After this preference test, each rat was returned to its home cage. This preference test was repeated for two subsequent sessions, during which the positions of the two temperature-controlled water sources were alternated on each day. This 3-day preference testing sequence was repeated for two more conditions: room temperature (25°C) water vs. cold (10°C) water; and cold (10°C) water vs. warm (40°C) water.
Results

For each preference sequence, a one-way ANOVA revealed that intakes did not vary across days of testing (ps > 0.05), so an ingestive score for each temperatures was calculated by averaging across the three days. A comparison between the mean ingestive scores for room temperature (25°C) water vs. warm 40°C water is seen in Figure 2a, where rats clearly prefer the room temperature water over the warm water. These mean intake scores were compared using a matched t-test for dependent samples, which showed that the rats had a significant preference for the water at room temperature over the warmer (40°C) water, t = -22.57, df = 12, p < 0.001.

When cold water at 10°C was compared with room temperature water, there was no clear preference for either temperature, which can be seen in Figure 2b. A matched t-test for dependent samples revealed no significant differences at 10°C vs. 25°C (p>0.05). In the last comparison between cold water (10°C) and warm water (40°C), rats showed a clear preference for the cold water over the warm water (see Figure 2c). A matched t-test for dependent samples, t=22.76, df=12, p < 0.001, revealed a significantly different preference for the cold water when warm water was the alternative (see Figure 2c). On the basis of these preference sequences, the data suggest that warm temperatures can be discriminated from temperatures at room temperature or colder.

Discussion

By comparing intake levels of water at different temperatures, preference for water at different temperatures has been shown. On the basis of these short-term intake tests, this experiment demonstrated a preference for room temperature water over warm temperature, but it failed to show such a preference for room temperature water when it was given simultaneously with cold water. It is not fully understood as to why there was no preference between room temperature water and cold water. One possibility may be that the rat’s ability to discriminate between these two temperatures is minimal at best. Although rats could clearly discriminate between 25° and 40°C water, it may have been due to the fact that 40°C water approaches the rat’s nociceptive threshold. Another possibility may be that rats can discriminate between 10°C and 25°C water, but rats simply do not show a preference at this thermal range. Although this question will be addressed in a subsequent experiment, it was shown that rats clearly preferred water at 10°C rather than at 40°C. With this finding, it is assumed that water at 10°C may be
used as a novel yet preferred thermal cue in the acquisition of a conditioned temperature aversion.
EXPERIMENT 2: EXPRESSION OF A CONDITIONED TEMPERATURE AVersion

The goal of Experiment 2 was to determine whether the rat could be conditioned to avoid a thermal cue that is normally preferred. Since Experiment 1 showed that rats preferred the cold water (at 10°C) over the warm water (40°C), the former was used as the conditioned stimulus (CS) in a conditioned aversion paradigm. By using a novel thermal cue that that was readily accepted by the rat in the earlier preference tests, a conditioned avoidance to such a cue could provide solid evidence that rats can acquire conditioned temperature aversions. Moreover, this experiment provides a replication of Nachman's work (1970), but the present experiment employed the use of a more effective stimulus (10°C) than the hot (45°C) stimulus used by Nachman to illustrate this point.

Methods

Subjects: Subjects were 16 naïve, male Sprague-Dawley rats that weighed between 300-400 grams.

Procedure: After the water training period, rats were divided into two groups (a saline-injected group and a LiCl-injected group, n=8 per group) with equivalent mean water intakes on the last two days of the training period. Rats then were then given 10-minutes access to water at 10°C (the conditioned stimulus, or CS) followed by an interperitoneal injection of either saline or LiCl.

The strength of the conditioned aversion was measured in three different ways. The first way measured the reduction of cold water intake between the conditioning day and the first day of post-conditioning. The second measure entailed preference comparisons between saline- and LiCl- injected rats on the first day of post-conditioning. Both total water intakes (from both water bottles) and preference scores for the CS on this first day of post-conditioning were compared to show that LiCl-injected rats suppressed only their CS intake rather than their total water intake. Finally, the rate of extinction was measured across 11 days of post-conditioning, where rats were given daily 10-minute preference tests between water at 10°C and water at 40°C.
Results

On the day of conditioning, the mean intakes for the cold water CS were 8.7 grams for the saline-injected controls and 9.1 grams for the LiCl-injected rats. A t-test for independent samples revealed that there was no significant difference in the CS intake of cold water between the saline- and LiCl-injected rats (p > 0.05). As seen in Figure 3, a comparison of the CS intake for both groups on the days of conditioning and the second day of conditioning, saline injected controls did not reduce their intake of the cold water. However, the LiCl-injected rats clearly avoided the cold on the first day of conditioning, where their mean intake of the cold water CS decreased from 9.1g to 0.4g across these days. To determine whether there was a reduction of intake after conditioning, a 2X2 factorial ANOVA for repeated measures was conducted on conditioning day and the the first day of post-conditioning (see Figure 3). There were significant main effects for both injection group, F (1,14) = 12.58, p < 0.01, as well as from the pre- (initial access to CS) to post-conditioning day, F (1,14) = 117.87, p < 0.0001. There was also a significant Group X Day interaction, F (1,14) = 118.55, p < 0.0001, from which a Tukey Honest Significant Difference Test was used. This post hoc test revealed that the saline-injected controls did not significantly reduce their intake of the CS between these two days. However, LiCl-injected rats significantly reduced their intake between these two days (p < 0.01). This clearly shows that LiCl-injected rats avoided the cold water after it was paired with a LiCl injection.

In order to show that this avoidance by LiCl-injected rats was specific to the cold water CS and not to both water sources, total intakes were compared, where it can be seen in Figure 4a that both groups drank comparable amounts of total water. A t-test for independent samples was conducted to compare the total water intakes, where no significant differences in total water intake were found between the two groups (p > 0.05). To determine each group's overall preference for the CS, preference scores were calculated by dividing the cold water intake by the total water intake. These preference scores (as seen in Figure 4b) reveal a strong preference for the cold water in saline-injected controls but a marked aversion (or extremely low) preference of the cold water in LiCl-injected rats. A second independent t-test revealed a significant difference in preference between saline- and LiCl-injected animals, t = 14.52, df=14, p < 0.0001. This would suggest that LiCl-injected rats reversed their preference for cold water on the basis of the aversive conditioning.
To determine if there were any differences in total intake across the post-conditioning period, total intakes were also compared by using a 2 X 11 factorial ANOVA (Group by Days) for repeated measures, in which there was neither a significant main effect nor a significant interaction (ps > 0.10). Similar to what was shown on the first day of post-conditioning, that the two injection groups demonstrated relatively similar total water intake across the entire post-conditioning period.

As seen in Figure 5, saline-injected rats showed a high preference for the cold water CS across all post-conditioning tests. However, LiCl-injected rats initially showed a clear aversion to the cold water CS that progressively diminished across the post-conditioning period. For the comparison between saline- and LiCl- injected rats during the extinction period, a 2 X 11 (Group by Days) factorial ANOVA for repeated measures was conducted on preferences scores for the cold water (see Figure 5). Significant main effects for both injection group and days were found, \( F (1,14) = 11.98, p < 0.01, \) and \( F (10,140) = 6.91, p < 0.01, \) respectively. A significant Group X Day interaction, \( F (10,140) = 4.11, p < 0.01, \) was also found. A Tukey honest significant difference (HSD) test revealed that LiCl-injected rats, when compared to saline-injected controls, demonstrated a significantly lower preference for the cold water for eight days of post-conditioning (ps < 0.05).

Discussion

These results show that a rat can avoid a thermal stimulus when paired with the effects of a LiCl-injection. Since the change in temperature of the water should not result in altering any gustatory or olfactory cues, it is assumed that the conditioned aversion was due to an association between a thermal stimulus and the illness-inducing effects of the LiCl. This experiment supports earlier work by Nachman (1970), who showed a similar aversion in rats conditioned to avoid warm water. LiCl-injected rats in Nachman's experiment did suppress intake of the warm water, but it is not clear as to whether this warm stimulus was partially aversive to these rats prior to conditioning. In the present study, LiCl-injected animals reduced intake of a more preferred, cold water CS, and actually consumed a warmer (and possibly innately aversive) stimulus. Moreover, these animals showed a significant aversion to the cold water for eight days of post-conditioning, which would suggest that this aversion to a thermal cue was relatively strong. This relative strength of such an aversion when compared to a taste aversion will be discussed in later experiments.
EXPERIMENT 3: DISCRIMINATION OF A THERMAL STIMULUS

The purpose of Experiment 3 was to determine the minimal range of thermal input that rats could discriminate. Since the previous experiment showed that rats could discriminate cold (10°C) water from warm (40°C) water when the former was paired with a LiCl injection, this experiment employed a similar procedure to assess a least discriminable range of temperature between a thermal CS and an alternative temperature.

Methods

Subjects: Subjects were 40 naïve, male Sprague-Dawley rats that weighed between 300-400 grams

Procedure: All animals followed the water training procedures similar to the previous experiments. Prior to conditioning, rats with equivalent mean baseline water intakes were assigned to one of four groups (two saline-injected control groups, two LiCl-injected groups, each n=10). Since the rats in this experiment were to be tested for several days after conditioning, effects from extinction were minimized by giving two CS-US pairings. On these two conditioning days, each rat was given 10 minutes access to 10°C water, followed by either a saline (controls) or a LiCl (experimentals) injection.

Post-conditioning testing consisted of 5 daily, 10-minute, two-bottle preference tests between the 10°C water CS and water at different temperature that varied across the post-conditioning testing sequence. One saline- and LiCl-injected group (n=10) were given one post-conditioning sequence, while the remaining saline- and LiCl-injected group (n=10) were given a different sequence of test (see Table 1). In the first sequence, the comparisons were made between 10°C water and water at 25°C, 22°C, 19°C, 16°C, and 13°C on each of the five days. For the second sequence, comparisons were made in the reverse order (i.e., between 10°C water and water at 13°C, 16°C, 19°C, 22°C, and 25°C on each of the five days).

Results

It can be seen in Figure 6 that saline-injected rats consumed similar amounts of the cold CS across the two conditioning trials, where LiCl-injected rats decreased their intake on the
second conditioning trial. A 2 X 2 (Group X Day) factorial ANOVA for repeated measures was used to compare intakes of the 10°C water consumed during the two conditioning pairings. This test revealed a significant main effect between injection groups, F (1,38) = 62.12, p < 0.001, as well as a significant main effect across days, F (1,38) = 67.60, p < 0.001. There was also a significant interaction, F (1,38) = 60.36, p < 0.001, where a Tukey HSD test was used for post hoc assessment. This test showed that LiCl-injected rats consumed significantly less of the 10°C water CS on the second conditioning day (p < 0.001).

Preference scores for all four groups (n=10) were compared across the post-conditioning period, where it can be seen in Figure (7a) that for each post-conditioning comparison, the two saline-injected groups showed a similar preference for the CS. In this comparison, the same can be said for the two LiCl-injected groups across the trials of post-conditioning. In order to compare any differences in the ascending and descending sequences of post-conditioning, a 2 X 2 X 5 (Group by Sequence by Day) factorial ANOVA for repeated measures was conducted on the preference scores for both the saline controls and LiCl-injected rats. While there was a significant main effect between injection groups, F (1,36) = 63.02, p < 0.001, there were no other significant main effects. There was, however, a significant interaction between the injection group and the post-conditioning trial, F(4,144) = 2.76, p < 0.03. A post hoc Tukey HSD test revealed that saline-injected controls (regardless of sequence) showed significantly higher preference scores for the cold CS on all post-conditioning tests except in the comparison between 10°C water and 13°C water.

Total water intake comparisons were also made, where it can be seen that LiCl-injected rats (regardless of the sequence) decreased their total water intake as the range of temperature between the CS and the alternative was decreased (Figure 7b). Similar to the analysis used to compare the preference scores, a separate 2 X 2 X 5 (Group by Sequence by Day) ANOVA for repeated measures was conducted on the total water intakes. This test showed significant main effects between injection groups, F(1,36) = 55.86, p< 0.0001, and across post-conditioning days F(4,144) = 16.12, p<0.001. There was also a significant Group X Day interaction, F(4,144) = 15.70, p<0.0001. Post hoc assessment using Tukey’s HSD revealed that saline-injected rats consumed significantly higher amounts of water than the LiCl-injected rats when the alternative water temperature was at both 16°C and 13°C (ps < 0.05). The LiCl-injected rats were unable to discriminate between the CS and the alternative as well when the difference in temperature was
smaller. As a consequence, these animals significantly decreased their total water intake on these last two post-conditioning tests. This would suggest that LiCl-injected rats generalized their aversion to the 10°C water to water when the alternative temperature was at 16°C and at 13°C.

In order to look more closely at the individual water intakes across the post-conditioning period, separate comparisons in intake for saline- and LiCl-injected animals were made. In the case of the saline-injected controls, the intake of the CS and the alternative were relatively similar, regardless of the post-conditioning day (see Figure 8a). A 2 X 5 (Temperature X Day) factorial ANOVA for repeated measures revealed no significant main effects and no significant interaction (ps > 0.05). However, in the case of LiCl-injected rats, there was a drastic change in intake of the alternative water temperature as the range between the alternative and the CS decreased (see Figure 8b). More specifically, as the two temperatures became closer to each other across the post-conditioning period, these animals maintained a low mean intake of the 10°C CS (never exceeding 2 ml) but drank progressively less of the alternative water source. A 2 X 5 (Temperature X Day) factorial ANOVA for repeated measures revealed significant main effects for both temperature, F (1,38) = 163.11, p < 0.001, and across days, F (4,152) = 17.27, p < 0.001. There was also a significant interaction, F (4,152) = 15.04, p < 0.001, from which a Tukey HSD test was used for further post-hoc assessment. From this test, it was shown that LiCl-injected rats did consume significantly higher levels of the alternative water temperature rather than the CS across all post-conditioning days (ps < 0.01). However, intakes of the alternative temperature were different across these trials, in which the following significant differences in intake were found (ps < 0.01): intake of 16°C water was significantly lower than intake of 25°C water and 22°C water; 13°C water intake was significantly lower than for all other alternative water intakes.

Discussion

The data suggest that rats were able to discriminate between different water temperatures in a 2-choice test. With a difference in temperature as low as 3°C, LiCl-injected rats did in fact show a significant difference in water intake between the CS and the alternative temperature. However, this preference for the alternative temperature diminished as the temperature difference between the two water sources was lessened. Moreover, the total water intake decreases when this thermal range is decreased across the post-conditioning period. This
discriminable range of temperature is broader than what has been previously shown in the literature regarding thermal discrimination on the rat's snout (Porter, Hecht, & Shaeffer, 1993), which was found to be less than 1°C. Nonetheless, it does show a rather narrow range of temperatures that can be discriminated in the oral cavity. This may be analogous to discrimination studies involving concentrations of different tastants (e.g., Scott & Giza, 1987), but such comparisons would have to be studied more thoroughly.
EXPERIMENT 4: GENERALIZATION OF AN AVERTION TO A THERMAL STIMULUS

The first three experiments have demonstrated temperature as an effective stimulus to the extent that a.) broad differences in temperature elicit preference in intake, b.) a thermal cue can be avoided when paired with a LiCl-injection, and c.) thermal cues can be discriminated by a rat within a rather narrow range of temperature. If an aversion to a thermal stimulus can be conditioned in the absence of gustatory input, then the next question to address is whether an aversion to a thermal stimulus can be generalized to a substance having taste input as well. Normally, the addition of a palatable substance (e.g., sucrose) to water increases an animal’s preference for the solution. However, whether such a preference would override an association between temperature and LiCl-induced illness is unknown. In Experiment 4, rats were conditioned to the 10°C cold water, but these animals were subsequently tested in a series of short-term post-conditioning tests that included the addition of taste cues to the original thermal CS.

Methods
Subjects: Subjects were 16 naïve, male Sprague-Dawley rats that weighed between 300-400 grams.
Procedure: All animals followed the same general training and conditioning procedures. Before conditioning, rats with equivalent mean water intake for the last two days of the training period were divided into two groups (one saline-injected group and one LiCl-injected group, n=8). Rats then were given 10-minutes access to water at 10°C, followed by either a saline or LiCl injection. Four daily, 10-minute post-conditioning tests were conducted, during which all rats received access to one substance having the thermal CS (water or a particular tastant) and an alternative of 40°C water. The substances paired with the conditioned thermal cue were as follows for each test day: water (Day 1), 0.125% saccharin (Day 2), 0.25 M sucrose (Day 3), and water once again (Day 4). These post-conditioning tests are summarized in Table 2.
Results

A t-test for independent samples showed that there were no statistical differences in intake of the 10°C water on conditioning day (p>0.05). In the first post-conditioning test, when 10°C water was paired with 40°C water, there was a clear difference in preference for the cold water between saline- and LiCl-injected rats. As seen in Figure 9a, saline-injected rats consumed mostly the cold water, where LiCl-injected rats showed a reversal of such a preference. A 2 X 2 (Group by Solution) factorial ANOVA was conducted to compared these intakes. From this test, only a significant interaction was found, F (1,10) = 27.52, p < 0.004. A Tukey Honest Significant Difference (HSD) test revealed that saline-injected rats consumed significantly higher amounts of the cold water when compared to LiCl-injected rats (p < 0.005). This finding was expected, as it showed that LiCl-injected rats clearly avoided the thermal cue that was paired with illness.

On the second day of post-conditioning, where 10°C saccharin was paired with 40°C water, LiCl-injected rats showed a profound avoidance of the cold saccharin when it was paired with the 40°C water (see Figure 9b). The saline-injected controls readily consumed the cold saccharin and avoided the warm water. The LiCl-injected rats, on the other hand, seemed to generalize their temperature aversion by consuming the warm water rather than the cold saccharin. A 2 X 2 factorial ANOVA revealed a significant interaction, F (1,10) = 144.90, p < 0.0001. Post hoc assessment using a Tukey HSD test showed that saline-injected rats consumed significantly more of the cold saccharin than LiCl-injected rats (p < 0.0002).

On the third day of post-conditioning, where 10°C sucrose was paired with 40°C water, it can be seen in Figure 9c that LiCl-injected rats show a relatively weak aversion to the cold sucrose. Although saline-injected controls consumed a greater amount of the cold sucrose when compared to LiCl-injected rats, the LiCl-injected animals consumed relatively equal amounts of the cold sucrose and warm water. Another 2 X 2 factorial ANOVA was conducted, whereby a significant main effect between injection groups was found, F (1,10) = 15.65, p < 0.03. There was also a main effect across solutions, F(1,10) = 12.33, p < 0.006, in which significantly more sucrose was consumed than water across all rats. However, a significant interaction was found, F (1,10) = 17.34, p < 0.002, where a Tukey HSD revealed that LiCl-injected rats consumed relatively similar amounts of the sucrose and water, but the cold sucrose intake of saline-injected
rats was significantly higher than either intake level demonstrated by LiCl-injected rats (ps < 0.04).

Finally, on the last post-conditioning test, a retest between cold and warm water, LiCl-injected rats still consumed more warm water than cold water (see Figure 9d), but their intake of the cold water on this last day was greater than their cold water intake on the first post-conditioning day (0.5 ml (Figure 9a) vs 3.3 ml (Figure 9d)). Although a 2 X 2 factorial ANOVA did not show a significant group main effect, there was a significant main effect for solution (or in this case, water temperature), $F(1,10) = 6.18, p < 0.03$. This shows that both groups are consuming the cold water to some extent. However, a significant interaction was also shown, $F(1,10) = 34.84, p < 0.002$, whereby post hoc assessment was conducted once again. A Tukey HSD test revealed that saline-injected rats consumed significantly more of the cold water than LiCl-injected rats ($p<0.005$). However, unlike the first day of post-conditioning, the LiCl-injected rats showed no significant difference in their intake of cold water and warm water.

**Discussion**

These data suggest that an aversive thermal cue, in some cases, may override (or generalize to) a palatable taste cue. This is most clearly seen in Figure 9b, where LiCl-injected rats avoided the saccharin when it was paired with a thermal cue. However, the same could not be said for sucrose (Figure 9c), where LiCl-injected rats consumed equal amounts of sucrose (paired with the aversive thermal cue) and water. This 50% preference for the CS may be considered to be a weak generalized aversion, and this may be so for a number of reasons. First, it is possible that the sucrose was more palatable than saccharin, whereby the gustatory input was not overridden by the presence of an aversive thermal cue. Second, the caloric difference between sucrose and saccharin may have affected differences in the generalized behavior. Because these rats are under a water-restricted regimen, the food intake (although not measured) was most likely lower than in ad libitum conditions. Since sucrose has a much greater caloric value when compared to saccharin, it is possible that the need for calories in sucrose (which is sought out with lower food intake) may have overridden the tendency to avoid a thermal cue. Finally, the most likely conclusion from this finding is that extinction may play a role in this weaker aversion. It is possible that such generalized aversions extinguish rather quickly, in which the order of the post-conditioning tests may have played a role in this finding. This may
have been the case, as the fourth post-conditioning test, a retest between warm and cold water (seen in Fig. 9d), showed a weakened aversion to the original CS as well.

This experiment has shown that, at least in some cases, a temperature aversion can generalize to taste mixtures. These results are similar to that found by Smith and Theodore (1984), who showed generalized aversions to different tastants when placed in mixtures with unconditioned tastants. In their experiment, it was shown that rats conditioned to avoid NaCl, HCl, or sucrose would also avoid any mixture containing the tastant that was paired with the illness-inducing agent. Moreover, the strength of the generalized aversion was a function of the respective CS concentration that was found in the mixture. Although the present study did not look at the relative intensity of the thermal CS, it does provide evidence that thermal cues are quite effective in generalization behavior.
EXPERIMENT 5: RELATIVE SALIENCY OF GUSTATORY AND TEMPERATURE CUES

Although Experiment 4 may demonstrate gustatory influences to a pre-established thermal CS, it does not describe the actual interaction between taste and temperature. It merely assessed whether gustatory input can override a thermal-specific aversion or vice versa. Ingestion of different substances in a more realistic setting usually entails exposure to foods with multimodal sensory stimuli. Previous work has shown that when a multimodal stimulus is used as a CS, such as a sucrose/corn oil emulsion, specific aversions to the individual components of the emulsion (i.e., sucrose and corn oil) can be shown in a series of post-conditioning tests (Smith et al., 2000). In this study, a more profound aversion to corn oil and little aversion to the sucrose was found after the rats were conditioned with a mixture of sucrose and corn oil, which suggests that the corn oil was more salient than the sucrose. Although there are many possibilities as to why the corn oil was the more salient substance from the original multimodal mixture (e.g., possible differences in gustatory, olfactory, or textural input between corn oil and sucrose components), this experiment provides an excellent paradigm in comparing the relative saliencies of components found in a mixture that was previously paired with a LiCl injection.

Similar to work by Smith et al. (2000), the present experiment employed the use of a multimodal CS (i.e., having both gustatory and thermal cues) in a conditioned aversion paradigm. However, this study attempted to look at two sensory modalities (i.e., taste and temperature) that could be easily separated into their individual sensory properties and assessed an animal’s tendency to avoid either or both sensory cues in subsequent post-conditioning tests. These post-conditioning tests consisted of 2-choice intake tests, in which each substance contained either, both, or none of the cues that were present in the CS. And like the work seen by Smith et al. (2000), the present study attempted to show that a relative association with each sensory component exists, but the present comparison was actually used to determine which sensory input is the more salient feature in an aversion to the original mixture.
Methods

Subjects: Subjects were 16 naïve, male Sprague-Dawley rats that weighed between 350-500 grams.

Procedure: All animals followed the same training procedure as the previous experiments. Before conditioning, rats were divided into two groups (one saline-injected group and one LiCl-injected group, n=8) with equivalent mean water intake for the last two days of the training period. Rats were then given 10-minute access to the 0.125% saccharin at 10°C, followed by either a saline or LiCl injection.

As seen in Table 3, five daily, 10-minute post-conditioning tests were administered to all animals, during which one or both of the substances, or choices, contained gustatory and/or thermal properties similar to the original CS. On the first day of post-conditioning, the multimodal CS (a 10°C saccharin mixture) was paired with warm (40°C) water. On the second day the saliency of the thermal component of the CS was tested in a comparison between 10°C water and 40°C water. One the third day of post-conditioning, the multimodal CS (i.e., cold saccharin) was paired with warm saccharin (i.e., the gustatory component of the CS). The fourth day of post-conditioning compared the relative saliency of the taste component of the CS, in which room temperature water was paired with room temperature (25°C) saccharin. Finally, in the last test, the gustatory component of the CS was compared to the thermal component of the CS in a test between 10°C water and 40°C saccharin.

Results

Before any comparisons were made on these post-conditioning tests, a t-test for independent samples showed that there were no significant differences in intake of the cold saccharin on conditioning day (p > 0.05).

For each post-conditioning test, a separate 2 X 2 factorial ANOVA was conducted on the mean intakes of the different solutions that were used. In the first post-conditioning test, where 10°C saccharin (the original CS) was paired with 40°C water, saline-injected controls prefer the cold saccharin. This was not surprising, as rats generally prefer saccharin over water in a short-term, 2-bottle test, and previous work from the present research also has shown that such animals prefer cold water over warm water (see Figure 10a). LiCl-injected rats, on the other hand, displayed a strong aversion to the cold saccharin, as they showed a reversal of preference compared to the saline-injected controls. A 2 X 2 factorial ANOVA did not show any significant
main effects (ps > 0.05), but there was a significant interaction, F (1,10) = 169.63, p < 0.0001. A post-hoc assessment was made, in which a Tukey HSD revealed that saline-injected rats consumed significantly more of the cold saccharin when compared to LiCl-injected rats (p < 0.01). This assessment also showed that LiCl-injected rats consumed significantly more of the warm water than that of the cold saccharin (p < 0.01).

In the second post-conditioning test, where 10°C water was paired with 40°C water, it can be seen in Figure 10b that an aversion to the thermal cue was apparent in LiCl-injected rats. While the saline-injected controls consumed mostly the cold water, the LiCl-injected rats avoided the thermal component of the CS found in the 10°C water. A 2 X 2 factorial ANOVA showed no main effects (ps > 0.05), but there was a significant interaction, F (1,10) = 166.44, p < 0.0001. Post-hoc assessment using a Tukey HSD test revealed that LiCl-injected rats consumed significantly lower amounts of the cold water when compared to their warm water intake as well as when compared to cold water intake of saline-injected animals. This test clearly shows that avoidance had been made on the basis of thermal input.

In the third day of post-conditioning, where 10°C saccharin was paired with 40°C saccharin, saline-injected controls preferred saccharin that was at 10°C rather than at 40°C (see Figure 10c). LiCl-injected rats, however, avoided both saccharin solutions, regardless of temperature. A 2 X 2 factorial ANOVA revealed a significant main effect between groups, F(1,10) = 130.92, p < 0.0001, as well as a main effect across solutions, F (1,10) = 51.83, p < 0.0001. There was also a significant interaction, F (1,10) = 60.09, p < 0.0001, after which a post hoc assessment was made. A Tukey HSD revealed that saline-injected animals consumed significantly more of the cold saccharin when compared to LiCl-injected rats. However, the LiCl-injected rats consumed virtually none of either saccharin solution. This would suggest that these animals are responding primarily to the taste properties during this test, making it difficult to assess any thermal effects on this avoidance behavior.

On the fourth day of post-conditioning, when room temperature saccharin was paired with room temperature water, it can be seen in Figure 10d that saline-injected controls showed a large preference for the saccharin over water when both were at room temperature. And a clear taste aversion was demonstrated by LiCl-injected rats, who virtually consumed none of the saccharin. A 2 X 2 factorial ANOVA revealed no main effects, but there was a significant interaction, F (1,10) = 67.73, p < 0.0001. A Tukey HSD test revealed that saline-injected
controls consumed significantly higher amounts of the saccharin when compared to LiCl-injected rats \( (p < 0.0006) \). A similar comparison also showed that LiCl-injected rats consumed virtually all water and no saccharin \( (p < 0.0002) \). This finding was expected, as it showed that these animals have a relatively strong aversion to the gustatory properties of the mixture (from conditioning day), similar to what was shown with the thermal aversion from the second day of post-conditioning.

Finally, the fifth day of post-conditioning was a test comparing the relative strengths of the thermal and taste aversions, in which the two choices were \( 10^\circ C \) water and \( 40^\circ C \) saccharin. When the two components were presented against each other in such a test, there were a couple of surprising findings, all of which can be seen in Figure 10e. First, saline-injected rats showed a clear preference for the cold water over the warm saccharin, which reversed the previous preference for saccharin when both substances were at room temperature. Second, LiCl-injected rats seemed to hold their aversion to both properties of the original mixture, as these animals drank very little of either substance. A 2 X 2 factorial ANOVA revealed a significant group main effect, \( F (1,10) = 12.10, p < 0.006 \), as well as across solutions, \( F (1,10) = 18.98, p < 0.001 \). The relatively high intake of warm saccharin and low intake of cold saccharin by saline-injected controls can best explain this, during which LiCl-injected rats avoided both of these substances. There was also a significant interaction, \( F (1,10) = 10.03, p < 0.01 \), from which a Tukey HSD test revealed that saline-injected rats consumed significantly higher amounts of the warm saccharin when compared to the LiCl-injected controls \( (p < 0.002) \). Although LiCl-injected rats consumed slightly more of the cold water than of the warm saccharin, there were no significant differences between these intakes \( (p > 0.05) \).

**Discussion**

In a series of preference tests between the temperatures of \( 10^\circ C \) and \( 40^\circ C \) and between distilled water and a particular concentration of saccharin, unconditioned rats (i.e., saline controls) preferred cold liquids over warm liquids, ignoring taste cues (seen in Figures 10a-c, 10e). Only when room temperature saccharin was paired with room temperature water did the unconditioned rats show a strong preference for saccharin over water (Figure 10d). The extent to which temperature has such an effect over taste is not well understood, but such a question awaits future research.
When the rats were strongly motivated by conditioning an aversion to the cold saccharin (i.e., the LiCl-injected rats), they appeared to show an aversion to both the thermal and the gustatory properties of the CS. It is clear that an aversion to cold saccharin was conditioned (Figure 10a), and such an aversion generalized to cold water (Figure 10b). However, when pairing warm saccharin with cold saccharin, the conditioned rats seemed to avoid both solutions. It is clear why they avoided the cold saccharin, as both sensory cues are found in this mixture. The thermal and gustatory components were also avoided, as the aversion to cold saccharin generalized to cold water (Figure 10b) and to room temperature saccharin (Figure 10d). When pairing warm saccharin with cold saccharin, the conditioned rats seemed to avoid both solutions. It is clear why they avoid the cold saccharin because it was the original CS that was followed by a LiCl injection. The aversion to the warm saccharin is not so clear, since unconditioned rats also avoided warm saccharin. When warm saccharin was paired with cold water, the conditioned rats again seemed to avoid both solutions. The assumption is made that they are responding to both thermal and gustatory cues. The aversion to the cold water is likely generalized from the original CS of cold saccharin, but again, the aversion to the warm saccharin is not so clear since the unconditioned rats also avoid the warm saccharin.

The present study has been relatively successful in separating the gustatory and primarily thermal components of the cold saccharin as well as demonstrating that both cues are readily associated with LiCl-induced sickness. However, this study was not successful in determining whether one sensory cue was more readily associated with the LiCl injection than the other sensory cue. It is possible that both cues together in a mixture may be a relatively stronger stimulus than its individual components, but intakes from a 10-minute post-conditioning test may not be an effective way to measure such an interaction in a conditioned aversion paradigm. The last experiment addressed a different approach to measure such an interaction when using a conditioned aversion paradigm.
EXPERIMENT 6: RELATIVE EXTINCTION TIMES OF THERMAL AND GUSTATORY AVERSIONS

The previous experiment addressed the importance of individual thermal and gustatory cues prior to conditioning an aversion to a multimodal CS. Although this experiment demonstrated that thermal cues may override gustatory cues without aversive conditioning, this experiment did not really show a similar interaction when both cues were paired with a LiCl injection. Furthermore, there was no way to assess whether the relative influence of both cues summates animal’s avoidance behavior to a multimodal stimulus. It has been demonstrated that both gustatory and thermal cues are sufficient cues in aversive conditioning, but it would be useful to understand the nature of a taste and temperature interaction at the behavioral level. One way to answer such a question was to compare the relative strength of aversions to a gustatory cue alone, a thermal cue alone, or the combination (i.e., by measuring the relative times of extinction for each aversion). By determining the time at which aversions to each stimulus extinguishes, the present experiment examined a.) aversions to which sensory cue (taste or temperature) extinguishes more quickly and b.) whether an aversion of a mixture having both sensory cues is enhanced.

Methods

Subjects: Subjects were 40 naïve, male Sprague-Dawley rats that weighed between 300-450 grams.

Procedure: After the water training period, rats were divided into four groups (a saline-injected control group and 3 LiCl-injected experimental groups, n=10 per group) with equivalent mean water intakes on the last two days of the training period. On conditioning day, each group was given a different conditioned stimulus that contained primarily thermal properties (distilled water at 10°C, or "T"), primarily gustatory properties (0.25 M sucrose at room temperature, or "G"), or both properties (0.25 M sucrose at 10°C, or "T+G") followed by a LiCl injection. A summary of these groups can be seen in Table 4. Although saccharin has been used almost exclusively in the previous experiments, sucrose was used as the taste stimulus in this case in
order to reduce the variability of intake between rats as well as across the days of post-conditioning. Although rats prefer both of these substances to water in a two-bottle test, there is a greater level of variability among rats for saccharin consumption than there is for sucrose consumption (Smith, 2000). Also, rats having a radiation-induced taste aversion to saccharin seemed to show large individual differences how long such an aversion lasted (Spector, Smith, & Hollander, 1981). Thus, sucrose seemed to be a more reliable stimulus for both the saline- and LiCl-injected rats.

The control group was given a 0.25 M sucrose solution at 10°C followed by a saline injection, while each LiCl-injected experimental group received a one of the three stimuli that were previously mentioned. Rather than having three different control groups (one for each CS), the choice of cold sucrose as the CS for only one control group was based on previous experiments. It has been shown by Smith and Wilson (1989) that preference for 0.25 sucrose does not change over a rat's life span, as it readily consumes higher amounts of sucrose over water over this time period. It has also been shown in the present study (Experiment 2) that saline-injected control rats clearly prefer cold water over warm water across many post-conditioning test days (refer to Figure 5). And since these are both "preferred" sensory cues over a warm water stimulus, there was only one control group that was given cold sucrose as its conditioned stimulus.

On each day of the post-conditioning period, each group was given 10-minutes access to its respective CS and warm water at 40°C. The post-conditioning period lasted a total of 20 days, where the positions of the two fluids were alternated on each subsequent test day.

Results

On conditioning day, intakes of the four groups were compared, where there all mean intakes were between 8-9 grams of fluid (refer to Figure 12). A one-way ANOVA revealed that there were no significant differences in the intake of the CS between any of the groups on conditioning day, \( F (3,36) = 0.75, p >0.50 \).

Similar to the intake comparison on conditioning day, all groups demonstrated relatively similar total intakes (from both two bottles) across the entire post-conditioning period, which can be seen in Figure 11. Regardless of which fluid the animal preferred, the overall amount of fluid for each group was relatively similar. These total intakes across the post-conditioning periods were compared by using a 4 X 20 factorial ANOVA (Group by Days) for repeated measures.
This analysis revealed no significant main effect between the groups, $F \ (3,36) = 0.73, \ p > 0.50$, but it did reveal a significant main effect across trials, $F \ (57,684) = 9.68, \ p < 0.01$. However, there was no significant interaction found in this comparison.

CS intakes were compared to determine the times of extinction for each group across all 20 days of the post-conditioning period. It can be seen in Figure 12 that while saline-injected controls showed a high intake of the cold sucrose (their CS) across the days of testing, each LiCl-injected group showed initially low intakes that gradually rose at different times across the post-conditioning period. LiCl-injected rats that were given the cold water as a CS (LiCL-T) increased its consumption most quickly, followed by LiCl-injected rats that were given room temperature sucrose (LiCl-G). Finally, it took the LiCL group that was given cold sucrose (LiCL-T+G) the longest time to increase its consumption. Since all groups consumed relatively similar amounts of fluid across the post-conditioning period, this would suggest that saline-injected controls exclusively drank the CS while all LiCl-injected groups consumed exclusively the alternative fluid (i.e., warm water). Moreover, each LiCl-injected group consumed more of the CS (and less of the warm water) after subsequent post-conditioning, albeit at different times.

A 4 X 20 factorial ANOVA (4 groups by 20 days) for repeated measures was conducted on the CS intakes across the post-conditioning period. From this analysis, there was a significant main effect between the groups, $F \ (3,36) = 18.65, \ p < 0.001$, and a main effect across post-conditioning days, $F \ (19,684) = 43.40, \ p < 0.001$. There was also a significant interaction, $F \ (19,684) = 4.67, \ p < 0.001$, from which a Tukey HSD was conducted. It was revealed from this test that on the first day of post-conditioning, all LiCl-injected groups consumed significantly less of its CS when compared to saline-injected controls ($p < 0.001$). It should be noted that on this first day of post-conditioning, there were no significant differences in CS intake between any of the LiCl-injected groups.

However, the relative times of when each group extinguished its aversion was different among the LiCl-injected groups. Rats were considered to have extinguished an aversion when their intake of their CS was no longer statistically different from the saline-injected controls. By this definition of extinction, rats that acquired an aversion to water at $10^\circ$C (i.e., the thermal cue) extinguished their aversion by the sixth day of post-conditioning ($p > 0.05$). Rats that were given sucrose at room temperature (i.e., the gustatory cue) that was paired with extinguished their aversion by the $10^{th}$ day of post-conditioning ($p > 0.05$). Finally, the LiCl group that
received cold sucrose as the CS did not extinguish its aversion until the 20th of post-conditioning (p < 0.05) when compared to saline-injected controls.

With relatively similar two-bottle intakes, the preference score comparison should be a direct function of the CS intake comparison (compare Figures 12 and 13). In can also been seen in Figure 13 that all LiCl-injected groups showed a profound aversion to their respective CS on the first post-conditioning day. While the control group displayed a high preference for the cold sucrose, each LiCl-injected group displayed preferences scores that barely exceeded 0. A one-way ANOVA between the four groups revealed a significant main effect across these groups, F (3,36) = 175.19, p < 0.0001. A Tukey HSD test revealed that while all three LiCl-injected groups showed a significantly lower preference for the respective CS when compared to the saline-injected controls (ps < 0.001), there were no significant differences in CS preference between the three LiCl-injected groups. Since all LiCl-injected groups showed a virtually no preference for their CS's but consumed relatively comparable amounts of fluid on this day (see Figure 11), then it can be noted that all three aversions began with almost total suppression of the CS on this first day of post-conditioning.

Similar to what was shown by the CS intake comparison, each LiCl-injected experimental group gradually increased its preference for the CS at different times along the post-conditioning period (also seen in Figure 13). In this comparison, a LiCl-injected group extinguished its aversion when its mean preference for the CS was no longer different from the saline-injected controls, who showed a reliably high preference for its CS. Rats that were given the 10°C water paired with LiCl extinguished their aversion most quickly, followed by the group that was given room temperature sucrose paired with a LiCl-injection. Finally, LiCl-injected rats that were given a CS with both cues (10°C sucrose) extinguished the slowest, where they still showed a relative indifference to the CS after 20 days of post-conditioning. A 4 X 20 factorial ANOVA (4 groups by 20 days) for repeated measures was conducted on preferences scores for the respective CS’s. Significant main effects for both group and days were found, F (3,36) = 23.75, p < 0.0001, and F (19,684) = 41.01, p < 0.0001, respectively. A significant drug X day interaction, F (57,684) = 5.88, p < 0.001, was also found, from which post hoc analysis using a Tukey honest significant difference (HSD) test was used.

As mentioned previously, rats were considered to have extinguished an aversion when their preference for their CS was no longer statistically different from the saline-injected
controls. By this definition of extinction, rats that acquired an aversion to water at 10°C (i.e., the thermal cue) extinguished their aversion by the seventh day of post-conditioning (p > 0.05), which is comparable to the temperature aversion found by LiCl-injected rats in Experiment 2 (see Figure 5). Rats that were given sucrose at room temperature (i.e., the gustatory cue) extinguished their aversion by the 14th day of post-conditioning. Finally, rats that received sucrose at 10°C (both thermal and taste cues) did not extinguish their aversion by the 20th post-conditioning day, by which the mean preference for the CS was still significantly different when compared to saline-injected controls (p<0.05).

Discussion

Each LiCl-injected group showed profound aversions to its respective CS on the first day of post-conditioning. Since there were no differences in overall intake (see Figure 11), the CS intakes and preference scores yielded very similar curves of extinction for each group over the post-conditioning period (see Figures 12 & 13). However, there were differences in the relative times of extinction between the LiCl-injected groups in the post-conditioning period. The data suggest that a thermal cue, although an effective stimulus in a conditioned aversion paradigm, does not produce as long-lasting an association with LiCl as a gustatory stimulus. However, the presence of both thermal and gustatory cues simultaneously produce an even longer time to extinguish. This extended period of extinction found in the last group provides evidence for an interaction of temperature and taste, resulting in summation of sensory cues in a conditioned aversion at the behavioral level. Based on such relative times to extinction, the following summarizes the effectiveness of each condition:

Thermal CS/LiCl < Gustatory CS/LiCl < Thermal + Gustatory CS/LiCl.

As far as the nature of such a relationship between taste and temperature, this point remains inconclusive. Most of the literature on the potentiation of conditioned aversions embodies the use of taste and smell as stimuli. In this literature, the addition of taste in the conditioning procedure enhances the aversive behavior to an odor that normally would not be avoided (e.g., Palmerino, Rusiniak, & Garcia, 1979). And in some cases, the presence of a preconditioned odor enhances the acquisition of a taste aversion (Batson & Batsell, 2000). In regards to taste and temperature, it is possible that a.) the presence of a taste cue may potentiate a thermal aversion, or b.) the presence of a thermal cue may potentiate a taste aversion. Future
studies using strong thermal cues with weak gustatory cues and vice versa may answer such a question, but much needs to be done to determine the quantitative values for such studies.
GENERAL DISCUSSION

The findings of this study provide a great deal of evidence to support an influential role of thermal input in short-term feeding behavior. It is important to first note that a testing apparatus has been developed to maintain the fluid temperature for such feeding experiments (see Figure 1). By controlling the amount of electrical current passing through each Peltier unit, thermal energy was transferred to a fluid for a resolution of 0.1°C. The development of such an apparatus was important for two reasons. First, this equipment allowed a more precise way to produce and maintain temperature of a fluid than those found in previous studies (e.g., Carlisle and Laudenslager, 1976). Second, this level of temperature control could be incorporated into two separate drinking sources, allowing two-choice comparisons to be demonstrated. Having an apparatus that can handle such a task, the present series of experiments showed that exclusively thermal input, like gustatory input, can elicit the following behaviors: general preference behavior, avoidance behavior, discrimination behavior, and generalization behavior.

The first of these behaviors, preferences for exclusively thermal cues, was shown between different temperatures of water. In a simultaneous short-term taste test between two broad concentrations of sucrose (e.g., 0.025 M vs. 0.25 M), rats will clearly consume more of the sucrose at the greater concentration. Using broad differences in temperature input, a similar finding was demonstrated. Rats clearly consumed more cold temperature water (10°C) when it was paired with warm water (40°C).

For the laboratory rat, cold water (10°C) is both novel and preferred, making it an ideal CS for conditioned aversion experiments. Using (10°C) water as a conditioned stimulus (CS) that was paired with an injection of LiCl, it was shown in a subsequent two-bottle test that rats clearly avoided the cold water and consumed a normally non-preferred warm water at 40°C. Furthermore, this aversion lasted for eight post-conditioning tests, which suggests that rats not only acquired a conditioned temperature aversion, but such an aversion is relatively strong. These findings support previous work by Nachman, (1970) who showed that LiCl-injected rats avoided hot water (43°C) when it was paired with an illness-inducing agent. However, the use
of hot water as a CS was probably not appropriate, for although hot water was novel to these rats, such a thermal stimulus is not normally preferred by unconditioned rats, especially in a two-choice test.

The conditioned temperature aversion model was then used in the present research to determine the minimal discriminable range where a rat showed avoidance behavior. When rats were given 10°C water followed by a LiCl injection, the rats avoided this cold solution in subsequent daily pairings with water at 25°C, 22°C, 19°C, 16°C, and 13°C subsequent two-bottle tests. In another group of rats these preference tests were run in reverse order and the results were the same, eliminating any role of extinction for these preference tests. During the tests between the 10°C and the 13°C or 16°C water, the total intake during these two preference tests diminished. This provided evidence that the rats were having more difficulty with those discriminations, drinking less of both the 16°C and 13°C waters.

Although the least discriminable range of temperature in the present study is not as narrow as the 1°C discriminable range on the rat’s snout reported by Porter, Hecht, & Sheaffer, (1993), it still provides solid evidence that a rat’s sensitivity for such a discrimination test is relatively high. Furthermore, it provides a finding analogous to taste discrimination studies that have employed a conditioned taste aversion procedure. Previous work by Nowlis (1974) and more recently by Scott and Giza (1987) showed that avoidance of a NaCl solution was a function of its proximity to the CS concentration. The present study suggests a similar effect: avoidance behavior of a thermal stimulus is a function of its proximity to the CS intensity (i.e., °C).

When rats were given cold water followed by a LiCl-injection, these rats not only acquired an aversion to the cold water, but they generalized this aversion to cold saccharin, a normally preferred solution. This conditioned aversion to cold water did not generalize as much to a cold sucrose solution. It is commonly known that rats consume more saccharin or sucrose than water in a two-bottle test, which was shown by saline-injected controls in this present finding. However, when these tastants are presented with a conditioned aversive thermal cue, such preferences are clearly changed. This would suggest that the establishment of a conditioned temperature aversion overrode any general taste preferences. This effect parallels research on generalized taste aversions that employed a post-conditioning procedure with mixtures containing the CS and other tastants. More specifically, Smith and Theodore (1984) showed rats that given a particular tastant (NaCl, sucrose, HCl) paired with LiCl would
subsequently avoid any mixture that contained the CS (but not mixtures without the CS). Thus, rats conditioned to avoid NaCl would avoid a mixture of sucrose and NaCl, but they would not avoid a mixture of sucrose and HCl. Although the present experiment was unable to have a thermal aversion generalize to another thermal concentration (which would be impossible), such an aversion did generalize to a mixture that had a different sensory cue. These data simply provided further evidence that thermal input is quite effective in short-term ingestive tests.

Having demonstrated all of these behaviors with exclusively thermal cues, the final goal of this research was to provide evidence that gustatory and thermal input can interact in short-term feeding behavior. Using a conditioned temperature aversion paradigm, a taste and temperature interaction was measured in two ways. The first way, as seen in Experiment 5, attempted to determine the relative saliency for thermal and gustatory cues when both were present in the CS. Depending on whether an animal was conditioned to avoid these cues or not, different relationships between the thermal and gustatory cues were shown.

In the case of unconditioned rats, cold temperature cues were preferred to warm temperature cues (Figures 10a-c,e), and a saccharin taste was preferred to water when temperatures were held constant between the fluids, (Figure 10d). However, when both gustatory and thermal properties were different between the solutions in a two-choice test, preferences in temperature seem to affect preferences in taste. This can be seen in two post-conditioning tests, the first of which paired warm saccharin with cold saccharin (see Figure 10c). In this test, saline-injected controls clearly prefer cold saccharin to warm saccharin. If room temperature were held constant, then these rats should have consumed relatively equal amounts of both saccharin mixtures. However, because the thermal cues were different, rats preferred the cold saccharin to the warm saccharin. Thus, the thermal cue influenced the taste preference. In the last test from this experiment, cold water was paired with warm saccharin, which pit a preferred thermal cue with the preferred gustatory cue. Similar to the last test, saline-injected rats consumed significantly more of the cold water than the warm saccharin (see Figure 10e). In this case, a preference for saccharin was not only changed, but it was completely reversed. This again provides evidence that in some cases, a thermal cue can override the effects from a gustatory cue.

When rats were conditioned to avoid a multimodal CS having the same gustatory and thermal cues, rats showed a profound aversion to the cold sucrose mixture (Figure 10a) as well
as to its thermal and gustatory components (Figures 10b and 10d, respectively). However, when these two sensory components were paired against each other in a two-choice test, it seems difficult to discern which cue is avoided more strongly. As seen in Figure 10c, when warm saccharin was paired with cold saccharin, intakes of both solutions were under 1 gram. A similar effect is seen in Figure 10e, where little was consumed between cold water (thermal component of CS) and warm saccharin (gustatory component of CS) in a two-choice test. In both of these tests, the thermal component of the CS is clearly avoided, but avoidance of warm saccharin (having the gustatory component of the CS) is difficult to interpret due to a strong avoidance by the control group as well. Thus, it would seem as though both gustatory and thermal cues display a strong relative saliency from a mixture that had been paired with illness, but there was no clear evidence to support that one cue was avoided more profoundly than the other.

The conditioning procedure from this study was modified from Smith et al. (2000), who examined relative strengths of a sucrose/corn oil emulsion as well as its components. When the emulsion was paired with a LiCl-injection, there was a profound aversion to the emulsion, but when the individual components were tested, a corn oil emulsion seemed to be stronger than a sucrose emulsion. Since corn oil has many salient properties, it was possible that the trigeminal (i.e., textural) properties influenced such an effect, but could not be determined. The present study was able to separate two sensory cues more cleanly from a multimodal CS, and the results showed that individual sensory aversions were just as profound as the aversion to the original CS (cold saccharin). This finding lends support that trigeminal (i.e., thermal) input from a food can affect the overall ingestive behavior, but more importantly, that thermal cues are relatively strong stimuli.

Since both thermal and gustatory cues were found to be relatively salient sensory components, the final experiment attempted to further demonstrate the relationship between taste and temperature in avoidance behavior. By conditioning rats to avoid either a thermal CS (10°C water), a gustatory CS (room temperature sucrose) or thermal/gustatory CS (10°C sucrose), the times of extinction demonstrated by each LiCl-injected group demonstrated the following: a.) a cold thermal aversion extinguished more quickly than a sucrose taste aversion and b.) an aversion to a multimodal CS (cold sucrose) was found to be more longer-lasting than individual temperature and taste aversions. These data provide even further evidence that both cues are
interacting and thus summating to form a stronger association between the ingested CS and illness-inducing US.

This last experiment seems to also support the notion of a taste and temperature interaction at the behavioral level, but the parameters of such an interaction are not too clear. For example, if a stronger aversion to a cold tastant is found compared to aversions to the individual sensory cues, is it due to a.) the thermal cue enhancing the taste aversion or b.) the taste cue enhancing the thermal aversion? A way to further understanding about this question is to adopt a procedure that measures potentiation of conditioned aversions. In such a study, such as by Palmerino, Rusiniak, & Garcia (1979), rats given a weak odor and a taste cue that was followed by LiCl injection displayed an aversion to the odor. This odor was not subsequently avoided the odor alone was followed by the illness-inducing agent. The same type of study may be conducted using temperature and taste as such cues in similar potentiation experiments. However, the nature of what is a “weak” temperature cue must be further determined.

Taken together, this dissertation demonstrates the importance of thermal input in feeding behavior. Temperature can be an effective stimulus independently, but it can also influence feeding behavior when taste cues are present. The present series of experiments lead to the conclusion that a taste and temperature interaction exists at the behavioral level. Although there has been a great deal of electrophysiological evidence to also support an interaction between temperature and taste, a great deal of work must be learned behaviorally to find similarities with the electrophysiology. For example, taste responses from the periphery increase depending on the temperature of the tastant (Contreras and Lundy, 2000), and responses from the peripheral trigeminal system increase when mild tastants are applied (Lundy and Contreras, 1994). In an attempt to show such an effect behaviorally, responses on the basis of stimulus detection must be accurately quantified. Behavioral measurements like pattern analyses of a rat’s licking behavior may show discrete differences between tastants that are maintained at different temperatures. Such work may be investigated in the future. Regardless of a lacking behavioral and electrophysiological relationship, the present study determined that short-term feeding is affected by more that gustatory (and possibly olfactory) cues. These experiments provide further evidence that thermal input can have a profound effect on such ingestive behavior as well.
APPENDIX A: TABLES
Table 1: Post-Conditioning Discrimination Tests in Experiment 3

<table>
<thead>
<tr>
<th>Test</th>
<th>Sequence 1</th>
<th>Sequence 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10°C vs 25°C</td>
<td>10°C vs 13°C</td>
</tr>
<tr>
<td>2</td>
<td>10°C vs 22°C</td>
<td>10°C vs 16°C</td>
</tr>
<tr>
<td>3</td>
<td>10°C vs 19°C</td>
<td>10°C vs 19°C</td>
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<tr>
<td>4</td>
<td>10°C vs 16°C</td>
<td>10°C vs 22°C</td>
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<td>5</td>
<td>10°C vs 13°C</td>
<td>10°C vs 25°C</td>
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Table 2: Post-Conditioning Generalization Tests in Experiment 4

<table>
<thead>
<tr>
<th>Test</th>
<th>Conditioned Stimulus (CS+, at 10°C)</th>
<th>Alternative Stimulus (CS-, at 40°C)</th>
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<tbody>
<tr>
<td>1</td>
<td>Distilled Water</td>
<td>Distilled Water</td>
</tr>
<tr>
<td>2</td>
<td>0.125% Saccharin</td>
<td>Distilled Water</td>
</tr>
<tr>
<td>3</td>
<td>0.25 M Sucrose</td>
<td>Distilled Water</td>
</tr>
<tr>
<td>4</td>
<td>Distilled Water (retest)</td>
<td>Distilled Water</td>
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Table 3: Post-Conditioning Saliency Tests in Experiment 5

<table>
<thead>
<tr>
<th>Test</th>
<th>Choice 1</th>
<th>Choice 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T+G+</td>
<td>T-G-</td>
</tr>
<tr>
<td>2</td>
<td>T+G-</td>
<td>T-G-</td>
</tr>
<tr>
<td>3</td>
<td>T+G+</td>
<td>T-G+</td>
</tr>
<tr>
<td>4</td>
<td>T-*G+</td>
<td>T-*G-</td>
</tr>
<tr>
<td>5</td>
<td>T+G-</td>
<td>T-G+</td>
</tr>
</tbody>
</table>

T+ (Thermal) -- Cold Temperature Cue at 10°C  
T- (Thermal) -- Warm Temperature Cue at 40°C  
G+ (Gustatory) -- 0.125% Saccharin  
G- (Gustatory) -- Distilled Water
Table 4: Post-Conditioning Groups in Experiment 6

<table>
<thead>
<tr>
<th>Group</th>
<th>US</th>
<th>CS Stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>T + G</td>
</tr>
<tr>
<td>2</td>
<td>LiCl</td>
<td>T</td>
</tr>
<tr>
<td>3</td>
<td>LiCl</td>
<td>G</td>
</tr>
<tr>
<td>4</td>
<td>LiCl</td>
<td>T + G</td>
</tr>
</tbody>
</table>

T (Thermal) -- Cold Distilled Water at 10°C  
G (Gustatory) -- 0.25 M Sucrose at Room Temperature (25°C)  
T+ G (Thermal + Gustatory Cues) -- Cold 0.25 M Sucrose at 10°C
APPENDIX B: FIGURES
**Fig. 1:** Schematic of the temperature apparatus. Electrical current was sent from the controller to Peltier refrigerators, which converted electrical energy to thermal energy. Aluminum blocks received this thermal energy and consequently transferred it to the aluminum blocks, the sipper tubes, and the fluid in the sipper tubes.
Fig. 2a: Mean (± SE) intake comparison of room temperature (25°C) water and warm (40°C) water.
Fig. 2b: Mean (± SE) intake comparison of cold (10°C) water and room temperature (25°C) water.
Fig. 2c: Mean (± SE) intake comparison of cold (10°C) water and warm (40°C) water
Fig. 3: Mean (± SE) intake comparison of the cold water CS on the pre-conditioning (CS prior to injection, clear bars) day and the first day of post-conditioning (colored bars) between saline- and LiCl-injected rats.
**Fig. 4a:** Mean (± SE) total water intake comparison between saline-injected controls and LiCl-injected rats on the first day of post-conditioning. Total intakes were the sum of water intake from both water sources.
**Fig. 4b:** Mean (± SE) preference scores for cold water between saline-injected controls and LiCl-injected rats on the first day of post-conditioning. Preference scores for the cold water were calculated by dividing the intake of the cold water by the total water intake.
Fig. 5: Mean (± SE) preference scores for cold water between saline-injected (clear diamonds) and LiCl-injected (colored squares) rats across the post-conditioning period. Preference scores for the cold water were calculated by dividing the intake of the cold water by the total water intake.
**Fig. 6:** Mean (± SE) intake comparison of the cold water CS on the two days of conditioning between saline- (clear bars) and LiCl-injected (colored bars) rats.
Fig. 7a. Mean (± SE) preference scores for cold water CS between saline-injected and LiCl-injected rats across the post-conditioning period. Based on differences in temperature between the CS and the alternative temperature, saline-injected controls either received the post-conditioning tests in a descending (Sal-1, clear bars) or ascending sequence (Sal-2, hatched bars). Likewise, LiCl-injected rats also received the tests in an descending (LiCl-1, gray bars) or ascending sequence (LiCl-2) black bars. Preference scores for the cold water were calculated by dividing the intake of the cold water by the total water intake.
Fig. 7b. Mean (± SE) total water intakes between saline-injected and LiCl-injected rats across the post-conditioning period. Based on differences in temperature between the CS and the alternative temperature, saline-injected controls either received the post-conditioning tests in an descending (Sal-1, clear bars) or ascending sequence (Sal-2, hatched bars). Likewise LiCl-injected rats also received the tests in an descending (LiCl-1, gray bars) or descending sequence (LiCl-2, black bars).
Fig. 8a: Mean (± SE) water intake comparison between the 10°C cold water CS (clear bars) and the alternative water temperature (black bars) for saline-injected rats across the post-conditioning period.
Fig. 8b: Mean (± SE) water intake comparison between the 10°C cold water CS (clear bars) and the alternative water temperature (black bars) for LiCl-injected rats across the post-conditioning period.
Fig. 9a: Mean (± SE) intake comparison of warm water (clear bars) and cold water (black bars) between saline-injected controls and LiCl-injected rats.
Fig. 9b: Mean (± SE) intake comparison of warm water (clear bars) and cold saccharin (black bars) between saline-injected controls and LiCl-injected rats.
Fig. 9c: Mean (± SE) intake comparison of warm water (clear bars) and cold sucrose (black bars) between saline-injected controls and LiCl-injected rats.
Fig. 9d: Mean (± SE) intake comparison of warm water (clear bars) and cold water (black bars) as a retest between saline-injected controls and LiCl-injected rats.
Fig. 10a: Mean (± SE) intake comparison of warm water (clear bars) and cold saccharin (black bars) between saline-injected controls and LiCl-injected rats.
Fig. 10b: Mean (± SE) intake comparison of warm water (clear bars) and cold water (black bars) between saline-injected controls and LiCl-injected rats.
Fig. 10c: Mean (± SE) intake comparison of warm saccharin (clear bars) and cold saccharin (black bars) between saline-injected controls and LiCl-injected rats.
Fig. 10d: Mean (± SE) intake comparison of room temperature (25°C) water (clear bars) and room temperature saccharin (black bars) as a retest between saline-injected controls and LiCl-injected rats.
**Fig. 10e:** Mean (± SE) intake comparison of warm saccharin (clear bars) and cold water (black bars) between saline-injected controls and LiCl-injected rats.
Fig. 11: Mean ($\pm$ SE) total intake comparison for all groups across the post-conditioning period. Saline-injected controls (open circles, solid line) received cold sucrose as a CS, while LiCl-injected rats either received one of the following CS's: an exclusive thermal stimulus of 10°C water (LiCl-T, open triangles with dashed line; an exclusive gustatory/taste stimulus of room temperature sucrose (LiCl-G, closed triangles, dashed line); and a temperature and thermal stimulus of 10°C sucrose (LiCL-T+G, closed triangles, solid line).
**Fig. 12:** Mean (± SE) cold water CS intake comparison for all groups from the day of conditioning (Pre) through the entire post-conditioning period. Saline-injected controls (open circles, solid line) received cold sucrose as a CS, while LiCl-injected rats either received one of the following CS's: an exclusive thermal stimulus of 10°C water (LiCl-T, open triangles with dashed line); an exclusive gustatory/taste stimulus of room temperature sucrose (LiCl-G, closed triangles, dashed line); and a temperature and thermal stimulus of 10°C sucrose (LiCL-T+G, closed triangles, solid line).
Fig. 13: Mean (± SE) cold water CS preference score comparison for all groups across the post-conditioning period. Saline-injected controls (open circles, solid line) received cold sucrose as a CS, while LiCl-injected rats either received one of the following CS's: an exclusive thermal stimulus of 10°C water (LiCl-T, open triangles with dashed line; an exclusive gustatory/taste stimulus of room temperature sucrose (LiCl-G, closed triangles, dashed line); and a temperature and thermal stimulus of 10°C sucrose (LiCl-T+G, closed triangles, solid line).
REFERENCES


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Smith PL, & Smith, JC. (2003). Preference and avoidance behaviors to solid foods in the rat based on orosensory stimulation of both taste and texture, manuscript under revision.


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