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Conditioned Taste and Flavour Preferences and Palatability Shifts in Rats.

Catherine A. Forestell

Submitted in partial fulfillment of the requirements

for the degree of Doctorate of Philosophy

at

Dalhousie University

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FACULTY OF GRADUATE STUDIES

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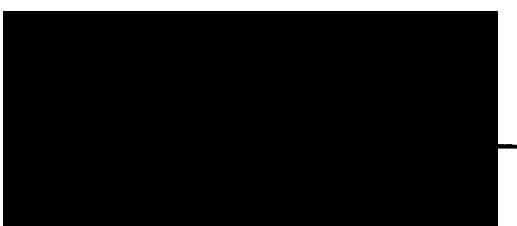

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Abstract

Animals acquire preferences for foods associated with nutrients or sweet tastes. The present set of ten experiments sought to determine first, whether rats would acquire conditioned preferences for tastes that are normally avoided (a bitter and a sour aqueous solution) and second, whether palatability shifts occur to these tastes and to other stimuli containing taste and odour components (i.e., flavours; grape and cherry Kool Aid) as a function of preference conditioning. In the first set of experiments, water and food restricted rats were conditioned with a differential reverse-order procedure. When rats were food and water restricted during conditioning and test, significant preferences were conditioned only when saccharin was added to each of the taste cues, thereby making them more acceptable. However, when rats were conditioned and tested while water but not food restricted, stronger preferences were expressed if rats received the taste cues without saccharin in test. In the second set of experiments, taste reactivity responses, which are considered to be a reliable measure of palatability, were also assessed to determine whether preference conditioning produces shifts in palatability. When rats were conditioned with the reverse-order procedure, preferences and palatability shifts were observed when the taste solutions were voluntarily consumed, but not when they were involuntarily consumed. When rats were conditioned with flavours, however, preferences and palatability shifts occurred when rats received involuntary oral infusions of the CS flavours, indicating that flavour preferences are more readily conditioned than taste preferences. Finally, in the last experiment rats were conditioned with a long-exposure simultaneous conditioning procedure in which they received differential taste or caloric reinforcement. This experiment indicated that although rats acquired conditioned flavour preferences regardless of the reinforcer used in training, only those that received differential calorific reinforcement demonstrated palatability shifts in test.

Abbreviations and Symbols used in Text

General

ad lib	*ad libitum;* freely available food
ANOVA	Analysis of Variance
B	Backward
CA	citric acid
CA^+	citric acid reinforced
CS	conditioned stimulus
CS^-	conditioned stimulus not reinforced
CS^+	conditioned stimulus reinforced
cm	centimetre
df	degrees of freedom
F	Forward
FAP	Fixed Action Pattern
G	gauge
I	Immediate
IG	intragastric
in	inch
ip	intraperitoneal
ISI	interstimulus interval
L:D	light:dark cycle
LiCl	lithium chloride
min	minute

µl	Microliter
ml	Milliliter
NR	Nonrestricted
NS	No Saccharin
R	Restricted
S	Saccharin
s	second
SEM	standard error of the mean
SOA	sucrose octaacetate
SOA^{+}	sucrose octaacetate reinforced
TR	Taste Reactivity
US	unconditioned stimulus
v/v	volume per volume
w/v	weight per volume

Taste Reactivity Responses:

Positive Responses

lf	lip flare
ltp	lateral tongue protrusion
mm	mouth movement
pl	paw lick
tp	tongue protrusion

Negative Responses

cr	chin rub
fw	face wash
g	gape
hs	headshake
pf	paw flick
pt	paw tread

Neutral Responses (albeit slightly aversive)

pd	passive drip
r	rear

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Chapter 1: Conditioned Taste Preferences in Rats[1]

In addition to their innate preferences and aversions for particular tastes, odours and flavours[2], animals rely on previous experience when deciding whether to accept or reject a potential food item. As an omnivore, the rat must choose from a rich array of foods, all of which vary in nutritional value, palatability, and potential toxicity. Given that rats are unable to vomit, it is to their benefit to exercise caution when foraging, by limiting consumption of novel foods (neophobia), learning about the consequences produced by these novel foods and finally, adjusting their consumption of these foods accordingly upon subsequent exposure.

It is well documented that learning about these foods can occur in a variety of ways. For example, through mere exposure rats learn that a stimulus is safe. As a result, preference for that food is increased over repeated exposures. It has been suggested that this increased familiarity mediates enhanced preferences for substance like coffee and chilli peppers (Zellner, 1991). However, this effect has been shown to be small relative to other types of associative learning (Fanselow & Birk, 1982, Mehiel, 1991). Foods can also become more preferred as a function of social conditioning (Rozin, 1988). That is, liking for certain foods may be enhanced if they are consumed in pleasant social situations or surroundings, or if social reinforcement is contingent upon the consumption of these foods (Galef, 1996). Of particular interest with respect to the present set of experiments is the rat's ability to acquire associations between various aspects of a food (such as its taste and/or odour) and post-ingestive consequences, such as nutritional reinforcement (see Sclafani, 1991 for a review).

Early research in the area of flavour conditioning concentrated mainly on the acquisition of flavour aversions. Animals were presented with a neutral flavour stimulus, followed by an illness-producing agent such as irradiation (e.g., Garcia, Kimeldorf & Koelling, 1955), or an injection of the emetic LiCl (Revusky & Gorry, 1973). As a function of the association formed between the flavour cue (CS) and the unpleasant gastrointestinal consequence (US), the animals avoided consumption of the flavour cue upon its subsequent presentation. Throughout the 1970s, an extensive literature arose which demonstrated this effect in many species and with a wide array of neutral flavours as the CS (see Riley & Tuck, 1985 for a bibliography of this literature).

It was only after the flavour aversion literature was well established that researchers began to concentrate on flavour preference conditioning. Initially, demonstration of this phenomenon was limited to paradigms in which a neutral flavour was paired with recovery from aversive situations such as illness or a specific nutritional deficiency (Zahorik, Maier & Pies, 1974, Booth & Simson, 1971). Later researchers demonstrated that pairing a flavour with palatable and/or calorific reinforcers increases both the acceptance (absolute consumption) (Drucker, Ackroff, & Sclafani, 1994) and the preference for a flavour relative to another concurrently presented solution that had not been reinforced (Holman, 1975, Mehiel & Bolles, 1984; Sclafani & Nissenbaum, 1988).

Most of the subsequent research in this area has employed sweet-tasting and/or calorific reinforcers to demonstrate flavour preference conditioning. Typically training paradigms involve a differential conditioning procedure in which animals receive one flavour (CS^+) paired with a highly calorific or palatable reinforcer and another flavour

(CS^-) paired with a nonnutritive, neutral-tasting source, such as water. After several trials of each kind, the animals' preference for the CS^+ relative to the CS^- is assessed with a 2-bottle preference test in which one bottle contains the CS^+ and the other contains the CS^-. During the test phase, animals consume more of the CS^+ than the CS^-, indicating that they have acquired a conditioned preference for the CS^+ relative to the CS^-. Depending on the type of reinforcer employed, conditioned flavour preferences can occur as a function of flavour-taste associations, which occur when a highly palatable reinforcer is employed, and/or flavour-calorie associations, which are a function of calorific reinforcement.

Flavour-Taste Associations

Typically flavour-taste associations occur only when the palatable reinforcer is mixed with or occurs immediately after presentation of the conditioned stimulus (CS). In one of the first demonstrations of conditioned flavour preferences, Holman trained rats to prefer either neutral banana extract or almond extract by pairing one flavour (CS^+) with concentrated saccharin (a palatable, but noncaloric US) and the other flavour (CS^-) with a dilute saccharin solution in a counterbalanced fashion. For groups in which the flavours were presented either simultaneously with, or immediately prior to the saccharin solutions, significant preferences were conditioned to the CS^+ flavour. However, if the CS^+ flavour was presented 30 min prior to saccharin, conditioned preferences did not occur. In a subsequent experiment, in which dextrose (a calorific and highly palatable reinforcer) tainted with quinine was the reinforcer instead of saccharin, conditioned preferences were formed to the dextrose-paired flavour when a 30-min ISI occurred between the presentation of the flavour cue and the reinforcer. Since the dextrose-quinine reinforcer

was calorific but not especially palatable, the ensuing preference was likely a function of a flavour-calorie association, rather than a flavour-taste association. These results suggest that the temporal relationship between the cue flavour and the reinforcer may interact with the type of reinforcer (be it calorific and/or highly palatable) in determining whether flavour-taste and/or flavour-calorie associations are conditioned.

Fanselow and Birk (1982) extended Holman's results by demonstrating that upward and downward shifts in preferences could be conditioned exclusively by flavour-taste associations. Experimental rats received almond and vanilla mixed with quinine and saccharin, respectively on different days, while control rats received unpaired presentations of almond, vanilla, quinine and saccharin in amounts similar to controls. When almond and vanilla were presented simultaneously with water in two-bottle tests, experimental rats displayed a preference for the saccharin-paired flavour and an aversion to the quinine-paired flavour relative to controls. Thus, flavour-taste conditioning is symmetrical and hence can play a role in determining flavour aversions as well as preferences.

Flavour-taste associations can also be formed between two hedonically neutral flavours. To demonstrate this, Lyn and Capaldi (1994) employed two conditioned stimuli; 0.15% saccharin (CS1) and 1.25% decaffeinated instant coffee (CS2), in a sensory preconditioning procedure (see also Rescorla & Freberg, 1978). In the first phase of training, three groups of animals received presentations of the CS1 and the CS2 separated by an interval of 0, 9, or 27 s. On each training day, animals received three trials separated by 1-min intertrial intervals. A control group received equivalent, but unpaired exposure

to the two CSs. In the second phase of training all groups were given 5 ml of CS2 followed 5 min later by 5 ml of a sucrose solution for 10 days. Finally, all groups were given 10 min, 2-bottle choice tests between the CS1 and water.

In the test phase all the groups, except for the control group, displayed a significant preference for CS1. Moreover, the preferences in the 0 and 9 s groups were significantly larger than those of the control group, whereas the preference for saccharin in the 27 s group was only marginally larger than the preference displayed in the control group. Therefore, as a function of the initial association between the two neutral stimuli, the associative strength acquired by CS2 in phase 2 transferred to CS1. In this experiment, flavour-taste associations between hedonically neutral substances occurred when there was an interval of 9 s but not 27 s between CS presentations. This is consistent with Holman's failure to get significant flavour-taste associations with delayed presentation of the reinforcer. It is possible, of course, that the CS1-CS2 interval in phase 1 may have interacted with the length of the CS-US interval or the strength of the US in phase 2 in determining the strength of the preference for CS1 in the test phase.

Overall, these studies suggest that nonnutritive flavours can become associated, regardless of their hedonic value, when a simultaneous differential reinforcement procedure is employed with animals. In situations in which a highly palatable or aversive flavour is mixed with a neutral flavour, preference for the neutral flavour will be shifted in a positive or negative direction, respectively. As indicated by sensory preconditioning studies, associative links can also be formed between neutral flavours.

Flavour-Calorie Associations

Although flavour-taste associations may not be as robust as flavour-calorie associations, they are a potential confound when a simultaneous conditioning procedure is used to study flavour-calorie associations. Thus, one of the main challenges involved in studying the effects of positive post-ingestive reinforcement is conditioning flavour-calorie associations while controlling for the formation of flavour-taste associations. In earlier work, this was accomplished by gastrically intubating the animals with the US (Sherman, Hickis, Rice & Garcia, 1983). However, there was some concern that the animals could detect the flavour of the US on the outside of the tube as it passed over the tongue during the intubation procedure on each training day.

More recently, Sclafani and his colleagues (e.g., Sclafani & Nissenbaum, 1988; Elizalde & Sclafani, 1988; Ackroff & Sclafani, 1994) have developed an intragastric (IG) infusion procedure, which convincingly demonstrates robust flavour-calorie associations independently of flavour-taste associations. In these studies, rats are fitted with two chronic IG catheters connected through a swivel device to two infusion pumps, which are controlled by computer-monitored drinkometers. These drinkometers allow the animals to control their infusions so that for each 1 ml of fluid consumed orally, 1 ml of fluid is infused. Throughout the training phase, two bottles containing different flavours are placed on the front of each animal's cage. Consumption of one of these flavours (CS^+) is paired with a highly caloric infusion such as 32% Polycose. The other flavour (CS^-) is paired with a water infusion. Animals receive *ad libitum* food and are trained 23h/day. Normally, when animals' preferences are assessed by a 2-bottle choice test consisting of

simultaneous exposure to both cue flavours, each of which is reinforced with the appropriate infusion, they show a robust preference for the CS^+. Moreover, this preference does not readily extinguish when both of the cue flavours are presented in a series of nonreinforced 2-bottle tests, (Drucker, Ackroff & Sclafani, 1994). Thus, preferences displayed in this 2-bottle test are a result of flavour-calorie learning.

Although generally less effective at conditioning strong preferences, experiments in which rats orally consume both the taste cue and the reinforcer have the advantage that they are technically less difficult and less expensive to conduct relative to the gastric reinforcement procedure. One disadvantage is that flavour-taste associations are often confounded with flavour-calorie associations (cf. Bolles, Hayward and Crandall, 1981), mainly because the caloric content of a solution is difficult to manipulate without altering its hedonic properties. Consider first the simultaneous conditioning paradigm. In this case, the animal receives one flavour cue (CS^+) mixed with a caloric reinforcer and another flavour cue (CS^-) mixed with saccharin. Rats acquire strong conditioned preferences in response to this training procedure (Fanselow & Birk, 1982; Holman, 1975; Mehiel & Bolles, 1988). This strong effect presumably occurs as a result of the combined effect of the palatable flavour of the reinforcer and the post-ingestive effects of its calories (Warwick & Weingarten, 1994).

In the present set of experiments, we were primarily interested in whether calorie-based conditioning alone can induce conditioned preferences for relatively unacceptable tastes. The simultaneous conditioning procedure is not appropriate for this purpose for a couple of reasons. First, as previously mentioned, in a simultaneous procedure the CS^+

flavour may become associated with the flavour of the US. This flavour-taste association would itself increase the acceptability of the CS (e.g., Fanselow & Birk, 1982; Holman, 1975). Second, the simultaneous procedure may also be construed as an example of taste-taste potentiation. Potentiation occurs when a strongly conditionable stimulus is presented in compound with a weakly conditionable stimulus prior to an unconditioned stimulus. Most commonly a strongly conditionable taste is presented with a weakly conditionable odour prior to illness. As a result, the taste potentiates rather than overshadows the conditioning of the aversion to the odour (Durlach & Rescorla, 1980; Lett, 1984; Palmerino, Rusiniak & Garcia, 1980, Rusiniak, Hankins, Garcia & Brett, 1979). There are also examples of strongly conditionable odours potentiating aversions to weakly conditionable tastes (Slotnick, Westbrook & Darling, 1997) and strong tastes potentiating aversions to other tastes (Bouton, Dunlap, & Swartzentruber, 1987). In simultaneous flavour preference conditioning, the animal experiences the taste of the caloric reinforcer with the CS^{+}. Therefore in training, the taste of the reinforcer may potentiate conditioning to the flavour CS^{+}. Potentiation of the CS^{-} by saccharin cannot occur since there is no contingent post-ingestive reinforcement following this cue.

Since flavour-taste associations are not readily conditioned with CS-US delays, one might expect to obtain a "clean" measure of flavour-calorie associations with a forward trace procedure, in which presentation of the CS solution is followed several minutes later by presentation of the reinforcer. Researchers have had mixed success obtaining conditioned preferences using such a procedure, however. Holman (1975) demonstrated that rats can acquire conditioned preferences for flavours followed by

calorific reinforcers in a forward trace procedure, and this effect has also been obtained in several subsequent studies (e.g., Capaldi, Campbell, Sheffer, & Bradford, 1987; Perez, Lucas, & Sclafani, 1995). However, in several other studies forward trace conditioning failed to produce a preference for the CS^+ (Boakes, Rossi-Arnaud & Garcia-Hoz, 1987; Lavin, 1976; Simbayi, Boakes & Burton, 1986, see also Dwyer, 2001).

According to Boakes and Lubart (1988; see also Elizalde & Sclafani, 1988), the difficulty in obtaining robust flavour preferences with delayed reinforcement may be a function of associative interference from the flavour of the reinforcer. That is, since the flavour of the reinforcer occurs between the presentation of the flavour of the CS and the post-ingestive effects of the reinforcer, it may interfere with the acquisition of associative strength by the CS. To overcome this difficulty, Boakes and Lubart reversed the order of stimulus presentation so that the reinforcer preceded rather than followed the presentation of the CS^+. They postulated that if the cue flavour fell between the presentation of the reinforcer and its post-ingestinal consequences, animals would behave as if they attributed the post-ingestive consequences of the reinforcer to the cue flavour rather than to the flavour of the reinforcer itself. In accordance with Boakes and Lubart's prediction, rats that received presentation of the US followed after 3 min by presentation of the CS^+ showed a significantly higher preference for the CS^+ relative to the CS^- than those in a control group that received the presentation of the reinforcer 1 h before the cue flavour.

The aim of the present set of experiments was to use Boakes and Lubart's (1988) backward conditioning procedure to determine whether we could use postingestive reinforcement to condition preferences to relatively unacceptable tastes. Very few

experiments, with the exception of a few studies of potentiation (Capaldi & Hunter, 1994; Holder, 1991; Lucas & Sclafani, 1995), have looked at the conditionability of tastes or odours alone. Moreover, in many flavour preference studies, either sucrose or saccharin is mixed with the flavour cue. Therefore, the compound CS solutions are initially highly acceptable before any conditioning occurs. Notable exceptions to this statement involve experiments using intragastric reinforcement (e.g., Drucker, Ackroff, & Sclafani, 1994; Perez, Lucas, & Sclafani, 1998), which have been successful in conditioning strong preferences to the initially unacceptable taste stimuli.

There is also evidence suggesting that rats do not readily acquire preferences to normally avoided tastes such as chili peppers (Rozin, Gruss & Berk, 1979). Mere exposure to the chili seasoning presented in rats' diet for the first 11 months of life failed to condition preferences for chili-seasoned chow vs. plain, unseasoned chow. The chili pepper aversion was eliminated (but not reversed) if they desensitized the animals to capsaicin or if they paired chili pepper seasoning with recovery from illness over multiple trials. This report is a dramatic example of rats' resistance to reverse their innate reactions to aversive stimuli. This failure is not surprising since there are few situations in the wild in which it is adaptive for a rat to learn to like sour or bitter tastes. In fact, avoidance of these tastes would perhaps be more adaptive since they are normally paired with toxic substances. Thus, it is of interest to determine whether rats have evolved a tendency or a predisposition to acquire preferences more readily for tastes that are neutral or somewhat acceptable relative to unacceptable tastes. In the next set of experiments we sought to determine whether we could get preferences for sour and bitter tastes paired with a

calorific reinforcer using Boakes and Lubart's (1988) reverse-order conditioning procedure.

General Method

Subjects: Sprague-Dawley rats obtained from Charles River, Canada were individually housed in 18 x 24 x 17.5 cm high Wahmann hanging cages. The colony room was maintained on a 16:8 light:dark cycle with lights on at 07:00. Rats received training and test trials in their home cages during the light part of the cycle.

Apparatus: Conditioned stimuli consisted of two taste solutions .03% (w/v) sucrose octaacetate (SOA; ICN Biomedicals) and .03% (w/v) citric acid (CA; Sigma-Aldrich) mixed in .2% (w/v) saccharin for Saccharin Groups (Sigma-Aldrich) or mixed in tap water for No Saccharin groups. The unconditioned stimulus was 8% (w/v) D-Glucose (Sigma-Aldrich) mixed in tap water. Unless otherwise specified, all solutions were presented in 40-ml nalgene conical centrifuge tubes placed on the front of each rat's cage with a spring.

Procedure: In general, rats were divided into Immediate and Delay groups, equated in mean weight[3]. For both groups, presentation of the glucose or water preceded presentation of the taste cues. Delay rats received 10 ml of either glucose or water for 5 min at the beginning of each training trial. Fifty-five minutes later, animals in the Immediate group received either glucose or water. Three min after the removal of this solution, the appropriate taste cue (CS^+ or CS^-) was presented for 10 min (unless otherwise specified) to rats in the Immediate and Delay groups. Thus, for rats in the Immediate

Group, the taste cue was presented 3 min after the glucose or water solutions and for the Delay group, 1 hour separated presentation of these solutions. In Experiments 1, 2, 6 and 7 Delay groups were not included.

Training lasted for 16-20 days, during which animals received 1 training trial per day. On a particular training day, half of the animals in each group received water and the remaining animals received the glucose solution; each was followed by the appropriate taste cue. Half of the animals within the Delay and Immediate groups received reinforced presentations of sucrose octaacetate (SOA^+) and the remaining animals received reinforced presentations of citric acid (CA^+). For SOA^+ rats, sucrose octaacetate was presented after the glucose solution and citric acid was presented after water, whereas CA^+ animals received the reverse pairings; citric acid followed the glucose solution and sucrose octaacetate followed water.

On the day after the last training trial, rats were given a 2-bottle water test in which they received two water bottles placed simultaneously on the front of their cages for 10 min. For each rat, each bottle was placed briefly on the cage alone until the animal approached the bottle and sampled the solution. Once each solution was sampled, the bottles were placed on the centre of the cage with approximately 1 cm separating the spouts. On each of the subsequent 4 days, animals were given one 10-min, 2-bottle test session in which one of the bottles contained SOA and the other contained CA. Each test consisted of 2 sessions, between which the positions of the bottles were reversed to counteract any side preferences. Before and after each training or test session all of the bottles were weighed to determine consumption. Unless otherwise specified, throughout

training and test, animals were maintained on a restricted food and water schedule in which they received 1 hour of food and water approximately (but no less than) 2 hours after the end of the conditioning phase.

Data Analysis: Consumption of the taste cues and of glucose and water in training was analyzed using separate repeated-measures Analyses of Variance (ANOVA).

In test, consumption of each of the test cues was combined across the test sessions and a preference ratio (amount of consumed $CS^+/(CS^+ + CS^-)$ was calculated for each rat. With the exception of Experiment 3, parametric tests were conducted on these preference ratios. Test data for these experiments were graphed using means and standard error of the means. For all analyses, the significance level was set at $p<.05$.

Experiment 1

In the present experiment, we attempted to condition preferences to a bitter (sucrose octaacetate) and a sour (citric acid) taste using either a forward or a reverse-order conditioning procedure (Boakes & Lubart, 1988). Rats in the Backward Immediate (BI) Condition received the reverse-order conditioning procedure described above in which the US preceded the CS. Rats in the Forward Immediate (FI) condition were treated exactly like those in Group BI, except presentation of the CS preceded rather than followed presentation of the US by three minutes.

Although some researchers have had little success with the Forward conditioning procedure, we decided to use it in the present experiment in an attempt to increase CS consumption relative to previous experiments from our laboratory (Forestell, 1997). Since animals in the Forward condition receive the CS^+ before glucose or water, consumption of

the CS solutions in that condition should not be affected by prior consumption of another solution.

Method

Subjects: Thirty-six naive male Sprague-Dawley rats weighing 350 g on average were used in the present experiment.

Procedure: Eight days prior to the beginning of the training phase, animals were placed on a restricted water and food access schedule. During this pretraining phase, water was made available for 20 min each day at 16:00, and water and food were presented together for one hour at 18:00. Animals were divided into 4 groups, which were matched according to their consumption of water at 16:00.

The training phase lasted for 20 days, during which rats received one training trial daily at approximately 16:00. Each trial consisted of a 20-min presentation of the CS and a 5-min presentation of the appropriate US. Group BI received the CS solution 3-min after the removal of the US solution, whereas Group FI received the US solution 3-min after the removal of the CS solution. Each CS was presented on 10 trials in a double alternation sequence. Throughout the training and test phases, animals continued to receive daily supplementary water and food for one hour at 18:00.

In the test phase, the 2-bottle tests were conducted at 16:00. In the present experiment, rats were adapted to the testing procedure on days 1 and 2 with the presentation of water in both bottles. On days 3 and 4, all rats received a 2-bottle test session in which the CS^+ was in one bottle and the CS^- in the other. This procedure was repeated on days 5 and 6.

Results

Training Data: With the exception of trial 1, rats in all groups consumed virtually all of the glucose available to them (daily mean = 8.36 ml). Consumption of water was lower than that of glucose for all groups, with the forward groups consuming less water (daily mean = 2.45 ml) than the backward groups (daily mean = 5.04 ml) throughout training. These claims were supported by the results of a Group (CA^+ vs. SOA^+) x Stimulus Order (BI vs. FI) x Stimulus (water vs. glucose) x Trials ANOVA, which revealed a significant effect of Stimulus ($F(1,243)=614$) and a significant Stimulus Order x Stimulus interaction ($F(1, 243)=82$).

Consumption of the CS solutions is shown in Figure 1. Animals in the FI groups (FI CA^+ and FI SOA^+; these are combined in the figure) consumed more of both CS solutions overall than those in the BI groups (BI CA^+ and BI SOA^+). Increased consumption of the CS^+ solution relative to the CS^- might be expected as a function of conditioning. This did occur in the BI groups, but not in the FI groups.

These claims were supported by the results of a repeated-measures ANOVA, in which Group (BI vs. FI) was a between-subjects factor and Stimulus (CS^+ or CS^-) and Trial were within-subjects factors. Significant main effects of Group ($F(1, 162) = 70.6$), and Stimulus ($F(1, 162) = 11.7$), were obtained. These main effects were qualified by a marginally significant Group x Stimulus interaction ($F(1, 162) = 4.1, p < 0.057$).

A post hoc analysis (Tukey's HSD) of group indicated that overall consumption of the CS solutions was greater in the FI groups than in the BI groups. The simple main effects analysis of the Group x Stimulus interaction yielded a significant effect of group on

consumption of the CS^+, F (3, 232) = 20.6, and the CS^-, F (3, 189)=19.0, with the FI group drinking more of both. The analysis of the effect of stimulus on group indicated that greater consumption of the CS^+ relative to the CS^- occurred only in Group BI, (F (1,18)=32.8).

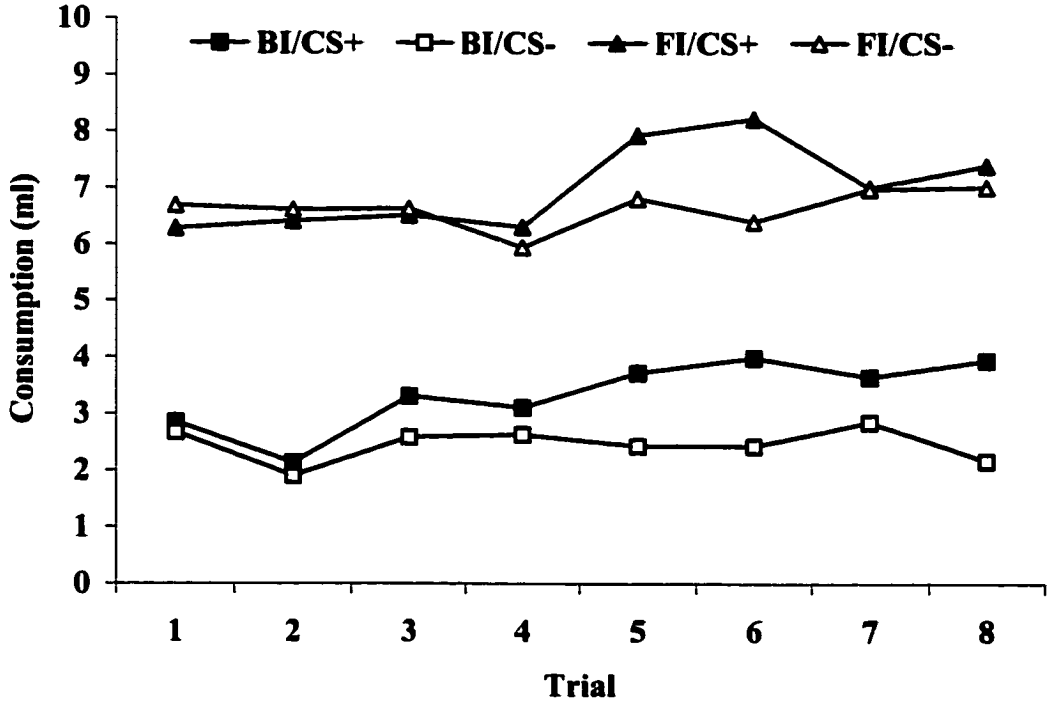


Figure 1: Mean consumption of the CS^- and CS^+ solutions during training for the Backward Immediate (BI) and Forward Immediate (FI) groups in Experiment 1.

Test Data: As displayed in Table 1, overall consumption of the taste cues in test did not differ between the BI and FI groups. Figure 2 displays the preference for SOA in each of the groups for Tests 1 and 2 combined. In neither test did rats that had received

SOA reinforced with glucose show a higher preference for SOA than those that had received CA paired with glucose.

This conclusion was supported by a repeated-measures ANOVA, in which Stimulus order (BI vs. FI) and Group (CA^+ and SOA^+) were between-subjects factors and Stimulus (CS^+ or CS^-) and Test were within-subjects factors. The main effect of Group failed to reach significance (F (1, 29) = .8) as did the Stimulus Order x Group (F (1, 29) = .3) interaction.

Table 1: Mean Consumption (and *SEM*) of the Taste Cues in Experiment 1

	SOA		CA		Total	
Group/Test	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*
BI SOA^+/1	11.4	*2.1*	6.4	*1.6*	17.8	*2.2*
BI CA^+/1	9.4	*2.6*	10.0	*2.0*	19.4	*3.5*
FI SOA^+/1	13.7	*1.7*	8.7	*1.8*	22.7	*1.9*
FI CA^+/1	10.4	*1.6*	7.7	*1.1*	18.1	*1.7*
BI SOA^+/2	10.3	*2.2*	5.7	*1.1*	16.1	*1.7*
BI CA^+/2	9.0	*2.1*	8.2	*2.4*	17.2	*3.5*
FI SOA^+/2	10.8	*2.5*	7.6	*2.3*	18.3	*2.8*
FI CA^+/2	10.8	*2.5*	7.9	*2.3*	18.7	*2.5*

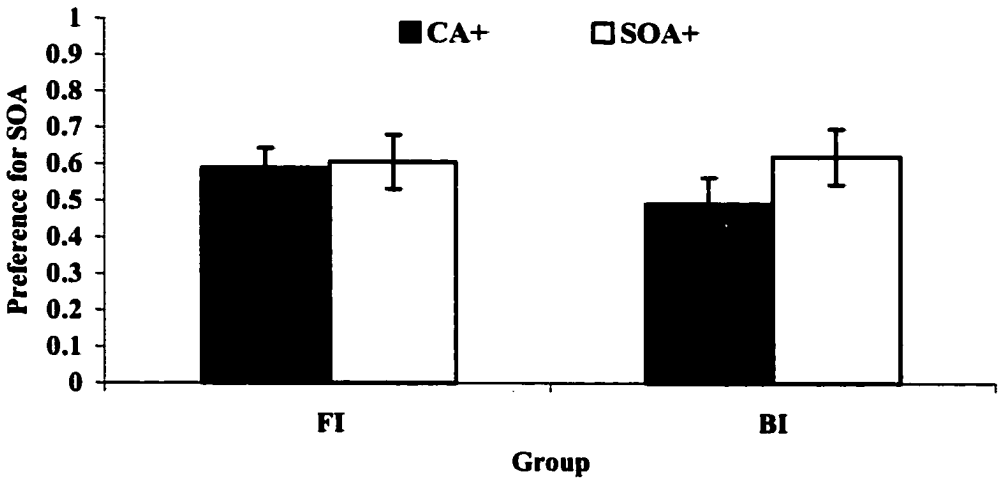


Figure 2: Mean preferences (+/- *SEM*) for sucrose octaacetate relative to citric acid in Group BI (A) and Group FI (B) in Tests 1 and 2 combined in Experiment 1.

Discussion

No evidence for conditioned preferences was observed in this experiment. Regardless of whether rats were conditioned with a forward or backward differential conditioning procedure, they did not develop a preference for the CS^+ over the CS^-.

These results are in contrast to those of Capaldi, Hunter and Lyn (1997), who obtained a significant conditioned preference with two relatively unacceptable conditioned stimuli, namely citric acid (of the same concentration used in our experiment) and quinine. However, the main difference between our experiment and that of Capaldi *et al* is that they mixed their CS solutions with an 8% sucrose solution in training and test. This difference is important to consider, since their procedure would have drastically increased the acceptability of the CS solutions. Therefore, in essence, Capaldi *et al* have shown that

preferences can be conditioned to stimuli that have an unacceptable component, but are not unacceptable overall. In the present experiment, we have failed to obtain conditioned preferences for stimuli that are relatively unacceptable overall, suggesting that rats' initial affective reactions, or their innate avoidance for certain tastes, are difficult to overcome with calorie-based preference conditioning.

It may be that the animals in this experiment failed to drink enough of the CS to acquire a conditioned preference. On average, animals in the BI groups consumed only about 3 ml/trial of the conditioned stimuli. This may have been an insufficient amount of exposure to the CS for conditioning of flavour preferences. However, if we consider the flavour aversion literature, we find that animals need to consume only about 3 ml of a CS paired with malaise in one trial to acquire a conditioned aversion (Domjan & Wilson, 1972). Not only did animals consume about 3 ml of the CS during a single conditioning trial in our experiment, but they did so over ten training trials.

The purpose of the next experiment was to determine whether Boakes and Lubart's (1988) reverse-order procedure is effective in conditioning taste preferences in our laboratory when more acceptable taste stimuli are used.

Experiment 2

One of the main differences between the previous experiment and that of Boakes and Lubart (1988) was that they mixed the taste stimuli with saccharin, whereas we presented them in water. Mixing these taste cues with saccharin appears to make them more acceptable. This may increase the rats' ability to acquire conditioned taste preferences. In the present experiment, we investigated whether rats would acquire

conditioned preferences for SOA and CA if they were mixed in saccharin during training and test, using Boakes and Lubart's reverse-order conditioning procedure only.

Another difference between Boakes and Lubart's (1988) design and ours was that we restricted the rats' access to water in an attempt to increase their consumption, whereas animals in Boakes and Lubart's study were given free access to water. Since water restriction is not typically employed in flavour preference conditioning experiments, it is possible that our failure to obtain conditioned preferences in the previous experiment was a result of water restriction rather than of the use of relatively unacceptable taste stimuli. Thus, we also looked at the effect of water restriction in training and in test on the formation and expression of conditioned taste preferences.

Method

Subjects: Thirty-six male Sprague-Dawley rats with a mean weight of 351 g were housed and maintained as previously described.

Apparatus: Both taste cues (CA and SOA) were mixed with 0.2% saccharin.

Procedure: Seven days prior to the beginning of the experiment, rats were divided into two groups matched on weight. These groups differed as to whether they were water restricted or not. Throughout the experiment rats in the restricted group were given water at 15:00 each day for 2 hours, whereas rats in the nonrestricted group received free access to water. For both groups, food was made available for 2 hours per day beginning at 15:00. Supplementary water consumption for all rats was recorded each day just prior to replacement of the water bottles on the cages of the restricted rats.

During the pretraining phase, animals received daily access to 0.2% saccharin at 12:30 to overcome any neophobic responses. On day 1, rats were free to consume as much as they wanted for one hour. Duration of access was gradually reduced to 20 min by day 4. Subsequently, the restricted and nonrestricted groups were further divided into 2 groups; Group SOA^{+} and Group CA^{+}, which were matched on saccharin consumption during the pretraining phase.

Training commenced on day 5. The Restricted Immediate (RI) and Nonrestricted Immediate (NRI) groups received reverse-order US-CS pairings as described in the General Methods. Each CS was paired with either glucose or water and presented on 8 trials in a double alternation sequence.

In the test phase, preference for the CS^{+} was assessed with a series of 2-bottle tests conducted at 12:30 h in which the taste cues were presented in 0.2 % saccharin. Animals received a 2-bottle water test session and two 2-bottle CS tests. For the first CS test, rats were maintained on the same water restriction schedule as in training (days 18 and 19). Water restriction states were reversed (the formerly NRI group received 2 hr/day of supplementary water and the formerly RI group received free water) 5 days prior to the second test (days 25-26).

Results

Training Data: Rats in all groups consumed more glucose than water ($F(1, 182) = 1885.0$), and Group RI drank more glucose and water (8.33 and 2.11 ml, respectively) than rats in Group NRI (7.97 and 0.83 ml, respectively), ($F(1, 182) = 17.8$).

Figure 3 shows consumption of the CS solutions during training. This figure indicates that both water restricted and nonrestricted groups increased their consumption of both CS solutions. Rats in both groups appeared to consume more of the CS^- than the CS^+ solution throughout training. These observations were supported by a repeated measures 2 x 2 x 8 (Group (RI vs. NRI) x Stimulus (CS^+ vs. CS^-) x Trial) ANOVA which revealed significant effects of Trial (F (7,84) = 33.8), and Stimulus (F (1,84)=5.7).

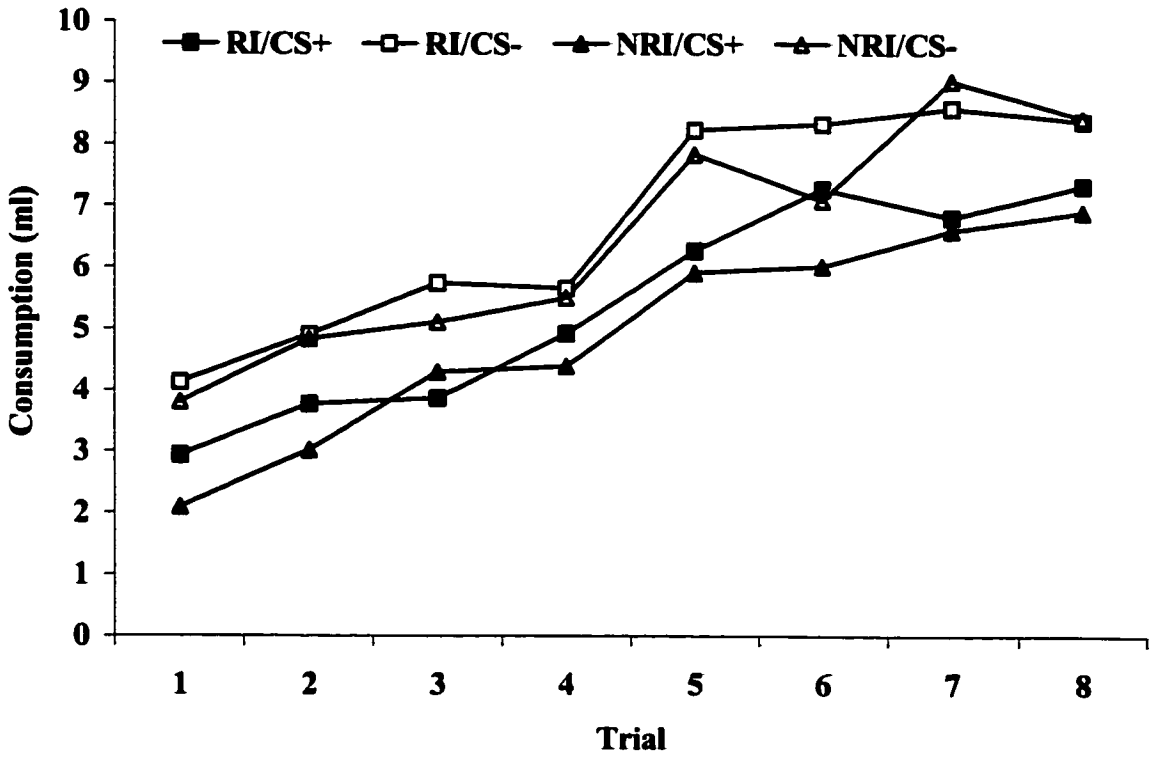


Figure 3: Mean consumption of the CS^+ and CS^- solutions during training for water restricted (RI) and nonrestricted (NRI) groups in Experiment 2.

Supplementary Water Consumption: Group NRI drank significantly more supplementary water throughout training (daily mean = 32.4 ml; this does not include consumption of the water US) than Group RI (daily mean = 23.2ml). After Test 1, when the water restriction conditions were reversed, this pattern of consumption also was reversed.

These claims were supported by the results of separate Group x Day ANOVAs of the data before and after Test 1. In both cases the effect of Group was significant (F (1, 612) = 78.52 prior to Test 1, and F(1, 165) = 75.9 after test 1).

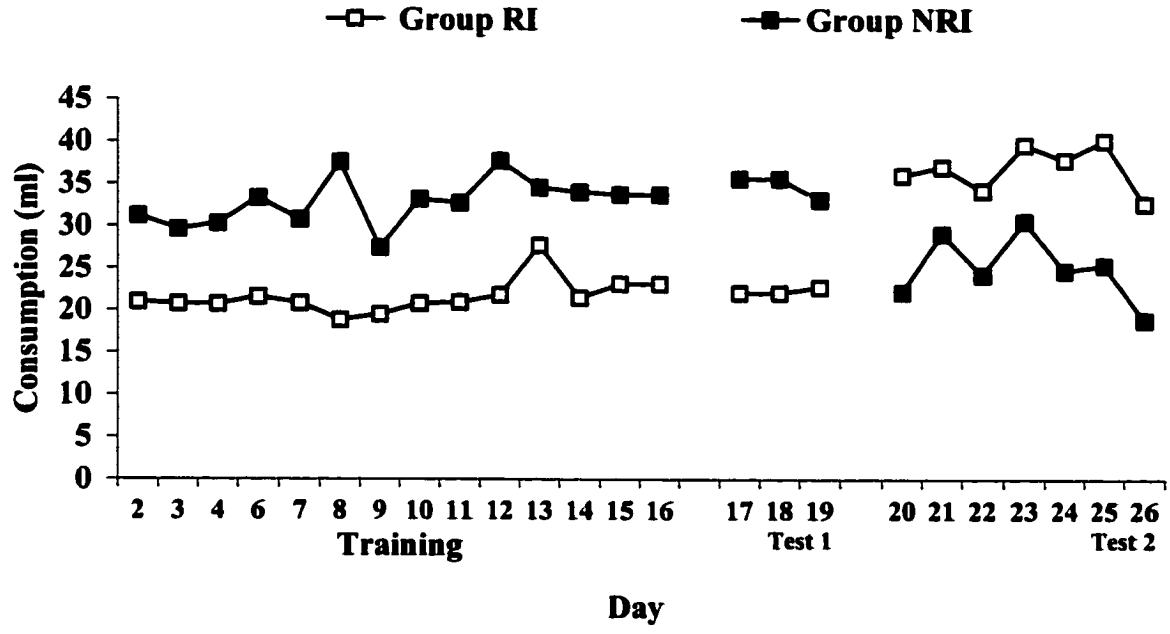


Figure 4: Mean consumption of supplementary water in Groups RI and NRI in Experiment 2. On Days 17-19, animals were maintained on the same water restriction schedule as in training. These restriction states were reversed from days 20-26.

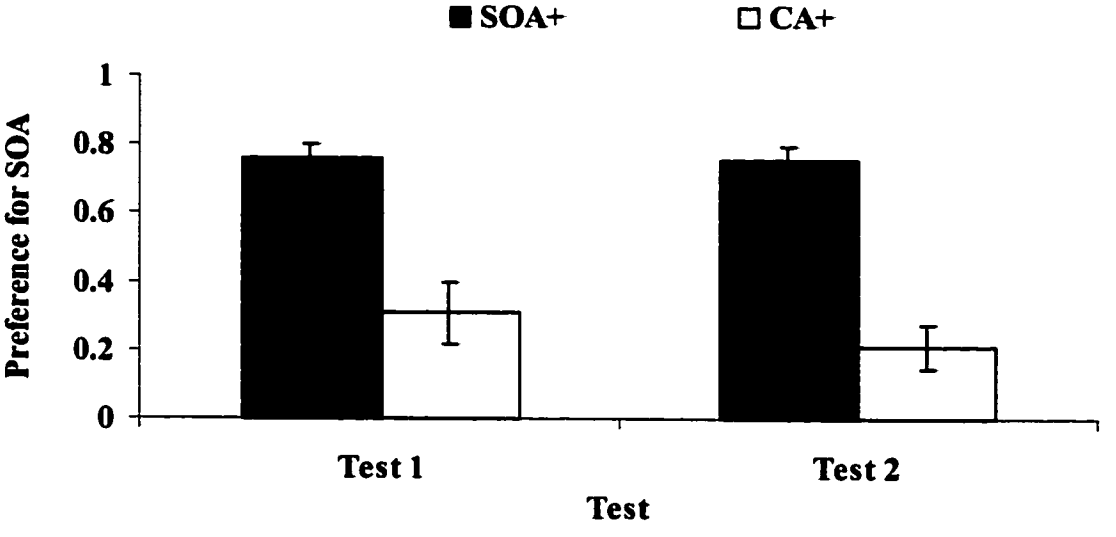
Test Data: Table 2 presents mean consumption of the two CS solutions in each of the tests. As revealed in this table, total consumption was similar between groups in and Tests 1 and 2 ($F<1$).

Figure 5A shows that preferences for SOA over CA were significantly higher in the SOA^+ than in the CA^+ group in Groups RI and NRI in Test 1 and Test 2. This was supported by a repeated-measures ANOVA in which restriction level in training and counterbalanced group (SOA^+ vs. CA^+) were the between subjects variables and test was the within subjects variable. This analysis revealed a main effect of counterbalanced group only (F (1, 30) = 102.7).

Table 2: Mean Consumption of the Taste Cues in Tests 1 and 2 of Experiment 2

	CA		SOA		Total	
Group/Test	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*
RI CA^+/1	27.4	*4.8*	9.0	*1.6*	36.4	*3.9*
RISOA^+/1	8.0	*1.7*	26.7	*3.3*	34.7	*2.8*
NRI CA^+/1	23.2	*2.6*	7.2	*2.0*	30.4	*2.7*
NRI SOA^+/1	6.7	*1.6*	29.8	*4.0*	36.5	*4.8*
RI CA^+/2	27.5	*4.3*	6.0	*1.5*	33.5	*3.7*
RISOA^+/2	7.8	*1.4*	24.7	*2.6*	32.5	*2.2*
NRI CA^+/2	21.0	*3.0*	9.1	*1.7*	30.1	*2.9*
NRI SOA^+/2	7.2	*1.8*	28.3	*2.9*	35.4	*2.4*

A. Group RI



B. Group NRI

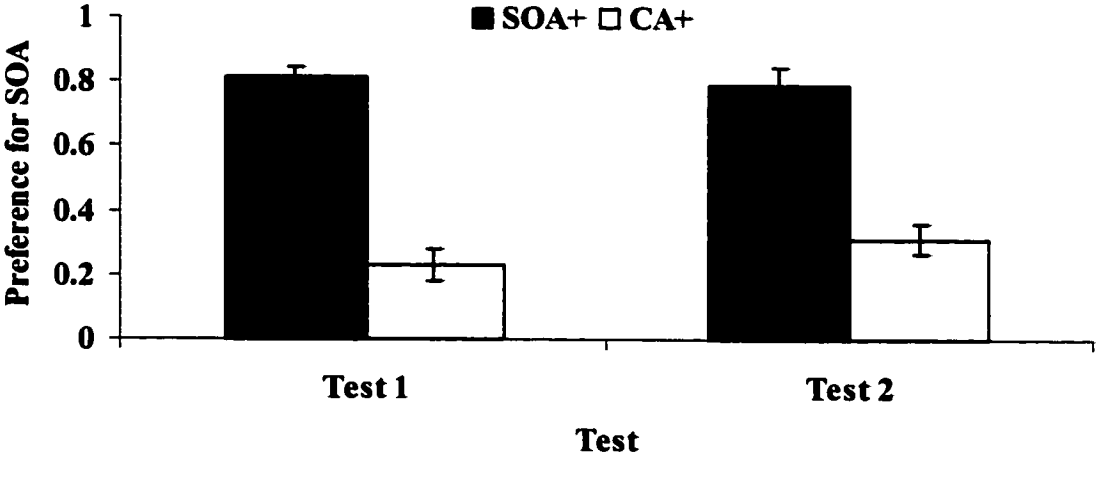


Figure 5: Mean preferences (+/- *SEM*) for sucrose octaacetate relative to citric acid in test for rats that were water restricted in training (Group RI) and those that were not water restricted in training (Group NRI) in Tests 1 and 2. In Test 1, the rats' water restriction state was the same as in training. In Test 2, the water restriction state was reversed.

Discussion

When saccharin was added to the CSs, a significant preference for the CS^+ solution was obtained in both the RI and NRI groups. Moreover, this preference did not significantly differ in magnitude between these two groups. These data suggest that the magnitude of taste preferences in test is unaffected by the level of water restriction during training or testing. Rats in the restricted group drank significantly more of the water US than those in the non-restricted group. The former also drank significantly less supplementary water than the latter. Thus, the water restriction manipulation was effective. However, water restriction does not appear to be a critical factor in the acquisition or expression of conditioned taste preferences, and so it is unlikely that our failure to obtain significant taste preferences in the previous experiment was a function of the rats' level of water restriction.

The absence of an effect of water restriction in test contrasts with Drucker, Ackroff and Sclafani (1994), who found that when rats were water restricted during test, their CS^+ preference ratio was reduced. A closer look at their data, however, indicates that this decrease in preference was accompanied by an overall increase in consumption of both the CS^+ and the CS^- after water restriction in test. In the present experiment, total CS consumption did not differ between tests in either group.

This experiment, in combination with Experiment 1, suggests that initial acceptability of the solutions may determine their conditionability. Thus pairing a taste with a source of calories in an oral conditioning procedure may be problematic for conditioning preferences for relatively unacceptable tastes. When these same tastes are

made more acceptable by the addition of saccharin, however, preferences for these stimuli are more readily increased by conditioning procedures.

It is possible that the significant preferences occurred in the present experiment and not in the last experiment because of differences in the water restriction state of the animals. Indeed, it should be noted that the restricted animals in this experiment had one more hour of supplementary water per day than those in the previous experiment. To ensure that these between-experiment differences in conditioned taste preferences were the result of the absence/presence of saccharin in the CSs and were not due to extraneous variables such as the level of water restriction, in the next experiment in otherwise identically treated groups, we manipulated whether or not the taste stimuli were mixed in saccharin.

Experiment 3

In this experiment, the CS solutions were mixed in either water or saccharin to determine whether the conditioned preference observed in the previous experiment was a function of the presence of saccharin in the CS.

The Saccharin (S) and No Saccharin (NS) conditions each contained an Immediate and a Delay group. The Delay groups were meant to serve as a baseline control with which to compare the CS^+ preferences obtained in the Immediate groups. In the previous experiments, preferences obtained in the two counterbalanced groups (CA^+ and SOA^+) were compared to each other within each condition. This comparison is often used in conditioned flavour preference experiments (e.g., Capaldi, Campbell, Sheffer & Bradford, 1987; Elizalde, 1990; Fanselow & Birk, 1982). However, this method reflects the sum of

2 preferences; that conditioned to CA plus that conditioned to SOA. As a result, the observed preference for the CS^+ is inflated. By comparing the Immediate experimental groups to the Delay control groups, we are able to look at the preference conditioned to the CS^+ relative to a neutral baseline.

If the addition of saccharin to the taste stimuli is required for animals to acquire conditioned taste preferences, then the Immediate group should have significantly higher preferences than the Delay group in Condition S, but not in the Condition NS.

Method

Subjects: Forty male Sprague-Dawley rats weighing 420 g on average were housed and maintained as described in the General Methods.

Apparatus: Rats in the NS condition received the CA and SOA stimuli mixed in tap water, whereas rats in the S condition received them mixed in a 0.2% (w/v) saccharin solution.

Procedure: In the pretraining phase, rats received seven days of restricted water and food access. On the first 4 days they received water for 30 min at 09:30 h and on subsequent days 40 min access to saccharin. Based on their weight and consumption at 9:30, rats were placed into four matched groups; Saccharin-Immediate (SI), Saccharin-Delay (SD), No Saccharin-Immediate (NSI) and No Saccharin-Delay (NSD).

Training lasted for 16 days, during which rats received one training trial per day at approximately 9:30. Each CS was presented on 8 of these trials in a double alternation sequence.

As in the previous experiments the rats' preferences were assessed with 2-bottle tests. In the first test each bottle was filled with water to acclimate the animals to the testing procedure. In subsequent test sessions, the bottles contained the CS solutions. Rats in Groups SI and SD received the taste stimuli mixed in 0.2% saccharin and Groups NSI and NSD received the taste stimuli in water.

Throughout the training and test phases, animals received 1 hour of supplementary water and food at least 2 hours after the end of each training and test trial.

Results

Training Data: Rats in all groups consumed more glucose (daily mean = 8.16 ml) than water (daily mean = 2.89 ml; $F(1, 231) = 334.6$).

Consumption of the CS solutions is shown in Figure 6. This graph shows higher CS consumption in the Saccharin condition than in the No Saccharin condition. Moreover, as training progressed, consumption in the Saccharin condition increased, whereas that of the No Saccharin condition did not appear to increase. These observations were supported by the results of a 2 x 2 x 2 x 8 (Condition (S vs NS) x ISI (Immediate vs. Delay) x Stimulus (CS^+ vs. CS^-) x Trial) repeated-measures ANOVA. This analysis revealed a significant main effect of Condition ($F(1,231) = 185.9$), Stimulus ($F(1, 231)=15.6$), and Trial, ($F(7, 231) = 8.4$). There was also a Condition x Trial interaction ($F(7,231) = 10.1$). Simple main effects analysis of the Trial x Condition interaction yielded a significant effect of Trial in the SI ($F(7, 49) = 7.9$), and SD ($F(7, 56)=3.6$), groups, but not in the NSI and the NSD groups.

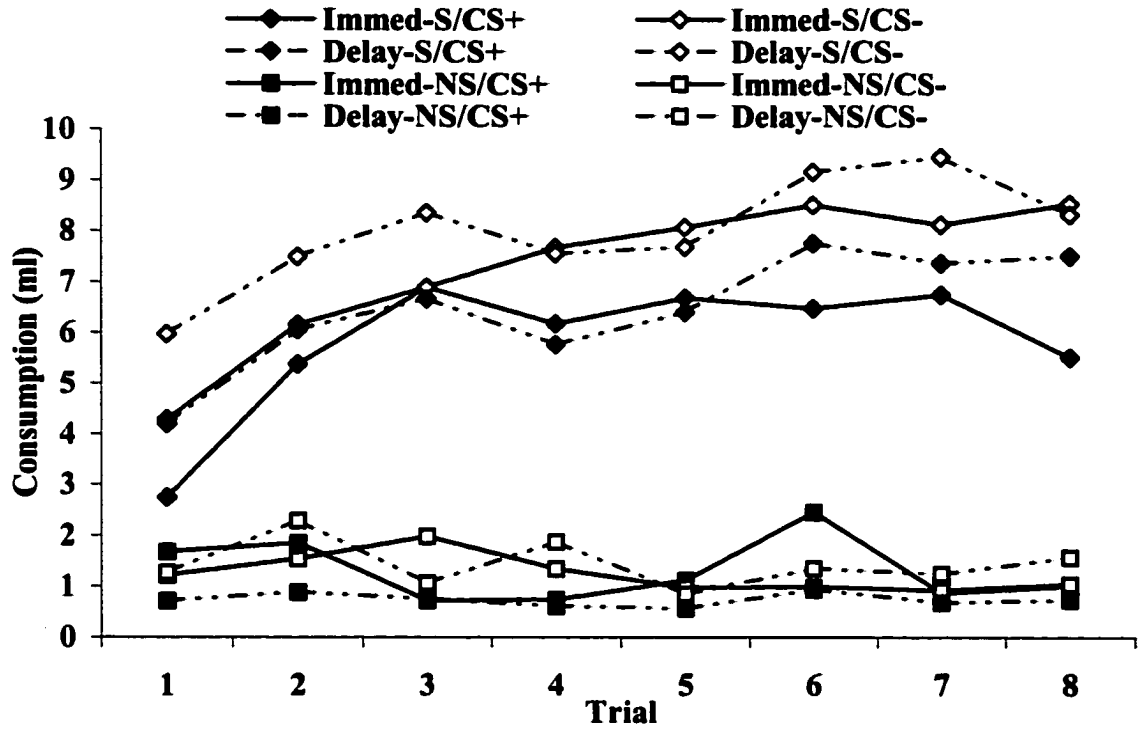


Figure 6: Mean consumption of CS^+ and CS^- during training in the Immediate and Delay Saccharin (S) and No Saccharin (NS) groups in Experiment 3.

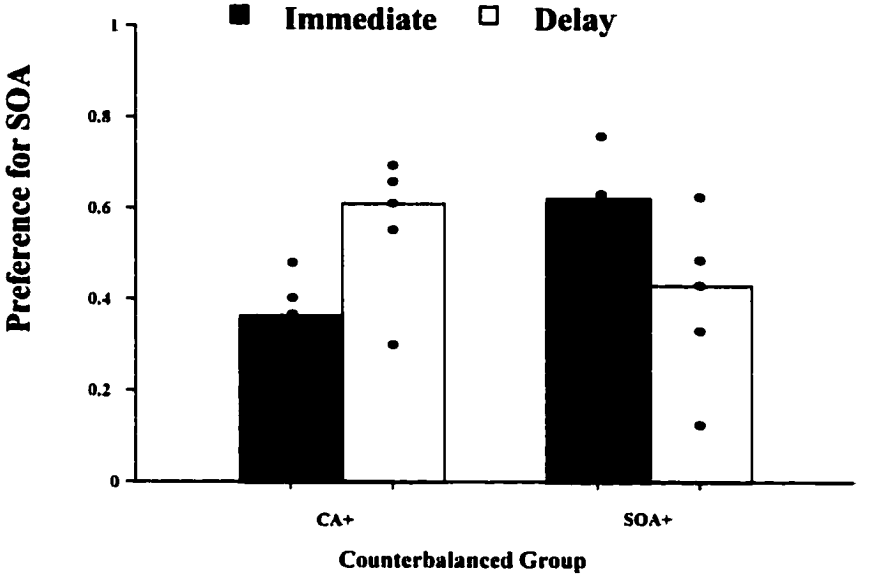
Test Data: Mean absolute consumption in Tests 1 and 2 combined is displayed in Table 3. As shown, total consumption in the S groups was higher than that of the NS Groups (F (1, 37) = 52.4) throughout the test phase. Because there was an SOA bias in the NSD groups, CS^+ preference ratios were not collapsed for the counterbalanced groups (CA^+ and SOA^+). Instead the SOA preference ratios for the CA^+ and SOA^+ groups within the SI and NSI conditions were separately compared to the appropriate Delay groups. Since group sizes were small (4-5 animals per counterbalanced group), nonparametric analyses were used.

Figures 7 A and B display mean CS^+ preferences for all of the groups for Tests 1 and 2 combined. This graph suggests that rats in the SI group, but not those in the NSI, group acquired a preference for the CS^+. Statistical analysis supported these claims. Animals in group SOA^+ of the SI group had a higher preference for SOA than the SOA^+ rats in the SD group ($U(5, 5) = 4$, 1 tailed test), whereas the CA^+ rats in the SI group had lower preferences for SOA than their counterparts in the CA^+ SD group ($U(5, 5) = 3$). There were no differences between the Immediate and Delay groups in the NS condition.

Table 3: Mean Consumption (and SEM) of the Taste Cues during the Test Phase of Experiment 3

	SOA		CA		Total	
Group	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*
CA^+SI	9.7	*2.6*	19.2	*3.2*	28.8	*4.3*
SOA^+SI	21.4	*2.1*	11.2	*1.1*	32.5	*2.2*
CA^+NSI	6.3	*3.8*	3.9	*2.6*	10.1	*3.7*
SOA^+NSI	12.2	*3.1*	4.4	*0.8*	16.6	*3.3*
CA^+SD	19.4	*3.2*	14.1	*1.4*	33.5	*2.6*
SOA^+SD	11.7	*2.5*	17.2	*3.4*	28.9	*4.3*
CA^+NSD	9.4	*12.9*	2.9	*2.5*	12.3	*14.1*
SOA^+NSD	12.1	*3.0*	2.0	*0.8*	14.1	*2.8*

A. Saccharin Condition



B. No Saccharin Condition

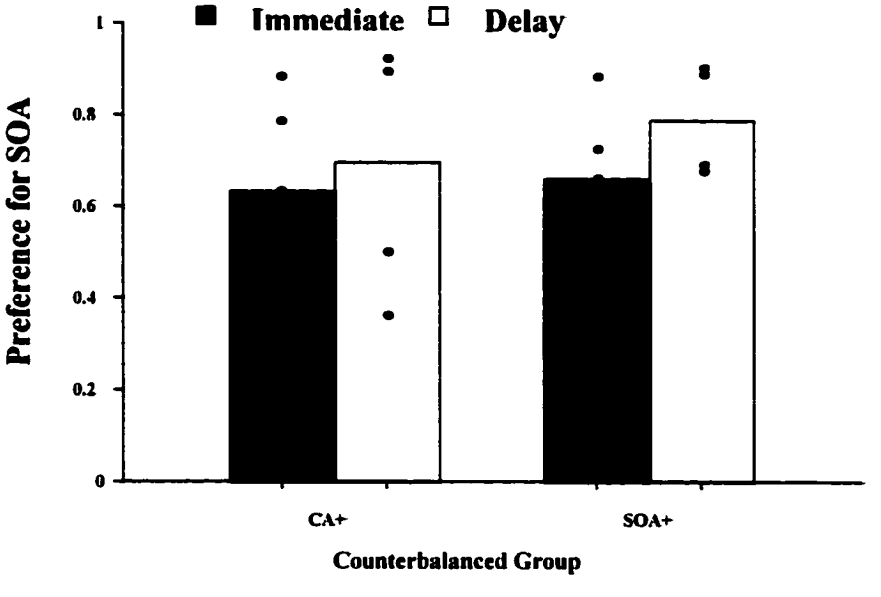


Figure 7: Median preference for SOA in the Saccharin Immediate and Delay (SI and SD) and No Saccharin Immediate and Delay (NSI and NSD) groups in Experiment 3. Data points refer to individual rats' preferences for SOA.

Discussion

When saccharin was added to the CS solutions during training and test, animals in the Immediate group showed a significantly higher preference for the CS^+ than animals in the Delay group. However, when animals were trained and tested with CA and SOA mixed in water only, there was no difference in preference for the CS^+ between Immediate and Delay groups. In this experiment, there was a SOA preference in both of the Delay groups in the No Saccharin condition. This indicated that there was a sucrose octaacetate bias in this condition. Thus in the CA^+ groups, there was plenty of room for the preference for CS^+ to be greater in the Immediate group than in the Delay group, but this did not occur.

Although other studies have succeeded in conditioning preferences to relatively unacceptable tastes such as unsweetened Kool Aid or CA and SOA mixed in water, they have employed different reinforcement procedures. For example, in Sclafani's experiments animals received intragastric, rather than oral, caloric reinforcement (e.g., Drucker, Ackroff & Sclafani, 1993; Elizalde & Sclafani, 1990; Sclafani, Cardieri, Tucker, Blusk & Ackroff, 1993). This difference is notable for several reasons. First, presentation of the taste cues for 23 h/day enabled animals to engage in many bouts of consumption. As a result, they received many trials each day. Moreover, when the two solutions are available concurrently during training, the rats' drinking appears to follow a pattern that allows them to efficiently determine which of the solutions was paired with reinforcement. For example, a 10-min interval normally occurred between the beginning of a drinking bout of one solution and that of another (Drucker *et al*, 1993). This interstimulus interval

was short enough for the animals to compare these postingestive effects, but long enough to prevent confusion between them. Moreover, in these experiments presentation of the reinforcer was proportional to the amount of CS consumed; for every ml of the taste cue consumed, they received 1 ml of reinforcer. Thus, there was a perfect positive contingency between the CS and the US.

Alternatively, in most oral trace procedures in which the rats drink the reinforcer, they receive only one trial per day. In this trial, their consumption of the US is typically not yoked to consumption of the CS. As a result, the amount of reinforcement received for each ml of CS consumed will vary from trial to trial. Hence, the oral trace procedure gives animals fewer trials, inconsistent reinforcement and less of an opportunity to compare the postingestive outcomes of the CS^+ and CS^- than do intragastric reinforcement procedures.

Although intragastric and drug USs are powerful, orally consumed calories are more widely employed as the US in flavour preference experiments. However, in most oral reinforcement conditioning experiments, the taste or flavour solutions are initially acceptable to the animals because the tastes are mixed with either a sweet reinforcer or saccharin. One exception is Rozin, Gruss and Berk (1979), in which they attempted and failed to condition a preference for chili seasoning in rats by exposing them to the seasoning in their food for the first 11 months of life.

When saccharin is added to the taste solutions, three things occur. First, the acceptability of the solutions is increased. Second, the solutions become more alike than when the stimuli are mixed in water, thereby decreasing any bias for one stimulus over the other. Third, according to our data, the solutions also become more conditionable.

Consumption during training in the present study would suggest that the sweetened solutions were much more acceptable than SOA and CA in water. Our failure to obtain conditioned preferences for the unsweetened taste cues, along with the data of Rozin *et al* (1979) suggests that, at least with orally consumed calories as the reinforcer, rats do not readily acquire conditioned preferences for foods that are initially relatively unacceptable. Taste stimuli range from highly acceptable through neutral to highly unacceptable. Perhaps the conditionability of a taste varies according to where it falls on this acceptability continuum, with stimuli on the lower end of the continuum being more resistant to preference conditioning than more acceptable stimuli. It is unclear why animals resist acquiring conditioned preferences to relatively unacceptable stimuli such as SOA, CA and chili peppers. It seems reasonable to assume that initial acceptability is determined to a large extent by the palatability of taste stimuli.

It is also possible that low consumption of the taste cues in water prevented rats from acquiring conditioned preferences to them in Experiments 1 and 3. However, although consumption of the taste cues in the No Saccharin groups of Experiment 3 was low, animals consumed an average of 3-4 ml/trial of SOA and CA in Experiment 1. Although it seems unlikely that our failure to obtain preferences for the unsweetened taste cues was merely a function of low consumption during training, we sought to rule out this possibility in Experiment 4.

Experiment 4

In the present experiment we manipulated rats' exposure to the taste cues in training to determine whether low consumption of the No Saccharin cues in our previous

experiments compromised their ability to acquire conditioned preferences. In the present study, all animals received the citric acid and sucrose octaacetate mixed in saccharin. For the 1 ml Immediate and Delay groups, consumption of these cues was limited to roughly the same amount (roughly 1 ml per training day) consumed by the No Saccharin Condition in Experiment 4. Similarly, the 3 ml Immediate and Delay groups drank 3 ml of the CS cues, which was similar to that consumed by rats in Group Backward in Experiment 1.

If our previous failure to get preferences in the No Saccharin groups was merely a function of the acceptability of the taste cues, then because the CS solutions contain saccharin, preferences should be obtained in both the 1 ml and 3 ml Immediate groups relative to their respective Delay groups. However, if our failure to get preferences occurred because of low consumption of the taste cues, then the rats trained with limited CS exposure in the present experiment should also fail to acquire a preference, regardless of the presence of saccharin in the taste cues.

Method

Subjects: Thirty six male Sprague Dawley rats with a mean weight of 343 g were housed and maintained as previously described.

Procedure: For one week before training, rats (N = 36) were exposed to saccharin alone for 3 days from 8:40 -9:00 am to acclimate them to drinking from the nalgene bottles. Starting on day 4, animals received one training trial per day at 9:30 am for 18 days (9 CS^+ trials and 9 CS^- trials). Groups 1 ml Immediate (n = 12) and 1 ml Delay (n = 12) received 2.5 ml per trial of the CS solutions, whereas animals in Groups 3 ml Immediate (n = 6) and Delay (n = 6) received 4.5 ml of the CS solutions per trial. Since

animals could access only a portion of the solution within each bottle, this amount ensured that they received roughly 1 ml and 3 ml of solution per trial, respectively.

Throughout the training and test phases, animals received 1 hour of supplementary water and food at least 2 hours after the end of each training and test trial.

Results

Training Phase: Animals consumed more glucose (overall mean = 8.01 ml/trial) than water (overall mean = 3.42 ml/trial) throughout conditioning regardless of their group designation. As training progressed, consumption of glucose increased, while consumption of water remained stable. These observations were supported by a repeated-measures 4 x 2 x 9 (Group x Solution (Glucose vs. Water) x Trial) ANOVA which revealed main effects of Solution (F (1,32) = 285.7), Trial (F (8, 256) = 8.0), and a Solution x Trial interaction (F (8, 256) = 5.4).

According to Figure 8, consumption of the taste cues appeared to be relatively stable throughout training for both the 1 ml and 3 ml groups. However, in addition to the main effect of Group (F (3, 30) = 155.28), the 4 x 2 x 9 (Group x Solution (CS^+ vs. CS^-) x Trial) ANOVA revealed a significant main effect of Trial (F (8, 240) = 13.6), which was qualified by a Group x Trial interaction (F (24, 240) = 4.57). Simple main effects of the Trial x Group interaction revealed that only Group 1 ml Delay failed to increase their consumption of the taste cues as training progressed.

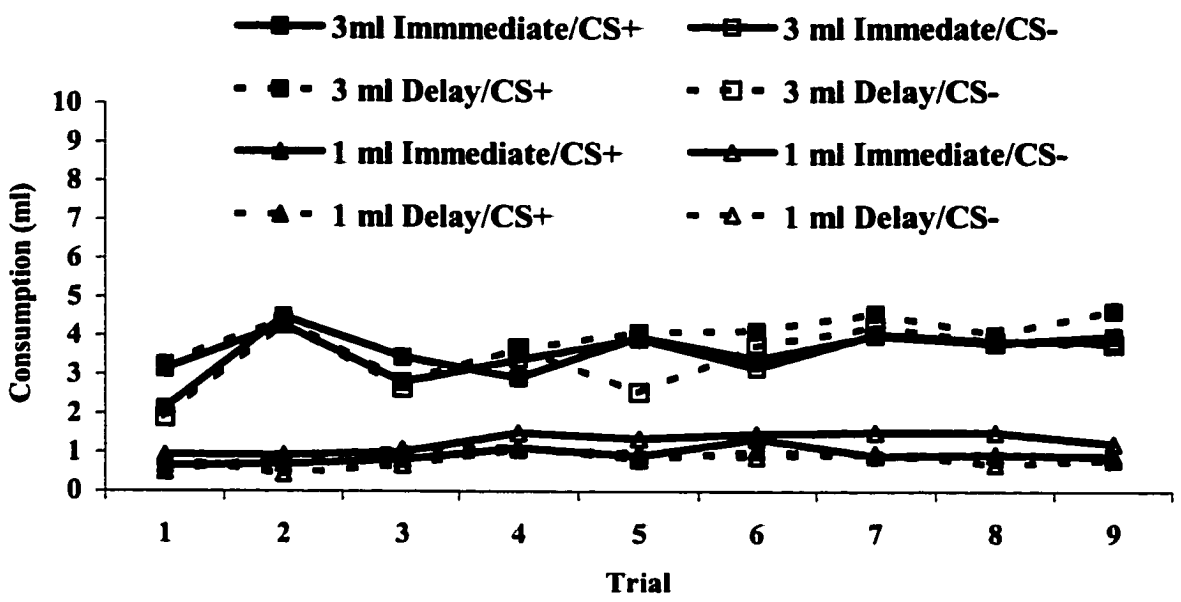


Figure 8: Mean consumption of CS^+ and CS^- solutions during training in the 3 ml Immediate and Delay, and 1 ml Immediate and Delay groups.

Test Phase: Consumption of each taste cue for tests 1 and 2 combined is shown in Table 4. Figure 9 displays mean CS^+ preference ratios for animals in each of the groups in Tests 1 and 2 combined. Group 3 ml Immediate acquired a preference for the CS^+ relative to Group 3 ml Delay, however Group 1 ml Immediate did not differ from Group 1 ml Delay. A Group (Immediate vs. Delay) x Amount (1 ml vs. 3 ml) ANOVA supported this claim by revealing a significant Group x Amount interaction ($F(1, 32) = 6.43$).

Table 4: Mean Consumption (and *SEM*) for the Taste Cues in Tests 1 and 2 Combined in Experiment 4.

	CS^+		CS^-		Total	
Group	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*
1 ml Immediate	28.6	*4.5*	26.6	*3.3*	55.2	*2.9*
1 ml Delay	20.3	*3.3*	28.0	*3.5*	48.3	*2.7*
3 ml Immediate	37.5	*2.6*	21.9	*5.2*	59.4	*4.3*
3 ml Delay	24.9	*4.2*	38.2	*5.6*	63.1	*6.6*

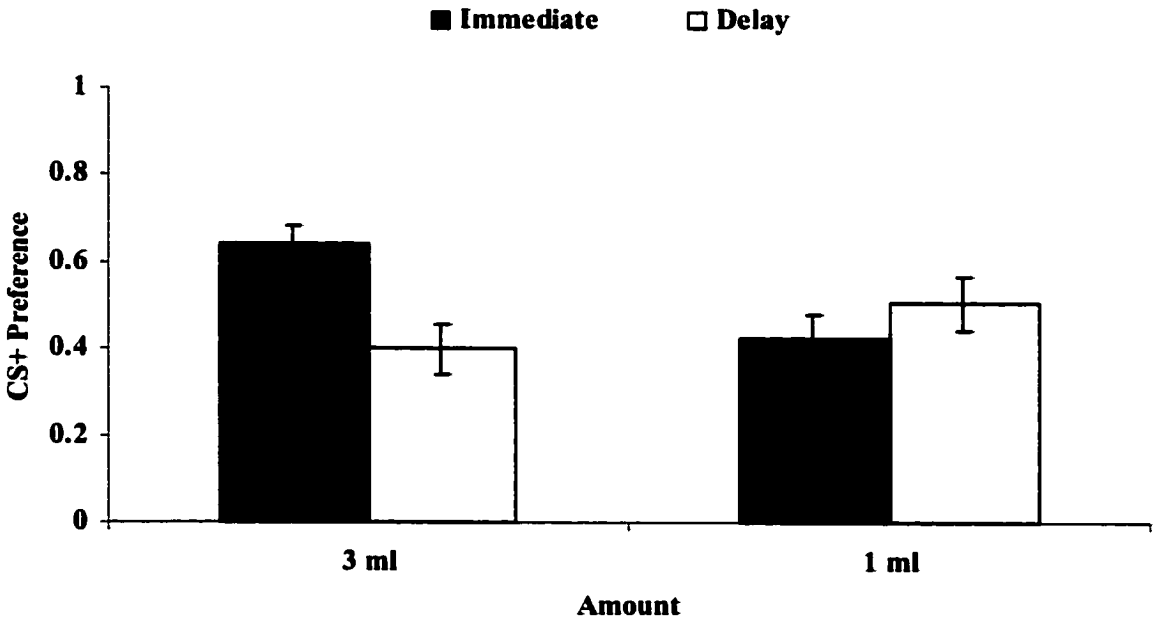


Figure 9: Mean preferences (+/- *SEM*) for the CS^+ relative to the CS^- in the 1 ml and 3 ml Immediate and Delay Groups in Experiment 1A.

Discussion

These results suggest that consumption of 3 ml, but not 1 ml per trial, of the taste cues is sufficient for animals to acquire conditioned preferences in a reverse-order conditioning procedure. Therefore, although our failure to condition preferences in the NS Group in Experiment 3 can be attributed to low consumption in this group, absence of conditioned preferences in Group BI in Experiment 1 cannot be explained by low consumption of the taste cues. Consumption of the taste cues in Group BI in Experiment 1 was roughly the same in the 3 ml groups in the present experiment. However, rats from Experiment 1 received the taste stimuli mixed in water instead of in saccharin, as in the present experiment. This difference between these groups suggests that when trained and tested thirsty and hungry in a reverse-order procedure, rats may acquire conditioned preferences less readily for SOA and CA when mixed in water than in saccharin.

Experiment 5

Rats in our previous experiments were maintained on restricted food rations because we believed that calories from the glucose reinforcer might be less effective in freely fed rats. In Experiment 5, rats were maintained on a different restriction state during conditioning in an attempt to increase consumption of the solutions of citric acid and sucrose octaacetate in water. All animals were thirsty as in previous experiments, however in this experiment they were not hungry[4]. Although this is an effective strategy for increasing consumption of unacceptable aqueous solutions, it has been shown that, with consumption equated, stronger preferences are conditioned when rats are food

deprived during training than when they are trained under *ad libitum* feeding conditions (Capaldi, Owens & Palmer, 1994).

A 2 x 2 factorial design was used in which Immediate or Delay groups received the taste cues mixed in saccharin or no saccharin solutions. No Saccharin Immediate and Delay groups were included to determine whether preferences for relatively unacceptable taste cues could be conditioned under this new restriction state. Immediate and Delay Saccharin groups were included so that if the No Saccharin Immediate group failed to acquire a conditioned preference, we would be able to determine whether this failure occurred because of some consequence of the free availability of food (in this case neither the Saccharin nor the No Saccharin groups should acquire preferences), or because rats do not readily acquire conditioned preferences for the unacceptable taste cues (in which case only the saccharin groups would acquire preferences).

To further minimize the possibility of interference from the free food, we removed the food from the animals' cages just prior to the conditioning trial and returned the food with the supplementary water two hours after each conditioning trial.

Method

Subjects: Thirty-six male Sprague-Dawley rats with a mean weight of 340 g were housed and maintained as described previously.

Procedure: Rats were randomly divided into 2 groups, one of which received saccharin alone while the other received water from 09:00 - 09:20 for three days prior to the conditioning phase. Based on consumption in this pre-training phase, animals that received saccharin were assigned to the Saccharin Immediate (SI) or the Saccharin Delay

(SD) groups. Those that had received water during the pretraining phase were placed in either the No Saccharin Immediate (NSI) or No Saccharin Delay (NSD) groups.

During training, the NS groups were presented with 10 ml of the taste cues. CS consumption in the SI and SD groups was yoked to that of the corresponding NS groups to ensure that animals in the S groups did not consume more than those in the NS groups. Thus, animals in the NS groups received each trial one day prior to those in the S groups. Throughout training, food was removed from the cages each morning prior to the conditioning trial. Two hours after each trial, food was returned to the cages and rats received 1 hour of supplementary water.

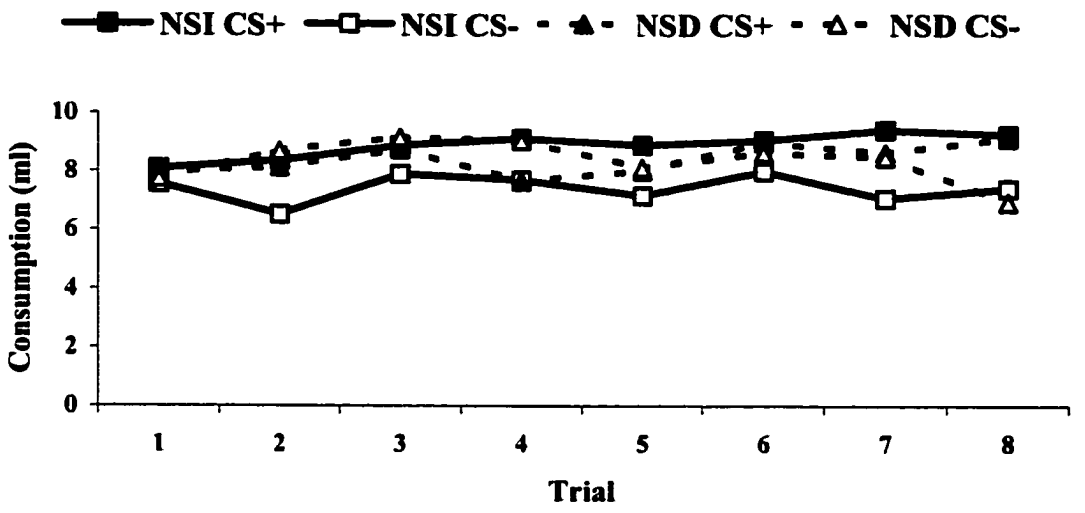
For the first 2-bottle CS test, animals were food deprived. This was accomplished by removing food with the supplementary water after the last training trial and again after the 2-bottle water test. Thus, animals were food restricted for the 2-bottle water test and the CS test. After the second session of the first 2-bottle CS test, food was left on the cages when supplementary water was removed so that animals were not food restricted for the second 2-bottle CS test. In test, animals in the S groups received the CS solutions mixed in saccharin, whereas animals in the NS groups received the taste cues in water.

Results

Training Phase: Rats within the NS groups drank less glucose (overall mean = 8.34 ml/trial) and water (overall mean = 8.16 ml/trial) than those in the S groups (overall means = 8.74 and 8.32 ml, respectively; $F(1,31) = 6.2$). In all groups, consumption of glucose was higher than that of water ($F(1,31) = 6.2$) and consumption of both solutions increased over training trials ($F(7, 217) = 7.6$).

As displayed in Figures 10A and B, overall consumption of the CSs was higher in the NS groups than in the S groups ($F(1,31)= 8.2$). In both groups, CS^+ consumption was higher than that of the CS^- ($F(1,31) = 13.9$) and consumption of the CS solutions increased over conditioning trials ($F(7, 217) = 6.4$).

A. No Saccharin



B. Saccharin

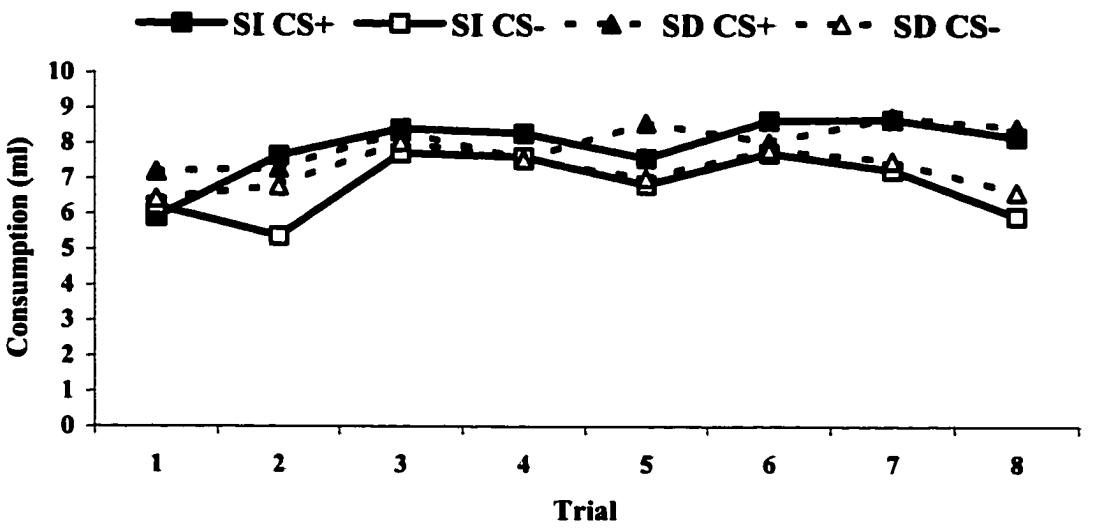


Figure 10: Mean consumption of CS solutions in the No Saccharin (A) and Saccharin (B) groups during training.

Test Phase: As shown in Table 5, when animals were food restricted in Test 1, overall consumption of the taste cues was lower than in Test 2, in which animals were thirsty but not hungry ($F(1, 30) = 19.0$). Moreover, rats in the S groups consumed more of both taste cues over the two tests than rats in the NS groups ($F(1, 30) = 30.9$).

Figure 11 suggests that in the first test, Group Immediate failed to display a preference for the CS^+ over the CS^- relative to the Delay Group in either the S or NS groups. However in Test 2, CS^+ preferences were displayed in both the SI and NSI groups, relative to their respective Delay groups. A Group (Immediate vs. Delay) x Condition (Saccharin or No Saccharin) x Test ANOVA revealed only a main effect of Group ($F(1, 30) = 10.6$). The Group x Test interaction was marginally significant ($F(1, 30) = 2.83$, $p < .1$) Therefore, a post hoc analysis was conducted on Test 1 to determine whether there was a difference between the Immediate and Delay groups. This analysis failed to reveal significant differences in CS^+ preference ratios between the Immediate and Delay groups ($F < 1$) when they were tested water and food restricted in Test 1.

Table 5: Mean Consumption (and SEM) of the Taste Cues in Tests 1 and 2 in Experiment 5

	Test 1						Test 2					
	CS^+		CS^-		Total		CS^+		CS^-		Total	
Group	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*
NS I	12.4	*1.5*	11.6	*2.5*	24.0	*2.9*	23.4	*3.1*	13.8	*2.8*	37.3	*4.1*
NS D	14.8	*2.0*	14.2	*2.5*	29.0	*3.0*	14.0	*2.4*	22.1	*3.8*	36.1	*2.2*
S I	20.7	*3.7*	18.9	*3.3*	39.6	*3.3*	31.1	*1.6*	15.3	*2.3*	46.4	*2.9*
S D	23.0	*2.2*	23.3	*3.3*	46.3	*3.2*	20.8	*4.0*	29.4	*4.2*	50.1	*1.9*

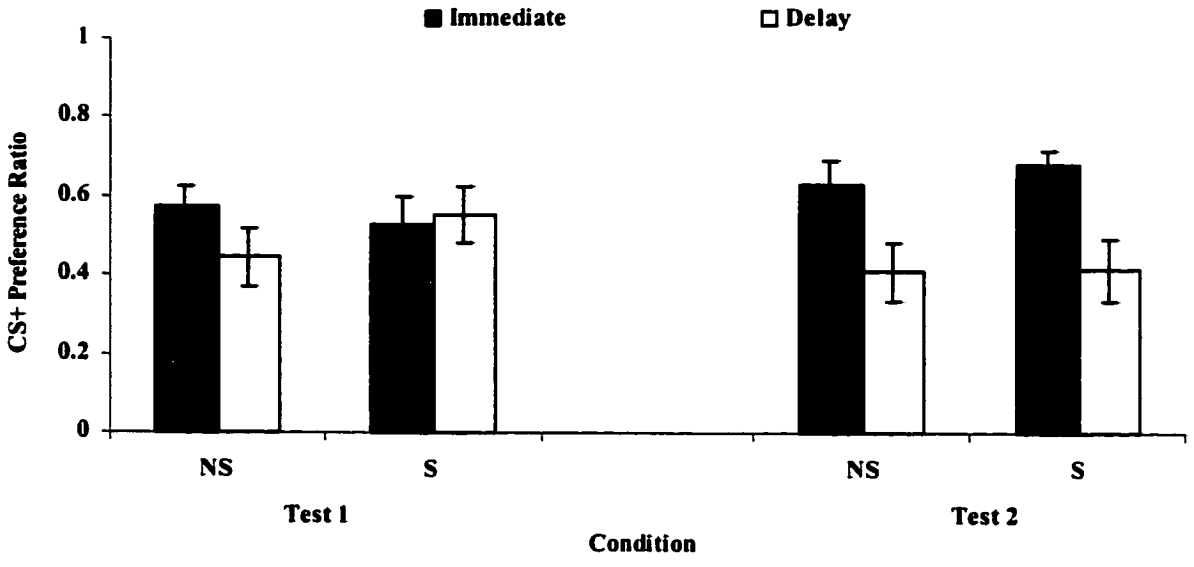


Figure 11: Mean (+/- *SEM*) preferences for the CS^+ in the Saccharin and No Saccharin conditions in the Immediate and Delay groups for Test 1 and Test 2. In Test 1, rats were food restricted, and in Test 2 they were not food restricted.

Discussion

This experiment differed from taste preference experiments previously conducted in our laboratory in that the rats were not food deprived during training. Animals in the NS group consumed all of the CS solution available to them on each conditioning trial and acquired a preference similar in strength to the animals that received the more acceptable taste solutions with saccharin. This result suggests that these animals readily acquired a conditioned preference to SOA and CA when thirsty but not hungry.

Although the Group x Test interaction was not significant, there was a trend for rats to display a preference for the CS solutions only when they were not food restricted.

It is possible that rats failed to exhibit their preference in Test 1 because they experienced generalization decrement between the training and test phases. These results do not support Fedorchak and Bolles (1987; see also Capaldi *et al*, 1994; Harris, Gorissen, Bailey, & Westbrook, 2000; Holder, 1991), which reports that hunger in test enhances the expression of conditioned preferences regardless of whether animals were trained under restricted or *ad libitum* food conditions.

This experiment suggests that animals are capable of acquiring preferences for citric acid and sucrose octaacetate under certain food and water restriction conditions. In Experiment 6, we assessed whether rats that are thirsty but not hungry are capable of acquiring preferences when they receive only 3 ml of the CS cues.

Experiment 6

The design of this experiment was a 2 x 2 factorial in which rats drank either 10 ml or 3 ml of the CS cues, mixed in either saccharin or water on each training trial. Since the previous experiment showed that animals would readily consume and acquire associations to CA and SOA when water but not food restricted, the purpose of the present experiment was to determine whether similar preferences could be conditioned regardless of whether the rats consumed 3 ml or 10 ml of the CS on each training trial.

Method

Subjects: Thirty six male Sprague Dawley rats with a mean weight of 341 g were housed and maintained as previously described.

Procedure: Rats were divided into 4 groups matched according to initial weight and consumption of water during 20-minute sessions in the morning between 9.30 and

10.00. Groups differed as to whether they received 3 ml or 10 ml of the CS solutions on each training trial, and whether these taste cues were mixed with saccharin or water. Thus, group designation was as follows; Group S/3 ml, Group S/10 ml for those that received 3 ml and 10 ml of the CSs mixed in saccharin, respectively. Group NS/3 ml and Group NS/10 ml designated those that received 3 ml and 10 ml of the CSs mixed in water, respectively. For the 3 ml groups, approximately 4.5 ml of the CS cues was put in each training bottle to ensure that animals would have access to 3 ml during training.

In test, animals in the S groups received the taste cues mixed in saccharin, whereas animals in the NS groups received the taste cues mixed in water. Throughout the training and test phases rats were thirsty but not hungry as in the training phase in the previous experiment.

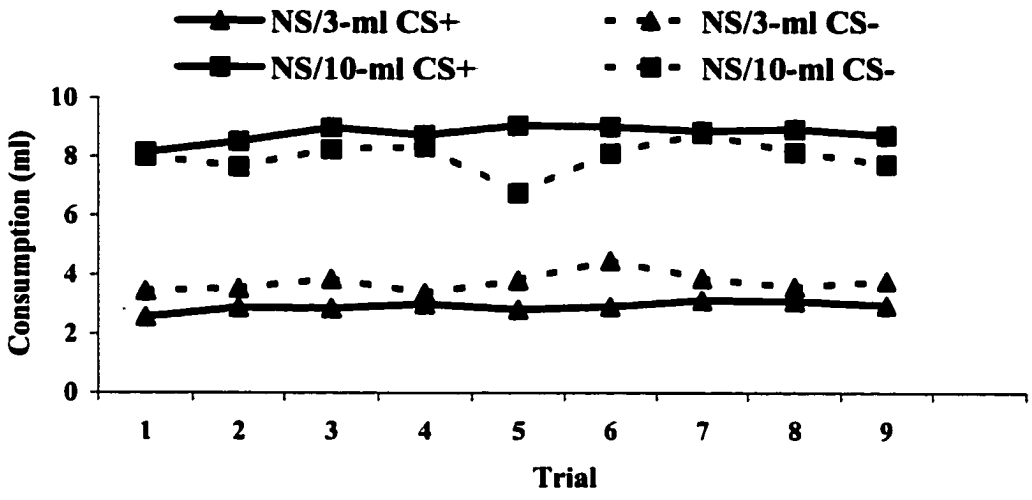
Results

Training Phase: Although consumption of both water (overall mean = 8.36 ml/trial) and glucose (overall mean = 8.76 ml/trial) was high throughout training, rats consumed significantly more glucose than water (F (1,28) = 7.0). As trials progressed, animals increased their consumption of both of these solutions (F (9,252) = 2.6).

The pattern of CS consumption was similar whether the taste cues were mixed in water (Figure 12 A) or in saccharin (Figure 12 B). Rats in the 10 ml condition consumed more than those in the 3 ml condition. Although consumption of the CS^+ and CS^- did not differ for the 10 ml rats, rats in the 3 ml condition consumed more of the CS^- than the CS^+. As training progressed, rats increased their consumption of the taste cues.

These statements were supported by the results from a Group (Saccharin vs. No Saccharin) x Amount (3 ml vs. 10 ml) x Solution (CS^+ vs CS^-) x Trial ANOVA, which revealed a significant main effects of Amount (F (1, 27) = 1035.2) and Trial (F (8, 216) = 3.1) and a significant Solution x Amount interaction (F (1, 27) = 6.8). Simple main effects analysis of this interaction indicated that rats in the 3 ml group consumed significantly more of the CS^- than the CS^+ (F (1,15) = 6.4), whereas consumption of the CS^+ and the CS^- did not differ in the 10 ml groups.

A. No Saccharin



B. Saccharin

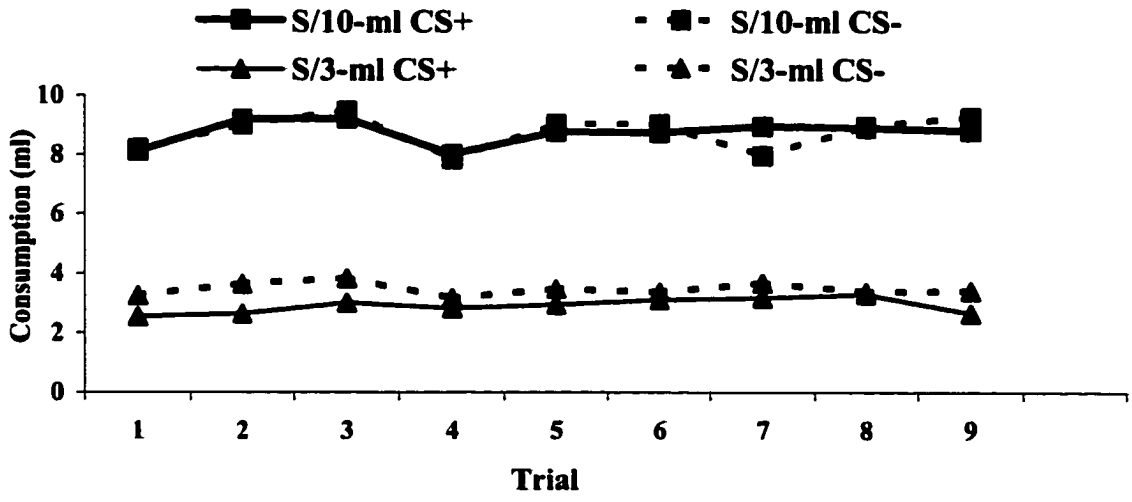


Figure 12: Training consumption of rats that received 10 and 3 ml of the CS in the No Saccharin Immediate (NSI) and Delay (NSD) groups (A) and the Saccharin Immediate (SI) and Delay (SD) groups (B) over 9 days of training in Experiment 6.

Test Phase: Data from Tests 1 and 2 were combined and displayed in Figure 13 and overall consumption of each of the taste cues is shown in Table 6. Inspection of Figure 13 indicates that overall, preferences in the Sacc groups appear to be lower than those in the No Sacc groups.

Preference ratios were analyzed with a 2 x 2 repeated measures ANOVA with Amount (3 ml or 10 ml) and Solution (Saccharin or No Saccharin) as the independent variables. This analysis revealed a main effect of Solution only (F (1, 31) = 8.14). Although there was not a significant Amount x Solution interaction, planned comparisons were conducted between the Saccharin and No Saccharin conditions within the 10-ml and 3-ml groups, since the previous experiment suggested that the presence of saccharin should not affect preferences in the 10-ml group. The difference between Groups S/10-ml and NS/10-ml ($t_{2\text{-tail}}$ (16) = 1.80) failed to reach significance, however in the 3-ml group preferences were higher in the NS rats than in the S groups ($t_{2\text{-tail}}$ (16) = 2.19).

Table 6: Mean Consumption (and SEM) for Tests 1 and 2 combined for all Groups in Experiment 6

	CS^+		CS^-		Total	
Group	Mean	SEM	Mean	SEM	Mean	SEM
S/3 ml	34.2	*4.9*	48.3	*6.3*	82.4	3.7
S/10 ml	44.1	*4*	36.7	*4.4*	80.8	4.2
NS/3 ml	38.3	*3.7*	25.5	*3*	63.9	1.7
NS/10 ml	47.6	*3.6*	25.2	*2.9*	72.8	2.9

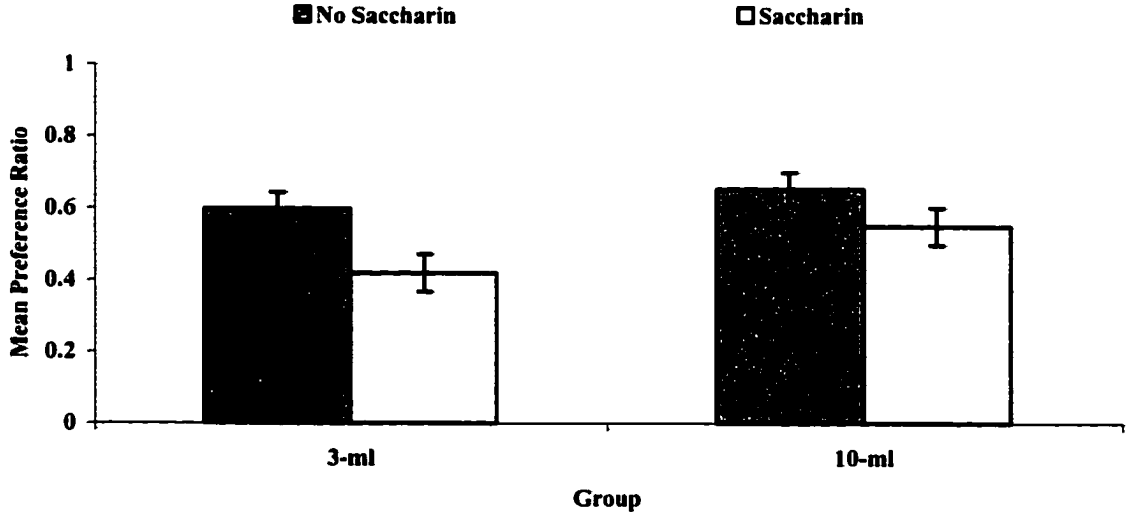


Figure 13: Mean (+/- *SEM*) preference ratios for the CS^+ in groups trained with 3 or 10 ml presentations of the taste cues. These cues were mixed in saccharin (S/3 ml and S/10 ml), or in water (NS/3 ml and NS/10 ml) during training and test.

Discussion

Surprisingly, when animals were thirsty but not hungry, preferences in the Saccharin groups were weaker than in the No Saccharin groups, especially when consumption of the solutions was restricted. This result may have occurred because the presence of the saccharin on both the CS^+ and CS^- trials in training made the discrimination more difficult. It is also possible that the presence of saccharin did not affect the acquisition of the discrimination, but its presence in test obscured expression of the discrimination by adding a common, positively valued stimulus to both choices.

Experiment 7

In an attempt to overcome the detrimental effects on conditioning of including saccharin in the taste cues, we pre-exposed animals to saccharin before conditioning them in the reverse-order procedure. Rats that got extensive exposure received saccharin on each of ten days in the pre-exposure phase. Those that did not receive extensive pre-exposure to saccharin received water for the first 8 days of pre-exposure and saccharin on the last 2 days only (which we have used in previous experiments to reduce neophobia on the first training trial in Saccharin groups). Additionally, we also manipulated the test solutions. Subgroups of rats in each of these groups received the taste cues mixed in water or in saccharin.

In this experiment, all animals received 3 ml of the CS solutions during training. If lower preferences for the CS^+ observed in the Saccharin groups from the previous experiment are truly a function of the detrimental effects of saccharin in training, and pre-

exposure to saccharin results in habituation, which makes the taste cues more distinguishable during training and test, then groups that received extensive pre-exposure to saccharin should show higher preferences than those that received limited pre-exposure to saccharin. Alternatively, if animals learn equally well about the taste cues during training when mixed in saccharin, but their performance in the test is affected by the presence of saccharin, then one might expect that the rats tested without saccharin would show stronger preferences for the CS^+ than those tested with saccharin.

Method

Subjects: Forty-five male Sprague-Dawley rats with a mean weight of 254 g were used in this experiment.

Procedure: Rats were divided into 5 groups based on weight. Two were designated as Saccharin pre-exposure groups and the remaining three groups were designated as No Saccharin pre-exposure groups. The pre-exposure phase lasted for 10 days, during which rats in the Saccharin pre-exposure groups received a 0.2 % saccharin solution, while animals in the No Saccharin pre-exposure groups received water for 10 min for 8 days. On the 9^{th} and 10^{th} days all groups received the saccharin solution for 10 min, except for one group that received only water on every trial.

Training began on day 11. All rats received 16 reverse-order conditioning trials as previously described, except that all groups received 4.5 ml of each of the taste cues on each trial. Additionally, all the groups received saccharin mixed in the taste cue solutions, except for the one group that received only water on all the pre-exposure trials. This group received the CA and SOA mixed in plain tap water throughout training.

The test was also conducted as in previous experiments. Depending on group designation, some rats were tested with saccharin mixed in the test cues, while others were tested with no saccharin in the taste cues. Out of the 4 groups that received saccharin during training (two of which received saccharin pre-exposure, while the remaining two received No saccharin pre-exposure), one from each pre-exposure group was tested with saccharin while the other was tested without saccharin in the taste cues. Thus there were 4 groups; S/S/S, S/S/NS, NS/S/S, and NS/S/NS. The first letter in each group designation refers to whether or not saccharin was mixed in the taste cues in pre-exposure, the second indicates that they all had the taste cues mixed with saccharin during training, and the third refers to whether or not saccharin was present during test. In addition to these four groups, there was also a NS/NS/NS group that received water during all 10 trials of the pre-exposure phase and had the taste cues mixed in water during training and test.

Throughout all phases of this experiment, animals were maintained on a restricted water schedule and had free food access as described in Experiment 5.

Results

Pre-exposure Phase: Rats pre-exposed to saccharin consumed a mean of 12.7 ml/trial over the initial 8 trials. Consumption increased over these 8 trials (F (7, 98) = 34.42), reflecting a reduction in neophobic response to the saccharin as trials progressed. Animals in the No Saccharin pre-exposure groups consumed a mean of 13.8 ml/trial of water over the initial 8 trials. Water consumption in this group was relatively consistent over trials (F<1). On trials 9 and 10, when animals in groups NS/S/NS and NS/S/S received their limited pre-exposure to saccharin, they consumed significantly less (mean

=11.43 ml/trial), than rats in groups S/S/NS and S/S/S (mean =18.9 ml/trial), which had consumed saccharin in the previous 8 trials ($F(1,34)=68.06$). This between-group difference was probably a function of neophobic responses in groups NS/S/NS and NS/S/S to the novel saccharin solution. Group NS/NS/NS, which continued to receive water on trials 9 and 10, consumed an average of 14.4 ml/trial.

Training Phase: Overall means of glucose and water consumption were 8.50 ml/trial and 8.02 ml/trial, respectively. Overall consumption of glucose was significantly higher than consumption of water ($F(1, 41) = 10.6$) in training. There was also a significant stimulus x trial interaction ($F(7, 287) = 3.2$) which occurred because as trials progressed, animals increased their consumption of glucose ($F(7, 238) = 2.7$), while consumption of water remained stable ($F(7, 245) = 2.0$).

Although not obvious in Figure 14, consumption of both taste cues increased as trials progressed, as indicated by a main effect of Trial ($F(7, 28) = 4.6$).

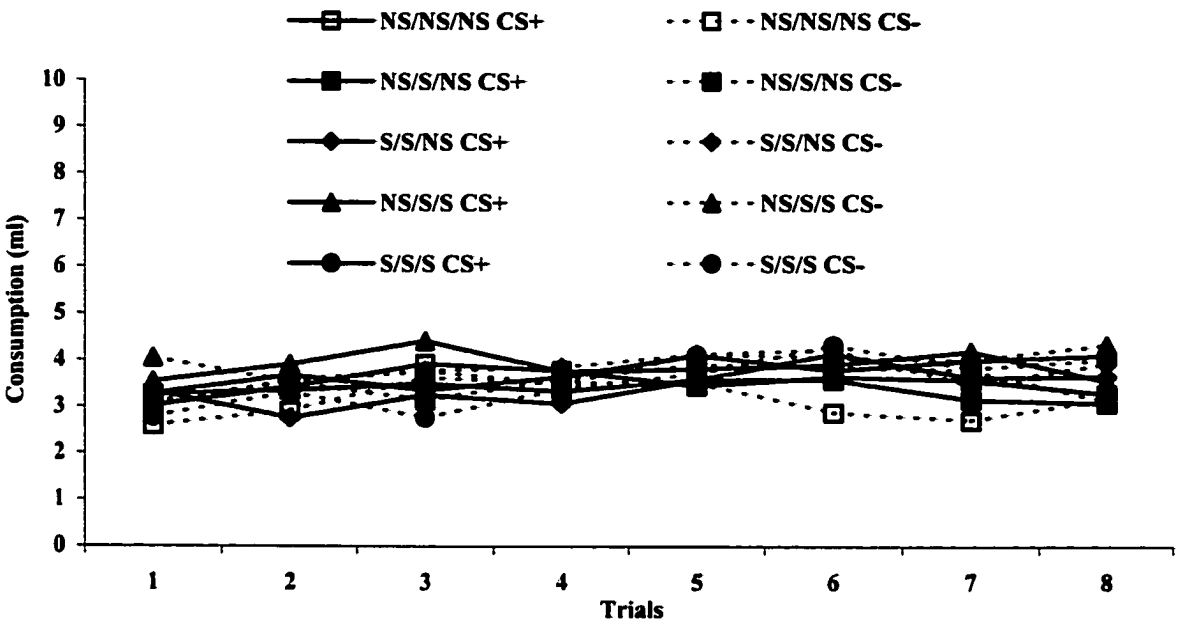


Figure 14: Mean consumption of the CS^+ and CS^- for each of the five groups during training in Experiment 7.

Test Phase: Mean overall consumption of each of the taste cues is displayed in Table 7. As displayed in the Table, overall test consumption was significantly higher in the rats tested with saccharin mixed in the taste cues than in the rats tested with taste cues in water ($t_{2\text{-}tail}(41) = 3.2$).

Observation of Figure 15 suggests that animals trained with saccharin in their taste cues expressed stronger taste preferences if tested without saccharin in the taste cues. Moreover, it appears that this effect was enhanced if rats received extensive pre-exposure to saccharin.

A 2 x 2 analysis of variance was conducted with Pre-exposure (Saccharin or No Saccharin) and Test (Saccharin or No Saccharin in test) as the independent variables. This analysis revealed a significant effect of Test only ($F(1,31) = 6.4$), indicating that preferences were stronger in rats tested without saccharin in the taste cues. Although there was some suggestion in Figure 15 that the interaction of Pre-exposure and Test might reach significance, this claim was not supported.

The mean preference ratio for group NS/NS/NS was .67 (SEM = .05). This group was compared to those rats that were tested with saccharin (NS/S/S and S/S/S combined) and to those tested without saccharin (NS/S/NS and S/S/NS combined). These groups were combined since saccharin pre-exposure had no effect in the previous analysis. This analysis indicated that preferences of rats trained and tested with saccharin were significantly lower than those of Group NS/NS/NS ($t_{2\text{-tail}}(24) = 2.5$), however rats trained with saccharin, but tested without saccharin in their taste cues did not differ from Group NS/NS/NS ($t_{2\text{-tail}}(23) = .8$).

Table 7: Mean Consumption of the Taste Cues (and SEM) for all groups in Experiment 7.

	CS^+		CS^-		Total	
Group	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*
NS/NS/NS	40	3.2	23.2	3.3	63.19	2.649
NS/S/S	41	3.9	39.6	4.7	80.6	2.721
S/S/S	43.6	6	43.2	5.5	86.79	3.885
NS/S/NS	40	4.7	28	3.9	67.7	3.309
S/S/NS	39.1	3.8	26.1	3.3	65.23	3.595

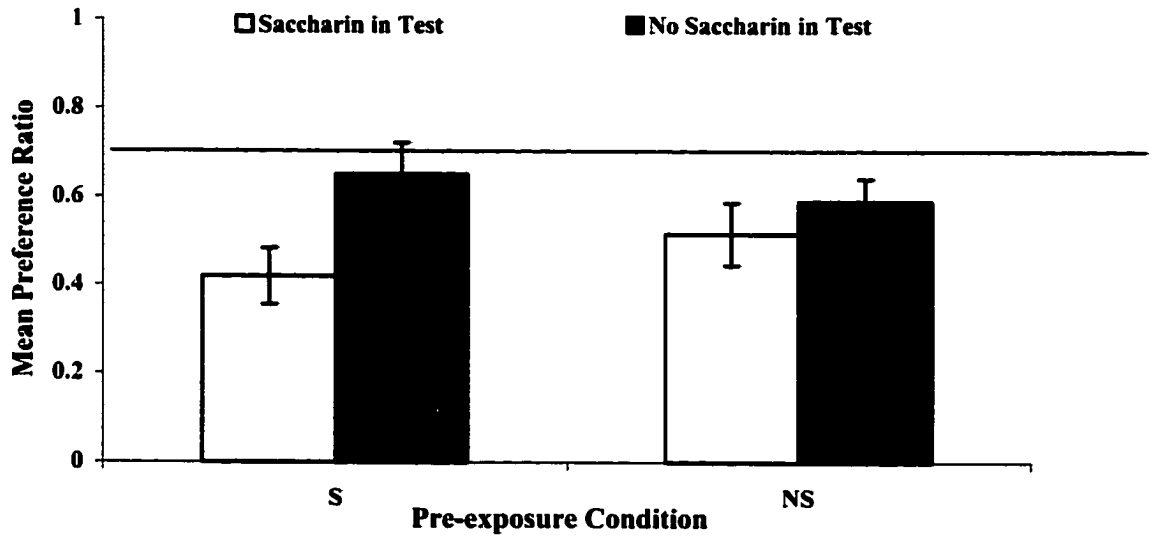


Figure 15: Mean preference ratio (+/- *SEM*) for the CS^+ for each of the four groups trained with saccharin mixed in their taste cues. The line indicates the mean preference ratio for Group NS/NS/NS.

Discussion

We have attempted to overcome the detrimental effects of including saccharin in the CS solutions by pre-exposing the animals to the common element, namely the saccharin, in the CS^+ and CS^- prior to conditioning. Pre-exposure to this common cue should result in its habituation, thereby making the taste cues more easily discriminated in training and test. This did not occur, however; conditioned preferences in test were unaffected by pre-exposure to saccharin. Instead, animals expressed stronger preferences if they were tested with taste cues without saccharin. This indicates that rats that are thirsty but not hungry will acquire similar conditioned taste preferences regardless of

whether saccharin is present or not in the taste cues during training, so long as saccharin is not presented in the test solutions.

The main effect of test solution indicated that the weaker preferences observed in saccharin animals in Experiment 6 were a function of performance rather than learning. As shown in the present experiment, the strength of the conditioned preferences expressed depends on whether saccharin is present in the taste cues during the test phase. This indicates that excitatory associative strength accumulates to the CS^+ (and possibly inhibitory associative strength to the CS^-) in training, but the difference in the values of CS^+ and CS^- in test is attenuated by the presence of the common saccharin element, whether that element has been extensively pre-exposed or not.

General Discussion

The focus of the first chapter was to determine whether rats acquire conditioned preferences for relatively unacceptable tastes when the reinforcer is orally consumed calories. In the first experiment, citric acid or sucrose octaacetate (both mixed in water) either preceded or followed presentation of a calorific reinforcer. In this experiment animals failed to acquire conditioned preferences for these taste cues. The addition of saccharin to the taste cues in Experiments 2 and 3 did result in conditioned preferences for the CS^+ solution. These conditioned preferences occurred regardless of the water deprivation state of the animals during training or testing. Although rats tended to consume more of the taste cues when they were mixed in saccharin than when they were mixed in water, it is unlikely that the preferences conditioned to the taste cues in saccharin were a result of higher consumption of these cues in training. Experiment 4 showed that

rats acquired conditioned preferences to the CS^+ in saccharin when they consumed as little as 3 ml per trial. These data suggest that our failure to condition preferences for CA and SOA in water in Experiment 1, in which rats consumed ~ 3 ml per trial, was not merely a function of insufficient consumption of the taste cues (although this may have been a contributing factor). It is true however, that the extremely low consumption (~ 1 ml) of the taste cues displayed in Experiment 3 probably prevented the occurrence of conditioned preferences.

There are a few possibilities why thirsty and hungry rats more readily acquire conditioned preferences to SOA and CA when trained in saccharin than when trained in water. It is possible that there is something special about saccharin itself that enhances the conditionability of the taste cues. For example, perhaps a cephalic phase response is induced to the CS^- when presented in saccharin. According to Tordoff and Friedman (1989) hungry animals experience discomfort when presented with a sweet taste that is not accompanied by calories, because metabolic fuels are shifted from oxidation and towards storage. This discomfort decreases their preference for the cue presented with the saccharin if calories are not present. Alternatively, cues presented with saccharin that also accompany calories become more preferred because the calories provide fuel to offset reduction in oxidation. This hypothesis predicts that our thirsty and hungry animals should increase their preference for the CS^+ and decrease their preference for the CS^-. The Delay groups should experience the cephalic phase response equally to both taste cues during training, and should therefore fail to acquire a preference. Although this hypothesis is in line with results from the thirsty and hungry animals, there is no reason to believe that

the cephalic phase response is involved in the formation of preferences in the thirsty, not hungry animals, especially for those that receive the taste cues in water. Thus, another mechanism must also be operating to explain the preferences in these relatively sated animals.

It is possible that the acceptability of the taste cues determines their conditionability. It is true that when animals were trained thirsty but not hungry, they acquired preferences for the taste cues mixed in water. In fact in some cases (i.e., when consumption was limited), preferences for the taste cues in water were stronger than preferences for the taste cues in saccharin. As indicated by Experiment 7, this was probably an effect of performance rather than learning, however. Although animals under both restriction states (thirsty and hungry, and thirsty but not hungry) in this study received the same amount of supplementary water each day (with the exception of Experiment 2), animals that were thirsty but not hungry consumed more food relative to water than those that were hungry and thirsty in Experiments 1 and 4. Therefore, animals in these experiments were probably not as thirsty as those in subsequent experiments. It is possible that the palatability of SOA and CA solutions increases when animals are thirsty. This, combined with the fact that animals readily consumed these taste cues when given the opportunity, suggests that SOA and CA in water became more acceptable as thirst increased. This suggests that conditioned preferences occurred to the taste cues in water because they were made more acceptable as a function of the water-restriction state of the rats. Thus, with all else equal, level of thirst appears to be an important factor in determining the acceptability of certain aqueous solutions.

Other factors may also interfere with conditionability. In the case of Experiment 6, the limited quantity of the taste cues available to the Saccharin groups might have decreased their ability to discriminate between the solutions in training relative to the No Saccharin animals, which acquired stronger preferences under these conditions. We tried to determine if this was the case in Experiment 7 by exposing the rats to saccharin prior to training, under the assumption that this exposure would produce habituation of the common saccharin element, thereby increasing the discriminability of the taste cues. Mackintosh, Kaye, and Bennett (1991) have shown that pre-exposure to the common element, lemon of lemon-sucrose and lemon-saline compounds, increased their subsequent discriminability. However, in our experiment, pre-exposure to saccharin failed to affect the strength of preferences observed in test.

Fortunately, the test data provided us with information that suggests that the stimuli were readily conditionable during training. Rats that received the taste cues without saccharin in test showed stronger preferences than those that received the taste cues in saccharin. Moreover, preferences in animals that were tested without saccharin did not differ from Group NS/NS/NS, which never received saccharin. Groups that received the taste cues with saccharin in test showed significantly weaker preferences than Group NS/NS/NS. This suggests that the presence of saccharin in training did not affect acquisition of conditioned preferences; instead the presence of saccharin in test attenuated performance in the 2-bottle test.

In combination, data from these 7 experiments suggest that acceptability of taste cues seems to be an important variable to consider when using the reverse-order procedure

to condition preferences for tastes in rats. Acceptability can be mediated not only by the sensory quality of the solution, but also the restriction state of the rats. Specifically, improvement of the taste quality of a solution by the addition of saccharin, or increasing the rats' level of thirst should increase the rats' performance in test and the conditionability of the taste cues, respectively. Levels of consumption are somewhat less important, so long as rats consume about 3 ml of the taste cues per trial on average. Finally, in some cases in which rats are conditioned with saccharin in the taste cues, it may be advantageous to present the taste cues without saccharin in test.

Chapter 2: Palatability Shifts in Conditioned Taste and Flavour Preferences[5]

The effectiveness of various training paradigms for conditioning flavour preferences has been extensively studied, however the mechanisms involved in the formation of these conditioned preferences have received far less attention. Researchers have identified various reinforcers that effectively condition flavour preferences as well as how they interact with particular physiological influences such as deprivation and satiety. We also know that given optimal training circumstances, conditioned flavour preferences can be extremely robust. Some laboratories condition preferences at the level of 90% that do not readily extinguish. There are at least two possibilities why conditioned animals in these experiments consistently choose to consume the CS^+ rather than the CS^-. According to Berridge (1996), higher consumption of the CS^+ over the CS^- in these preference tests may be governed by at least 2 factors; the degree to which they *want* and/or *like* one cue over the other.

Although intuitively it seems that these two terms are one and the same, in reality these two mechanisms can be affected by different physiological, and pharmacological manipulations (See Berridge, 1996 for an extensive review), indicating that they are governed by separate neural substrates. Wanting corresponds to an animal's motivation to acquire a food item (or its appetite). This is believed to reflect a food item's incentive value; i.e, its ability, and that of its associated cues, to elicit attention and goal-directed behaviour. Liking, on the other hand, refers to the pleasure derived from consuming the particular flavour or the hedonic evaluation of a flavour. This is believed to reflect a flavour's intrinsic value. Thus in conditioning experiments, animals may choose to

consume or avoid a cue because of anticipated positive or negative consequences that were previously associated with the cue, and/or because the intrinsic value of the cue itself has changed.

The goal of the present set of studies was to determine whether changes in liking; i.e., palatability shifts, occur in flavour preference conditioning by employing the taste reactivity measure to assess shifts in liking or palatability.

Palatability and Taste Reactivity

The term palatability is widely used in everyday language, however it is not an easy concept to characterize. Loosely defined, it refers to how much a taste or flavour cue is liked. Most researchers agree that in addition to the sensory qualities of a taste cue, its palatability is dependent on the physiological state and prior experience of the organism consuming it (Grill & Berridge, 1985; Le Magnen, 1987). Although Kissileff (1990) generally agrees with this claim, he asserts that these components should be defined separately rather than under the umbrella term palatability for the sake of clarity.

Although there are various reports in the literature that make claims about palatability, not all of them are based on results from taste reactivity tests. Traditionally, researchers have assessed palatability of cues by whether and how much they are consumed (Ackroff & Sclafani, 1999; Warwick & Weingarten, 1994). It is true that palatability probably does partially determine consumption, however other variables such as an animal's level of motivation and expectations based on previous experience also affect this measure. Moreover, as pointed out by Ossenkopp, Parker and Spector (1995) consumption measures are indirect measures of palatability in that they are *consequences*

of behaviour. A more direct and widely accepted measure of palatability is the taste reactivity test, which has proven to be a useful tool in furthering our understanding of the involvement of neural and pharmacological controls of ingestive behaviour.

Devised in 1978 by Grill and Norgren at Rockefeller University, the taste reactivity (TR) measure consisted of nine quantifiable orofacial behaviours that are elicited in response to solutions infused directly into the animal's mouth via an intraoral cannula. By placing the rat in a Plexiglas cage with a mirror mounted at an angle beneath the cage, these behaviours are readily observed ventrally. Each of these nine behaviours (or fixed action patterns; FAPs) falls into either the positive or the negative category. The negative behaviours that are elicited by typically avoided solutions, such as quinine, include chin rubs, gapes, headshakes, face washes, paw flicks (termed forearm flails) and paw treads (or paw pushes). Positive behaviours, elicited by infusions of solutions that are normally preferred, such as sucrose, include midline tongue protrusions, lateral tongue protrusions and mouth movements. Members of each category are typically elicited in close temporal proximity to each other, indicating that members of each category originate together from hedonic or aversive affective states (Berridge, 2000, Grill & Berridge, 1985). Since Grill and Norgren's original article, a few additional behaviours have been added; namely paw licks and lip flares to the ingestive category. Additionally passive drips are considered to be a neutral behaviour (see appendix for photos and operational definitions of all behaviours).

Although not required for the TR measure (as in Gray & Cooper, 1996; Pelchat, Rozin, Grill & Jacobs, 1983), the introral infusion of solutions is a valuable feature of the

taste reactivity test. As opposed to consumption measures, in which the animal voluntarily drinks the solution, control over the amount and timing of fluid delivery is in the hands of the experimenter. This is very useful for assessing palatability in flavour aversion paradigms and in nondeprived rats, because in both of these situations rats do not readily consume the flavour cues.

According to Grill and his colleagues (eg. Breslin, Spector & Grill, 1992), these TR behaviours likely occur along a single continuum, with paw flicks induced by strongly aversive solutions at one end of the continuum, and lateral tongue protrusions induced by highly palatable solutions at the opposite end of the continuum. The remaining behaviours fall somewhere in the middle of the continuum, with lip flares and mouth movements induced by mildly hedonic solutions and gapes by mildly aversive solutions. They have further shown that as the frequency of positive behaviours increases, the frequency of negative behaviours decreases over 5 minutes following an infusion of a sucrose solution that had been previously paired with LiCl. This indicates that positive and negative qualities of a flavour are mutually exclusive.

This is in disagreement with earlier work by Berridge and Grill (1983; 1984), which showed that the probability of occurrence of positive and negative behaviours could be changed independently of one another. In Berridge and Grill (1984), the probability and quantity of ingestive FAPs elicited by various isohedonic pairs of a sucrose + quinine mixture and sucrose alone were assessed. They found that the incidence of negative responses was greater to the sucrose and quinine mixture relative to the isohedonic sucrose solution within the isohedonic pair of lower concentration. However, ingestive FAPs did

not differ between these two solutions. This suggests that positive and negative categories are orthogonally organized, which is an idea that Berridge continues to support (1996). Thus, an animal may make two independent "decisions" with respect to the negative and positive aspects of a flavour's palatability and the processing of these decisions may be separate rather than integrated (Berridge & Grill, 1984). Although reciprocal changes may occur between positive and negative FAPs in many situations, this does not necessarily mean that they are mediated by a unidimensional mechanism. Examples do exist in the literature in which positive and negative FAPs change independently (see Berridge, 1991; Thiele, Kiefer & Bailey, 1996).

One such example has been demonstrated by manipulating the food deprivation state of rats. In Berridge (1991), rats' positive reponses were suppressed by satiety resulting from voluntarily consumed cereal mash prior to TR tests or involuntarily consumed sweetened milk delivered via gastric intubation. Positive taste reactivity responding was further suppressed by sensory specific satiety; i.e., suppression of positive TR responses to a particular substance (milk or a sucrose solution) was greater if the rats were sated on that particular substance before test. Conversely, rats that were 48 hours food deprived displayed enhanced positive TR responses relative to baseline animals. Negative responses were unaffected by these manipulations, providing further support for Berridge's hypothesis that positive and negative behaviours are orthogonally organised.

Dissociations between Incentive Value and Intrinsic Value of Conditioned Flavours

As previously mentioned, changes in liking and wanting are the result of separate mechanisms. Neural manipulations have shed some light on the neurotransmitters and

brain areas that are responsible for these processes. For example, considerable evidence suggests that opioid neurotransmitters affect palatability (Parker, Maier, Rennie & Crebolder, 1992; Doyle, Berridge, & Gosnell, 1993; Mehiel, 1996). Alternatively, while the mesolimbic dopamine system appears to affect the incentive salience of a reward (Berridge & Robinson, 1998), it does not appear to affect palatability of the reward. Taste reactivity studies in which animals received 6-OHDA lesions that depleted mesolimbic dopamine failed to affect positive taste reactivity responses (Berridge, Venier, & Robinson, 1989). Also injections of the GABA agonist muscimol into the nucleus accumbens increase preferences for sweet food, but do not affect rats' hedonic responses to sweet taste (Berridge, 2001). Thus, while some manipulations affect liking without wanting, others affect appetite and wanting without changing liking. This section will review the few studies involving classical conditioning that have exhibited dissociations between these processes.

Garcia was probably the first study to report this distinction (Garcia, Hankins & Rusiniak, 1974). They indicated that the type of reinforcer was an important determinant of whether palatability shifts or anticipation of a negative outcome would govern avoidance behaviour. When a flavour was paired with gastrointestinal distress, rats avoided the conditioned flavour and reacted to the conditioned flavour with distaste, suggesting that it had become less palatable. When a flavour was paired with an external aversive stimulus such as footshock, only avoidance of the flavour occurred. They suggested that the aversion that occurred for a flavour paired with gastrointestinal distress was a function of a change in the intrinsic value of the flavour, whereas avoidance of a

flavour paired with footshock was a function of a conditioned expectation of danger upon the consumption of the flavour cue.

Subsequently Pelchat, Rozin, Grill and Jacobs (1983), extended the results of Garcia *et al* (1970) by employing the taste reactivity test of Grill and Norgren (1978) to reliably assess palatability. In this study, rats that received a sugar solution paired with footshock subsequently avoided the sugar when it was presented with water in a 2-bottle test, but continued to display positive TR responses to the sugar solution in a TR test. However, rats that received the sugar solution paired with LiCl, not only avoided the sugar solution in a 2- bottle test, but they also displayed increased negative responding (i.e., gapes) in the TR test. In this study, Pelchat *et al* more convincingly demonstrated that avoidance of a sucrose solution paired with footshock was a function of anticipated danger and not a change in the palatability of the sucrose solution, whereas avoidance of sucrose that had previously been paired with nausea was a function of acquired dislike for sucrose.

More recently, research in Parker's laboratory has revealed further dissociations in flavour avoidance learning. In these studies, Parker and colleagues showed that when paired with sucrose, high doses of rewarding drugs such as methylphenidate, cocaine and amphetamine produce conditioned avoidance but do not condition a shift in palatability to sucrose (see Parker, 1995 for a review). In combination, these studies show that at least in conditioned taste avoidance studies, changes in wanting or incentive value of a conditioned stimulus can occur without associated changes in liking or intrinsic value of the CS.

Palatability Shifts in Flavour Preference Conditioning

Since conditioned taste aversion paradigms sometimes result in changes in incentive value without corresponding changes in intrinsic value, it is of interest to determine whether palatability shifts accompany conditioned flavour preferences as measured by 2-bottle consumption tests. Although few studies have assessed palatability shifts as a result of flavour preference conditioning, there are precedents for this in the literature. For example, it has been shown that some drug reinforcers are capable of conditioning increases in shifts to palatability. In one such study, Zellner, Berridge, Grill and Ternes (1985) exposed rats to a bitter morphine solution as their only source of fluid repletion for 9 months. Extended forced exposure to this initially unacceptable drug produced a significantly higher preference for morphine over water, relative to rats raised with access only to tap water. This preference persisted across drug replete and drug withdrawal states. Moreover, in taste reactivity tests rats that had received repeated exposures to morphine displayed significantly fewer aversive responses and more ingestive responses than rats that were raised on tap water. Similarly, Flynn Webster and Ksir (1989) found increased preference ratios for nicotine over water accompanied by increased positive TR responses to nicotine in rats previously exposed to a nicotine solution and water for 96 days, relative to a control group that received only water. Interestingly, although nicotine was avoided at concentrations of 5 μg or greater in the 2-bottle, long-term intake tests, positive responses were induced by concentrations of nicotine up to 50 μg. These data suggest that the postingestinal effects of nicotine at least partially affect consumption in long-term tests.

In addition to drug reinforcers, a few examples exist in which nutritional reinforcers; i.e., those containing calories or those that provide nutritional repletion, have successfully conditioned upward shifts in palatability. For example, Breslin, Davidson and Grill (1990) have demonstrated conditioned increases in palatability to initially unacceptable flavour cues such as hydrogen chloride (HCl) and quinine as a function of sucrose reinforcement. In this paper, Breslin *et al* demonstrated a conditioned increase in positive TR responses and a conditioned decrease in negative TR responses to the CS^+ relative to the CS^-. Since animals received only .5 ml of the US; a 32% sucrose solution, these palatability shifts may have been a result of taste-taste rather than taste-calorie associations. Animals were not given a 2-bottle preference test in this experiment, so it is impossible to determine if a conditioned increase in flavour preference accompanied these palatability shifts.

Sclafani's laboratory has also measured palatability shifts to flavour cues paired with intragastric reinforcement, albeit with mixed success. In their first attempt (Elizalde, 1990) rats in the Experimental Group were given 24 h presentations of taste cues (0.03% SOA and 0.05% CA), the consumption of which was paired with IG infusions of 32% Polycose or water, whereas a Control group received the same amount of exposure to the same taste cues, both of which were paired with water infusions. Strong conditioned preferences were observed; the Experimental Group showed a 97% conditioned preference for the CS^+ over a CS^-. This preference was significantly higher than that of the Control Group, which drank similar amounts of the CS^+ and CS^-. However, there was no evidence

of conditioned shifts in palatability to these taste cues, although more mouth movements to both CS solutions were observed in the Experimental Group than in the Control Group.

Until recently these were the only existing reports that assessed whether palatability shifts occurred in flavour preference conditioning. Their discrepant results could have been due to various methodological differences between the studies. As previously noted, Breslin *et al* (1998) used a very small amount of a calorically dense reinforcer. Therefore palatability shifts observed in this study may have been a function of taste-taste associations, whereas Elizalde's (1990) animals acquired taste-calorie associations. Could it be possible that taste reinforcers are more effective at conditioning palatability shifts than calorie reinforcers?

According to a recent report by Myers and Hall (2000), both flavour-taste and flavour-calorie associations are capable of conditioning increases in palatability to flavour cues. In this study food restricted rats that received flavours paired with either sweet, low calorie or nonsweet, high calorie reinforcers showed conditioned preferences for and more positive TR responses to these flavours relative to a flavour paired with a nonsweet, low calorie reinforcer.

More recently Myers and Sclafani, 2001a provided further support that calories alone are capable of conditioning increases in palatability of flavour cues. In this paper, food restricted animals received 20 h/day access to grape or cherry Kool Aid sweetened with .05% saccharin and paired with 16 % IG reinforcement over several days. As in Elizalde's (1990) experiment, animals showed strong preferences for the CS^+ in two-bottle tests. They also exhibited an overall increase in hedonic FAPs to the CS^+, but not to the

CS^-, when the individual hedonic behaviours were combined. Further, they compared taste reactivity responses to the taste cues with those of various concentrations of fructose (a sweet tasting saccharide that has delayed reinforcing effects relative to glucose; Ackroff, Touzani, Peets & Sclafani, 2001). They found similar levels of ingestive taste reactivity responses to the CS^+ and 16% fructose, and between the CS^- and 3% fructose. Interestingly, although the sum of the ingestive FAPs was the same for the CS^+ and the 16% fructose, the pattern of behaviours differed between them, indicating that although the level of palatability was comparable between these two solutions, the rats did not consider the CS^+ "sugar-like".

One of the aims in the present set of experiments was to determine if oral calorific reinforcement is capable of conditioning preferences to relatively unacceptable taste stimuli in the reverse-order procedure. Further, similar to Myers and Hall (2000) we sought to directly compare the strength of palatability shifts produced by flavour-taste and flavour-calorie associations in a simultaneous flavour preference conditioning paradigm using flavours rather than tastes as CSs. In addition to shifts in positive TR responses, we also assessed changes in aversive responses.

General Method

Subjects: Sprague-Dawley rats obtained from Charles River Canada were housed and maintained as described in the General Methods section in Chapter 1 for Experiments 8-10 unless otherwise specified.

Apparatus:

Stimuli: In Experiments 8 and 9 conditioned stimuli were the same as in Chapter 1; 0.03% Citric Acid and 0.03% Sucrose Octaacetate. Additionally in Experiment 9, some animals received flavour stimuli; 0.05% (w/v) Cherry Kool Aid and 0.05% (w/v) Grape Kool Aid (unsweetened, Kraft Canada, Inc.). For all rats in Experiment 10, the Kool Aid (0.1% Grape and Cherry) flavour stimuli served as CSs. Unless otherwise specified, the US was an 8% (w/v) glucose solution.

Taste Reactivity: The taste reactivity chamber consisted of a clear acrylic chamber (45cm long x 23.5 cm wide x 21.5 cm high), which was divided in 2 equal compartments. For Experiments 9 and 10, the ceiling was lowered to a height of 13 cm to reduce rearing. Each compartment had an acrylic lid with a hole in the centre to allow the tubing to pass from the animal to the infusion pump (Harvard Apparatus Pump 22). The chamber sat on an acrylic base under which a mirror was positioned at a 45° angle. A video camera with a telephoto lens was positioned approximately 90 cm from the surface of the mirror and was connected to a video-cassette recorder (VCR, Panasonic AG-2560). A 13-inch monitor was connected to the VCR and was used as a guide to position of the camera during testing.

Procedure:

Surgery: Animals were first treated with atropine (.4 mg/kg, ip) and then anaesthetized 15 min later with rompin (10 mg/kg, ip) and ketamine (80 mg/kg, ip). A 3 in, 15 G hypodermic needle (Harvard Apparatus) was inserted under the skin in the back of the neck and guided subcutaneously under the skin until the tip of the needle reached

the area behind the animal's first molar. The needle was then pushed through the inner wall of the animal's mouth through the buccal muscle, where it exited near the rear molars. Polyethylene Tubing (PE90, Becton Dickinson), was then inserted into the shaft of the needle, and the needle was carefully removed while holding the end of the tubing outside the rat's mouth. The tubing was secured by inserting the metal shaft of a .5 in, 20 G needle (Small Parts, Inc.) at the neck and a Buna-N o-ring (1/16" ID x 3/16" OD, Small Parts, Inc) in the mouth. The o-ring was secured by heat flaring the end of the tubing in the mouth. Three subcutaneous injections of warmed physiological saline were administered to each animal to replenish fluid levels. Animals were given a week to recover from surgery before any behavioural testing occurred.

Data Analysis: All taste reactivity video was analysed at ½ the regular playback speed, with the aid of observation software on an IBM PC. Frequency of tongue protrusions and mouth movements were scored by hand, frame-by-frame. All scoring was done by an observer who was blind to the experimental designation of each animal. The following positive behaviours were measured: frequency of tongue protrusions (tp), lateral tongue protrusions (ltp), mouth movements (mm), lip flares (lf) and paw licks (pl). The negative behaviours measured included: frequency of headshakes (hs), paw flicks (pf) chin rubs (cr), paw treads (pt), gapes (g), and 2-s bouts of face washes (fw). Additionally duration of rearing (r) and frequency of passive drips (pd) were recorded. See Appendix 1 for description of each of the behaviours scored in the present study.

Experiment 8

The purpose of the present experiment was to determine whether palatability shifts could be conditioned to relatively unacceptable taste cues such as CA and SOA using the reverse-order conditioning procedure. If animals' conditioned preferences, as measured in 2-bottle tests, are a result of conditioned shifts in palatability, we should see an upward shift in positive TR and a downward shift in negative TR responses to the CS^+ in the Immediate Group. The conditioning procedure should not affect responding to the CS^+ in the Delay group.

In this experiment, animals' unconditioned responses to the CS solutions were measured before and after conditioning. Differences between the pre and post conditioning measures were assessed to determine whether palatability of the taste cues had been shifted as a function of conditioning. Although animals were thirsty but not hungry during training and the 2-bottle tests, they were neither thirsty nor hungry during the TR tests. We shifted the animals' restriction state for the TR tests because rats tend to show high levels of positive responses when they are thirsty but not hungry. Since they show fewer ingestive responses when they are neither thirsty nor hungry (Forestell & LoLordo, 2000), we chose this restriction state for the TR test to optimize our chances of seeing upward shifts in palatability between the pre and post tests.

Method

Subjects: Thirty-three male Sprague-Dawley rats obtained from Charles River, Canada, were housed as described in the General Method. The mean weight of the rats at the beginning of the experiment was 316 g. Throughout training animals were maintained

on restricted water and free food as described in the General Methods section of Experiment 1, however during the taste reactivity test, all rats received free access to both food and water.

Procedure:

This experiment consisted of three phases: a pretest, conditioning and a posttest.

Pretest Phase: Prior to receiving their taste preference conditioning trials, rats received one trial per day for three days in the taste reactivity chamber. On all trials they received a 2-minute infusion of solution at a rate of 1 ml/min. Day 1 was a habituation trial in which all animals received water infusions, whereas days 2 and 3 were test trials in which they received either CA or SOA presented in a counterbalanced fashion. Throughout this pretest phase, animals were not food or water restricted.

Conditioning Phase: Animals were conditioned as described in the General Methods section of Experiment 1. They received 8 training trials in which the CS^+ was paired with glucose and the CS^- was paired with water. The two types of trials were presented in a double alternation sequence (ABBA). Prior to each training and test trial, food was removed from the cages. It was returned with supplementary water each day. Animals received 1 hour of supplementary water, 2 hours after the end of each training trial.

Test Phase: On the first three days of the test phase, animals received a 2-bottle preference test (consisting of one 2-bottle water acclimation session and two 2-bottle CS sessions). For these three days rats were maintained on the same water and food restriction schedule as in the training phase. On day 3 of the test phase, supplementary food and

water were left on the cages. Animals were given 3 days to acclimate to free access to water. On day 6 of the test phase, animals were exposed to the TR chamber for 3 min, during which time they received a 2 min infusion of water at a rate of 1 ml/min. On days 7 and 8 animals received infusions of the CS solutions, in a counterbalanced order, at a rate of 1 ml/ min for 2 min. During the CS tests, the animal's orofacial responses were recorded. At the end of the second TR test on day 8, water was removed from the animals' cages. Again they were given 3 days to adapt to the new water restriction schedule. On days 11 and 12, they were given another 2-bottle preference test with the taste cues.

Results

Training Data: With the exception of trial 1, rats in all groups consumed virtually all of the glucose available to them (daily mean = 8.95 ml). Consumption of water was less than that of glucose (daily mean = 8.18; F (1, 26) = 31.0). Although consumption of both solutions increased (F (7, 182) = 26.2) as training progressed, this increase was greater for glucose consumption than for water consumption, as indicated by the Group x Stimulus interaction (F (7, 182) = 7.2).

Consumption of the CS solutions during training is shown in Figure 16. For animals in the Immediate group, consumption of the CS^+ appeared to be higher than that of the CS^-, whereas the Delay groups appeared to consume more of the CS^- than the CS^+. These observations were supported by a repeated-measures Group (Immediate vs. Delay) x Stimulus (CA and SOA) x Trial ANOVA. This analysis yielded a main effect of Trial (F (7, 196) = 44.6), and a significant Group x Stimulus interaction (F (1, 28) = 10.7). Simple main effects analysis revealed that while the Delay group consumed more CS^- than

CS^+ (F (1,14)=7.8), the Immediate group consumed more of the CS^+ than the CS^- (F(1,13)=3.3).

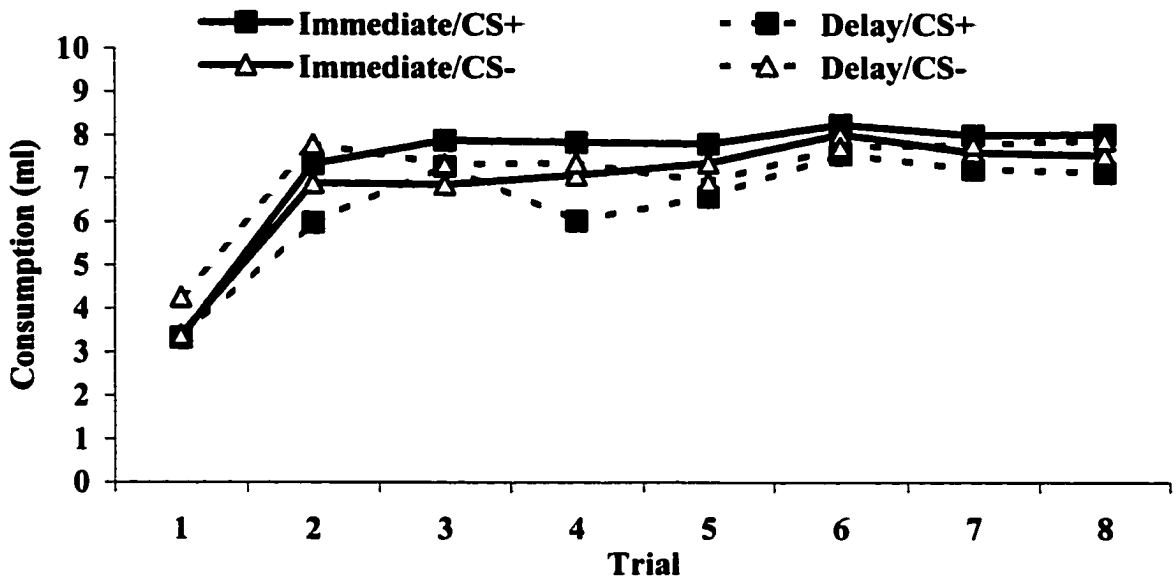


Figure 16: Mean consumption of the CS^+ and CS^- for the Immediate and Delay groups throughout training in Experiment 8.

Two-bottle test data: Table 8 presents mean absolute consumption of the two taste solutions in each of the tests. As displayed in the Table, overall consumption of the taste cues did not differ between the Immediate and Delay groups, nor did it differ between the pre and post TR tests. Figure17 displays CS^+ preference ratios for animals in the Immediate and Delay groups in 2-bottle preference Tests 1 and 2. In each test, preferences for the CS^+ were greater in the Immediate group than in the Delay group. This claim was supported by a Group x Test (Pre vs. Post TR tests) repeated measures ANOVA which

revealed a main effect of Group ($F(1, 27) = 19.4$). Thus the intervening TR test had little impact on performance in the second 2-bottle test.

Table 8: Mean absolute consumption (+/-SEM) of the CS^+ and CS^- before (Pre TR Test) and after (Post TR Test) the taste reactivity test in Experiment 8.

	CS^+		CS^-		Total	
Group/Test	Mean	SEM	Mean	SEM	Mean	SEM
Immediate/Pre TR Test	23.6	2.3	12.2	1.6	35.8	1.3
Delay/Pre TR Test	12.8	2.3	19.7	2.4	32.5	1.6
Immediate/Post TR Test	20.1	2.3	10.8	2.0	30.8	1.5
Delay/Post TR Test	10.7	1.5	20.8	2.3	31.5	2.0

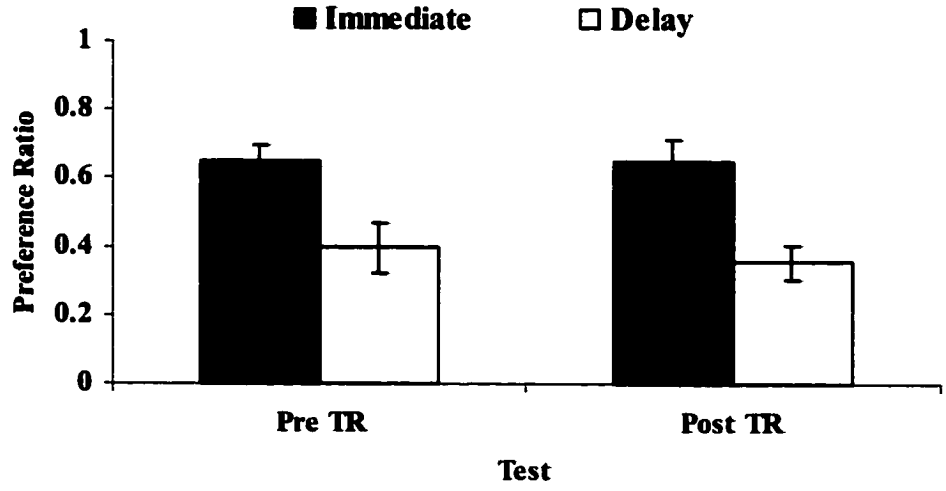


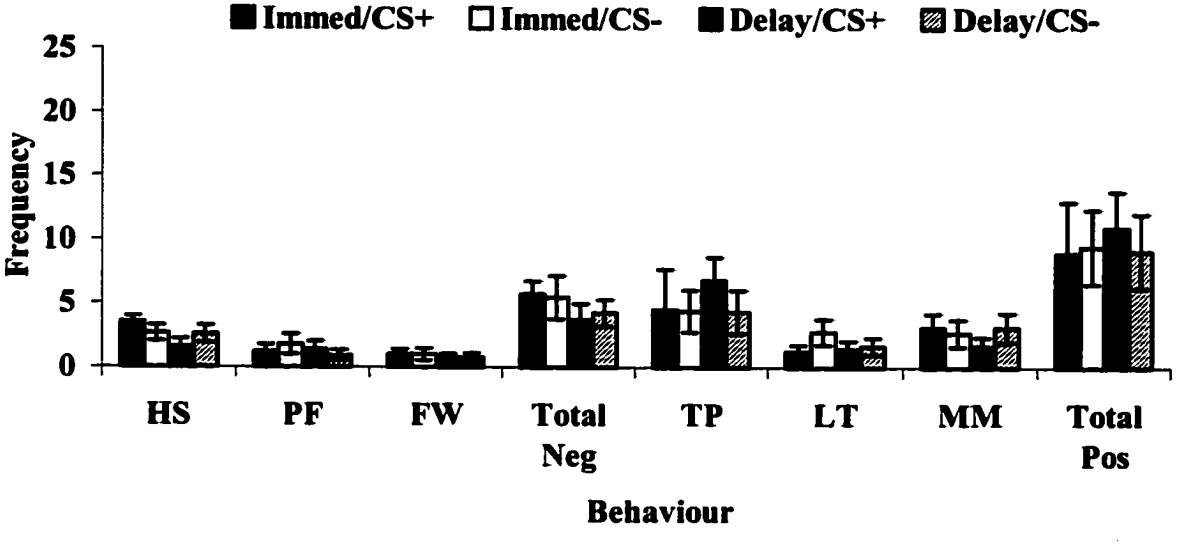
Figure 17: Mean (+/- *SEM*) preference ratio for the CS^+ in the Immediate and Delay groups both before (Pre TR) and after (Post TR) the taste reactivity test in Experiment 8.

Taste reactivity data: The absolute frequency of each of the positive and negative orofacial reactions is displayed in Figures 18 A and B. Orofacial responses prior to conditioning (Figure 18A) did not differ between groups, nor did they appear to differ between taste cues. However, after the rats were conditioned (Figure 18B), the Immediate group displayed more tongue protrusions to the CS^+ than to the CS^-, whereas rats in the Delay group did not tongue protrude differentially to the CS^+ and CS^-.

These assertions were supported by a series of repeated-measures ANOVAs, in which group (Immediate vs. Delay) was the between-subjects variable and Time (pre conditioning vs. post conditioning) and Stimulus (CS^+ vs. CS^-) were within-subjects variable for the total of the positive and negative TRs and for each orofacial reaction. This analysis yielded a significant Group x Time x Stimulus interaction for total positive behaviours ($F(1, 52) = 4.27$) and for tongue protrusions ($F(1, 24) = 7.7$). Simple main effects analysis revealed an effect of Stimulus in the Immediate Group in the post conditioning TR test only for total positive behaviours $F(1, 12) = 5.0$) and for tongue protrusions $F(1, 12) = 6.4$). For the Delay group, the simple main effect of stimulus failed to reach significance in both the pre and post conditioning TR tests.

Duration of rearing (not shown in graph) was also included in the above series of analyses. This analysis failed to yield any significant effect, indicating that rearing to the CS^+ relative to the CS^- did not differ between groups.

A. Pre Conditioning



B. Post Conditioning

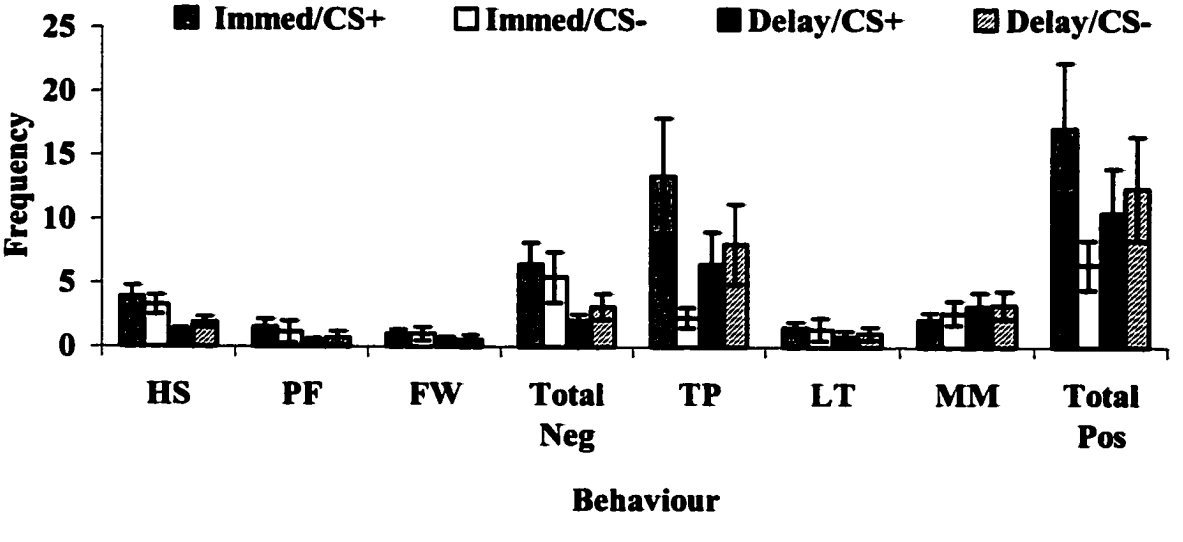


Figure 18: Mean frequency (+/- *SEM*) of positive and negative TR responses before and after conditioning in Experiment 8.

Discussion

In the present experiment, animals trained with a reverse-order conditioning procedure displayed more tongue protrusions to the CS^+ than to the CS^- after conditioning. The Delay Group, which had a long interval between the glucose and the CS^+ during conditioning did not show this effect. This shift in ingestive behaviour suggests that the palatability of the CS^+ cue may have increased as a function of its pairing with glucose in the Immediate Group. It is worth noting, however that we failed to see any significant differences in the aversive behaviours as a result of the reverse-order conditioning procedure. This suggests that although the Immediate animals may "like" the CS^+ more as a function of conditioning, there are components of the taste solutions that the animals still dislike. It is also possible that we failed to observe differences in the aversive behaviours because their frequency was not high enough. On average, each of these behavioural responses was displayed only between 1 and 5 times per 2-min session. Although our failure to see differences within the individual aversive behaviours may have been due to a floor effect, this is unlikely since there was no difference in *overall* aversive responding to the CS^+ and CS^-.

Since the animals' food deprivation state differed between the taste reactivity tests and conditioning, they may not have generalized between these two situations. Perhaps the rats perceived the taste cues to be very different under the two deprivation states. If this is the case, it may explain why we did not observe shifts in more of the orofacial responses measured in this experiment. Our effect may have also been weakened by pre-

exposure to the taste cues during the pre conditioning TR test. Perhaps latent inhibition weakened conditioning to the CS^+.

Several procedural changes were made in the next experiment in an attempt to enhance the taste reactivity effect observed in the previous experiment.

Experiment 9

In the present experiment half of the animals received the same reverse-order conditioning procedure as in the previous experiments, except that the CS solutions were orally infused during each training session. Additionally a Forward group was included that also received oral infusions of each of the CS solutions, immediately followed by either glucose or water. Although we have failed to condition taste preferences to cues presented in a forward conditioning procedure in previous experiments (cf. Experiment 1), this procedure is similar to that of Breslin *et al* (1990), which successfully conditioned palatability shifts; i.e., positive behaviours were shifted upward while negative behaviours were shifted downward as a function of forward pairings of CS and US infusates. One remaining difference between the training procedure in the present experiment and that of Breslin *et al* is that animals in this experiment voluntarily consumed the glucose or water on each conditioning trial, whereas the US was orally infused in Breslin *et al*.

An additional change made in this experiment was that animals were not exposed to the CS solutions in a TR test prior to conditioning. Since the post test in the previous experiment was sufficient to show differences between responding to the CS^+ and CS^-, we chose not to risk the possibility of latent inhibition occurring as a function of pre exposure to the CS solutions. Moreover, animals were water but not food restricted throughout the

conditioning and test phases to avoid any generalization decrement that might have occurred in the previous experiment as a function of changing the animals' deprivation state for the TR tests.

The present experiment included a group that received the usual taste cues; citric acid and sucrose octaacetate as CSs and another group that received flavour cues; grape and cherry Kool Aid as CSs. As previously discussed, Kool Aid flavours have been shown to be very effective cues in flavour preference conditioning experiments (Myers & Hall 1998, Myers & Sclafani, 2001b, Sclafani, Cardieri, Tucker, Blusk, Ackroff, 1993). According to A. Sclafani (personal communication, November, 2000), these flavour cues may be more amenable to flavour preferences and palatability shifts than the citric acid and sucrose octaacetate taste cues. Indeed this may partially explain Elizalde's (1990) failure to condition palatability shifts (even though she successfully conditioned taste preferences for these taste cues).

Finally, the taste reactivity test procedure was altered in an effort to make it more sensitive. Grill Roitman and Kaplan (1996; also see Kaplan, Roitman & Grill, 1995) asserted that the procedure as usually conducted is subject to ceiling and floor effects on mouth movements, which can be avoided if the taste is infused for 15 s, and taste reactivity responses are measured not only during the infusion, but for the 45 s following the infusion. Grill *et al* found that post infusion mouth movements were more affected than were mouth movements during the infusion by food deprivation. Moreover, when glucose concentration was varied, mouth movements after infusion showed a bigger dynamic range than mouth movements during infusion, indicating that assessment of

mouth movements after a short infusion is a valuable tactic. In the present experiment rats were water but not food restricted during the training and test phases. Since we know that under this restriction state rats show many positive responses, we were concerned that a ceiling effect would prevent us from detecting differential responding to the CS^+ and CS^- if rats received a full 2-minute infusion in each TR session.

These changes were made in the present experiment to determine whether we would observe shifts in more of the positive and negative TRs in test as a function of conditioning than in the previous experiment.

Methods

Subjects: Forty-eight male Sprague-Dawley rats with a mean weight of 390 g were housed as described in the General Methods. Rats were maintained on free food, however they received 1 hour of supplementary water each day as described below throughout training and testing.

Apparatus: Rats were trained in plastic tub cages with wire covers. Each of the rats' adaptors was connected to PE 90 tubing, the distal end of which was connected to one of five 10-ml syringes within a Razel infusion pump. A sixth syringe was connected to a Harvard apparatus infusion pump. Glucose and water were presented in glass bottles on the top of the rats' cages on each conditioning trial.

Procedure: All rats were cannulated according to the protocol described in the General Methods and given 1 week to recover from surgery. Two days prior to the training phase, animals were acclimated to the training cages and the infusion procedure by placing them into the plastic tub cages and infusing water into their cannulas for 4 min

at a rate of 1 ml/min. Training began on day three. Rats were each placed in the training cages and connected to the infusion pumps in batches of six; 3 rats from Group Backward (Group BI) and 3 rats from Group Forward (Group FI), on each trial. Group BI was presented with either glucose or water for 5 minutes. Three minutes after the removal of the glucose and water solutions, the infusion pumps were turned on and the appropriate CS solution was delivered to all rats at a rate of 1 ml/min for 4 minutes. Within each group, reinforcement of SOA and CA was counterbalanced. Immediately after the infusion, rats from Group FI received either water or glucose for 5 min. Subsequently, animals were removed from the training cages and returned to their home cages.

Approximately 2 hours after the end of the training trial for each batch, food was returned to the rats' cage hoppers and supplementary water was presented for one hour. Rats received 16 training trials; 8 reinforced trials and 8 nonreinforced trials.

On days 18, rats were individually placed in the taste reactivity chamber for 2 min and given a water infusion at a rate of 1 ml/min for the first 15 s in each of 2 min. This procedure was repeated on days 19 and 20, except that the rats received one of the CS solutions on each day in a counterbalanced order.

On each of the subsequent 5 days, rats were tested as described in the General Methods section of Chapter 1. Briefly, rats received a 10-min 2-bottle test session in the training cages. On day 1, the bottles contained water and on the following days each bottle contained one of the CS solutions. Animals were maintained on the same water and food schedule as in training throughout the test phase.

Results

Training consumption: One of the animals in Group FI/Taste was removed from the experiment during the training phase because of a hole in its cannula. Consumption of the glucose (daily mean = 8.21 ml) was significantly higher than that of water (daily mean = 6.85) for all groups (F (1, 43) = 4.4). Moreover, animals in Group BI drank more of the glucose and water overall than Group FI (F (1, 43) = 43.5). Consumption of both glucose and water increased across trials for both groups (F (7, 301) = 13.1).

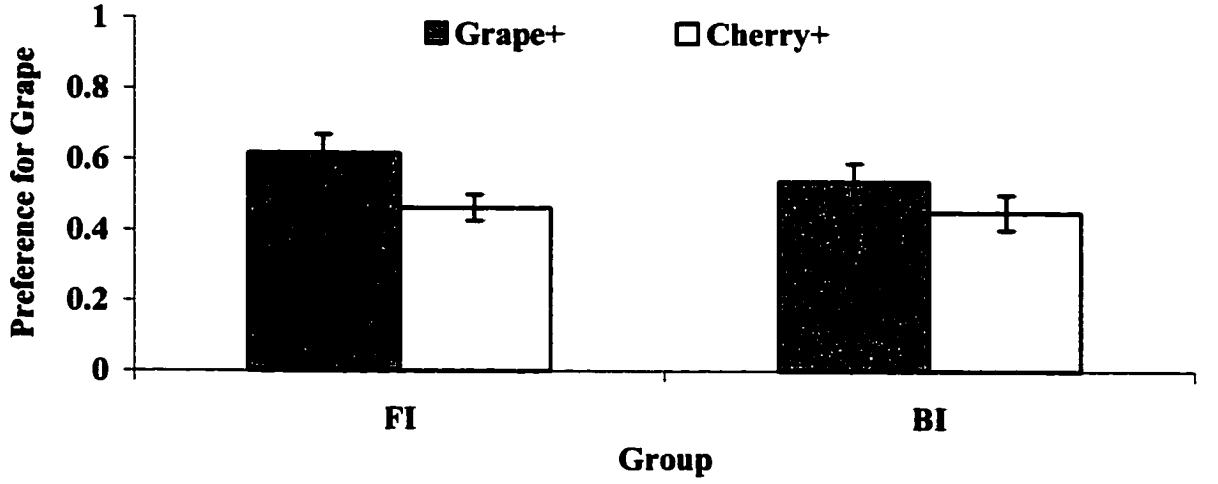
Two-bottle tests: Mean consumption of the taste cues is presented in Table 9. Overall consumption did not differ between groups. According to Figures 19A and B, both the FI and BI rats within the Flavour group appear to prefer the CS^+ to the CS^-, however none of the rats in the Taste group appear to have conditioned preferences. Moreover, the preference in the FI/Flavour group appears to be stronger than that of the BI/Flavour group.

These assertions were supported by Condition (FI vs. BI) x Counterbalanced group (SOA^+ vs. CA^+ for the taste group and $Grape^+$ vs. $Cherry^+$ for the flavour group) ANOVAs conducted separately for the taste and flavour groups. These analysis revealed only a significant main effect of counterbalanced group in the rats conditioned with the flavour cues ($F(1, 22) = 6.3$). The Condition x Counterbalanced group interaction did not reach significance ($F < 1$), indicating that the conditioned preferences observed in the FI and BI groups given flavour CSs did not differ in strength.

Table 9: Mean consumption of the taste and flavour cues during Tests 1 and 2 combined in Experiment 9.

	CS^+		CS^-		Total	
Group	Mean	*SEM*	Mean	*SEM*	Mean	*SEM*
FI/Flavour	25.2	*2.0*	18.3	*1.5*	43.5	*2.1*
BI/Flavour	25.1	*1.9*	20.9	*1.6*	46.0	*2.0*
FI/Taste	25.2	*2.9*	23.8	*2.3*	49.0	*3.2*
BI/Taste	27.0	*2.1*	22.5	*2.0*	49.5	*1.6*

A. Flavour Group



B. Taste Groups

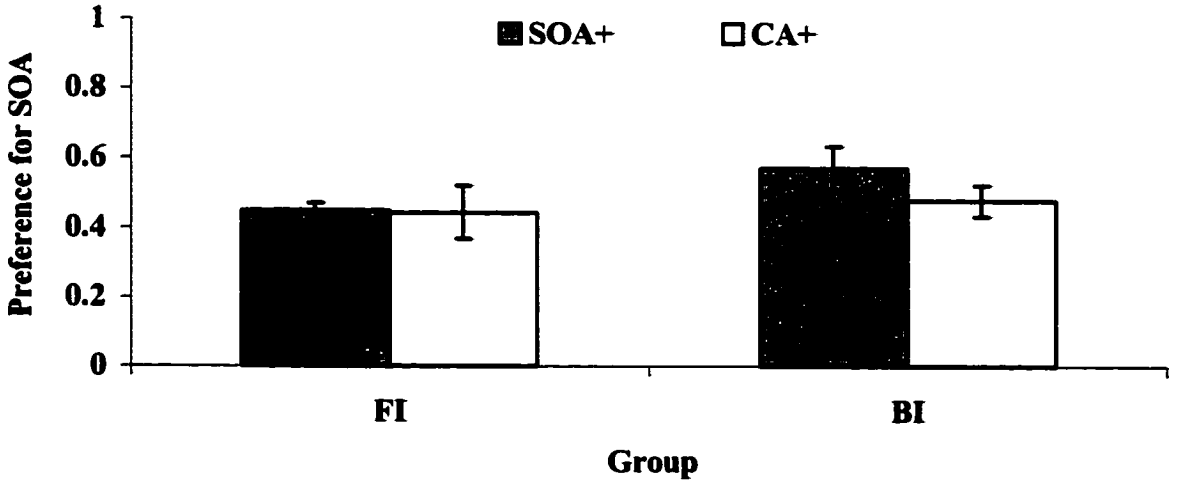


Figure 19: Mean preferences for grape in the Forward (FI) and Backward (BI) groups that received flavours as CSs during conditioning (A), and mean preferences for SOA in the groups that received tastes as CSs during conditioning (B) in Experiment 9.

Taste reactivity test: In this experiment rats exhibited mainly positive behaviours. Further, most of these behaviours occurred during the 15-s infusion trial. Few behaviours were exhibited during the subsequent 45 s of each minute. Figures 20A and B suggest that the palatability of the flavour cues increased as a function of the reverse-order conditioning trials, but not forward trials. Moreover, conditioned palatability shifts did not occur to the taste cues as a function of either the reverse-order or the forward conditioning procedure.

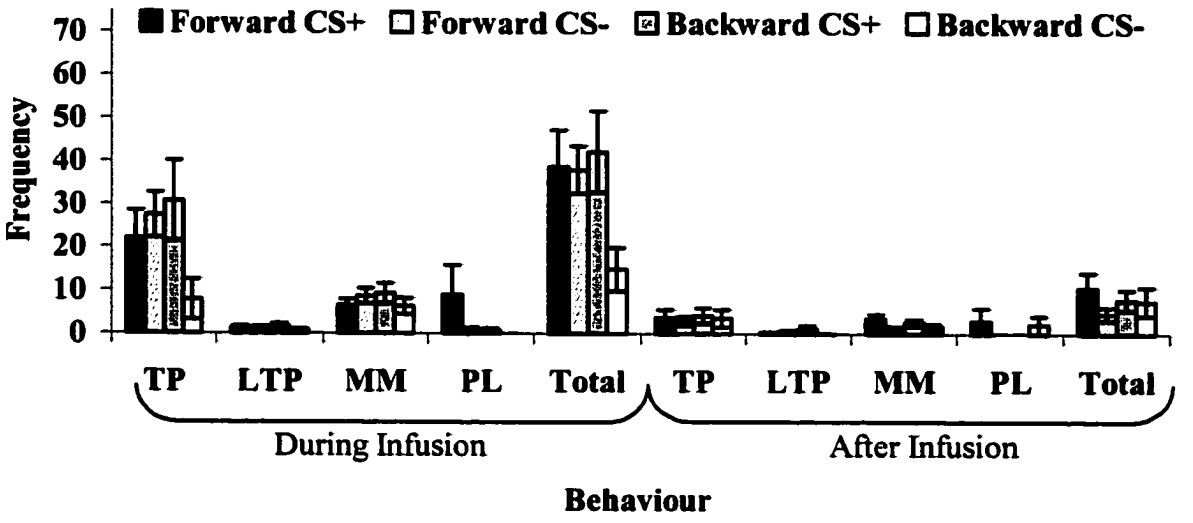
These assertions were supported by series of Condition (FI vs. BI) x Stimulus (CS^+ vs. CS^-) x Time (during infusion vs. after infusion) x Min (first vs. second min of TR test) ANOVAS. Separate analyses were conducted for the Flavour and Taste groups.

For the sum of all hedonic responses, analysis of the Flavour Groups revealed significant main effects of Stimulus (F (1, 22) = 6.7) and Time (F (1, 22) = 77.5), which were qualified by a marginal Condition x Stimulus x Time x Min interaction (F (1, 22) = 3.53, p<0.074). Simple main effects analysis of the four-way interaction yielded an effect of Stimulus for Group BI during the infusion in the first min (F (1, 11) = 6.9), whereas Group FI responded similarly to both flavour cues. For the second minute only the main effect of Time (F (1, 22) = 52.8) reached significance. For tongue protrusions, a Group x Stimulus interaction (F (1, 22) = 4.8) was obtained during the CS infusion in the first minute in the Flavour Group. Simple main effects analysis revealed an effect of stimulus in Group BI (F (1, 11) = 5.7), but not in Group FI. Finally, the amount of time spent rearing was similar for the CS^+ and CS^- flavour cues in the FI/ and BI/Flavour groups at both time points in both minutes, so no correction for rearing was made.

The analysis of the sum of all the hedonic responses for the taste groups yielded only a main effect of Time (F (1, 21) = 136.0). Therefore, rats conditioned with the taste cues did not respond differentially to the CS^+ and the CS^- cues in either the first or second minute of the TR test regardless of whether they were conditioned with a forward or reverse-order conditioning procedure.

The amount of time spent rearing was similar for the CS^+ and CS^- taste cues in the FI and BI conditions within the Flavour and Taste groups at both time points in both minutes. The BI condition did rear more than the FI condition (F (1, 21) = 5.6) in the Taste group. Reanalysis of the converted frequency scores (i.e. frequencies/min) for the Taste Group revealed the same pattern of result reported above.

A. Flavour Groups



B. Taste Groups

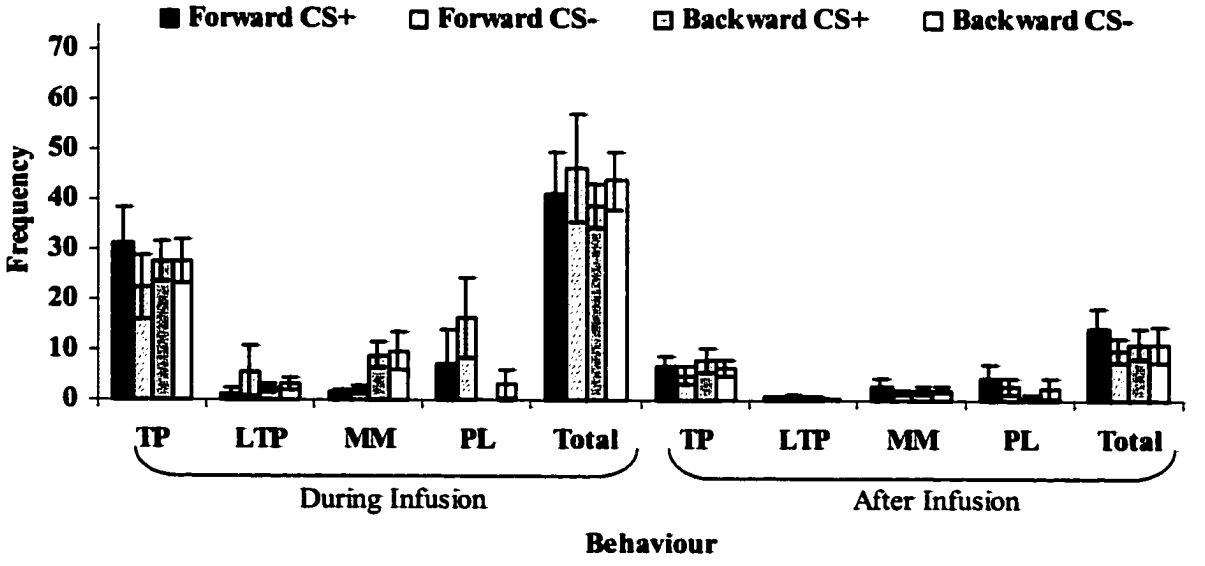


Figure 20: Frequency of positive behaviours during the 15-s infusion and the 45 s following the infusion in the first minute of the TR test in Experiment 9.

Discussion

In the present experiment, conditioned preferences and palatability shifts occurred in the Flavour groups but not in the Taste groups, suggesting that preferences and palatability shifts occur more readily to flavour CSs than to taste CSs. This result is somewhat surprising, since we reliably conditioned preferences with this procedure in experiments in which the rats voluntarily consumed the US (cf. Experiment 8). Although flavour animals acquired 2-bottle preferences for the CS^+ relative to the CS^-, regardless of whether they were trained with a forward or reverse-order conditioning procedure, only those animals conditioned with the reverse-order conditioning procedure shifted their preferences to the CS^+ relative to the CS^-. This is interesting given that the 2-bottle preference data revealed a trend (although not significant) for stronger preferences in the forward relative to the reverse-order group.

In combination with data from Experiment 8, these data suggest that oral infusion (i.e., involuntary consumption) of the CS solutions may be less effective than voluntary consumption at conditioning preferences and palatability shifts in rats, since rats that received taste CSs in the present experiment failed to acquire preferences. It is possible that the oral infusions in the present experiment were less effective at conditioning preferences because rats consumed less of the CS solutions than they would if they had voluntarily consumed the CS solutions. Although the rats tasted the CS solutions during conditioning sessions, they did not necessarily consume the CS solutions; passive dripping, or active expulsion of the fluid could also occur. There is evidence in the aversion literature that rats form weaker aversions to flavour cues that are merely passed

over the tongue and not consumed, relative to those that are consumed (Domjan & Wilson, 1972; Revusky, Parker, Coombes & Coombes, 1976). It is true that animals in Group BI showed more of this behaviour in response to the CS solutions than did Group FI (unsystematic observation). This was likely because the CS was preceded by rather than followed by consumption of another solution (either glucose or water) in Group BI. However, strength of preference did not differ between Group BI and Group FI. Moreover, palatability shifts were stronger in group BI than in Group FI. As suggested by Domjan and Wilson (1972), perhaps learning about an involuntarily consumed substance is attenuated because animals have not evolved a strategy to learn about flavours they do not approach and freely ingest. Indeed, this situation would rarely present itself in the wild.

Although we failed to get conditioned palatability shifts to orally infused taste stimuli, Breslin *et al* (1990) have shown that it is possible for palatability shifts to occur to orally infused taste stimuli. In their experiment positive responses to the CS^+ increased relative to the CS^-, while negative responses to the CS^+ decreased as conditioning progressed. One striking difference between results of our previous experiment (Experiment 8) and those of Breslin *et al* is the difference in total frequency of positive responses to the CS cues before conditioning. In the pre test of Experiment 8, frequency of positive responses in nonrestricted rats to Citric acid and Sucrose Octaacetate was less than 20 in a 2 min period, whereas in Breslin *et al* the frequency of responding on the first trial was about 40 in a 30 sec period. This would suggest that although these concentrations of QHCl and HCl are normally avoided, they are more palatable to

nonrestricted rats than the concentrations of citric acid and sucrose octaacetate presented in these experiments. Perhaps it is the combination of the low palatability of our taste CS cues and their involuntary consumption that prevented the acquisition of conditioned preferences and conditioned palatability shifts in the Taste animals in the present experiment.

It is also worth noting that few aversive behaviours were observed in the present experiment to either the flavour or the taste cues. This was probably a function of the restriction state of the animals. Thirsty animals tend to show more positive and fewer negative TR behaviours to stimuli (Forestell & LoLordo, 2000) than animals that are not thirsty. For this reason, we infused smaller amounts of the CS solutions into the animals' mouths during the TR tests and observed the animals' behaviour during and after the end of each short infusion. As discussed previously, this procedure has been used previously by Grill *et al* (1996) and Kaplan *et al* (1995) when infusing highly palatable sucrose solutions. However, the pattern of results in our experiment was different from that reported in Grill *et al*. In our experiment within and between group differences were confined to the 15-s infusion procedure. Frequency of behaviours dropped off dramatically in the ensuing 45 s of each min in our experiment. Alternatively, Grill *et al* observed group differences in mouth movements in the post infusion period. It is possible that had we been using more palatable CS cue, the frequency of behaviours would have been higher in the post-infusion period, thereby increasing our chances of detecting significant differences in responding to the stimuli between groups.

As in Experiment 8, the only behaviour to show any shift was tongue protrusions. Evidently, in our lab this behaviour is the most sensitive measure of conditioned palatability shifts. This pattern of results differs from that reported by Myers and Sclafani (2001a) who failed to find differences between groups in individual behaviours, instead however, they found differences when all the behaviours were added into a composite frequency score. It would be interesting to know what factors affect these differences in response patterns (over and above between-laboratory differences in scoring).

Experiment 10

Experiment 8 demonstrated that a differential conditioning procedure in which rats freely consume the taste CSs is capable of conditioning palatability shifts to tastes without the aid of either oral or gastric infusions. Further, Experiments 8 and 9 together suggest that voluntary oral consumption of the CS solutions more effectively conditions preferences and palatability shifts than do oral infusions.

In all of the previous conditioning experiments we have employed a reverse-order conditioning procedure. According to Boakes and Lubart (1988) this procedure conditions preferences by means of taste-calorie associations (or flavour-calorie associations, depending on the type of CS employed). Since the taste cue follows the presentation of the sweet-tasting reinforcer, it is unlikely that taste-taste or flavour-taste associations contribute significantly to taste and flavour preferences conditioned with this procedure. This suggests that taste- or flavour-calorie associations result in increased palatability of the CS taste or flavour.

In the present experiment we were interested in whether flavour-taste associations also produce palatability shifts. Although it has been suggested that the palatability shifts conditioned in Breslin *et al* (1990) for quinine and HCl were a function of flavour-taste associations (see Myers & Sclafani, 2001a), it is possible that flavour-calorie associations also played a role in producing these palatability shifts, since Breslin *et al* infused a small amount of a calorifically potent reinforcer on each CS trial.

Additionally Myers and Hall (2000) have shown that increases in palatability occur to a flavour paired with a "sweet/low cal" solution consisting of a small oral infusions of a 30% sucrose or a flavour paired with a "nonsweet/high cal" solution consisting of a 26% sucrose gastric infusion, relative to a flavour paired with a "nonsweet/low cal" gastric infusion of 5.1% sucrose. These data suggest that palatability shifts do occur in response to both flavour-taste and flavour-calorie associations. In this experiment, however calories were present in both of the reinforcers. Although there was an equal number of calories presented in the sweet/low cal reinforcer and the nonsweet/low cal reinforcer, perhaps the presence of calories potentiated the effect of the flavour-taste association on the response to the cue paired with the sweet/low cal reinforcer.

In the present experiment, we used an overnight simultaneous conditioning procedure, in which animals received two flavours of Kool Aid as flavour cues. This method of extended exposure has been used extensively by Sclafani (eg., Sclafani & Nissenbaum, 1988; Elizalde & Sclafani, 1989; Lucas & Sclafani, 1989) in rats that receive intragastric reinforcement. According to Lucas and Sclafani, these extended exposures are effective because: (1) they allow multiple training trials; i.e., rats consume the CS

solutions in many bouts over these extended presentations, and (2) the rat can voluntarily control the size and duration of each of these reinforced bouts.

For one group (Group Calorie) one flavour was paired with a sweet, calorific reinforcer (sucrose), and the other with a sweet noncalorific reinforcer (saccharin). For another group (Group Taste), one flavour was paired with saccharin, and the other was presented in water. The remaining animals (Group Taste/Cal) received one flavour paired with a super-sweet, calorific reinforcer (saccharin + sucrose), while the other flavour was paired with sucrose only. In addition to assessing the strength of flavour preferences and palatability shifts resulting from flavour-taste and flavour-calorie associations, we also sought to determine whether these shifts would be affected by manipulations of food restriction state during test.

Methods

Subjects: Thirty male Sprague-Dawley rats with a mean weight of 339 g were housed in plastic tub cages (dimensions: 44 cm long x 22 cm wide x 22 cm high) with wire tops. After one week of recovery from surgery, rats were food restricted and maintained at 85% their free feeding weight throughout training and test 1. Rats were fed a restricted amount of rat chow each morning and were given 1 hour of supplementary water at this time. After the end of the session of the first TR test, food was returned to the rats' cages and they were maintained on free food for the second 2-bottle and TR tests. Animals continued to receive 1 hour of supplementary water while *ad lib* food was available, except where noted below.

Stimuli: Grape and cherry Kool Aid were used as the flavour cues. Since these flavours were mixed with the reinforcer in the present experiment the concentration was doubled to .1% (w/v) relative to our previous experiments, so that the flavours would not be masked by the taste of the reinforcers. The US solutions included 4% (w/v) sucrose, .4% saccharin (w/v), and a mixture of 4% sucrose and .4% saccharin, all of which were mixed in tap water. These sucrose and saccharin concentrations were used because they have previously been shown to be isohedonic for nondeprived rats in a very brief preference test (Young & Madsen, 1963) and to be equally effective in conditioning a preference to an associated flavour, also in nondeprived rats (Harris *et al*, 2000). Animals in the Taste/Cal group received the CS^+ mixed with sucrose and saccharin, and the CS^- mixed with sucrose. Young and Madsen (1963) also showed that a mixture of 4% sucrose and 0.4% saccharin was isohedonic to 8% sucrose in non-deprived rats. Therefore, this group would receive two flavours paired with reinforcers containing equal calories, however one of these reinforcers would be paired with a sweeter taste. The Calorie group received the CS^+ mixed with 4% sucrose and the CS^- mixed with .4% saccharin, and the Taste group received the CS^+ in .4% saccharin and the CS^- in water.

Procedure: Rats were cannulated as described in the General Methods. Conditioning began once the rats' weights stabilized at 85% of their free feeding weights. Rats were placed into groups equated in weight. Within each group, half of the animals received grape as the CS^+ and the remaining animals received cherry as the CS^+. On the morning of the first conditioning trial, the water bottles were removed from all the cages at approximately 11:00. At 19:00 a bottle containing approximately 100 ml of one flavour

mixed with the appropriate reinforcer was placed on the animals' cages. Bottles were removed the next day at approximately 11:00. Rats were fed their restricted ration of food and given 1 hour of supplementary water at 12:00. In total, rats received 6 conditioning trials; 3 trials with each flavour in a double alternation sequence. On each conditioning trial, half of the animals within each group received grape Kool Aid and the remaining animals received cherry Kool Aid.

Since rats in the Flavour group voluntarily consumed a limited quantity of the CS^- flavour solution when it was presented in water on the first trial, the amount of the CS^+ saccharin solution made available on trials 2 and 3 was limited to 20 ml and then to 10 ml for this group in an effort to minimize differences in consumption of the CS^+ and CS^-.

One day following the last conditioning trial, animals were given a 2-bottle test to assess their conditioned preferences. Bottles containing the two flavour cues were placed on the front of their cages at 19:00 h. These bottles remained on their cages until 11:00 the following day. This procedure was repeated once with the position of the bottles reversed. On the following three days, animals were given taste reactivity test sessions as described in the General Methods section; briefly, rats received water on the first session and one of the taste cues on each of the next two sessions. On the last day of taste reactivity testing, food and water were returned to the rats' cages. They were maintained on free food for one week prior to and during another 2-bottle test. On the day of each of the 2-bottle tests, animals did receive the hour of supplementary water, to ensure that consumption of the flavour cues would be sufficient during the ensuing 2-bottle test sessions.

Results

Training consumption: As displayed in Figure 21, rats in the Taste/Cal and Calorie groups consumed more of the CS-US mixtures than animals in the Taste group, which had restricted access to the CS^+ on the second and third trials. Moreover, consumption of the CS^- and CS^+ mixtures did not appear to differ in the Taste/Cal and Calorie groups. For the Taste group, consumption of the CS^+ was greater than that of the CS^- on trials 1 and 2.

These assertions were supported by a 3 x 2 x 3 (Group x Solution (CS^+ vs. CS^-) x Trial) repeated-measures ANOVA. This analysis revealed main effects of Group (F (2, 27) = 952.8) and Solution (F (1, 27) = 44.3) and a Group x Solution x Trial interaction (F (4, 54) = 15.2). Simple main effects analysis of the three-way interaction confirmed our observation that the Taste group consumed more of the CS^+ than the CS^- (F (1,9) = 74.4). In that group, consumption of the CS^+ decreased over trials relative to the CS^- as a function of its limited presentation in Trials 2 and 3, as indicated by a Stimulus x Trial interaction in this group (F (2,18) = 22.9). Consumption of CS^+ and CS^- did not differ in the other groups on any trial.

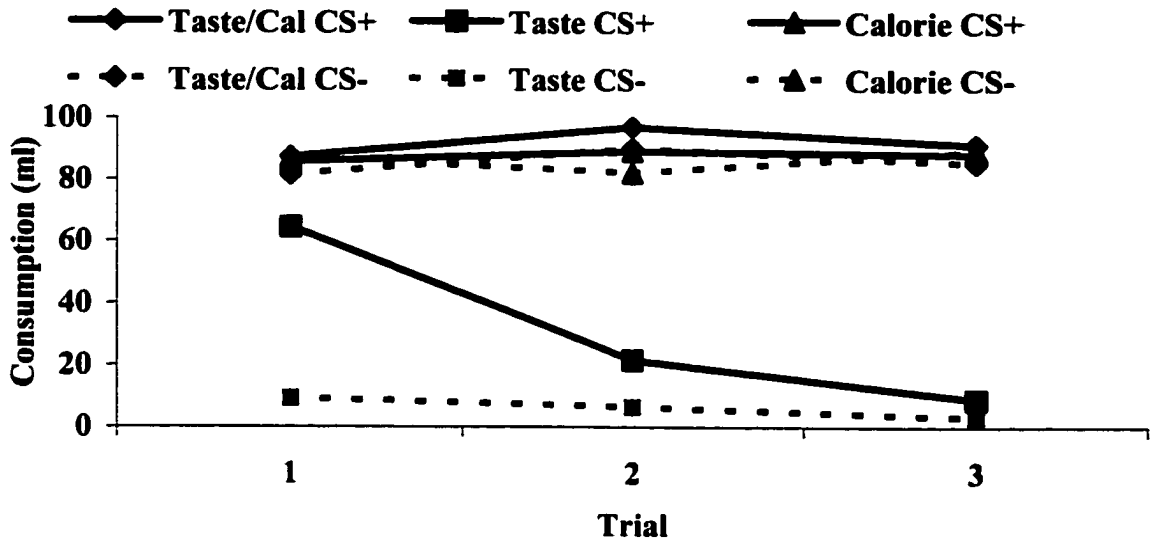


Figure 21: Consumption of the CS^+ and CS^- cues during training for the Taste/Cal, Taste and Calorie Groups in Experiment 10.

Two-bottle tests: As shown in Table 10, total mean consumption of the CS cues did not differ between groups on either of the two 2-bottle tests, however total consumption in Test 2 was larger than that in Test 1 for all groups (F (1,26) = 848.2).

Preferences within each of the three groups were assessed by comparing the two counterbalanced halves of each group on their preference for the grape solution. These preferences are displayed in Figures 22 A and B for the first test, in which animals were food restricted and for the second, in which they were not food restricted. These graphs suggest that when food restricted, the Taste and Calorie groups expressed conditioned preferences for the CS^+, however the Taste/Cal group did not. When tested under *ad lib* feeding conditions, this pattern changed. Conditioned preferences for CS^+ were observed in the Taste and Taste/Cal groups, but not in the Calorie group.

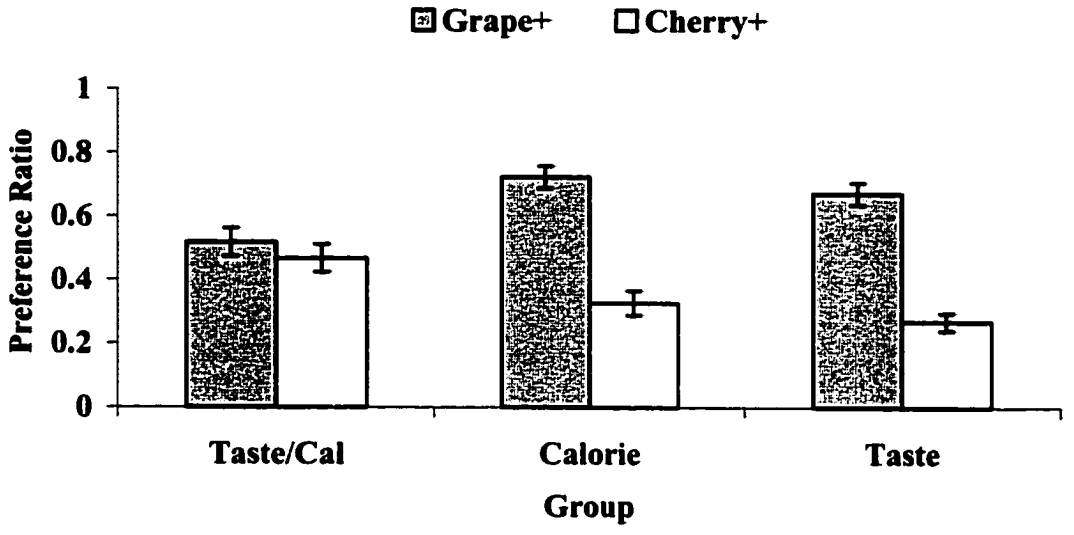
These observations were supported by a Group (Taste/Cal, Calorie and Taste) x Counterbalanced group (Cherry$^+$ vs. Grape$^+$) x Test (Restricted test vs. Nonrestricted) ANOVA on preference for grape. This analysis revealed a main effect of Counterbalanced Group ($F(2, 24) = 78.2$) and a Test x Group x Counterbalanced Group interaction ($F(2, 24) = 11.4$). Simple main effects of Conterbalanced Group x Test for each group indicated that preferences of the Grape$^+$ rats in Group Taste were significantly higher than those of the Cherry$^+$ rats for both tests ($F(1, 8) = 143.2$). However, preferences of the Grape$^+$ rats were higher in the Calorie group only when they were tested food restricted ($F(1, 8) = 29.5$). On the other hand, in the Taste/Cal group differences in grape preferences between the Grape$^+$ and Cherry$^+$ group occurred only when the animals were tested under *ad lib* feeding conditions ($F(1, 8) = 9.3$).

To determine whether differences in exposure to the two CS solutions in the Taste Group were correlated with preferences in this group, Pearson correlation coefficients were calculated on the differences between CS$^+$ and CS$^-$ consumption during conditioning and preference ratios in Tests 1 and 2 combined. This analysis failed to reveal a significant correlation ($r = .36$).

Table 10: Mean Consumption of the Flavour cues during Tests 1 and 2 for Experiment 10.

	Taste/Cal		Taste		Calorie	
Stim/Test	Mean	SEM	Mean	SEM	Mean	SEM
$CS^+/1$	26.3	2.1	34.0	3.8	42.4	3.6
$CS^-/1$	24.2	2.7	12.6	1.6	18.1	2.2
Total	50.5	2.6	46.6	4.6	60.5	3.3
$CS^+/2$	81.2	4.8	91.6	4.7	64.9	4.7
$CS^-/2$	45.3	2.7	28.8	4.6	67.0	7.0
Total	126.5	5.1	120.4	4.0	121.9	3.4

A. Food Restricted



B. Free Fed

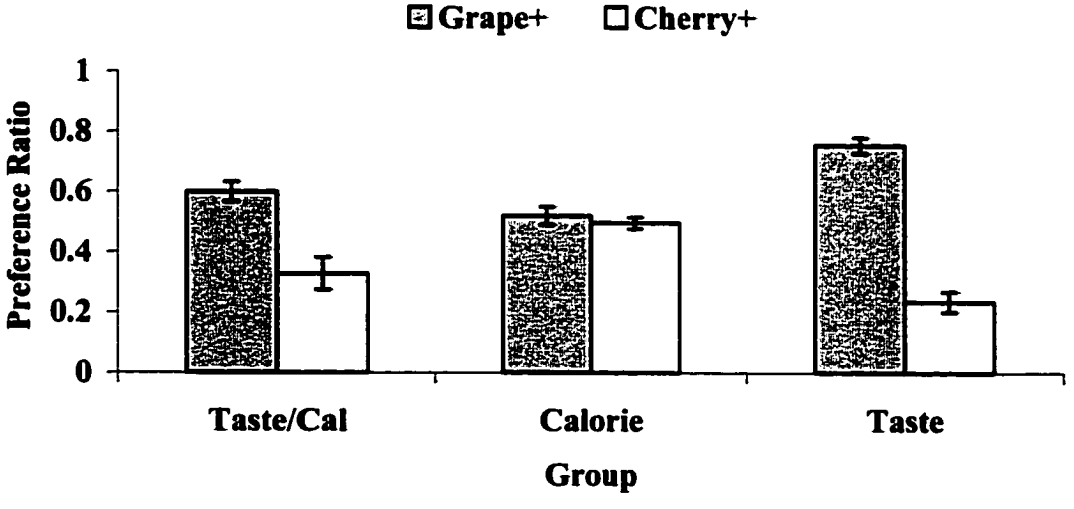


Figure 22: Mean Grape preference ratios in Test 1, when animals were food restricted and Test 2, when animals were free fed in Experiment 10.

Taste Reactivity Tests: Many of the cannulas were damaged as the present experiment proceeded. We suspect this occurred because animals were catching them on the wire cage tops. Also many of the animals chewed holes in their cannulas, which was likely a function of their food restriction state in training. Twenty of the animals had functional cannulas by the first TR test (n = 7, 7, and 6 in the Taste, Calorie, and Taste/Calorie groups, respectively). By the second TR test, only 14 cannulas were functional. Because of the restricted sample size in the second TR test, this test was omitted from the subsequent analyses.

In this experiment, the amount of time spent rearing to the CS^+ relative to the CS^- differed between groups. These claims were supported by a repeated-measures Group x Stimulus ANOVA, which revealed a Group x Stimulus interaction (F (2, 11) = 4.0). Therefore, all of the behavioural frequencies were converted by dividing each of the frequency scores by the number of seconds the animals were not rearing and multiplying by 120 seconds (to extrapolate the behaviours observed during non rearing to 2 minutes).

Looking first at the positive behaviours, Figure 23 suggests that hungry rats responded differentially to the CS^+ and CS^- flavour cues depending on the type of reinforcer used during conditioning. Group Calorie and Group Taste/Cal appear to respond more to the CS^+ than to the CS^-. This effect appears to be mainly due to differential levels of tongue protrusions to the CS^+ and CS^- in Group Calorie. Group Taste however, does not appear to respond differentially to the flavour cues.

These claims were assessed with Group x Stimulus repeated-measures ANOVAs for all of the behaviours combined and for each individual behaviour. The analysis of all

the behaviours combined ("Total" in graph) revealed a main effect of stimulus (F (1, 17) = 6.1) and a marginal Group x Stimulus interaction (F (2, 17) = 2.7, p<.1). This marginal interaction occurred because there was an effect of stimulus in Group Calorie (F (1, 6) = 6.4), but not in the other groups. Similarly, for tongue protrusions there was a main effect of stimulus (F (1, 17) = 5.9), and a marginal Group x Stimulus interaction (F (2, 17) = 3.3, p<.054). Simple main effects of this marginal interaction revealed a marginal effect of stimulus (F (1, 6) = 5.74, p<.054) in the Calorie Group only.

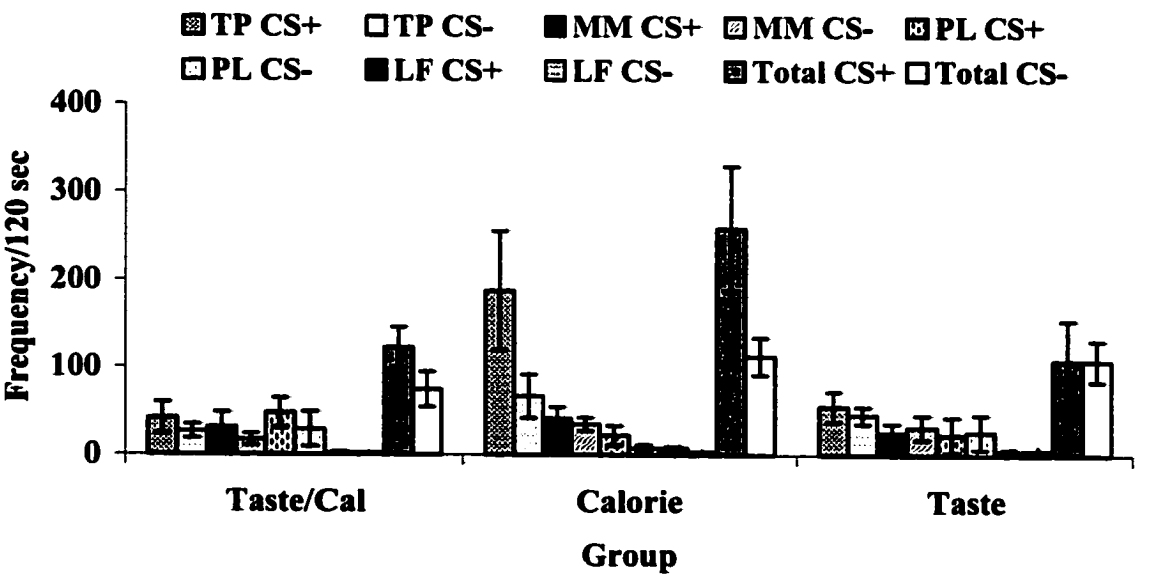


Figure 23: Positive taste reactivity behaviours elicited in Group Taste/Cal, Group Calorie and Group Taste when tested food-restricted in Experiment 10.

Figure 24 displays the negative behaviours observed in each of the TR tests. This Figure suggests that the Calorie group responded more to the CS^- than to the CS^+, whereas

the other two groups did not. Analyses similar to those conducted above for the positive behaviours revealed a significant main effect of Stimulus (F (1, 17) = 6.2) and a Group x Stimulus interaction (F (2, 17) = 13.3) for all the negative behaviours combined. Simple main effects analysis of this interaction revealed that overall negative responding for the CS^- was greater than for the CS^+ (F (1, 6) = 33.3) in the Calorie Group only. Additionally, there was a Group x Stimulus interaction for face washes (F (2, 17) = 5.5) and for paw flicks (F (2, 17) = 6.4). Simple main effects analysis of each of these interactions revealed effects of Stimulus (F (1, 6) = 6.0 and 6.5, respectively) in the Calorie group only.

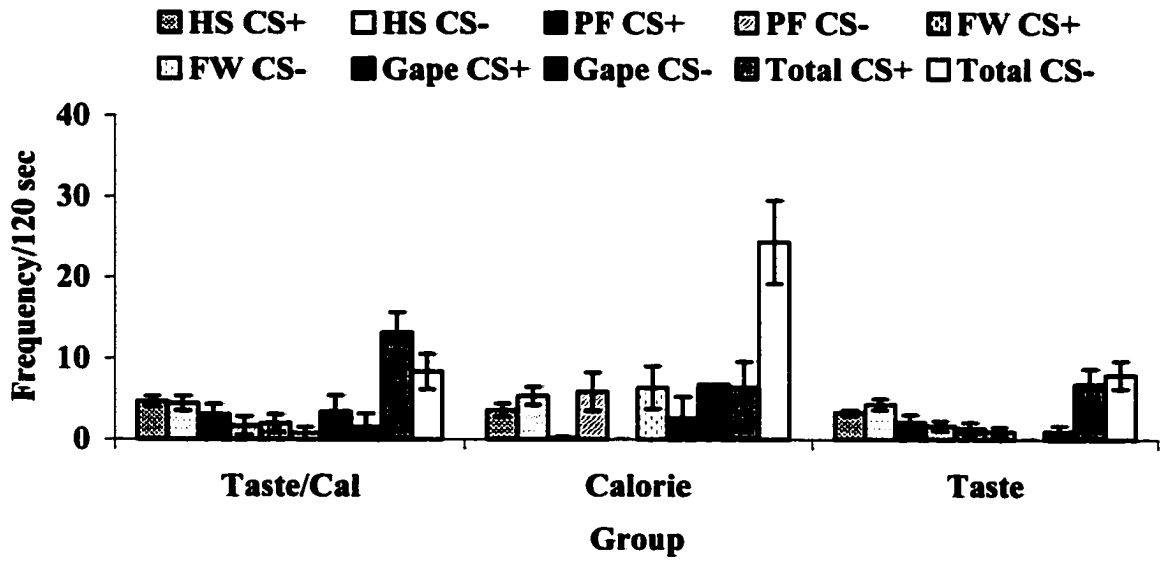


Figure 24: Negative taste reactivity behaviours elicited in Group Taste/Cal, Group Calorie and Group Taste when tested food-restricted in Experiment 10.

Discussion

In the present experiment, rats in all groups acquired significant preferences for the CS^+ flavour over the CS^- flavour, as assessed by 2-bottle tests. Rats in the calorie group received the two taste cues paired with concentrations of sucrose and saccharin that have been reported to be equally hedonic for non-restricted rats (Young & Madsen, 1963; Harris, Gorissen, Bailey & Westbrook, 2000). Therefore their preference for the CS^+ should have been a function of a flavour-calorie association. For the other 2 groups, the caloric content of the reinforcer paired with the CS^+ was the same as that of the reinforcer paired with the CS^-. Neither cue was paired with calories in the Taste group, whereas both cues were paired with the same number of calories for the Taste/Cal group. Since these reinforcers differed only in their sweetness in both groups, the conditioned preferences should have been a result of a flavour-taste association.

When tested hungry, rats in the Calorie Group and the Taste Group expressed significant 2-bottle preferences for the CS^+ relative to the CS^-, whereas the Taste/Calorie Group did not. This pattern of results was not mirrored in the taste reactivity test, where only Group Calorie tongue protruded marginally more to the CS^+ than to the CS^- and expressed more negative behaviours (specifically face washes and paw flicks) to the CS^- than to the CS^+. Differential taste reactivity responding was not observed in the Taste Group, even though this group expressed a preference in both 2-bottle tests, which was at least as strong as that expressed in Group Calorie. In previous experiments (i.e., Experiments 8 and 9), we have shown that in our laboratory, tongue protrusions are more sensitive to pairings of flavours with reinforcers than other positive TR responses. This

experiment extends these results by suggesting that the pattern of responding to flavour CSs may differ depending on whether they have been reinforced with calories or sweet tastes. Further, these results suggest that palatability shifts do not always coincide with conditioned preferences as measured by 2-bottle tests.

It is possible that the conditioned preferences observed in the Taste group were a function of differential consumption of the CS flavours during conditioning. Perhaps rats in the Taste Group consumed more of the CS^+ than the CS^- in the 2-bottle preference test because they were more familiar with its flavour than that of the CS^-, not because it had been paired with the sweet taste. It is true however, that there was no relation between how much more of the CS^+ than of the CS^- rats in Group Taste drank in training and performance on the 2-bottle tests (even though the ranges in consumption were sufficient to detect a correlation). Instead it appears that flavour-taste associations may change how much an animal "wants" a stimulus more readily than how much it "likes" a stimulus. On the other hand, changes in wanting appear to coincide with changes in liking in response to flavour-calorie associations.

Thus far in all of the reports in the literature, palatability shifts have been conditioned with reinforcers that contain calories (Breslin *et al*, 1990, Myers & Hall, 1999, Myers & Sclafani, 2001a). It has been assumed in some cases (eg., Myers & Hall; Breslin *et al* as discussed in Myers & Sclafani) that these palatability shifts are a result of flavour-taste associations because of the small amount of reinforcer employed on each trial in these experiments. In Breslin *et al*, palatability shifts were conditioned when only .5 ml

of a 30% reinforcer was infused into the animals' mouths on each trial. Therefore animals in that study received approximately .15 g of sucrose dissolved in water on each CS^+ trial.

Although animals only received a small amount of sugar per trial in the Breslin *et al* study (1990), it has been shown that the effectiveness of a reinforcer is not necessarily a function of the absolute number of calories presented, but instead of the caloric density of the reinforcer (Bolles, Hayward & Crandall, 1981). Indeed, concentrated carbohydrate solutions (16-32%) have been shown to be especially effective at conditioning robust flavour preferences (i.e., > 90%) in rats (e.g. Elizalde & Sclafani, 1990, Ackroff & Sclafani, 1994, Sclafani, Fanizza & Azzara, 1999). Moreover, Ackroff and Sclafani (1994) have shown that nondeprived rats can acquire preferences for flavours paired with IG 1% Polycose solutions. On average, in training animals' consumption consisted of 16 bouts of 1.3 ml. Since the ratio of CS consumption to US infusion was 1:1, these animals were receiving approximately 21 ml of reinforcer per day, which converts to .2 g of Polycose reinforcement per day. Remarkably, rats were able to acquire conditioned preferences for the CS^+ flavour based on the small amount of reinforcement provided by the 1% Polycose reinforcer, even though their freely available chow provided 98% of the of total number of calories on days in which the CS^+ was presented. Therefore it is possible that the calories present in the reinforcer presented in Breslin *et al* (1990) contributed to the palatability shifts observed in this study.

Myers and Hall (2000) reported conditioned preferences for, and a greater duration of mouthing to 0.5 ml infusions of a flavour paired with a sweet, low calorie reinforcer relative to an equally caloric nonsweet reinforcer in food restricted rats. However, Myers

and Sclafani (2001a) noted that Myers and Hall measured only the duration of oromotor responding, rather than recording the frequency of all the behaviours typically assessed in the TR test. They concluded that Myers' and Hall's results which suggested that flavour-taste associations can enhance the palatability of flavours, were not conclusive on that point.

Accepting this argument, neither Breslin *et al* (1990) nor Myers and Hall (2000) provides conclusive evidence for enhanced palatability based on flavour-taste associations. Nonetheless, it is possible that the 30% sucrose reinforcer used in these studies, which is much sweeter than the 0.4% saccharin consumed by the Taste Group in the present study, was more effective in conditioning flavour-taste associations.

In addition to the dissociations between the 2-bottle preferences and TR results in the present study, our data also indicate that the content of the association acquired in training and the restriction state in test interact to affect expression of conditioned preferences. Animals in Group Calorie displayed significant preferences only when they were food restricted, suggesting that the conditioned preference for the CS^+ was a function of a flavour-calorie association. On the contrary, Group Taste/Cal failed to express 2-bottle test preferences when tested hungry. However, when tested sated these rats expressed a 2-bottle preference for the CS^+ indicating that they had acquired a flavour-taste association in training. These animals probably acquired both flavour-taste and flavour-calorie associations between the CS^+ and the CS^- and their respective reinforcers. However, the flavour-taste association accrued to the flavour paired with the sucrose + saccharin was probably stronger than that of the cue paired with sucrose only solution

because of the sweeter taste of the sucrose + saccharin mixture. These data suggest that flavour-calorie associations override flavour-taste associations when animals are tested hungry. Therefore, when animals acquire flavour-taste and flavour-calorie associations in training, flavour-taste associations control choice of CS in 2-bottle preference tests when rats are sated, whereas flavour-calorie associations control choice of flavour in 2-bottle preference tests when they are hungry. It is also possible that flavour-calorie associations occur to both CS solutions, and when the rats are tested hungry, both CSs are so positive on the basis of these that the differential strengths of the two flavour-taste associations do not affect performance.

Harris *et al* (2000) have also reported that hunger levels in test can control the expression of flavour-taste and flavour-calorie associations. In their experiments, hungry rats received pairings of almond and sucrose in training. Some of these rats received additional presentations of sucrose by itself intermixed with the almond-sucrose pairings, whereas others received additional presentations of saccharin intermixed with almond-sucrose pairings. These additional presentations of sucrose and saccharin were meant to degrade the contingency between the almond flavour and the sucrose reinforcer. That is, extra presentations of the sucrose and saccharin were meant to decrease almond's ability to predict presentation of the calories and/or the sweet taste of sucrose. Separate presentations of saccharin were meant to degrade the contingency between almond and the sweet taste of sucrose, thereby decreasing the strength of flavour-taste associations. Similarly, separate presentations of sucrose were meant to degrade the contingency

between almond and calories as well as almond and the sweet taste of sucrose, thereby decreasing the strength of flavour-taste and flavour-calorie associations formed in training.

In test, rats that had received presentations of saccharin in addition to the almond-sucrose reinforcer expressed a preference for almond only when they were tested hungry, not when they were tested under *ad lib* feeding conditions. Rats that received additional presentations of sucrose, rather than saccharin, in addition to almond-sucrose pairings, expressed attenuated preferences regardless of whether they were tested hungry or thirsty. The fact that expression of conditioned preferences for almond was attenuated by saccharin only when rats were tested sated, whereas expression of preferences for almond was attenuated by sucrose regardless of the food restriction state during test, suggests that both flavour-calorie and flavour-taste associations contribute to preferences reinforced by sucrose in animals trained hungry. Which of these associations is expressed depends on the animals' deprivation state in test. When the rat is hungry in test, preferences for a flavour previously paired with sucrose are controlled by flavour-calorie associations. However, when rats are not hungry, these preferences are controlled by flavour-taste associations.

These results are also consistent with those reported by Fedorchak and Bolles (1987; see also Capaldi *et al*, 1994; Holder, 1991). In that study rats that received an odour paired with ethanol when hungry expressed a much larger preference for that odour only when they were tested hungry. Since the taste of ethanol has an aversive taste to rats, it is assumed that their preference for the odour associated with ethanol was based primarily on a odour-calorie association. Additionally in Fedorchak and Bolles, expression

of odour-taste associations was unaffected by restriction state in test. The results from the Taste group in our experiment are also consistent with this conclusion.

In summary, our results indicate that conditioned preferences are more readily expressed than are conditioned palatability shifts in response to flavours reinforced by a sweet taste in hungry rats. Additionally, in combination with those reported by others (eg. Fedorchak & Bolles, 1987; Harris *et al*, 2000), our results suggest that expression of conditioned preferences are often affected by the deprivation state of the animal in test. For example, flavour-calorie associations are expressed in hungry animals whereas flavour-taste associations, if conditioned in the absence of calories are unaffected by the restriction state of the animal in test. However, if flavour-taste associations are conditioned in the presence of calories, they contribute only to the preference expressed by an animal that is not food restricted in test. Future research in our lab will focus on how restriction state during conditioning affects expression of taste reactivity responses to flavour-taste and flavour-calorie associations.

General Discussion

In the present set of 3 experiments, we successfully conditioned palatability shifts to tastes and flavours in rats using differential oral conditioning procedures that differed methodologically in several ways. Conditioning procedures included reverse-order, forward and simultaneous long-exposure paradigms. The conditioned stimuli were either voluntarily consumed or involuntarily infused directly into the mouth whereas the US, which was either a sweet taste or a caloric reinforcer, was always voluntarily consumed. Further, rats were maintained on various food and water restriction states in training and

test in these experiments. Assessment of the data in light of these methodological differences provides some information about how each of these variables affects acquisition and expression of conditioned preferences and the pattern and strength of TR responses. Moreover, they begin to specify the relation between palatability shifts and flavour preference conditioning.

In Experiment 8 we observed significant conditioned preferences and palatability shifts to the CS^+ relative to the CS^- taste. However, the TR effect was mainly due to a shift in tongue protrusion frequency to the CS^+ relative to the CS^- between the pre and post tests. None of the other positive or negative TR behaviours were significantly shifted as a function of the reverse-order conditioning procedure in this experiment. Changing the mode of delivery of the taste solution (i.e., involuntary rather than voluntary) failed to enhance differential TR responding to the taste cues in Experiment 9. In fact, rats that received oral infusions of the taste cues paired with voluntary consumption of the US failed to express conditioned preferences or differential TR responses to the taste cues in test.

It is true that rats in Experiment 8 consumed more of the taste cues on each training trial than rats in Experiment 9 (i.e., 10 ml vs. a maximum of 4 ml per trial). However, it is unlikely that differences in training consumption of the taste cues would cause weaker conditioning in Group Taste in Experiment 9 relative to rats in Experiment 8. Previous experiments in our lab have shown that preferences can be conditioned when only 3 ml of the CS is voluntarily consumed on each conditioning trial.

A more plausible reason for the discrepant results between these two experiments was the difference in the way the CS was presented during training. For example, rats may acquire weaker preferences for tastes that are involuntarily consumed relative to those that are voluntarily consumed. It is also possible that the expression of conditioned preferences for the tastes was differentially affected by generalization decrement as a function of the voluntary vs. involuntary consumption in training. Since the mode of administration of the CS cues was consistent in training and in the 2-bottle test in Experiment 8, these rats should have experienced more generalization decrement in the TR test than in the 2-bottle test. However, for rats in Experiment 9 the mode of administration of the CS cues was consistent in training and the TR test. Therefore these rats should have experienced more generalization decrement in the 2-bottle preference test than in the TR test. Although generalization decrement may explain why animals in Experiment 8 demonstrated conditioned taste preferences in the 2-bottle tests, whereas those in Experiment 9 did not, generalization decrement does not explain the differences in the TR responses between these 2 experiments. If our discrepant results were a function of generalization decrement, then the taste group should have displayed palatability shifts in Experiment 9. Therefore, based on our results it is likely that acquisition rather than expression of the conditioned taste preference was attenuated in Experiment 9 by involuntary consumption of the taste cues.

In contrast to the Taste group, rats in the Flavour group in Experiment 9 acquired conditioned preferences regardless of whether they received reverse-order or forward pairings of the flavour with the US in training. First, these data suggest that flavours are

more conditionable than tastes. A. Sclafani also claims that Kool Aid flavours are more conditionable than tastes (personal communication, November, 2000). In Chapter 1 our data suggested that the Citric Acid and Sucrose Octaacetate in water are not readily conditioned because they are unacceptable tastes to the rat. Following this logic, it is possible that the Kool Aid flavours are more conditionable than the CA and SOA because they are more acceptable than these tastes. This is unlikely however, since the main component of the Kool Aids' taste is citric acid (Elizalde, 1990). Therefore these Kool Aid flavours should be as unacceptable to the rats as the taste cues.

Discrimination of the Kool Aid flavours is based on their odour rather than on their taste (A. Sclafani, personal communication, November 2000). Therefore in the case of the Flavour Group, the conditioned preferences were probably based on odour-calorie associations. It is true that odour associations are reported to be weaker than taste associations in the aversion literature. However, the presence of the citric acid taste may have potentiated the strength of the odour-calorie association in our studies. Potentiation typically occurs when a CS is presented in compound with a weakly conditioned CS during conditioning. Most commonly strongly conditionable taste cues are presented with weakly conditionable odours prior to illness. Although learning theory might predict that the presence of the taste would overshadow (or attenuate) the conditioned aversion to the odour (Resorla & Wagner, 1972), instead the presence of the taste in training enhances the strength of the conditioned aversion to the odour (Durlach & Rescorla, 1980; Lett, 1984; Palmerino, Rusiniak & Garcia, 1980, Rusiniak, Hankins, Garcia & Brett, 1979). There are

also examples of potentiation of odours by tastes in the flavour preference literature (Holder, 1991, Lucas & Sclafani, 1995).

Although all the animals in the Flavour Group acquired conditioned preferences as indicated by the 2-bottle preference tests, palatability shifts were only conditioned in the BI/Flavour rats. Those conditioned with the forward procedure failed to express differential TR responses to the CS^+ and the CS^-. This is surprising, especially since the preference in the Forward group looks stronger than that of the Reverse-Order Group (graphically, though not statistically). It is true that the forward procedure has been reported to produce weak flavour preference conditioning effects by several research groups (Boakes, Rossi-Arnaud & Garcia-Hoz, 1987; Lavin, 1976; Simbayi, Boakes & Burton, 1986, see also Dwyer, 2001). According to Boakes and Lubart this is because the taste of the reinforcer is more readily associated with its postingestive effects than the flavour or taste of the CS because the taste of the reinforcer occurs more closely in time to the postingestive effects than the CS cue. The reverse-order procedure is supposed to overcome this problem because presentation of the CS occurs after the presentation of the US. Therefore, it is assumed that the animal experiences the taste of the reinforcer, then the flavour or taste of the CS and finally, the postingestive effects of calories. Given the fact that the palatability of the flavour cue paired with glucose had not increased as a function of the forward conditioning procedure, it is remarkable that the strength of the conditioned preferences in Group FI/Flavour did not differ from those conditioned with the reverse-order procedure (Group BI/Flavour). This suggests that another mechanism in

addition to palatability shifts must play a role in the formation of conditioned flavour preferences.

Finally, in the last experiment we used a simultaneous long-exposure conditioning procedure to condition preferences to flavours based on flavour-taste associations and flavour-calorie associations. Regardless of the content of the association, animals acquired conditioned preferences. Moreover, the strength of these conditioned preferences and the conditioned palatability shifts in the Calorie Group were greater than those observed in our previous conditioned preference studies, mainly because of the small amount of variability in the former. The increased strength of the preferences in this experiment relative to the previous experiments may have been a function of the longer presentations of the conditioning solutions in training, which provided the opportunity for animals to consume the solutions in many bouts. In the previous experiments animals had only one opportunity to consume a relatively small amount of the solutions per day. Further in all of these bouts, the amount of reinforcer was perfectly correlated with the amount of CS consumed because it was a simultaneous conditioning procedure; i.e., the CS and US were mixed. In the other conditioning procedures the CS and US were presented separately, therefore the correlation between consumption of the CS and consumption of the US would be less than perfect. The long test session in Experiment 10 may have also contributed to the stronger preferences observed in this experiment. These factors, combined with our use of voluntarily consumed flavours rather than tastes as CSs, probably enhanced the conditioned preferences in this experiment.

A notable finding of this experiment was the dissociation between the conditioned preferences as measured by the 2-bottle tests and the TR responses in the Taste group. Although these animals acquired a significant conditioned preference, they did not show any evidence that the CS^+ had become more palatable than the CS^- as a function of conditioning. In contrast, the Calorie group displayed both conditioned preferences and conditioned palatability shifts to the CS^+ flavour and the Taste/Cal group showed neither effect. The results of this experiment, along with those reported by Elizalde (1990) are the only examples of dissociations between conditioned preferences and palatability shifts reported in the literature. Further, all of our results support the conclusion that flavour-calorie or taste-calorie associations can shift the palatability of flavour and taste cues. Although we failed to observe palatability shifts in Group FI/Flavour in Experiment 9, this may have been because these animals had acquired flavour-taste and not flavour-calorie associations. Although our studies do not provide any evidence that flavour-taste associations produce shifts in palatability, the results of Myers and Hall (2000) are consistent with this claim, though they measured only duration of mouthing and not the full range of taste reactivity behaviours.

In terms of the pattern of TR results in the three experiments, tongue protrusions appeared to be the most sensitive measure of palatability shifts since they increased to the CS^+ in all of the experiments, while none of the other behaviours changed in Experiments 8 and 9. Since many researchers only report the total positive and negative behaviours, it is difficult to determine whether this is a common pattern of results. Myers and Sclafani (2001a), do mention that mouth movements were the most frequent behaviour scored in

the positive category, however they did not see evidence of differential responding to the CS solutions for mouth movements or any of the other individual behaviours. Significant group differences emerged only when all of the positive behaviours were totalled in their experiment.

As for negative responses, behaviour shifts were observed only in the Calorie group in the last experiment. In this experiment paw flicking and face washing occurred more to the CS^- than to the CS^+. In the other groups in this experiment and in experiments 8 and 9, the negative behaviours did not shift as a function of conditioning. Although aversive responding was at a floor in Experiment 8, the mean frequency of all of the responses combined was about 5-6 prior to conditioning. If preference conditioning increases aversive responding to the CS^- (which would suggest that conditioned inhibition occurs to the CS^- during preference conditioning), then there should have been lots of room for aversive responses to the CS^- to increase in all of the experiments. Alternatively, if preference conditioning decreases negative behaviours to the CS^+ (suggesting conditioned excitation to the CS^+), it is possible that a floor effect prevented us from seeing a difference in negative responses between the pre and post conditioning tests in Experiment 8. It is true however, that differences in individual behaviours were observed in the Calorie Group in Experiment 10, even when the individual negative behaviour frequencies were at about the same level as the total frequency of negative behaviours in Experiment 8. Therefore, there was probably enough room to detect changes in negative responses in Experiment 8. Perhaps stronger conditioning is required than that observed in Experiment 8 for shifts in negative responses to occur.

A hierarchy of TR responses has been suggested by Breslin, Spector & Grill (1992). They found that the rate of change was different for individual positive and negative behaviours, as a function of sucrose-LiCl pairings. They ordered the responses from extreme acceptance to extreme rejection in the following manner: tongue protrusions, mouth movements, lip flares, gapes, chin rubs & paw flicks. In their experiments as the total number of ingestive behaviours increased, the total number of aversive behaviours decreased, and vice versa. Therefore, they did not postulate that positive and aversive behaviours might be differentially sensitive. Our data seem to suggest that positive and negative behaviours change independently of one another. This is consistent with Berridge's orthogonal hypothesis of positive and negative TR responses (Berridge & Grill, 1983, 1984; Berridge 2001).

Flavour-taste Associations in Humans

Studies of human evaluative conditioning suggest that Pavlovian flavour-taste associations may play a similar role in the determination of acquired likes and dislikes for foods by humans. We view these studies as analogous to those that evaluate palatability shifts in animals, because humans are asked to rate their liking of particular taste cues after these cues have been repeatedly paired with either a sweet tasting or an aversive tasting reinforcer.

In an early study by Zellner, Rozin, Aaron and Kulish (1983), enhanced preferences for flavoured teas were conditioned in humans by pairing them with sugar solutions. Throughout conditioning, participants were exposed to two flavours of herbal tea, one of which was sweetened with sucrose while the other remained unsweetened. In

the test phase, between-subject comparisons revealed that the flavours that had previously been presented unsweetened received a higher rating than those that had not been previously experienced at all, suggesting that mere exposure was involved in enhancing flavour preferences. Moreover, ratings were stronger for flavours that had previously been presented sweetened than for those that had been presented unsweetened. This implied that flavour-taste associations were operating in addition to mere exposure effects. Although it was also possible that the calorific properties of the sucrose contributed to the flavour preference shift observed, this seems unlikely because the sugar was presented in small quantities to participants who were not hungry. Hence, these results suggest that although mere exposure to the CS solutions was capable of increasing flavour preferences, the flavour-taste associations also contributed to shift of flavour ratings in this experiment. It is interesting to note that in Experiment 10, even though animals in the Taste group consumed more of the CS^+ solution than the CS^- solution in training, they still failed to show palatability shifts to the CS^+.

Shifts in liking for flavours paired with sweet tastes are not always readily conditioned in humans, however. In a more recent study, Baeyens, Eelen, Van den Bergh and Crombez (1990) were unable to obtain evidence of flavour-taste associations with a sweet taste in humans. Alternatively, they did observe decreased preferences for neutral flavours that were paired with a hedonically negative reinforcing flavour. In this study, separate groups received the CS^+ mixed with either a sucrose solution or an aversive tasting Tween 20 (Polysorbate 20) solution, and the CS^- in water. In the test phase, participants evaluated how much they liked or disliked the CS^+ and CS^- flavours.

Although participants that had been reinforced with the aversive Tween 20 solution displayed an evaluative discrimination between the two flavour cues, in which they rated the CS^- more positively than the CS^+, there was no evidence of differential conditioning in the group that was reinforced with sugar.

Because of the procedural differences between this study and that of Zellner *et al* (1983), it is difficult to determine why Zellner *et al*, but not Baeyens *et al* (1990), were able to enhance flavour preferences using a palatable flavour reinforcer. Baeyens and his colleagues (Baeyens, Crombez, Hendrickx & Eelen, 1995) suggested that this inconsistency may have occurred because a large number of their subjects did not evaluate the sugar solution positively. The existence of a significant positive correlation between the ratings of the sugar reinforcer and the strength of the preference for the CS^+ relative to the CS^- indicated that if a more effective positive US had been employed, the positive flavour-taste effect might have been observed. However, Baeyens (in press) reports difficulties at increasing the effectiveness of sugar reinforcers by increasing the number of trials or by the use of individual determination of sugar concentrations.

In a subsequent study, Baeyens, Crombez, Hendrickx, and Eelen (1995) further demonstrated the robust nature of evaluative flavour-Tween conditioning by manipulating the number of acquisition trials during training. People received either 6 or 12 presentations of the CS solutions mixed with the appropriate reinforcer in a differential conditioning procedure. In test, the size of the conditioned effect was similar regardless of whether there were 6 or 12 conditioning trials indicating that this negative flavour-taste association was learned rapidly; i.e., in less than 6 trials. Moreover, during the test phase

this flavour-taste association did not readily extinguish; the conditioned effect was as strong in the second block of 8 non-reinforced trials as in the first block.

Thus, although Baeyens' lab reliably conditions robust dislikes for flavours paired with Tween 20, they consistently fail to condition preferences paired with sweet tastes even for subjects that report liking the taste of the sugar reinforcer (Baeyens, 2002). These studies are interesting in light of the results from our experiments in that they suggest that like our rats, humans do not readily learn to like flavours paired with sweet tastes. It remains to be seen whether rats would show shifts in palatability to flavours paired with an aversive-tasting US.

Expectancy Learning in Flavour Preference Conditioning

In the present set of experiments, the animals that were conditioned with a calorific reinforcer expressed palatability shifts in the TR test. These results suggest that flavour-calorie associations are capable of conditioning shifts in palatability. Additionally our data suggest that flavour-taste associations did not lead to palatability shifts as readily as flavour-calorie associations, yet animals still acquired conditioned preferences when flavours or tastes were paired with sweet tastes. If the animals were not actually learning to "like" the flavour better as a function of the flavour-sweet taste pairings, then what other mechanism may be involved in the formation of the flavour preference in the Taste group in the first experiment?

It has been postulated that expectancy learning may also be involved in flavour preference conditioning. However, very little research has been conducted to investigate the involvement of expectancy learning in flavour preference conditioning. One study by

Campbell, Capaldi, Sheffer and Bradford (1988) used a go/no go discrimination training procedure in which animals were presented with one of two flavour stimuli on the left side of their cage. One flavour cue was followed by access to sucrose while the other was followed by access to quinine, both on the right side of the cage. After several conditioning trials, the animals' latencies to approach the right side of the cage were shorter for the sucrose-paired flavour than for the quinine-paired flavour. Moreover, at the end of the training phase, when the animals were given a 2-bottle preference test, they showed a preference for the flavour paired with sucrose, relative to the other flavour paired with quinine. This go/no go discrimination effect was not mediated by odours emanating from the sucrose and quinine consequences, since random pairings of the cue flavours and consequences failed to result in discriminative approach behaviour in the Control group in the second experiment.

Although it was clear that expectancy learning played a role in this conditioning paradigm, it is worth noting that the conditioning procedure employed in this paper was very different from the typical flavour preference experiment. One major difference was that this task required the animal to instrumentally respond to the US, whereas in most flavour preference conditioning experiments, the animal is exposed to a Pavlovian conditioning procedure. Thus, it is possible that the results of this experiment do not reflect the conditioning mechanisms involved in the formation of flavour preferences conditioned by a typical Pavlovian flavour preference procedure.

It is worth noting however, that Delamater, LoLordo and Berridge (1986) demonstrated that when rats receive pairings of a tone and sucrose in a Pavlovian

differential conditioning procedure, presentation of the tone enhanced responding to water more than another auditory stimulus that had not been conditioned. Thus, presentation of the tone produced an expectancy of sucrose, which induced a change in the palatability of water during the TR test. This experiment is interesting because it demonstrates that palatability shifts may not always occur directly in flavour preference conditioning, instead these shifts may occur as a function of expectancy learning.

Future Directions

One of the main themes that has run through the experiments within Chapters 1 and 2 is how an animal's restriction state interacts with preference conditioning. Our data have provided some insight on how food and water restriction states can affect acquisition and expression of conditioned preferences. Our data have shown that an experimenter's choice of food and water restriction parameters and CS solutions can affect animals' ability to acquire preferences. For example, when food and water deprived, animals do not readily acquire preferences for relatively unacceptable tastes unless they are already somewhat palatable. However, animals that are thirsty but not hungry will acquire preferences readily for these relatively unacceptable tastes. Whether a preference is expressed in test depends on what type of association animals have acquired as well as their deprivation state. We, in addition to others, have shown that the expression of flavour-calorie associations is stronger when animals are tested hungry than when they are tested sated (e.g., Fedorchak & Bolles, 1988; Harris *et al*, 2000). However the expression of flavour-taste associations in a preference test does not appear to be affected by restriction state in test unless calories are present during conditioning. If calories are

present during conditioning, they will override the flavour-taste association in test when the animals are tested while food restricted. However, if rats are tested while not food restricted, they will display preferences based on their flavour-taste association.

Unfortunately in the last experiment we were unable to assess how restriction state affects the expression of conditioned shifts in palatability, due to the loss of cannulas. It would have been especially interesting to see how expression of palatability would have been affected in animals that received calorific reinforcers during conditioning (i.e., Group Calorie and Group Taste/Cal). One might expect Group Calorie to show a weaker palatability effect when not food restricted in test. It is difficult to determine how Group Taste/Cal would respond in the TR test when sated. It is possible that the results from the TR test would mirror those of the 2-bottle test. That is, rats in the Taste/Cal group would show palatability shifts when tested sated. However, given the fact that the Taste Group, whose flavour preferences were also based on flavour-taste associations, failed to show palatability shifts when they showed strong 2-bottle preferences, it is possible that the Taste/Cal group would also fail to demonstrate conditioned palatability shifts when tested sated.

It would be interesting to replicate Experiment 10 and manipulate hunger levels in Test 1 and 2. Half of the animals would be tested while food restricted and the other half while free fed in Test 1. For the second test, their food restriction state would be reversed. This design would eliminate the confound of test order that occurred in Experiment 10. Additionally, to reduce the high rate of attrition observed in Experiment 10, the wire tops of the shoebox cages could be adapted by covering the food hopper that dips into the

animals' cages with a steel plate. This would prevent the rats from catching and damaging their cannulas on the hoppers.

It would also be interesting to condition sated animals using the long-exposure simultaneous conditioning paradigm. Although rats may not express conditioned preferences when tested sated, this does not mean that they will not acquire conditioned preferences when trained sated. In fact, previous work has shown that deprivation state in training does not affect the strength of the conditioned preference acquired in either rats (Fedorchak & Bolles, 1988) or mice (Forestell, Schellinck, Boudreau & LoLordo, 2000). According to a report by Berridge and Schulkin (1989), this may also be the case for palatability shifts in rats. In this experiment when taste cues such as quinine and citric acid are paired with a salt in sodium-replete animals upon subsequent sodium depletion, these animals will display an increased liking for these taste cues relative to sodium-replete animals.

Given our failure to observe evidence of palatability shifts in response to a flavour paired with a sweet taste, a logical next step would be to determine whether conditioned palatability shifts occur to flavours that have been paired with aversive tastes, such as quinine. We already know from Fanselow and Birk that flavour aversions can be conditioned by pairing a flavour with an aversive taste. Further, if analogies exist between the palatability shifts in rats and evaluative conditioning in humans, then one might predict that the bitter taste of quinine would be more effective at shifting palatability downward than sweet tastes at shifting palatability upward.

Finally, assuming that palatability shifts did occur in response to flavour-taste associations in Myers and Hall (2000), it would be informative to determine whether our failure to observe a similar effect in the Taste and Taste/Cal groups in Experiment 10 occurred because our reinforcer was less sweet than that of Myers and Hall. Rats in Myers and Hall received a 30 % sucrose solution, whereas ours received a .4% saccharin solution, which is equihedonic to a 4% sucrose solution. Although it might seem intuitive to merely increase the concentration of the saccharin solution, Young and Madsen (1963) have shown that beyond .4%, saccharin actually becomes less preferred in a test too short for post-oral factors to matter. Therefore, in order to employ a highly sweet tasting reinforcer, the flavour cue would have to be paired with a concentrated sham-fed glucose or sucrose solution.

In conclusion, although we have a good understanding of the types of reinforcers that produce conditioned flavour preference, there has been considerably less work investigating the mechanisms involved in the formation of flavour preferences. Our work in combination with Breslin *at al* (1990), Myers and Hall (2000) and Myers and Sclafani (2001a), suggests that palatability shifts play an important role in the formation of conditioned flavour preferences. Additionally, our work demonstrates situations in which palatability shifts do not accompany flavour preference learning. The fact that flavour preferences can be conditioned in the absence of palatability shifts suggests that palatability shifts are not the sole mechanism operating in flavour preference conditioning.

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Footnotes

[1] Data from Experiments 1-3 of this chapter have been published in Learning and Motivation, Volume 31, pages 153-179. Data from Experiments 4-7 have been submitted to Animal Learning and Behavior.

[2] Throughout this document the term flavour will be used to define stimuli that have taste and odour components.

[3] Additionally, in Experiment 1 forward groups were included in which presentation of the CS preceded presentation of the US.

[4] Although rats had free access to food over night throughout training, they were probably somewhat food restricted because they did not have access to water. Compared to the previous experiments however, in which rats had only 1 hour access to food, these animals were less hungry.

[5] Data from Experiments 8-10 of this chapter have been submitted to Quarterly Journal of Experimental Psychology.

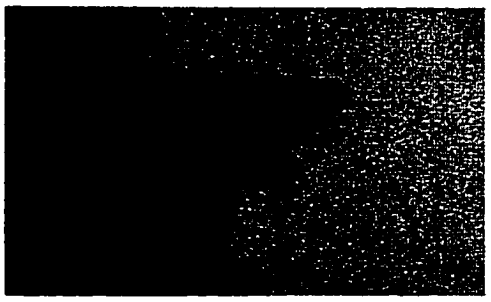
Appendix 1

Positive Behaviours

Tongue Protrusion (tp):
Rhythmic midline protrusions of the tongue, including floor licking. In all experiments frequency of individual tongue protrusions was recorded.

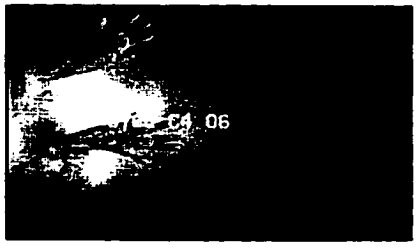
Lateral Tongue Protrusion (ltp):
Tongue protrusions in which the tongue emerges in either corner of the mouth and pushes the upper lip laterally as it moves forward. In all experiments, frequency of individual lateral tongue protrusions were recorded.

Mouth Movements (mm):
Rhythmic movements of the mouth without tongue protrusions. In all experiments, frequency of individual mouth movements
were recorded

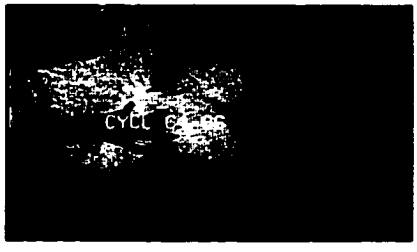


Paw Licks (pl):
Continuous licking of the paws and circular wipes of the face bringing fluid to the mouth. In all experiments individual paw licks were measured by observing movement of the paws or movement of the jaw.

Negative Behaviours

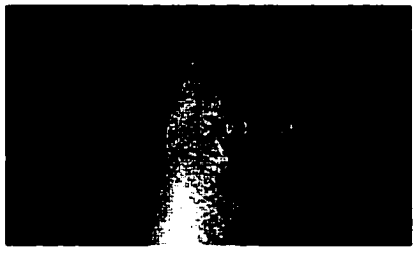


Headshakes (hs):
A burst of rapid side-to-side movements of the head and neck.

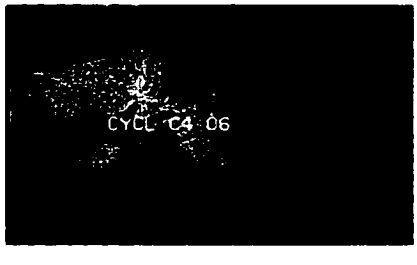


Paw Flicks (pf):
A burst of rapid movements of one or both of the forelimbs

Paw Treads (pt):
Forward and backward movement of the forepaws in sychronous alternation



Gapes (g):
Large opening of the mouth with the corners of the lips retracted; the mouth has a triangular shape.



Face washes (fw):
Wipes over the face with forepaws while rearing; often alternate with paw flcks.