CHAPTER 1

Rats that are poisoned after consuming a novel flavour will show an aversion to that flavour (Garcia, Ervin, & Koelling, 1966; Garcia & Koelling, 1966). This aversion to the flavour is called a conditioned taste aversion (CTA). It can be viewed as a Pavlovian process, where the flavour acts as a conditioned stimulus (CS) and the poisoning/illness acts as the unconditioned stimulus (US).

There are some unique procedural variations in taste aversion experiments that are not used in other kinds of preparations. Two important differences include the use of long-acting, interoceptive, gastrointestinal nausea as the US instead of exteroceptive application of electric shock, and secondly, the use of a taste CS that has motivational properties instead of a biologically neutral event such as a light or tone. The violation of biological neutrality does not pose an important methodological problem since the taste CS does not initially elicit the nausea UR. Nevertheless, the use of an appetitive reinforcer as a signalling stimulus stands apart from traditional Pavlovian conditioning procedures, and it is this fact that has led some authors to exclude taste aversion learning from classical conditioning (e.g. Spiker, 1977).

One alternative offered by Garcia (1989; 1990) hypothesizes that taste aversion learning involves CS-US-FB relations. After a taste has been paired with a lithium injection, a hypothetical feedback mechanism (FB) selectively modulates the hedonic value of the taste. Garcia and colleagues have established a taste aversion when the

animal is anaesthetised before the lithium injection (Bermudez-Rattoni, Forthman, Sanchez, Perez, & Garcia, 1988). They take this to demonstrate that the feedback mechanism operates without awareness of the relation between the CS and the US. The primary function of such feedback in taste aversion conditioning is to reduce the hedonic value of the taste. The negative after-effects of nausea-paired food emanate from the gut and selectively seek out the taste of the food in the mouth debasing its palatability. According to this view, the taste stimulus plays a dual role. As a CS it signals the onset of nausea, and this role is greatly facilitated by attention and memory. But after conditioning it operates as a US because it has acquired its own aversive properties due to a hedonic shift in palatability; in other words it now tastes disgusting. The evidence for this is presented below.

Despite alternative conceptions of taste aversion learning, CTAs have been shown to be amenable to many of the laws of Pavlovian conditioning (for a review see Logue, 1979). For the sake of parsimony, in this thesis taste aversion conditioning will be viewed as a class of Pavlovian conditioning with a few exceptions.

There appear to be at least three important differences between the learning expressed as a result of taste aversion conditioning and other forms of conditioning. Firstly, taste aversions are acquired despite extremely long delays between the CS and the US. For example, Revusky (1968a) established an aversion to sucrose in rats with a 7-h delay, and Smith and Rolls (1967) used saccharin to

establish an aversion over a 12-h delay. Secondly, there appears to exist a proclivity for animals (rats and humans) to associate tastes and not other stimuli with illness (Garcia & Koelling, 1966). The third important difference in taste aversion learning is suggested by research showing that these procedures appear to produce a hedonic shift.

An explicit demonstration of a hedonic shift has been provided by Pelchat and colleagues (Pelchat, Grill, Rozin, & Jacobs, 1983). Rats trained to avoid a sugar solution by pairing it with lithium (nausea) showed orofacial responses indicative of distaste when presented with the solution again. Other rats trained to avoid the solution by pairing it with shock or lactose (lower gastrointestinal tract discomfort) showed a complete absence of such responses. This qualitatively different response remained despite equally low levels of observed aversion in all groups, and considerably longer training times for the shocked rats (up to 25 days compared to 2 days for other groups). The hedonic shift produced by lithium toxiphobia has been replicated in studies since (Parker, 1988, 1995), and unique neuroanatomical substrates underlying the hedonic shift produced by lithium toxicosis have been identified in the amygdala (Simbayi, Boakes, & Burton, 1986).

Despite suggestions the palatability shift is responsible for the avoidance response, there is some reason to believe that a palatability shift is not the sole mechanism responsible. For example, Parker (1988) found that equally-avoided conditioned (sucrose-LiCl) and unconditioned (quinine) aversive flavoured solutions did not produce rejection responses of equal magnitude in a taste reactivity test. In

particular, the rejection responses to quinine were stronger than for equally-avoided lithium-paired sucrose. Apparently the lithium-paired sucrose was avoided not solely because it had become unpalatable, but due to an additional mechanism perhaps similar to that suggested by traditional learning theories (e.g. Rescorla & Wagner, 1972).

More than a decade after Garcia demonstrated most of the unique properties of taste aversion learning known today, most researchers had taken the view that the data can be incorporated into existing, or somewhat modified, general laws of learning (e.g. Logue, 1979; Mackintosh, 1974; Revusky, 1977). For example, despite the selectivity of associations between tastes and illness, exteroceptive cues such as visual and tactile cues, as well as contexts can be associated with illness (Best, Best, & Henggeler, 1977; Boakes, Westbrook, Elliot, & Swinbourne, 1997), although such learning requires repeated trials, closer temporal contiguity between cue and toxicosis, and the absence of interfering interoceptive cues. And in accordance with other forms of learning experiments, it has been consistently found that the strength of a CTA is less with longer intervals between the CS and US (Garcia & Koelling, 1966; Logue, 1979; Nachman, 1970; Revusky, 1968a). Furthermore, experiments have shown that the short delay between the CS and US in traditional experiments can be lengthened (up to two hours) if potential sources of interference are removed (Lett, 1977; Revusky, 1977), though 2 hours is well short of the 24 hour interval shown in some cases of taste aversion learning (e.g. Etscorn & Stephens, 1973).

In sum, although there are some large and obvious parametric differences between taste aversion learning and other forms of learning, there remains very little to distinguish taste aversions on qualitative grounds. One possible exception is the case of potentiation of odour and illness associations by tastes. Potentiation of an odour occurs after it is presented in combination with a taste and illness, and subsequently an aversion to the odour is established (Lolordo & Droungas, 1989). This may represent an exception to the general law of overshadowing. Apart from this exception, which does not directly concern the subject of this thesis, what reason is there to conclude that a different set of principles are necessary to correctly explain performance in a CTA task? Another possible exception might be provided by the studies described above showing taste aversions result in a hedonic shift to the CS. However, a categorical distinction between taste aversion learning and other forms of learning is not endorsed here, but rather a more conservative view that conditioning phenomena are made up of many components and that the hedonic component is one strong and pervasive aspect of taste aversion learning. Another component is the predictive value of the CS in relation to the US, but it is the role of the hedonic component that may be important in this thesis.

Extinction.

Modern descriptions of extinction have developed from the account given by Konorski (e.g. Konorski, 1967). Konorski's views on

conditioning emphasised the importance of the predictive value of the CS in relation to the US. A general assumption of Konorski's view was that representations of the CSs and USs can enter into associations, but only those CSs that accompany increases or reductions in the activation of the US representation develop associations and thus become conditioned.

According to this view, extinction depends on the formation of associations between the CS and a centre activated by the absence of the US, called the no-US centre. The activation of the no-US centre occurs as a consequence of the activation of the US centre in the absence of the US itself, and it is assumed to have inbuilt inhibitory connections with the US centre. Thus, omission of the US results in a response decrement to the CS. In a similar manner, the Rescorla-Wagner model (Rescorla & Wagner, 1972) claims that only stimuli that predict the non-occurrence of an expected US develop inhibitory properties during extinction. Konorski (1967) also permitted contextual determination of the activation of the no-US Gnostic unit which parallels Bouton's more recent view (e.g. Bouton, 1993) that the context of extinction comes to gate an inhibitory association with the US. Despite differences between modern theories most explanations of extinction today recognise along with Konorski that the response decrement is partly due to the loss of predictive value of the CS with respect to the US.

If extinction is due to the changed predictive status of the CS in relation to the US, what factors would produce resistance to extinction?

Research on the partial reinforcement effect (PRE) suggests that another factor governing the rate of extinction appears to be the amount of generalization between acquisition and extinction.

Both the frustration theory of Amsel (1958, 1967, 1992) and the sequential theory of Capaldi (1967) called attention to the generalization between learning and extinction, pointing to the importance of viewing extinction in terms of the transfer from acquisition. They note, in one language or another, how different conditions of acquisition bear different similarity relations to the circumstances of extinction. They depend on the observation that treatments that encourage learning about features of the acquisition that would also be present in extinction will result in the most behaviour during extinction.

It seems clear from the evidence presented for such theories that a major contributor to the response-decrement is the changed stimulus situation in which the animal is asked to exhibit its learning. Omitting the outcome or US clearly removes from the situation a major stimulus in the presence of which initial learning took place. Capaldi (1967) argues that this change may account for a large portion of the loss of responding due to generalization decrement. Similarly, the repeated occurrence of non-reinforcement gives rise to different emotional states such as frustration or relief. To the degree that these states were absent in acquisition, and the animal has been denied the opportunity to learn to respond in their presence, we can expect their appearance to disrupt performance (Amsel, 1967). Thus, the absence of the shock

after tone-shock conditioning may result in relief to the tone. This new emotional reaction would disrupt the fear of the shock once elicited by the tone. However, in the case of the extinction of taste aversions, if the original stimulus (CS) undergoes a hedonic shift in the palatability of the taste such that it now tastes disgusting (e.g. Garcia, 1989) the overall changes to emotional state from acquisition to extinction will be less. To the extent that disgust is similar to the initial emotional reaction to nausea during conditioning, the emotional conditions experienced during initial conditioning (nausea) will resemble the conditions under which extinction takes place, when extinction is dominated by disgust reactions to the CS alone. For the point to be made here, it must be seen that in tone-shock conditioning relief during extinction would interfere with the fear response, whilst in taste-nausea conditioning disgust may not necessarily interfere with nausea. Thus, unlike other forms of learning, extinction of CTAs may not arrange for a new emotional response to occur following the CS that might be necessary to produce the decrement in response.

Of course, under extinction the CS no longer predicts the occurrence of the US, and this loss in predictive value may undermine any effect of maintaining the emotional state between acquisition and extinction. Yet, taste aversions may be less vulnerable to the loss of predictive value. Most extinction schedules are characterised by the absence of the US. Unlike an explicitly presented US, which presumably contains sensory, affective and response components, the absence of the US contains little sensory information and is dominated

by responses generated by the animal itself (e.g. Rescorla, 1973). In the case of taste aversions it has been argued that the response to the CS is dominated by a powerful disgust reaction which may be even more salient during an extinction schedule. There is substantial evidence that emotional responses themselves condition stimuli paired with them. Both Wagner (1963) and Daly (1974) found that neutral stimuli present during the time of omission of an anticipated reward take on aversive properties, becoming themselves capable of promoting escape learning. Thus, it is possible that the disgust reaction to the taste after conditioning is maintaining the aversion and the taste's original predictive relation with nausea is no longer important. If the stimulus conditions during extinction are dominated by the animals own disgust response, rather than the absence of nausea then the effect of the loss of predictive value may be less. In sum, the principles described by Capaldi (1967) and Amsel (1967) to explain extinction might also be used to account for resistance to extinction after taste aversion conditioning. On the other hand, the principles described by Konorski (1967) and others (e.g. Rescorla & Wagner, 1972) clearly predict that extinction should occur when the CS no longer predicts the US.

Evaluative conditioning.

Pavlovian conditioning may involve at least two separate components. On the one hand, the subject can learn about the contingency relationship between the CS and the US, i.e. that the CS

predicts or signals the US. This kind of learning is referred to as signal learning. On the other hand, the subject's evaluation of the CS can be altered intrinsically in a classical conditioning situation; i.e. learning to like or dislike the CS itself (Garcia, Kovner & Green, 1970; Levey & Martin, 1987). In human learning research this kind of learning is referred to as affective – evaluative learning or evaluative conditioning (Levey & Martin, 1975; Levey & Martin, 1983; Martin & Levey, 1985), and it is used to describe the process by which an affective evaluative reaction evoked by a significant stimulus is transferred to a previously neutral stimulus, presented contiguously with the significant stimulus. Viewed this way it has been compared to the idea that conditioned responses may be linked directly to the CS, as in S-R type associations (Baeyens, Eelen, & Crombez, 1995). In this view, the US representation plays little or no role in producing the conditioned response after the initial stages of conditioning.

Evidence for human evaluative conditioning comes from experiments that have paired sugar with neutral tea flavours and found a flavour preference for the unsweetened tea on subsequent test (Zellner, Rozin, Aron & Kulish, 1983) and other experiments that have paired Tween20 (bitter) with neutral fruit flavours and found an aversion to the once neutral flavour on subsequent test (Baeyens, Eelen, Van den Bergh & Crombez, 1990). What is interesting for the purposes of this thesis is that such preferences have been shown to be resistant to extinction. For example, Baeyens, Crombez, Hendrickx and Eelen (1995) first exposed subjects to 6 or 12 pairings of neutral fruit

flavours and either Tween20 or sugar as the US. This produced reliable shifts in CS evaluations, but more importantly, these evaluations were unaffected by 8 non-reinforced CS presentations. The explanation of this resistance to extinction relies upon the assumption that the acquired (dis)liking of the CS is not mediated by reference to the US.

Levey and Martin (1983) proposed that, unlike signal-learning, the evaluative response should be resistant to extinction. In signal-learning the subject essentially acquires the proposition "CS predicts US" during the acquisition phase. Hence, the CS acquires its significance through reference to the US-representation (Konorski, 1967). During extinction then, the CS-generated expectation of the US is disconfirmed: the proposition would become "CS no longer predicts the US". The same does not necessarily apply to evaluative learning. If the acquired (dis)liking of the CS is not mediated by reference to the US, then the fact that the US is no longer contingent with the CS might well leave the acquired value of the CS unaltered.

The failure to detect an extinction effect in experiments by Baeyens and his colleagues may simply reflect the small number of extinction trials that were given. Alternatively, the evaluative conditioning effect may not be a form of associative learning at all (Field & Davey, 1999; Shanks & Dickinson, 1990). However, some recent evidence from animal research has demonstrated similar resistance-to-extinction that is not so vulnerable to such criticisms. For example, Sclafani and colleagues have demonstrated that a flavour paired with post-ingestional, polycose infusions produced a

conditioned taste preference that was not at all affected by 14 days of a standard extinction procedure. Even more surprisingly, the conditioned preference survived – although in weakened form – an additional non-reinforced exposure to the positive CS over a period of 40 additional extinction days in the home cage (Elizade & Sclafani, 1990). Similarly, work with flavour-flavour conditioned preferences has shown that long extinction periods (14 days) do not noticeably remove the conditioned preferences or aversions to flavours (e.g. Fanselow & Birk, 1982; Harris, Shand, Carrol, & Westbrook, 2001). At present there appears to be no reports indicating unusual persistence of hedonic conditioning involving stimuli other than flavours. Nevertheless, given this evidence from both rats and humans on the persistence of hedonic or evaluative conditioning of flavours, the problem of resistance to extinction of such preferences has become a special problem. It is unknown whether this reflects a general property of hedonic reactions in other classical conditioning procedures such as taste-aversions.

Counter-conditioning.

" . . .the evaluative response, once it is conditioned to a neutral stimulus, cannot thereafter be extinguished through non-reinforcement, but can only be altered by counter-conditioning" (p. 122, Levey & Martin, 1987). Apart from extinction per se, another source of response decrement seen after acquisition of an aversion might be counter-conditioning. A counter-conditioning procedure is one in which a CS is first paired with an aversive US and then paired with an appetitive US,

or vice versa. If effective, the second appetitive US is said to have *counter-conditioned* the CR to the first aversive US. A widely accepted account for such effects is that the two motivational systems tend to inhibit one another reciprocally (Dearing & Dickinson, 1979; Konorski, 1967; Rescorla & Solomon, 1967). Within the terms of the Rescorla-Wagner model, they have opposite algebraic signs and transformation of the CS into an appetitive excitor would cancel out its initial aversive association, representing the loss and replacement of the old learning with new learning about the appetitive consequences.

Both counter-conditioning and extinction involve the attenuation of the original CR. Stimuli after counter-conditioning also show other similar properties to stimuli after extinction. Reinstatement after both counter-conditioning (Brooks, Hale, Nelson, Bouton, 1995) and extinction (Schachtman, Brown, & Miller, 1985) produces an increment in the response. Consumption of flavours in taste aversion experiments show renewal after both extinction (Rosas & Bouton, 1997, 1998) and counter-conditioning (Peck & Bouton, 1990); as well as spontaneous recovery after both counter-conditioning (Bouton & Peck, 1992) and extinction (Brooks, Palmatier, Garcia, & Johnson, 1999; Rosas & Bouton, 1996). As the responses observed in CTA experiments display the same general phenomena after both counter-conditioning and extinction, the possibility arises that any performance loss observed in CTA experiments may be due to either counter-conditioning processes, extinction processes, or both.

CHAPTER TWO

Seligman (1970; Seligman & Hager, 1972) claimed that taste aversions represent prepared forms of learning because such aversions can be established over relatively long delays, and also because tastes seem particularly associable with illness. Animals are naturally prepared to associate events that are more likely to have occurred together in the organism's evolutionary history or its previous experience (such as tastes and illness), and unprepared, or even contra-prepared to learn others (such as tones and shock). Traditional learning theories are based on data from experiments that may exploit the features of unprepared or contra-prepared learning. An example of this might be extinction. When Pavlov (1927) demonstrated experimental extinction by measuring the reduction in salivation to an arbitrary clicker that used to signal meat powder but no longer did, he hoped that this was an instance of extinction that would have application beyond clicking metronomes, meat powder, and salivation. But the response decrement observed may be due to the unnatural and arbitrary conjunction of a clicker and meat powder. So, when researchers pair events that are more likely to occur in nature, exceptions to this law of extinction might be found. Whether or not taste aversions show such resistance to extinction, as some authors have claimed (e.g. Garcia, Hankins, & Rusiniak, 1974; Mitchell, Scott, & Mitchell, 1977) is examined below.

If the extinction of CTAs has sometimes been difficult to demonstrate, it may primarily be because rats do not voluntarily re-

expose themselves to the taste once an aversion to it has been produced. For example, there is some evidence that rats will avoid approaching the spout once an aversion is established (Mitchell, Kirschbaum, & Perry, 1975), implying that the lack of extinction observed in this case could be due to learned avoidance. The in-extinguishability of learned avoidance is not equivalent to resistance-to-extinction, because if the CS is avoided, then CS re-exposure will not occur, and if the CS is not re-exposed by itself then the aversion (CR) will not subside, and extinction cannot be observed. It is worthwhile noting that repeated 2-bottle tests were often used in early experiments, which allowed such avoidance of the adverse spout and this may have led to researchers mistaking such avoidance for resistance to extinction. Nevertheless, numerous experiments have shown that extinction of a taste aversion will occur when the CS is repeatedly presented alone (e.g. Baum, Foidart & Lapointe, 1974; Colby & Smith, 1977; Domjan, 1975; Garcia & Koelling, 1966; Nolan, McGaughey, Giza, Rhinehart-Doty, Smith & Scott, 1997; Nowlis, 1974). In addition, it has been found that extinction is faster if exposure to the CS is not dependent on the rat approaching the spout. For example, when CS fluid presentation occurs through an oral cannula surgically mounted to the rat's cheek rather than in a one-bottle test, extinction occurs relatively quickly (e.g. Wolgin & Wade, 1990). Extinction of taste aversions also shows similarities with other forms of learned aversions. For example, the rate of extinction of taste aversions varies with the characteristics of acquisition; the amount of

CS-alone presentations; and the context of learning, as does extinction in more traditional learning tasks (Abelson, Pierrel-Sorrentino, & Blough, 1977; Mikulka & Klein, 1980; Rosas & Bouton, 1997). In addition, there is such a large range in the resistance to extinction of tasks acquired in standard conditioning procedures that trying to establish the case that taste aversions are resistant to extinction by making comparisons across widely different procedures is fruitless. In sum, without some alternative explanation provided, the fact that in Garcia's classic experiment published in Psychonomic Science (Garcia & Koelling, 1966), extinction was complete after three 10-min sessions is evidence against any argument that taste aversions are resistant to extinction compared with other forms of learning.

An alternative explanation: The role of counter-conditioning.

An important common feature of the above demonstrations of extinction of a CTA is that in order to get rats to drink an aversive fluid or food, experimenters have deprived their subjects of water or food. This introduces the possibility that the loss of aversion observed might be due to thirst relief counter-conditioning the aversion, rather than extinction per se. As stated before, counter-conditioning can occur when a once aversive CS is paired with a rewarding US, and a new CR appears. Forcing thirsty rats to drink an aversive solution in extinction conditions may relieve their thirst and if the relief from thirst acts as a positive US, counter-conditioning may entirely account for an observed increase in consumption, which would then be mistaken for extinction.

With few exceptions, using deprived animals has been the common practice in such experiments. Only two experiments have examined the possible role of such a confound in extinction. Grote and Brown (1973) showed that rats under high water-deprivation conditions (23-hr) exhibited more rapid extinction than rats under low water-deprivation conditions (10-hr). Similarly, Balagura & Smith, (1970) found an aversion to a sodium chloride solution was extinguished faster when a need for sodium was produced by injections of formalin or by adrenalectomy. However, simply manipulating the motivational state of rats is not enough to avoid other potential confounds. The faster rate of extinction seen in the above studies could have been due to the fact that the thirsty rats drank more of the CS fluid, and thus they were exposed to the CS for longer than the less-thirsty rats. The fact that the rate of extinction increases with the amount of exposure to the CS is already well established (e.g. Abelson et al., 1977; for a review see Mackintosh, 1974), but it does not constitute evidence against a strong evaluative account of taste aversions. The effect of mere exposure of a stimulus may produce positive hedonic shifts in humans and preferences in animals, regardless of initial stimulus valence (Revusky & Garcia, 1970; Zajonc, Markus, & Wilson, 1974). Thus, the decrease in aversion may be the consequence of a shift in the pervasive hedonic component of the CTA, and only by controlling the amount of fluid exposure during extinction would this potential confound be controlled for.

What evidence is there that thirst relief can act as a potential source of counter-conditioning during extinction of CTAs? Studies have shown that relief from thirst can be associated with flavours as an appetitive US. (Revusky, 1967, 1968b, 1974). For example, Revusky (1974) established an increased preference for an otherwise neutral flavour in rats after just three pairings with thirst relief from 42-h water-deprivation. This preference lasted through 39 days of extinction testing (when rats were not water deprived) and was still present 60 days after initial conditioning in a retention test. It was specific to the flavour and did not depend on differences in familiarity between the target flavour and a control flavour. Capaldi and colleagues have extended such results to conditioned food preferences using relief from hunger as the US (Caampbell, Capaldi & Myers, 1987; Capaldi & Myers, 1982; Capaldi, Myers, Campbell & Sheffer, 1983). In sum, it appears that flavour preferences conditioned by relief from deprivation may have a strong hedonic component, as indicated by resistance to extinction, and little retention loss. This fact, taken with the fact that most studies on extinction of CTAs have used deprived animals, means the possibility remains that the observed extinction of conditioned taste aversions is at least in part due to counter-conditioning by relief from deprivation.

Summary.

CTAs may represent a unique form of learning involving naturally prepared associations or a hedonic shift as in evaluative conditioning.

Under such views, CTAs may represent an exception to extinction. In spite of this, CTAs do show classical extinction functions but there is some reason to believe that the extinction observed could be due to counter-conditioning. Previous demonstrations of extinction of CTAs have confounded deprivation state with extinction, or failed to control for differences in CS exposure during extinction. Both these factors could be responsible for the observed increase in intake of a previously -aversive taste.

It is worth distinguishing between two sorts of claims arising from the preceding review. A strong claim might be made from human evaluative conditioning, namely, that all the observed loss of aversion observed during extinction trials of CTAs is due to counter-conditioning (Levy & Martin, 1987; Baeyens et al., 1988). This claim would be based upon the principle that evaluative conditioning is entirely due to a hedonic shift to the CS in order to produce the observed aversion. Extinction should not occur in that case because the hedonic value of the CS remains unchanged when it is presented alone. A second, weaker claim might be made from suggestions derived from Seligman (e.g. Seligman, 1970). Under this more conservative view CTAs represent a naturally prepared form of association that is quickly and strongly formed and also slow to remove. Thus extinction should occur as the predictive relation between the CS and illness is lost with CS-alone trials, but counter-conditioning processes would accelerate the aversion loss.

The present set of experiments attempts to examine the role of deprivation state on the extinction of CTAs whilst controlling for the amount of exposure to the CS. The results should reveal important features of taste aversions, and by implication, help distinguish taste aversions from other forms of learning or alternatively, provide a rapprochement between these forms.

Immediate Background to Present Study.

Whitfield (1999) obtained results in two experiments in this laboratory suggesting that thirsty rats undergo faster extinction than sated rats. Below is a summary of those results.

His first experiment used two groups of rats (n=8) on a daily 30-min water schedule. Each rat was injected with 0.15M LiCl (i.p.) at 5ml/kg after drinking a 0.1% saccharin solution (w/v). Conditioning was followed by the first 2-bottle choice test between saccharin and water. After this test (and two days rest) extinction training occurred. One group of rats was manually infused with 1-ml saccharin before the daily 30-min water access (Group Thirsty) while the other group was manually infused after this 30-min water access (Group Sated). These infusions occurred once a day for four days, followed by a choice test and two days rest. This 7-day cycle occurred three times, and the final cycle was immediately followed by a fifth and final choice test. The results of the five choice tests are shown in Figure 2.1 (pp. 27). As can be seen, group Thirsty showed more extinction to saccharin than Group Sated across the extinction test sessions. This difference was

significant (group main effect, $p < 0.05$, two-tailed $t_{0.05, 8} = 2.35$), but there was no statistical evidence that the rate of extinction was faster in Group Thirsty than Group Sated as the linear interaction between groups failed the 0.05 criterion ($F_{1, 14} = 4.31$, $p = 0.057$).

The significant main effect reported above has several alternative explanations apart from the theoretically interesting one. Firstly, despite equivalent handling and treatment, the Thirsty group consumed more saccharin at the initial test than the Sated group. It is possible that prior to the extinction procedure a weaker aversion was produced in the Thirsty group than the Sated group. A main effect without a significant linear interaction between groups is consistent with this alternative. Secondly, Whitfield did not control for the effect of the infusion procedure itself. Infusions only occurred with the CS during extinction, rather than as a within-subject water control as well. Sated rats struggle more and show more signs of stress to the intrusive infusion procedure than thirsty rats. This might mean the sated rats were more stressed than the thirsty rats. Higher levels of stress in these rats might have maintained the aversion relative to the less stressed rats. One way to reduce the possibility of this happening would have been to infuse both groups of rats when both thirsty and sated. This would ensure that both groups of rats were equally stressed.

Whitfield's second experiment also provided evidence for an effect of counter-conditioning on extinction, but again there are problems with these data. The experiment involved four groups (n=8) run in two replications. As in the previous experiment rats were

infused with 1-ml of saccharin when either thirsty (Dep-1) or sated (Sated) during extinction. No attempt was made to control for the effect of the infusion procedure. An additional control group was added which was only ever infused with water (Water), and a final group was only infused with 0.3-ml of saccharin (Dep-0.3). The extinction treatment lasted 12 days with no testing during that time. The results from a pair of planned 2-bottle choice tests failed to reveal any differences between groups, but the results from a further 1-bottle test did reveal an interesting difference, but in the first replication only. When the subjects from the second replication were added, all differences were no longer significant. The results from the first replication and both replications (combined), from the third 1-bottle test are presented in Figure 2.2 (pp. 27). In the top panel, the group which received 1-ml of saccharin when thirsty (Dep-1) shows a much higher level of consumption of saccharin than any of the other groups. This difference was confirmed in a Tukey HSD test of all possible comparisons. That is, Dep-1 drank significantly more saccharin at test in comparison to any other group. There were no significant differences between any of the other groups. However, as can be seen in the bottom panel of Figure 2.2, when the subjects from the second replication were added the large difference between Dep-1 and the other groups was somewhat reduced, and there were no significant differences between any of the group

The Effect of Thirst On The Course of Extinction

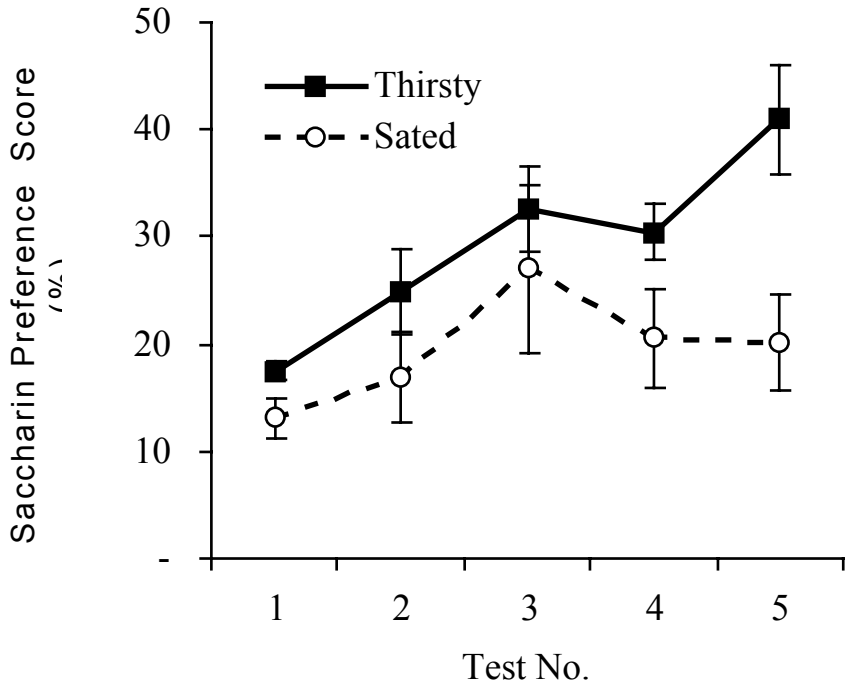

Figure 2.1 From Whitfield (1999), Experiment 1.

A conditioned taste aversion was established in 16 rats, and then half the rats received saccharin exposures when thirsty, whilst the other half received exposures when sated. A faster rate of extinction was observed in the thirsty rats. Vertical bars represent SEM.

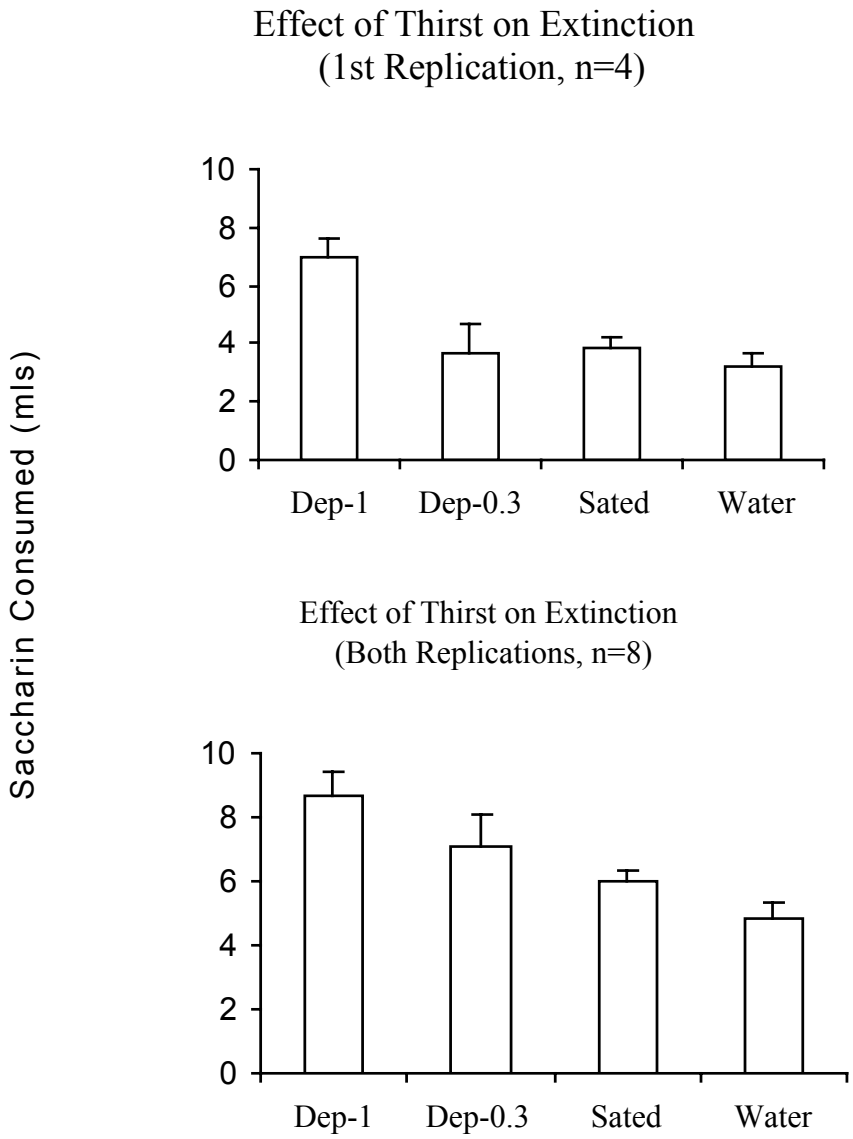

Figure 2.2 From Whitfield (1999), Experiment 2.

Mean saccharin consumption after 12 days of extinction of a CTA to saccharin under four different conditions. Dep-1 was re-exposed to 1-ml sac when thirsty, Dep-0.3 was re-exposed to 0.3-ml sac when thirsty, Sated was re-exposed to 1-ml sac when sated, and Water was not re-exposed to sac. Rats were then tested with a 1-bottle sac test. Vertical bars represent SEM.

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Taken together, the results of Whitfield's experiments suggest that the effect of counter-conditioning on the rate of extinction is minimal but detectable.

Some comments on the methods used in this thesis.

As stated in the above section, the present set of experiments attempted to examine the role of thirst on the extinction of a conditioned taste aversion to some discriminable flavour, whilst controlling for the amount of exposure to the CS.

Previous experiments examining the role of deprivation state on the acquisition or extinction of a taste aversion have usually manipulated the deprivation state between groups. For example, Grote and Brown (1973) compared the consumption rates of two groups of rats, one group maintained on a 23-h water deprivation schedule and the other group maintained on a 10-h deprivation schedule. Under such a method, both groups of rats were water deprived and so any potential differences due to deprivation state would be reduced. This drawback is usually taken as a necessary consequence of the fact that rats must be thirsty before they will drink. Another drawback is that it also introduces any potential (as yet unidentified) confounds arising from the fact that each group is on a different daily 30-min water schedule. To eliminate these drawbacks in the current set of experiments all groups are kept on the same daily water schedule. Deprivation state is manipulated by providing the CS either before daily water (Thirsty

condition) or after daily water (Sated condition). Presumably, thirsty rats drink their fill during their daily watering and they will be sated on fluid immediately afterwards. The potential problem with this method, which has already been alluded to above, is how does the experimenter convince sated rats to drink?

Previous studies examining the effect of fluid deprivation have usually failed to control for the different amount of fluid exposure (e.g. time spent drinking, amount consumed) between groups of rats on different deprivation schedules. Not surprisingly, thirstier rats drink more than less thirsty rats and are usually exposed to the stimulus for longer as well. The current methodology attempted to avoid the problem of getting sated rats to drink as well as the problem of preventing thirsty rats from drinking more of the CS by hand-feeding predetermined amounts of the CS fluid (see Fanselow & Birk, 1982). In order to do this; a 5-ml syringe (without needle) was used with the required amount of fluid in it. Each rat was then held against the experimenter's chest and the tip of the syringe was gently forced into the rat's mouth. The plunger was depressed in a slow and continuous manner, taking a set amount of time to empty the syringe (25 sec). Fluid infusion invariably elicited licking movements when the syringe was positioned in such a manner. If the rat would not voluntarily lick the fluid, the tip was gently forced behind the rat's upper front teeth. Fluid would only be released when the rat was licking the syringe tip or the tip was in the rat's mouth (so that the rat tasted it).

Because there is some suggestion that the infusion procedure may have differential effects on thirsty and sated rats, each subject received infusions when both thirsty and sated. So, for example, the thirsty groups received water infusions when sated and CS infusions when thirsty. This within-subject control was designed to eliminate the possibility that the infusion procedure itself was maintaining the observed aversion in some groups and not others.

The above methodology was designed to manipulate thirst by using the same water deprivation schedule for all animals and at the same time control for different amounts of fluid exposure between groups. This should avoid or eliminate potential confounds arising from the use of different water schedules between groups and from different levels of stimulus exposure between groups.

A brief note on statistics.

All the ANOVAs carried out in this thesis were performed using the Contrast statistical package (Peter S. Horne © 1993). These ANOVAs are equivalent to the MANOVA approach to repeated measures data (Harris, 1985; O'Brien & Kaiser, 1985) rather than the univariate ANOVA mixed model approach. At the level of contrast testing, the difference between these approaches is reflected in the choice of the error term for within-subject tests: The MANOVA approach results in a separate error term for each within-subject contrast, while the ANOVA approach (with or without the various corrections that have been proposed to rid that approach of its notorious lack of robustness when

the sphericity assumption is violated) provides a common error term for all within-subject tests. Since non-sphericity implies that different within-subject contrasts have different variances, the ANOVA approach to testing such contrasts is difficult to defend. Unless otherwise indicated, contrasts tested were planned to be orthogonal (Hays, 1963). When a contrast analysis is fully planned, overall tests are irrelevant.

CHAPTER 3

Experiment 1

The first experiment examined the influence of deprivation level on the preference for flavours in rats. Flavours that are ingested when hungry or thirsty are consequently preferred, even after satiation has occurred (Campbell et al., 1987; Capaldi & Myers, 1982; Capaldi et al., 1983; Revusky, 1967, 1968b, 1974). Thus the flavour becomes associated with relief from hunger or thirst, resulting in an observed preference for that flavour. This experiment attempted to replicate this basic result using the methods of this laboratory. Earlier research in this lab has suggested that relief from thirst accelerates the habituation of neophobia in rats (Experiment 3, Whitfield, 1999), implying that thirst-relief associations were formed. The experiment reported here involved four groups in a 2 x 2 factorial design. Rats were exposed to a flavour when thirsty (eg. almond) and a different flavour when sated (eg. vanilla), for six or twelve days in succession. The first independent variable (IV) was whether the almond or vanilla was presented to the rat when it was thirsty or sated, and the second IV was whether the rat received six or twelve presentations of the flavours. The second IV was included in order to reveal how many flavour presentations are necessary to establish the effect. The dependent variable was the rat's preference for almond, tested in a pair of 2-bottle preference tests between almond and vanilla. The four groups are labelled Almond-Thirsty-6, Almond-Thirsty-12, Almond-Sated-6 and Almond-Sated-12. The hypothesis was that if flavour-relief associations are formed, then

the animals should show a preference for the flavour presented to them when thirsty. Thus, evidence for this would be obtained if the Almond-Thirsty groups (Almond-Thirsty-6, Almond-Thirsty-12) displayed a stronger preference for almond than the Almond-Sated groups (Almond-Sated-6 and Almond-Sated-12). Due to a null result in the planned tests, a second series of tests were conducted between each flavour solution and water. This series of tests was included as a more sensitive measure of flavour preferences.

Method

Subjects. Thirty-two male inbred albino Wistar rats were obtained from the colony of Specific Pathogen Free rats maintained by the Combined Universities Laboratory Animal Services (CULAS), at Little Bay, Sydney. Rats had previously served in an experiment involving the extinction of a conditioned taste aversion to saccharin. At the start of the experiment, rats were about 110 days old with weights ranging from 393 to 501 g, and mean weight equal to 448 g. They were housed in squads of eight in four large white plastic boxes (65 x 40 x 22 cm) which served as home-cages and were kept in a colony room maintained under a 12:12 light/dark cycle. All phases of the present experiment were conducted in the light portion of this cycle. Unlimited access to standard laboratory rat chow was available through the wire mesh lids and water was provided from metal spouts inserted through the same lids. During the experiment and five days before training began, access to water outside of training or testing

times (supplementary water) was limited to 30 min in the home-cages when in the colony room, which occurred 30 min after daily training in the laboratory.

Apparatus. 1% almond and 1% vanilla flavour solutions (vol/vol) were prepared by adding 5-ml of flavour essence (Aeroplane ™, Sydney) to 500-ml of tap-water. Note the almond essence contained alcohol, whilst the vanilla essence contained none. During training, these two CSs were delivered directly to the mouth by a plastic 3-ml handheld syringe. Eight clear acrylic cages (33 x 21 x 18 cm) with wire mesh lids and sawdust on the floor were set up in a separate room under dim natural light as the drinking cages in which all training and testing occurred. The cages themselves were placed in unpainted chipboard cubicles, which blocked vision between cages. Through the wire lids of the cages a metal spout of a 200-ml plastic water bottle could be inserted on the left and the right side. During training and testing, fluids were delivered by inserting the plastic bottles to either the right or left side of the cage. Fluid consumption was measured by weighing the bottles before and after each session.

Counterbalancing. Within each 8-rat home-cage squad, 2 rats were allocated to each of the 4 experimental conditions. To counter any possible confounding with timing of the procedures (e.g. greater delay between end of drinking and infusion in later rats in a squad) or differences between drinking-chambers, this allocation was systematically varied across squads. Allocation was also varied to counterbalance for prior experience.

Procedure. At the start of the experiment, rats were assigned to four equal groups (n=8), so that within each home-cage squad, there were equal numbers from each group, and they were assigned to drinking cages in a counterbalanced fashion. Rats were run in their home-cage squad of eight at a time. No pre-training was given because rats were already accustomed to the drinking cages and the infusion procedure from their previous experiment.

Conditioning. On conditioning days, all rats were given 15 minutes access to water in the drinking cages. Immediately prior to this, the Almond-Thirsty groups received a 1-ml infusion of almond solution, while the Almond-Sated groups received a 1-ml infusion of vanilla solution. Immediately after the 15-min drinking session Almond-Thirsty groups received a 1-ml infusion of vanilla, whilst the Almond-Sated groups received a 1-ml infusion of almond. Water bottles were weighed before and after each drinking session. Position of the water bottle in the drinking cage was counterbalanced across days. Conditioning took place on Days 1-12 in the Almond-12 groups. In the Almond-6 groups conditioning took place on Days 1 to 6, and Days 9 to 14.

Testing. There were three series of tests. The first series involved a pair of two-bottle preference tests between almond and vanilla after 6 or 12 days training. On Days 7 – 8, Almond-Thirsty-6 and Almond-Sated-6 were tested for a preference between almond and vanilla solutions. Rats in each group were placed in the drinking cages. A bottle of flavour solution was inserted on the left, until the rat licked

the spout, then it was removed and the second flavour was inserted on the right until the spout was licked, and it was removed. Finally, the first bottle was reinserted on the left and then the other bottle was inserted on the right, and the animals were left for 15 min. On the first day of testing, the almond solution was presented on the left, and on the second day it was presented on the right. On Days 13 – 14, testing occurred in the same manner for the Almond-12 groups. Testing occurred again on Days 15 – 16 for the Almond-6 groups after their further 6-days of training (so they had a total of 12 days training) and are referred to as the 6+6 tests in Table 3.1 (pp. 38) and Table 3.2 (pp. 39). Due to the absence of significant results, four more testing days were carried out, on which animals received a 2-bottle preference test between the flavour solution (almond or vanilla) and water. These tests intended to provide more sensitive measurements of the flavour preferences. On Days 24 – 27, each day a 2-bottle test between a flavour solution and water was given. Thus, each animal had an almond preference score and a vanilla preference score. Half the rats in each group were tested with the almond solution on the first two days, and with the vanilla solution on the last two days. The order was reversed for the other half of the rats. The flavour solution was presented on the left on Days 24 and 26, and on the right on Days 25 and 27. Insertion of the bottles into the drinking cages was carried out in the manner described above.

Data Analysis. For the results from the first two series of tests between almond and vanilla, almond preference was arbitrarily chosen

as the DV. It was defined as almond consumption divided by total fluid consumption across two test days. Almond preference from these tests was examined in two separate MANOVAs. The first tests were analysed in a 2 x 2 MANOVA, where the first factor was amount of training, either 6 days or 12 days and the second factor was type of training, either almond presented when thirsty and vanilla when sated, or the reverse. The results from the retest (after a further six days training on the Almond-6 groups, so all groups had a total of 12-days of training) were examined in a second 2 x 2 MANOVA. The first factor was whether testing had occurred after six days or not, and the second factor was whether almond was presented when thirsty or sated during training. The results from the final series of tests between each flavour and water were analysed in a 2 x 2 x (2) MANOVA. This analysis included amount and type of training as the first two factors, as well as an additional factor, almond preference score and vanilla preference score. The main effects and their interactions were tested. The important result from this series of tests is not the main effect of type of training (almond thirsty or almond sated), but rather the possible interaction between type of training and test type (almond preference test or vanilla preference test). Such an interaction would indicate a higher preference for the flavour rats were trained with when thirsty than the flavour trained with when sated, versus water. In all cases, F-critical $_{1, 28}$ = 4.26.

Results

Results from the planned tests are shown in Table 3.1 below.

Table 3.1 Almond Preference Scores.

	Sessions Prior to Test		
	6	6+6	12
Almond-Thirsty	59.7 (4.2)%	57.9 (3.5)%	44.2 (5.1)%
Almond-Sated	51.6 (3.8)%	55.5 (3.0)%	43.9 (3.3)%

Note: Figures represent mean percent preference scores (± SEM).

Although the Almond-Thirsty group consistently showed a higher preference for almond over vanilla than the Almond-Sated groups, these differences were not statistically significant. The first two series of tests failed to obtain evidence that the Almond-Thirsty groups displayed a stronger preference for almond than the Almond-Sated groups ($F_{1, 28} < 1.00$ in both MANOVAs). Results from these tests did reveal a significant almond preference that interacted with length of training ($F_{1, 28} = 6.12$) but not training condition. The direction of the interaction means that an almond preference existed in those groups tested after 6 days of training, compared to those groups tested after 12

days of training, regardless of whether almond was presented when thirsty or sated. In other words, the presentation of almond when thirsty or sated had no influence on preference for almond versus vanilla, but a short training interval before testing apparently did. This preference was still present after an additional six days of training ($F_{1, 28} = 7.61$). No other differences in this analysis were significant. Results from the final tests are shown in Table 3.2 (below).

Table 3.2 Almond Preference and Vanilla Preference Scores.

	Almond-Thirsty		*Almond-Sated*	
	Almond Test	Vanilla Test	Almond Test	Vanilla Test
6 + 6	51.3 (3.9)%	40.2 (3.0)%	47.4 (4.5)%	52.7 (3.6)%
12	47.6 (3.3)%	43 (5.1)%	45.5 (3.2)%	45.6 (2.1)%

Note: Figures represent mean percent preference scores (± SEM).

The Almond-Thirsty groups showed a higher preference for almond over water than vanilla over water; whilst the Almond-Sated groups displayed a higher preference for vanilla over water than almond over water. This interaction was statistically significant ($F_{1, 28} = 4.30$, $p = 0.047$). There was no main effect for type of training; for amount of training; or for type of test and no other interactions were significant.

Discussion

Some support was obtained for conditioning of a preference by relief from thirst associations. Animals that had been exposed to a flavour when thirsty displayed a preference for that flavour over water, relative to a flavour that had been presented when sated. This is consistent with earlier research on the formation of flavour preferences (Campbell et al., 1987; Capaldi & Myers, 1982; Capaldi et al., 1983; Revusky, 1967, 1968b, 1974).

Some qualifications must be made about the above result. Firstly, by chance an almond preference may have existed in some groups and not others. The first series of tests showed that an almond preference existed in the six-day training groups compared to the 12-day training groups when almond was tested against vanilla. This preference was not evidence for thirst-relief associations as it was obtained across both Almond-Thirsty and Almond-Sated groups. The source of this preference is unclear, as it was only apparent in the groups tested after six days of training. Why six days of training before testing would produce an almond preference rather than 12 days of training is unknown, and the preference may simply represent chance differences between groups (Type I error). However, these differences were controlled for in the results from the final tests where each flavour was tested against water. This is because the almond preference in these tests interacted significantly with training condition, and not length of training. This means that the preference for almond that was revealed was dependent upon whether almond had been presented when thirsty

or sated, once the effect of the length of training condition was accounted for. As such, the almond preference does represent evidence for conditioning of a preference by relief from thirst.

The above interaction was only just significant following extensive training, whereas results from other studies suggest that a clear preference can be obtained with relatively little training (e.g. Revusky, 1967, 1968b, 1974). The lack of a clear preference may have been due to the relatively modest amount of thirst relief produced in the rats by the flavours in this experiment compared to similar experiments (e.g. Revusky, 1968b, Experiment 2). There are two reasons for this. Firstly, smaller amounts of the flavour (CS) were provided each day in this experiment (1-ml per day) whereas Revusky used larger quantities of the flavour each day (5-ml per day). Secondly, the rats in this experiment were only deprived for 23.5 hours, whereas in Revusky's experiment, the animals were deprived for 44 hours. Given the greater thirst, in addition to the greater relief from thirst provided by the methods in Revusky's experiment, it might be possible that the clearer effect obtained there can be attributed to these differences. Any concern arising from the slim statistical results might be balanced against the observation that the trend in the means shown in Table 3.1, although unreliable, is in the appropriate direction for the hypothesis being tested. That is, almond-thirsty groups show a higher preference for almond than almond-sated groups.

One of the objectives of this experiment was to identify how many training days with the method used here would be necessary to

produce an effect. Given the slim results, it is tempting to conclude that more than 12 days training is necessary. However, if the results from the initial tests are examined closely (Table 3.1), it can be seen that the obtained differences between the groups tested after 6 days of training were larger than when tested after 12 days of training (although the interaction between groups and training was non-significant). This suggests that flavour preferences may develop very early in the animal's training experience, and further testing may remove them. Other research supports the idea that establishment of preferences occurs after only a few training trials. For example, Revusky (1967b) used 4-hour exposures to the flavours per day for only 2 days. Similar training schedules have been used by other researchers (e.g. Capaldi & Myers, 1982; Fanselow & Birk, 1982).

Apart from greater amounts of CS and fewer training sessions, there are other methodological differences between Revusky's basic research design and the methods used here. For example, Revusky (1968b) used longer training and testing sessions, 4 and 2 hours respectively, whilst this experiment used around 25-sec exposures, and then tested consumption over 15 min. These differences might be responsible for the lack of a clear effect in this experiment as well. Indeed, unless such differences are systematically investigated, it is impossible to attribute the marginal results of this experiment to any one factor.

Nevertheless, it should be reiterated, despite the small amounts of CS given during training, an effect was obtained. Given larger

amounts, it is possible that the effect would be much clearer. The amounts usually consumed under deprivation during the extinction of CTAs are usually much greater than 1-ml. Thus, it is likely that if counter-conditioning is occurring during extinction training, it would be having a clearer effect than that reported here.

CHAPTER 4

Experiment 1 demonstrated that a flavour preference can be established by providing exposure to the flavour when thirsty. Assuming that this also happens during an extinction procedure, Experiment 1 supports the hypothesis that counter-conditioning contributes to the extinction of CTAs. The next experiment was designed as a direct test of this hypothesis. It was essentially a replication of Experiment 2 from Whitfield (1999).

Experiment 2

Experiment 2 examined the role of counter-conditioning on the observed extinction of CTAs in rats. By using manual infusions of the CS during extinction and using the same deprivation schedule for all rats, it was designed to control for differences in CS exposure between groups. The previous experiment, in line with other research (Revusky, 1968b, 1974), demonstrated that flavours which are ingested when thirsty are consequently preferred. The present experiment attempted to manipulate the deprivation state and measure whether thirst deprivation accelerates the extinction of a CTA whilst controlling for the amount of exposure to the flavour (CS).

The design was based upon Experiment 2 from Whitfield (1999). In both Whitfield (1999) and the present experiment, a conditioned taste aversion to saccharin was established in four groups of eight rats and then an extinction phase occurred under four different conditions:

Group Dep-1 received 1-ml of saccharin prior to daily water; Group Dep-0.3 received 0.3-ml saccharin prior to daily water; Group Sated received 1-ml saccharin after daily water; and the Water control received no exposure to saccharin. After a number of such pairings, all rats were tested for an aversion to saccharin in a one-bottle test. The following procedural changes were unique to the present experiment: 1) During pre-training and throughout the experiment all rats were given oral infusions before and after every session, except for conditioning and test days. Whitfield only provided infusions of saccharin, either before or after daily water; 2) Infusions were given in the laboratory before and after the 15-min water access in the test cages there. During extinction Whitfield gave infusions in the colony room, either before or after the 30-min supplementary drinking period and gave no drinking sessions in the test cages during this phase.

The independent variable was the condition under which the extinction phase occurs, and the dependent variable was saccharin consumption measured in 1-bottle tests before and after conditioning and extinction. The predictions based upon Whitfield's results were as follows: If after extinction, Group Dep-1 showed less aversion to saccharin than Sated this would replicate Whitfield's result from Experiment 1 and 2 (Whitfield, 1999) and suggest that thirst relief is involved in extinction; if Group Dep-0.3 showed more aversion to saccharin than Groups Dep-1 and Sated, this would show that the extinction effect is a function of saccharin exposure; and finally if Group Water shows more aversion than Sated, this would show that

extinction is not entirely due to thirst relief but also due to exposure alone.

Method

Subjects. Thirty-two experimentally naïve, male, inbred, Wistar rats about 90 days old at the start of the experiment were obtained from the same source as Experiment 1. Mean weight was 393 g with a range from 337 to 438 g. Rats were housed in the same manner as Experiment 1, and as in Experiment 1, water access outside of training was restricted to 30 min of supplementary water provided 30 min after daily training. Four equal groups (n=8) were assigned evenly from each home-cage squad in the same manner as Experiment 1 and rats were run in their home-cage squads.

Apparatus. The 0.1% saccharin solution (weight/volume) consisted of 1-g of pure saccharin dissolved in 1000-ml of tap water. As in Experiment 1, the eight clear acrylic cages set up in a separate room from the colony room, served as drinking cages where all testing and training occurred. Saccharin solution was provided in the drinking cages during conditioning and test; otherwise tap water was provided. The bottles were weighed and recorded before and after each drinking session. Saccharin solution and tap water were also manually infused to the animal's mouth from 3-ml syringes during pre-training and extinction. LiCl was mixed in distilled water the day before injections (5 ml/kg of 0.15M LiCl). All injections were intraperitoneal (i.p.).

Counterbalancing. Within each 8-rat home-cage squad, 2 rats were allocated to each of the 4 experimental conditions. To remove any possible confounding with timing of the procedures or differences due to drinking chambers, this allocation was systematically varied across squads.

Procedure. In order to avoid the need to inject all 32 rats on the same day, this experiment was run as two replications of sixteen rats each with the second replication running just 1 day behind the first. Groups were evenly represented in each replication and daily training occurred at the same time each day.

Pre-training. On Days 1 – 5, rats were taken, one squad of eight at a time, to the experimental room. Each rat received an oral infusion of 1-ml of water from a handheld syringe, prior to placement in the drinking cages. Whilst the rats were in the drinking cages, they were given ad lib access to water. A further 1-ml infusion of water occurred upon being taken out of the drinking cages. Rats were then returned to the colony room. Amount of water consumed during drinking sessions was measured and recorded for each rat. On Day 5, all rats were weighed.

Conditioning. There was one conditioning trial. On Day 6 rats were taken in squads of eight to the experimental room and given 15 min access to saccharin solution (0.1%) in the drinking cages. Consumption was measured and recorded as the acquisition test. Upon removal, each rat was injected with LiCl, and returned to the colony

room. Day 7 was a recovery day. On this day, only supplementary water was given.

1st test. On Day 8, an extinction test occurred in the drinking cages to measure the strength of the aversion established. One-bottle of saccharin solution was presented for 15 min. No infusions or injections were given.

Extinction. On Days 9 - 16 rats were taken to the experimental room for their daily drinking session. Rats in Group Dep-1 were infused with 1 ml of saccharin solution before the drinking session, and 1 ml of water afterwards. Group Dep-0.3 were infused with 0.3 ml of saccharin solution before and 1 ml of water afterwards. Group Sated were infused with 1 ml of water before and 1 ml of saccharin afterwards. Group Water were infused with 1 ml of water before and after the drinking session. After this, all rats were returned to the colony room and received supplementary water.

2nd and 3rd test. On Days 17 and 18, another extinction test occurred. These tests occurred in exactly the same manner as the first test.

4th test. On Day 19, a final test occurred when the rats were satiated. After the daily water drinking session, rats received a 1-bottle saccharin test for a further 15 min in the same drinking cages. This test was provided to reveal any group differences still remaining that may have been obscured by water deprivation.

Results

Results from pre-training (left panel) and the first four tests (right panel) are shown in Figure 4.1 (pp. 49). As the left panel in Figure 4.1 shows, pre-training produced stable levels of drinking that did not differ between the groups. Mean water intakes on the last day of pre-training was Dep-1, 11.7 ml; Dep-0.3, 12.9 ml; Sated, 12.5 ml; Water, 12.1 ml.

The right panel of Figure 4.1 shows saccharin consumption over the first four tests. Differences between groups and across test sessions were tested. As can be seen in the graph, conditioning produced a reduction in intake but consumption returned to Conditioning-Day levels by the end of Test 3. A significant quadratic trend confirmed this U shaped pattern ($F_{1, 28} = 251.49$, $p < 0.01$). However, no significant differences between groups were obtained ($F_{1, 28} < 1.00$).

Results from Test 4, performed when the rats were sated, are shown in Figure 4.2 (pp. 49). The planned analysis confirmed what is suggested by the graph. The Water group, which received no extinction training, consumed significantly less saccharin during this test than the other groups ($F_{1, 28} = 4.90$, $p = 0.04$). No other tests in the planned analysis were significant ($p > 0.05$). When the replication factor was added into any of the above analyses, it did not have a significant main effect ($F_{1, 28} < 1.00$), and it did not interact significantly with any of the other tests ($p > 0.05$).

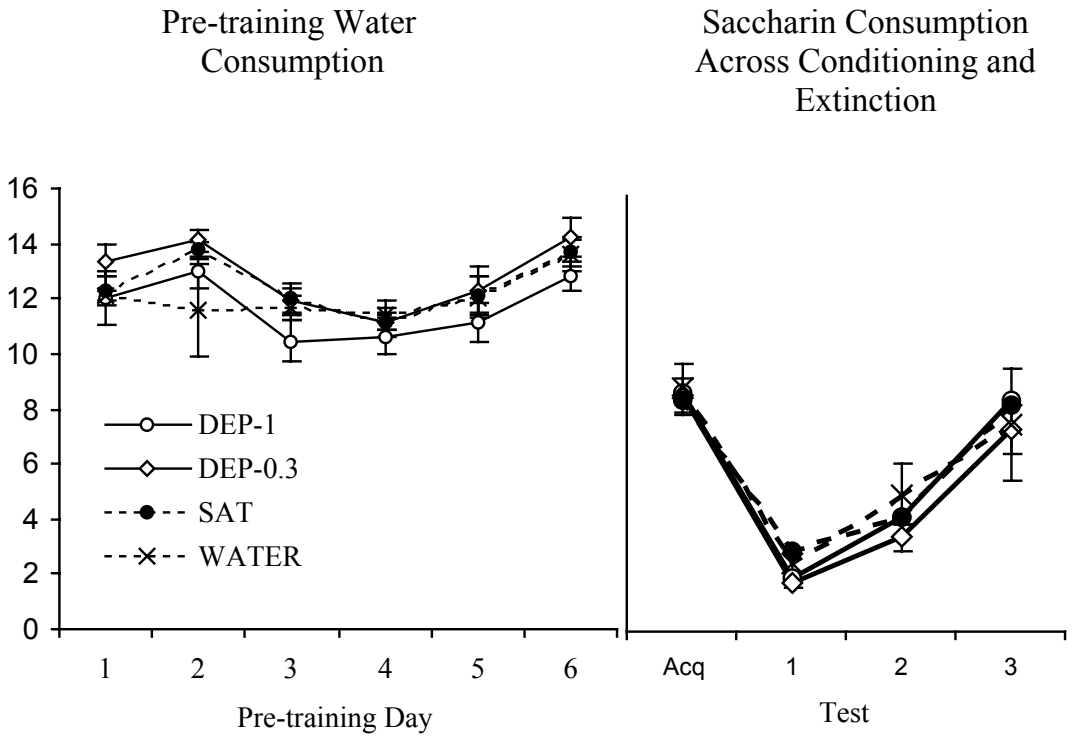

Figure 4.1 Experiment 2 mean consumption scores.

The left panel shows water consumption in the drinking chambers during pre-training. Rats received 15 minutes of water in the drinking chambers daily. The right panel shows the change in saccharin consumption across conditioning and extinction. Saccharin consumption at Acq was followed by LiCl injections that established a CTA at Test 1. Between Test 1 and Test 2, eight days of extinction occurred under four different conditions. Group Dep-1 received 1-ml sac infusions when prior to daily watering, Group Dep-0.3 received 0.3-ml sac infusions prior to daily watering, Group Sated received 1-ml sac infusions after daily watering, Group Water received no sac exposures except during test sessions. Vertical bars represent SEM.

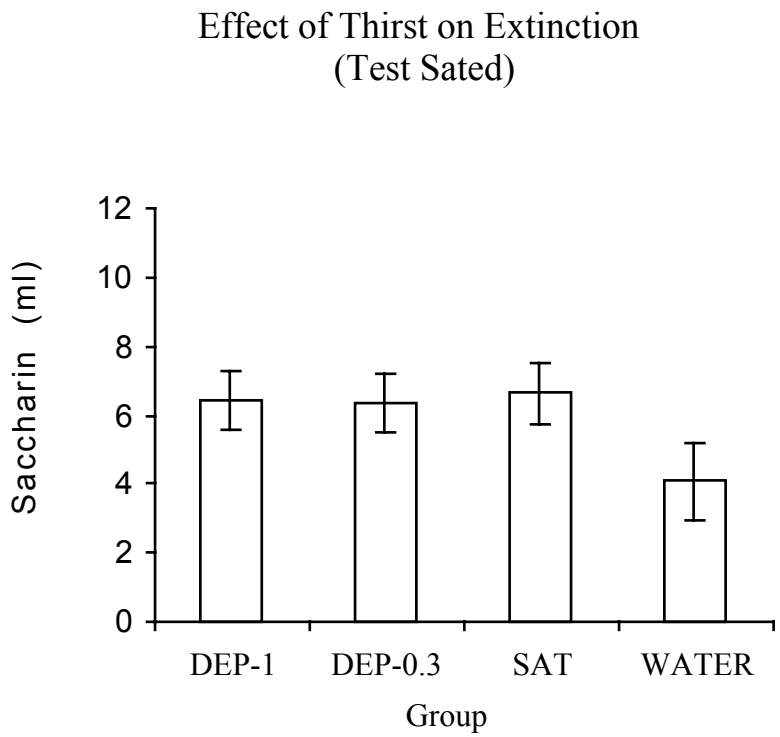

Figure 4.2 Experiment 2. Test 4 (Sated).

The mean consumption of saccharin in Test 4 when sated, after extinction of a taste aversion to saccharin. Rats were given 15 min access to 1-bottle saccharin solution (0.1%) after receiving 15-min water access. Vertical bars represent SEM.

Discussion

There was no evidence from the above results that deprivation affected the rate of extinction. No statistically significant group differences were found until Test 4, when the Water control drank less saccharin than the other groups. The result from this test confirms that the extinction methods used reduced the observed taste aversion, without any suggestion that thirst relief was involved.

One surprising aspect of the results was the absence of any differences between the Water control and other groups on Tests 1, 2, and 3. Group Water received no saccharin during extinction training, and so the biggest difference between groups would be expected to be observed between the Water group and the other groups at Test 2. However, it was only in the satiation test (Test 4, shown in Figure 4.2) that the Water control drank less saccharin than the other groups. Extinction training, then, had not produced an observable effect on consumption levels by the time testing had begun, and more extinction training must be required before differences between groups will emerge. A similar conclusion was reached Whitfield (1999). He found that 12 days of extinction was necessary before a reliable difference could be detected between sated and thirsty rats.

The absence of a reliable difference between the Water control and the other groups at Tests 2 and 3 also suggests the 1-bottle saccharin test was extinguishing more of the aversion than the extinction training. As the rats, on average, drank 10 to 14 times more of the CS in each extinction test than during extinction training, it is

likely that the test procedure contributed to far more observed extinction than the extinction training. This would mask any differences between groups that were due to the carefully controlled experimental conditions and would explain the absence of group differences at Tests 2 and 3. As Experiment 1 demonstrated only a very small effect on thirst preferences, using the manual infusion methods also used here, combined with a series of 2-bottle tests it is likely that 2-bottle test procedures are more sensitive to taste preferences in some cases relevant here, than 1-bottle tests.

Another possible masking source may have come from the fact that the rats were well trained to drink in the test chambers by the time testing occurred. Both pretraining and extinction phases involved daily water training in the test chambers, whereas in Whitfield's (1999) experiments, only conditioning and testing occurred in the test-chambers. If rats develop a strong habit of drinking whenever they are placed in the test chambers, any differences produced by the IV may be masked, especially as a weak aversion was used.

A third alternative is the risk that differences between groups may have been lost due to the social transmission of flavour preferences. Galef has shown that a conditioned aversion or preference to food can be established in rats when in close contact to other rats eating the food (Galef, Whiskin, & Bielavska, 1997). As groups were counterbalanced in each 8–rat home-cage squad, it is possible that such social transmission may have occurred by the scent of the CS-fluid on the other rats.

Experiment 3

This experiment attempted to replicate Experiment 2 from Whitfield (1999), and improve upon Experiment 2 in this thesis. It incorporated the following changes from the previous experiment: 1) Unlike Experiment 2 where multiple training sessions were given in the test cages, there was only one 15-min drinking session given in the test cages during pre-training and extinction. Otherwise daily drinking occurred in the home-cages during pre-training and extinction. This was designed to reduce the development of a drinking habit in the rats whenever they were placed in the test cages and is more similar to the method used by Whitfield (1999) where no drinking sessions were provided in the test cages until testing. 2) 2-bottle tests were used, both immediately following conditioning and on test. This was to minimise the disruption which exposure to the CS produces during testing and may also be more sensitive to small group differences. Note that Whitfield used 2-bottle tests after conditioning and after extinction, but only his final 1-bottle test detected significant group differences. 3) Vanilla solution (1%) was used instead of saccharin solution (0.1%). Vanilla is an odour, and as such may be more malleable to extinction than a flavour. Odours were successfully used in establishing a flavour preference in Experiment 1. 4) Twelve extinction sessions were given, and then a pair of two-bottle tests. The results from this test session were used to decide whether further training occurred or a 1-bottle test. 5) Rats were housed in cages of two, rather than eight. Each pair was in the same experimental group,

so that pairs are treated equivalently. This change attempted to prevent the social transmission of flavour preferences.

As in the previous experiment, four groups of eight rats were used; the independent variable was the four different extinction conditions: 1-ml vanilla presented before daily water (Dep-1); 1-ml vanilla presented after daily water (Sated); 0.3-ml vanilla presented before daily water (Dep-0.3); 1-ml of water presented before and after daily water (Water). The dependent variable was vanilla preference obtained by dividing vanilla consumption by total consumption from the 2-bottle tests both before and after extinction.

The predictions were the same as for the previous experiment: If after extinction, the Dep-1 group showed less aversion to saccharin than Sated this would replicate Whitfield's result his from Experiments 1 and 2 and suggest that thirst relief is involved in extinction; if the Dep-0.3 group showed more aversion to saccharin than Dep-1 and Sated groups, this would show that more familiarity results in more observed extinction; and finally if the Water group showed more aversion than Sated, this would show that extinction is not entirely due to thirst relief but also due to exposure alone.

Method

Subjects. Thirty-two experimentally naïve, male, inbred, Wistar rats were obtained from the same source. At the start of the experiment, rats were about 120 days old, with a mean weight of 474 g and with a range from 325 to 555-g. Rats were housed in groups of two

in blue plastic boxes (38 x 25 x 28: L x W x D) with wire lids and kept under the same conditions as both Experiment 1 and 2. During the experiment, daily water access was restricted to 30-min of supplementary water provided during daily training in the experimental room. At the start of the experiment, rats were assigned to four equal groups (n=8) and the experiment was run in squads of eight at a time, with groups evenly represented in each squad. However, unlike previous experiments, both rats in each home-cage were from the same experimental group.

Apparatus. The 1% non-alcoholic vanilla solution (v/v) was prepared in the same manner as Experiment 1. As in Experiment 1, the same eight clear acrylic cages were used for all tests, whilst extinction training occurred in the same room from the home-cages. Vanilla solution was provided in the test cages from the same bottles as used in Experiment 1, and bottles were weighed and recorded before and after each test session. Vanilla solution and tap water was also manually infused to the rat's mouth in the same manner as Experiment 1. LiCl was prepared and delivered in the same manner as Experiment 2 (0.15M at 5-ml/kg; i.p. injections).

Counterbalancing. Within each 8-rat experimental squad, 2 rats were allocated to each of the 4 experimental conditions in the same systematic variation as for previous experiments.

Procedure. This experiment was run as two replications, with the second replication running just one day behind the first. There were 16

rats in each replication with groups evenly represented in each replication.

Pre-training. On Days 1 – 5 rats were taken in their home-cages to the experimental room. Each rat received an oral infusion of 1 ml of water from a handheld syringe, before being returned to the home-cage. There they were given ad lib access to water for 30 min. A further 1-ml infusion of water occurred before being returned to the colony room.

Conditioning. There was one conditioning trial. On Day 6 eight rats at a time were taken to the experimental room and given 15-min access to vanilla solution in the drinking cages. Consumption was measured and recorded as the conditioning test. Upon removal each rat was injected with LiCl and returned to the colony room. Day 7 was a recovery day. On this day, only supplementary water was given.

1st test. On Day 8 the first 2-bottle choice test between vanilla and water was given. Eight rats were taken at a time to the experimental room and placed in the test cage. The left-side bottle was inserted until the rat licked the spout, then it was withdrawn and the right-side bottle was inserted until the spout was licked. It was then withdrawn and the left bottle was reinserted followed by the right. Vanilla bottles were presented on the left side for half the rats and on the right side for the other half. No infusions were given.

Extinction. On Days 9 - 21, rats were taken to the experimental room for their daily infusion session. Rats in the Dep-1 group received 1 ml of vanilla solution before the 30-min supplementary water session, and 1 ml of water afterwards. Dep-0.3 received 0.3 ml of

vanilla solution before and 1 ml of water afterwards. The Sated group received 1 ml of water before and 1 ml of vanilla afterwards. The Water group received 1 ml of water both before and after the supplementary session. After this, all rats were returned to the colony room.

2nd and 3rd test. On Days 22 and 23 all rats were tested in the manner described for Test 1. Vanilla bottles were presented on the left side for half the rats and on the right side for the other half, and the order was reversed the next day.

4th test. On Day 24 all animals were given a single 1-bottle test with vanilla solution.

Results

No differences between groups were obtained from any statistical tests. On the day before conditioning, water intakes in the test cages were Dep-1, 12.4 ml; Dep-0.3, 14.0 ml; Sated, 14.4 ml; Water, 14.8 ml. On the day of conditioning, vanilla intakes were Dep-1, 14.2 ml; Dep-0.3, 14.0 ml; Sated, 12.7 ml; Water, 13.8 ml. A planned one-way MANOVA revealed no differences between groups on these days ($p > 0.05$).

Results from the first three tests are shown in Figure 4.3 (pp. 57, left panel). Planned orthogonal contrasts were used to test for differences between groups in a repeated MANOVA. There was a significant linear trend ($F_{1, 28} = 22.45$, $p < 0.001$) as consumption increased for all groups, but no other tests were significant. There were no group difference ($F_{1, 28} < 1.00$), no quadratic trend ($F_{1, 28} < 1.00$),

and no interactions were significant. Results from a post-hoc MANOVA including only the data from the 3rd test (shown in Figure 4.3, right panel) failed to reveal any significant group differences ($p > 0.05$). When the different replications were added as a second factor in the analysis, there was no significant main effect ($F_{1, 28} < 1.00$), and no significant interactions with any of the other tests. Results from the 4th test (1-bottle) failed to reveal any significant group differences in a Tukey HSD comparison. Group consumption means from this test ranged from 14.7 ml to 16.1 ml.

Discussion

This experiment failed to reveal any statistical evidence for the influence of counter-conditioning on extinction of taste aversions. Figure 4.3 (left panel) shows that the effect of 12 days (12 ml) of extinction training does not have a clear effect on the consumption of sucrose beyond that of water controls. The clearest difference is seen at Test 3, shown in Figure 4.3 (right panel), where both the water deprived groups consume more than the other two groups. However, this difference was not significant and further testing failed to confirm the pattern of differences.

The present results fail to confirm the results of Whitfield (1999) Experiment 2, despite the procedural similarities. The most important difference between the present experiment and the latter is that the present method involves infusing all rats when both thirsty and sated. This removes any possibility that rats may develop an aversion to the

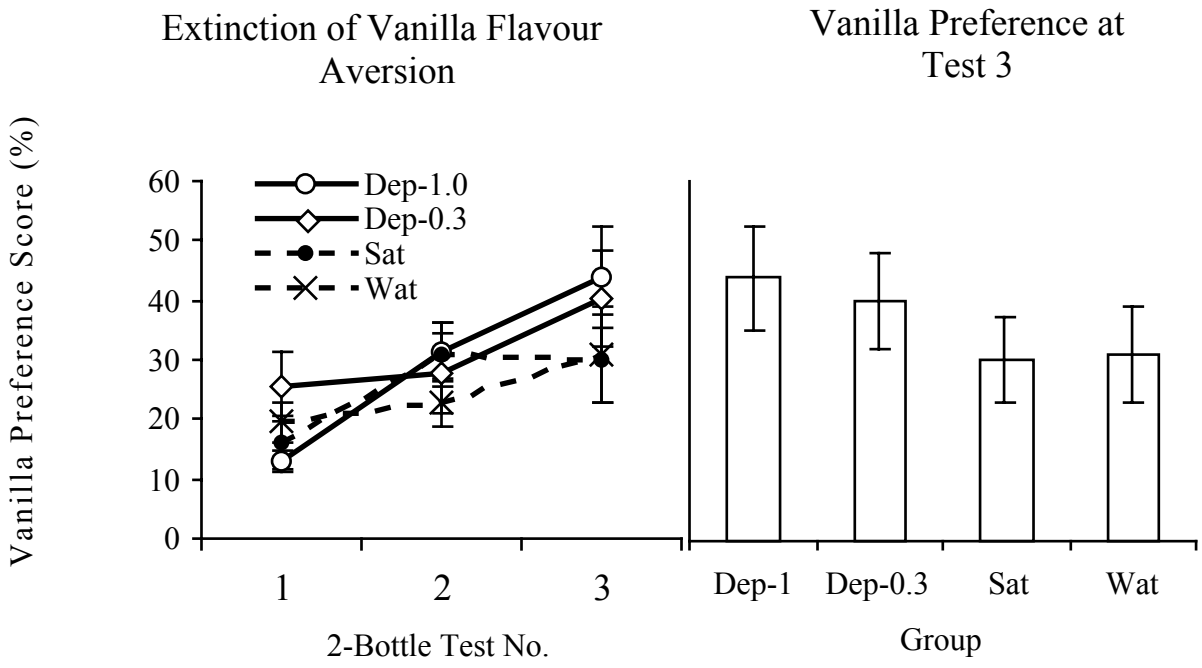

Figure 4.3 Experiment 3 results.

The left panel shows the mean vanilla preference score for each group across three post-conditioning test sessions in Experiment 3. All rats had an aversion to vanilla (1%) established by pairing it with LiCl (5 ml/kg, 0.15M). Then extinction occurred under four different conditions. Dep-1 was re-exposed to 1 ml vanilla when thirsty, Dep-0.3 was re-exposed to 0.3 ml vanilla when thirsty, Sated was re-exposed to 1 ml vanilla when sated, and Water was not re-exposed to vanilla. 12 days of extinction occurred between Test 1 and Test 2. The right panel shows the results from Test 3 alone, which occurred the day after Test 2.Vertical bars represent SEM.

flavour associated with the stressful infusion procedure. Given that when such a control is included, all previous indications of an effect disappear, it seems likely that the infusion procedure may be responsible for Whitfield's (1999) results. More comment on this factor will be provided in the general discussion of this chapter.

As mentioned in previous discussions, the amount of stimulus exposure during extinction was limited to no more than 1-ml per day. It is possible that this does not produce enough thirst relief for a measurable effect in these experiments. Most demonstrations of extinction involve much higher levels of CS consumption and presumably more thirst relief than the methods used here allow. Thus the possibility remains that counter-conditioning plays a measurable role under different conditions.

Experiment 4

Experiment 4 attempted to demonstrate the influence of thirst-relief associations on flavour preferences and conditioned taste aversions in a 2 x 2 factorial design, shown in Table 4.1 (pp. 59). Experiment 4 is a more thorough replication of Experiment 1 from Whitfield (1999) and Experiment 1 of this thesis. Experiment 1 (Whitfield, 1999) found that extinction occurred more rapidly under thirsty conditions than sated conditions in a repeated measured two-group design. The current experiment incorporated a similar comparison between two groups, both of which had a CTA to vanilla, and then either received extinction under thirsty or sated conditons. Experiment 1 of this thesis

demonstrated that flavours given to rats when thirsty are preferred to flavours when sated. This comparison was repeated in the present experiment by including two additional groups. These groups received vanilla when thirsty or sated but were not given paired lithium injections. Furthermore, the current experiment attempted to provide a slightly more controlled method of infusion procedure. One concern is the manual infusion procedure cannot control for differences between groups in the amount of the CS consumed. In spite of evidence the important variable in producing extinction of taste aversions is not the consumption of the CS fluid, but rather the length of time tasted (Nachman, 1970), it may be prudent to provide some measure of differences in consumption between groups that exists. For this reason, the amount of fluid spilled (not consumed) during infusions was recorded by a set of scales underneath the restrained animal.

Table 4.1. Design of Experiment 4.

Conditioning	Test 1	Extinction (9 Days)	Test 2
PS: Van-LiCl	Van	Wat→Van	Van
PT: Van-LiCl		Van→Wat	
US: Van/LiCl		Wat→Van	
UT: Van/LiCl		Van→Wat	

Note: Van : Vanilla 2%; LiCl : lithium chloride (injected 0.15M at 5ml/kg); Wat : 30 min water access; → : order of event.

The present experiment doubled the amount of fluid infused from previous experiments (increased from 1-ml to 2-ml) as well as increasing the concentration of vanilla from 1% to 2%. In addition to these changes, younger rats were used. Capretta reported that obtaining thirst-relief preferences is easier in younger rats (e.g. Capretta, Moore & Rossiter, 1973; Revusky, 1968b). Thus, younger rats (40 days) were used in order to increase the likelihood of any effect.

Four groups of eight rats were used in a 2 x 2 factorial design shown in Table 4.1 (above). The first factor was whether lithium injections were paired with vanilla, (including groups labelled Paired-Thirsty and Paired-Sated abbreviated to PT and PS respectively), or unpaired with vanilla, (including groups labelled Unpaired-Thirsty and Unpaired-Sated abbreviated to UT and US respectively); the second factor was whether or not rats received vanilla before (PT and UT) or after supplementary water (PS and US) during the extinction phase. Thus, an aversion to vanilla was established in half the rats, then vanilla was presented either before or after daily drinking to the orthogonal halves. The primary dependent variable was the vanilla preference score for each rat, measured before and after the extinction phase. Results from the final pair of tests were combined to provide a single post-extinction score, so each rat had a pre-extinction score and a post-extinction score. The spillage data, which consisted of the total amount of vanilla spilt by each rat over all infusion sessions during extinction was also analysed. The expected outcome from the primary data was a main effect of infusion condition; that is, infusing vanilla

when thirsty should produce an increase in vanilla preference in those rats, regardless of whether an aversion had been established to vanilla or not.

Following Whitfield (1999) Experiment 1, all training and infusions occurred in the colony room rather than the laboratory. The only noteworthy difference was infusions were given both before and after daily drinking.

Method

Subjects. Thirty-two experimentally naïve male Wistar rats about 40 days old at the start of the experiment with weights ranging from 144 to 215 g and a mean weight of 181 g, were obtained from the same source. Rats were housed in the same manner as in Experiment 3. As before, water access during the experiment was restricted to 30 min provided during daily training, which occurred in the colony room. At the start of the experiment, rats were assigned to four equal groups (n=8), and the experiment was run in squads of eight at a time, with groups evenly represented in each squad. As in Experiment 3, each homecage pair was assigned to the same group.

Apparatus. The 2% vanilla solution (v/v) consisted of 20 ml of non-alcoholic vanilla essence dissolved in 1000 ml of tap water. Conditioning and testing took place in the same eight acrylic drinking cages set up as in previous experiments. However, unlike in previous experiments, extinction training occurred in the colony room and the amount of fluids manually infused was increased to 2 ml. LiCl was

prepared and delivered in the same manner as Experiments 2 and 3 (0.15M at 5 ml/kg; i.p. injections).

Counterbalancing. As in Experiment 3, within each 8-rat experimental squad two rats were systematically allocated to each of the 4 experimental conditions in order to remove any confound with timing of the procedures or differences between test chambers. As before, to prevent any influence of social contact with rats from other groups, both rats in each homecage were in the same condition.

Procedure. Pre-training. Days 1 – 3 in the colony room rats were removed from their home-cages and infused with water over a dish on a scale that measured the amount of spillage from the rat's mouth. The time taken for each infusion was measured and carefully controlled at 45 sec for each rat. This was maintained throughout the experiment whenever infusions occurred. After the first infusion, rats were returned to their homecage. Supplementary water was immediately provided, after which another infusion of water occurred. On Day 4 rats were taken to the experimental room in squads of eight and placed in the drinking cages where they were given access to water for 15 min. Supplementary water was provided afterwards.

Conditioning. There was one conditioning trial. On Day 5 eight rats at a time were taken to the experimental room and given 15-min access to vanilla solution in the drinking cages. Upon removal, each rat from the paired condition was injected with LiCl. Rats were then returned to the colony room. Day 6 was a recovery day. On this day, all rats were taken to the experimental room and the unpaired rats were

injected with LiCl, after which all rats were returned to the colony room. On Day 7 all rats were taken to the experimental room and provided with 15-min water in the test cages.

1st test. On Day 8 the first 2-bottle choice test between vanilla and water was given. Eight rats were taken at a time to the experimental room and placed in the test cage. The same procedure as in Experiment 3 was used to insert both bottles. Vanilla bottles were presented on the left side for half the rats and on the right side for the other half. No infusions were given.

Extinction. On Days 9 - 17, rats were given infusions in the colony room of water and vanilla. Rats in the Thirsty condition received vanilla infusions before the supplementary water session, and water infusions afterwards. Rats in the Sated condition received infusions of water before supplementary water and infusions of vanilla solution afterwards.

2nd and 3rd test. On Days 18 and 19, after 9 days of extinction training, all rats were tested in the manner described for Test 1. Vanilla bottles were presented on the left side for half the rats and on the right side for the other half, and the order was reversed across days.

Results

The day before conditioning water intakes in the drinking cages were Paired-Thirsty (PT), 7.8; Paired-Sated (PS), 10.4; Unpaired-Thirsty (UT), 10.4; Unpaired-Sated (US), 9.9. On the day of conditioning,

vanilla intakes were PT, 14.3; PS, 13.3; UT, 13.4; US, 13.2. After conditioning, average water intakes in the test context were PT, 13.5; PS, 13.5; UT, 14.4; US, 14.6. Planned MANOVAs failed to reveal any significant differences on these days ($p > 0.05$).

Figure 4.4 (pp. 64) shows the pre-extinction and post-extinction vanilla consumption. The data were examined in a 2 x 2 x (2) planned factorial analysis. The first and most important factor was whether vanilla was provided when thirsty or sated, and there was no evidence that infusions of vanilla given when thirsty produced any effect on vanilla preference, as compared to sated controls ($p > 0.1$). The second factor was whether vanilla was paired with LiCl injections, and pairing successfully produced an aversion to vanilla, relative to unpaired injections across both tests ($F_{1, 28} = 26.89$, $p < 0.01$). The interaction of this factor across test sessions revealed that the paired groups increased their consumption of vanilla after extinction, relative to the unpaired controls ($F_{1, 28} = 30.62$, $p < 0.01$). No other main effects or interactions from this data were significant.

Across all extinction training the mean summed total amount of vanilla spilled (ml) for each group was: PT, 6.2 (0.2); PS, 12.1(0.4); UT, 6.0 (0.2); US, 11.6 (0.2), SEM in parenthesis (see also Figure A.3 in the Appendix). Animals in the Thirsty condition clearly spilled less vanilla (and thus presumably consumed more) than animals in the Sated condition ($F_{1, 28} = 447.30$, $p < 0.01$).

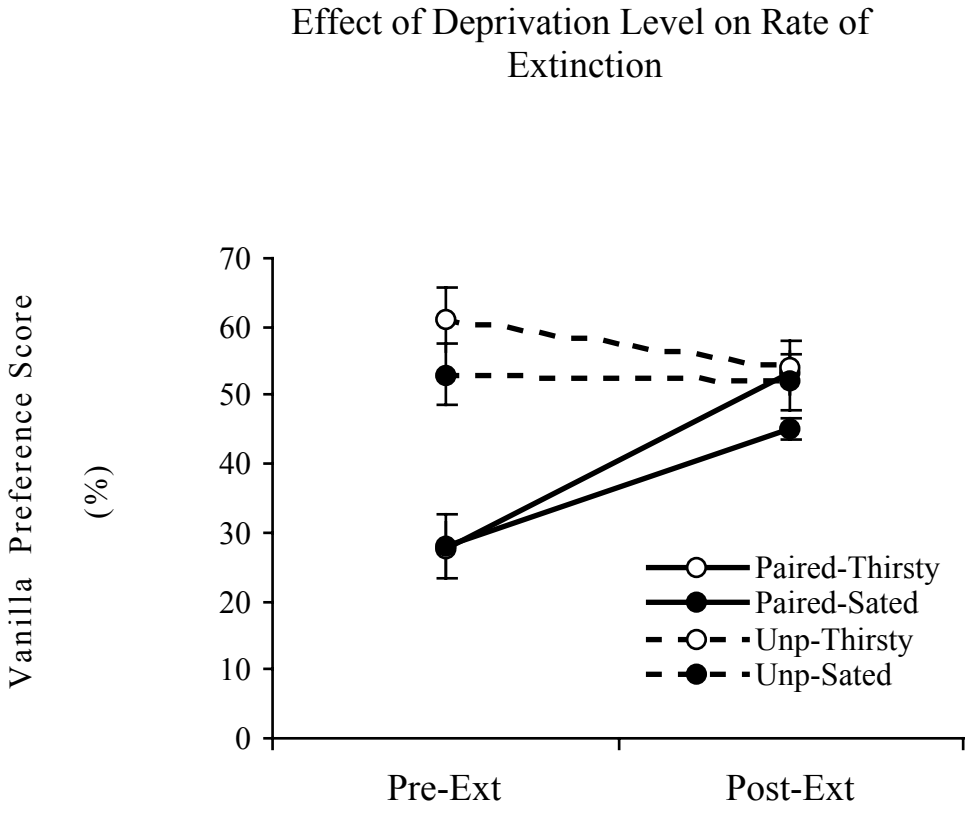

Figure 4.4 Experiment 4 results.

Mean vanilla preference score for each group in Experiment 4, before extinction and after. Rats either received vanilla paired with LiCl or unpaired, then during an extinction phase, rats were either re-exposed to vanilla before daily watering (thirsty) or after daily watering (sated). 2-bottle preference tests between water and vanilla occurred before the extinction stage and afterwards. Vertical bars represent SEM.

Discussion

This experiment failed to reveal any evidence for the influence of thirst on accelerating the extinction of taste aversions. Animals that received vanilla when thirsty did not subsequently display a preference for vanilla or display faster extinction of an aversion to vanilla, relative to animals that received vanilla when sated. Thirsty animals consumed more of the vanilla when it was infused than sated animals. However, this confound had no effect on flavour preferences.

Using younger rats and greater exposures to a stronger CS failed to produce any noticeable effect on the establishment of thirst-relief preferences or their possible role in increasing the rate of extinction. In addition the null result suggests more consumption of the CS by the rats in the Thirsty condition is not an important confound because it was not effective in producing a preference for vanilla. Some evidence suggests the important variable in producing extinction of taste aversions is not the consumption of the CS fluid, but rather whether the CS fluid is tasted and the length of time tasted (Nachman, 1970). This experiment found higher levels of consumption without an equivalent increase in flavour preference, which suggests that more consumption of the CS did not produce a preference for that flavour. In other words, the amount of the CS consumed under these conditions was not sufficient to produce a flavour preference. As this experiment and all previous ones ensured that the CS fluid was passed over the tongue and inner cheeks of the rats mouth for a controlled amount of time, the ineffectual influence of the consumption difference reported here is

consistent with the literature. The implication taken from these results is that consumption differences are unlikely to have had any effect in previous experiments.

The absence of evidence for thirst-relief counter-conditioning a conditioned taste aversion during extinction training in this experiment is consistent with the results of the previous two experiments. Indeed, the observation of extinction, using only small quantities of the CS over many days in sated rats is the most convincing evidence that relief from thirst does not influence the rate of extinction in any important way. In relation to Whitfield's (1999) positive results, it appears that once the aversive effect of the infusion procedure is controlled for, any effect on the rate of extinction disappears. As a demonstration of extinction, this result is also consistent with the view that conditioning is due to the CS predicting or signalling US, and extinction training reduces this signal value. A conditioned taste aversion then is not entirely due to a change in the hedonic status of the taste.

CHAPTER 5

If the delivery of sucrose is paired with a tone, then sucrose is paired with lithium injection so that a conditioned taste aversion is established to sucrose, and finally the tone is presented alone across many trials, the aversion to sucrose is subsequently attenuated on later test as if extinction of the conditioned taste aversion has occurred (Holland & Forbes, 1982; see Table 5.1, pp. 68 for the experiment design of representation-mediated extinction). The explanation offered by Holland and Forbes (1982) for the decrease in the aversive response to sucrose was that after initial tone-sucrose pairings, the tone evoked a representation of sucrose, such that during extinction this tone-mediated representation of sucrose acted in a similar manner to non-reinforced presentations of actual sucrose. In this case, the extinction observed was less than that of direct extinction, but it was specific to the sucrose taste. Holland has labelled this effect as representation-mediated extinction of conditioned taste aversions.

The possibility that the evoked representation of events can stand in for the actual presentation of the events themselves is of theoretical interest to associative learning theory. Previous research on second order effects such as second-order conditioning and sensory preconditioning has usually interpreted the results in terms of the formation of an associative chain (Hall, 1996). Table 5.1, below, describes each of the above phenomena:

Table 5.1 Associative Phenomena

Stage 1	Stage 2	Stage 3	Test
Sensory Preconditioning E1→E2	E2→US		E1
Second-order conditioning E2→US	E1→E2		E1
Representation-mediated conditioning E1→E2	E1→US		E2
Representation-mediated extinction E1→E2	E2→US	E1 alone	E2

Note – E1 and E2 represent neutral stimuli; US = unconditioned stimulus.

In both sensory pre-conditioning and second-order conditioning, each stage of training establishes one of the links in the chain E1→E2→US, so that on final test E1 is able to elicit the CR by way of that chain. However, in the case of representation-mediated effects, this chain is not established and so such effects are not amenable to the same explanation (e.g. Holland, 1981; Ward-Robinson & Hall, 1999).

Representation-mediated effects are important because they suggest that associative changes can take place to absent stimuli. The explanations of representation-mediated effects are usually in terms of stimulus-substitution (Hall, 1996). Under this S-S view, the

associatively-activated representation of an event can serve as a substitute for the event itself in associative learning (e.g. Konorski, 1967). In contrast to the S-S view, the stimulus-response (S-R) view is that exposure to the CS results in the elicitation of only the most efferent motor activity formerly elicited by the US. Consider two extreme possibilities (Holland, 1990). After tone-sucrose pairings, under the S-S view the CS activates all the sensory features of the US. The rat will not only salivate when the tone is presented, but also it will taste, smell, feel and otherwise experience the US, as if the sucrose was present. In fact, it salivates because of this perceptual processing of the absent US. The tone serves as a perfect surrogate for sucrose, invoking the same perceptual experiences as sucrose itself. In short, the rat imagines or hallucinates the absent food. On the other hand, under the S-R view, the CR reflects a changed potential of the CS to activate only the output mechanism that controls the UR. No perceptual feature is engaged by the CS alone. Consequently, although the S-R rat salivates when the tone is present, there is no perceptual experience of the absent US.

There is some evidence that S-R type connections explain the effects of second-order conditioning. For example, after second-order conditioning, extinction or devaluing of E2 does not change the CR to E1 (Rescorla 1973; 1977). During Stage 2, E1 is paired with the concurrently present CR to E2, which allows S-R associations to form between E1 and the CR to E2. The CR elicited by E1 is a result of such pairings. According to some theorists (e.g. Rescorla), the content of

such associations do not include the stimulus properties of E2. This explains why changing the value of E2 by devaluation or extinction has no effect on responding to E1 because it now elicits the CR independently from E2 (Rescorla, 1973, 1977; but see Rashotte, Griffin & Sisk, 1977). This view has been compared to evaluative conditioning where the CS takes on the hedonic properties of the US and no longer refers to the US itself (Baeyens et al., 1995). According to both views, responding to E1 should not extinguish because it is directly associated with its own CR without reference to the US. But the S-R view cannot explain representation-mediated effects where the response to E2 is changed in the absence of E2 itself. This fact is usually taken to mean that E1 evokes the stimulus properties of E2 (Holland, 1990).

This brief review of some aspects of representation-mediated effects leads to a few worthwhile conclusions. The explanations of such effects are not amenable to associative-chain accounts, and there is evidence that such effects rely upon S-S associations rather than S-R associations. This distinguishes representation-mediated effects from other forms of conditioning such as second-order and by implication evaluative conditioning.

The possibility that a representation of the CS can stand in for the event itself provides an opportunity to separate out the role of counter-conditioning in extinction. It is unlikely that any associatively-activated representation of sucrose would relieve the thirst of fluid-deprived rats in the same manner as presentation of the fluid itself during extinction. From this argument, we can predict that, if

representation-mediated extinction occurs despite no actual presentation of fluids during extinction then it is unlikely that thirst relief is a necessary factor in the extinction of flavour aversions.

There have been very few demonstrations of representation mediated-extinction. Apart from the original experiment by Holland and Forbes (1982), Holland and Ross (Experiment 2, 1981) extinguished a light CS after serial light-tone-food compound conditioning in rats and found considerable mediated-extinction of the tone stimulus, which again was highly stimulus specific. Stimulus-specificity encourages the view that the stimulus properties mediate the effects. One other area in which S-S associations are likely to be involved is sensory pre-conditioning (Table 5.1). All the stages of sensory pre-conditioning are contained within representation-mediated extinction experiments but the latter involves an additional test stage to E2. What is usually observed in sensory pre-conditioning is that in the test stage to E1, the once neutral E1 is now able to elicit the CR previously only elicited by E2, presumably by way of the chain E1→E2→US. This requires S-S associations, as the response to E1 is specifically due to E1-E2 pairings, and E1 was never paired with the changed CR to E2 after E2-US training (Hall, 1996). To the extent that sensory pre-conditioning shares common processes with representation-mediated extinction, it might be expected that obtaining evidence for one would produce evidence for the other. At present, evidence for both phenomena have not been obtained in a single experiment. For example, Holland and Forbes (1982) did not take the relevant measures

to reveal sensory pre-conditioning in their demonstration of representation-mediated extinction.

Due to the absence of experimental data, it is not clear under what conditions representation-mediated extinction can occur. Obviously, conditions that support sensory pre-conditioning might be expected to produce representation-mediated extinction. One parameter proven to be important in sensory pre-conditioning, and also likely to be important in representation-mediated conditioning is the number of first stage trials. Mackintosh (1974) reports that initial experiments showing sensory pre-conditioning effects used up to 200 E1-E2 pairings (e.g. Brogden, 1939c cited in ibid), but other researchers found the greatest effect with fewer trials (e.g. 4 in Hoffeld et al., 1958, cited in ibid). The same conditions appear to be important in representation-mediated conditioning studies. Holland (Experiment 10, 1990) reported that 16 tone-food pairings produced a larger affect on later consumption than 28 or 40 such pairings. Holland takes this to suggest that extended pairings might produce a gain in S-R links that are not amenable to representation-mediated effects. Another factor that might be important in obtaining such effects, at least in taste aversion preparations, is whether water is provided when the animal is expected to 'hallucinate' the taste CS (Holland, 1990). Water may promote the experience of the stimulus properties of the associatively activated taste and augment the effects, or in the case of representation-mediated extinction, counter-condition the associatively-activated aversion by relieving thirst, and thus augment

the effects again. The provision of water during the critical stage has not been examined in any published experiments, but Holland claims that providing fluids during representation-mediated conditioning augments the effect (personal communication). However, Holland and Forbes (1982) did not provide fluids during the representation-mediated extinction phase and still observed an effect.

The next two experiments attempt to provide a demonstration of representation-mediated extinction, which would verify that thirst-relief is not critically involved in the extinction of taste aversions.

Experiment 5

Experiment 5 was designed to obtain the representation-mediated extinction effect using contexts rather than tones to signal sucrose. The design is shown in Table 5.2 below. In an initial Training Phase (Stage 1) rats were first trained to associate sucrose with one distinctive context (E-sucrose) and water with another distinctive context (E-water). Then sucrose was paired with lithium in a third context (E-test) during Stage 2, before an Extinction Phase (Stage 3) in which half the rats were placed back in the E-sucrose context for mediated extinction, and the other half were placed back in the E-water context as a no-extinction control. Finally all rats were tested in E-test with sucrose. The main question of interest was whether the associatively activated representation of sucrose by the E-sucrose context would produce extinction of the aversion relative to E-water. If so, then thirst-relief is unlikely to be responsible for the decrement in aversion.

The presentation of water during the Extinction Phase was added as a second factor in order to examine the influence of water provision during this stage. Half the groups received water during the Extinction Phase in their respective contexts whilst the other half received empty water bottles. It might be expected that providing water during this critical stage would augment the effect of representation-mediated extinction by either facilitating the experience of the associatively-activated stimulus properties of the sucrose, or by counter-conditioning the associatively-activated aversion. Thus, if water increases the rate of extinction in the experimental groups then it is still unclear whether relief of thirst plays a role in reducing the aversion.

Water consumption during the Extinction Phase (from those rats receiving water) provided a secondary dependent variable. If pairing the presentation of sucrose and the E-sucrose context in Stage 1 established S-S associations between these two events, then water consumption in that context may be less than that in E-water after conditioning. On the other hand, if the appetitive CR to sucrose (S-R associations) rather than the stimulus properties of sucrose were attached to the E-sucrose context then water consumption in E-sucrose might be higher than in E-water.

The third factor was whether the Training Phase involved 2 pairings or 4 pairings of the context with the flavour, and it was included because there is some evidence (Experiment 10, Holland, 1990) to suggest that the number of initial context-sucrose pairings may be critical in establishing S-S associations rather than S-R

associations, something on which sensory pre-conditioning and representation-mediated effects are deemed to depend. It is critical that enough discrimination training is provided so that only the sucrose context brings to mind a representation of sucrose and little or no generalisation to other contexts occurs, in order to obtain a difference between groups. On the other hand, too many pairings between the context and flavour may reduce the likelihood of S-S associations (Holland, 1990).

Thus, the present experiment used a 2 x 2 x 2 factorial design. The first factor was whether subjects received the Extinction Phase in the E-sucrose context or E-water. Group membership of this factor is denoted by X ('extinction') or C ('control') respectively. The second factor was whether subjects are provided with water or empty bottles during the Extinction Phase, denoted by W ('water') or E ('empty') respectively; the third factor was whether the Training Phase involves 2 pairings of the context with the flavour, or 4 pairings, denoted by S ('short training') or L ('long training').

Therefore, with eight groups representing each factorial cell (XWL, XWS, XEL, XES, CWL, CWS, CEL, CES), the predictions for the final test were that: 1) evidence for representation-mediated extinction would be obtained if X groups drank more sucrose than C groups; 2) evidence that thirst relief is involved in extinction of taste aversions would be obtained if the provision of water (E or W groups) interacted with the first factor; and 3) evidence for S-R associations would be obtained if the training factor interacted with the first factor.

Table 5.2 Design of Experiment 5.

Training Phase	Conditioning	Extinction Phase	Test 2
2 x E-sucrose:suc 2 x E-water:wat XWS, XES, CWS, CES	E-test:suc+ and Test 1. All groups	E-sucrose:wat XWL, XWS E-sucrose: 0 XEL, XES	E-test:suc. All groups
4 x E-sucrose:suc 4 x E-water:wat XWL, XEL, CWL, CEL		E-water: wat CWL, CWS E-water: 0 CEL, CES	

Method

Subjects. Thirty-two experimentally naïve, male, inbred, Wistar rats about 6 months old at the start of the experiment were obtained from the same source as previous experiments. Mean weight was 625 g and ranged from 520 to 695 g. Rats were housed in four squads of eight (A, B, C and D) under the same conditions as Experiment 1, with restricted access to water.

Apparatus. The experiment used three different contexts in which the spouts from the same water bottles used in previous experiments could be inserted. One context (E-test) consisted of eight white plastic buckets (290 x 435-mm : D x H) with white plastic lids and kitty litter on the floor. The spouts could be inserted into holes in the wall so that their tips were 55-mm from the floor. The buckets were in a separate

room under fluorescent light. The other two contexts (E-water and E-sucrose) were eight place-preference chambers and eight acrylic boxes set up in separate rooms. Each place-preference chamber measured 295 x 297 x 293-mm (L x W x H), with black cardboard sides and a wire mesh lid, while the floor was bare, painted chipboard. The chambers were kept in almost complete darkness, the only source of light into the room coming from a crack under the door. The spouts could be inserted into holes in the wall, 90-mm from the midline, so that their tips were 70-mm from the floor. The eight acrylic cages were the same as used in Experiment 1. These were kept under natural light conditions, with background sound coming from an exhaust fan. As described for Experiment 1, the bottle spouts could be inserted through the wire lids. E-sucrose and E-water were counterbalanced between the place preference chambers and the acrylics cages, whilst E-test was the buckets for all rats. The sucrose used was a solution of 10% castor sugar in tap-water (w/v), which was prepared the day before and served at room temperature.

Counterbalancing. Counterbalancing across homecage squads was achieved to represent each squad position evenly in both the X (extinction treatment) and C (control treatment) groups, and equate the strength of the aversion at Test 1 between groups on this factor. The E-sucrose and E-water contexts were also counterbalanced. Squads A and C were given sucrose in the place-preference chambers and water in the acrylic cages, while B and D received the opposite pairings. The order

of discrimination training was fixed to control for the sequential effects of training.

Procedure. The experiment was run in the home-cage squads of eight (A, B, C and D) in alphabetical order, and there were five phases of training, Pre-training; Training Phase; Conditioning; Extinction Phase; Test. During the experiment, squads were carried by hand to each of the three contexts. All sessions in these contexts lasted 10 min and training was given once a day. Rats were assigned to equal sized groups (n=4) on the basis of results from the conditioning phase, so as to equate the average strength of aversion between groups, as detailed below.

Pre-training. On Days 1 to 3 all rats were given water in E-test for 10 min after which they were returned to the colony room in their homecages and received supplementary water 30 min after training. This schedule of supplementary water was continued throughout the experiment.

Context Training Phase. This phase lasted either four days (S groups) or eight days (L groups) and each squad received 10-min access to water in their E-water context and 10-min access to sucrose in their E-sucrose context in the following sequence: ABABBAAB, where A indicates a water day and B indicates a sucrose day. Squads A and D only received the first four days of this schedule (short-trained condition). After each session, rats were placed back in their home-cages and returned to the colony room.

Conditioning. This phase lasted 5 days in the E-test context. In order to accommodate for the prior exposure to sucrose, the lithium dosage was doubled to 10ml/kg from previous experiments. On the first day squads received sucrose in the E-test context and upon removal were immediately injected (i.p.) with 0.15M LiCl at 10 ml/kg. They were then placed back in their homecages and returned to the colony room. On the next two days 10-min access to water was provided in the E-test context in order to remove any context aversion which might have accrued due to conditioning. The conditions during these sessions were identical to pre-training and no injections were given. On the fourth day, all rats were given sucrose in the E-test context as an initial extinction test (Test 1) before the Extinction phase. The results of Test 1 were used to allocate subjects in a manner that equated the aversion between the X and C groups. On the fifth and final day of this phase, all squads were given 10-min access to water in E-test.

Extinction Phase. All rats were then either returned to the E-water context or the E-sucrose context for the next six days. In each context, half the rats either received water filled bottles or empty bottles. Due to experimenter error, squads A and D received an extra, 7^{th} day of the extinction phase. This means that the difference between the short discrimination-phase group and the long discrimination-phase group was confounded with a difference in length of Extinction Phase.

Test. For four days immediately after the extinction phase, rats were returned to the E-test context and given water, as during pre-

training. On the fifth day of this phase, rats were given a 1-bottle sucrose test in E-test for 10 minutes.

Results

The results from Test 1 in the E-test context (after conditioning but prior to the Extinction Stage) revealed that the short-trained groups drank significantly less sucrose than the long-trained groups ($F_{1, 24} = 23.13$, $p < 0.01$). Thus, sucrose consumption after conditioning increased with the number of sucrose pre-exposures (latent inhibition). No other main effects or interactions were significant ($p > 0.05$).

Sucrose consumption for each group at Test 2 in the E-test context (the primary DV) is shown in Figure 5.1 (pp. 82). The planned orthogonal analysis revealed there was no main effect of the E-sucrose context during the Extinction Phase on later sucrose consumption and so no clear evidence for representation-mediated extinction was obtained ($p > 0.05$). In addition, there was no main effect of the provision of water during the Extinction Phase on later sucrose consumption ($p > 0.05$). The long-trained groups consumed significantly more sucrose than the short-trained groups at this test ($F_{1, 24} = 7.72$, $p = 0.01$), which verifies that more sucrose pre-exposures prior to conditioning results in a weaker aversion (latent inhibition). The XE groups drank more sucrose than the XW groups relative to the C groups (CES, CEL vs CWS, CWL). This interaction was significant ($F_{1, 24} = 4.63$, $p = 0.04$) and suggested that the absence of water

during representation-mediated extinction produced a loss of the aversion.

When Test 1 scores were subtracted from Test 2 scores to remove any pre-existing differences that may have existed between groups prior to the Extinction Phase, the resulting scores for each group are shown in Figure 5.2 (pp.82). The pattern of results from Test 2 (shown in Figure 5.1) are only clearly present in the short-trained groups difference scores in Figure 5.2, however no main effects or their interactions were significant ($p > 0.05$). This means that the results reported from Test 2 were probably due to pre-existing differences rather than any IV in the Extinction Phase.

Figure 5.3 (pp.82) shows water consumption of groups XWL, XWS, CWL, CWS in the E-test context on the day prior to the extinction phase and during the six days of the extinction phase. Rats consumed significantly less water in the E-sucrose context (XWL, XWS) than the E-water context (CWL, CWS) across the six days of the extinction phase ($F_{1, 12} = 5.41$, $p = 0.04$). This main effect did not interact with the length of training factor or the course of the extinction phase ($p > 0.05$), which means that there was no evidence that the difference between groups was being reduced by the Extinction Phase. Group differences in water consumption between the E-test context on the day prior to the Extinction Phase and the on the first two days in the extinction contexts (E-sucrose or E-water) changed significantly ($F_{1, 12} = 6.10$, $p = 0.03$). This means that the difference in water consumption between the XW groups and CW groups was

specific to the extinction contexts. The results from the Extinction Phase provide the best evidence obtained in Experiment 5 that associations between the E-sucrose context and sucrose were established and these subsequently affected water consumption. However, the results failed to provide evidence that representation-mediated extinction took place.

Discussion

The results from Experiment 5 are summarised here. There was no evidence that representation-mediated extinction occurred. The rats that received the representation-mediated extinction treatment (X groups) did not drink reliably more sucrose at Test 2 than the control rats (C groups). Although the provision of water significantly interacted with the extinction treatment at Test 2 (in the opposite direction to that expected), differences were removed once pre-existing differences were accounted for by subtracting Test 1 scores (see Figure 5.2). However, evidence was obtained that associative processes may have been influencing water consumption during the Extinction Phase. Results from this phase revealed that water consumption in the E-sucrose context was significantly lower than in the E-water context (Figure 5.3). This difference was specific to the context, which means the difference between groups was only apparent between the E-sucrose and E-water contexts and not in the E-test context beforehand. And the effect was not due to differences in the physical attributes of the contexts used, as the distinctive chambers used for these two contexts

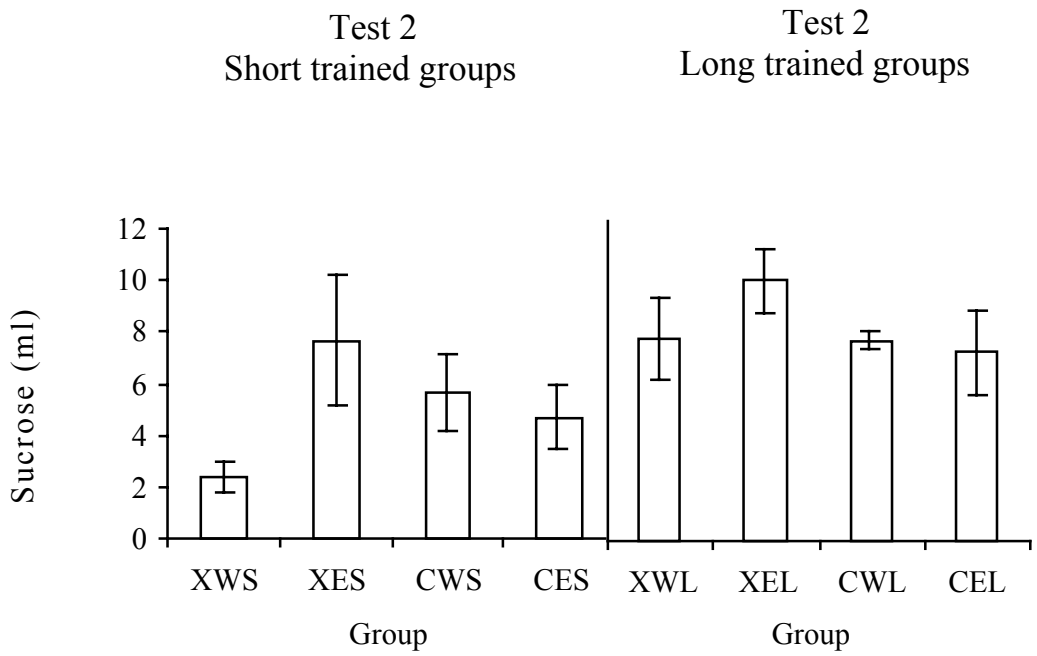

Figure 5.1 Experiment 5. Mean sucrose consumption at Test 2. The left panel shows those groups which received only two context-flavour pairings, whilst the right panel shows groups which received four such pairings. In both cases, a conditioned taste aversion was established to sucrose, and an extinction stage was spent in either E-sucrose (XWS, XES, XWL, XEL) or E-water (CWS, CES, CWL, CEL), with water provided (XWS, CWS, XWL, CWL) or without (XES, CES, XEL, CEL). Rats were then tested for an aversion to sucrose in E-test. Vertical bars represent SEM.

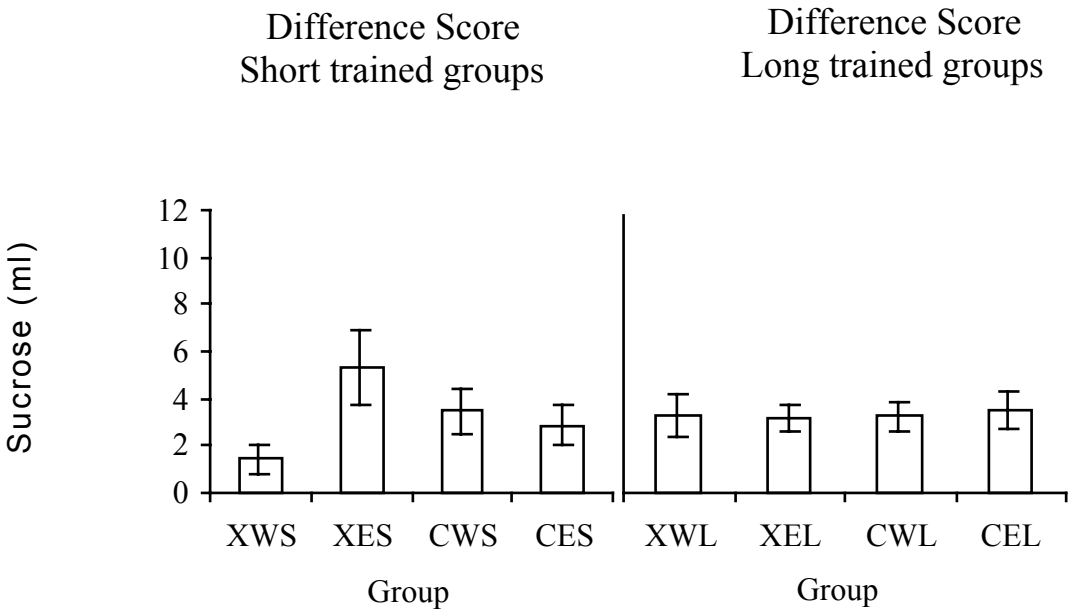

Figure 5.2 Experiment 5. Mean difference scores (Test 2 – Test 1). The left panel shows those groups which received only two context-flavour pairings, whilst the right panel shows groups which received four such pairings. Test 1 occurred after sucrose-LiCl pairings in E-test. Test 2 occurred in E-test after an extinction stage either in E-sucrose with water (XWS, XWL) or without water (XES, XEL); or in E-water with water (CWS, CWL) or without water (CES, CEL). Differences apparent in the short trained groups (left panel) are absent in the long trained groups (right panel).Vertical bars represent SEM.

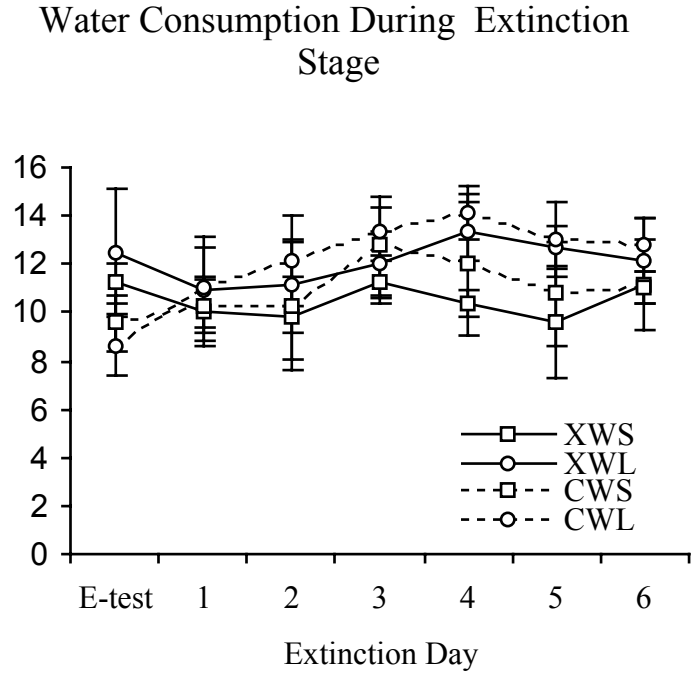

Figure 5.3 Experiment 5, Extinction Phase results.

Mean water consumption in E-sucrose and E-water during the extinction stage in Experiment 5. E-sucrose had been previously paired with sucrose prior to sucrose pairings with LiCl in a separate context (E-test). E-water had been previously paired with water. Rats in E-sucrose (XWS, XWL) consumed less water than rats in E-water (CWS, CWL) across the six days ($p < 0.05$). Vertical bars represent SEM.

were counterbalanced. In addition, there was no evidence that the difference was being removed across the Extinction Phase.

The results from the final test in the E-test context are considered first. Figure 5.2 shows that once pre-existing differences are accounted for by subtracting Test 1 from Test 2 scores to obtain a difference score for each subject, (non-significant) group differences most clearly existed within the short-trained groups (S groups). XWS appears to show more aversion than XES, relative to the control groups (CWS, CES), whereas this is not so apparent in XWL, XEL, CWL, CEL groups. This pattern of differences is also apparent in the Test 2 data (Figure 5.1), where the interaction was significant. The null result obtained from the difference scores may be due to lack of power as group size was limited to 4 subjects (n=4), and increasing the group size would more clearly indicate whether the provision of water is critical in producing representation-mediated effects. If water prevents the mediated-extinction of the sucrose aversion, then the differences observed may be due to the intensity of the representation evoked. In theoretical terms, E-sucrose without water may excite a partial representation of sucrose without its associated consequences such as lithium toxicosis. This would then be equivalent to representation-mediated extinction. On the other hand, E-sucrose with water may excite the complete chain of associations with sucrose, including a representation of the toxicosis. This evoked representation would maintain the aversion to sucrose for longer periods. In the same manner, Holland (1990) suggests that differences between

representation mediated effects and real effects may be due to differences in intensity.

The results from the Extinction Phase are now considered. Water consumption during the Extinction Phase revealed that rats consumed less water in the E-sucrose context than rats in the E-water context. This occurred despite the fact that the lithium US was never contiguous with the E-sucrose or E-water contexts and neither was sucrose ever presented in the E-sucrose context after it was paired with lithium, so the aversion cannot be due to direct associations between the E-sucrose context and the CR or CER established by the lithium US. This rules out any possibility that S-R connections were formed between the context and the response to nausea (Rescorla, 1973, 1977). S-R type associations could have only been established with the E-sucrose context if the CR to sucrose (a CTA) was paired with the context, either by presenting sucrose in that context after conditioning so that a CR is elicited, or by pairing the context with the lithium US. Neither of these two possibilities occurred in this experiment.

The difference in consumption was specific to the extinction contexts and remained constant across the six extinction days. The result by itself does not show whether the difference in consumption between the two contexts is due to increased water consumption in the E-water context or decreased water consumption in the E-sucrose context. The first possibility is difficult to explain, whilst at least three plausible alternative explanations can be offered for the second possibility. Firstly, a decrease in consumption in E-sucrose may be due

to generalization decrement (Capaldi, 1967). The sucrose and the context were always presented together (not serially) during Stage 1 and sucrose may have been the most salient feature of the context to thirsty rats. If the E-sucrose context and the presentation of sucrose were encoded as one event during Stage 1, then presenting water in E-sucrose during the Extinction Phase could have changed the stimulus conditions controlling the appetitive consumption response. In a similar manner, the lower intakes in the E-sucrose context could be a case of surprise-induced neophobia. After conditioning with lithium toxicosis, rats show increased neophobia (Nachman, 1970). The unexpected delivery of water in E-sucrose during the Extinction Phase may be equivalent to a novel flavour. Consequently, rats may display a mild aversion to the water, as in neophobia. Unlike neophobia and generalization decrement though, the effect appears robust over the six days of extinction (Figure 5.3). Nevertheless, without the relevant unpaired control group which would be expected to show an increase in water consumption in the sucrose associated context, it is impossible to decide the matter.

The second alternative and more interesting explanation is that the lower consumption of water was a result of representation-mediated conditioning. During Stage 1, bidirectional associations might have formed between sucrose and the E-sucrose context. During Stage 2, presenting sucrose again before LiCl injections, may have activated a representation of the E-sucrose context, which in turn may have been directly associated with the lithium US. This association would have

reduced fluid intake in that context upon subsequent test during the Extinction Phase. The advantage of this explanation is that it explains why no representation-mediated extinction effect was seen after the Extinction Phase. According to this view, the E-sucrose context would have activated a representation of the lithium US rather than the intended representation of the sucrose CS. Activating the US representation would further explain why the lower water consumption in E-sucrose was not removed by the end of the six days of the Extinction Phase.

The third alternative and equally interesting explanation for the lower consumption levels of water in E-sucrose is that due to sensory pre-conditioning via the associative chain E-suc → Suc → LiCl. In this experiment, the E-sucrose context was paired with sucrose in the first stage. At this stage, an appetitive CR to sucrose was established, as indicated by the higher sucrose consumption in E-sucrose than at any other stage in the experiments. This represents the first link in the chain E-suc → Suc. In the following stage, sucrose was paired with lithium injections (in a separate context: E-test) to produce a sucrose aversion, as indicated by the drop in sucrose consumption upon re-exposure. This represents the second link in the chain Suc → LiCl. Finally, a reduction in water intake was observed in E-sucrose relative to E-water due to the first-order associatively-activated representation of sucrose and the second-order association with the lithium US.

The design of the present experiments means it is impossible to decide between these alternatives. All that can be said is that the lower

consumption in E-sucrose is evidence that associations were established between that context and the presentation of sucrose. The exact associative mechanism that is then responsible for the lower water consumption is not revealed. Further experiments examining issues not directly related to the topic of this thesis would be necessary before a conclusion could be drawn. What can be emphasized though, is that representation-mediated extinction was not obtained despite evidence that the necessary associations had formed.

If the lower water consumption in the E-sucrose context was due to the associatively-activated representation of sucrose, why was there no evidence of representation-mediated effects on later sucrose consumption at Test 2? One possibility is that any effect at Test 2 may have been removed in the long-trained groups (L groups), leaving too few subjects in the short-trained groups (S groups) to reveal the effect. The pattern of group differences shown in Figure 5.3 suggests that differences are clearer in the short-trained groups. The conclusion proposed here is that the short-training phase produces the best chance of obtaining representation-mediated effects. This is consistent with Holland's (1990) conclusion that extended S1-S2 pairings results in a loss of ability for S1 to evoke a representation of S2. This conclusion must be qualified by noting that no effect of short or long training was seen on water consumption during the Extinction Phase. If there was a loss of event representation with more training, then the long-trained groups might have been expected to show no sensory pre-conditioning

at this stage, yet length of training did not significantly interact with the observed effect.

One feature of the current design that may be responsible for the lower intakes in the E-sucrose context is that an additional sucrose exposure occurred in the E-test context after conditioning. There is some evidence to suggest that the associative effects of devaluing a stimulus are only observable if the devalued stimulus is re-exposed to the animal (Balliene & Dickinson, 1991, 1992; Bermudez-Rattoni, et al., 1988; Garcia, 1989). That is, the aversion to sucrose is only apparent after the animal experiences the loss of palatability. In this case, the reduced water intake observed in the E-sucrose context may have required the re-exposure to sucrose at Test 1 which established that sucrose was now disgusting. This experience may have been required before the reduction in water consumption was seen. Under this view, there is no role for the US representation in the associative chain. One way to test this theory would be to re-expose half the rats to sucrose prior to the Extinction Phase and the other half to water as a control. Water intake in the E-sucrose context would not be expected to decrease without re-exposure to sucrose.

Experiment 6

Experiment 6 re-examined the effect of providing water during mediated extinction of a sucrose aversion. A 2 x 2 x 2 factorial design was used based upon Experiment 5 (Table 5.3, pp. 91). The first factor was whether the Extinction Phase occurred in the E-sucrose context or

E-water context (X or C groups respectively). So, the X groups receive the mediated extinction treatment and the C groups receive a control treatment. The second factor was whether water was provided during the Extinction Phase (W or E groups). W groups received water, whilst E groups received the empty water bottle. The training factor from Experiment 5 was removed and replaced with a re-exposure factor in this experiment, because the results from Experiment 5 suggested that a further two context-CS pairings removed the effect of the other factors.

The current experiment examined the role of re-exposure in producing the group differences seen in Experiment 5. According to Dickinson (1994; see also Garcia, 1989) aversions conditioned by lithium toxicosis are not based on the knowledge of the relation between food and illness. Rather, he argues that conditioning brings about a *latent* change in the affective reaction elicited by a taste, which is then manifest when the animal is re-exposed to the taste. Thus, following taste aversion conditioning, the rat does not avoid eating the food because it knows it causes illness, but rather because it now tastes disgusting. Balleine and Dickinson have shown that rats will still perform an instrumental action for food (lever pressing for sucrose) even after it is devalued by pairing the sucrose with lithium, as long as the animals have not been re-exposed to the food after lithium pairings. They explain this by arguing that the change in value of an outcome does not occur at the time of aversion conditioning but rather when the rat is subsequently re-exposed to the food and experiences that it is now distasteful or disgusting (Balleine & Dickinson, 1991, 1992).

Based on these results, the associative effects of devaluing the sucrose may only be seen when the animals are re-exposed to the sucrose after conditioning with lithium and before representation-mediated extinction. Unless re-exposure occurs at this stage, the hedonic value of sucrose may remain rewarding rather than aversive, and extinction of the aversive effects of sucrose will not be seen. Indeed, in Holland's experiment demonstrating representation-mediated extinction (Holland & Forbes, 1982), the CS fluid was paired with lithium across multiple conditioning session, providing ample opportunity for the rats to taste it as disgusting.

It is possible that such experience is necessary before representation-mediated extinction can take place. However, the role of this experience has never been examined in representation-mediated effects. For this reason, the present experiment included a third factor, such that half the rats were re-exposed to sucrose after conditioning in E-Test, whilst the other half received water instead.

In summary, the following major changes from Experiment 5 were introduced in the current experiment: 1) The training factor was removed. Rats were given 2 pairings of the context and sucrose over four days; 2) An additional factor was included. Half the rats were re-exposed to sucrose (after conditioning in E-Test) on day 12. 3) The group size was increased from 4 rats per factorial cell to 5 rats per factorial cell. 4) Counter-balancing within homecage squads ensured that each group was represented in each homecage.

The predictions were that the X groups, which received representation-mediated extinction treatment, would consume more sucrose at final test than the C groups (control). Based on results from Experiment 5, the water factor may interact with the extinction factor. If re-exposure is necessary for sucrose devaluation to occur after conditioning, the re-exposure factor may interact with the extinction factor so that only those rats that received re-exposure (R versus N) show the effects of the representation-mediated extinction treatment. In addition, the re-exposure should determine whether a CR to water is observed in E-sucrose during the Extinction Phase.

Table 5.3 Design of Experiment 6.

Training Phase	Conditioning	Extinction Phase	Test
2 x E-sucrose:Suc 2 x E-water:Wat All groups	E-test:Suc+ then re- exposed to Suc or Wat XWR XWN XER XEN CWR CWN CER CEN	E-sucrose:Wat XWR, XWN E-sucrose: 0 XER, XEN E-water: Wat CWR, CWN E-water: 0 CER, CEN	E-test:Suc All groups

Method

Subjects. Forty experimentally naïve, male, outbred, Wistar rats were obtained from the same source. Rats were about 2 months old at the start of the experiment with weights ranging from 172 to 254 g and a mean weight of 211 g. They were housed in five squads of eight (A, B, C, D and E) under the same conditions as Experiment 5, with restricted access to water.

Apparatus. The experiment used the same three contexts as Experiment 5, and the 10% sucrose solution was prepared the same way.

Counterbalancing. Each factor was counterbalanced within squads so there was one rat from each group in each squad. Unlike Experiment 5, the first factor and second factors were both counterbalanced and rotated within squads. Finally, the presentation of sucrose or water in the place-preference chambers and the acrylics was counterbalanced between squads.

Procedure. As in Experiment 5, all transport between the colony room and the laboratories occurred by hand (not trolley); training occurred by squad in alphabetical order; and all squads received 30-min supplementary water in the colony room, 30 min after training. Training was given once daily, and all training sessions lasted 10 min. The experiment was run in five phases: Pre-Training; Training Phase; Conditioning; Extinction Phase; Test.

Pre-training. On Days 1 and 2, all rats were given water in E-test for 10 min after which they were returned to the colony room in their

homecages and received supplementary water 30 min after training. This schedule of supplementary water was continued throughout the experiment.

Context Training Phase (Days 3 - 6). All squads received context discrimination training, with water on the 1st and 3rd day and sucrose on the 2nd and 4th day. Squads received 10-min access to water in their E-water context and 10-min access to sucrose in their E-sucrose context. Days 7 and Day 8 were rest days. All squads received supplementary water only.

Conditioning (Days 9 - 14). Conditioning and re-exposure were run over six days. On Day 9 rats were given water in the buckets. On Day 10 sucrose was given in the buckets and upon removal each rat was immediately injected (i.p.) with 0.15M LiCl (10-ml/kg). On the next two days water was given in the buckets to help remove any contextual conditioning that accrued. Re-exposure (or non re-exp) occurred on Day 13. Half the rats were given sucrose in the buckets (R: re-exposed treatment), whilst the half were given water (N: non-reexposure treatment). On Day 14, all rats were given water in the buckets again.

Extinction Phase (Days 15 - 20). For the next six days rats were either returned to the E-water context (C groups) or the E-sucrose context (X groups). There, half the rats received water (W groups) while the other half simply received the empty spouts (E groups). Water consumption for the W groups was measured.

Test (Days 21 – 23). For two days immediately after the extinction phase rats were returned to E-test and given water, as during pre-training. This was provided in order to re-establish baseline drinking levels. On Day 23 rats were given a 1-bottle sucrose test in E-test for 10 min.

Results

On the day of re-exposure in E-test, mean consumption levels in mls were XWR, 7.7 (1.0); XER, 9.4 (1.3); CWR, 6.7 (1.4); CER 10.2 (1.9); XWN, 12.8 (0.8); XEN, 13.6 (1.0); CWN, 14.7 (0.6); CEN, 14.7 (1.0), SEM in parenthesis. Rats given sucrose (R groups) drank significantly less than rats given water (N groups; $F_{1, 32} = 40.50$, $p < 0.01$), indicating that conditioning successfully produced an aversion. This difference disappeared the following day when all rats were given water in E-test, prior to the extinction phase ($p > 0.05$). Thus, it appears that in this experiment there were no residual group differences produced by conditioning prior to the Extinction Phase of the kind found in Experiment 5.

Sucrose consumption of each group at the final test in the E-test context is shown in Figure 5.4 (pp.95). The R groups, which received re-exposure to sucrose after conditioning consumed more sucrose at this final test than the N groups ($F_{1, 32} = 21.42$, $p < 0.01$). No other results from this test were significant. In particular, there was no evidence of representation-mediated extinction provided by the

extinction treatment main effect ($p > 0.05$) or interactions with either the re-exposure factor ($p > 0.05$) or the water factor ($p > 0.05$).

Water consumption during the Extinction Phase and the day prior in the E-test context is shown in Figure 5.5 (pp.95). Rats in the E-sucrose context drank significantly less water than rats in the E-water context across the six days of extinction ($F_{1, 32} = 10.31$, $p < 0.01$). This main effect did not interact with the linear trend ($p > 0.05$), which means there was no evidence that the difference between the groups was reduced by repeated extinction sessions. Furthermore, it did not interact with the re-exposure factor ($p > 0.05$), so there was no evidence that outcome devaluation requires re-exposure. Group differences in water consumption between the E-test context on the day prior to the Extinction Phase and the on the first two days in the extinction contexts (E-sucrose or E-water) did not change significantly ($p > 0.05$). This means that the difference in consumption between the two extinction contexts may not have been specific to the extinction contexts.

Discussion

No evidence for representation-mediated extinction was found. The interaction between the water factor and extinction treatment suggested by Experiment 5 was not reproduced. The problem of pre-existing differences in Experiment 5 was successfully removed in this experiment by the addition of a water day in the E-test context prior to conditioning there. This procedural variation may be responsible for

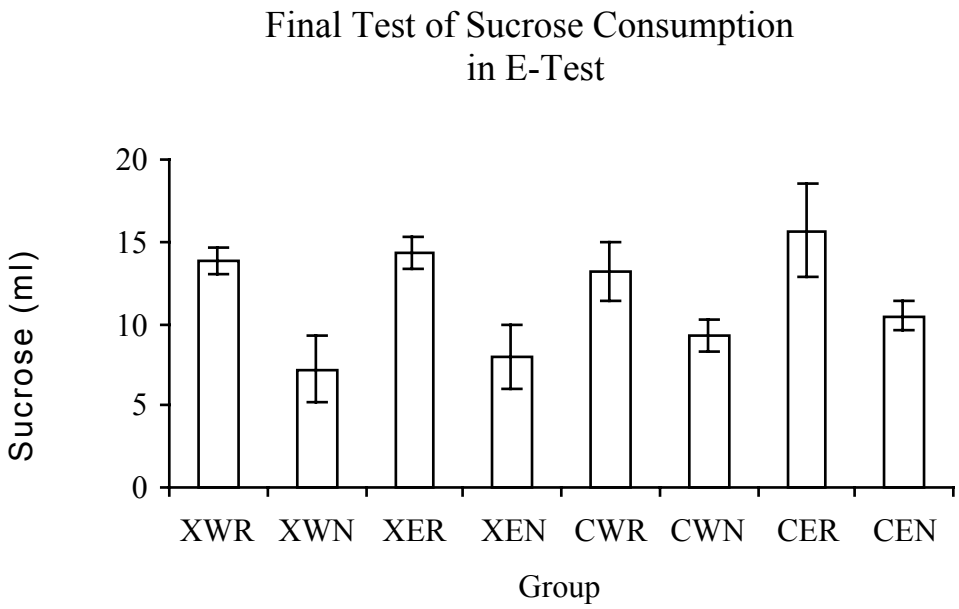

Figure 5.4 Experiment 6. Test 2 mean sucrose consumption. Results from the final 1-bottle test in the E-test context are shown here. Rats which received re-exposure to sucrose after conditioning (XWR, XER, CWR, CER) consumed more sucrose in E-test relative to rats that had not received such re-exposures (XWN, XEN, CWN, CEN). Vertical bars represent SEM.

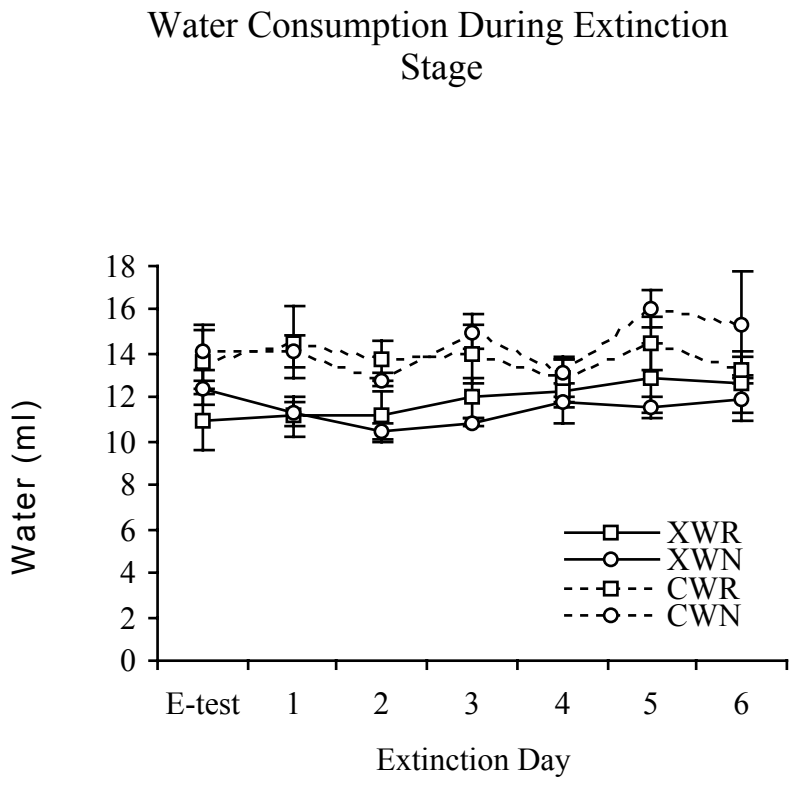

Figure 5.5 Experiment 6. Extinction Phase water consumption. Mean water consumption on the final day in E-test and during the six days of the Extinction Phase in either E-sucrose or E-water. All rats had received training with sucrose in E-sucrose and water in E-water, prior to pairing sucrose with LiCl in E-test and then half were re-exposed to sucrose (XWR, CWR) or water in E-test. Subsequently, rats in E-sucrose (XWR, XWN) consumed less water than rats in E-water (CWR, CWN) across the six days ($p < 0.05$). Vertical bars represent SEM.

the clear results of this experiment relative to Experiment 5. In fact, the only effect obtained in the final test was that re-exposing sucrose after conditioning resulted in a greater loss of the aversion than no re-exposure. This effect clearly accounted for the majority of between-group variance in this experiment (Figure 5.4). Consequently, the only available conclusion is that the suggestion of a water or extinction effect in Experiment 5 was a Type-I error, and due to accidental pre-existing differences.

The main result consistent with that in Experiment 5 was the lower water consumption in the E-sucrose context relative to the E-water context (Figure 5.5). As in Experiment 5, this difference was not due to contextual features, as the E-water and E-sucrose context were counterbalanced within groups. In addition, there was no evidence that the differences between groups were disappearing across the six days of extinction. Unfortunately, the results here did not confirm that the effect was context specific (as in Experiment 5). As the effect size is so small (only detected across repeated measures on six days in both experiments) unambiguous demonstrations of context-specificity are vulnerable to chance pre-existing differences.

As before, there are a number of alternative explanations for the lower consumption in the E-sucrose context, but some uninteresting explanations have already been ruled out (See discussion to Experiment 5). In particular, S-R associations between the context and the conditioned response decrement could not be responsible. However, in the absence of appropriate control groups, there is no clear way to tell

whether the surrogate aversion seen in E-sucrose is specifically due to general, non-associative effects of lithium induced nausea. Against this possibility, and for the sake of argument, it seems implausible that the same effect would have been observed in a group that received non-contingent lithium. Rather, the lower intakes appear to be due to the association between sucrose and the E-sucrose context established at Stage 1. How these associations could reduce water intake in the Extinction Phase can be explained in more than one way.

Some general observations can be made about the measured effect. Firstly, any aversive response to water expressed in the E-sucrose context during the Extinction Phase (Stage 3) must have been larger than any appetitive response established in E-sucrose during the Training Phase (Stage 2). This means the size of the effect may be under-represented in the results.

Secondly, half the animals in Experiment 6 had not received re-exposure to sucrose between conditioning and extinction stages, and this did not interact with performance in the extinction context. This means that animals do not need to experience any loss of palatability to sucrose in order to express an associated aversion. As such, the result contrasts with claims made by Garcia (1989) and Dickinson (1994) and suggests that the aversion is not due to the loss in palatability of sucrose itself.

Thirdly, there was no evidence in the data from either Experiments 5 or 6 that the course of consumption differences between the groups were following different linear trends or otherwise being

removed over the course of the extinction phase. As such, the result appears to be resistant to extinction which is a hallmark of evaluative conditioning. Further testing beyond six days would help establish whether the absence of evidence is due to an absence of extinction effect or merely lack of statistical power.

Fourthly, there was no evidence that the effects were measurable outside the E-sucrose context. If these results are taken as evidence for sensory pre-conditioning, then they may dissociate sensory pre-conditioning and representation-mediated extinction. Different processes may be involved in representation-mediated extinction, over and above those sufficient for sensory pre-conditioning. The processes likely to be involved in this demonstration of sensory pre-conditioning include the associatively-activated representation of sucrose by the E-sucrose context, and a subsequent aversion in E-sucrose via the associative chain that is not mediated by a loss of palatability to sucrose or S-R connections between sucrose and nausea. If this analysis is correct then merely associatively-activating event representations is not sufficient to produce representation-mediated effects.

Although Experiment 6 demonstrated that a loss of palatability of sucrose does not have to be experienced for an aversion to be expressed, without data from taste-reactivity tests it is impossible to say how the sucrose-lithium association affected the consumption of water. It may be the case the aversion was the result of the E-sucrose context reducing the palatability of all fluids consumed within it.

Alternatively, the E-sucrose context could have been directly or indirectly signalling the lithium US. All that can be emphasized is that experiencing a loss in palatability to sucrose itself is not necessary before an aversion is observed.

This discussion of the results from Experiments 5 and 6 leads to no very satisfactory conclusion regarding the topic of this thesis. Because representation-mediated extinction could not be demonstrated, the results do not reveal what role counter-conditioning plays in the extinction of taste aversions. The experiments here do provide some evidence that the associatively-activated representation of an aversive event can produce a reduction in fluid intake, and this does not require experiencing the conditioned taste. The results also may dissociate sensory pre-conditioning from representation-mediated extinction. Further experiments would be required to test whether the result is specifically dependent upon the sucrose-LiCl associations at Stage 2, and whether it is mediated by a shift in palatability to fluids in the associated context. Unfortunately, there is no compelling evidence to decide on the broader issue of whether a loss of palatability is responsible for the observed CTA, and in turn whether counter-conditioning is necessary for a decrement in aversion under extinction training.

CHAPTER 6

The present studies do not support the view that relief from thirst counter-conditions taste aversions but they do not compel a rejection of that view either. The view taken in this discussion is that counter-conditioning is not necessarily involved in the extinction of the aversive response, but it may still contribute under different conditions to the ones used in this thesis.

Chapter 3 showed that thirst-relief preferences could be obtained with the methods used in this thesis. However, Chapter 4 found that extinction of a conditioned taste aversion could be obtained under thirsty or sated conditions, relative to water controls (Experiment 2 and 3) or unpaired controls (Experiment 4). The experiments in Chapter 5 failed to show whether extinction could occur under conditions that removed all influence of thirst-relief because representation-mediated extinction could not be obtained. But the results did suggest that the sucrose-context associations could produce a conditioned aversion to water consumed in the associated context, regardless of whether the aversion had ever been expressed to sucrose or not. This result suggests that experiencing a loss in palatability to the aversive taste is not responsible for the CTA. Overall, the results failed to support the view of conditioned taste aversions described under evaluative conditioning (Baeyens, Eelen & Crombez, 1995; Levey & Martin, 1987), or the conditioning of prepared associations (Seligman, 1970; Seligman & Hager, 1972) because extinction of a CTA was obtained

and there was no evidence of resistance despite controlling for possible sources of counter-conditioning.

According to the evaluative conditioning account, pairing the CS with the US produces a hedonic shift in the value of the CS, such that the conditioned response, once it is established to the once neutral CS, cannot thereafter be extinguished through non-reinforcement, but can only be altered by counter-conditioning (Levey & Martin, 1987). At variance with this prediction is the fact that in Experiments 2, 3, and 4 extinction of a conditioned taste aversion occurred relative to water controls (2 and 3) or unpaired controls (4), regardless of whether the CS fluid was presented to rats in a thirsty state or a sated state. These experiments were performed under highly controlled conditions that equated amount of exposure to the CS fluid, any effect of the infusion procedure itself, and differences in deprivation-state between groups. Despite controlling for these factors, the aversions in this thesis did not indicate any sign of resistance to extinction. Statistical tests were not used to demonstrate equivalence between groups, yet it is apparent from Experiments 2 and 4 (Figures 4.1 and 4.4) that there is very little difference between extinction in thirsty rats and sated rats. Clearly, relief from thirst is not entirely responsible for counter-conditioning the taste aversion or accelerating extinction in the experiments reported here.

The pattern of results accords, however, with suggestions by other authors (e.g. Parker, 1988) that conditioned taste aversions are not entirely due to a palatability shift, but rather some other

mechanism. Such mechanisms might be those described by Konorski (1967) or Rescorla (Rescorla & Wagner, 1972), which accord with more traditional views that conditioning involves the CS acquiring signal value after pairing it with the US and extinction involves the loss of this predictive value. These conclusions reinforce the view presented above that relief from thirst is not counter-conditioning the aversion, and that CTAs undergo extinction for the same reasons as other forms of learning.

Experiment 1 found that the influence of thirst-relief might be very small, at least when only small amounts of fluid are provided as in the first four experiments. This result is consistent with previous research by Revusky and Capaldi, that relief from thirst or hunger deprivation can establish preferences for specific tastes. However, unlike those demonstrations, Experiment 1 showed only a small effect that was not replicated in Experiment 4. The most likely reason for this is that the methods used here involved only very small amounts of the CS fluid, while other studies used much larger amounts. This suggests that thirst-relief preferences are not due to the initial relief of fluid consumption. There are two a priori possible sources of thirst relief. Firstly, such relief may stem from the initial few mouthfuls of water (wetting the whistle, so to speak) or, alternatively, relief may stem from the delayed consequences of complete thirst satiation, after drinking is completed. Given that only small amounts of the CS were provided at any one time during extinction in these experiments, it is unlikely that the source of thirst-relief preferences are the first

alternative. This conclusion is consistent with results from experiments which used much more severe deprivation schedules (48 hours compared to 23.5 used here) and larger amounts of CS exposure at one time (5-ml in Revusky 1968b compared to 2-ml in Experiment 4) in order to produce thirst-relief preferences. As most extinction studies allow the animals free access to the CS fluid during extinction, the CS fluid is probably consumed until the animal is sated. This fact means it is impossible to rule out the role of counter-conditioning in accelerating the extinction of taste aversions. All that is suggested is that relief from thirst is not necessary for extinction to occur.

The experiments in Chapters 3 and 4 were beset with the methodological problem of infusing by hand uncooperative and sated rats, with fluid they did not want to drink. A struggling and uncooperative rat disrupts the infusion procedure, which creates problems with controlling the amount and duration of fluid exposure for each rat, and also when comparing to thirsty (and more co-operative) rats. For this reason, it is difficult to resolve the discrepancy in results between Experiment 1, where a slim (but significant) flavour preference was established, and Experiment 4, which failed to find the effect using essentially the same technique and method. Apart from discarding the first result as a Type I error, the discrepancy may also have been due to the differences in success of the infusion procedure between experiments. In future, the use of surgically implanted oral cannulas in the cheeks of the subjects is recommended. This technique would remove almost all the methodological problems referred to

above and in addition, larger amounts of the CS fluid could be provided.

It is sometimes claimed that absence of evidence for an effect is not evidence of absence because there always exists the possibility that under different conditions an effect may emerge. The parameters that generated the null results reported in Chapter 3 were quite narrow. The group size was always eight (n=8), only small amounts of the CS were used during extinction (0.3 to 2-ml), the aversions established were always quite mild (0.15M at 5ml/kg produced about a 70% reduction in experiment 4), and the range of CSs tested were also small – saccharin and aqueous odours. It is always open to the experimenter to alter the parameters, and the lack of effects seen here may simply be that the particular training parameters used in the study were less than optimal. Nevertheless, the strong position claimed for the purposes of this thesis, that CTAs represent an evaluative form of conditioning which should be exempt from the laws of extinction, is clearly contradicted by these results. On the other hand, there is always room for the weaker position that counter-conditioning processes contribute to the observed extinction, even if they are not entirely necessary for it. It seems likely given flavour preferences can be established by thirst-relief using only small amounts of the CS fluid as shown in Experiment 1, that the contribution of this factor would be increased as the amounts consumed under deprivation during extinction of CTAs is increased also, as is the case when fluid consumption is not controlled. Future exploratory work could well reveal a set of parameters such as

increased thirst-relief that would generate a powerful deprivation effect on extinction.

Hence, the ideal future study examining the same question would provide 10-ml of sucrose through surgically implanted oral cannulas, to thirsty rats and sated rats. Larger amounts of the CS would be more likely to relieve thirst in the thirsty rats; sucrose would provide a taste-based flavour that would produce stronger conditioning effects than odour-based flavours; and the oral cannula would prevent the rat from disrupting infusions and help remove the aversive conditions involved with providing the CS. Alternatively, using different concentrations of saline as the CS (hypotonic, isotonic, and hypertonic) in order to manipulate the thirst-relieving properties may be another way at manipulating the thirst-relieving nature of the CS fluid, while avoiding the difficulty of infusing sated rats.

Having dispensed with the strong claim of evaluative conditioning, what else can be learnt about conditioned taste aversions from these experiments? In Chapter 5, it was shown that water consumption in a context associated with a now averse taste is lower than in another control context. This effect suggested that context-sucrose associations were established, though the experiments did not reveal how these associations may have produced the lower water consumption during the Extinction Phase. But the result does suggest that E1→E2 associations are not sufficient to produce representation-mediated effects.

The above result is also consistent with previous demonstrations that the palatability of water can be controlled by exteroceptive Pavlovian signals once paired with flavours, such as sucrose or quinine (Delamater, LoLordo, & Berridge, 1986) although the results here do not reveal whether a palatability shift was responsible for the reduced water consumption. Without further experiments, the role of the palatability shift in producing the aversions in this thesis cannot be completely discounted. Nevertheless, the evidence presented in Experiment 6 contrasts with explanations of taste aversions that refer to the role a non-cognitive palatability shift that must be experienced before an aversion is seen, such as Garcia's hypothetical feedback mechanism (Garcia, 1989). It is also inconsistent with the devaluation effect seen in instrumental learning (Balleine & Dickinson, 1991, 1992). According to such views, the hypothesised relation between the CS and lithium toxicosis established during taste aversion learning is formed by an affective processing system resulting in hedonic (or incentive) modification that does not impact on performance until re-exposure to the taste occurs (Balliene & Dickinson, 1991, 1992; Bermudez-Rattoni, et al., 1988; Garcia, 1989). The devaluation effect seen in Experiment 6 should only have occurred in animals re-exposed to the stimulus after conditioning. There was no evidence that re-exposure was having such an effect. The view here, that the associatively-activated representation of sucrose mediated the reduced fluid consumption in the sucrose- associated context accords with evidence for sensory pre-conditioning and the reinforcer revaluation

effect in the Pavlovian case. For example, Best, Davis and Grover (1989) have reported that the effect of reinforcer devaluation on simple approach to food source does not depend upon re-exposure to the reinforcer after aversion conditioning. In addition, Dickinson and Dawson (1987) found that the motivational properties of Pavlovian stimuli in a Pavlovian-instrumental transfer task were immediately affected by a transition from hunger to thirst, even when animals had no prior contact with the reinforcer when thirsty.

This thesis began by emphasising the hedonic shift that is involved in taste aversion learning. It was argued that such a shift might provide reasons to distinguish taste aversion learning from other forms of learning. However, the results of six experiments have indicated that conditioned taste aversions do not exhibit properties unique from other forms of learning. Taste aversions can be extinguished and can result from an associatively-activated representation of an aversive event. In addition, it is unlikely that the aversion is due to experiencing the loss in palatability of the taste. Taken together, the results imply that taste aversions are governed by the signal value acquired by the CS in relation to the US as a result of contingent presentation. In other words, principles developed and described in models such as the Rescorla-Wagner model (Rescorla & Wagner, 1972).

In final conclusion, the major contribution of this thesis has been to establish that conditioned taste aversions do undergo extinction despite the removal of potential sources of counter-conditioning. The

theoretical implications of this are to help place CTAs under the same principles as other forms of learning, despite differences in the arbitrary nature of the stimuli used and the hedonic consequences of such learning. These principles include that knowledge of the predictive relationship between paired stimuli underlies associative learning rather than solely a hedonic shift.

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APPENDIX

Experiment 1 Results.

Table A.1 Planned Orthogonal Contrasts.

	Almond-Thirsty (6+6)		Almond-Sated (6+6)		Almond-Thirsty (12)		Almond-Sated (12)	
	Alm. Pref.	Van.Pref.	Alm. Pref.	Van.Pref.	Alm. Pref.	Van.Pref.	Alm. Pref.	Van.Pref.
A1	1	1	1	1	-1	-1	-1	-1
A2	1	1	-1	-1	1	1	-1	-1
A1A2	1	1	-1	-1	-1	-1	1	1
B1	1	-1	1	-1	1	-1	1	-1
A1B1	1	-1	1	-1	-1	1	-1	1
A2B1	1	-1	-1	1	1	-1	-1	1

A1 contrasts the 6+6 groups against the 12 groups.

A2 contrasts the Thirsty groups against the Sated groups.

A1A2 tests for the interaction between the testing factor (A1) and the training factor (A2).

B1 is the repeat contrast between the Almond preference test and the Vanilla preference test.

A1B1 tests for the interaction between the testing factor (A1) and the flavour test factor (B1).

A2B1 tests for the interaction between the training factor (A2) and the flavour test factor (B1).

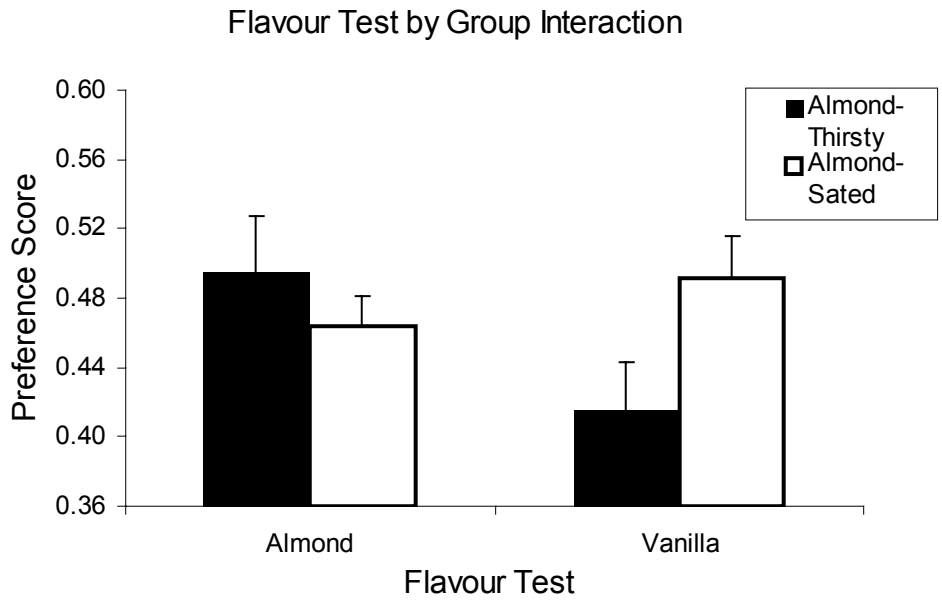
Table A.2 Results of the Planned Orthogonal MANOVA (Hays, 1963).

Summary of Analysis of Variance					
Source	*SS*	*df*	*MS*	*F*	*p*
Between					
A1	0.010	1	0.010	0.843	0.366
A2	0.008	1	0.008	0.675	0.418
A1A2	0.006	1	0.006	0.506	0.483
ERROR	0.332	28	0.012		
Within					
B1	0.011	1	0.011	1.051	0.314
A1B1	0.000	1	0.000	0.000	1.000
A2B1	0.045	1	0.045	4.300	0.047
ERROR	0.293	28	0.010		

Note: Hays F_c (1, 28) was 4.2.

The only significant effect was the interaction A2B1 between the type of training (Almond when thirsty or sated) and the type of flavour tested against water (Almond or Vanilla), shown in Figure A.1 below.

Figure A.1 Experiment 1 Results. Flavour vs Water tests.



Experiment 2 Results.

Table A.3 Group Means from the five test sessions in Experiment 2.

	DEP-1	DEP-0.3	SAT	WATER
ACQ	8.8	8.7	8.5	8.9
1	2.0	1.8	3.0	2.5
2	4.2	3.5	4.2	5.1
3	8.5	7.4	8.3	7.6
SATED	6.4	6.4	6.6	4.1

Table A.4 Planned Orthogonal Contrasts tested.

	DEP-1	DEP-0.3	SAT	WATER
A1	1	1	1	-3
A2	1	1	-2	0
A3	1	-1	0	0
B1	-3	-1	1	3
B2	1	-1	-1	1

A1 contrasts the extinction groups against the Water control.

A2 contrasts the thirsty groups against the Sated group.

A3 compares the Dep-1 group to the Dep-0.3 group.

B1 is the linear trend across test sessions.

B2 is the quadratic trend across test sessions.

Table A.5 Results of the Planned Orthogonal MANOVA (Hays, 1963).

Summary of Analysis of Variance					
Source	*SS*	*df*	*MS*	*F*	*p*
Between					
A1	2.042	1	2.042	0.186	0.669
A2	3.658	1	3.658	0.334	0.568
A3	4.569	1	4.569	0.417	0.524
ERROR	307.042	28	10.966		
Within					
B1	0.196	1	0.196	0.019	0.892
A1B1	0.645	1	0.645	0.062	0.805
A2B1	0.312	1	0.312	0.030	0.863
A3B1	2.096	1	2.096	0.202	0.656
ERROR	289.895	28	10.353		
B2	823.165	1	823.165	251.491	0.000
A1B2	3.488	1	3.488	1.066	0.311
A2B2	2.363	1	2.363	0.722	0.403
A3B2	0.045	1	0.045	0.014	0.907
ERROR	91.648	28	3.273		

Note: Hays F_c (1, 28) was 4.2.

The only significant effect was the quadratic trend across test sessions (B2).

Table A.6 Results of the Planned Orthoganal MANOVA (Hays, 1963) on data from the Sated test.

Summary of Analysis of Variance					
Source	*SS*	*df*	*MS*	*F*	*p*
Between					
A1	34.440	1	34.440	4.899	0.04
A2	0.285	1	0.285	0.041	0.842
A3	0.016	1	0.016	0.002	0.962
ERROR	196.824	28	7.029		

Note: Hays F_c (1, 28) was 4.2.

The only significant effect was between the extinction groups versus the water control (A1).

Experiment 3 Results.

The same planned orthogonal contrasts shown in Table A.4 were used to test the data from the three tests in Experiment 3. The results of the MANOVA are shown below.

Table A.7 Results of the Planned Orthogonal MANOVA (Hays, 1963).

Summary of Analysis of Variance					
Source	*SS*	*df*	*MS*	*F*	*p*
Between					
A1	0.030	1	0.030	0.731	0.400
A2	0.017	1	0.017	0.414	0.525
A3	0.000	1	0.000	0.000	1.000
ERROR	1.149	28	0.041		
Within					
B1	0.534	1	0.534	22.450	0.000
A1B1	0.025	1	0.025	1.051	0.314
A2B1	0.043	1	0.043	1.808	0.190
A3B1	0.031	1	0.031	1.303	0.263
ERROR	0.666	28	0.024		
B2	0.003	1	0.003	0.131	0.720
A1B2	0.009	1	0.009	0.394	0.535
A2B2	0.016	1	0.016	0.700	0.410
A3B2	0.010	1	0.010	0.438	0.514
ERROR	0.640	28	0.023		

Note: Hays F_c (1, 28) was 4.2.

The only significant effect was for a linear trend across test sessions (B1).

Experiment 4 Results.

The data from Experiment 4 was analysed as a 2 x 2 x (2) factorial. The repeated-measures factor was included in order to reveal an effect of extinction on the conditioned taste aversion. The between-group contrasts are shown below.

Table A.8 Planned Orthogonal Contrasts Experiment 4.

	Paired-Thirsty	Paired-Sated	Unpaired-Thirsty	Unpaired-Sated
A1	1	1	-1	-1
A2	1	-1	1	-1
A1A2	1	-1	-1	1
B1	Linear trend contrast			

A1 contrasts the Paired groups with the Unpaired groups.

A2 contrasts the Thirsty groups with the Sated groups.

A1A2 is the interaction between them.

B1 compares the pre-extinction scores with the post-extinction scores.

Table A.9 Results of the Planned Orthogonal MANOVA (Hays, 1963).

Summary of Analysis of Variance					
Source	*SS*	*df*	*MS*	*F*	*p*
Between					
A1	0.438	1	0.438	26.895	0.000
A2	0.033	1	0.033	2.026	0.166
A1A2	0.000	1	0.000	0.000	1.000
ERROR	0.456	28	0.016		
Within					
B1	0.122	1	0.122	14.536	0.001
A1B1	0.257	1	0.257	30.621	0.000
A2B1	0.001	1	0.001	0.119	0.733
A1A2B1	0.021	1	0.021	2.502	0.125
ERROR	0.235	28	0.008		

Note: Hays F_c (1, 28) was 4.2.

Significant differences occurred between Paired and Unpaired groups (A1), between pre-extinction scores and post-extinction scores (B1) and the interaction between these two factors (A1B1). The important contrasts for the purposes of the experiment were the A2 contrasts that were designed to reveal any differences between Thirsty and Sated groups.

Figure A.3

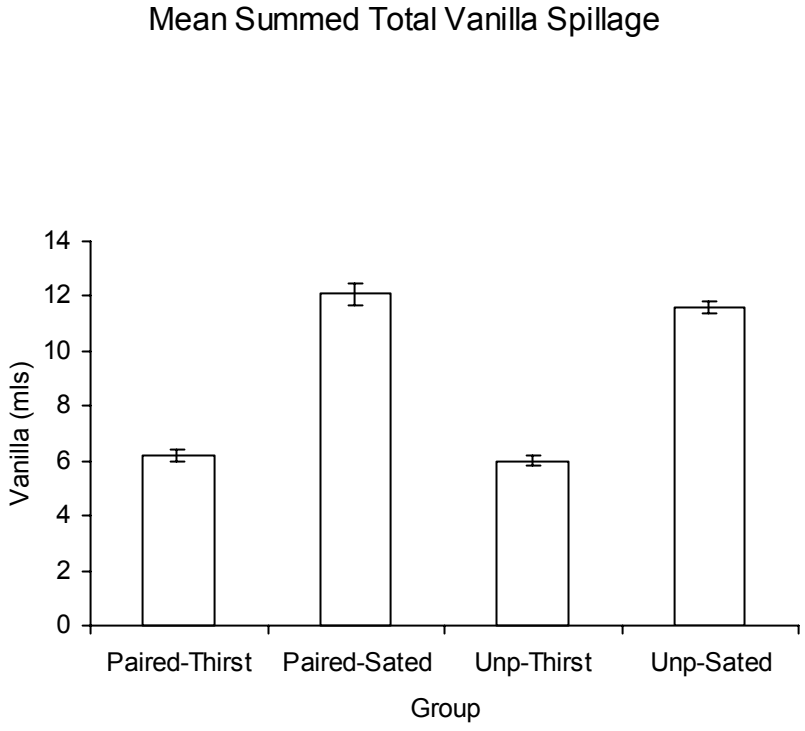


Figure A.3 shows the mean amount of vanilla spilt during extinction when infusing into the rat's mouth. Amount spilt for each rat on each day was summed across the nine days and the mean for each group was calculated. Vertical bars represent SEM.

Experiment 5 Results.

Test 1 and Test 2 in Experiment 5 was a 2 x 2 x 2 factorial. The first factor was the Extinction factor (X or C) representing whether the Extinction phase occurred in the E-sucrose context or the E-water context; the second factor was the Water factor (W or E) representing whether water was presented during the Extinction Phase or not; the third factor was the Training factor (S or L) representing whether initial Stage 1 Context Discrimination Training occurred for four days or eight. The following planned and orthogonal contrasts were tested for differences between groups.

Table A.10 Planned Orthogonal Contrasts used at Test 1 and Test 2.

	XWS	XWL	XES	XEL	CWS	CWL	CES	CEL
A1	1	1	-1	-1	1	1	-1	-1
A2	1	1	1	1	-1	-1	-1	-1
A3	1	-1	1	-1	1	-1	1	-1
A1A2	1	1	-1	-1	-1	-1	1	1
A1A3	1	-1	-1	1	1	-1	-1	1
A2A3	1	-1	1	-1	-1	1	-1	1
A1A2A3	1	-1	-1	1	-1	1	1	-1

A1 is the Water factor.

A2 is the Extinction factor.

A3 is the Training factor.

The other contrasts are the interactions between the above three factors.

Test 1 occurred immediately after conditioning. The results were used to reveal chance differences between groups that may have existed prior to the important Extinction Phase. These results are shown below.

Table 17. Results from the Planned Orthogonal MANOVA at Test 1 (Hays, 1963).

Summary of Analysis of Variance					
Source	*SS*	*df*	*MS*	*F*	*p*
Between					
A1	3.445	1	3.445	1.265	0.272
A2	2.703	1	2.703	0.993	0.329
A3	63.000	1	63.000	23.134	0.000
A1A2	11.163	1	11.163	4.099	0.054
A1A3	0.138	1	0.138	0.051	0.824
A2A3	6.938	1	6.938	2.548	0.124
A1A2A3	0.633	1	0.633	0.232	0.634
ERROR	65.358	24	2.723		

Note: Hays F_c (1, 28) was 4.2.

The only significant difference was between the short and the long trained group. This is most likely due to the fact that the long-trained group received more sucrose pre-exposures than the short-trained group prior to conditioning. Worth noting is that the Extinction-Water factor interaction approached the criterion.

Table A.11 below shows how these differences changed after extinction at Test 2.

Table A.11 Results from the Planned Orthogonal MANOVA at Test 2 (Hays, 1963).

Summary of Analysis of Variance					
Source	*SS*	*df*	*MS*	*F*	*p*
Between					
A1	18.3	1	18.3	2.138	0.157
A2	2.9	1	2.9	0.336	0.567
A3	66.1	1	66.1	7.724	0.010
A1A2	39.6	1	39.6	4.626	0.042
A1A3	3.4	1	3.4	0.395	0.536
A2A3	4.7	1	4.7	0.543	0.468
A1A2A3	6.7	1	6.7	0.778	0.386
ERROR	205.455	24	8.560625		

Note: Hays F_c (1, 28) was 4.2.

Significant differences occurred between the short and the long trained groups (A3), as in Test 1, and the Extinction-Water factor interaction (A1A2) now exceeded the criterion level for significance.

Experiment 5 also examined whether water consumption during the Extinction phase was different between groups. The following contrasts were used to reveal these differences.

Table A.12 Planned Orthogonal Contrasts used on the Extinction Phase data.

	XWS	CWS	XWL	CWL
A1	1	1	-1	-1
A2	1	-1	1	-1
A1A2	1	-1	-1	1
B1	linear			
B2	quadratic			

A1 contrasts the main effect of the Training factor, whether the Context-discrimination phase lasted four days or eight.

A2 contrasts the main effect of the Extinction factor, whether Extinction Phase occurred in the E-sucrose context or E-water.

A1A2 is the interaction between them.

B1 is the linear trend across the 6 days of extinction

B2 is the quadratic trend across the 6 days of extinction.

Table A.13 The results of the Planned Orthogonal MANOVA (Hays, 1963) from the Extinction Phase data.

Summary of Analysis of Variance					
Source	*SS*	*df*	*MS*	*F*	*p*
Between					
A1	14.18	1	14.18	1.22	0.29
A2	62.57	1	62.57	5.36	0.04
A1A2	0.05	1	0.05	0.00	0.95
ERROR	140.04	12	11.67		
Within					
B1	15.159	1	15.159	9.127	0.011
A1B1	0.007	1	0.007	0.004	0.949
A2B1	3.761	1	3.761	2.265	0.158
A1A2B1	0.000	1	0.000	0.000	1.000
ERROR	19.930	12	1.661		
B2	18.480	1	18.480	12.023	0.005
A1B2	4.667	1	4.667	3.036	0.107
A2B2	2.106	1	2.106	1.370	0.264
A1A2B2	0.724	1	0.724	0.471	0.506
ERROR	18.444	12	1.537		

Note: Hays F_c (1, 28) was 4.2.

The main effect contrast for the Extinction factor (A2) was significant, along with linear (B1) and quadratic trends (B2) across this phase.

The data from the stage immediately prior to the Extinction Phase was then included in a planned orthogonal analysis in order to determine whether the significant main effect obtained above was specific to the Extinction contexts. In order to do this, water

consumption from the day prior to the Extinction Phase in the E-test context was included with water consumption from the first two days of the Extinction Phase which occurred in either E-sucrose or E-water. The same between-group contrasts were used as in Table A.12, and the linear and quadratic trends were adjusted to three days. The results are shown below.

Table A.14 Results from the change in water consumption between E-test and the Extinction contexts.

Summary of Analysis of Variance					
Source	*SS*	*df*	*MS*	*F*	*p*
Between					
A1	4.441	1	4.441	0.380	0.55
A2	8.501	1	8.501	0.727	0.41
A1A2	0.963	1	0.963	0.082	0.78
ERROR	140.338	12	11.695		
Within					
B1	0.845	1	0.845	0.210	0.66
A1B1	24.500	1	24.500	6.075	0.03
A2B1	4.205	1	4.205	1.043	0.33
A1A2B1	3.645	1	3.645	0.904	0.36
ERROR	48.395	12	4.033		
B2	0.060	1	0.060	0.029	0.87
A1B2	3.682	1	3.682	1.808	0.20
A2B2	0.027	1	0.027	0.013	0.91
A1A2B2	0.327	1	0.327	0.161	0.70
ERROR	24.442	12	2.037		

Note: Hays F_c (1, 28) was 4.2.

Again, there was a significant main effect for the Extinction factor (A2), and this time it significantly interacted with the linear (B1) and quadratic trends (B2). This reveals that the differences in water consumption between groups were changing between the E-test context and the two Extinction contexts.

Experiment 6 Results.

The final test in Experiment 6 used the same planned orthogonal contrasts shown in Table A.10. The only necessary change is that A3 now contrasts for the main effect of the re-exposure factor. The results are shown below.

Table A.15 Results of the final test in Experiment 6.

Summary of Analysis of Variance					
Source	*SS*	*df*	*MS*	*F*	*p*
Between					
A1	17.6	1	17.6	1.237	0.274
A2	14.8	1	14.8	1.041	0.315
A3	304.2	1	304.2	21.4	0.000
A4	3.7	1	3.7	0.258	0.615
A5	8.7	1	8.7	0.616	0.438
A6	0.6	1	0.6	0.039	0.845
ERROR	454.0	32	14.2		

The only significant result is the main effect of re-exposure. It clearly accounts for most of the between-group variance.

Experiment 6 also found the same reduced water intake in the E-sucrose context relative to the E-water context as Experiment 5. The results pertaining to this effect are presented here.

Data from the Extinction Phase was analysed with the same planned orthogonal contrasts as shown in Table A.12. The only changes necessary to keep in mind are that A1 now contrasts the main effect of the re-exposure factor. A2 is still the main effect of the Extinction factor. The results from the six days of the Extinction phase are shown below.

Table A.16 Results of the Planned Orthogonal MANOVA (Hays, 1963) from the Extinction Phase data.

Summary of Analysis of Variance					
Source	*SS*	*df*	*MS*	*F*	*p*
Between					
A1	0.1	1	0.085	0.005	0.945
A2	177.6	1	177.63	10.285	0.005
A1A2	12.7	1	12.675	0.734	0.404
ERROR	276.3	16	17.271		
Within					
B1	15.6	1	15.646	2.714	0.119
A1B1	2.3	1	2.288	0.397	0.538
A2B1	3.4	1	3.440	0.597	0.451
A1A2B1	10.2	1	10.217	1.772	0.202
ERROR	92.2	16	5.765		
B2	1.6	1	1.560	0.341	0.567
A1B2	0.9	1	0.869	0.190	0.669
A2B2	0.8	1	0.754	0.165	0.690
A1A2B2	0.2	1	0.180	0.039	0.845
ERROR	73.2	16	4.576		

Note: Hays F_c (1, 28) was 4.2.

There was a main effect of the Extinction factor (A1). This did not interact with the re-exposure factor (A1A2).

As in Experiment 5, this effect was analysed to reveal whether it was specific to the Extinction contexts. In order to do this, water consumption data from the E-test context was included in the analysis, along with the first two days of data from the Extinction phase. The same between-group contrasts were used as in Table 19, and the linear and quadratic trends were adjusted to three days. The results are shown below.

Table A.17 Results from the change in water consumption between E-test and the Extinction contexts in Experiment 6.

Summary of Analysis of Variance					
Source	*SS*	*df*	*MS*	*F*	*p*
Between					
A1	5.163	1	5.163	0.607	0.447
A2	67.841	1	67.841	7.970	0.012
A1A2	22.571	1	22.571	2.652	0.123
ERROR	136.187	16	8.512		
Within					
B1	39.800	1	39.800	11.312	0.004
A1B1	1.722	1	1.722	0.489	0.494
A2B1	7.310	1	7.310	2.078	0.169
A1A2B1	0.600	1	0.600	0.171	0.685
ERROR	56.292	16	3.518		
B2	4.370	1	4.370	0.421	0.526
A1B2	0.102	1	0.102	0.010	0.922
A2B2	0.030	1	0.030	0.003	0.958
A1A2B2	0.154	1	0.154	0.015	0.905
ERROR	166.245	16	10.390		

Note: Hays F_c (1, 28) was 4.2.

There was a significant main effect of Extinction factor across the three days (A2), but this did not interact with linear (A2B1) or quadratic trends (A2B2).