# Conditioned Side Effects of Cancer Chemotherapy

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# Abstract

The evidence for the occurrence of two side effects of cancer chemotherapy, taste aversions and anticipatory nausea and vomiting (ANV) was reviewed. It was suggested that both taste aversions and ANV occur as a result of classical conditioning. Thus, investigation of possible interventions for the control of these side effects using a rat model was proposed.

First, two pharmacological interventions were tested for their efficacy in the attenuation of CTA. The compounds tested were $5\text{-}HT_3$ and $NK_1$ receptor antagonists both of which have been found to have antiemetic properties. Two $5\text{-}HT_3$ receptor antagonists, ondansetron and granisetron, failed to attenuate cisplatin-induced CTA in rats. Two $NK_1$ antagonists, CP-99,994 and L-742,694, were tested against cisplatin in the formation of CTA. High dose L-742,694 partially blocked CTA, probably through blocking the activity of substance P in the nucleus of the solitary tract or the parabrachial nucleus.

A previously tested psychological intervention for the control of CTA in cancer patients is the presentation of a novel "scapegoat" flavour before drug infusion, which is thought to overshadow aversions towards normal dietary items. Using a rat model, it was found that the scapegoat flavour, in addition to overshadowing CTA to previously consumed flavours, may potentiate an aversion to the context in which it was presented. However, further experiments suggested that potentiation may not occur if the flavour presented in the context is varied across each trial. Therefore, presentation of different scapegoat flavours on each visit to the clinic may lead to a reduction in CTA with no accompanying increase in the context aversion (ANV).

It was concluded that $NK_1$, but not $5\text{-}HT_3$, receptor antagonists may lead to an attenuation of CTA, and possibly ANV, in cancer chemotherapy patients. Further interventions for the control of ANV and CTA based on learning theoretic principles were suggested.

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# Chapter One

# Taste Aversions and Anticipatory Nausea and Vomiting in Cancer Chemotherapy Patients

## 1.1: Introduction

Cancer patients undergoing chemotherapy suffer from a number of treatment-related side effects, most notably acute nausea and vomiting. In addition to direct effects of cytotoxic drug infusion, patients also develop side effects which are thought to have a psychological etiology. The most common psychological side effects of cancer chemotherapy are anticipatory nausea and vomiting (ANV) and taste aversions.

ANV is the term used to describe the nausea and vomiting which is experienced by a large proportion of chemotherapy patients before administration of the treatment. Patients often experience ANV on entering the clinic, or while merely thinking about treatment. It is possible that ANV is a result of heightened anxiety due to the aversiveness of previous treatments; entering the clinic or thinking about chemotherapy may induce sufficient anxiety to provoke nausea and vomiting (Andrykowski, 1990). Alternatively, ANV may result from classical conditioning. According to this hypothesis, previously neutral cues, such as sights and smells which are present in the clinic, become associated with gastrointestinal malaise. Thus, on entering the clinic on subsequent occasions, the cues present in the clinic will re-elicit a representation of illness and, therefore, nausea and vomiting may occur in the clinic before treatment is undertaken.

Some of the taste aversions experienced as a result of therapy are also thought to be a product of a classical conditioning process similar to that which occurs in nonhuman animals. There is an extensive animal literature on the effects of pairing previously neutral flavours with illness. It has been found that animals which are subject to such a procedure will develop a conditioned taste aversion (CTA) towards that flavour, as demonstrated by a reluctance to consume the flavour on test (Garcia, Kimeldorf & Koelling, 1955).

An alternative hypothesis is that the reduction in consumption of foods by patients is a direct, result of the ongoing experience of nausea resulting either from chemotherapy, or from the tumour

itself. This mechanism can be distinguished from that of CTA. In the case of CTA, the flavour will lead to nausea through a previously learned association between the flavour and nausea, and, therefore, be perceived as unpleasant. In the case of the rejection of foods as a result of the experience of nausea, a general loss of appetite may result. Alternatively, specific foods may be perceived as unpleasant as a result of the effect of that food stuff on the gut; although this itself may lead to the learning of an association between the flavour and nausea, on the first exposure to the flavour, no such association is present and yet the food is perceived as unpleasant.

The present chapter will present a survey of the findings with respect to ANV and CTA in cancer chemotherapy patients. The evidence for an associative account of both of these phenomena will be presented, and potential psychological and pharmacological interventions will be discussed.

## 1.2: Conditioned Taste Aversion (CTA)

### 1.2.1: The prevalence of CTA

Two approaches which have been taken in the investigation of the possible occurrence of CTA in cancer-chemotherapy patients. The first is to take an inventory of the meals eaten at or around the time of treatment and compare changes in preference for these foods with any changes in preference for foods eaten at other times. The second is to present the patients with some distinctive food item or meal before treatment and measure their willingness to consume this food at a later date. Studies which use the first methodology have assessed the prevalence of CTA in the chemotherapy patient population. Results of studies of the second kind relate to the cause of CTAs and will be discussed in section 1.2.2.

Three experiments investigating the prevalence of CTA in chemotherapy patients have used a longitudinal design (Jacobsen, Bovbjerg, Schwartz, Andrykowski, Futterman, Gilewski, Norton, & Redd, 1993; Mattes, Arnold & Boraas, 1987b; Mattes, Curran, Alavi, Powlis & Whittington, 1992), rather than a retrospective questionnaire design which might be thought to be less accurate due to failures in the recall of the relevant events. In these experiments, food consumption questionnaires were administered during the 24 hours before treatment and some time after the 24 hours following treatment. In addition, a food preference questionnaire was presented on each visit to the clinic in which the previously consumed items were rated a second time. The food preference ratings were then compared with those made at the treatment session before which the

food was consumed, and a reduction in preference of a pre-specified magnitude was taken to indicate the development of a CTA. Both Jacobsen et al (1993) and Mattes et al (1987b; 1992) used a nine point rating scale to measure food preferences both before the foods were paired with treatment and on test, at the following visit to the clinic.

Although they used different criteria for the development of a CTA (a five point reduction in preference in the case of Mattes et al (1987b; 1992) and a four point reduction in the case of Jacobsen et al), these studies showed surprisingly good agreement as to the prevalence of CTA. Mattes et al (1987b, 1992) found that 55 and 56% of the patients they surveyed developed at least one CTA, while Jacobsen et al (1993) found a 46% prevalence rate. Among the patients who developed a CTA, the mean number of foods towards which an aversion developed ranged from 3.0 to 3.3 across these studies. Jacobsen et al also found that the majority of aversions developed across the first two treatment sessions (approximately 75%). In none of these studies was the drug regime examined in detail, and a range of cancer types were represented in the patient samples.

As well as agreement on the number of CTAs which developed in these samples, the foods which became the target of CTAs were very similar across the samples. The most common targets of a CTA were sweet foods (especially chocolate), meats and caffeinated beverages. In a survey of CTA in normal human subjects, Midkiff and Bernstein (1985) found that over 30% of aversions developed towards protein-rich foods such as meat and eggs. This study used a questionnaire in which subjects were asked to remember occasions on which the consumption of a specific food had been followed by illness resulting in subsequent avoidance of that food. There are obvious methodological limitations of retrospective questionnaire studies such as that carried out by Midkiff and Bernstein. However, the implication that protein-rich foods are more easily associated with illness is further supported by both human questionnaire studies elsewhere (Logue unpublished data) and animal studies (Bernstein, Goehler & Fenner, 1984). Bernstein et al (1984) found that rats on a dietary self-selection regime developed aversions towards protein-rich foods significantly more often than towards carbohydrates. More recently, it has been established that the flavour, rather than any post-ingestional consequences of consumption, of protein-rich foods is the factor responsible for their increased associability with illness (Brot, Braget & Bernstein, 1987).

Although meats are highly represented in both the studies carried out on CTA in cancer patients and normal human subjects, cancer patients develop a large proportion of their aversions towards chocolate and caffeinated beverages, while no such tendency is apparent in normal subjects. The absence of aversions towards chocolate and caffeinated beverages in normal subjects may be due to extensive preexposure to these stimuli prior to pairing with illness. Preexposure to a conditioned stimulus (CS) before pairing with an unconditioned stimulus (US) commonly leads to an attenuation in the strength of the conditioned response (CR) towards the CS on test. This effect has been termed latent inhibition (Lubow, 1973). It is, therefore, interesting that cancer chemotherapy patients have been found to develop aversions towards these foods and raises the possibility that these aversions do not have an associative basis. This possibility will be investigated further in section (1.2.2).

The final consistent finding related to the prevalence of CTA in cancer chemotherapy patients is that the aversions which develop have a very short duration. Mattes et al (1987b) found that the mean duration of CTAs developed after the first treatment session was less than the period between the first and second treatment. Jacobsen et al (1993) collected data on the duration of the CTAs developed after the first, second and third treatment session. Each CTA was examined across the four treatment sessions following that on which the aversion first occurred. It was found that only 35% of those which occurred after the first infusion were still present four infusions later. Eighty and 100%, respectively, of CTAs following the second and third infusion were still present four infusions later.

It would seem that a large number of the CTAs which develop in cancer chemotherapy patients are likely to be very short lived. In combination with the finding that the number of CTAs which develop is unrelated to treatment outcome or weight loss (Mattes et al, 1987), it may seem that further study of the occurrence of CTA as a result of cancer chemotherapy is not warranted. However, there are four reasons for further investigation of the development of CTAs in cancer chemotherapy patients. First, there is the unexplained development of aversions towards highly familiar foods which invites a non-associative explanation and is therefore interesting in its own right. Second, there are quality of life implications for the affected patients. Third, there may be a close relationship between the formation of CTA and that of ANV in cancer patients, and a full understanding of this relationship may lead to the development of a psychological intervention for both. Finally, evidence will be presented in section 1.2.2 which suggests that the tests used by Mattes et al (1987; 1992) and Jacobsen et al (1993) may have underestimated the prevalence of

CTA as a result of cancer chemotherapy.

## 1.2.2 The cause of CTA

There are two possible reasons why cancer patients might find a number of foods aversive as a result of their chemotherapy treatment. The first is that a learned aversion develops as a result of the pairing of the food consumed before treatment with the illness experienced during and following treatment. Thus, through a classically conditioned association, the flavour will re-elicit illness at a later date, and will therefore be rejected. This effect has been demonstrated in rats using a wide variety of toxins (see Riley & Tuck, 1985 for bibliography).

However, there is also an account which does not rely on an associative mechanism. Since the nausea experienced by cancer patients may last for a number of days, a range of foods may be aversive due to their direct post-ingestional effects; many of the most problematic items are potent stimulators of gastric acid secretion (American Dietetic Association, 1981). Jones, Hill, Soukop, Hutcheon, Cassidy, Kaye, Sikora, Carney & Cunningham (1991) found that, even when receiving the antiemetic ondansetron, 37% of the patients that they tested, who were receiving cisplatin therapy, reported nausea eight days following treatment. The protracted nature of the emetic effects of this drug allow that, across the period during which patients are receiving therapy, some effect of this drug is always present. Even when there is no reportable experience of nausea, it is possible that the patients have a lower threshold for experiencing nausea as a result of gastric stimulation. If patients are sensitized in this way, foods which normally stimulate the gut to some extent may now become aversive and therefore be rejected.

Since the only food items which were surveyed in the experiments in section 2.1 (Jacobsen et al, 1993; Mattes et al 1987b; Mattes et al 1992) were those consumed directly prior to, or directly following, chemotherapy it is not possible to determine whether their contiguity with illness was necessary for the development of an aversion and, therefore, whether a non-associative account is sufficient to explain the development of aversion towards specific flavours in the patients' diets. Information about foods eaten at some time other than the period around treatment would be required to determine whether the aversions recorded were the result of conditioning. The other foods would then constitute an unpaired control condition.

Another procedure which has been developed to investigate chemotherapy-induced taste aversions is the presentation of a specific foodstuff before therapy, which is then offered to the patient sometime after therapy (Bernstein, 1978; Bernstein & Webster, 1980). If a choice of the paired food and some other similar food is presented on test, and the patient is found to prefer the non-paired flavour over the paired flavour, it is clear that the non-associative account of this aversion outlined above would be insufficient to explain the results.

Bernstein (1978) presented paediatric cancer patients with a novel flavoured ice cream (maple and black walnut flavour: "Mapletoff") before emetic chemotherapy (session 1). Two control groups were used. In one of these, children due to receive non-emetic treatment were presented with the ice cream before drug infusion (Vincristine), while in the other, children due to receive emetic chemotherapy were given a toy with which to play. On test (session 2), two to four weeks later, all groups were given a choice of either Mapletoff ice cream to eat or a game to play. The results of this experiment suggested that the experimental group was significantly less likely to choose the ice cream over the game than the two control groups combined. It must be assumed, since the control groups were combined for analysis, that neither control group alone was more likely to choose the ice cream over the game than the experimental group.

A second test was carried out on the same subjects at a mean time of 4.5 months following session 1. On this occasion, a choice was presented to the same patients, this time of Mapletoff ice cream and a second novel flavoured ice cream Hawaiian Delight. There was no difference between the groups in terms of total ice cream consumption, but the control groups combined ate significantly more Mapletoff ice cream than did the experimental group. Although, again, combination of the data from the control groups was required to show a statistically reliable treatment effect, these data support the hypothesis that conditioned taste aversions may occur as a result of cancer chemotherapy.

A weakness of the data collected from the first test (Session 2) in the Bernstein (1978) study is that the subjects receiving nausea-inducing chemotherapy, who had been given either a game to play or ice cream to eat during conditioning, were given a choice between playing the game and consuming ice cream on test. Thus, a preference for playing the game in the group which received ice cream during conditioning, as compared to the group which received the game during conditioning, might either be the result of a decrease in palatability of the ice cream in the former group, or a decrease in the preference for the game in the latter group. However, it has been

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suggested that, in toxicosis conditioning, flavour cues become associated with toxin reinforcers more readily than do cues from other sensory modalities (Domjan & Wilson, 1972). On the basis of these data, it would seem more likely that the flavour, rather than the game, acquired aversive properties as a result of its pairing with chemotherapy in the Bernstein (1978) study.

A second, and possibly more damaging weakness of this study is that no correction was made for the number of patients who experienced nausea following treatment on session 1; post treatment nausea was higher in the experimental group (78% of patients) than in the poisoned control group (67% of patients). Therefore, it is possible that the reduction in consumption of ice cream in the experimental group was the result of a general suppression in appetite leading to a preference for playing the game on test. However, these two objections are addressed by the second test in which a choice between two different flavoured ice creams were presented. Although, in both tests, combination of the data from the control groups was required to show a statistically reliable treatment effect, these data support the hypothesis that conditioned taste aversions may occur as a result of cancer chemotherapy.

This result of Bernstein (1978) was replicated using adult cancer chemotherapy patients (Bernstein & Webster, 1980). In this experiment, an experimental group, which received either Maple Nut or Hawaiian Delight ice cream (counterbalanced) before emetic chemotherapy, and a control group which also consumed one of these ice creams before either non-emetic treatment, or no treatment at all (session 1) were compared. On test (session 2), all patients were presented with both ice creams and asked to taste both flavours and give a preference rating for each flavour. They were then asked to consume as much of each flavour as they wished. It was found that the flavour which had been presented on session 1 had no effect on preference scores in session 2 in the control group; Hawaiian Delight was preferred regardless of which ice cream had been consumed in session 1. In the experimental groups, however, those patients who had eaten Hawaiian Delight in session 1 were significantly more likely to reject this flavour on test, in preference for Maple Nut, than those who had eaten Maple Nut in session 1, and vice versa.

Taken together, the data collected in the questionnaire studies, and those in which a specific target food was presented to the patients before therapy, suggest that a range of foods show a decline in palatability as a result of chemotherapy and that it is possible that this decline is the result of classical conditioning. However, although classical conditioning can be demonstrated in the chemotherapy clinic (Bernstein, 1978; Bernstein & Webster, 1980) the data that are currently

available do not indicate unequivocally that such conditioning is responsible for the food aversions that actually develop in the clinic (e.g. Jacobsen et al, 1993).

If there is a non-associative basis for the taste aversions which are experienced by cancer chemotherapy patients, then this would readily explain the fact that a large number of the aversions which have been recorded are towards highly familiar foods such as chocolate and caffeinated beverages. Alternatively, the aversions may be due to conditioning, and the ease with which the familiar foods become associated may be due to a context change between preexposure and conditioning. Latent inhibition of CTA in rats is context specific (Hall and Channell, 1986). One possible source of such a context change might be the enduring experience of nausea brought about either by the chemotherapy or by the cancer itself.

In section 1.2.3, a possible intervention for the alleviation of taste aversions is discussed which is based on learning theoretic principles. The success of this intervention would support the conclusion that a substantial proportion of taste aversions which develop in cancer chemotherapy patients result from classical conditioning.

## 1.2.3: An intervention for CTA

In a study carried out by Bernstein, Webster and Bernstein (1982), three groups of paediatric chemotherapy patients completed a pre-therapy dietary inventory covering the five hours before arrival at the clinic. The experimental group received Mapletoff ice cream prior to drug infusion, while control group 1 did not. Control group 2 did not receive emetic chemotherapy or ice cream. It was predicted that, in accordance with the findings discussed in 2.1, a number of the food items consumed before therapy would be rated as less palatable on a subsequent visit to the clinic. It was further predicted that the patients who received the ice cream before drug infusion would be subject to fewer such aversions. The rationale for the second prediction was that, in animal flavour aversion learning, if a novel and salient flavour is presented in addition to the target flavour before the animal experiences illness, the aversion conditioned towards the target flavour is attenuated. This effect is known as overshadowing (Mackintosh, 1976). It was, therefore, expected that the Mapletoff ice cream would overshadow the aversions which developed towards the food items consumed in the five hours before therapy.

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Both of the predictions were borne out. On test, the control group receiving chemotherapy tended to show a reduction in preference for more foods than both the group which did not receive chemotherapy, and the experimental group in which the overshadowing stimulus (the ice cream) was presented before drug infusion. In fact, there was no difference between the overshadowing group and the unpoisoned controls in the number of aversions which developed towards the previously consumed foods. The implication from this study is that the presentation of a novel and salient food item prior to drug infusion may serve to alleviate the problem of taste aversions in cancer chemotherapy patients.

These results (Bernstein et al, 1982) also suggest strongly that the taste aversions which develop towards the food items consumed before therapy are the consequence of a conditioning process similar to that observed in animals. If the reduction in palatability of these food items were a result of their direct effects on the digestive system, in combination with a lowered threshold for nausea induced by the presence of toxins in the blood, then it would be difficult to explain why the overshadowing stimulus had a palliative effect.

However, it is possible that what was observed in this experiment was a recall effect rather than an attenuation in the number of aversions presumed to result from a classical conditioning process. In order to rate the foods on test, some mental re-elicitation of the flavours of these foods would be necessary; the foods were not presented to the patients on test for consumption, and they may not have sampled these foods during the period between 'conditioning' and test. Balleine and Dickinson (1991) presented animal data which suggest that the sampling of a flavour which has been paired with illness is necessary, in order for the animal to 'know' that food is unpalatable. This process is known as incentive learning. If this were the case, then, in the absence of first hand experience of the flavour, the patients would have to imagine what that flavour would taste like were it now to be presented.

Patients who received the overshadowing stimulus during training are likely to have made the assumption that the presentation of the overshadowing stimulus was the important aspect of the experiment and failed to re-elicit a strong enough representation of the target food items for a true measure of their palatability to be made. This may have led these patients to rate the foods on the basis of well-established pre-therapy preferences. By contrast, the group which did not receive the ice cream may have been aware that the important aspect of the experiment was the palatability of the target foods, which might have led them to process this relevant information to

a greater extent and. therefore, rate the paired foods as aversive. Although it is not immediately clear that such an analysis provides an accurate account of the pattern of data presented by Bernstein et al (1982), which was said to suggest that patients in the experimental group were protected from developing aversions as a result of the presentation of a novel flavour before therapy, there would certainly seem to be potential problems related to the use of retrospective palatability questionnaires in the absence of re-exposure to the target flavours.

A further experiment, by Broberg and Bernstein (1987), used a within-subjects procedure. The measure of taste aversions towards targets in this case was the presentation of the target food items for consumption on test. All patients received two 'conditioning' trials; on one trial, a packet of novel flavoured sweets were consumed following the last meal before treatment, while on another occasion no sweets were consumed (the order was counter balanced). On test, the target food items, the items eaten in the meal before treatment, were significantly more likely to be consumed if they had been followed by the overshadowing stimulus during training. Interestingly, there was little correlation between the results of a preference rating questionnaire which was given before the consumption test was carried out, and the pattern of responding when the food items were actually presented for consumption. This suggests that, consistent with the hypothesis above, preference rating questionnaires have limited validity in the measurement of the palatability of drug-paired foods and, more specifically, may underestimate the level of CTA occurring in this patient population.

Thus, there is some evidence that conditioning of taste aversions occurs towards normal dietary items as a result of cancer chemotherapy, and that the use of an overshadowing stimulus may serve to alleviate this problem. It is not clear, however, to what extent conditioning is responsible for the aversions which have been documented in the questionnaire studies. If the target food is not presented on test, then it is possible that some of the ratings reflect the sampling of certain memories of the palatability of these flavours, and not the preference for those flavours when the test is carried out.

In combination with a classical conditioning account of taste aversions, an account in terms of memory retrieval of some of the taste aversions measured by questionnaire studies might help to explain some of the data presented in the study carried out by Jacobsen et al (1993). They found that the majority of taste aversions occurred after the first and second treatment session (75%), and that these aversions had a shorter duration than those which developed at the third treatment

session. Since many of the aversions were towards chocolate and caffeinated beverages which are both highly latently inhibited, and stimulate gastric secretion, it is possible that these aversions did not result from conditioning, but from their ability to elicit nausea directly, due to a toxin-induced lowered threshold for nausea. If this were the case, then most of these aversions would be expected to occur after the first treatment sessions, provided that the patients consume these foods on a regular basis. However, the duration of such gut-sensitivity to these foods may be shorter than the period between treatment sessions, allowing a window in which, if they are consumed, they will be found to be palatable. It might be expected that, over a number of treatment sessions, the patients will become aware of the correlation between treatment and the aversiveness of chocolate and caffeinated drinks, and therefore rate their preference on test as reflecting their 'normal' reaction to these foods, which is to find them palatable. Since the awareness of this contingency would require a number of samples of the foods, and the aversions do not require close contiguity of the flavour and treatment, one would expect many such aversions to occur at early treatment sessions and to decline across subsequent sessions. This is what was found in the study carried out by Jacobsen et al (1993). Thus, it may be the case that the taste aversions found by Mattes et al (1987; 1992) and Jacobsen et al (1993) have a combination of etiologies, some associative, some non-associative.

## 1.3: Anticipatory Nausea and Vomiting (ANV)

### 1.3.1: The prevalence of ANV

A large number of studies of ANV have estimated the prevalence of anticipatory nausea and vomiting (ANV) in cancer chemotherapy patients. They suggest that between 25 and 50% of all cancer chemotherapy patients develop ANV (e.g. Burish & Carey, 1986; Carey & Burish, 1988; Morrow & Dobkin, 1988), and that a number of factors are correlated with ANV: extent of post chemotherapy nausea and vomiting (e.g. Andrykowski, Jacobsen, Marks, Gorfinkle, Hakes, Kaufman, Currie, Holland & Redd, 1988), state anxiety (e.g. Andrykowski, 1990), susceptibility to motion sickness (Morrow, Lindke & Black, 1991) and the experience of flavours in the mouth as a direct result of drug infusion (Nerenz, Leventhal, Easterling & Love 1986). Age has been found to correlate negatively with development of ANV (Morrow et al, 1991).

All of these correlations have been found using longitudinal questionnaire designs (e.g. Andrykowski, Redd and Hatfield, 1985). The procedure involves the completion of a

questionnaire before each of a number of therapy sessions, beginning with the first session, in which the levels of nausea and vomiting are recorded. In addition, if a variable factor is being investigated such as state anxiety, this is also measured. In studies in which post-therapy nausea and vomiting were investigated, a second nausea and vomiting questionnaire is presented following therapy.

The results of a study by Andrykowski and Redd (1987) indicated that, like taste aversion, the development of ANV largely occurred across the first few treatment sessions. They found a rapid accumulation in the number of patients reporting ANV across the first four treatment sessions, followed by a sudden drop in the number of new occurrences at the fifth infusion. Andrykowski and Redd separated their patients into two groups on the basis of early and late onset of ANV. The first group included those cases of ANV which occurred before the fifth infusion, while the second group included those cases which occurred at or after the fifth infusion. Comparison of the data from these two groups suggested that their ANV had different etiologies. Early onset of ANV was associated with stable levels of anxiety across all infusions, while the late onset group showed an increase in anxiety across infusions. More specifically, the late onset group suffered a significant increase in both post-treatment nausea and anxiety at infusion minus one (the infusion before that at which the first ANV was recorded). Furthermore, Andrykowski and Redd found that, while the two groups showed equivalent post therapy nausea and vomiting, the initial expectations of treatment side effects were lower in the late onset group. Thus, it is possible that these violated expectations gave rise to an increase in arousal (Mandler, 1991), and, therefore, anxiety. Since anxiety has been found to be correlated with ANV, this might explain the occurrence of ANV late on in the treatment. The possible reasons for the relationship between anxiety and ANV will be discussed in section 1.3.2.

## 1.3.2: The cause of ANV

There are two possible explanations for the occurrence of ANV in cancer chemotherapy patients. Either the stimuli present during therapy (the sights and sounds of the clinic) become associated with illness through classical conditioning and thus become able subsequently to elicit nausea, or alternatively, the anxiety experienced by these patients in anticipation of treatment induces nausea directly. It is not possible to choose between these two hypotheses on the basis of the data that are currently available. The factors associated with a high prevalence of ANV are consistent with both hypotheses (e.g. high post-therapy nausea and vomiting, high state anxiety and susceptibility

to motion sickness).

If post-therapy nausea and vomiting were high, then an increase in ANV would be expected if ANV is the result of classical conditioning or an increase in anxiety. It is widely accepted that the level of responding to a conditioned stimulus (CS) is, to some degree, determined by the intensity of the unconditioned stimulus (US) with which it has been paired (e.g. Rescorla and Wagner, 1972). If ANV is the result of intense anxiety, and the anxiety is due to the expectation of nausea and vomiting as a result of treatment, then an increase in the post-treatment nausea and vomiting would lead to an increase in ANV. Thus, the finding that the measured level of post-therapy nausea and vomiting determines the level of ANV (Andrykowski et al, 1988) is consistent with both the classical conditioning and the anxiety hypotheses.

Anxiety may lead to an increase in ANV directly, as suggested by the anxiety hypothesis, through increasing the rate of conditioning (Spence, 1964) or by increasing the salience of the stimuli present in the clinic through an increase in the amount of attention paid to them during the visit (Dolgin, Katz, McGinty & Siegal, 1985). In general, it is widely accepted that levels of arousal have an effect on learning (e.g. Spence, 1964), and it is possible that the level of arousal experienced in the more anxious patients is optimal for the learning of this kind of association.

The finding that the susceptibility of a patient to motion sickness is a predictor of ANV (Morrow et al, 1991) is not surprising. Susceptibility to motion sickness may be the result of a general sensitivity to nausea-inducing experiences. It is known that vestibular information passes through the chemoreceptor trigger zone and into the vomiting centre where it induces an emetic reflex (e.g. Borison, 1983), thus following a similar pathway to that necessary for other toxins to induce an emetic reflex. Individuals with a highly sensitive mechanism for the detection of motion-induced sickness might, therefore, also be expected to be highly sensitive to chemotherapy-induced nausea and vomiting. Again this would lead to an increase in the level of ANV experienced by such individuals.

The finding that the experience of tastes in the mouth as a result of drug infusion is a predictor of ANV is explicable in one of two ways. It is possible that the experience of these tastes merely reflects the intensity of the chemotherapeutic drug, the hypothetical US. However, it is also possible that a flavour in the mouth leads to an increase in the conditioning of an aversion towards the context. Such an effect, known as potentiation (e.g. Durlach and Rescorla, 1980), in which

the presence of a novel flavour increases the conditioning of an association between a second stimulus and illness, has been demonstrated in animals. This potentiation hypothesis will be discussed more fully in Chapters 6 and 7.

The final predictor of ANV is age (Morrow et al, 1991). Older patients were found to be less likely to develop ANV than younger patients. One speculative possibility is that this is due to latent inhibition in the older patients who are likely to have had more experience of hospital settings. Thus, the learning of an association between these cues and the consequence of illness would be expected to be retarded in older patients.

Thus, the findings from studies of cancer chemotherapy patients are consistent with a conditioning model, which assumes that the cues present in the clinic become associated with illness, and on subsequent occasions are able to re-elicit nausea and vomiting. However, under an alternative analysis, they are also consistent with the hypothesis that ANV is a direct result of intense treatment-related anxiety. If high levels of post-therapy nausea are responsible for high levels of anxiety, then the predictors of high post-therapy nausea and vomiting would also be predictors of high levels of ANV. However, under this analysis, it is not immediately clear why age is negatively correlated with ANV.

Two approaches might allow the classical conditioning and anxiety hypotheses to be distinguished empirically. A demonstration that cues present in the clinic elicit nausea in the absence of any expectation of treatment, would imply that these cues have become conditioned stimuli. Alternatively, if it could be shown that some procedure which induces a comparable degree of anxiety to that induced by chemotherapy does not lead to nausea and vomiting, then the hypothesis that therapy-induced anxiety is responsible for ANV would be untenable. The results of two studies applying these principles render the conditioning hypothesis more plausible.

Katz, Kellerman and Siegal (1980) tested a sample of bone marrow aspiration patients for ANV and found that only 2% of these patients developed ANV. It has been claimed that bone marrow aspiration can be as stressful as chemotherapy (Redd, Burish & Andrykowski, 1985) implying that anxiety alone cannot be responsible for ANV in cancer chemotherapy patients. However, to reach a firm conclusion, it would be necessary to compare cancer chemotherapy and bone marrow aspiration patients in a single study using a common measure of anxiety.

Finally, in a study by Bovbjerg, Redd, Jacobsen, Manne, Taylor, Surbone, Crown, Norton, Gilewski, Hudis, Reichman, Kaufman, Currie, & Hakes (1992), a novel flavoured beverage was presented to chemotherapy patients before each of a number of chemotherapy treatments and then later in their own home. It was found that, when given the beverage in their own home, patients who had received it before treatment experienced significantly higher nausea than those who did not. These data were argued to support the conditioning model of ANV. This finding is similar to those of Bernstein (1978) in which a novel flavoured ice cream was found to be more aversive as a result of its pairing with chemotherapy. However, in light of the relative ease with which flavour cues are thought to become associated with illness (Domjan & Wilson, 1972), stronger evidence for the conditioning hypothesis of ANV would be a demonstration of chemotherapy-induced conditioned nausea towards a non-flavour cue.

These last studies are the closest in procedure to those which might answer the question as to whether ANV is anxiety or conditioning based. However, further studies are required using the proper stimuli and proper controls in order to provide sound evidence for one of these hypotheses. An experiment in which bone marrow aspiration patients were directly compared with chemotherapy patients, in both the aversiveness of the treatment and levels of ANV, would allow an evaluation of the anxiety hypothesis of ANV. In addition, a demonstration of the conditioning of an aversion towards a non-flavour cue in the chemotherapy clinic, using a procedure such as that used by Bovbjerg et al (1992), would support the conditioning hypothesis of ANV.

## 1.3.3: An intervention for ANV

Since the etiology of ANV is not clear, there are a number of possible interventions which may or may not be effective in the attenuation of this chemotherapy side effect. It has been demonstrated that, even if ANV is the result of classical conditioning, anxiety may play a role in its development. Thus, interventions designed to reduce anxiety in the clinic would be expected to lead to a reduction in ANV and there are, indeed, a substantial number of studies which support this prediction (see Andrykowski, 1990 for a review). Thus, procedures such as progressive muscle relaxation and guided imagery have been found to reduce both the development and expression of ANV (Burish, Carey, Krozely & Greco, 1987; Lyles, Burish, Krozely & Oldham, 1982). However, such procedures are costly and time consuming, and, therefore, if it can be shown that ANV is due to classical conditioning, a more simple, cost effective intervention may be possible based on learning theoretic principles.

Two candidate interventions would be based on overshadowing (Pavlov, 1927) and latent inhibition (Lubow, 1973). Neither of these has been tested for its efficacy. As discussed above, overshadowing has been found to be effective in the attenuation of chemotherapy-induced taste aversions. However, since it has been found in animals that the presentation of a novel flavour stimulus in a novel context (in this case the chemotherapy clinic) can lead to an increase in the level of the aversion towards that context when it is paired with illness (Best, Brown & Sowell, 1984), the nature of the second stimulus may be important in determining whether it will be effective in attenuating, rather than exacerbating, ANV. On the basis of other animal experiments, the presentation of a novel odour prior to drug infusion might be expected to reduce ANV in cancer patients. Taukulis and St George (1982) found that, in rats, the presence of a novel odour reduced the level of conditioning towards a novel context when it was paired with toxicosis. The odours used would have to be synthesized such that they did not resemble odours present in the normal environment in order to avoid the experience of nausea and vomiting in locations other than the clinic. In combination with preexposure to the clinic, which should lead to latent inhibition of the cues present there, such an overshadowing procedure may prove highly effective. However, initial studies should concentrate on establishing whether such interventions might be possible through testing the conditioning hypothesis of ANV.

## 1.4: Conclusions

There is some evidence (Bernstein, 1978; Bernstein & Webster, 1980) that the taste aversions suffered by chemotherapy patients occur as a result of a classically conditioned association between certain foods eaten prior to treatment and the nausea experienced as a result of drug infusion. This is largely based on evidence (Bernstein et al, 1982; Broberg & Bernstein, 1987) that such aversions may be attenuated by the presentation of a novel, overshadowing, flavour before treatment. The data regarding the status of ANV is less clear cut. Anxiety has been found to play an important role in the development of ANV, either through inducing nausea and vomiting directly, or through an interaction with a classical conditioning mechanism. However, evidence that procedures which are as stressful as chemotherapy (Katz et al, 1980) do not give rise to ANV suggest that anxiety alone cannot account for ANV. Therefore, it will be assumed that ANV and a large number of CTAs which develop in chemotherapy are the result of classical conditioning

It is interesting that most recorded taste aversions occur during the first three visits to the clinic. It is possible that this is due to many of these having a non-associative basis. However, the evidence from the studies carried out on ANV suggest another possible cause. Since most cases of ANV occur across the first four visits to the clinic, it is possible that the reduction in the number of new taste aversions which develop after this period is due to blocking (Kamin, 1969). It has been shown in animals that a flavour presented in a context which has been previously paired with illness shows retarded conditioning (Willner, 1978). Thus it is possible that, following the fourth treatment session, the clinic is strongly associated with illness, and this association blocks the formation of further taste-illness associations.

There are, then, two ways in which the tastes (either of foods previously consumed or as a direct result of drug infusion) and contextual cues present during chemotherapy treatments might interact. First, the context might block new taste aversions after a number of context-illness pairings. Second, the presence of novel flavours in the clinic may potentiate an aversion towards contextual cues in the clinic (Durlach and Rescorla, 1980). This possibility would suggest that further investigations of the psychological side effects of cancer chemotherapy would be enhanced through the measurement of both of these phenomena simultaneously. Such an experiment would combine questionnaires recording the pre- and post-treatment nausea and vomiting across the initial treatment sessions, and a record of pre-treatment consumption of foods followed by a test in which these flavours are presented to the patients for consumption.

The analysis above would suggest that, if both ANV and CTA result from classical conditioning, then patients will fall into one of two distinctive categories on each of two dimensions. First, individual differences in the susceptibility to post-therapy nausea and vomiting (as a result of levels of anxiety, susceptibility to state anxiety and age) may lead to overall differences in the conditionability of patients to both tastes, and exteroceptive cues present in the chemotherapy clinic. Second, patients may develop CTAs early on in treatment which may lead to the potentiation of the context in which that flavour is consumed, or ANV might develop quickly in its own right and, therefore, block the development of CTAs. Thus, there is the possibility that the occurrence of CTA and ANV is determined by a complex interaction of the predisposition of the patients and the salience of the stimuli presented before treatment. The first would determine the general level of conditioning, while the second would determine which stimuli (flavours or contextual cues) become associated with illness.

In the chapters which follow, both pharmacological and psychological interventions for the control of CTA and ANV are investigated. In Chapter 2, a background for the pharmacological studies is presented, which is followed by a series of experiments in which a range of compounds are tested for their effect on CTA in rats (Chapters 3 to 5). In Chapters 6 and 7, the possibility that a scapegoat intervention, such as that used by Broberg and Bernstein (1987), may lead to the potentiation of a context aversion, and therefore ANV, is investigated. The findings from both the pharmacological and psychological experiments are then discussed in Chapter 8.

# Chapter Two

# The Efficacy of Antiemetics in the Attenuation of CTA

## 2.1: Introduction

Since ANV and CTA, the psychological side effects of cancer chemotherapy reviewed in Chapter 1, are thought to be the result of treatment-induced nausea, reduction in the severity of this nausea by antiemetic drugs, should result in the reduction of CTA and ANV in this patient population. CTA, and the effect of antiemetics on CTA have been widely studied, whereas no work has addressed the issue of the effect of antiemetics on the conditioning of context aversions. Therefore, the discussion below will be limited to the possibility of pharmacological interventions for the control of CTA. Implications for ANV of the effects of antiemetics on CTA will be discussed in Chapter 8.

There are a large number of pharmacological interventions which have been used in the control of chemotherapy-induced nausea and vomiting (Revusky & Martin, 1988). Of these interventions, the most effective are corticosteroids such as dexamethasone, and $5\text{-HT}_3$ receptor antagonists such as ondansetron (Jones et al, 1992). In addition to the drugs which are used in the clinic, $NK_1$ receptor antagonists such as CP-99,994 and L-742,694 have been found to block emesis very effectively in the ferret (Bountra, Bunce, Dale, Gardner, Jordan, Twissell, Ward, 1993; Tattersall, personal communication). The review presented here examines the possible role of $5\text{-HT}_3$ and $NK_1$ receptor antagonists in the attenuation of cisplatin-induced CTA. The focus of interest is the receptor systems, rather than any particular antagonists at these receptor sites. However, the experiments which follow use the $5\text{-HT}_3$ receptor antagonist ondansetron, and the $NK_1$ receptor antagonists CP-99,994 and L-742,694 in order to block these pathways, and therefore reference will be made to these compounds in particular rather than to $5\text{-HT}_3$ and $NK_1$ receptor antagonists in general.

Dexamethasone and ondansetron have both been tested for their ability to attenuate CTA in the rat. Using one procedure, dexamethasone, but not ondansetron, was found to be effective in the attenuation of cisplatin-induced CTA (Mele, McDonough, McLean & O'Halloran, 1992). However, the procedure used was not necessarily appropriate to the demonstration of ondansetron-induced CTA attenuation (see Chapter 3 for a full discussion of this issue). Ondansetron has been

found to attenuate nicotine-induced CTA (Mitchell & Pratt, 1990) and, although few methodological details were given for the experiment, this provides some evidence that ondansetron is able to reduce CTA. CP-99,994 has not been tested for its ability to attenuate CTA.

This chapter examines the evidence, from human and animal studies, favouring the prediction that the novel antiemetics, ondansetron, CP-99,994 and L-742,694, will reduce the level of CTA induced by one common chemotherapeutic drug cisplatin which is known to induce nausea and vomiting in approximately 90% of patients (Jones & Cunningham, 1993). Three lines of evidence are considered. The first two concern the identity of the US in CTA, while the third is an examination of the binding site density of the two antiemetics. Since the emetic drug which is injected is a 'stimulus' in this procedure, it may be inappropriate to refer to the illness that results from drug infusion as a US rather than a unconditioned response (UR). However, it is common to use the term US in discussion of the question of what effective property of the drug is responsible for the formation of CTA (eg Grant, 1987) and this convention will be used here.

The first candidate for the role of the US in CTA is emesis (Garcia, Hankins & Rusiniak, 1974). The strongest prediction from this theory is that vomiting and CTA are the result of the action of the same mechanism. It is clear that, if this were the case, then any drug which reduces vomiting (eg ondansetron and CP-99,994) will also attenuate CTA. A second and related claim has been made that, more specifically, nausea is the US in CTA (Revusky and Martin, 1988). If so, drugs that reduce nausea (e.g. ondansetron) should also reduce CTA. Finally, since ondansetron and CP-99,994 are both highly specific antagonists at the $5-HT_3$ and $NK_1$ receptors respectively, the binding site density of these two receptor types, in areas which are known to be important in CTA formation, will be outlined. High receptor-site density in areas which have been found to be involved in CTA formation would imply a possible role for these receptors in CTA formation, and therefore antagonists at these receptors may attenuate CTA.

It will be argued that the reduction of nausea, but not vomiting, by a drug may be an indication that that drug will also attenuate CTA. In addition, the brain localisation of the receptors at which the two drugs in question bind, implies that ondansetron, CP-99,994 and L-742,694 should attenuate CTA induced by a wide range of drugs. Although the specific route of action of cisplatin in the induction of CTA is not known, many drugs share common pathways in CTA formation. It can be inferred from this evidence that ondansetron, CP-99,994 and L-742,694 may

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attenuate CTA induced by cisplatin as well as by drugs which have a known route of action.

## 2.2: Emesis as the US in CTA

Garcia et al (1974) claimed that if a stimulus, such as a toxin, is detected by the "gut-defense system", then a hierarchy of emetic responses will result. This hierarchy is composed of CTA, nausea, retching and vomiting. CTA is the most sensitive and emesis is the least sensitive response; most emetic substances will lead to CTA, but only the strongest will give rise to the vomiting reflex. If this relationship could be demonstrated, then any drug which blocks vomiting, such as ondansetron, CP-99,994 or L-742,694, since it suppresses the gut-defense system, should also attenuate CTA. Some evidence against Garcia et al's (1974) claim comes from antiemetics which do not affect CTA but are used to control vomiting in the clinic (Revusky and Martin, 1988). However, it is possible to argue that a drug which blocks the vomiting reflex to a strong emetic drug, may not block the more sensitive response of CTA formation due to a difference in the threshold of activation; complete suppression of the emetic system may be required to attenuate CTA. A dissociation of this sort would not indicate that different mechanisms are responsible for vomiting and CTA. In order to discount the claim that there is one mechanism which is responsible for both vomiting and CTA, a double dissociation must be demonstrated in which one procedure leads to CTA but not vomiting, and another leads to vomiting but not CTA.

There are many studies in which the neural substrates of CTA and vomiting have been investigated through the lesioning of specific brain areas. From these it should be possible to determine directly whether CTA and vomiting develop as a result of activation of the same physiological system. A problem which arises in the comparison of the effects of brain lesions on CTA and vomiting is that most CTA lesion studies have been carried out on rats which do not have a vomiting response. The studies of vomiting have, in the main, been carried out on cats, dogs and ferrets. It may be argued that this sort of cross-species comparison cannot be valid. However, the brain areas which have been found to be responsible for both of these responses are present in all of the species which have been tested, regardless of whether or not that species has the vomiting reflex. Therefore, it might be argued that it is the response, not the mechanism which gives rise to that response, which differs across species. In the absence of the necessary within-species comparisons of the vomiting and CTA reflexes, the similarities in brain structures across species will be taken as evidence that these species share neural mechanisms for the detection of toxins.

## 2.2.1: The physiology of the emetic system

Garcia's (e.g. Garcia et al, 1974) gut-defense system was derived from a model of the emetic system first proposed by Borison and Wang (1953), and now widely accepted. The Borison-Wang model is comprised of four elements (see Figure 2.1): emetic receptors located in various parts of the body, including the gut and the brain, which detect toxic chemicals; afferent nerve fibres, including the vagus nerve, which transmit the information from the receptors to the emetic centre; the emetic, or vomiting, centre, thought to be located in the nucleus of the solitary tract (NST), which receives input from the afferent neural circuitry and integrates the emetic response syndrome (Borison and McCarthy); the output generated by the emetic centre (nausea, retching and vomiting). The receptors which detect the presence of toxins are located both centrally and peripherally. Central receptor areas include the chemoreceptor trigger zone which is associated with the area postrema (AP) and is probably the primary site of blood-borne toxin detection; the AP has no blood-brain barrier. Since the AP is located near the floor of the fourth ventricle, cerebrospinal fluid may also be monitored. There are also other receptors in the CNS which are linked directly to the emetic centre. Peripheral receptor areas include the gastrointestinal tract, which is connected to the emetic centre via the vagus nerve, and the vestibular apparatus of the inner ear which is linked to the emetic centre via the AP.

In summary, toxins may be detected in the CNS, in the blood through the AP, or in the gut, while motion stimulates the vestibular system. Information from these areas passes along the afferent nerve fibres to the emetic centre where the emetic response is produced. It is Garcia's claim that CTA is the most sensitive of the emetic responses, and is the result of stimulation of the emetic system in exactly the same way as are nausea, retching and vomiting. This hypothesis predicts that a manipulation which reduces vomiting will also reduce CTA, since it must be acting on the emetic system which is responsible for both of these responses.

## 2.2.2: Testing the emesis hypothesis

Since there are a number of emetic substances which induce vomiting as a result of the stimulation of one or more of the receptor areas in the emetic system, it is possible to test for concordance between the brain areas which detect a toxin in order to stimulate vomiting, and those which give rise to the formation of CTA. Grant (1987) reviewed the literature on lesions of the emetic system and their effects on vomiting and CTA, to determine whether lesions that lead to a blockade of

emetic reflex

NST

AP

abdominal vagus

GUT
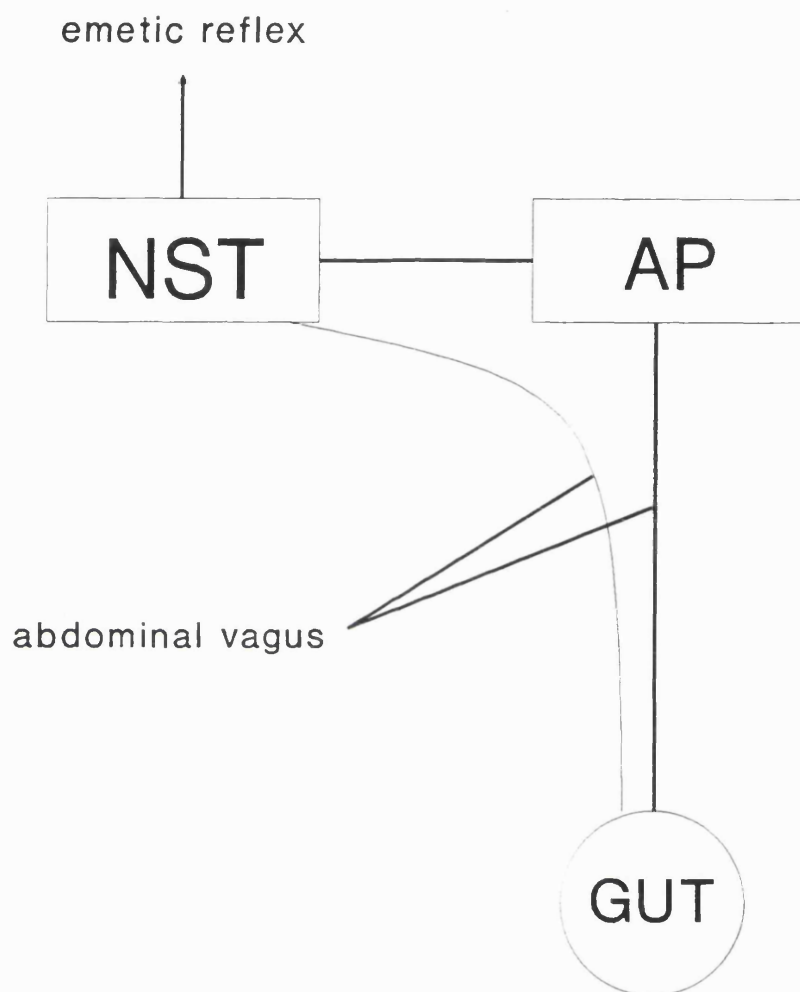
Figure 2.1. The Borison-Wang model of emesis. Toxins are detected in the gut by the vagus nerve, or in the blood by the area postrema (AP), while motion is detected by the vestibular system. Information is passed to the nucleus of the solitary tract (NST) which gives rise to the vomiting reflex.

the vomiting response to a particular toxin also lead to the blockade of CTA and vice versa. Any exception to this would disconfirm the emesis-as-US hypothesis. Of the studies which she reviewed, those in which copper sulphate (CuSO4), and those in which morphine were used as the emetic are of particular interest in the evaluation of the emesis-as-US hypothesis; work using copper sulphate as the toxin indicates that there are some parallels between vomiting and CTA, while the data from morphine studies provide strong evidence that these parallels are likely to be toxin-specific.

Intragastric (IG) injection of CuSO4, at moderate doses, gives rise to emesis mainly through the vagus nerve. Lesions of the AP have no effect on CuSO4-induced emesis (eg. Wang and Borison, 1952) and, although sympathectomy alone results in no attenuation of emesis, simultaneous sympathectomy and vagotomy attenuate emesis more completely than does vagotomy alone (Wang and Borison, 1951). Very high IG doses of CuSO4 behave differently to moderate IG doses of the same compound in that they activate the chemoreceptor trigger zone (CTZ) in the AP, and vagotomy is ineffective at high doses unless the AP is also lesioned (Wang, 1980). It would seem, therefore, that CuSO4 is absorbed into the blood at these high doses. This is substantiated by the finding that intravenous (i.v.) CuSO4-induced emesis is attenuated by AP lesions (Wang, 1980).

Coil, Rogers, Garcia et al (1978) found that vagotomy leads to attenuation of CuSO4-induced CTA, but Rabin, Hunt and Lee (1985) found the converse, that IG CuSO4 failed to give rise to CTA unless vagotomy had been carried out. In addition, Rabin et al (1985) found that no CTA was formed if rats were given both vagotomy and AP lesions. They concluded that, although the vagus nerve may mediate CuSO4-induced CTA, vagotomy itself delayed stomach emptying, thus allowing more time for the CuSO4 to be absorbed into the blood, and hence detected by the AP. One final result relevant to the present issue is that removal of the celiac ganglia (which cuts off sympathetic input to the brain from the gastrointestinal tract) has been found to attenuate CTA to IG CuSO4 (Martin, Cheng & Novin, 1978) whereas sympathectomy alone does not affect emesis.

The results of CuSO4 studies therefore suggest that the vagus nerve may mediate both CTA and vomiting, although there are some contradictory results in the experiments on CTA. The experiments in which the AP has been lesioned showed strong support for the hierarchy of response in the emesis-as-US hypothesis. Low dose CuSO4 induced CTA through the AP, while only high-dose CuSO4 induced vomiting through this area. This would suggest that CTA is a more sensitive response than vomiting, but is mediated by the same neural mechanism. However,

the sympathectomy data suggest that CTA and vomiting are mediated by two distinct neural pathways.

In the case of morphine, lesions of the AP lead to an attenuation of the vomiting response (Wang and Glaviano, 1954), suggesting that morphine induces vomiting at the chemoreceptor trigger zone. However, AP lesions fail to attenuate morphine-induced CTA (Van der Kooy, 1984). Morphine-induced CTA is attenuated by vagotomy (Bechara and Van der Kooy, 1985), while no study has implicated the vagus nerve in the emetic action of morphine. Morphine may also induce CTA by acting behind the blood brain barrier since only opiate antagonists which cross the barrier are effective in attenuating morphine-induced CTA (Le Blanc and Cappel, 1975; Corrigall, Linseman, D'Onofrio et al 1986).

Although questions remain as to the route of action of morphine-induced CTA, it is clear from these data that the chemoreceptor trigger zone, although vital to the vomiting reflex, does not play a role in morphine-induced CTA formation; AP lesions do not attenuate morphine-induced CTA. The finding that only opiate antagonists which cross the blood-brain barrier attenuate morphine-induced CTA is also inconsistent with a role for the AP in morphine-induced CTA, since the AP lacks a blood-brain barrier. Thus, the evidence from studies of morphine does not support Garcia's model.

### 2.2.3: Conclusion

There is evidence that activation of the mechanism which is responsible for vomiting is not necessary or sufficient for the formation of CTA. It would seem that, when morphine is used as the toxin, the AP mediates the vomiting response and not CTA, while the vagus nerve mediates CTA but not vomiting. This is strong evidence that CTA and vomiting are mediated by different pathways. It can be concluded that the effectiveness of both ondansetron and CP-99,994 in blocking cisplatin-induced vomiting cannot be taken as an indicator that these drugs will be effective in the attenuation of cisplatin-induced CTA.

### 2.3: Nausea or "Gut-related distress" as the US in CTA

Although the strong claim that all emetic reflexes result from the operation of one mechanism received little support from the lesion data, it is still possible that some component of the emetic

response is responsible for CTA formation. The lesion studies reviewed by Grant (1987) indicate that the neural substrates responsible for CTA formation are part of the emetic system; lesions of areas such as the AP and vagus nerve which are known to be involved in vomiting block CTA induced by a range of drugs. Thus, there is some relationship between CTA and vomiting on the physiological level, in that components of the emetic system play a role in both. The similarities which exist between the emetic and CTA system have led other researchers to conclude that, although vomiting is not a good indicator that the CTA mechanism is active, some other component of the emetic reflex might serve as the US in CTA.

Revusky and Martin (1988) suggested that 'gut-related distress' is the US in CTA, and that this distress is mediated by some part of the emetic system as outlined by Garcia et al (1974). Revusky and Martin appear to use the term gut-related distress in referring to nausea, since the data they cite come from experiments in which nausea was measured. The term nausea will be used here. Thus, although Revusky and Martin accept that vomiting may not correlate perfectly with CTA, they predicted that a drug which is found to reduce the emetic response of nausea, would also reduce CTA formation. Direct testing of this hypothesis with respect to the underlying neural substrates responsible for the two responses in question through lesion studies is not possible since there is no measure of nausea in animals (in humans, such effects are measured through verbal report e.g. Andrykowski et al(1985)). The approach adopted by Revusky and Martin was to take a range of drugs which are used in the clinic as antiemetics, only a subset of which are known to reduce nausea as well as vomiting, and test them for their efficacy in the attenuation of CTA in rats. Concordance between the effects of a wide range of drugs on these two responses would be suggestive evidence that the same mechanism is responsible for both, while any exception would implicate distinct mechanisms.

## 2.3.1: Testing the gut-related distress hypothesis

Revusky and Martin compared the effectiveness of a range of antiemetics in the attenuation of CTA. They identified two types of drug which are used as antiemetics and which have also been shown to reduce distress induced by cancer chemotherapy: the glucocorticoids and prochlorperazine. The glucocorticoids tested were cortisol, methyl-prednisolone, and prednisolone. In addition, a number of drugs were tested which are used in the control of vomiting in the clinic, but which are thought not to alleviate nausea: $\Delta 9$THC, domperidone, haloperidol, metoclopramide and scopolamine. These drugs are thought to have a general palliative effect, but not have to have

a specific effect on nausea.

In the studies carried out by Revusky and Martin, animals were allowed to drink saccharin flavoured water and were then injected with a toxin or vehicle, 60 minutes following saccharin consumption on six or seven occasions. In addition, 30 minutes following consumption and 30 minutes prior to toxin infusion, either an antiemetic or its vehicle was administered. Thus, animals were exposed to a number of pairings of saccharin solution with vehicle, emetic or emetic plus antiemetic. Saccharin consumption on each conditioning trial was recorded, this constituted a measure of the development of a flavour aversion across trials.

Revusky and Martin demonstrated that the glucocorticoids and prochlorperazine, the compounds thought to reduce nausea in the clinic, were effective in attenuating cyclophosphamide-induced CTA while the remaining drugs tested did not alter the animals' level of saccharin consumption. It would seem then, that there is some agreement between the data collected from studies on cancer patients and those carried out on rats, with respect to the effects of various antiemetics on nausea and the effects of these drugs on CTA.

A further prediction can be made from the hypothesis that the reduction of nausea will result in an attenuation of CTA. This is that the CTA induced by all emetics will be attenuated by the glucocorticoids and prochloperazine. Revusky and Martin tested this claim by taking one glucocorticoid, dexamethasone, and investigating its effect on CTA induced by a number of other emetics using the same procedure as that used in the previous experiment. The other emetics used were carmustine, cisplatin, cytarbine, dactinomycin, doxorubicin, lithium chloride, mechlorethamine and copper sulphate. The CTA induced by all of these agents was found to be significantly attenuated by treatment with dexamethasone. The effect of dexamethasone on cisplatin-induced CTA is of particular interest here since it demonstrates that it is possible to reduce cisplatin-induced CTA. This finding has been replicated using a single conditioning trial procedure (Mele et al, 1992).

## 2.3.2: Conclusion

There is clear agreement between the effects of the antiemetics used in the Revusky and Martin (1988) study on both nausea in humans and CTA in rats. This would suggest that ondansetron will be effective in the attenuation of cisplatin-induced CTA, since it has been found to control

nausea in humans (Jones et al, 1991). No conclusion can be drawn with regard to the effectiveness of CP-99,994 or L-742,694 on cisplatin-induced CTA since no clinical trials have yet been carried out on these compounds.

The Revusky and Martin hypothesis predicts that ondansetron will attenuate CTA. Thus, testing this compound for its efficacy in the attenuation of CTA will serve as a test of the Revusky and Martin hypothesis. In addition, neither CP-99,994 or L-742,694 has been tested for their effects on nausea or CTA, but are effective in reducing vomiting (Bountra et al, 1993; Tattersall et al, 1994). If it is found that CP-99,994 and L-742,694 reduce CTA in rats, then it can be predicted that it will also reduce nausea in the clinic. It would seem that investigation of the behaviour of these two types of novel antiemetic, the 5-$HT_3$ and $NK_1$ receptor antagonists, with respect to CTA might serve as a good test of Revusky and Martin's hypothesis.

One last point must be made with respect to the testing of Revusky and Martin's hypothesis. If it were found that ondansetron is ineffective in the attenuation of CTA, it might be argued that this is not due to nausea and CTA having distinct neural mechanisms, but to suppression of CTA requiring more complete suppression of the mechanism which they share in order to show attenuation at the behavioral level. Consistent with the model proposed by Garcia et al (1974), Revusky and Martin allow that a lower threshold of activation of this mechanism might be necessary for the formation of CTA than that required for the experience of nausea. Thus, Revusky and Martin's hypothesis may be supported but not falsified.

## 2.4: Implications from the Identity of the US in CTA

The first section of this chapter attempted to investigate the possibility that two types of antiemetic, 5-$HT_3$ and $NK_1$ receptor antagonists, might attenuate CTA through the analysis of the relationship between CTA and two other responses to toxin infusion on which these drugs are known to have a suppressant effect. It is known that ondansetron reduces both vomiting and nausea in humans (Jones et al, 1991), and that CP-99,994 and L-742,694 reduce vomiting in the ferret (Bountra et al, 1993; Tattersall et al, 1994). Evidence demonstrating the presence of a common mechanism which is responsible for CTA and vomiting or nausea would be a strong indicator that ondansetron and/or CP-99,994 and L-742,694 would attenuate CTA. However, no strong evidence was found, and therefore no prediction may be made confidently as to the effectiveness of these drugs in the reduction of CTA.

Studies of the effect of some antiemetics on CTA in rats, most notably the glucocorticoid dexamethasone, suggest that drugs which reduce nausea may also reduce CTA. Ondansetron reduces nausea in human cancer patients and may, therefore, be predicted to attenuate CTA.

## 2.5: Localization data

The general antiemetic effects of ondansetron, CP-99,994 and L-742,694 do not allow a firm prediction to be made regarding their ability to attenuate CTA. In this section, a closer look will be taken at the neuropharmacology of the receptors at which these substances are thought to be active. The density of $5\text{-}HT_3$ and $NK_1$ receptors in brain areas thought to be responsible for CTA will be presented. If either of these receptor types are found to have high density in areas related to CTA then it might be hypothesised that there is a role for these receptors in CTA and that antagonists at these receptors will attenuate CTA.

This analysis cannot provide conclusive evidence of the sort required since the localization of receptors in brain areas relevant to CTA can only be an indirect indicator that their function might be necessary to the formation of CTA. However, a closer analysis of the neuropharmacology of the $5\text{-}HT_3$ and $NK_1$ receptor systems will provide a more complete picture of the possible roles that ondansetron, CP-99,994 and L-742,694 might play with respect to CTA. It will be argued that ondansetron may be effective in attenuating CTA through blockade of the actions of 5-HT on the vagus nerve, and that CP-99,994 and L-742,694 may block CTA through blockade of the action of substance P in the NST. CP-99,994 and L-742,694 may also attenuate CTA through activity at the parabrachial nucleus (PBN). In addition, a brief discussion will be presented of possible indirect effects on CTA of areas which are known to mediate brain states which affect learning in general. For example, it is known that level of arousal affects the rate of learning (Spence. 1964), and therefore the presence of receptors in areas controlling arousal may be of importance in CTA formation.

It has already been shown that a number of brain areas which have been found to play a role in emesis are also responsible for CTA (see Grant, 1987 for review). Thus, localization of $5\text{-}HT_3$ and $NK_1$ receptors in the vagus nerve, AP and NST may indicate a role for these receptors in CTA. In addition, the parabrachial nucleus (PBN), which is connected to the NST, has been found to play an important role in the formation of CTA. The PBN is also connected to the amygdala (see Figure 2.2). The implication here is that the PBN may be an area in which outputs from the
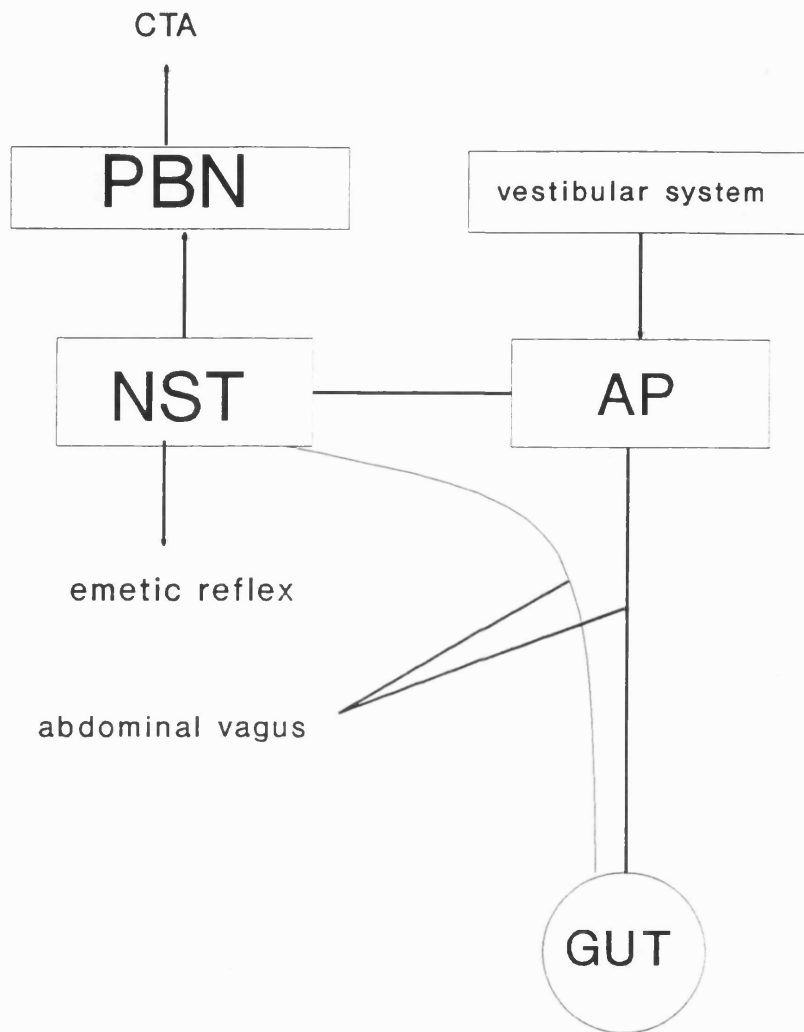
Figure 2.2. The emetic system and connections with the parabrachial nucleus (PBN). Toxins are detected in the gut by the vagus nerve, or in the blood by the area postrema (AP), while motion is detected by the vestibular system. Information is passed to the nucleus of the solitary tract (NST) which gives rise to the vomiting reflex. Information from the NST passes to the PBN which is thought to play a role in the formation of CTA.

NST are processed for entry into higher cortical areas, and areas in which their affective relevance are assessed. It is possible that the experience of nausea results from activation of neurones in the PBN.

Bielavska and Bures (1994) carried out a number of experiments on animals in which activation of the PBN was temporarily blocked by the infusion of tetrodotoxin. This allowed them to assess the importance of the PBN on CTA formation without contamination by effects that the lesion might have on performance. They found that CTA induced by all of the drugs which they tested (LiCl, D-amphetamine and carbachol) was attenuated by a PBN lesion during training. It is not clear whether the taste aversion was completely abolished since the appropriate control group was not run. However, CS (0.9% NaCl solution) consumption was around 50% total consumption (CS + water) on test for the lesioned animals. Thus, there are four areas which have been implicated in the formation of CTA: the AP, the NST, the vagus nerve and the PBN.

## 2.5.1: Localization of 5-$HT_3$ and $NK_1$ receptors in the emetic system

Using tritium-labelled GR65630, a radio ligand with high affinity for the 5-$HT_3$ receptor subtype, three of the areas most important to this investigation, the vagus nerve, the AP and the NST, have been found to have a higher density of 5-$HT_3$ receptors than any other area in the nervous system in the rat (Kilpatrick, Jones & Tyers. 1987; Pratt and Bowery, 1989). Other areas found to have an appreciably high 5-$HT_3$ receptor site density were the human amygdala and hippocampus, which are concerned with emotion and memory, and the rat entorhinal cortex (Kilpatrick et al, 1987).

In a recent study, the distribution of the $NK_1$ receptor in the rat brain was investigated by in situ hybridization histochemistry (Maeno. Kiyama & Tohyama, 1993). $NK_1$ receptors were identified in many brain areas, including the NST and PBN. Thus, two brain areas thought to mediate CTA, the NST and PBN, have a high density of $NK_1$ receptor sites, suggesting that CP-99,994 and L-742,694 will be active in the blockade of the action of substance P in these areas.

If normal functioning of these two receptor systems is necessary for the functioning of the vagus, AP and NST in the case of 5-$HT_3$, and the NST and PBN in the case of $NK_1$, then antagonists at both of these receptors will block CTA induced by most drugs. Ondansetron can be expected to block CTA since no drug has been found to induce CTA in a simultaneously vagotomized and AP

lesioned animal (Grant, 1987). CP-99,994 can be expected to block CTA since there is some evidence that PBN lesions lead to a disruption of the CTA mechanism which is non toxin-specific (Bielavska and Bures, 1994). In addition, the NST is thought to play a non-specific role in CTA, although no tests of this hypothesis have been undertaken (Grant, 1987).

## 2.5.2: 5-HT$_3$ receptors in the area postrema

The picture with respect to the 5-HT$_3$ receptor system is complicated by the fact that the AP has been found to connect directly to the vagus nerve in the cat (Leslie, 1985). Since 5-HT$_3$ receptors are known to lie on the vagus nerve, the presence of 5-HT$_3$ receptors in the NST and AP may merely reflect the close proximity of vagal terminals with these areas. The route of action of cisplatin-induced CTA is not known, but it is likely to be through the AP and/or vagus nerve; all known drugs which induce CTA do so through one or both of these pathways. It is therefore imperative to establish whether the presence of 5-HT$_3$ receptors in the AP and NST are likely to be active in these areas independently of the vagus nerve.

Evidence can be gathered as to the possible localization of 5-HT$_3$ receptors in the AP and on the vagus nerve, from comparing the effects of ondansetron and vagotomy on the vomiting response to a number of toxins. If ondansetron acts only on the vagus nerve, then it would be predicted that drugs which are blocked by vagotomy in their induction of an emetic response will also be blocked by ondansetron. Also, those drugs which induce emesis through some other route, such as the AP, will be unaffected by ondansetron.

Andrews, Davis, Bingham, Davidson. Hawthorn, Maskell (1990) listed 16 emetic drugs the effects of which are attenuated by 5-HT$_3$ receptor antagonists (including cisplatin and low to medium dose radiation), and 11 interventions which are unaffected by 5-HT$_3$ receptor antagonists (including morphine and motion). Erythromycin was unique in that it was found to be unaffected by ondansetron and is thought to induce emesis through the vagus nerve. However, the remaining 26 interventions showed a clear correlation; those inducing emesis which is blocked or reduced by 5-HT$_3$ receptor antagonists were unable to support emesis after vagotomy or a combination of vagotomy and greater splanchnic nerve lesion (which increases the effect of vagotomy but has no effect when carried out alone), while those which are unaffected by vagotomy were also unaffected by ondansetron. In addition, although medium to low dose radiation-induced vomiting is attenuated by both vagotomy and 5-HT$_3$ receptor antagonist administration, there is an emetic

response to high dose radiation (800cGy) in vagotomized and splanchnectomized animals, and this is unaffected by 5-HT$_3$ receptor antagonism (Carpenter, Briggs, Knox & Strominger, 1988).

The concordance between the effect of 5-HT$_3$ receptor antagonist administration and vagotomy on vomiting in the ferret suggests that 5-HT$_3$ receptor antagonists are effective in blocking the vagus nerve but not the AP. This hypothesis is supported by data on the effects of 5-HT$_3$ receptor antagonist administration and vagotomy on cisplatin-evoked induction of c-fos protein in the brainstem of the ferret (Reynolds, Barber, Grahame-Smith & Leslie, 1991). Reynolds et al (1991) used immunocytochemistry to detect the expression of c-fos protein in the ferret brainstem as a result of cisplatin (10 mg/kg i.p.) administration, and thereby identify the brainstem pathways mediating cisplatin-induced vomiting. The results suggested that cisplatin administration leads to vomiting through activation of the vagus nerve and not the AP. Both vagotomy and administration of granisetron (a 5-HT$_3$ receptor antagonist) led to a reduction in c-fos expression in the NST, while c-fos expression in the AP was not significantly affected by either of these manipulations. It is interesting to note, however, that cisplatin-induced vomiting was attenuated by granisetron but not by vagotomy in this experiment. Thus, although there was a parallel between the two interventions neuronally, their behavioural effects were quite different. However, other evidence suggests that cisplatin (10 mg/kg i.p.) induces vomiting in the ferret which is attenuated by vagotomy (Andrews et al, 1990). Andrews et al (1990) have suggested that some plasticity in the emetic system may allow an increase in sensitivity of the AP as a result of vagotomy which may explain the variability in the results of the effects of vagotomy on vomiting.

The suggestion that 5-HT$_3$ receptor antagonists act on the vagus nerve and not in the AP is important in establishing whether or not ondansetron might attenuate cisplatin-induced CTA. Although the route of action of cisplatin-induced CTA is not known, the AP or vagus nerve, or both of these areas, is likely to play a major role. That 5-HT$_3$ receptors have been found to be present in both of these areas would suggest that, regardless of the route of action, cisplatin-induced CTA will be attenuated by treatment with ondansetron. However, that the presence of 5-HT$_3$ receptors in the AP is thought to be the result of vagal terminals lying close to this area, allows the possibility that cisplatin-induced CTA, if it occurs through activation of the AP and not the vagus nerve, will not be affected by ondansetron.

## 2.5.3: The route of action of cisplatin-induced CTA

There are certain parallels between the effects of interventions such as cisplatin administration and radiation therapy, allowing that data regarding the route of action of radiation-induced CTA, might have a bearing on the route of action of cisplatin-induced CTA. Both radiation and cisplatin cause cellular damage, both release endogenous emetic agents (probably from the gut), and both radiation- and cisplatin-induced vomiting are attenuated or blocked by 5-HT$_3$ receptor antagonists (Andrews, Rapeport & Sanger, 1988).

It is known that radiation-induced CTA in rats is attenuated by lesions of the AP (Rabin, Hunt & Lee, 1983), but not lesions of the vagus nerve (Rabin et al, 1983). Therefore, if cisplatin induces CTA through the AP and not the vagus nerve as does radiation, and ondansetron is active on the vagal terminals and not the AP, ondansetron would not be expected to attenuate cisplatin-induced CTA. However, as was noted above, Andrews et al (1990) have suggested that vagotomy may lead to the sensitization of the AP, allowing toxins, which may ordinarily be detected by the vagus nerve, to be detected by the AP. In addition, a lesion to the AP is also a lesion to the vagus nerve (Andrews et al, 1990). Therefore, the finding that AP lesions, but not lesions to the vagus nerve, result in the attenuation of radiation-induced CTA, is consistent with either or both of these areas being responsible for the detection of radiation in the intact animal. Since there is no clear evidence as to the route of action of radiation-induced CTA, and therefore cisplatin-induced CTA, no precise prediction can be made as to the possible effects of ondansetron on cisplatin-induced CTA.

## 2.5.4: Conclusions from the localisation of 5-HT$_3$ and NK$_1$ receptors in the emetic system

In summary, the presence of NK$_1$ receptors in areas such as the PBN and NST suggest that CP-99,994 and L-742,694, two NK$_1$ receptor antagonists, may be effective in the attenuation of CTA. The NST and the PBN are not thought to be toxin-specific in their role in the formation of CTA and, therefore, a range of drugs, including cisplatin, might be expected to show attenuated effectiveness in the induction of CTA as a result of administration of CP-99,994 or L-742,694. The presence of 5-HT$_3$ receptors on the terminals of the vagal afferents suggests that cisplatin-induced CTA will be attenuated by ondansetron if cisplatin induces CTA through the vagus nerve. If cisplatin induces CTA through the AP then ondansetron would not be expected to attenuate cisplatin-induced CTA.

## 2.5.5: Remaining brain areas

There are three areas, unrelated to the emetic system, which have been found to have an appreciable density of 5-HT$_3$ receptors and which may have an effect on CTA. These are the amygdala, the hippocampus and the entorhinal cortex (Kilpatrick et al 1987). Two of these areas, the hippocampus and the amygdala, have also been found to be high in NK$_1$ receptor site density (Maeno et al, 1993). From these findings, 5-HT$_3$ and NK$_1$ receptors may be involved in the control of emotion and memory, but only the role of 5-HT$_3$ receptors in emotion, specifically anxiety, and memory have so far been investigated.

Memory and anxiety, which are unrelated to the emetic system, may have general effects on learning and thereby affect CTA formation. For example, in Chapter 1, it was suggested that high state anxiety might increase the rate of acquisition of ANV through an increase in the rate at which the stimuli become associated (Spence, 1964). This may also be the case in the acquisition of CTA. If high anxiety leads to quicker learning, then an anxiolytic drug will reduce the rate of learning. It has been suggested that ondansetron has anxiolytic properties (Costall, Domeney, Gerrard et al, 1988), raising the possibility that it might reduce CTA through a reduction in anxiety. However, with respect to effects on memory, ondansetron has also been thought to enhance cognitive function (e.g. Domeney, Costall, Gerrard, Jones, Naylor & Tyers, 1991) which might be expected to increase the rate of learning.

The experiments which have been carried out on the non-emetic effects of 5-HT$_3$ receptor antagonists must be treated with scepticism. Positive findings have invariably been followed by failures to replicate in other laboratories, and some of the doses of 5-HT$_3$ receptor antagonist which are purported to be effective are very much lower than those found to block emesis. Experiments which have investigated the possible anxiolytic effects of ondansetron will be discussed here, since they illustrate many of the difficulties encountered in trying to resolve the functional relevance of the central 5-HT$_3$ receptor.

The effects of ondansetron have been tested on a wide variety of animal models of anxiety. Gleeson, Ahlers, Mansbach, Foust & Barret (1989) found that ondansetron failed to attenuate punished drinking in a conditioned suppression procedure with pigeons as subjects. More positive results have been demonstrated in which the spontaneous exploratory behaviour of mice is measured in a black/white box. A greater amount of time spent in the white half of the box is

thought to be a measure of reduced anxiety. Costall, Jones, Kelly, Naylor & Tomkins (1989) found that 0.00005-0.01 mg/kg ondansetron increased exploratory behaviour of the mouse. However, Mos, Heyden and Olivier (1989) found, using this test, that ondansetron either had no effect, or an effect opposite to that of diazepam.

In a test in which the level of social interaction is measured in rats, Jones, Costall, Domeney, Kelly, Naylor, Oakley & Tyers (1988) found that ondansetron (0.0005-1.0 mg/kg) increased social contact (thought to be indicative of a reduction in anxiety) while File (1990) found no effect of ondansetron on social interaction across a wide range of environments. Dunn, Corbett, Hubbard, Tobiasz, Nordstrom, Carlezon, Cornfeldt, and Fielding (1990) found that 0.01-0.1 mg/kg of ondansetron had anxiolytic effects in rats in the elevated plus maze. However, Piper, Upton, Thomas and Nicholass (1988) found no such effects across comparable dose ranges. Finally, Broekkamp, Berendsen, Jenck and Van Delft (1989) found no effect of ondansetron on defensive burying in the mouse. An ethological approach to the testing for anxiolytic effects of ondansetron has been taken with similar results. Shepherd, Rodgers, Blanchard, Magee, and Blanchard (1993) failed to show any effect of ondansetron (0.001 - 0.1 mg/kg) on antipredator defensive behaviour in rats, and Rodgers, Cole and Tredwell (1993) failed to find consistent effects of ondansetron in an extended range of behaviours in the elevated plus maze.

There are two conclusions to be drawn from these data. First, all positive findings have rarely been shown to be replicable by independent groups of workers. Second, the dose ranges which have been found to be effective have been extremely wide, showing flat or bell-shaped dose-response relationships. The bell-shaped response relationships could be due to secondary effects of ondansetron suppressing the 'appropriate' responding at high doses. However, the nature of these secondary effects remains elusive. Furthermore, the lowest doses which have been found to be effective are around 0.0001 mg/kg, and it seems highly unlikely that, under these circumstances, sufficient drug could accumulate in brain to block the receptor.

Overall, regardless of the relative merits of the animal models of anxiety which have been tested, the evidence that ondansetron has any effect in these models is equivocal. Similar criticisms can be applied to the animal models of memory. A small number of workers have produced unreplicable effects at very low doses of ondansetron in a range of tests of memory, including the Wisconsin test in marmosets (Domeney et al, 1991), a habituation test in mice (Barnes, Costall, Coughlan, Domeney, Gerrard, Kelly, Naylor, Onaivi, Tomkins & Tyers 1990) and a food

reinforced alternation task in rats (Costall, Naylor & Tyers, 1990). It is clear that, if ondansetron is able to attenuate CTA, it is more likely to have its effect through the emetic system, where its activity is well established, than via any effects on memory or emotion.

## 2.6: Conclusions

It has been shown that the effects of ondansetron, CP-99,994 and L-742,694 on vomiting do not constitute good reason to suppose that these drugs will reduce CTA. However, there is some evidence that a reduction of nausea may lead to a reduction in CTA (Revusky and Martin, 1988), although this model is largely based on results from only two drugs, prochlorperazine and glucocorticoids, which both lead to a reduction in toxin-induced nausea in humans and CTA in animals. If this relationship holds, ondansetron might be expected to attenuate CTA. Since it is not known what effect CP-99,994 or L-742,694 might have on nausea, no prediction can be made with respect to these drugs on the basis of any relationship there may be between nausea and CTA.

The brain localization data suggest that CP-99,994 and L-742,694 will attenuate CTA; $NK_1$ receptors have been found in high density in the PBN, and lesions to this area block CTA induced by a range of toxins. Also, the NST, which is thought to play a non-toxin specific role in the generation of emetic reflexes, is high in $NK_1$ receptor site density. Ondansetron might be expected to have its effect on the vagus nerve, thus whether it has any effect on CTA will depend on the toxin used to induce the aversion. In the case of cisplatin, the route of action in the induction of CTA is not known.

If it were found that cisplatin-induced CTA is unaffected by ondansetron, CP-99,994 or L-742,694, the dissociation between the emetic and CTA mechanisms could be explained in terms either of two mechanisms or a hierarchy of response. Specific neural connections within the emetic system could be responsible for the responses of vomiting and nausea and these may be little related to those neurones mediating CTA induced by cisplatin. The latter, hierarchy hypothesis, would assume that ondansetron, CP-99,994 and L-742,694 reduce activation of the NST and the PBN (either directly in the case of CP-99,994 and L-742,694 or by reducing the amount of information passing into this area from the vagus nerve in the case of ondansetron) such that the level of activation is below the threshold for vomiting and nausea, but above that for CTA. There is some evidence for both of these explanations. The different mechanism hypothesis is supported by the

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lack of correlation between the emetic system and the CTA system (Grant, 1987), while the hierarchy hypothesis is supported by evidence that extremely low levels of toxin, which are unable to induce vomiting, are able to induce a CTA (Revusky and Martin, 1988).

# Chapter Three

# 5-HT₃ Receptor Antagonists in the Attenuation of Cisplatin-induced CTA

## 3.1: Introduction

In this chapter, six experiments are described investigating the efficacy of the $5\text{-HT}_3$ receptor antagonist ondansetron in the attenuation of cisplatin-induced CTA. During the course of these studies, Mele et al (1992) showed that dexamethasone but not ondansetron was effective in the attenuation of cisplatin-induced CTA in rats. However, it would be premature to accept the conclusion that cisplatin-induced CTA is not mediated by $5\text{-HT}_3$ receptor mechanisms, since there are a number of respects in which the procedure used by Mele et al could be varied to produce a more sensitive test of the hypothesis.

There is one respect in which the procedure used by Mele et al was followed in the experiments presented here, despite a possible lack of sensitivity, for reasons of clinical validity. This is that only one conditioning trial was used. Revusky and Martin (1988) argued that multiple conditioning trials may be necessary to show an effect of an antiemetic on CTA. In the latter experiments, the animals received six or more CS-US pairings, and it was shown that the difference in consumption of the test fluid between animals given an antiemetic and those given the toxin alone tended to increase across trials. Although multiple conditioning trials might provide a more sensitive test of the effectiveness of ondansetron in the attenuation of CTA, such aversions have been found to occur in the clinic following one conditioning trial (Bernstein, 1978). Therefore, it is important that one-trial CTA can be attenuated by the antiemetics used in the clinic in order for them to make a significant impact in the control of this problem. It may be the case that the role for $5\text{-HT}_3$ receptors in the induction of CTA is very small and only detectable when multiple conditioning trials are used, thus, it is possible that the experiments presented here will, in attempting to model CTA in the clinic, fail to detect an effect of $5\text{-HT}_3$ antagonism on cisplatin-induced CTA.

There are two further aspects of the procedure used by Mele et al which may be responsible for their failure to detect an effect of ondansetron. First, it can be assumed that, if an antiemetic

suppresses activity of the mechanism which gives rise to CTA, then suppression of this mechanism for a large proportion of the period during which the emetic is active would lead to a attenuation of CTA. The greater the overlap between the activity of the emetic and antiemetic, the larger the expected attenuation of CTA. This is especially important with respect to cisplatin-induced CTA, due to the long duration of action of cisplatin; data from experiments on cisplatin-induced vomiting in ferrets suggest that cisplatin is active for the 24 hours following drug infusion (Andrews et al, 1988). CTA can occur across CS-US intervals of many hours (Smith & Roll, 1967). Thus, if cisplatin stimulates the brain areas responsible for CTA over a period of hours, it is important that the antiemetic is active during a large part of this period.

Mele et al (1992) administered both ondansetron and dexamethasone on one occasion, following CS consumption and prior to cisplatin administration. For the comparison between these two drugs in terms of efficacy in the attenuation of CTA to be a fair one, it must be assumed that they have an equivalent duration of action in their suppression of the CTA mechanism. Experiments testing the effects of ondansetron on both vomiting and nausea indicate that it has a short half-life in the control of both of these responses. In particular, Jones et al (1991) found that dexamethasone was more effective than ondansetron in the control of delayed nausea as a result of emetic chemotherapy in man. In conclusion, it is possible that dexamethasone was found to attenuate CTA as a result of its long duration of action (Mele et al, 1992), and that repeated administration of ondansetron across a period of time might demonstrate an effect of ondansetron on cisplatin-induced CTA where acute administration did not.

The second respect in which the test of CTA in the procedure used by Mele et al might have been insensitive is that a two-bottle choice test was used. That is, animals were presented with a single flavour during training and on test, two days later, were given a choice between the training flavour and tap water. The measure of CTA was the suppression of consumption of the training flavour on test, as a percentage of total consumption, compared to vehicle controls. A choice test is thought to be extremely sensitive to CTA, but not alterations in the strength of CTA, thus the subtle effects of some antiemetics may not be detected. Batsell and Best (1993) found that a single-bottle test was more sensitive in the detection of differences between levels of CTA than a two bottle test in an overshadowing procedure in rats. Thus, whatever the reason for this difference, it is clear that the use of a single-bottle test may yield results where a two-bottle test did not.

Since a two bottle choice test is though to be more sensitive to CTA, and a single bottle test is thought to be more sensitive to detection of alterations in the level of CTA, of the experiments presented here, those in which an antiemetic was tested in the attenuation of CTA, a single-bottle test was utilized, while in those in which a drug was tested as to its effectiveness as a US, a choice test was given. In this chapter, and in the experimental chapters that folllow, the method used, which is largely identical across experiments, will be presented first. Each individual experiment will then be detailed in turn. This will include any deviations from the standard method, and the results and discussion of the data.

In Experiment 3A, a dose response analysis was carried out on cisplatin in the induction of CTA. In Experiments 3B a range of doses of ondansetron were paired with sucrose solution in order to test whether ondansetron could serve as the US in this CTA procedure. An attempt was then made to attenuate a cisplatin-induced aversion using dexamethasone in Experiment 3C. Finally, three experiments, Experiments 3D - 3F, tested ondansetron for its ability to attenuate cisplatin-induced CTA. It was concluded that, with the procedure used here, a dose-dependent attenuation of cisplatin-induced CTA by dexamethasone is demonstrable. However, ondansetron does not reliably reduce a cisplatin-induced CTA to sucrose solution when it is administered acutely, or repeatedly across a 1.5 hour period.

## 3.2: General Method

### 3.2.1: Subjects

Male Sprague Dawley rats (200-300g) were housed in fours in plastic, wire-topped cages (53 x 38 x 18 cm) in a temperature controlled room on a 12 hour light/dark cycle, lights on at 0800 hours. All animals were experimentally naive and were allowed food ad libitum throughout the experiment. Water consumption was controlled as below.

### 3.2.2: Apparatus

A rack with 12 cages in an illuminated experimental room was used for all experimental procedures. The experimental cages were identical to the home cages except that they had wire instead of sawdust floors. Water bottles (500 ml) were used to allow access to liquid in the experimental cages. Each of these bottles had a rubber stopper and a long metal spout with a ball

bearing to minimize leakage.

## 3.2.3: Procedure

On Day 1, all animals were taken off water at 1400 hours, weighed and marked. On Day 2, 20 hours later, the animals were weighed and placed in the experimental cages in batches of 12, one rat to each cage. Tap water was immediately presented, and the rats were allowed to drink for 20 minutes. The bottles were then removed and weighed, and the animals were replaced in the home cages. Liquid consumption in the experimental cages was recorded on each day of the experiment. Three hours after training to drink in the experimental cages, tap water was presented in the home cages for 1 hour and 10 minutes free drinking. This procedure was repeated on Day 3. Day 4 was the conditioning day. Animals were assigned to groups pseudo-randomly with equal numbers of animals from each experimental group in each batch when possible. The animals were given 20 minutes free access to a flavoured solution in the experimental cage and then each rat was removed, injected, and replaced in its home cage. Three hours after injection, tap water was made available in the home cage for 1 hour and 10 mins. On Day 5, the rats were treated as on Day 2. Day 6 was the test day. The drug-paired flavour, or that flavour plus an alternative solution, was presented in the experimental cages for 10 or 20 minutes. The animals were then replaced in the home cages and allowed free access to water.

## 3.3: Experiments

## 3.3.1: Experiment 3A Cisplatin as a US in CTA

The first experiment in this series was a dose-response analysis of the induction of CTA by cisplatin. A pilot study had established that 10 and 3 mg/kg cisplatin, but not 1mg/kg, led to a general suppression of consumption of liquid on test. In addition, these three doses were all shown to lead to a greater reduction in consumption of the paired flavour (0.1% saccharin solution) than to the alternative which was tap water. When tap water is the alternative in a choice test, it is not clear whether reduced consumption of the paired flavour is due to conditioning or enhancement of neophobia due to a sensitizing effect of drug infusion (Best & Batson, 1977); the animals may have rejected the saccharin solution due to its novelty, and not because it was aversive as a result of prior pairing with cisplatin. In the present experiment, animals were conditioned with either 0.5% saline solution or 3% sucrose solution and were given a choice

between these flavours on test in order to rule out an explanation in terms of enhanced neophobia.

Other pilot studies using different flavours (3% w/v sucrose solution and 4% v/v cider vinegar solution) indicated that cisplatin at doses of 0.3 and 0.1 mg/kg is able to support CTA. Thus, the doses tested in Experiment 3A were in this range. Five groups of animals were used (n=8). Groups 3-5 drank either saline or sucrose and were then injected with 0.03 mg/kg cisplatin (Group 3), 0.1 mg/kg cisplatin (Group 4) or 0.3 mg/kg cisplatin (Group 5). Group 1 was a vehicle control that received sterile water instead of cisplatin, and Group 2 was a positive control that received 0.15 M LiCl instead of cisplatin. All drugs were administered in a volume of 10 ml/kg, i.p.

**Results and Discussion**

Preference for the drug-paired flavour was taken as a percentage of the total consumption of both flavours on test. A criterion for inclusion in the statistical analysis was that the animal drank at least 5 mls of sucrose solution on Day 4 (conditioning), a criterion which was applied to all experiments in Chapters 3-5. One animal was dropped from further analysis in the present experiment due to a failure to consume sufficient flavoured solution on Day 4. Examination of Figure 3.1 suggests that 0.15 M LiCl led to a reduction in consumption of the paired flavour compared to the vehicle controls. In addition, Group 5 (0.3 mg/kg cisplatin) but not Groups 3 or 4 (0.03 or 0.1 mg/kg cisplatin) showed suppressed consumption compared to controls. The variance in percentage consumption increased with the mean, therefore the data were subjected to a square-root transformation before analysis. This transformation was carried out on all the data in this series of experiments before statistical analysis, although the figures show untransformed data. The criterion set for significance in the analyses carried out on these data, and all subsequent analyses presented in this thesis, was $p < 0.05$. A one-way ANOVA on the transformed data revealed a significant effect of treatment ($F_{(4,34)} = 15.08$) and Dunnet post hoc control test confirmed that 0.15 M LiCl and 0.3 mg/kg cisplatin, but not 0.1 or 0.03 mg/kg cisplatin, led to a reliable suppression of consumption of the paired flavour.

The results presented here indicate that 0.3 mg/kg cisplatin is able to support a CTA. Although 0.1 mg/kg cisplatin was shown to induce CTA by Mele et al (1992), and in some pilot studies forming part of the present investigation, it would seem that 0.3 mg/kg cisplatin is the lowest dose with which a reliable CTA can be induced. This effect was not due to a non-associative enhanced neophobia since the alternative flavour in the choice test was also novel. Thus 0.3 mg/kg cisplatin
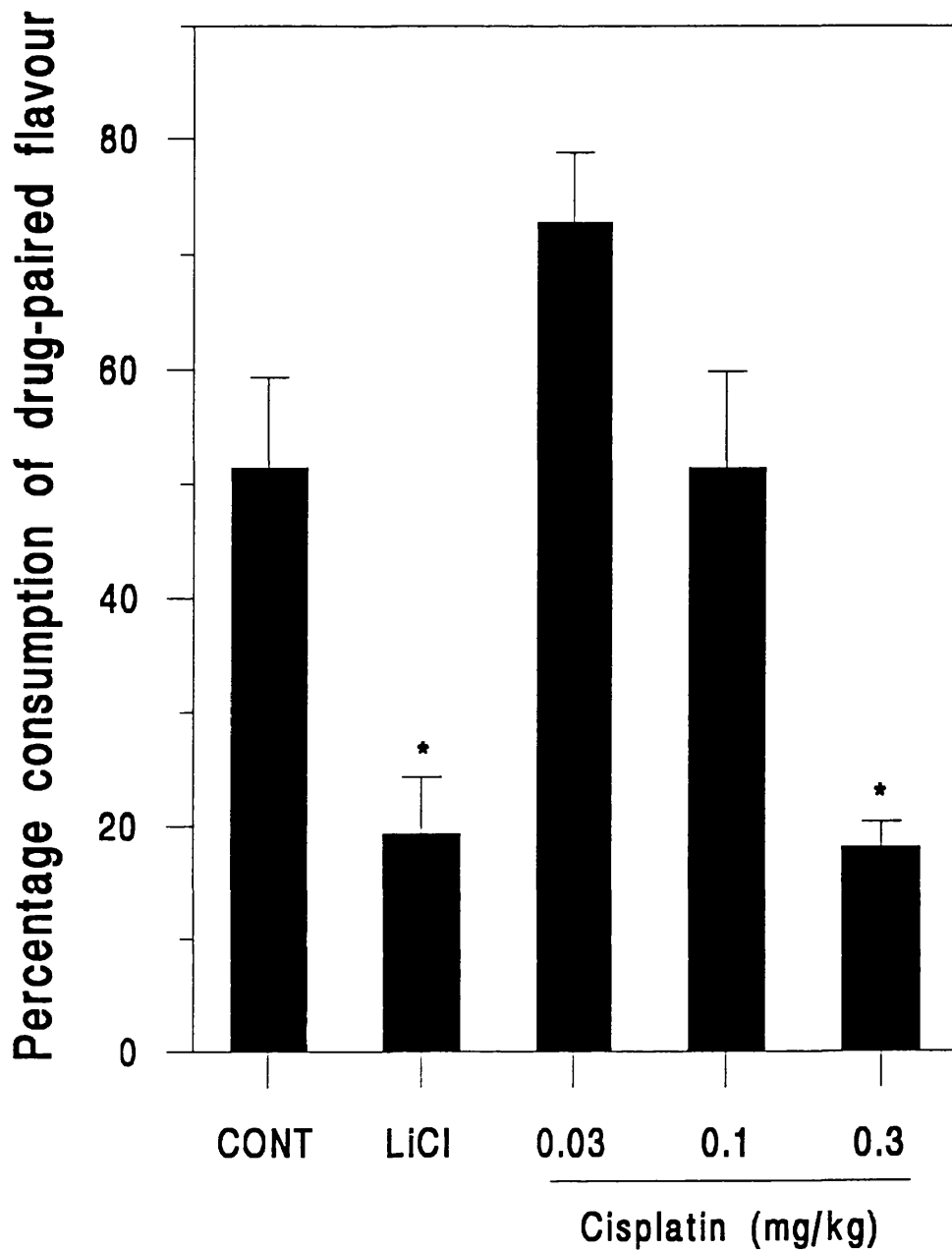
Figure 3.1. Mean percentage of target flavour consumed on test in Experiment 3.A. The target was paired with sterile water, LiCl (0.3M) or cisplatin during training. Error bars indicate SEMs. * Significantly different from controls.

was used in the following experiments testing the efficacy of ondansetron in the attenuation of cisplatin-induced CTA.

### 3.3.2: Experiment 3B Ondansetron as a US in CTA

Before testing the efficacy of ondansetron in the attenuation of cisplatin-induced CTA, this compound was tested for its potential to induce CTA in a procedure identical to that used in Experiment 3A. Establishing the potential of ondansetron as a US in CTA is important for interpretation of the data from subsequent experiments. A choice test procedure was used in which saline (0.5% w/v) or sucrose (3% w/v) were paired with a drug, and animals were presented with a choice of these two flavours for 20 minutes on test. All drugs were administered 1 ml/kg i.p. Seven groups were used (n=8). Groups 3 - 7 drank either sucrose or saline solution and were then injected with ondansetron (0.0001, 0.001, 0.01, 0.1 and 1.0 mg/kg respectively). Group 1 was a vehicle control that received an injection of sterile water instead of ondansetron and Group 2 was a positive control that received 0.3 mg/kg cisplatin instead of ondansetron.

A wide range of drug doses was used because, although the antiemetic effects of ondansetron are first evident at doses of about 1.0 mg/kg (Andrews et al, 1990), behavioral effects of the compound have been noted to occur at much lower doses (see Chapter 2 for examples). A complete test of the efficacy of ondansetron with respect to CTA thus involved investigation of the entire dose range at which it has been found to be effective in other tests.

### Results and Discussion

Figure 3.2 shows the consumption of paired flavour as a percentage of total consumption on test. It would seem that, as in the previous experiments, cisplatin (Group 2) gave rise to a suppression of consumption of the paired flavour compared to vehicle controls. The consumption levels in Groups 3-7 given ondansetron during training, were, in general, lower than those of the vehicle control animals, but the magnitude of the suppression was not as great as that in the cisplatin-treated animals. A one-way ANOVA carried out on the square-root transformed data revealed a significant effect of treatment ($F_{(6,49)} = 3.63$) and the Dunnets post hoc test, with Group 1 as the control, revealed a reliable difference between the unpoisoned animals (Group 1) and the cisplatin-treated animals (Group 2), but no other differences were significant. These results indicate that 0.3 mg/kg cisplatin, but not ondansetron (0.0001-1.0 mg/kg) induce CTA when paired with a novel
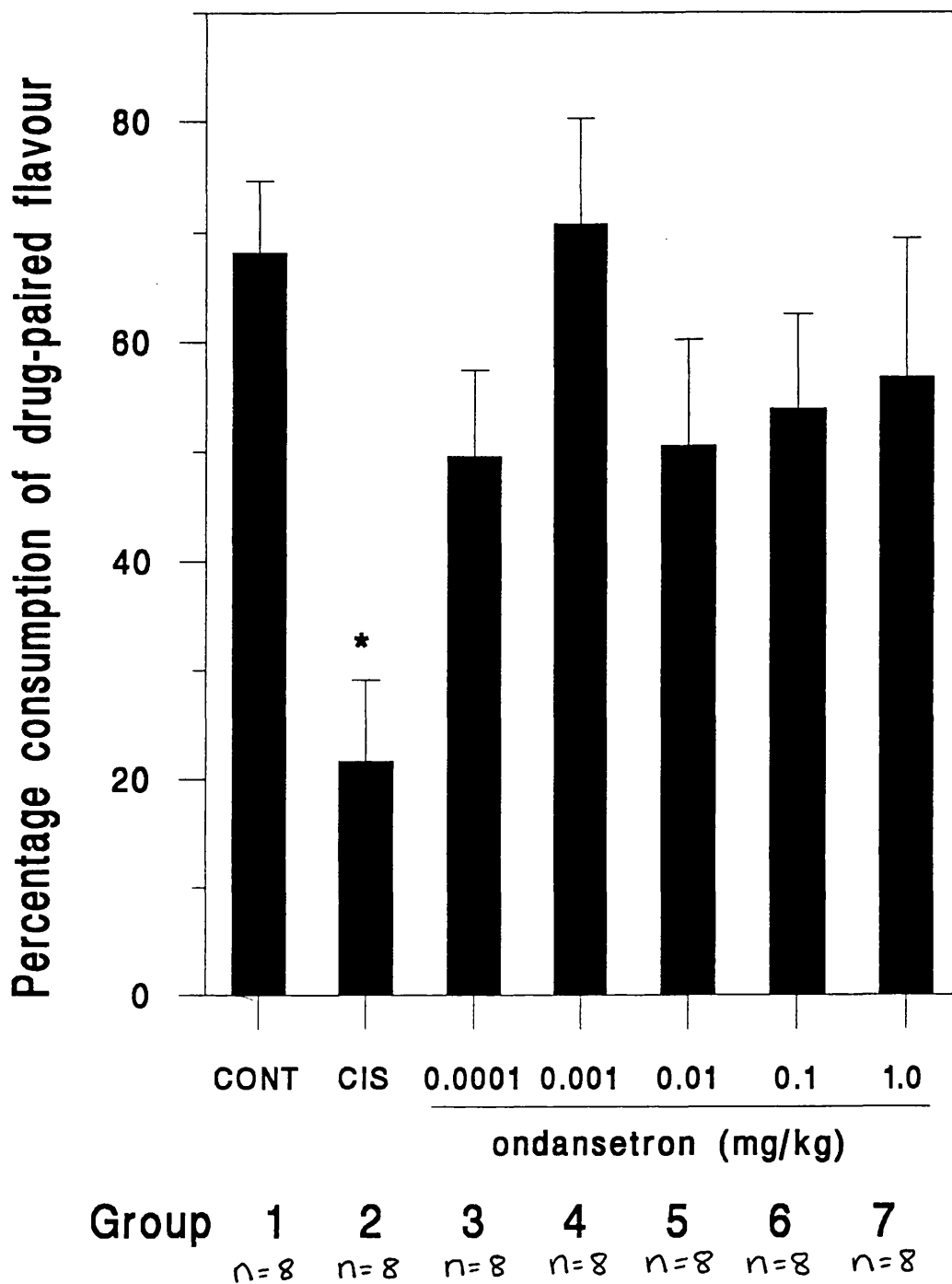
Figure 3.2. Mean percentage of target flavour consumed on test in Experiment 3.8. The target was paired with water (CONT), cisplatin (0.3 mg/kg) or ONS during training. Error bars indicate SEMs. * Significantly different from controls.

flavour.

In a further experiment that will not be reported in detail here, ondansetron (1 mg/kg) was found not to induce a taste aversion towards sucrose solution when administered repeatedly across a period of 1.5 hours (Experiment A(i), see Appendix 1). In this experiment, ondansetron was administered three times, once immediately following sucrose consumption, and on two further occasions, 45 and 90 minutes after removal from the experimental cages. This result is pertinent to some of the experiments in which ondansetron was tested against cisplatin-induced CTA (Experiments 3D and 3E). Repeated administration using the procedure outlined above was used with a maximum dose of 1.0 mg/kg.

Overall, these experiments suggest that ondansetron is not aversive across a wide range of doses and administration regimes. Thus, any attempt to show an effect of ondansetron on cisplatin-induced CTA is unlikely to be affected by ondansetron serving as an additional US in this procedure. The finding that administration of ondansetron does not lead to CTA at the doses tested in Experiment 3C replicates some findings of Mele et al (1992) who found that 10 mg/kg, but not 0.0001 - 1.0 mg/kg, ondansetron served as an effective US in their CTA procedure.

### 3.3.3: Experiment 3C Attenuation of cisplatin-induced CTA by Dexamethasone

In order to establish that the procedure used here is sensitive to possible reductions in CTA brought about by administration of antiemetic drugs, dexamethasone, a drug which previously has been shown to be effective in the attenuation of cisplatin-induced CTA, was tested in Experiment 3C. Revusky and Martin (1988) found that a wide dose range of dexamethasone (0.05 - 1.35 mg/kg) was effective against CTA, and that 0.4 mg/kg dexamethasone alone did not induce CTA. In order to show dose dependency, 0.001, 0.01 and 0.1 mg/kg (s.c.) dexamethasone were tested against cisplatin-induced CTA in Experiment 3C. Cisplatin or vehicle (sterile water) was administered 1 ml/kg i.p., while dexamethasone or vehicle (sterile water) was administered 1 ml/kg s.c. Five groups were used (n=8), all of which received two injections: Group 1 received sterile water; Group 2 received cisplatin; Groups 3 - 5 received cisplatin and dexamethasone (0.001, 0.01 and 0.1 mg/kg respectively). A single bottle test was utilized in order to optimize sensitivity to possible reductions in the level of CTA as a result of administration of dexamethasone (Batsell & Best, 1993). The CS was 3% sucrose solution, which was presented to the animals for 10 minutes on test.

Figure 3.3. Mean consumption of sucrose on test in Experiment 3.C. Sucrose was paired with water (CONT), cisplatin (0.3 mg/kg), or cisplatin and DEX during training. Error bars indicate SEMs. * Significantly different from cisplatin group.

**Results and Discussion**

The datum from one animal in Group 5 was considered an outlier, as its sucrose consumption was more than two standard deviations from the mean consumption of that group, and was excluded from further analysis. The sucrose consumption of the remaining animals on test is shown in Figure 3.3. It can be seen that 0.3 mg/kg cisplatin (Group 2) suppressed sucrose consumption on test compared to controls (Group 1). In addition, a dose-dependent attenuation of the aversion was observed in the animals which received dexamethasone (Groups 3-5). A one-way ANOVA on the square-root transformed data revealed a main effect of treatment ($F_{(4,34)}$ = 4.69). A Dunnet post hoc analysis, with Group 2 taken as the control group, confirmed that animals in Group 1 consumed more sucrose on test than those in Group 2, suggesting that an aversion towards sucrose developed in Group 2. Furthermore, animals treated with 0.1 mg/kg dexamethasone (Group 5) drank more sucrose on test than those given cisplatin alone (Group 2), suggesting that, at this dose, dexamethasone attenuated a cisplatin-induced CTA. No other doses of dexamethasone (Groups 3-4) were effective.

The results of Experiment 3C provide evidence that the procedure used in this series of experiments is sensitive to changes in the conditioning of a taste aversion brought about by the administration of an antiemetic prior to toxin infusion. They also replicate the findings of Revusky and Martin (1988) and Mele et al (1992) showing that the steroid dexamethasone is effective in the reduction of cisplatin-induced CTA, thus indicating that cisplatin-induced CTA is amenable to reduction by the administration of an antiemetic. The following experiments aimed to investigate the role of selective $5-HT_3$ receptor antagonists in the attenuation of cisplatin-induced CTA.

### 3.3.4: Experiment 3D Attenuation of cisplatin-induced CTA by ondansetron

In an initial dose-finding study, in which, following a sucrose-cisplatin pairing, ondansetron was administered 3 times at 45 minute intervals across a 1.5 hour period, there was some suggestion of an effect of ondansetron on cisplatin-induced CTA at a dose of 0.1 mg/kg. Consumption of the flavour which was paired with cisplatin was higher in a group which received 0.1 mg/kg ondansetron, than in the control group which was given cisplatin alone, but this difference only emerged in the course of a number of extinction sessions. The doses tested were 0.0001, 0.001, 0.01, 0.1 and 1.0 mg/kg, and no dose other than 0.1 mg/kg showed any effect. On the basis of

this result it was decided that the following studies would concentrate on the upper end of the ondansetron dose range.

In the present study, male hooded Lister rats were used (400-550g). All animals received 3% (w/v) sucrose solution in the experimental cages on the conditioning day and were then given two injections immediately: cisplatin (0.3 mg/kg) or vehicle (sterile water (i.p.)), and ondansetron or vehicle (sterile water (s.c.)). All animals were then given two further injections of ondansetron or vehicle, 45 and 90 minutes following cisplatin administration. All injections were administered in a volume of 1 ml/kg. Six groups were used (n=6-8): Group 1 received vehicle; Group 2 received cisplatin and vehicle; Groups 3-6 received cisplatin plus ondansetron (0.03, 0.1, 0.3 and 1.0 mg/kg respectively). On the test day (Day 6) all groups were allowed 10 minutes access to 3% sucrose solution in the experimental cages. Again the single bottle test was used in order to maximize sensitivity to changes in the strength of the CTA.

**Results and Discussion**

Sucrose consumption on test is presented in Figure 3.4. Unfortunately, it proved impossible to administer a controlled dose of ondansetron to five animals in this experiment, two each from Groups 4 and 6 and one from Group 5. Thus, these animals were excluded from further analysis. Examination of Figure 3.4 indicates that cisplatin led to a large reduction in sucrose consumption on test compared to vehicle controls. It would also appear that 0.3 mg/kg ondansetron, but not 0.03, 0.1 or 1.0 mg/kg, led to an attenuation of this suppression in consumption. A one-way ANOVA was carried out on the square-root transformed data and a significant effect of treatment was found ($F_{(5,37)} = 14.12$). A Dunnets post hoc test, with Group 2 (the animals treated only with cisplatin) as controls, revealed that Group 2 drank less sucrose on test than unpoisoned controls (Group 1). In addition, animals treated with 0.3 mg/kg ondansetron (Group 5) drank more sucrose on test than the animals in Group 2. Sucrose consumption in the remaining groups (Groups 3,4 and 6) was not different from that of Group 2.

It would seem that the repeated administration of 0.3 mg/kg ondansetron across a period of 1.5 hours, gave rise to an attenuation in the cisplatin-induced suppression of consumption of sucrose solution. Similar results were obtained in a further experiment in which Sprague Dawley, rather than hooded Lister, rats (200-250g) were used (Experiment A(ii), see Appendix I). It is interesting to note that the higher dose of 1.0 mg/kg ondansetron failed to attenuate the aversion towards
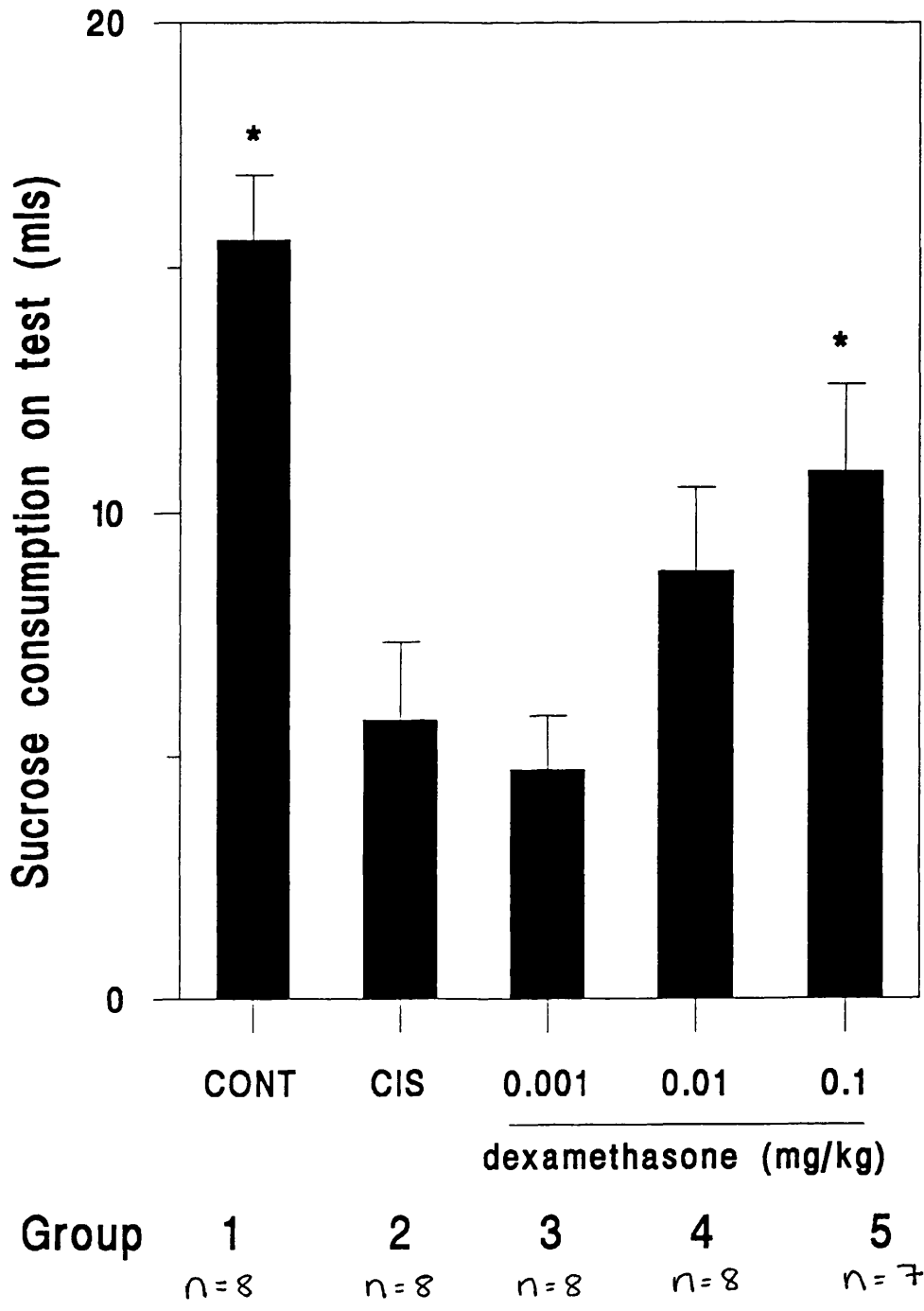
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Figure 3.4. Mean consumption of sucrose solution on test in Experiment 3. D. Sucrose was paired with water (CONT), cisplatin (0.3 mg/kg) or cisplatin and ondansetron. Error bars indicate SEMs. * Significantly different from cisplatin controls (Gp2).

sucrose. It is possible that this bell-shaped response curve resulted from some effect that ondansetron has at this higher dose, that might lead to a suppression in consumption of sucrose solution. However, the simplest hypothesis, that 1.0 mg/kg ondansetron induced a CTA which masked any effects it might have had on the CTA induced by cisplatin, is not supported by the results of a study discussed in Experiment 3B. It was found there that 1.0 mg/kg administered three times across 1.5 hours following cisplatin-sucrose pairing failed to produce a CTA (Experiment A(i), see Appendix I).

It is apparent that, in addition to the factors identified in the introduction to this chapter, Mele et al (1992) may have failed to find an effect of ondansetron on cisplatin-induced CTA simply because the effect of ondansetron is capricious and poorly dose-related. They tested 0.01, 0.1 and 1.0 mg/kg ondansetron against 0.32 mg/kg cisplatin and found no reliable effects at any of these doses. These results are consistent with those of the present experiment. Since, in Experiment 3D, 0.3 but not 0.1 or 1.0 mg/kg ondansetron led to an attenuation of CTA, one would not expect to obtain an effect of ondansetron at the doses tested by Mele et al. It is unclear, however, whether the use of repeated administration, or a single bottle test played any part in allowing an effect of ondansetron on cisplatin-induced CTA to be detected in Experiment 3D, but not in the studies by Mele et al. Experiment 3E examined the first of these possibilities, the role of repeated administration of ondansetron.

### 3.3.5: Experiment 3E Repeated versus single administration of ondansetron in the attenuation of cisplatin-induced CTA

The aim of Experiment 3E was to replicate the effect demonstrated in Experiment 3D, and to establish whether repeated administration was necessary to show an effect of ondansetron on cisplatin-induced CTA. Male Sprague Dawley rats were used (250-350g). All animals received 3% sucrose solution in the experimental cages on the conditioning day and were then immediately given two injections: cisplatin (0.3 mg/kg (i.p.)) or vehicle (sterile water (i.p.)), and ondansetron (s.c.) or vehicle (sterile water (s.c.)). All animals were then given two further injections of ondansetron or vehicle, 45 and 90 minutes following cisplatin administration. All injections were made in a volume of 1 ml/kg. Eight groups were used (n=6): Group 1 received vehicle; Group 2 received cisplatin and vehicle; Groups 3-5 received one dose of cisplatin and one dose of ondansetron (0.1, 0.3 and 1.0 mg/kg, respectively) immediately following sucrose consumption, and were then injected with sterile water 45 and 90 minutes following sucrose consumption;

Groups 6-8 received one dose of cisplatin plus three doses of ondansetron (0.1, 0.3 and 1.0 mg/kg respectively), 0, 45 and 90 minutes after sucrose consumption. On the test day (Day 6) all groups were allowed 10 minutes access to 3% sucrose solution in the experimental cages.

## Results and Discussion

The data are presented in Figure 3.5. It would seem that cisplatin led to a reduction in consumption of sucrose on test (Group 2) compared to the unpoisoned controls (Group 1). There was also some indication that ondansetron led to an attenuation of this effect; sucrose consumption in the ondansetron treated groups (Groups 3-8) was higher than that of Group 2. A one-way ANOVA on the square-root transformed data showed a significant effect of treatment ($F_{(7,40)}$ = 4.90). A Dunnets post hoc test was used to compare the cisplatin treated controls (Group 2) with each of the other groups. A reliable difference was demonstrated between the cisplatin treated animals and the vehicle control group (Group 1), and between the cisplatin treated animals and those given cisplatin and 3 x 0.3 or 3 x 1.0 mg/kg ondansetron (Groups 7 and 8). No other differences were reliable.

These results replicate, to some extent, those found in Experiment 3D; 0.3 mg/kg ondansetron, administered repeatedly across a period of 1.5 hours was able to attenuate a cisplatin-induced CTA. However, unlike Experiment 3D, 1.0 mg/kg ondansetron was also found to attenuate CTA when administered repeatedly. 0.1 mg/kg ondansetron was not effective when administered according to this regime, and no dose of ondansetron, including 0.3 mg/kg, was effective in the attenuation of CTA using an acute administration regime. Thus, Experiment 3D and 3E suggest that, across a narrow dose range, and using repeated administration across a 1.5 hour time period, ondansetron was effective in the attenuation of cisplatin-induced CTA.

The evidence with respect to the efficacy of 1.0 mg/kg ondansetron on cisplatin-induced CTA is mixed. In Experiment 3D it was found to be without effect, whereas an attenuation of CTA was obtained with both 0.3 and 1.0 mg/kg ondansetron in Experiment 3E. It is possible that the use of different strains of rats in Experiments 3D and 3E (hooded Lister rats and Sprague Dawleys, respectively) is responsible for this discrepancy. However, this hypothesis was not explicitly tested. In summary, there would seem to be some evidence that $5\text{-HT}_3$ receptor mechanisms are involved in the mediation of cisplatin-induced CTA.
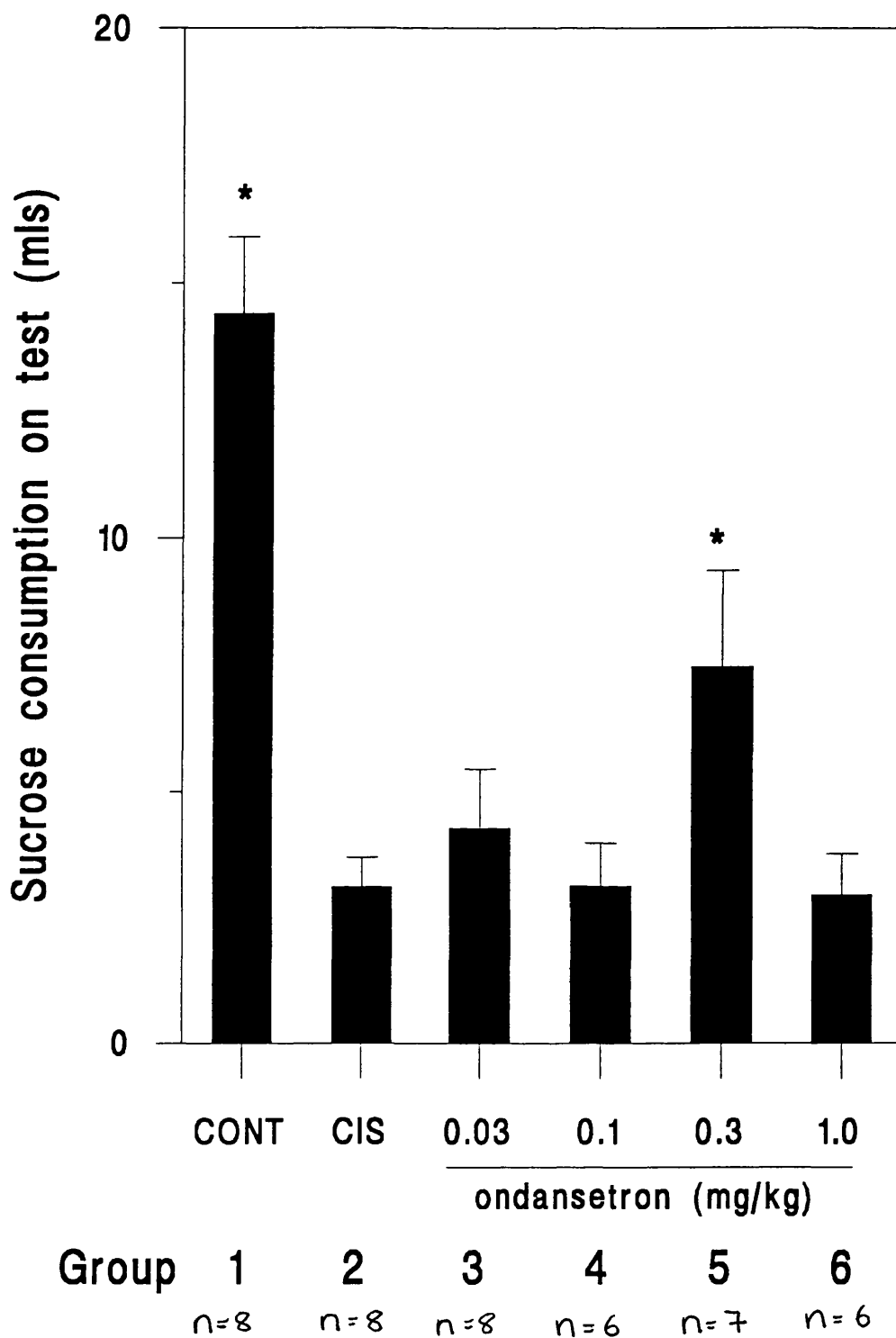
Figure 3.5. Mean consumption of sucrose solution on test in Experiment 3.E Sucrose was paired with water (CONT), cisplatin (0.3 mg/kg), or cisplatin and ondansetron (acute or repeated dos). Error bars indicate SEMs. * Significantly different from Group 2.

The effect of 0.3 mg/kg ondansetron on CTA induced by 0.3 mg/kg cisplatin was demonstrated in three consecutive replications (Experiment 3D, Experiment A(ii) - see Appendix I, and Experiment 3E). However, the effect was not replicated on any further occasions. Nine unsuccessful replications were run, all of which used the repeated administration regime for ondansetron employed in Experiment 3D. Two experiments, using Sprague Dawley rats, which were designed to demonstrate dose-dependency of the effect of ondansetron observed in Experiments 3D and 3E, failed to show an effect of repeated administration of ondansetron at doses of 0.0625, 0.125, 0.25, and 0.5 mg/kg on 0.3 mg/kg cisplatin-induced CTA.

Following these two failures to replicate, the effective dose of ondansetron found in Experiment 3D (0.3 mg/kg) was used in a three group experiment (a vehicle control, a poisoned control and an ondansetron group) in order to re-establish the effect. In this experiment, which used hooded Lister rats, no effect of ondansetron was observed. This dose of ondansetron was then tested in two further experiments using Sprague Dawley rats. For these experiments, a new batch of ondansetron was used in order to test whether the failures in the previous experiments were due to the use of a degraded compound. Again, administration of ondansetron was without effect.

It was observed that, in the experiments in which ondansetron reduced the level of CTA observed (e.g. Experiment 3D), the suppression of sucrose consumption, or level of CTA, as a result of cisplatin administration was greater than in those cases where ondansetron was ineffective. One possibility was, therefore, that a strong aversion was necessary in order to demonstrate an attenuation of that aversion. However, an experiment in which a dose of 0.6 mg/kg cisplatin was used (instead of 0.3 mg/kg cisplatin), to induce a stronger aversion to sucrose, did not show any effect of administration of ondansetron (0.15, 0.3 and 0.6 mg/kg).

Finally, it was thought that more success might be obtained through the testing of another selective 5-HT$_3$ receptor antagonist, granisetron. It would be premature to rule out a role for 5-HT$_3$ receptors in the mediation of cisplatin-induced CTA on the basis of tests of a single 5-HT$_3$ receptor antagonist. However, if it were found that other 5-HT$_3$ receptor antagonists are equally ineffective in reducing CTA, then it could be confidently concluded that, using this procedure, cisplatin-induced CTA is not attenuated by 5-HT$_3$ receptor antagonism. In two experiments, doses of granisetron ranging from 0.125 to 5.0 mg/kg were found not to attenuate cisplatin-induced CTA. A third experiment in this series, Experiment 3F below, tested both ondansetron and granisetron.

### 3.3.6: Experiment 3F Failure to attenuate cisplatin-induced CTA by ondansetron and granisetron

The example presented here tested two doses of granisetron and two doses of ondansetron (injected 1 ml/kg s.c.) against a CTA induced by 0.3 mg/kg cisplatin (injected 1 ml/kg i.p.), and the antiemetics were again administered three times over a 1.5 hour period. Male Sprague Dawley rats were used (350-650 mg/kg). Six groups were tested (n=8): animals in Group 1 were injected with vehicle; Group 2 received cisplatin (0.3 mg/kg) and vehicle; Groups 3 and 4 received cisplatin (0.3 mg/kg) and granisetron (1.0 and 2.0 mg/kg, respectively); Groups 5 and 6 received cisplatin (0.3 mg/kg) and ondansetron (0.3 and 0.5 mg/kg, respectively). With the exception of the drugs used, and the doses at which they were administered, this experiment was procedurally identical to Experiment 3D.

### Results and Discussion

The consumption of sucrose solution is presented in Figure 3.6. Two animals, one from Group 3 and one from Group 4, failed to consume 5mls of sucrose during conditioning and were thus excluded from further analysis. In addition, administration of a controlled dose of antiemetic was not possible with two animals and these were also excluded from further analysis. The pairing of sucrose with cisplatin seems to have given rise to a suppression of consumption of this flavour on test. In addition, granisetron (1.0 mg/kg) and ondansetron (0.3 mg/kg) would seem to have given rise to an attenuation of this suppression. A one-way ANOVA was carried out on the square-root transformed data and a significant effect of treatment was found ($F_{(5,38)} = 11.49$). A Dunnets test was applied to the data in which the cisplatin treated animals (Group 2) served as the control group. It was found that animals in Group 2 drank significantly less sucrose than the unpoisoned animals (Group 1), but no other differences were reliable.

### 3.4: General Discussion

First, an effective dose of cisplatin (0.3 mg/kg) in the induction of CTA was established (Experiment 3A). It was also established that a wide range of doses of ondansetron were unable to support CTA (Experiment 3B and Experiment A(i), see Appendix I). Dexamethasone attenuated cisplatin-induced CTA in a dose-dependant manner (Experiment 3C). Following this, data were presented from which it was suggested that ondansetron at a dose of 0.3 mg/kg was also able to
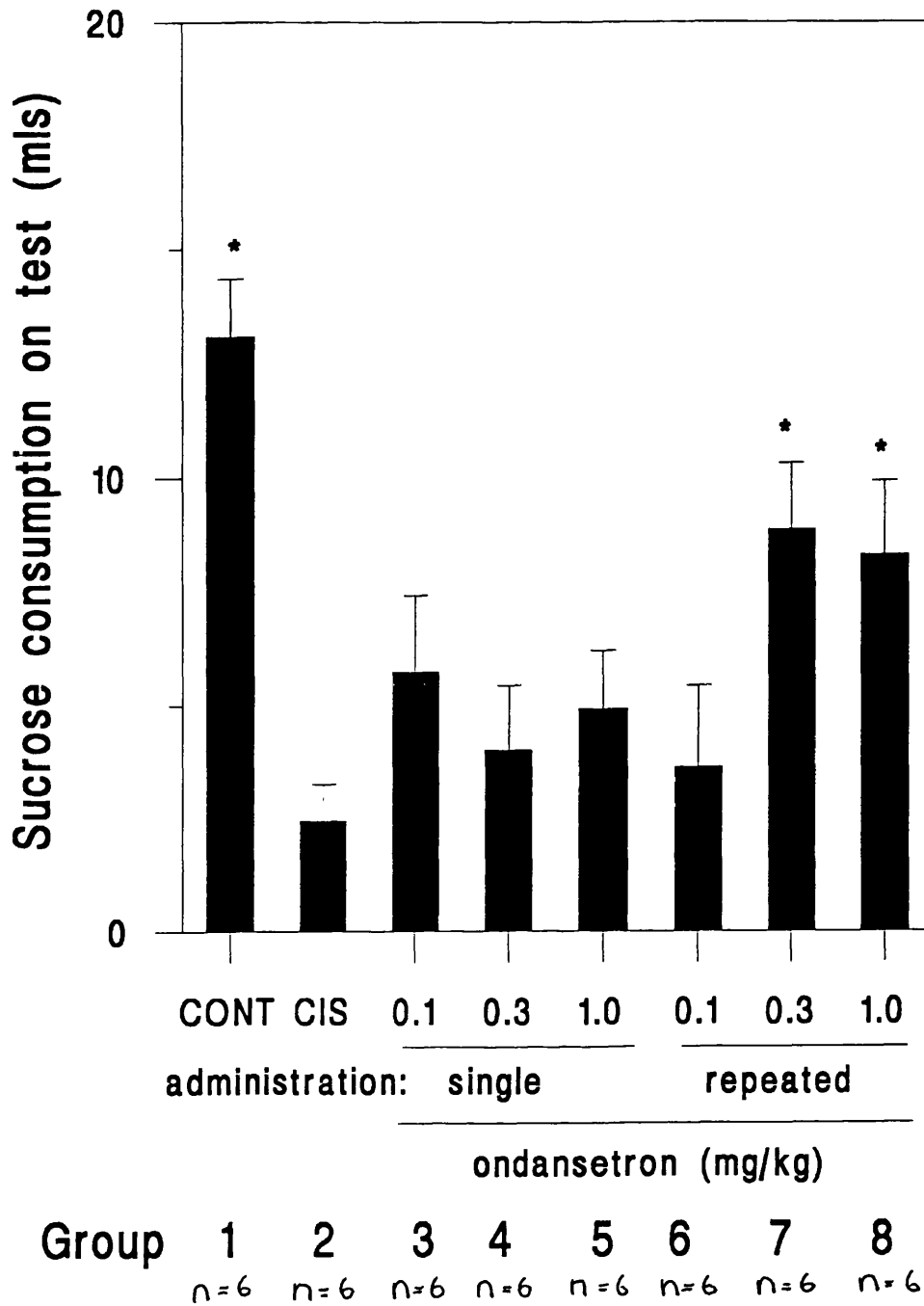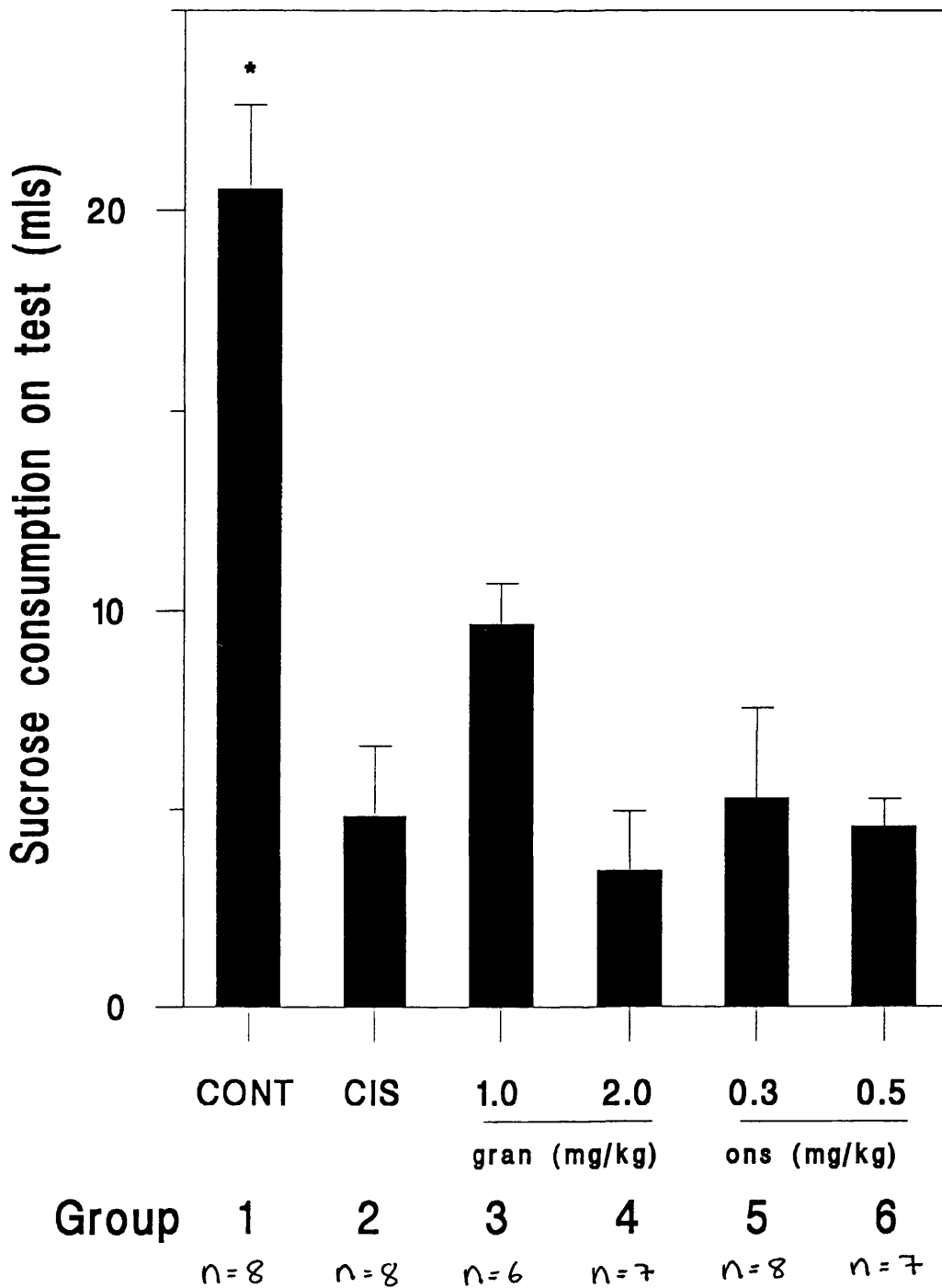
Figure 3.6. Mean consumption of sucrose solution on test in Experiment 3.F. Sucrose was paired with water (CONT), cisplatin (0.3 mg/kg) or cisplatin and either ondansetron or granisetron. Error bars indicate SEMs. * Significantly different from Group 2.

attenuate cisplatin-induced CTA (Experiment 3D and Experiment A(ii), see Appendix I), and that this effect relied on repeated administrations of ondansetron across a period of 1.5 hours (Experiment 3E). However, further experiments (e.g. Experiment 3F) failed to replicate this effect. The experiments presented in this chapter suggest that ondansetron and granisetron do, to some extent, suppress the functioning of the mechanism which gives rise to cisplatin-induced CTA. However, with the procedure used here, the effect is small and unreliable.

The procedure used in these experiments was shown to be sensitive to the effects of antiemetics on the conditioning of CTA, by the demonstration that dexamethasone attenuated cisplatin-induced CTA in a dose-dependent manner (Experiment 3C). In the analysis of the data from Experiment 3C, one datum had to be eliminated from Group 5 (0.1 mg/kg dexamethasone), the group which demonstrated an attenuation of cisplatin-induced CTA, because it lay more than two standard deviations below the mean of that group. It is unfortunate that an animal in this group should have to be eliminated post hoc, although the rejection criterion was that suggested by Tukey (1977). It is, therefore, possible that the reason for the failure to replicate the effect observed in Experiments 3D and 3E was not that the effect is unreliable but that the test was not sensitive enough for the effect to be robust.

There are two reasons why the rejection of the outlier in Group 5 of Experiment 3C should not cast doubt on the sensitivity of the procedure. First, no other data points in all of the studies presented here lay outside 2 standard deviations from the mean of the group, suggesting that the excluded animal behaved very unusually in this experiment. Second, in addition to Experiment 3C, data to be presented in Chapter 5 on the effect of $NK_1$ receptor antagonists on cisplatin-induced CTA suggest that the procedure used here is sensitive to alterations in the level of CTA brought about by antiemetic administration.

Assuming that the procedure used here was appropriate, there are two reasons why ondansetron and granisetron might have failed reliably to attenuate cisplatin-induced CTA. First, it was argued in Chapter 2 that cisplatin might induce CTA through activation of the vagus nerve and/or the area postrema (AP). It has been suggested that $5-HT_3$ receptor antagonists are active at the vagal terminals alone, and not in the AP (Andrews et al, 1990). Therefore, it is possible that the failure to report a reliable effect of ondansetron and granisetron on cisplatin-induced CTA here is due to cisplatin inducing CTA through the AP which is unaffected by $5-HT_3$ receptor antagonism. If, on the other hand, the vagus nerve plays a partial role in cisplatin-induced CTA, an effect of

ondansetron and granisetron would be expected. However, this may be difficult to detect if the AP remains active in the formation of CTA.

The second explanation for the results presented here is that antagonism of 5-HT$_3$ receptors is effective in attenuating CTA induced by cisplatin, but that the duration of action of ondansetron, even when administered three times across 1.5 hours, is not long enough to demonstrate the effect. This hypothesis was tested in the experiments reported in the next chapter. In this experiment, ondansetron was infused from subcutaneously implanted osmotic mini-pumps for a period of 24 hours following cisplatin administration.

# Chapter Four

# 5-HT$_3$ Receptor Antagonists Infused Across a 24 Hour Period in the Attenuation of Cisplatin-induced CTA

## 4.1: Introduction

Although CTA learning has been demonstrated across a CS-US delay of up to 24 hours, it is well established that a flavour presented in close contiguity with a toxin will become more aversive than one which has been trained using a procedure in which a delay is introduced between the flavour and illness (e.g. Kalat & Rozin, 1973). Thus, if the ondansetron administered in Experiment 3D was effective in blocking the vagus nerve for a period of hours, and if the vagus nerve is important in the formation of cisplatin-induced CTA (see Chapter 2 for discussion of this issue), some attenuation of cisplatin-induced CTA would be expected as a result of ondansetron administration. Of course, if cisplatin is inducing CTA through multiple routes (e.g. both through the vagus nerve and through the area postrema), then the attenuation of this CTA through the blockade of one route for a limited period of time would not be expected to be large.

Given these assumptions, one way of maximizing the impact of ondansetron administration might be to increase the length of time for which it is active. The present series of experiments was designed to test whether 5-HT$_3$ receptors mediate cisplatin-induced CTA by infusing ondansetron into the animal at a constant rate across a period of 24 hours following cisplatin administration in the CTA procedure described in Chapter 3.

## 4.2: General Method

### 4.2.1: Subjects

Male Sprague Dawley rats were used (200-300g). Housing and feeding conditions were as detailed in Chapter 3. Water consumption was controlled as below.

## 4.2.2: Apparatus

A rack with 12 cages was used for all experimental procedures. These experimental cages were identical to the home cages except that they had wire instead of sawdust floors. Twelve, 500 ml water bottles were used to allow access liquid in the experimental cages. Each of these bottles had a rubber stopper and a long metal spout with a ball bearing to minimize leakage. It was necessary to transfer the experimental cages to a separate experimental room on conditioning days, in preparation for surgery. The room used on conditioning days was very similar to the standard experimental room in terms of illumination and sound level, although the odours may have differed to some extent as a result of the proximity of the surgery. Osmotic minipumps were implanted behind the neck and used to administer a total of 221 µl of ondansetron dissolved in sterile water, across a time period of 24 hours at a rate of 9.2 µl per hour.

## 4.2.3: Procedure

On Day 1, all animals were taken off water, weighed and marked. On Day 2, the animals were weighed and placed in the experimental cages in batches of 12. Tap water was immediately presented, and the rats were allowed to drink for 20 minutes. The bottles were then removed and weighed, and the animals were replaced in the home cages. Liquid consumption in the experimental cages was recorded on each day of the experiment. Three hours following drinking training in the experimental cages, tap water was presented in the home cages for 1 hour and 10 minutes free drinking. This procedure was repeated on Day 3.

On Day 4, conditioning was carried out in the surgical recovery room in batches of twelve animals. Animals were assigned to groups pseudo-randomly with equal numbers of animals from each group in each batch. Sucrose solution (the concentration varied across experiments, see below) was presented in the experimental cage for 20 mins. Between 0 and approximately 18 minutes following sucrose consumption, the animals were removed, anaesthetized with isoflourane, and a minipump was surgically implanted. Immediately after implantation, between 2 and 20 minutes following sucrose consumption, all animals were injected with cisplatin or vehicle, and placed in a recovery cage for 30 minutes until completely mobile. Three hours after injection, tap water was presented in the home cage for 1 hour and 10 mins consumption. On Day 5, the rats were treated as on Day 2. Day 6 was the test day. On this day, sucrose was presented in the experimental cages for 20 minutes. The animals were then replaced in the home cages and

allowed free access to water.

## 4.3: Experiments

### 4.3.1: Experiment 4A Ondansetron infused across a 24 hour period: The effect on cisplatin-induced CTA

The initial study in this series used a 3% (w/v) sucrose solution, which was paired with 0.3 mg/kg cisplatin. This procedure has been shown to induce a CTA after a single pairing (see Experiment 3A). The doses of ondansetron tested were 0.5, 0.13 and 0.05 mg/kg/hour across a 24 hour period. Because of the solubility of ondansetron, the dose of 0.5 mg/kg/hour was the highest that could be given. Five groups were tested: Group 1 received vehicle both in the minipumps and by injection following surgery; Group 2 received vehicle and cisplatin; Groups 3-5 received ondansetron (0.5, 0.13 and 0.05 mg/kg/hour) in the minipumps, and cisplatin following surgery. The vehicle used in the preparation of cisplatin and ondansetron in all of the experiments presented in this chapter was sterile water.

## Results and Discussion

During the experiments presented in this chapter, some animals were discarded because of failure of the minipumps following implantation. Thus, two criteria applied for inclusion of animals in the statistical analyses: evidence that the pump had emptied during the infusion period and consumption of sucrose on Day 4 greater than 5 mls. Two animals were discarded from the analyses presented here, one each from Groups 1 and 2 due to failure of the minipumps. Sucrose consumption on test is presented in Fig 4.1. It appears that the animals in Group 1 drank more than those in Group 2 (the cisplatin-treated animals) and, furthermore, that animals in Groups 3-5 also drank more sucrose on test than those in Group 2. However, a one-way ANOVA carried out on these data found no effect of treatment ($F_{(4,33)}$ = 1.7). It would seem then, that administration of cisplatin did not induce an aversion towards the paired sucrose solution.

In Experiment 3A, and in a number of subsequent replications, a single pairing of 3% sucrose solution with 0.3 mg/kg cisplatin gave rise to a reduction in consumption of sucrose on test compared with controls. One possible explanation for this discrepancy is that the anaesthetic procedure used here interfered with the formation of an aversion to sucrose in some way. It is not

Figure 4.1. Mean consumption of sucrose on test. Sucrose was paired with water (CONT), cisplatin (0.3 mg/kg) or cisplatin and ondansetron. Ondansetron was infused from ninipumps across a 24 hour period. Error bars indicate SEMs.

clear how anaesthesia alone could have disrupted the CTA towards sucrose in this experiment. It has been found that the administration of a toxin to an anaesthetized animal will lead to the development of an aversion towards a previously presented flavour (Buresova & Bures, 1977), even when the effects of the toxin are experienced while the animal remains under anaesthetic. In the present experiment, cisplatin was administered, on average, ten minutes before the animal regained consciousness. Thus, since the sucrose was presented before the anaesthetic was administered, it is likely that both the flavour and the illness were experienced while the animal was conscious.

However, the use of anaesthetic in this experiment altered the parameters from those used in Experiment 3D in two other respects: there was a longer average delay between sucrose presentation and cisplatin administration (approximately ten minutes compared to an average of five minutes in Experiment 3D), and there was the experience of the distinctive odour of isoflorane between sucrose presentation and cisplatin administration. Increasing the CS-US delay may have lead to a weakening of the association between these two stimuli which may have reduced the strength of the aversion on test. Instead or in addition, the odour may have overshadowed the aversion towards the sucrose solution. Westbrook. Homewood. Horn and Clarke (1983) found that a novel odour overshadowed an aversion towards a novel flavour in a toxicosis conditioning procedure. The experiments which follow aimed to increase the potential for an association between sucrose and cisplatin-induced illness

### 4.3.2: Experiment 4B Ondansetron infused across a 24 hour period: Increasing the CS intensity

In an attempt to increase the strength of the flavour-illness association using a similar procedure to that of Experiment 4A. the concentration of the sucrose solution used in the present experiment was increased to 6% w/v. In most other respects the procedure was similar to that used in Experiment 4A. The doses of ondansetron used were altered in order to increase the possibility of detecting dose dependency in the administration of ondansetron.   In Experiment 4A, consumption of sucrose solution was similar across all groups given ondansetron (Groups 3-5). This would indicate that. were an aversion to sucrose to be demonstrated, and an attenuation of this aversion to be shown as a result of the administration of ondansetron, the attenuation would be equal across all ondansetron-treated groups. Five groups were used in the present experiment: Group 1 received vehicle: Group 2 received vehicle and cisplatin (0.3 mg/kg); Groups 3-5 received

Figure 4.2. Mean consumption of sucrose on test. Sucrose was paired with water (CONT), cisplatin, or cisplatin and ondansetron. Ondansetron was infused from minipumps across a 24 hour period. Error bars indicate SEMs.

ondansetron (0.005, 0.05 and 0.5 mg/kg/hour, respectively) and cisplatin (0.3 mg/kg).

## Results and Discussion

Two animals in Group 5 were excluded from the analysis due to failure of the minipumps. The consumption of sucrose solution on test is presented in Fig 4.2. Again, it would seem that some reduction in consumption of sucrose solution occurred in Group 2 compared to Group 1, suggesting that an aversion developed towards sucrose in this group. A dose dependant attenuation of this aversion is suggested by the sucrose consumption of Groups 3-5. However, a one-way ANOVA on these data found no effect of treatment ($F_{(4,33)}$ = 2.44). Thus, increasing the concentration of sucrose solution paired with cisplatin was unsuccessful in producing a reliable taste aversion using this procedure.

### 4.3.3: Experiment 4C Ondansetron infused across a 24 hour period: Increasing the US intensity

It has been suggested that there are two determinants of the rate of learning of an association between a CS and US: as well as the intensity of the CS, the intensity of the US is thought to determine the learning rate (Rescorla and Wagner, 1972). Thus, in the final experiment in this series, a further attempt was made to demonstrate a CTA using a procedure in which isoflorane anaesthesia was induced following sucrose consumption, and prior to an injection of cisplatin. In this experiment, the intensity of the US was increased.

The sucrose solution used in Experiment 4B (6% w/v) was used. In addition, the concentration of cisplatin was increased from 0.3 to 0.6 mg/kg in an attempt to demonstrate a reliable aversion towards sucrose solution and thereby any effects of administration of ondansetron by minipump. The method was the same as that used in Experiment 4B, except that the dose of cisplatin was 0.6 mg/kg.

## Results and Discussion

Minipump failure led to the rejection of five animals from the statistical analysis, two from each of Groups 2 and 5, and one from Group 4. The test day data are shown in Figure 4.3. It can be seen that consumption of sucrose on test in Groups 2 - 5 is lower than that of Group 1. This
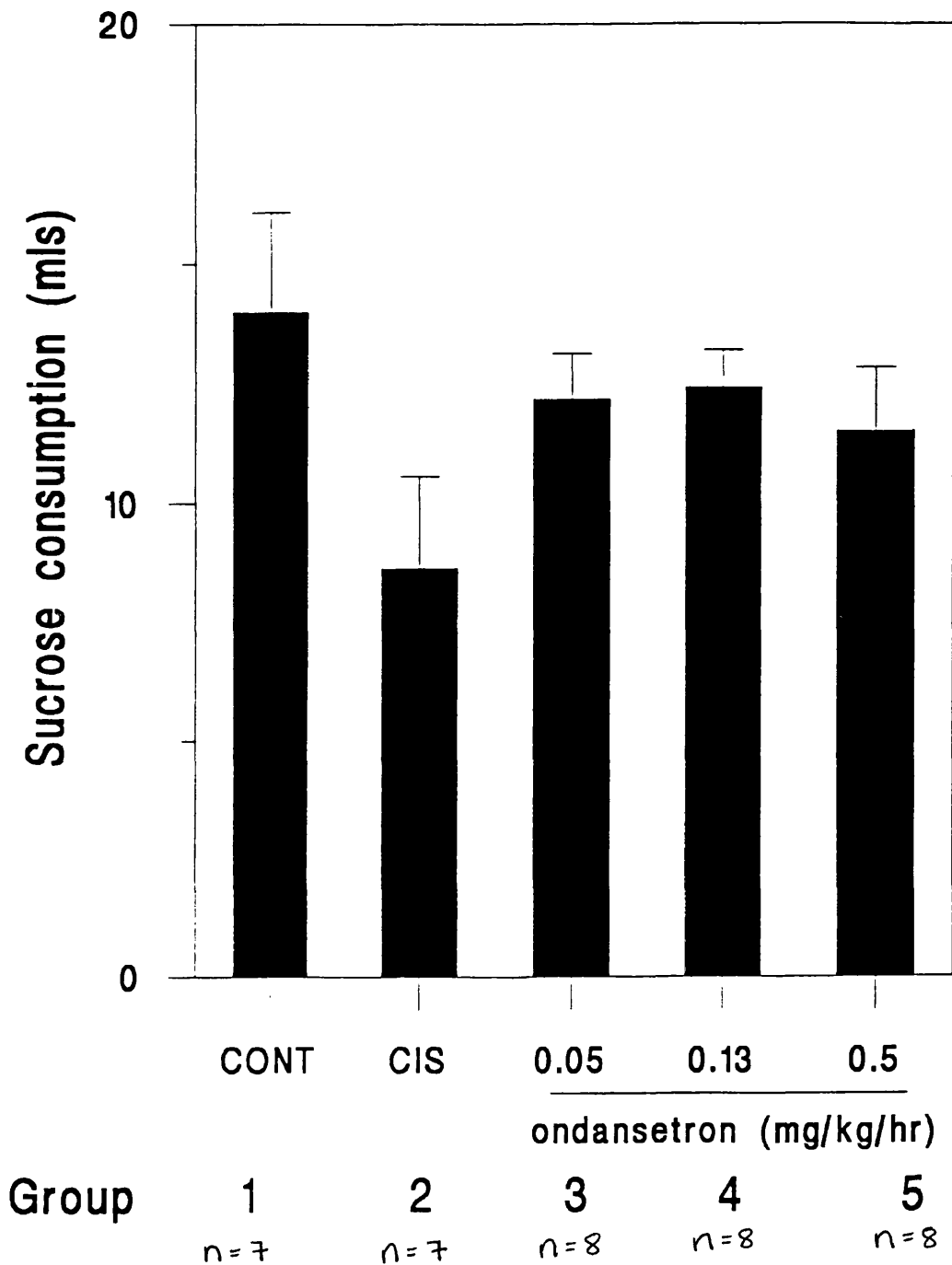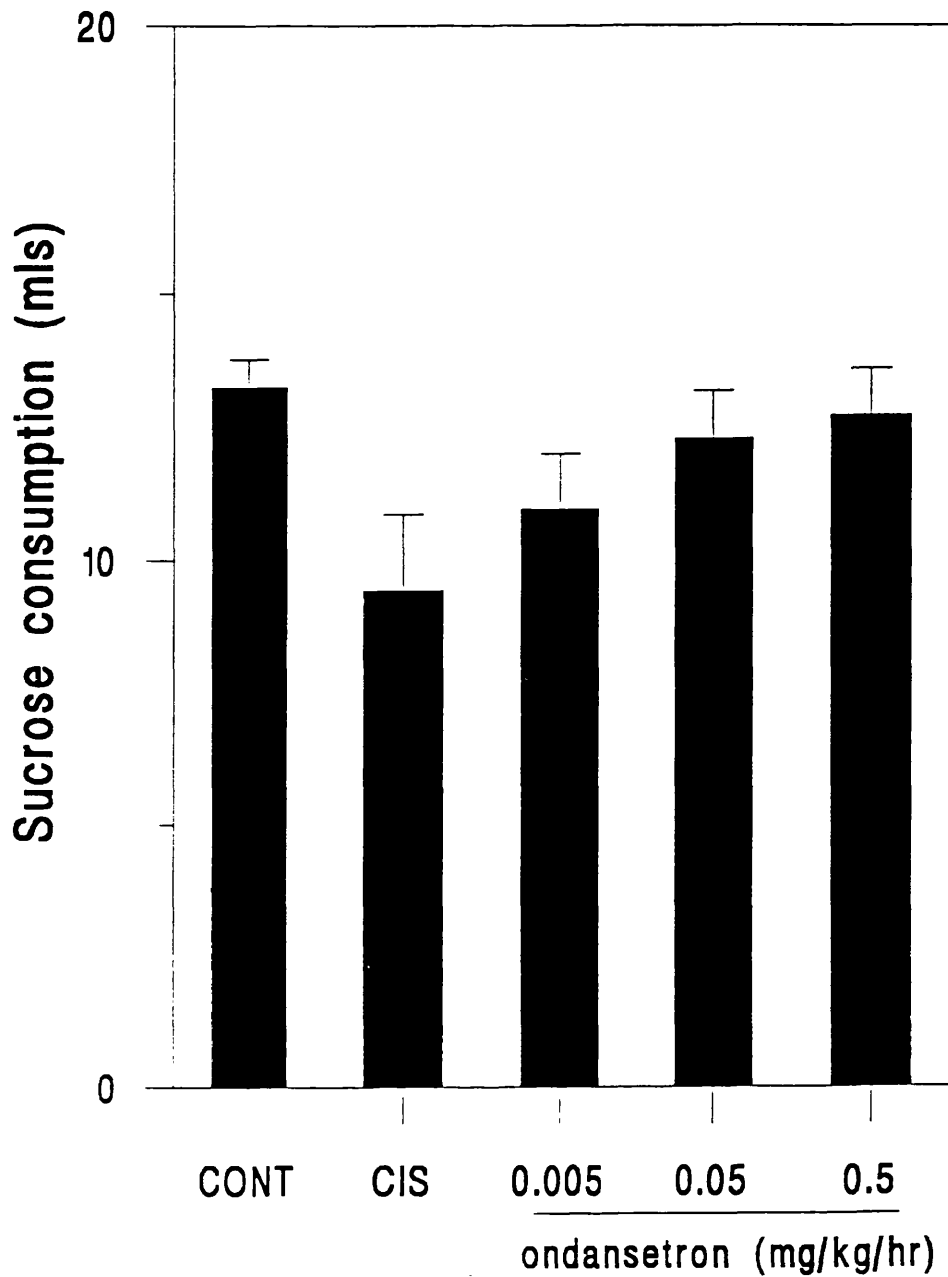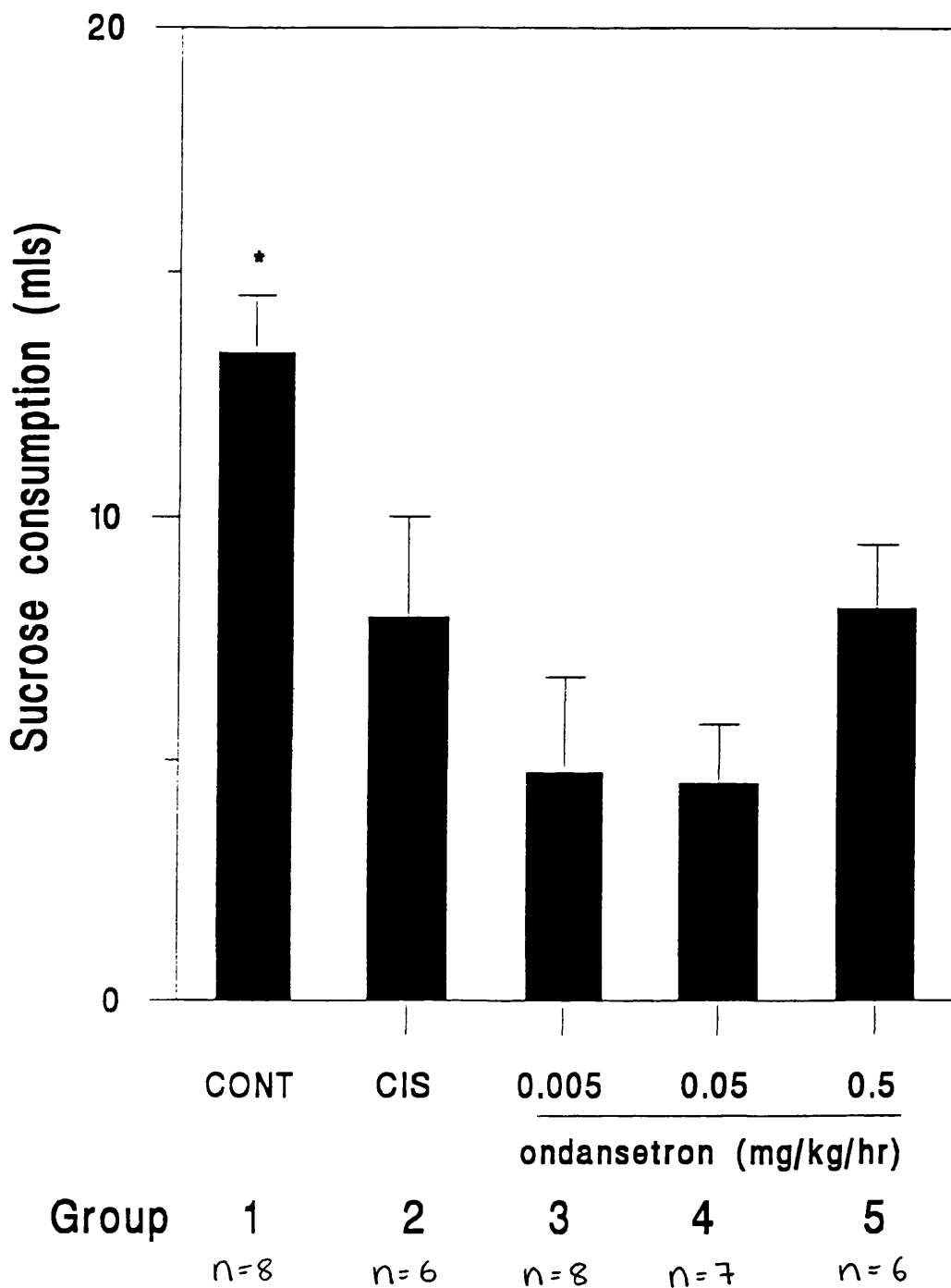
Figure 4.3. Mean consumption of sucrose on test. Sucrose was paired with water (CONT), cisplatin (0.6 mg/kg), or cisplatin and ondansetron. Ondansetron infused across 24 hours. Error bars indicate SEMs. * Significantly different from Group 2.

would suggest that an aversion towards sucrose developed in the groups treated with cisplatin. Indeed, a one-way ANOVA on the data revealed an effect of treatment ($F_{(4,30)}$ = 6.04). However, there does not appear to be any difference in sucrose consumption between the animals treated with ondansetron and cisplatin (Groups 3 - 5) and those treated with cisplatin alone (Group 2). A Newman Keuls post-hoc analysis indicated that Group 1 (the vehicle controls) consumed more sucrose on test than all other groups. No other differences were reliable. These data suggest that an aversion developed towards sucrose solution as a result of cisplatin administration during conditioning, but that treatment with ondansetron did not attenuate this aversion.

It is clear from these data that the problems encountered in the induction of CTA in the previous experiments in which anaesthesia was used (Experiments 4A and 4B) were overcome; a strong and reliable suppression of consumption of sucrose occurred in all groups which received cisplatin during training. However, the data showed that ondansetron was ineffective in attenuating cisplatin-induced CTA when a dose of 0.6 mg/kg cisplatin was used as the US in this procedure. These data therefore suggest that the mechanism of action of cisplatin in the induction of CTA is not mediated by 5-HT$_3$ receptors.

## 4.4: General Discussion

Three experiments were presented in which ondansetron was administered through subcutaneous osmotic minipumps in an attempt to demonstrate an attenuation of cisplatin-induced CTA by the blockade of 5-HT$_3$ receptors across a period of 24 hours. In Experiment 4A, cisplatin (0.3 mg/kg) failed to induce a CTA. It was thought that the anaesthetic used in the implantation of the minipumps (isoflorane) disrupted the formation of a CTA towards 3% sucrose solution when it was paired with cisplatin. A second attempt was made at testing the efficacy of ondansetron in the attenuation of cisplatin-induced CTA using the same procedure but increasing the CS intensity; 6% sucrose solution was paired with 0.3 mg/kg cisplatin (Experiment 4B). Again, no effect of cisplatin on sucrose consumption was observed on test. In the final experiment, both a high intensity CS (6% sucrose solution) and a high intensity US (0.6 mg/kg cisplatin) were used in order to demonstrate a CTA using the anaesthetic procedure. Although a reliable taste aversion was demonstrated using these stimuli, no effect was demonstrated of ondansetron on the CTA which was formed towards sucrose solution.

In ferrets, vomiting is initiated approximately 1 hour following i.v. administration of cisplatin

(Andrews et al, 1988), and therefore, it is unlikely that unconsciousness during the 10 minutes following i.p. injection of cisplatin is sufficient to disrupt a cisplatin-induced CTA. Thus, the disruption of CTA in these experiments is unlikely to have been due to a reduction in the level of nausea experienced by the animal as a direct result of anaesthesia, since the administration of cisplatin occurred approximately 10 minutes before the animals recovered consciousness. In addition, there is some evidence suggesting that CTA formation is not dependant on the conscious state of the animal (Buresova & Bures, 1977), rendering such an explanation for the disruption effect found in the present experiments even more implausible.

It is more likely that disruption of CTA through anaesthetic administration was due to overshadowing of the aversion towards the taste by the stimuli present in the anaesthetic box. The most salient of the cues in this context would appear from casual observation to be the odour of the anaesthetic. Westbrook et al (1983) found that a novel odour attenuated an aversion towards a taste with which it was presented before poisoning. In the present case, the animals were exposed to the odour following sucrose consumption and before cisplatin administration, and therefore the odour was more contiguous with illness than was the flavour. These would seem to be conditions in which the odour might overshadow an aversion towards sucrose. However, Experiments 4B and 4C do not speak to this issue. Both an increase in CS and an increase in US intensity might be expected to overcome the disruptive effects of the anaesthetic, regardless of the reason for the disruption. Thus, it is not surprising that increasing the CS and US intensity in Experiment 4C led to the demonstration of a reliable CTA towards sucrose in this experiment.

Of course, the aspect of these experiments which is of greatest interest is the effect of ondansetron on the cisplatin-induced CTA. It is clear that the only experiment in which the effect of ondansetron on the induction of CTA by cisplatin can be assessed is Experiment 4C, since this is the only experiment in which a CTA formed towards sucrose. In Experiment 4C, no attenuation of the CTA by ondansetron was demonstrated. This result would seem to indicate that cisplatin does not, in fact, induce CTA through 5-HT$_3$ receptor mediated pathways. However, it is possible that the effect of ondansetron is dependent on the dose of cisplatin used. There are two lines of reasoning which support the notion that ondansetron may attenuate low-dose cisplatin-induced CTA, while having no effect on CTA induced by higher doses of cisplatin.

The first argument comes from work carried out on radiation-induced vomiting. It has been argued that effects of lesions on vomiting do not necessarily imply similar effects on CTA (Grant,

1987). However, in the case of radiation, there is some evidence for a parallel between the mechanism underlying vomiting and that underlying CTA; Rabin and Lee (1986) found that lesions to the AP attenuated both vomiting and CTA in cats. Therefore, some data will be presented here relating to the effect of the dose level of radiation in the induction of vomiting. It was pointed out in Chapter 2, that it is possible that high dose radiation is mediated by action on the area postrema (AP), whereas the response to low dose radiation is detected only by the vagus nerve in the induction of emesis. Since the emetic responses to both cisplatin and radiation are thought to be pharmacologically similar (Andrews et al, 1988), and the anti-emetic effect of ondansetron is vagally mediated, administration of ondansetron might be expected to have the effect of attenuating the emetic response to low dose cisplatin to a greater extent than it would the emetic response to high dose cisplatin. This would occur because, at high doses of cisplatin, both vagal and AP mechanisms would be recruited and the latter would be relatively unaffected by 5-HT$_3$ receptor antagonists. Overall, however, it appears that 5-HT$_3$ receptors play little, if any, role in the genesis of the CTA induced by cisplatin.

The second line of argument comes from the experiments reported in this Chapter. Although no individual experiment using 0.3 mg/kg cisplatin showed a reliable effect of cisplatin on sucrose consumption on test, all three experiments which were carried out using this dose of cisplatin in this procedure (Experiment 4A, 4B and a replication of Experiment 4B which is not reported) showed that, numerically, the mean consumption of sucrose of the animals which received cisplatin (Group 2) was lower than that of those that were not poisoned (Group 1), and the sucrose consumption of the animals which received the highest dose of ondansetron (0.5 mg/kg/hour; Group 3) was higher than the poisoned controls (Group 2). This would suggest an attenuation of a cisplatin-induced CTA by 0.5 mg/kg/hour ondansetron. It is possible that an effect of ondansetron on cisplatin-induced CTA would be demonstrable through the use of a procedure in which a low dose of cisplatin is paired with sucrose solution on a number of occasions, thus allowing the conditioning of a strong aversion while maintaining a low dose level of cisplatin.

A number of experiments have been presented here and in the preceding chapter which suggest that 5-HT$_3$ receptors might play some role in the formation of cisplatin-induced CTA. However, there is no procedure used here that has demonstrated a reliable effect of this sort. It is possible that ondansetron does not have an effect on cisplatin-induced CTA. However, it is also possible that the use of a procedure such as that employed by Revusky and Martin (1988), in which animals receive multiple flavour-toxin pairings with a low dose of toxin, might reveal some, albeit

small, effect of ondansetron in the attenuation of cisplatin-induced CTA.

# Chapter Five

# NK$_1$ Receptor Antagonists in the Attenuation of Cisplatin-induced CTA

## 5.1: Introduction

Research investigating the localization of NK$_1$ receptors has highlighted two brain areas which may be especially important in CTA formation which also have a high density of tachykinin NK$_1$ receptors (Maeno et al, 1993): the nucleus of the solitary tract (NST) and the parabrachial nucleus (PBN). The PBN has not been seen as significant in the induction of vomiting, but lesions of this area block the formation of CTA induced by a number of different toxins (Bielavska & Bures, 1994). In addition, the NST is thought to be important in the induction of the vomiting response towards all emetic stimuli (Borison & Wang, 1953). Thus, there is strong circumstantial evidence that selective NK$_1$ receptor antagonists may block the formation of CTA. Two such compounds have been examined, CP-99,994 ((+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine (McLean, Ganong, Seymour, Snider, Desai, Rosen, Bryce, Longo, Reynolds, Robinson, Schmidt, Siok & Heym 1993) and L-742,694 (structure withheld by Merck Sharp and Dohme).

The method used in the three experiments presented in this chapter was the same as that used in the experiments in Chapter 3. Thus, rats were exposed to a single pairing of sucrose and cisplatin, and the dose of cisplatin used was 0.3 mg/kg. Evidence from the experiments carried out on the efficacy of NK$_1$ receptor antagonists (including CP-99,994 and L-742,694) on vomiting in ferrets, suggests that, unlike ondansetron, these compounds suppress the action of the emetic system for a number of hours. Ferrets receiving ondansetron and cisplatin showed a vomiting response after a two hour period following cisplatin infusion (Rycroft, personal communication), while those treated with CP-99,994 demonstrated little or no vomiting for the duration of a 7 hour observation period following infusion of cisplatin (Bountra et al, 1993). These data would suggest that the half life of the NK$_1$ receptor antagonists is longer than that of ondansetron.

In the experiments presented in Chapter 3, ondansetron was administered repeatedly across a period of 1.5 hours following cisplatin infusion in order to increase the effective half life of the compound without increasing the dose administered on any one occasion. If the NK$_1$ receptor

antagonists tested in the experiments in this chapter have a longer duration of action in suppression of the emetic response than ondansetron, it is possible that the repeated administration of these compounds will not be necessary to demonstrate an attenuation of cisplatin-induced CTA. Thus, in the experiments presented here, the antiemetics were administered acutely, directly following cisplatin infusion.

The procedure used in all of these experiments, including those from Chapters 3 and 4, and the present chapter, used rats as subjects. It is clear that this species is convenient for the demonstration of CTA; the majority of experiments in the area of CTA have been carried out on rats. This aspect of the experiments reported below presents a particular problem due to the nature of the $NK_1$ receptor system of the rat. It has been found that the $NK_1$ receptors of the rat are different from those of the guinea pig (Saffroy, Beaujouan, Petitet, Torrens & Glowinski, 1994), for which CP-99,994 has a high affinity ($K_i$ = 0.25 nM (McLean et al, 1993)). The corresponding affinity of CP-99,994 for the rat $NK_1$ receptor is therefore low ($K_i$ = 1.0 $\mu$M). Other species which share the guinea pig $NK_1$ receptor type are man, ferret and pig. Resources precluded the use of other species, and it was decided that a test using the previously developed rat model was potentially informative; an increase in the dose level from that found to be effective against vomiting in ferrets should allow effective $NK_1$ receptor antagonism by CP-99,994 in the rat.

Three experiments were carried out, the first tested CP-99,994 against cisplatin-induced CTA while the second and third tested L-742,694 at different doses.

## 5.2: General Method

The general method used was the same as that used in the experiments presented in Chapter 3 except in the following respects. All the subjects used were experimentally naive, male Sprague Dawley rats (200-300g). In all of the experiments presented here, the CS was 3% sucrose solution which was presented twice, first on the conditioning day (Day 4) and then for 20 minutes on the test day (Day 6).

## 5.3: Experiments

### 5.3.1: Experiment 5A The effect of CP-99,994 on cisplatin-induced CTA

In this experiment, CP-99,994 was tested in order to determine its efficacy in the attenuation of cisplatin-induced CTA. The doses of CP-99,994 used were 1, 3 and 10 mg/kg. These doses were tested against 0.3 mg/kg cisplatin. Five groups were used. Group 1 received vehicle, Group 2 received vehicle and cisplatin, while Groups 3-5 received CP-99,994 (1, 3 and 10 mg/kg respectively) and cisplatin. Cisplatin and its vehicle (sterile water) were administered in a volume of 1 ml/kg i.p. CP-99,994 and its vehicle (5% methyl cellulose) were administered in a volume of 1 ml/kg s.c.

## Results and Discussion

Consumption of sucrose solution on test is presented in Figure 5.1. Examination of the sucrose consumption data suggests that the animals in Group 2 drank significantly less sucrose on test than those in Group 1, indicating that an aversion developed towards sucrose in Group 2. A one-way ANOVA found a reliable effect of treatment ($F_{(4,35)}$ = 17.35). A Dunnets post-hoc comparison, with Group 2 as the control group, revealed a difference between Group 2 and Group 1, indicating that a reliable aversion developed towards sucrose in Group 2. However, no other differences were significant. Thus, it would appear that CP-99,994 did not give rise to an attenuation of cisplatin-induced CTA.

As was suggested in the case of ondansetron, it may be the case that CP-99,994 successfully suppressed the activity of the mechanism underlying cisplatin-induced CTA, but that this suppression had too short a duration of action for an effect on the expression of CTA to be demonstrated. However, this explanation of the present results is unlikely. CP-99,994 is effective in the blockade of cisplatin-induced emesis in the ferret (at a dose of 3 mg/kg) for at least 4 hours following administration (Rycroft, personal communication). Thus, it would seem more likely that the doses used here were too low to demonstrate an effect of CP-99,994 in the rat. It was concluded that the failure to demonstrate an effect of CP-99,994 on cisplatin-induced CTA was either due to the low affinity of this compound to the rat $NK_1$ receptor, or that the $NK_1$ receptor is not important in the formation of cisplatin-induced CTA.

Figure 5.1. Mean consumption of sucrose on test. Sucrose was paired with water (CONT), cisplatin (0.3 mg/kg), or cisplatin and CP-99,994. Error bars indicate SEMs. * Significantly different from Group 2.

## 5.3.2: Experiment 5B The effect of L-742,694 on cisplatin-induced CTA

The second experiment in this series tested the ability of the $NK_1$ receptor antagonist L-742,694 to block cisplatin-induced CTA. Six groups were used. Group 1 received vehicle, Group 2 received cisplatin (0.3 mg/kg) and vehicle. Groups 3 - 6 were treated with cisplatin (0.3 mg/kg) and L-742,694 (1, 3, 10 and 30 mg/kg, respectively). All drugs were administered in a volume of 1 ml/kg. The vehicle for cisplatin was sterile water, while L-742,694 was suspended in 0.5% methyl cellulose. The experiment was carried out in two replications (n=8 per replication).

## Results and Discussion

Two animals in Group 6 were excluded from the analysis because they consumed less than 5 mls of sucrose solution during the conditioning phase of the experiment. A two-way ANOVA carried out on the remaining animals failed to show any effect of replication, or any replication by treatment interaction (F < 1 in both cases). Thus, the data from the two replications was pooled for further analysis. The consumption data from the present experiment are shown in Figure 5.2. Examination of the figure suggests that cisplatin-treated animals consumed less sucrose on test than those in Group 1. A one-way ANOVA was carried out on the data which revealed a significant main effect of treatment ($F_{5,88}$ = 23.21). A Dunnets post-hoc comparison, in which Group 2 was taken as the control, showed a reliable difference between Groups 1 and 2, suggesting than an aversion towards sucrose solution developed in Group 2. However, no other groups differed from Group 2. It would seem, therefore, that an aversion towards sucrose resulted from the pairing of this flavour with cisplatin, and that L-742,694 failed to attenuate this aversion. However, a final attempt to establish the efficacy of L-742,694 was made in which the compound was given at the high dose of 100 mg/kg.
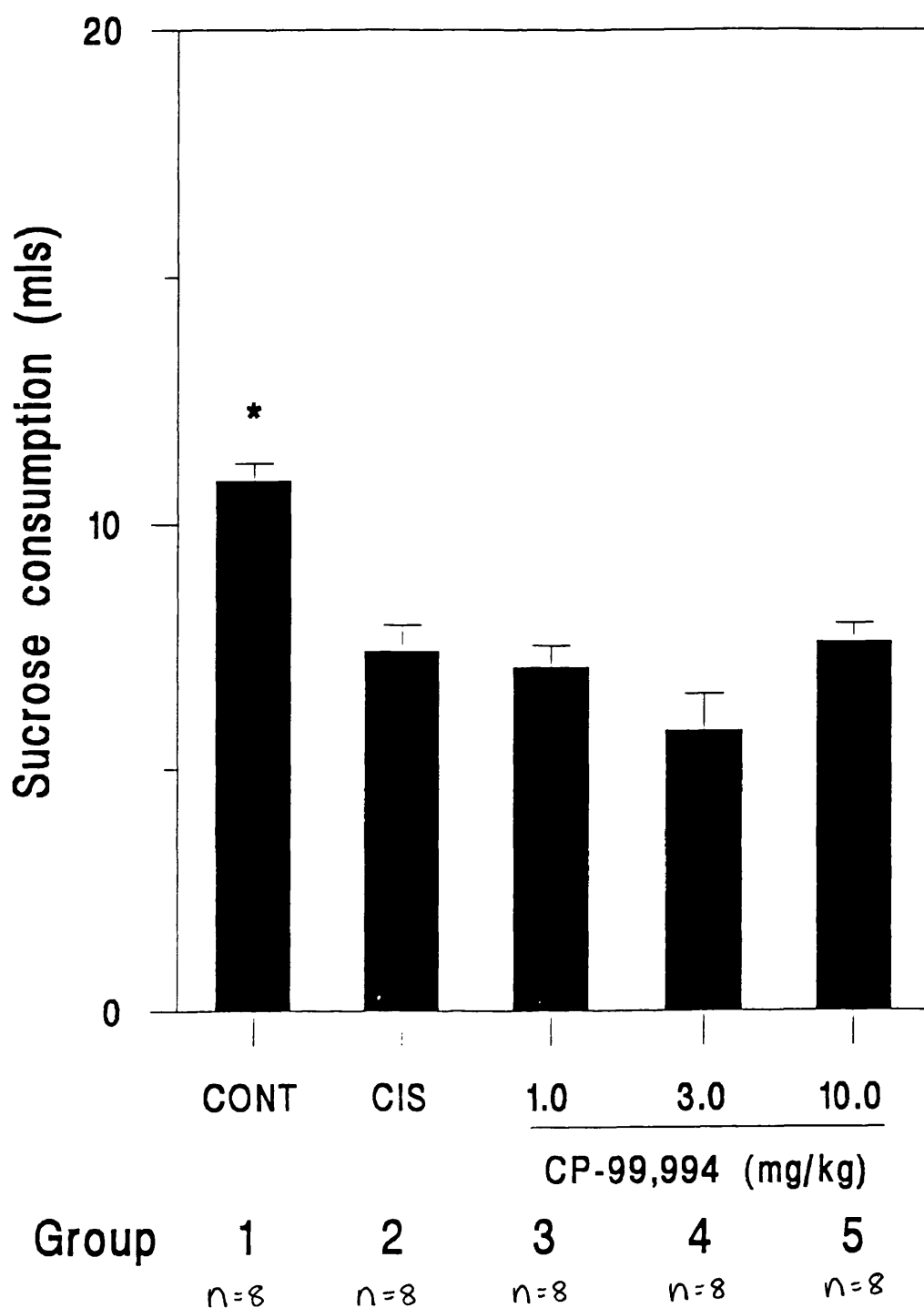
Figure 5.2. Mean consumption of sucrose on test. Sucrose was paired with water (CONT), cisplatin (0.3 mg/kg), or cisplatin and L-742,694. Error bars indicate SEMs. * Significantly different from Group 2.

### 5.3.3: Experiment 5C High-dose L-742,694 attenuates cisplatin-induced CTA

Three groups were used: Group 1 received vehicle following sucrose consumption; Group 2 received vehicle and cisplatin (0.3 mg/kg); Group 3 received 100 mg/kg L-742,694 and cisplatin (0.3 mg/kg). All drugs were administered in the same manner as in Experiment 5B.

### Results and Discussion

The sucrose consumption data are presented in Figure 5.3. Both Groups 2 and 3 drank less sucrose on test than Group 1, consistent with the formation of a cisplatin-induced CTA. A one-way ANOVA revealed a significant effect of treatment $(F_{(2,21)} = 35.36)$. Two pre-planned orthogonal contrasts indicated that Groups 2 and 3 consumed less sucrose on test than Group 1 $(F_{(1,21)} = 65.3)$, and that Group 3 drank more sucrose on test than Group 2 $(F_{(1,21)} = 5.41)$. Thus, L-742,694 (100 mg/kg) attenuated a cisplatin-induced CTA towards sucrose in this experiment. However, the attenuation is not very large; the mean consumption of sucrose on test for the L-742,694 treated animals was 7.9 mls compared to 5.6 mls in the poisoned controls, whereas the non-cisplatin treated controls consumed 13.9 mls of sucrose solution.

### 5.4: General Discussion

The three experiments presented here showed that CP-99,994 (1-10 mg/kg) and L-742,694 (1-30 mg/kg) did not attenuate cisplatin-induced CTA, but that the higher dose of 100 mg/kg L-742,694 gave rise to a small but statistically significant increase in sucrose consumption on test when administered with cisplatin following sucrose presentation during training. These data suggest that $NK_1$ receptors play some part in mediating cisplatin-induced CTA. Although L-742,694 has very high affinity for the human, ferret and gerbil $NK_1$ receptor $(K_i = 0.1nM)$, its affinity for the rodent homologue is considerably lower $(K_i = 80 Nm)$. In functional assays in vivo in the gerbil and ferret, the active dose range of L-742,694 is 0.1 - 1 mg/kg and extrapolation to the rat indicates that a dose range of 80 - 800 mg/kg would be required for an equivalent degree of receptor occupation.

A similar argument can be applied to CP-99,994 $(K_i$ human = 6 nM; rat = 1 μM). The active dose range for CP-99,994 in the gerbil and ferret is 1-10 mg/kg, and so doses in excess of 100 mg/kg would be required in the rat. Higher doses were not admitted, however, since the compound has
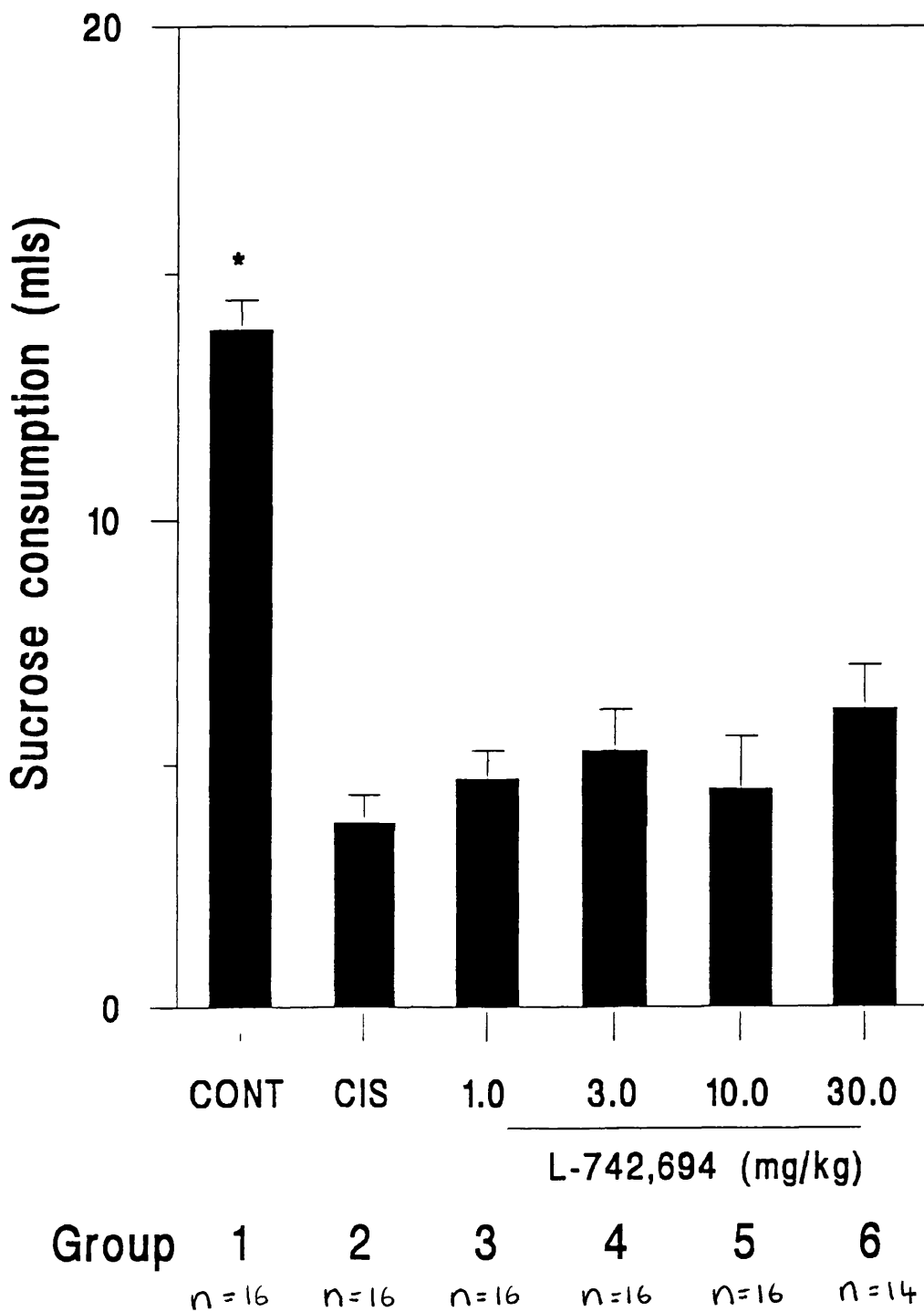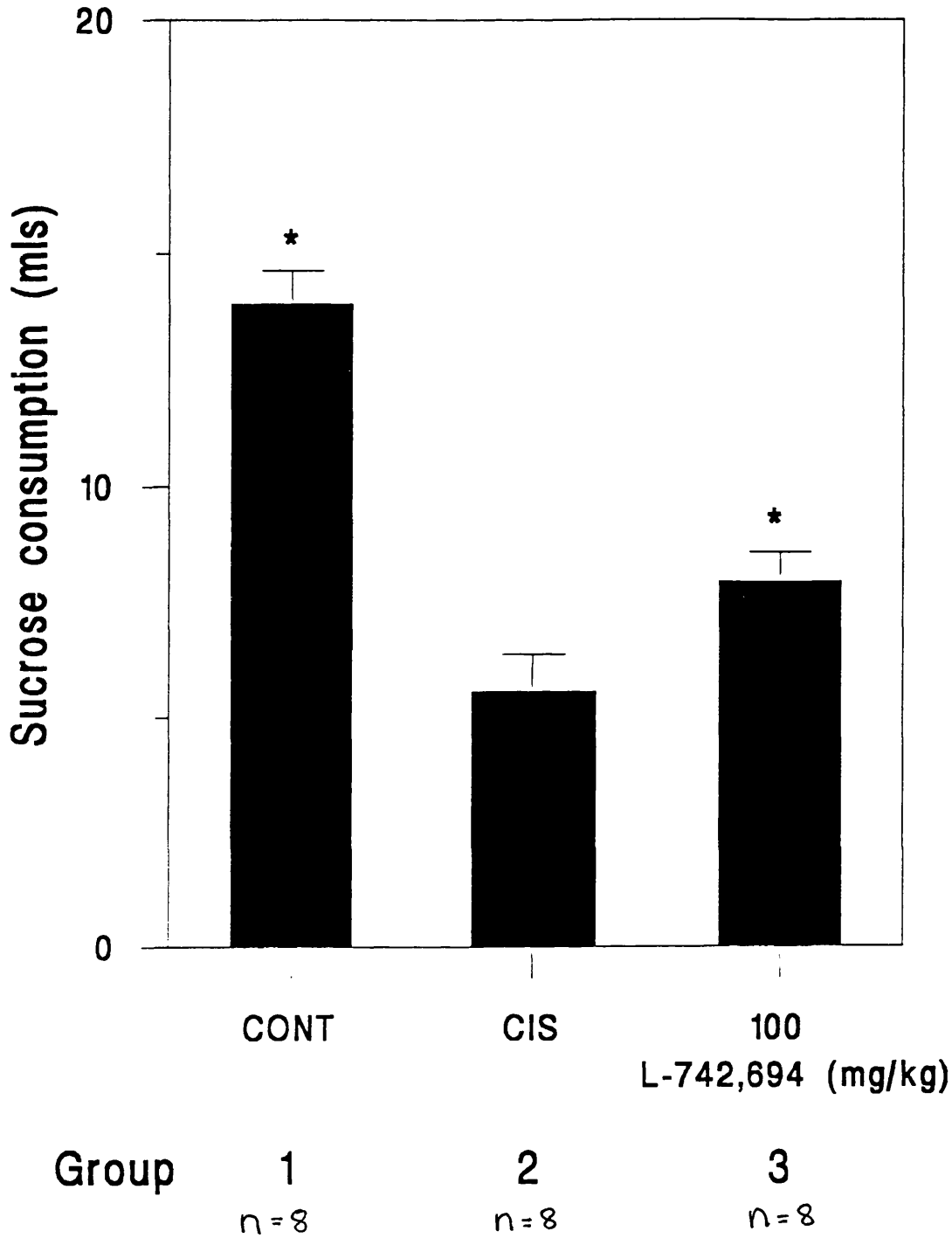
Figure 5.3. Mean consumption of sucrose on test. Sucrose was paired with water (CONT), cisplatin (0.3 mg/kg), or cisplatin and L-742,694 (100 mg/kg). Error bars indicate SEMs. * Significantly different from Group 2.

affinity for verapamil-sensitive $Ca^{2+}$ channels similar to its affinity for the rat $NK_1$ receptor (McLean et al, 1994) and induces behavioral activation at doses in excess of 10 mg/kg. Thus, until a compound with high affinity for the rat receptor with good brain penetration and bioavailability becomes available, it will not be possible to test properly the involvement of the $NK_1$ receptor in the formation of cisplatin-induced CTA

It was argued in Chapter 2 that if antagonism of $NK_1$ receptors interferes with cisplatin-induced CTA, then the most likely sites of action are the NST and the PBN. The high density of $NK_1$ receptors in these areas (Maeno et al, 1993), and the fact that these areas have been shown in lesion studies to be involved in the production of CTA (Bielavska & Bures, 1994) supports this claim. There is, of course, no conclusive evidence from the experiments presented here that either of these brain areas are responsible for the effect of L-742,694 on cisplatin-induced CTA. One way to test this hypothesis would be to monitor the cisplatin-induced c-fos protein expression in the NST and PBN as a result of cisplatin or cisplatin plus L-742,694 administration. A reduction in cisplatin-induced c-fos expression in either or both of these areas might be expected as a result of L-742,694 administration. This technique was used by Reynolds et al (1991) in the investigation of the route of action of $5$-$HT_3$ receptor antagonists (described in Chapter 2).

It is interesting to note that, if $NK_1$ receptor antagonism is responsible for the attenuation of CTA indicated in Experiment 5C, it is likely that L-742,694 will be effective in the attenuation of CTA induced by a number of preparations which give rise to this response. Bielavska & Bures (1994) have demonstrated that temporary blockade of the PBN, by infusion of tetrodotoxin into this area, gives rise to an attenuation of CTA induced by a range of drugs (lithium chloride, D-amphetamine and carbachol). In addition, in experiments in which manipulations such as area postrema (AP) lesions and vagotomy have led to a decrease in the intensity of CTA induced by a specific drug (see Grant, 1987 for review), it is assumed that the detection of that drug through one of these areas leads to activation of the NST which, in turn, leads to the formation of CTA. Therefore, it is possible that stimulation of the NST, and stimulation of the PBN, is necessary for formation of CTA, regardless of the manipulation which gives rise to this response. It might then be concluded that, if L-742,694 is effective in attenuating cisplatin-induced CTA through its activity at either the NST or PBN, antagonism of $NK_1$ receptors will attenuate CTA induced by any drug.

Further implications of the finding that L-742,694 attenuates CTA are suggested by work carried out by Revusky and Martin (1988). They noted a concordance between the antiemetics which give

rise to a reduction in gut-related distress in the clinic and those which attenuate CTA in the rat. They found that dexamethasone and prochlorperazine reduced both gut-related distress and CTA, while none of the other antiemetics they tested were effective in either of these respects. They concluded that CTA is induced as a result of the association between the novel flavour and gut-related distress, and that drugs which reduce CTA in the rat will reduce gut-related distress in the clinic. The finding that L-742,694 reduces CTA in the rat is very interesting in this respect. This compound has not been tested in the clinic for its effects on gut-related distress, and may therefore be used to test Revusky and Martin's hypothesis. They would predict that this compound will reduce gut-related distress in the clinic.

The absence of complete suppression of CTA by L-742,694 in Experiment 5C suggests one of four things: i) the half life of this compound may not be long enough to demonstrate complete blockade of CTA; ii) the low affinity of this compound for the rat $NK_1$ receptor may be too low, even at the high doses of compound used here, to suppress completely the activation of these receptors; iii) CTA may be mediated by a number of receptor types, and blockade of one of these is not sufficient to block CTA completely; iv.) L-742,694, at the high doses used here, may itself induce CTA.

i) Some of the suppression of sucrose consumption on test may have been due to cisplatin activating the CTA mechanism after the plasma levels of the antagonist dropped below that at which the antagonist is effective. The time course of the antiemetics used in these sorts of experiments is of vital importance, especially when cisplatin is being used as the toxin with which the flavour is paired because it is possible that cisplatin is active for a period of 24 hours following infusion (Andrews et al, 1988). Blockade of the CTA mechanism for a short period following cisplatin infusion will allow some CTA to form as a result of later activation of the mechanism. This temporary blockade would be expected to lead to an attenuation of the aversion as measured on test, but this may only be small. Experiments in which L-742,694 is administered across a number of hours might establish whether the duration of action of this compound is a factor in determining the magnitude of its effect on cisplatin-induced CTA.

ii) The low affinity of the $NK_1$ receptor antagonists used in these experiments for the rat $NK_1$ receptor (Saffroy et al, 1994) necessitates the use of high doses of this compound in order to demonstrate an effect of $NK_1$ receptor antagonism in this species, and in other species with the same receptor type. This gives rise to the possibility that effects non-specific to $NK_1$ receptor

antagonism may be detected as a result of drug administration. These may enhance CTA through possible aversive properties of such high doses of compound or attenuate CTA through disruption of some non-$NK_1$ receptor mediated mechanism.

It is clear that the effect demonstrated in Experiment 5C requires replication in a species with the guinea pig $NK_1$ receptor type, testing doses such as those normally required to reverse $NK_1$ receptor agonist effects in this species, in order to establish that the attenuation of CTA by L-742,694 is the result of $NK_1$ receptor antagonism. An alternative to the use of a different model for cisplatin-induced CTA to test CP-99,994 and other $NK_1$ receptor antagonists of this type, is to test a rat $NK_1$ receptor selective antagonist. RP 67580 has high affinity for the rat $NK_1$, but was not tested due to its low brain penetration and bioavailability (Garret, Carruette, Fardin, Moussaoui, Peyronel, Blanchard & Laduron, 1991).

iii) If other receptor types are partially responsible for the formation of CTA, possibly others present in the NST and PBN, the antagonism of the $NK_1$ receptor would not be expected to lead to complete suppression of CTA. This allows the possibility that combinations of drug treatments would be required to give complete protection from gut-related distress in the clinic.

iv.) It is possible that high doses of L-742,694 are aversive to the rat, and therefore induce CTA. Thus, the small attenuation in cisplatin-induced CTA demonstrated in Experiment 5C may represent a combination of the suppression of cisplatin-induced activation of the CTA mechanism by L-742,694 and direct L-742,694-induced activation of this mechanism. If L-742,694 were found to support CTA it could be concluded that the limited effect that L-742,694 has on cisplatin-induced CTA may be due to L-742,694 acting both as an antiemetic and an aversive reinforcer.

In conclusion, although further studies are required in order to establish the exact nature of the effect (see i-iv), there are a number of implications of the detection of an attenuation of cisplatin-induced CTA by administration of L-742,694 (100 mg/kg), and the finding that this blockade of CTA is not complete. The most interesting of these are that clinically-induced CTA may be attenuated through the selective antagonism of $NK_1$ receptors, and that an $NK_1$ receptor antagonist may be used to test Revusky and Martin's (1988) hypothesis that the attenuation of CTA in the rat by a compound indicates that such a compound will also attenuate clinically-induced gut-related distress.

# Chapter Six

# Simultaneous Overshadowing of a Taste Aversion and Potentiation of a Context Aversion

## 6.1 Introduction

Studies of the potential for a pharmacological intervention for the attenuation of CTA in cancer chemotherapy patients have not, thus far, provided a means of complete control for this side effect (see Chapters 2-5). Whether or not a pharmacological intervention will become available, it might seem preferable to take a psychological approach in the development of an intervention for CTA. A psychological intervention based on learning theoretic principles is likely to be less costly and will avoid the use of further drugs which may have detrimental side effects.

It was suggested in Chapter 1 that one way of controlling CTAs in cancer chemotherapy patients might be to present a novel and salient 'scapegoat' flavour to the patients before treatment in order to protect the normal dietary items from becoming aversive. This would be expected as a result of the overshadowing of the aversion towards the normal dietary items by the added novel flavour (e.g. Revusky, 1971). This intervention was investigated and found to be effective in human cancer patients by Broberg and Bernstein (1987). However, it was also suggested in Chapter 1 that interactions may occur between the development of CTAs and that of ANV. In particular, the presentation of a novel flavour in the clinical setting may increase ANV, as a result of the potentiation of an aversion to that context. In this chapter, a brief summary of the mechanisms which have been thought to be responsible for the phenomena of overshadowing and potentiation will be presented followed by a demonstration that the presentation of a single flavour cue, such as that used as the 'scapegoat' by Broberg and Bernstein (1987) can both overshadow an aversion towards a previously presented flavour, and, simultaneously, potentiate an aversion towards the context in which it is presented.

Overshadowing (Mackintosh, 1976; Pavlov, 1927; Revusky, 1971) is a common phenomenon of compound conditioning procedures in which an added CS, presented during conditioning, leads to a decrement in conditioned responding to the target cue on test. Several accounts of overshadowing have been proposed. Rescorla and Wagner (1972) suggested that, in the

conditioning of an association between a CS and US, a limit is set by the US as to the amount of associative strength which can accrue to a CS. Once this asymptote is reached, no further learning can occur. In compound conditioning, overshadowing is expected because the associative strength made available by a US is shared between all of the CSs which are present. Thus, each individual CS of a compound accrues less associative strength than a CS which is conditioned in isolation.

An alternative, configural, position (Pearce, 1987) suggests that animals possess a limited capacity buffer containing the overall pattern of stimulation to which that animal is currently exposed. It is this stimulus array, not each individual element (as held by Rescorla & Wagner (1972)), which serves as the CS on any given trial. Overshadowing is predicted by this model because a compound CS (for example a light and a tone) presented during training, will not generalize well to the single CS which is presented on test (e.g. the light), and thus the test CS will elicit a weak CR. This generalization decrement will be greater for a CS trained in compound with a second CS and tested in isolation, than for one which is trained in isolation and tested in isolation. Other theories of learning have attributed overshadowing to the reduction in attention which is paid to the less salient stimulus in the compound (Mackintosh, 1975a; Pearce & Hall, 1980) and to competition for limited processing resources (Wagner, 1981).

In contrast to the overshadowing effect, the presence of one stimulus may potentiate conditioned responding to another (e.g. Rusiniak, Hankins, Garcia & Brett, 1979). Potentiation is particularly pronounced in toxicosis conditioning. For example, when exposure to a novel context is followed by the injection of lithium chloride, the aversion developed towards that context is greater if the animal is given access to novel sucrose during context exposure (Best, Brown & Sowell, 1984). It is possible then, that the presentation of a novel flavour in the chemotherapy clinic before treatment may potentiate an aversion towards the context in which it is presented. If ANV is the result of a classically conditioned association between the chemotherapy clinic and treatment-induced illness, the procedure used to overshadow CTA towards normal dietary items may, in addition, potentiate ANV.

Three mechanisms have been proposed to explain potentiation. First, Garcia and his colleagues (e.g. Rusiniak et al, 1979) suggested that non-food cues presented contiguously with consumption of a novel flavour are 'gated' into the feeding system. It is claimed that illness is a US which is specific to the feeding system. Thus, stimuli which gain access to this system, foods, and cues presented contiguously with foods, are able to form strong associations with illness. A second,

related hypothesis is that the presentation of a novel flavour increases the amount of attention paid to contiguous stimuli and thus increases their associability (Galef & Osborne, 1978). These first two hypotheses differ in that the former postulates a domain-specific learning mechanism, while the latter attributes potentiation to the properties of a general attentional mechanism. They are, however, similar in assuming that processes in addition to those thought to be acting in other associative learning phenomena are responsible for potentiation.

Unlike the hypotheses proposed by Galef and Osborne (1978) and Rusiniak et al (1979), Durlach and Rescorla (1980) have suggested that potentiation does not result from flavour-mediated strengthening of a context-illness association, but from a form of second order conditioning. Thus, cues which are present when the animal is consuming a novel flavour become associated with that flavour. The flavour, in turn, becomes associated with illness. When the cues which were present during consumption of the flavour are presented on test, they elicit a representation of the flavour which, in turn, elicits a representation of illness and leads to avoidance behaviour. This associative perspective allows that a flavour can overshadow contextual cues, but that the associative strength which accrues to the contextual stimuli through second order conditioning will outweigh the effect of any decrement resulting from overshadowing. One reason for the primacy of the context-taste and taste-illness associations over the context-illness association in determining responding in the context might be that the direct association between context and illness would be expected to be very weak because of cue-to-consequence specificity; whereas exteroceptive stimuli such as lights, tones and contextual cues easily become associated with other exteroceptive stimuli such as shock, associations with interoceptive reinforcers such as illness form less readily (Domjan & Wilson, 1972). All three of these hypotheses relating to the underlying mechanism of potentiation have received some empirical support (see Lolordo & Droungas, 1989 for review).

The experiments reported in this chapter assessed the likelihood that use of Broberg and Bernstein's (1987) overshadowing intervention to attenuate CTA in cancer chemotherapy patients would increase the risk of ANV in these patients, assuming that both ANV and CTA occur as a result of classical conditioning. Although a novel flavour has been shown to be able to overshadow a second flavour using a toxicosis procedure (Revusky, 1971), and a novel flavour has also been shown to potentiate an aversion towards contextual cues (Best et al, 1984) in a toxicosis procedure, the assertion that these two effects may occur concurrently requires validation. An experiment will be presented in which simultaneous overshadowing of a flavour cue and potentiation of contextual cues by a flavour is demonstrated in rats. Thus, it will be argued that

the procedure used by Broberg and Bernstein to overshadow CTA may lead to an increase in ANV due to the flavour-induced potentiation of an aversion towards the contextual cues present in the clinic.

In the experiments carried out in this chapter, the model of ANV used is similar to the contextual conditioning paradigm used by Best et al (1984). It has been argued that ANV is a classically conditioned association between the chemotherapy clinic and the illness induced by the treatment (e.g. Morrow, 1992). Similarly, suppression of drinking in a context which has been paired with lithium chloride injections is also thought to reflect an aversion towards that context (Best et al, 1984). Thus, in the model used here, rats were given exposure to a novel context and then injected with LiCl in order to induce a conditioned aversion towards that context. The context aversion was tested by the reluctance of animals to drink a familiar saline solution in that context.

In Experiment 6A, the development of a context aversion was demonstrated and this aversion was enhanced by the presence of a novel sucrose solution in the context during conditioning. Experiment 6B provided evidence that the sucrose solution presented in the novel context is an appropriate stimulus for the overshadowing of an aversion towards a previously presented vinegar solution. It was demonstrated that the sucrose solution was able to overshadow a vinegar aversion at the same time as enhancing an aversion towards the context in which it was presented. It was shown in Experiments 6C(i) and 6C(ii) that the enhancement of the aversion to the context demonstrated in Experiments 6A and 6B is unlikely to have been due to the generalization of an aversion from sucrose solution to the flavour used to test drinking suppression in the novel context (Mitchell & Heyes, 1994).

## 6.2 Experiments

### 6.2.1: Experiment 6A Potentiation of a context aversion by sucrose presentation

Evidence of potentiation of a context aversion by sucrose was sought using a procedure similar to that used by Boakes, Westbrook and Barnes (1992, Exp3). Three groups of rats were allowed to drink in a novel context and then received an injection immediately after being taken out of the context. Two of the groups were injected with lithium chloride. Of these groups, one drank sucrose in the context while the other drank tap water. The third group was given access to tap water in the context and then injected with saline solution. It was predicted that the poisoned

animals would develop an aversion towards the context as measured by a reduction, relative to the unpoisoned controls, in consumption of familiar saline solution in the context. It was further predicted that the animals given sucrose solution in the context would show potentiation by consuming less saline on test than poisoned animals given tap water in the context during conditioning.

## Method

### Subjects

Twenty four, experimentally naive, male Sprague Dawley rats (250 - 350g) were used. They were housed in groups of four in wire topped plastic cages (53 x 38 x 18 cm) in a temperature controlled room on a 12 hour light/dark cycle, lights on at 0700 hours. All animals were experimentally naive and were allowed food ad libitum throughout the experiment. Water consumption was controlled as shown below.

### Apparatus

A rack with 12 cages in a dark experimental room was used for the animals daily access to water. The cages were similar to the home cages in all respects except that they had wire instead of sawdust floors.These cages constituted context 1. In context 1, water was made available from 500 ml water bottles with rubber stoppers and ball bearing spouts to minimize leakage. The conditioning context (context 2) consisted of operant chambers with clear plastic walls, housed in open-fronted, sound attenuating chambers. The room containing the operant chambers was brightly lit by a combination of natural and florescent light, and two noisy fans were placed on the floor in front of the boxes. Thirty ml water bottles were used to allow access to water and sucrose solution. Each of these had a rubber stopper and a long metal spout, again with a ball bearing, which protruded through a hole in the wall of the box. Thus, context 1 was similar to the home cages in construction, and had a low level of lighting and no background noise. Context 2 was brightly illuminated and noisy, and was different in construction from the home cages.

## Procedure

On Day 1, the water bottles in the subjects' home cages were filled with 0.9% saline solution, and remained in place for 72 hours. On Day 4, the saline bottles were removed from the home cages. All of the animals were then allowed 15 mins access to tap water each day for seven days in context 1 (Days 5 - 11). The first conditioning trial occurred on Day 12. The animals were assigned to groups by equating mean group water consumption on Days 10 and 11 as closely as possible. They were placed in context 2 for 15 minutes and given access to sucrose solution (Group SUC-LI) or tap water (Groups WAT-LI and WAT-SAL). An injection (10 ml/kg i.p.) of 0.3 M LiCl or 0.9% saline solution was administered immediately on removal from context 2. The rats were then replaced in their home cages. Days 13 and 14 were recovery days when the animals were allowed 15 mins access to tap water in context 1 each day. This three day cycle (Days 12 - 14) was repeated twice across the following six days (Days 15-17 and Days 18-20) so that there were three conditioning trials in total. Testing was carried out on Day 21, when each animal was given 15 mins access to 0.9% saline solution in context 2.

## Results & Discussion

Fluid intake in context 2 on test is shown in Figure 6.1. It is apparent that both poisoned groups (WAT-LI and SUC-LI) showed suppressed consumption of saline on test compared with controls (Group WAT-SAL). Moreover, Group SUC-LI drank less than Group WAT-LI. Due to inequality of variance across groups, non parametric statistics were used in the analysis of these data. A Kruskal-Wallis (H) test confirmed that the groups differed in their consumption of saline on test (H(24) = 20.28), and follow up tests showed that Group WAT-SAL consumed more than Group WAT-LI (Z = 2.33, 1-tailed), and that Group WAT-LI consumed more than Group SUC-LI (Z = 2.17, 1-tailed). These results are consistent with the development of an aversion towards the context in groups WAT-LI and SUC-LI, and a stronger aversion towards the context in Group SUC-LI than in Group WAT-LI.

This experiment replicated the results of Boakes et al (1992, Exp3) in demonstrating a stronger aversion towards a context in which a novel sucrose solution was presented than one in which tap water was presented. The enhanced aversion was particularly pronounced since the animals in Group SUC-LI effectively failed to drink on the test trial. Consequently this procedure was taken to be a valid model of the development of conditioned context aversions and used as such in the
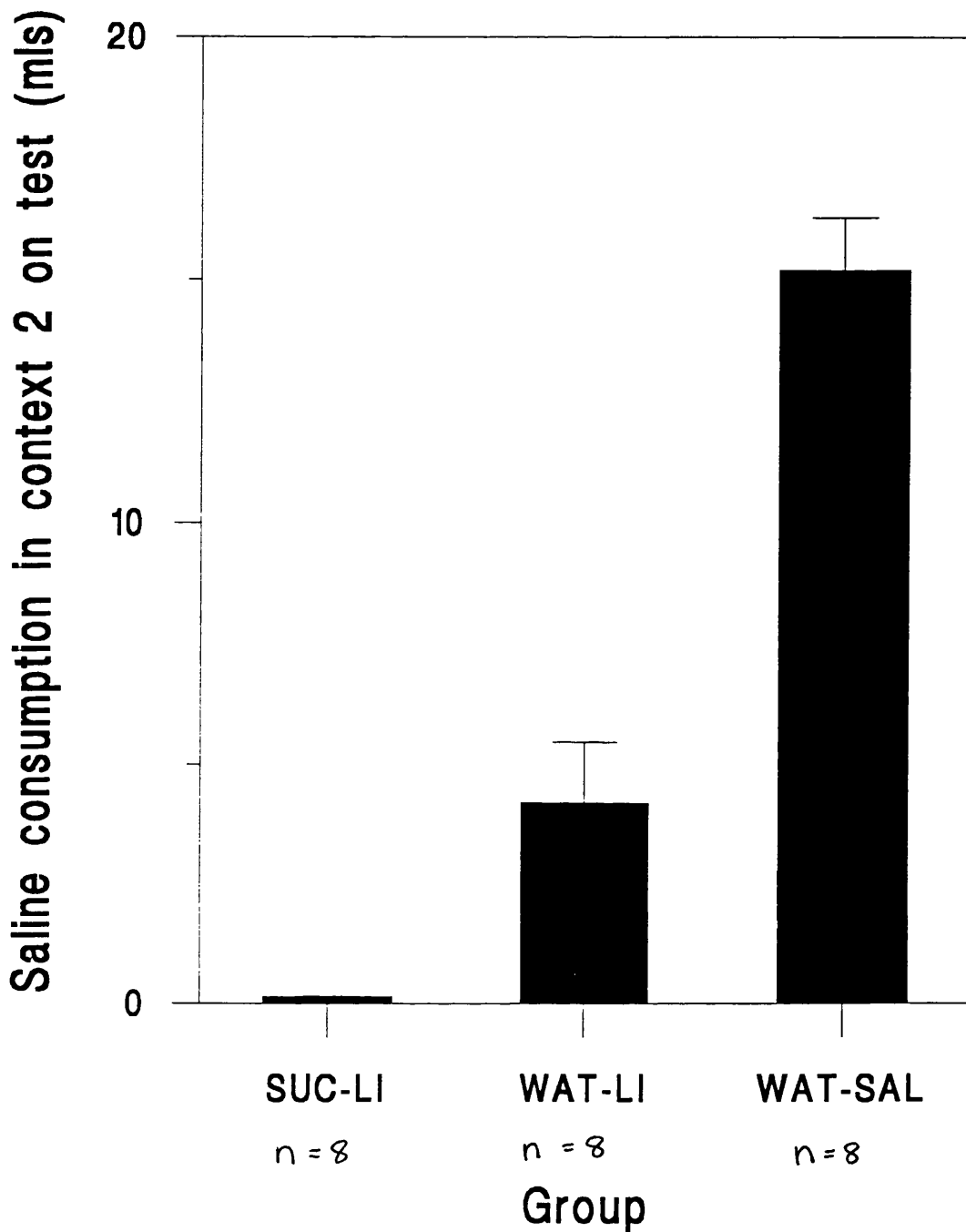
Figure 6.1. Mean consumption of famillar saline in context 2 on test. Sucrose or water (SUC or WAT)were presented in Context 2, followed by an injection of LiCl or water (LI or WAT) during training. Error bars indicate SEMs.

## 6.2.2: Experiment 6B Simultaneous overshadowing and potentiation of taste and contextual cues by a second taste

In an attempt to obtain simultaneous overshadowing and potentiation, a third stimulus, novel vinegar solution, was chosen to be the overshadowed stimulus. One-trial overshadowing of a novel vinegar solution by a novel sucrose solution in a CTA procedure has been demonstrated by Kaye, Gambini and Mackintosh (1988), and there seems no reason to suppose that the use of a multi-trial procedure would abolish the overshadowing effect. If the sucrose solution presented in the novel context is able to overshadow an aversion towards a previously presented vinegar solution, and at the same time enhance the aversion towards the context as it did in Experiment 6A, this will demonstrate that simultaneous overshadowing and potentiation can occur.

The procedure used in Experiment 6B to demonstrate simultaneous potentiation and overshadowing was similar to that of Experiment 6A. On conditioning days, however, five minutes access to novel vinegar solution was given to all animals, 3 hours and 15 minutes before exposure to context 2 and injection. It was thought that the presentation of sucrose in the context would again potentiate the aversion to the context and, in addition, reduce the magnitude of the aversion developed towards the vinegar solution. Since overshadowing is known to be a reciprocal effect (Mackintosh, 1976), it was also possible that the vinegar would overshadow the sucrose. If this were the case, the magnitude of the potentiation effect may be expected to be less than that observed in Experiment 6A. However, this prediction was not tested explicitly. The design of Experiment 6B is presented in Table 6.1.

| Table 6.1 The design of Experiment 6B | | | | |
|---|---|---|---|---|
| | Conditioning | | Test | |
| Group | context 1 | context 2 | context 2 | context 1 |
| 1 SUC-LI | 5 mins vin | 15 mins suc => LiCl | saline | vinegar |
| 2 WAT-LI | 5 mins vin | 15 mins wat => LiCl | saline | vinegar |
| 3 WAT-SAL | 5 mins vin | 15 mins wat => NaCl | saline | vinegar |

## Method

The experiment was run in two replications, and half of the animals in each replication were tested for their aversion to vinegar, while the other half were tested for their aversion to the context.

## Subjects and Apparatus

Forty-eight male, experimentally naive, Sprague Dawley rats (250 - 400g). Twenty four animals were used in each of the two replications. The apparatus was as described in Experiment 6A.

## Procedure

On Days 1-10, all animals were treated as in Experiment 6A. On the Days 11, 14 and 17 (the days before conditioning took place) all of the animals which were not due to receive the sucrose solution in context 2 during conditioning (Group WAT-LI and WAT-SAL), were given 15 mins access to sucrose solution in context 1 in place of tap water in order to equate sucrose consumption between groups. On each of the three conditioning days (Days 12, 15 and 18), each animal was given 5 minutes access to 3% v/v vinegar solution (distilled malt vinegar) in context 1 in the morning, and then replaced in its home cage. Three hours later, they were placed in context 2 for 15 minutes access to sucrose solution (Group SUC-LI) or tap water (Groups WAT-LI and WAT-SAL). An injection (i.p.) of 0.3 M LiCl (Groups SUC-LI and WAT-LI) or 0.9% saline solution (Group WAT-SAL) was administered immediately upon removal from context 2, and the rat was replaced in its home cage.

Two recovery days followed each of the first two conditioning days. On the first of these recovery days, animals were given 15 mins access to tap water in context 1, while on the second day, animals received either tap water or sucrose solution in this context, depending on which of these would be presented on the following day (see above). Following the final conditioning day, seven 15 minute exposures to context 1 were given in order to allow any aversion to context 1 to extinguish before testing for a vinegar aversion in context 1. Testing was carried out on Day 26. On this day, half the animals from each group were given 15 mins access to 0.9% saline solution in context 2. The other half were given 15 mins access to 3% v/v vinegar solution in context 1.

## Results & Discussion

The vinegar consumption data were first tested for an effect of replication and treatment in a two-way ANOVA. There was no effect of replication and no replication by treatment interaction, and therefore the data from the two replications were pooled. The data relating to vinegar consumption in context 1 are given in the left-hand panel of Fig 6.2. It would appear that Group WAT-SAL drank more than Group WAT-LI. In addition, consumption of vinegar in group SUC-LI was higher than that of Group WAT-LI. A one-way ANOVA revealed a significant difference in vinegar consumption across groups ($F_{(2,21)}$ = 30.94), and Tukeys post-hoc comparisons indicated a difference between Groups WAT-SAL and Group WAT-LI and a difference between WAT-LI and SUC-LI. These data suggest that an aversion developed towards vinegar in Group WAT-LI due to the pairing of this flavour with lithium chloride, and that the development of this aversion was attenuated by consumption of sucrose on the conditioning trials in Group SUC-LI.

The results for saline consumption in context 2 on the test day are presented in the right hand panel of Figure 6.2. These data were first tested for an effect of replication and treatment. Although the effect of replication was significant ($F_{(1,17)}$ = 7.9), as was the effect of treatment ($F_{(2,17)}$ = 13.7), there was no replication by treatment interaction ($F_{(2,17)}$ = 2.0). Therefore, since the effect of replication was not different across groups, the data from the two replications were pooled. Consumption of saline appears to have been suppressed in both poisoned groups (Groups WAT-LI and SUC-LI) compared to that of unpoisoned controls (Group WAT-SAL). However, suppression was greatest in Group SUC-LI. It would seem then, that the pattern of data from this experiment with regard to saline consumption in context 2 is similar to that shown in Experiment 6A; poisoned groups showed a suppression of consumption in context 2 which was strongest in Group SUC-LI. A one-way ANOVA indicated a reliable difference between the three groups ($F_{(2,21)}$ = 8.7). Tukeys post-hoc comparisons showed that there was a difference between groups SUC-LI and WAT-LI, and between SUC-LI and WAT-SAL. No difference was found between Groups WAT-LI and WAT-SAL.

In combination, the results of the vinegar and saline tests suggest that a novel sucrose solution can simultaneously potentiate and overshadow other stimuli which are present on conditioning trials, and that the effect is dependent on the nature of those stimuli; contiguous contextual cues were potentiated while a non-contiguous flavour cue (vinegar solution) was overshadowed. Poisoned animals given vinegar and sucrose solution on the conditioning days showed a greater tendency to drink vinegar when it was offered than those given vinegar and water, thus demonstrating

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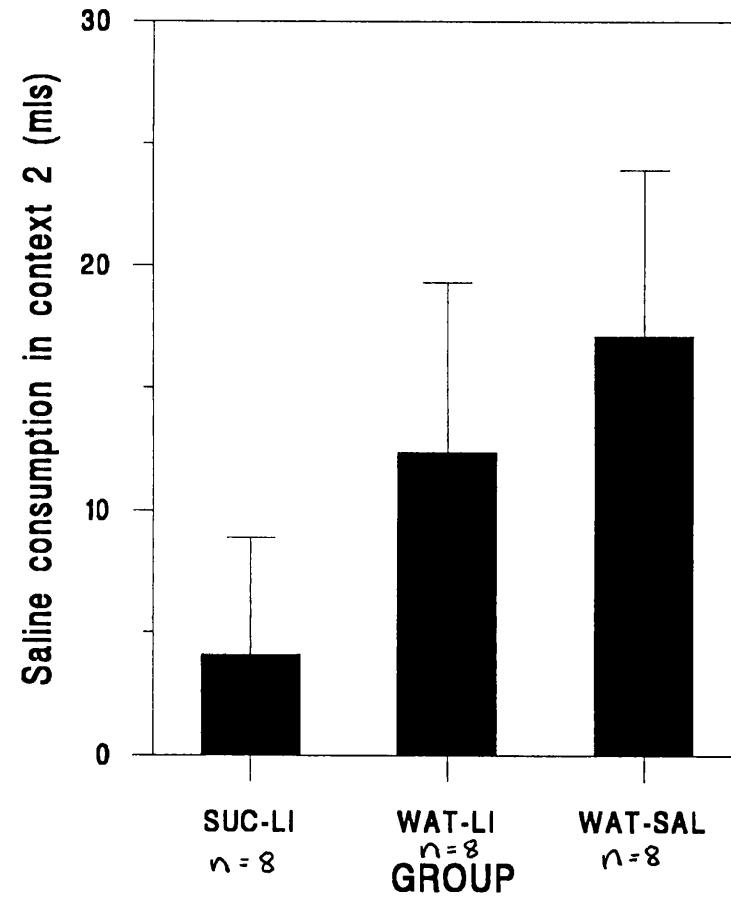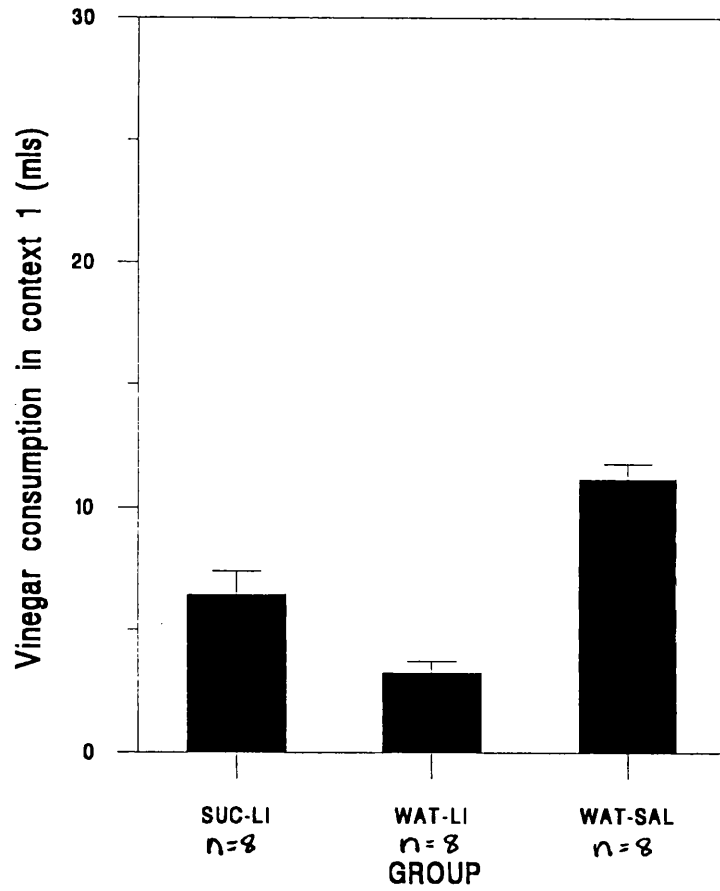
Figure 6.2. Left-hand panel shows mean consumption of vinegar on test in context 1. Animals received sucrose or water (SUC or WAT) in context 2 during conditioning followed by an injection of LiCl or water (LI or WAT).

The right hand panel shows mean consumption of saline solution in context 2 following the same training. Error bars indicate SEMs.

overshadowing. In contrast, animals treated in an identical manner showed a greater aversion to the context in which the sucrose was presented as measured by their reluctance to drink familiar saline in context 2, compared to animals which received water in this context, thus demonstrating potentiation.

### 6.2.3: Experiments 6C(i) and 6C(ii) Generalisation from aversive sucrose solution to familiar saline solution

The results of Experiment 6B have been interpreted as showing that a novel sucrose solution may simultaneously give rise to overshadowing of a flavour cue and potentiation of contextual cues. However, if an aversion to novel sucrose solution generalises to familiar saline solution, then it is possible that the difference between Groups SUC-LI and WAT-LI in Experiment 6B is due, not to potentiation of a context aversion by sucrose, but to simple generalization of a sucrose aversion to the familiar saline solution presented on test. Although the saline solution used here was familiar and the sucrose novel, it is still possible that sucrose-lithium pairings in group SUC-LI led to a greater saline aversion than did the water-lithium pairings in group WAT-LI.

Two experiments were carried out in order to test whether the potentiation effect apparently found in Experiment 6B could, in fact, be the result of generalization of a sucrose aversion. Experiment 6C(i) sought to establish whether sucrose-lithium pairings in one context would lead to suppression of consumption of a familiar saline solution presented in another context. Two groups of animals were used. In one group, on three occasions, sucrose presentation was followed by LiCl in context 1. In the second group, three water-lithium pairings were given in context 1. The rats received the same amount of context 2 exposure as did the animals in Experiments 6A and 6B (15 minutes three times) before being tested for saline consumption in this context. If an aversion to novel sucrose generalizes to familiar saline solution, a suppression in consumption of saline would be expected to be demonstrated in the animals which received sucrose-lithium pairings in training.

Experiment 6C(ii) sought to confirm that a sucrose-mediated enhancement of context 2 aversion would occur when the control and experimental groups received an equivalent number of sucrose-lithium pairings. Both groups received three sucrose-LiCl pairings and a further three water-LiCl pairings. The difference between the two groups was that group POT (the group expected to show potentiation of an aversion towards context 2) was given sucrose in context 2 before an injection of lithium, while group CONT (the control group) received water in context 2 before lithium

injection. Both groups were injected with lithium a further three times after exposure to context 1 in which they received either water (group POT) or sucrose (group CONT) before injection. Context 2 aversions in both experiments were measured by the level of consumption of familiar saline solution in that context. A summary of the designs of Experiments 6C(i) and 6C(ii) is presented in Table 6.2.

| Table 6.2 The designs of Experiments 6C(i) and 6C(ii). | | | |
|---|---|---|---|
| Experiment 6C(i) | Conditioning | | Test |
| Group | Context 1 | Context 2 | Context 2 |
| 1 SUC | SUC= > LiCL | WAT | Saline |
| 2 WAT | WAT= > LiCl | WAT | Saline |
| | | | |
| Experiment 6C(ii) | | | |
| Group | | | |
| 1 POT | WAT => LiCl | SUC => LiCl | Saline? |
| 2 CONT | SUC => LiCL | WAT => LiCl | Saline? |

## Method

### Subjects and Apparatus

Thirty two experimentally naive male. Sprague Dawley rats (400 - 600g) were housed as described in Experiment 6A. The apparatus was that described in Experiment 6A.

### Procedure

The procedure for Days 1 to 11 in Experiments 6C(i) and 6C(ii) was the same as that used in Experiment 6A, and assignment to groups was on the same basis. Thus, all animals were familiarized with 0.9% saline solution and given seven days drinking experience in context 1. Experiments 6C(i) and 6C(ii) differed in the procedure applied from Day 12 onwards.

Experiment 6C(i)
On Day 12, half of the animals from each group were placed in context 2 and received 15 minutes access to tap water before they were replaced in their home cages. The other half were placed in context 1 for 15 minutes access to 3% sucrose solution (Group SUC) or tap water (Group WAT), and then injected with 0.3M lithium chloride (10 ml/kg) and replaced in the home cage. A second

conditioning trial followed on Day 13. On this day, the animals which had been given context 2 exposure on Day 12 were placed in context 1 and presented with 3% sucrose solution (Group SUC) or tap water (Group WAT) and then injected (0.3M LiCl, 10 ml/kg). Those animals that were conditioned in context 1 on Day 12 were placed in context 2 for 15 minutes access to tap water before being replaced in the home cage. Following the second conditioning day, there were two recovery days (Days 14 and 15) in which 15 minutes access to tap water was given in context 1. This four day cycle (Days 12-15) was repeated on two further occasions (Days 16 to 19 and Days 20-23). Thus, all animals received three non-reinforced exposures to context 2, and three injections of lithium chloride following either sucrose consumption (Group SUC) or tap water (Group WAT) in context 1. Testing was carried out in context 2. All animals were given familiar saline solution to drink for 15 minutes.


Experiment 6C(ii)

On Day 12, half of the animals in each group were placed in context 2 for 15 minutes access to sucrose solution (Group POT) or tap water (Group CONT). An injection of 0.3 M LiCl (10 ml/kg i.p.) was administered immediately upon removal from context 2, and the rats were replaced in their home cages. The other half of the animals from each group were given 15 mins access to water (Group POT) or sucrose solution (Group CONT) in context 1 followed by an injection of 0.3 M LiCl (10 ml/kg i.p.). Days 13 and 14 were recovery days on each of which the animals were allowed 15 mins access to tap water in context 1. On Day 15, the procedure for Day 12 was repeated. However, the animals that had been conditioned in context 1 on Day 12 were conditioned in context 2 on Day 15 and vice versa. Again, animals in group POT received sucrose if they were in context 2 and water if they were in context 1, while animals in group CONT received the opposite treatment. Another two days recovery followed this conditioning day (Days 16 and 17).


Thus, in Experiment 6C(ii), both groups received one sucrose-LiCl pairing and one water-LiCl pairing, and both were given one context 1 - LiCl pairing and one context 2 - LiCl pairing; Group POT received sucrose in context 2 and water in context 1, while Group CONT received opposite treatment. This six day cycle (two conditioning days and four recovery days) was repeated on two further occasions. Thus the animals received a total of six conditioning trials. It was necessary to give supplementary water in the home cage on the recovery days during the final cycle in order to keep the animals above 95% of their free feeding weight. Thus, each animal was allowed 1

hour access to tap water in the home cage on each of these four days in addition to the 15 mins access they received in context 1. On test (Day 28), animals were given familiar saline to drink in context 2.

## Results and Discussion

The test data from Experiment 6C(i) are presented in the left hand panel of Figure 6.3. Both Group SUC and Group WAT readily consumed saline solution in context 2. While Group SUC appears to have consumed less than Group WAT, the difference was not reliable (Mann Whitney U = 20, p > 0.1). Thus, it would appear that sucrose-lithium pairings do not lead to an aversion to familiar saline, and therefore the effect on saline consumption observed in Experiment 6B is unlikely to have been due solely to generalisation from sucrose to saline rather than potentiation of a context aversion by sucrose.

The test day data from Experiment 6C(ii) are presented in the right hand panel of Figure 6.3. It is clear that all animals were reluctant to drink saline in context 2 but that animals in Group POT were less willing to drink than those in Group CONT (mean consumption in group POT = 0.1 mls, mean consumption in group CONT = 1.6 mls). The difference in saline consumption between the groups was reliable (Mann-Whitney U = 11.5), and consistent with a potentiated context aversion.

The results of Experiments 6C(i) and 6C(ii) indicate that the difference between Groups SUC-LI and WAT-LI in Experiment 6B is unlikely to have been due to generalisation rather than potentiation. In Experiment 6C(i), in context 1 sucrose-LiCl pairings did not lead to a reliable aversion towards familiar saline solution presented in context 2. Consistent with this result, even when they received equal numbers of sucrose-lithium pairings, rats which drank sucrose in a context before poisoning subsequently consumed less saline in that context than rats that drank water in that context.

## 6.3: General Discussion

The data presented here provide evidence that the same flavour cue presented in a novel context before poisoning can both enhance an aversion towards that context and, at the same time, reduce an aversion towards a previously presented flavour cue. This finding has implications for the adoption of the intervention for chemotherapy-induced CTA proposed by Broberg and Bernstein
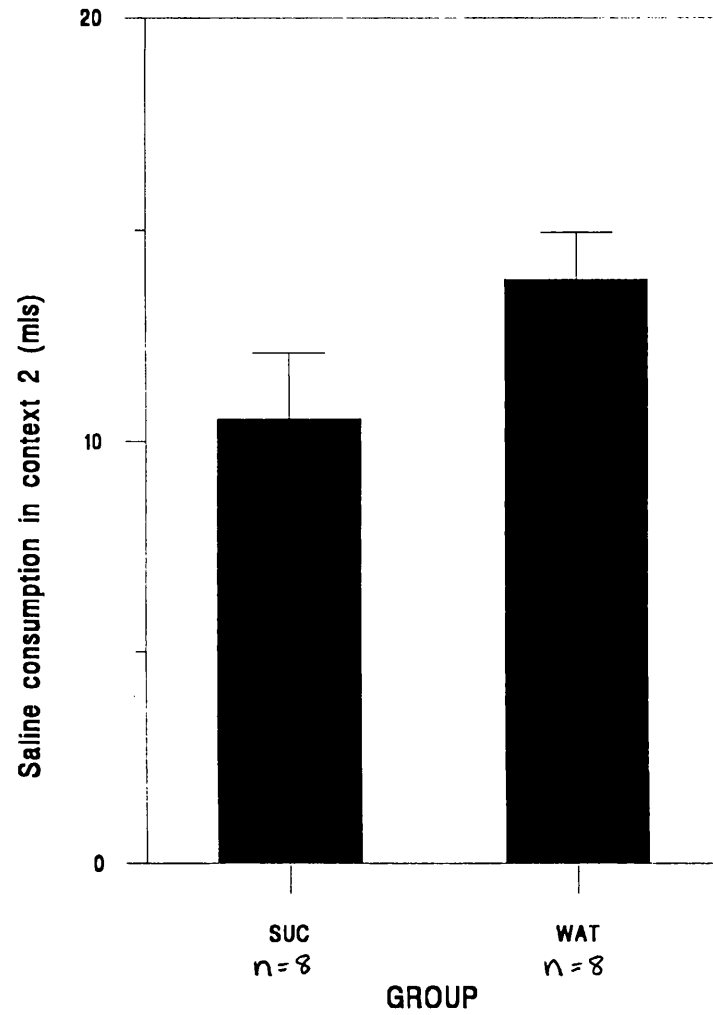
Figure 6.3. The left hand panel shows mean consumption of saline solution in context 2 in Experiment 6C(i) on test. Animals were presented with sucrose or water followed by LiCl in context 1 during training.
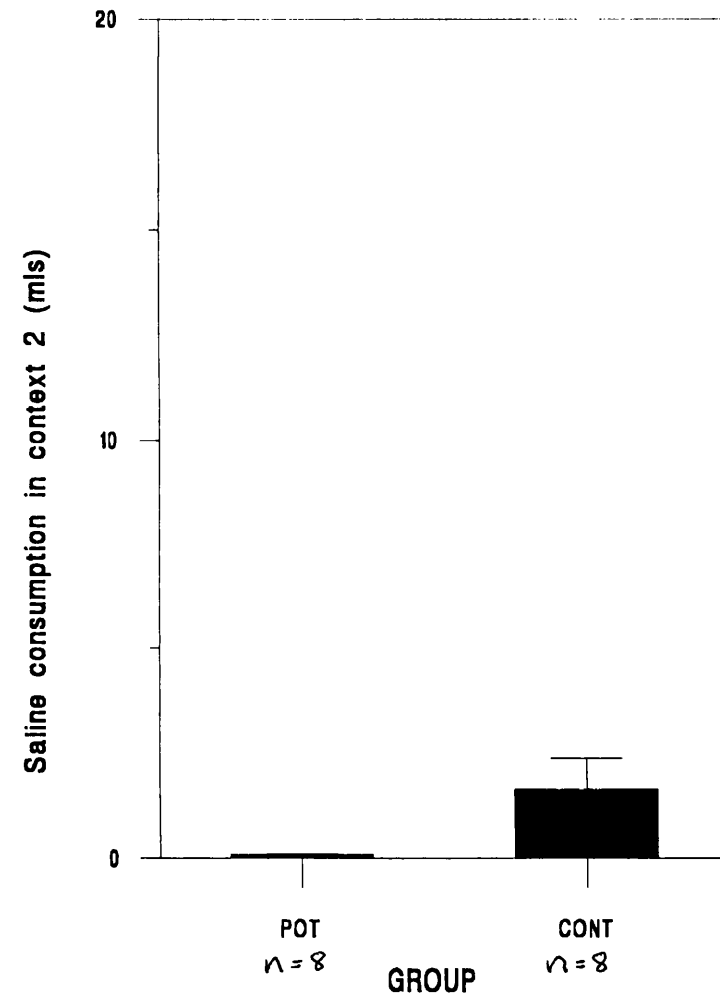
The right-hand panel shows mean consumption of saline in context 2 ontest in Experiment 6C(ii). Context 2 was reinforced in the presence of water (CONT) or sucrose (SUC). Error bars indicate SEMs.

(1987). Broberg and Bernstein suggested that novel flavour cues be used to overshadow aversions towards foods given to chemotherapy patients before treatment. These Experiments suggest that the scapegoat flavour, as well as reducing CTA, might increase the level of ANV in these patients.

From Experiment 6A, it can be seen that an aversion can be conditioned towards a novel context through the administration of lithium chloride, and that this aversion can be potentiated by the presentation of a novel sucrose solution in that context before injection. Simultaneous potentiation and overshadowing was demonstrated in Experiment 6B; the novel sucrose solution both overshadowed a novel vinegar solution and potentiated an aversion towards a novel context. Finally, the potentiated context aversion was shown not to be the result of generalization between the aversive sucrose solution and saline, the flavour used to test the magnitude of the aversion towards the context.

In one respect, the results of Experiment 6B do not seem to be consistent with those of Experiment 6A. In Experiment 6A, not only was a potentiated aversion towards the context demonstrated in Group SUC-LI, but an aversion was also demonstrated towards context 2 in group WAT-LI. However, in Experiment 6B, the difference in saline consumption in context 2 between groups WAT-LI and WAT-SAL was not found to be significant. Procedurally, the major difference between Experiments 6A and 6B was the presentation of vinegar solution before exposure to context 2 in Experiment 6B. It is possible that the vinegar overshadowed the aversion towards context 2 in Group WAT-LI. The novel vinegar flavour was not presented contiguously with context 2, thus it might have been expected to overshadow, rather than potentiate, an aversion to context 2. Westbrook et al (1983) found that a simultaneous odour-flavour cue led to potentiation of an aversion to the odour, while the same compound cue presented sequentially led to overshadowing of the odour.

Alternatively, context 1 may have overshadowed the aversion towards context 2 in Group WAT-LI. Although all animals received 7 days preexposure to context 1 in Experiment 6B, and context 1 would not therefore have been expected to condition well due to latent inhibition (Lubow, 1973), it is possible that the presentation of vinegar solution in this context on conditioning days represented a context change. Thus, novel vinegar might have become the context for the conditioning of context 1. Since latent inhibition is known to decline with transfer across contexts (e.g. Hall & Channell, 1986), this would allow context 1 to become associated with toxicosis and

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therefore overshadow context 2. In addition, conditioning of context 1 might be expected to be greater than that of context 2 due to the potentiation of context 1 by the presence of the novel vinegar solution in that context. If context 1 became more associable as a result of the presence of novel vinegar (potentiation), then its ability to overshadow context 2 would have been enhanced (Rescorla & Wagner, 1972).

There remains the question of whether the results of Experiments 6C(i) and 6C(ii) rule out a generalization explanation of the sucrose effect demonstrated in Experiments 6A and 6B. Experiment 6C(i) demonstrated that pairings of sucrose solution with lithium in context 1 did not lead to a reliable reduction in saline consumption in context 2, and Experiment 6C(ii) showed that, when sucrose-lithium pairings were equivalent across groups, the presence of sucrose in context 2 before lithium administration was able to support an enhancement effect similar to that demonstrated in Experiments 6A and 6B. However, it might be argued that, in both Experiments 6A and 6B, the sucrose aversion resulting from pairing with LiCl was context-specific; CTA is known to be context specific (Bonardi, Honey & Hall, 1990; Sjoden and Archer, 1989). The implication of this analysis is that, in Experiment 6C(i), any aversion which developed towards sucrose in context 1 did not generalize to saline in context 2 due to the context change. The same argument can be made for Experiment 6C(ii): animals in Group POT received conditioning with sucrose in context 2 while those in Group CONT received conditioning with sucrose in context 1. If the test stimulus, saline, generalized to sucrose, and an aversion towards sucrose was context-specific, a suppression of consumption of saline in Group POT compared to Group CONT would be expected.

There are two reasons why the potentiation effect demonstrated is unlikely to be due to a context-specific sucrose aversion which generalizes to familiar saline solution. First, in demonstrations of the context-specificity of CTA, Sjoden and Archer (1989) found that the decrement in responding due to a change in contexts was largely due to the aversion which developed towards the contextual cues in the groups which did not undergo a context change. It may be the case that, in Sjoden and Archer's experiments, the presence of a flavour during conditioning led to the potentiation of the contextual cues which were tested in the same-context groups (groups which did not undergo a context change). Second, in an experiment in which the two contexts received an equal amount of conditioning, thus controlling for the effect of any context-illness associations, Bonardi et al (1990) found that the context specificity of CTA was not revealed immediately but after a number of extinction sessions. The effect observed in Experiment 6C(ii) was apparent on

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the first test session. Although it is not possible to compare the present experiment to that of Bonardi et al (1990) directly, it seems likely that the effect observed in Experiment 6C(ii) was the result of something more than mere generalization from sucrose solution to saline solution.

Assuming the validity of the rat model of learned side effects of cancer chemotherapy used in the present studies (see Chapter 8 for a discussion of this issue), it seems clear that the use of an overshadowing stimulus to reduce CTA in human cancer patients may, in addition, lead to an increase in the level of ANV in this clinical population. There is some evidence from a study carried out on cancer patients by Nerenz et al (1986), that potentiation may occur in the chemotherapy clinic. When a measure of ANV was taken, it was found to correlate with the incidence of the experience of novel tastes in the mouth as a direct result of the treatment drug. Just such a correlation would be expected if these drug-induced tastes led to an increase in contextual conditioning similar to that demonstrated in the experiments presented in this chapter.

It is clear from the data presented here and elsewhere (e.g. Best et al, 1984) that the consumption of a novel flavour in a context before toxicosis leads to an increase in the aversion which is conditioned to that context. It is therefore possible that the contextual conditioning which underlies the majority of cases of ANV is potentiated by the experience of novel flavours in the clinic, either as a result of consuming novel-flavoured foods or as a direct result of drug infusion (Nerenz et al, 1986). The predictor variables of ANV (e.g. anxiety and susceptibility to motion sickness) which have been isolated thus far have commonly only accounted for 25% of the variance in the data (Andrykowski and Redd, 1987), and the experience of flavours in the clinic has not generally been monitored. Although the hypothesis is speculative, Nerenz et al's findings allow the possibility that potentiation of a context aversion, by a flavour, is required in order for measurable ANV to develop. Given the data presented in this chapter, there is clearly a need to carry out clinical studies in which the development of ANV is investigated, and the experience of flavours while in the clinic is monitored.

In a study of human cancer patients, Bovbjerg et al (1992) found that a flavour presented on a number of occasions before drug infusion elicited nausea when subsequently presented in the home environment. This finding suggests that, in addition to any effects that repeated flavour presentation before chemotherapy might have on ANV (see Chapter 6), the presentation of a previously drug-paired flavour would, itself, increase nausea.

An alternative to the repeated presentation of a novel flavour before therapy would be to present a different novel flavour on each visit to the clinic. However, it might be argued that repeated presentation of sucrose solution was necessary for the demonstration of the overshadowing effect observed in Chapter 6 (Experiment 6B). This might suggest that simultaneous overshadowing and potentiation would not be observed when a different flavour is used on each trial due to a failure to demonstrate overshadowing using this procedure. In Experiment 6B, the overshadowed stimulus (vinegar solution) was also presented repeatedly. Since overshadowing has been observed in one trial using identical stimuli to those used in Experiment 6B (sucrose and vinegar solutions) in a previous study (Kaye et al, 1988), it is likely that overshadowing will occur when a different flavour is used as the overshadowing stimulus on each conditioning trial if the overshadowed stimulus is similarly varied. Before testing this hypothesis, it is necessary to establish that potentiation will occur when the flavour presented in the context is different on each conditioning trial. The experiments presented in Chapter 7 were designed to test this hypothesis.

# Chapter Seven

# Varying the Flavour Across Conditioning Trials in the Potentiation of a Context Aversion

## 7.1: Introduction

Evidence presented in Chapter 6 and elsewhere (e.g. Boakes et al, 1992) suggests that the presentation of a novel flavour in a novel context in a toxicosis procedure may lead to the potentiation of an aversion towards that context. Although presentation of the same flavour stimulus across multiple trials has been shown to potentiate a context aversion in toxicosis conditioning, it is not known what effect varying the flavour between trials may have, and , as suggested in Chapter 6, procedure is likely to be used in the clinic in the overshadowing of CTA in cancer patients. One simple way of demonstrating that the presentation of different flavours in the context on each conditioning trial might lead to potentiation of the aversion towards that context, would be to establish that one-trial potentiation occurs using this procedure.

There are examples of potentiation in which only one conditioning trial took place (e.g. Lett, 1984). However, these involved a compound stimulus which was made up of a flavour and an odour using rats as subjects. or a flavour and a colour using pigeons as subjects. It is possible that the salience of the stimuli used in these experiments was crucial in allowing one-trial potentiation to be observed.

It has been suggested that the relative salience of the two stimuli which are presented in this sort of compound conditioning procedure is important in determining whether potentiation or overshadowing takes place. Bouton, Dunlap and Swartzentruber (1987) found that 0.1% saccharin solution potentiated an aversion towards 0.03% NaCl, but not 0.6% or 1.2% NaCl solutions when the compound was paired with lithium chloride. From these data, it would seem that aversions towards stimuli of low salience are more likely to be potentiated by the presence of a flavour during conditioning than are stimuli of high salience. In contrast, it has been suggested that the target cue must be conditionable in its own right in order to be subject to taste-mediation in the conditioning of an aversion (Westbrook, Clarke & Provost, 1980). In the light of the apparent primacy of the associability of the target cue in determining whether potentiation will result from

compound conditioning with a flavour cue, it is difficult to extrapolate from data derived from conditioning procedures using odours in rats and colours in pigeons (e.g. Lett, 1984) to the procedure in which contextual cues are used with rats as subjects.

It is not claimed here that the demonstration of one-trial potentiation of contextual cues in rats would indicate that potentiation will occur in human cancer patients as a result of the introduction of an overshadowing flavour before treatment. Rather because the data presented in Chapter 6, demonstrating simultaneous overshadowing and potentiation, rely on multiple presentations of a single flavour in the context, it is supposed that, in order for this model to be clinically valid, it must be demonstrated that the same phenomenon (simultaneous overshadowing and potentiation) occurs when the flavour is altered between conditioning trials. The simplest way to achieve such a demonstration would be to establish the potentiation, and simultaneous potentiation and overshadowing, effects using a one-trial procedure.

It is clear that a single conditioning trial is unlikely to lead to as much associative strength accruing to the CSs (the context and flavour), as is a three conditioning trial procedure. However, there is a further difference between one- and three-trial procedures in that, in the latter case, an aversive substance is sampled in the context on a majority of the conditioning trials (all trials following the first). An effect of the presentation of an aversive (through prior pairing with LiCl) versus a non-aversive flavour in a context before toxicosis was observed by Westbrook and Brookes (1988). They found that presentation of an aversive flavour during compound conditioning led to a reliable increase in the aversiveness of the context on test, compared to the presentation of a non-aversive flavour.

One reason for the difference observed by Westbrook and Brookes (1988) may be that the aversive flavour is more salient than the non-aversive flavour. All three theories of potentiation which have been considered here (Durlach & Rescorla, 1980; Galef & Osborne, 1978; Rusiniak et al, 1979) predict that the potentiation which arises as a result of the pairing of a salient flavour with a context before illness will be stronger than if this flavour were non-salient. A more salient flavour may be better able to increase the amount of attention which is paid to the exteroceptive cues which are spatially contiguous with that flavour (Galef & Osborne, 1978) or to 'gate' the exteroceptive stimuli into the feeding system, allowing them to become aversive as a result of subsequent illness (Rusiniak et al, 1979). In support of their theory, Rusiniak et al (1979) provided evidence suggesting that the intensity of the taste component of a taste-odour compound

was directly related to the degree of potentiation of the odour cue which took place. Thus, if a flavour which has been previously paired with illness is more salient than an unpaired flavour, a greater degree of potentiation would be expected as a result of the conditioning of a taste-odour compound in which the taste is aversive.

The within-compound association analysis of potentiation (Durlach & Rescorla, 1980) might predict that a flavour of high salience will be more effective in producing potentiation rather than overshadowing, due to an increase in the strength of the context-taste association. This theory postulates that the exteroceptive stimuli become associated with the taste, which, in turn, becomes associated with illness. On test, the exteroceptive stimuli re-elicit a representation of the taste, which then leads to avoidance behaviour as a result of its association with illness. According to Rescorla and Wagner (1972), the rate of learning is thought to be directly related to the salience of the stimuli to be associated. Thus, in a context-taste compound, the association between the two stimuli might be expected to be stronger if the salience of the taste stimulus is high.

The three experiments presented in this Chapter are designed to assess the possibility that the presentation of a non-aversive flavour in a novel context may potentiate an aversion to that context when it is paired with illness. Experiment 7A is a replication of Experiment 6A, but with only one conditioning trial. Experiment 7B was a further attempt to demonstrate one-trial context-potentiation. However, in this case, the context was used to block an aversion towards a second taste stimulus. In addition to the possibility of demonstrating one-trial potentiation, such a procedure may rule out an account of context potentiation in terms of instrumental conditioning.

The suppression of consumption of the familiar saline solution on test as a result of sucrose presentation during training (e.g. Experiment 6A) may be accounted for in terms of instrumental conditioning. Such an account can be contrasted with the classical conditioning account presented above, in which the context becomes aversive as a result of being paired with illness, and the contextual cues re-elicit a representation of the illness on test. According to this interpretation, it is the experience of illness on test which is thought to lead to a reduction in consumption of familiar saline. However, it is possible that, instead or in addition, instrumental learning mediates the observed reduction in consumption. Thus, subjects may learn that approach and licking at the water bottle has aversive consequences. It is not thought that such consummatory responses readily come under instrumental control (Mackintosh, 1983), and therefore only a brief analysis of the implications of this hypothesis will be made here.

If the drinking response in this context is under instrumental control, then knowledge of the outcome of the response will affect the level of responding on test (Dickinson & Balleine, 1994). On the first conditioning trial of a potentiation procedure (e.g. Experiment 6A), a palatable flavour is presented in a novel context. Outside of this context, illness is experienced as a result of administration of an emetic substance. Garcia, Brett and Rusiniak (1989) suggested that the association between the flavour and illness which occurs in CTA is detected only when the flavour is encountered on test. That is, although the flavour is aversive, the subject has no explicit knowledge that this is the case. Thus, on the second trial the animal learns that the liquid which is available in this context is aversive. This learning of the value of the outcome of an instrumental response has been termed incentive learning (e.g. Dickinson & Balleine, 1994; but see Rescorla, 1994). If only one conditioning trial were to occur, or if, on each trial, a different flavour were to be presented, then the animal would not receive an aversive substance as a result of drinking in this context, and thus the response of drinking would not be depressed.

Some evidence in support of the hypothesis that the flavour only becomes aversive when presented on test comes from experiments carried out on the effect of reinforcer devaluation on instrumental responding. Balleine and Dickinson (1991) trained rats to chain pull and lever press concurrently for either sucrose solution or saline solution in a single session. Following this training, the animals were injected with lithium chloride. Between this training session and testing, all animals were presented with one of the solutions in the absence of both of the manipulanda. They found that, on test, the rats responded less in extinction on the manipulandum which had been associated with the flavour which was re-exposed. This suggested that experience of the flavour after conditioning with lithium chloride, leading to the learning that the outcome of the instrumental response was aversive, was necessary for the suppression of instrumental responding.

If an aversion for the taste is necessary for potentiation (Durlach & Rescorla, 1980), then evidence for one-trial potentiation is inconsistent with the necessity for incentive learning in the learning of a taste aversion. The occurence of one-trial potentiation (Lett, 1984), implies either the case that the potentiation does not arise from the development of within-compound associations as suggested by Durlach & Rescorla (1980), or that a taste aversion is learned without re-exposure to the taste, as suggested by Rescorla (1994). A procedure in which the aversiveness of the context is tested in terms of its ability to block the development of an aversion towards another stimulus, may rule out such an account (see Experiment 7B).

The same analysis can be applied to the instrumental account of potentiation; either incentive learning is not necessary for suppression of responding following outcome devaluation, or potentiation is not a result of the devaluation of the outcome of an instrumental response. Of course, if what is being learned by the animals which undergo this procedure is that the bottle presented in this context contains an aversive liquid, rather than that the context is aversive, then the procedure used in Experiment 6A is not a good model of ANV in cancer chemotherapy patients. It is clear that, if ANV is a conditioning phenomenon, what is being learned by cancer patients is not that a particular instrumental response will lead to an aversive outcome. Altering the response to a nausea questionnaire (Andrykowski et al, 1985) will not lead to a reduction in the subjective experience of nausea in these patients.

The experiments presented in this chapter assessed whether the presence of an aversive taste in the context during conditioning is necessary for potentiation to occur and, if so, to what extent the aversiveness of this stimulus affects the level of conditioning to the context. If it were the case that an aversive flavour is necessary for the demonstration of flavour-mediated context conditioning, and that a different flavour were used as the overshadowing stimulus on each visit to the clinic, then the use of an overshadowing stimulus in the control of CTA in chemotherapy patients would not be expected to lead to an increase in the level of context aversions which develop in these patients. Two experiments tested whether context-potentiation might occur following a single conditioning trial (Experiments 7A and 7B), while the third experiment tested for potentiation as a result of the presentation of a novel flavour on each of three conditioning trials (Experiment 7C).

## 7.2: Experiments

### 7.2.1: Experiment 7A One-trial potentiation of a context aversion

The first experiment used the same procedure as that of Experiment 6A, except that there was only one conditioning trial, and only two groups of animals. Both groups received one context-illness pairing, and for one group (Group SUC-LI) sucrose solution was presented in the context during the conditioning trial, while for the other group (Group WAT-LI) water was available. It was predicted that, if potentiation of the contextual cues used in this procedure occurs at the first conditioning trial, then animals which drink sucrose solution in the context before being poisoned will drink less familiar saline in this context on test than those which consumed water in the

context during conditioning.

## Method

### Subjects

Sixteen, experimentally naive, male Sprague Dawley rats (250 - 350g) were housed in groups of four in wire-topped plastic cages (53 x 38 x 18 cm) in a temperature controlled room on a 12 hour light/dark cycle, lights on at 0700 hours. All animals were experimentally naive and were allowed food ad libitum throughout the experiment. Water consumption was controlled as shown below.

### Apparatus

The apparatus used was the same as that in Experiment 6A. Thus animals were given water to drink in a dimly illuminated cage similar in construction to that of the home cages (context 1), whereas conditioning occurred in operant chambers which were brightly illuminated and noisy (context 2).

### Procedure

The procedure was the same as that for Experiment 6A except that only one conditioning trial took place. Thus, animals were familiarized with saline solution in the home cages for three days (Days 1-3), and then familiarized with context 1 across the following 7 days (Days 4 - 11). One conditioning trial took place in context 2 on Day 12, on which sucrose (Group SUC-LI) or water (Group WAT-LI) were presented followed by an injection of lithium chloride (0.3 M, 10 ml/kg). The conditioning day was followed by two recovery days on which water was made available in context 1 (Days 13 - 14), and testing took place on Day 15, when all animals received saline solution in context 2.

## Results and Discussion

Saline consumption in context 2 on test is shown in Fig 7.1. There was no difference in saline consumption between Group SUC-LI and Group WAT-LI (F < 1). There is, therefore, no evidence from this experiment that the presentation of sucrose in a novel context has any effect
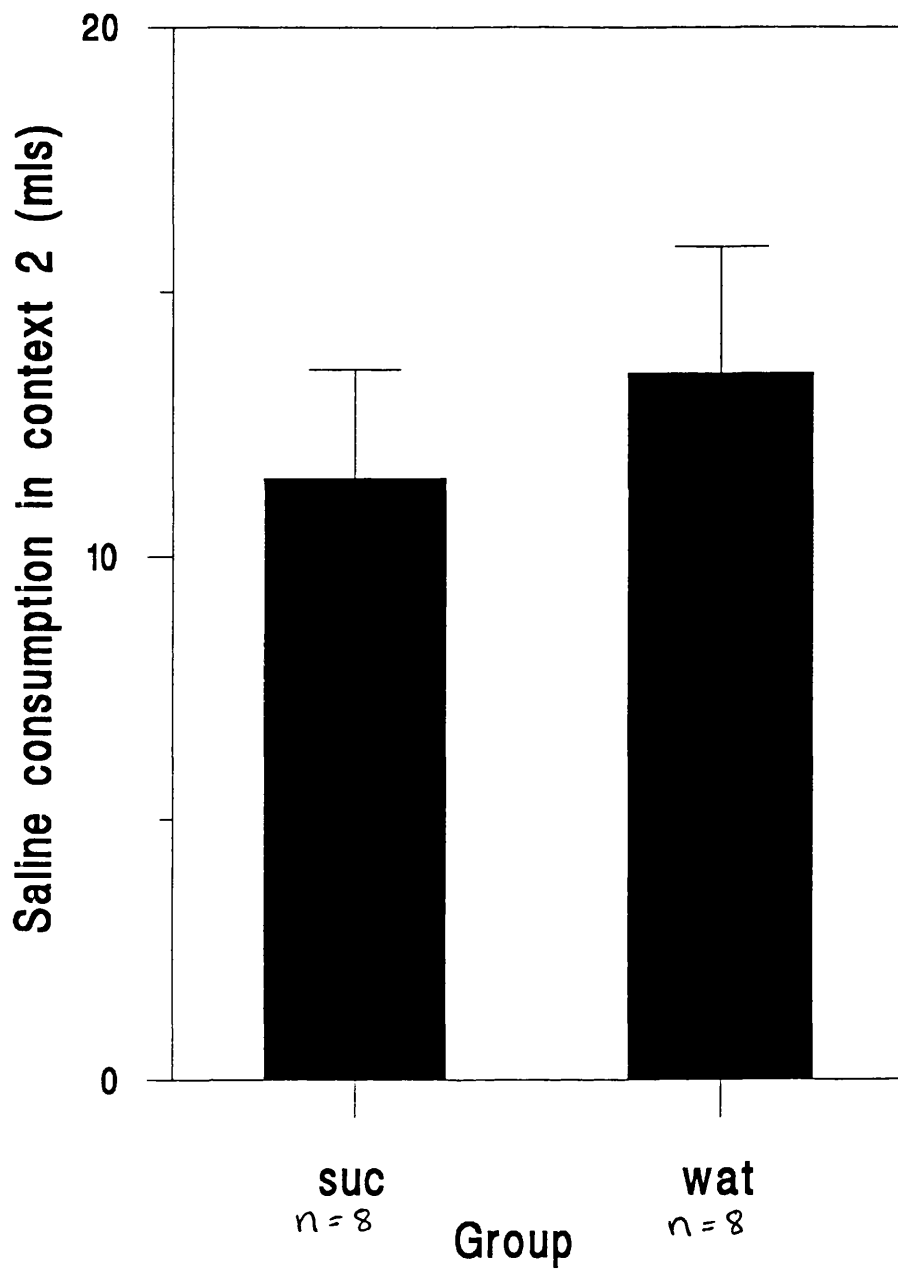
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Figure 7.1. Mean consumption of saline in context 2 on test in Experiment 7A. Context 2 was paired with LiCL in the presence of water (WAT) or sucrose solution (SUC) on one occasion. Error bars indicate SEMs.

on the aversiveness of that context when only one conditioning trial takes place. It would be premature to conclude from this that one-trial potentiation does not occur using this procedure. It may be that the test used for a context aversion in this procedure is not sensitive to the small changes in the aversiveness of the context which result from the use of only one conditioning trial. In Experiment 7B, a potentially more sensitive test was used.

## 7.2.2: Experiment 7B Blocking of a taste aversion by a context which was subject to one-trial potentiation

Experiment 7B used a similar procedure to that of Experiment 7A, except that the aversiveness of the context was not directly tested following conditioning, but was used to block an aversion towards a second novel flavour. It has been suggested (Lolordo & Droungas, 1989) that such a test might be sensitive to small differences in the aversiveness of a potentiated stimulus where a direct test is not. In addition to a possible increase in sensitivity, this procedure has the advantage that it may rule out the instrumental account of potentiation outlined above, regardless of whether incentive learning is necessary for a taste aversion to occur (Balleine & Dickinson, 1991). If the presence of sucrose in the context during conditioning leads to an increase in the ability of that context to block an aversion towards a second flavour, it is difficult to see how this could arise due to a suppression of an instrumental response; the more aversive context was predicted to lead to an increase in consumption of the second flavour on test.

Four groups were used, two of the groups received a context-LiCl pairing with sucrose present in the context, while a further two groups received a context-LiCl pairing with water in the context. All four groups then received a further context-LiCl conditioning trial during which no liquid was available in the context. On this second conditioning trial, two groups, one of which had received sucrose on the initial trial (SUC-VIN), and one of which had received water (WAT-VIN), received a vinegar solution before entering the context. The remaining groups (SUC-WAT and WAT-WAT) drank water rather than vinegar before entering the context on the second conditioning trial. All groups were then tested for an aversion to vinegar.

It was predicted that, of the groups which received vinegar on the second trial, the one in which sucrose was presented in the context during the first conditioning trial (SUC-VIN) would show an attenuation in the aversion to vinegar compared to that in which water was presented in the first conditioning trial (WAT-VIN). If sucrose presentation in the context during the first conditioning

trial led to an increase in the aversion which developed towards the context, then the degree to which the context is likely to block an aversion towards vinegar would be expected to increase. The remaining two groups which received water rather than vinegar on the second conditioning trial represented control groups both for the conditioning of an aversion towards vinegar (WAT-WAT) and the possibility that an aversion towards sucrose generalised to vinegar (SUC-WAT).

## Method

### Subjects and Apparatus

Thirty two, male Wistar rats (350 - 550g) were used. All of the animals had previously served in an experiment on latent inhibition in which they received lights as CSs and food pellets as reinforcers. They were housed, fed and watered as in Experiment 7A. The apparatus was also the same as that used in experiment 7A.

### Procedure

The design of Experiment 7B is presented in Table 7.1. All of the animals were taken off water on Day 1. On each of the following seven days (Days 2 - 8), the animals received 15 minutes access to water in context 1. All groups were equated for water consumption on Days 7 and 8 and assignment to groups was carried out on day 8. The first conditioning trial took place on Day 9. On this day, all animals were placed in context 2 for 15 minutes. They were then removed and injected with 0.3M LiCl (10 ml/kg, i.p.). Of the four conditions, two (Group SUC-VIN and Group SUC-WAT) received sucrose solution in context 2, while the other two (Group WAT-VIN and Group WAT-WAT) received water. The animals were replaced in the home cage following injection. Days 10 and 11 were recovery days on which all animals were placed in context 1 for 15 minutes access to water.

The second conditioning trial took place on Day 12. All animals were placed in context 1 where Groups SUC-VIN and WAT-VIN were given 5 mls of 4% (v/v) vinegar solution to drink, while Groups SUC-WAT and WAT-WAT were given 5 mls of water. The animals were then replaced in the home cages for three hours. Following this period, the animals were put in context 2 for 15 minutes, with no liquid available. Removal from context 2 was followed immediately by an injection of 0.3 M LiCl (10 ml/kg, i.p.), and the animals were then replaced in the home cages.

| Table 7.1 The design of Experiment 7B. | | | | |
|---|---|---|---|---|
| | | Conditioning | | Test |
| | Day 9 | Day 12 | | |
| Group | context 2 | context 1 | context 2 | context 1 |
| SUC-VIN | SUC => LiCl | VIN | Ø => LiCl | VIN |
| WAT-VIN | WAT => LiCl | VIN   3hrs | Ø => LiCl | VIN |
| SUC-WAT | SUC => LiCl | WAT | Ø => LiCl | VIN |
| WAT-WAT | WAT => LiCl | WAT | Ø => LiCl | VIN |

Days 13 and 14 were recovery days on which the procedure was the same as that on Days 10 and 11. Day 15 was the test day. On this day, all animals were placed in context 1 and offered 4% (v/v) vinegar solution.

## Results and Discussion

Vinegar consumption on Day 15 (the test day) is shown in Figure 7.2. The data indicate that animals given vinegar solution in context 1 at conditioning (Groups SUC-VIN and WAT-VIN) consumed less vinegar on test than those given water in context 1 during conditioning (Groups SUC-WAT and WAT-WAT). A two-way ANOVA revealed a main effect of flavour (vinegar vs water) on the second conditioning day ($F_{(1,28)}$ = 16.3), but there was no effect of flavour presentation (sucrose vs water) on the context conditioning day (F<1) and no interaction (F<1).

These data suggest that an aversion developed towards vinegar in those animals which received vinegar solution before being placed in the context and then poisoned on Day 12 (Groups SUC-VIN and WAT-VIN). However, exposure to the context on this day, a context which had either previously been conditioned in compound with sucrose (Groups SUC-VIN and SUC-WAT) or water (WAT-VIN and WAT-WAT) did not affect vinegar consumption on test. It can be concluded that animals which received sucrose in context 2 during context conditioning (Group SUC-VIN) did not develop a stronger aversion to that context compared to animals which received water at this time (WAT-VIN), as measured by the ability of the context subsequently to block
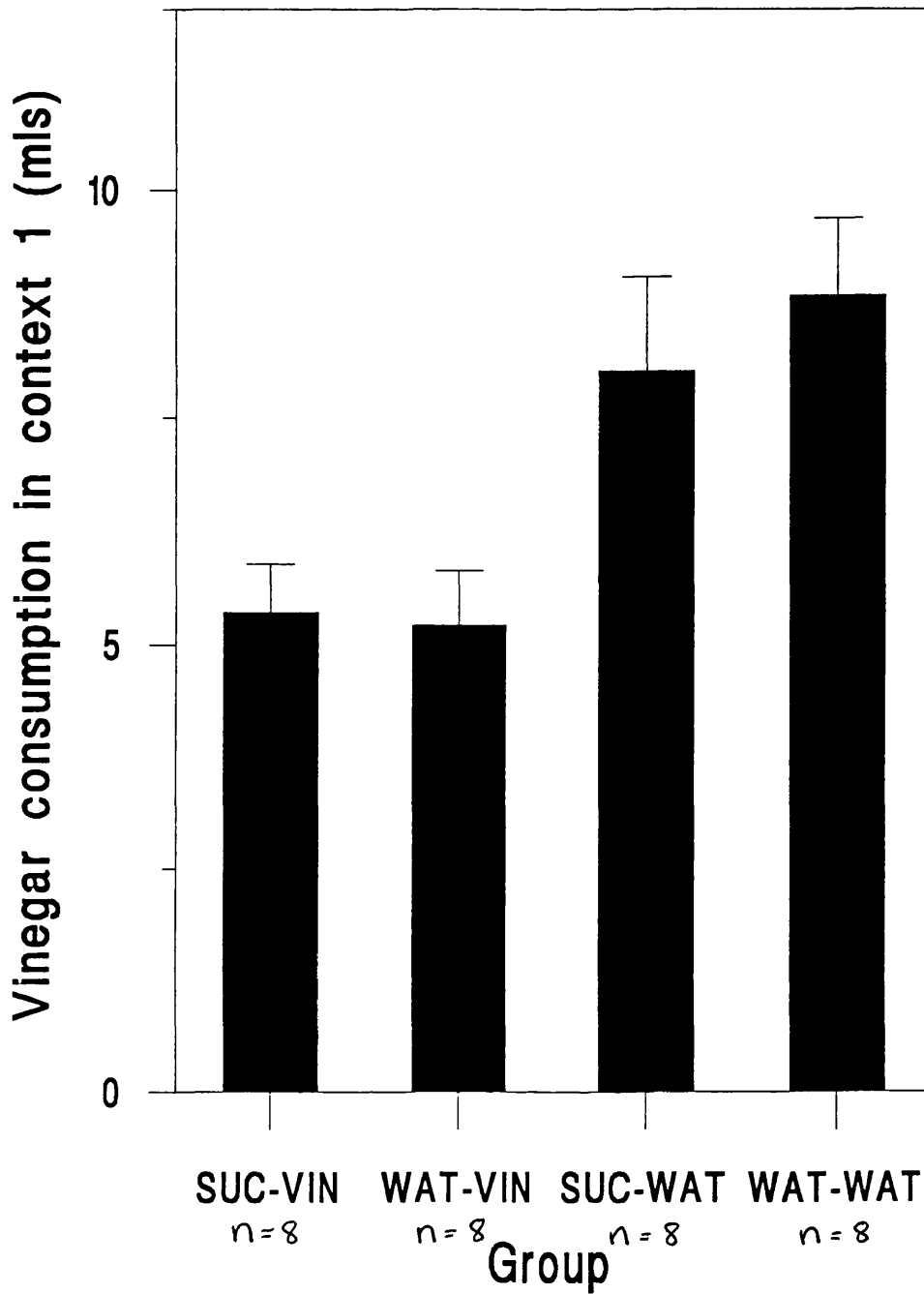
Figure 7.2. Mean consumption of vinegar in context 1 on test in Experiment 7B. Vinegar or water was paired with LiCL in compound with context 2, which had been previously paired with LiCl in the presence of water or sucrose. Error bars indicate SEMs.

an aversion to vinegar solution. In addition, conditioning with sucrose did not lead to an aversion to vinegar; Group SUC-WAT did not show suppressed consumption of vinegar on test compared to Group WAT-WAT.

There is no evidence from either Experiment 7A or 7B that a single conditioning trial led to the potentiation of an aversion to the context in which sucrose was presented compared to that in which water was presented. However, the simplest account of this failure is that insufficient conditioning occurred in order to detect such a difference using these test procedures. The final experiment in this series addressed this question using a similar procedure as that used in Experiments 6A and 7A but instead of one conditioning trial, the animals in the crucial experimental group were subject to three conditioning trials with a different flavour being presented on each trial.

### 7.2.3: Experiment 7C Potentiation of a context aversion following varied flavour presentation in that context

The present experiment assessed whether the presentation of a different flavour in a novel context on each of three conditioning trials would lead to potentiation of an aversion to that context. In an initial study, the three flavours used were sucrose, coffee and dilute hydrochloric acid. It was found that sucrose and coffee, but not HCl, presented in the novel context three times during conditioning led to potentiation of an aversion to that context. Therefore, it was not possible to interpret the data from a group which received all three of these flavours, one on each conditioning day. This group did not provide any evidence of potentiation of a context aversion but this could have been due to a failure of one of the flavours to lead to potentiation rather than the failure of three different flavours to lead to potentiation. In the present experiment, the HCl solution was replaced with vinegar solution. Pilot studies suggested that aversions towards the coffee, vinegar and sucrose solutions used in this experiment do not generalise significantly to each other.

As well as testing whether repeated reinforced pairings of a flavour-context compound are necessary for the demonstration of potentiation, this experiment aimed to determine the relative strength of the context aversion resulting from this procedure when either the same flavour or different flavours are used on each conditioning trial. It was argued in the introduction to this chapter that an aversive flavour might be more salient than a non-aversive flavour and therefore lead to a greater degree of potentiation, a prediction which is consistent with all three models of

potentiation (Durlach & Rescorla, 1980; Galef & Osborne, 1978; Rusiniak et al, 1979). Since, according to Galef and Osborne (1978) and Rusiniak et al (1979), taste-mediated potentiation is not dependent on an aversion developing towards the flavour, but on a direct context-illness association, these theories only predict a difference between these two groups on the basis of the relative distinctiveness or salience of aversive and non-aversive flavours.

In contrast, the hypothesis proposed by Durlach and Rescorla (1980) makes the prediction that the degree to which potentiation occurs is directly related to the strengths of the context-taste and the taste-illness associations. Thus, an analysis of the strengths of these two associations will allow predictions to be made as to the strength of the conditioned response to the potentiated stimulus. Since, according to Rescorla and Wagner (1972), the associative strength which accrues to a CS during training is greatest on the first trial of learning, it might be predicted that potentiation would be greater if a different flavour were presented in the context on each conditioning trial. This is predicted because the strength of the context-taste associations in a procedure in which a different flavour is presented on each trial, summed across three trials, will be greater than the context-taste association which forms as a result of multiple conditioning trials with the same flavour. In the former case, each pairing is the first pairing with that flavour and thus the learning rate is at a maximum. The same is true for the taste-illness associations; in total, the associative strength of three separate flavours, when paired with illness, will be greater than that of a single flavour paired three times.

Thus, although the associative strength of each of the context-taste-illness associations will be less in the case when a separate flavour is used on each conditioning trial, the sum of these associations will be greater than that which forms when a single flavour is conditioned three times. There are, however, reasons why the sum of the context-flavour associations in a potentiation procedure in which the flavour varies across trials may be lower than when the flavour remains constant across trials. It is possible that the context-flavour association formed on the initial trials extinguishes on later trials in which the context, but not that particular flavour, is presented. Also, the context-flavour associations formed during the early trials may interfere with the learning of the association between the context and the flavours presented on later trials. Therefore, although the Durlach and Rescorla model of potentiation allows that varying the flavour across conditioning trials may lead to an enhancement of the potentiation effect, examination of the strengths of the context-flavour and flavour-illness associations which may form during a multiple flavour potentiation procedure, does not allow a firm prediction to be made as to the outcome of such a

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procedure. Finally, if it is the case that a taste aversion is not learned until the flavour is re-exposed (Dickinson & Balleine, 1994), then, according to Durlach and Rescorla (1980), no taste aversion learning will take place in the multiple-flavour procedure, and therefore no enhancement of the context aversion should be seen.

The present experiment used a procedure which was similar to that used in Experiment 6A. All three groups received three flavour-context compound conditioning trials. One group was presented with a single novel flavour in the context on each of the three occasions (Group SAME), while a second group received water in the context (Group WAT). In the experimental group, three different flavours were presented in the context, one on each conditioning trial (Group DIFF).

## Method

### Subjects and Apparatus

Twenty eight experimentally naive, male Sprague Dawley rats (250 - 350g) were used. They were housed, and fed and watered as in Experiment 7A. The apparatus was also as in Experiment 7A.

### Procedure

The procedure was the same as that in Experiment 7A for the first 11 days. Thus, all animals received four days access to 0.9% saline solution in the home cage at the beginning of the experiment, and seven days on which they were presented with water for 15 minutes in context 1. The animals were assigned to groups by equating mean group water consumption on Days 10 and 11 as closely as possible. The animals from Group SAME were then divided into three batches of three, each batch would receive one of the three flavoured solutions during conditioning, the same flavour presented to each batch from this group on each conditioning trial. The animals from Group DIFF were to receive all three flavours, one on each conditioning day. The order of flavour presentation in Group DIFF was counterbalanced as far as possible; of the six possible flavour orders, three were presented to six animals while the remaining three were presented to only three animals.

The first conditioning trial occurred on Day 12. All the animals were placed in context 2 for 15 minutes and given access to 0.3% (w/v) sucrose solution, 4% (v/v) distilled vinegar solution or

10% (w/v) decaffeinated coffee solution (Groups SAME and DIFF) or tap water (Group WAT). An injection (10 ml/kg i.p.) of 0.3 M LiCl was administered immediately on removal from context 2. The rats were then replaced in their home cages. Days 13 and 14 were recovery days when the animals were allowed 15 mins access to tap water in context 1 each day. This three day cycle (Days 12 - 14) was repeated twice across the following six days (Days 15-17 and Days 18-20) so that there were three conditioning trials in total. Group SAME received the same flavour in context 2 on each of the conditioning trials, whereas Group DIFF received all three flavours across the three conditioning trials (order counterbalanced). Testing was carried out on Day 21, when each animal was given 15 mins access to 0.9% saline solution in context 2.

## Results and Discussion

The saline consumption from each of the groups is shown in Fig 7.3. It is apparent that both of the groups which received flavours in context 2 during conditioning (Groups SAME and DIFF) showed suppressed consumption of saline solution on test compared to the group which received water in the context during conditioning (Group WAT) suggesting that potentiation of a context aversion occurred in both Group SAME and Group DIFF. Due to inequality of variance across groups, non-parametric statistical analyses were carried out on these data. A Kruskall Wallis test revealed a significant main effect of group (H(28) = 10.39) and follow up test indicated a difference between Group SAME and Group WAT (Z = 3.22), but no other differences were reliable. These data, like those of Experiment 6A suggest that the reinforced presentation of a single flavour in a novel context on three occasions led to an enhancement in the aversion which developed towards that context compared to a group which received water in the context. However, although the animals in Group DIFF apparently showed suppressed consumption of saline on test, the difference between this group and the water controls (Group WAT) was not reliable. These results extend those of Experiment 6A by showing that, when presented during each of three context-LiCl pairings, not only sucrose, but also coffee and vinegar, support enhancement of a context aversion.

The absence of a reliable potentiation effect in Group DIFF may indicate that it is necessary to present the same flavour on each of the three conditioning trials in order to demonstrate such an effect. The fact that the mean consumption of saline in context 2 in this group was lower than that in Group WAT may have been due to some generalization between the three flavours, possibly on the basis of novelty. Honey (1990) suggested that novelty is one stimulus dimension
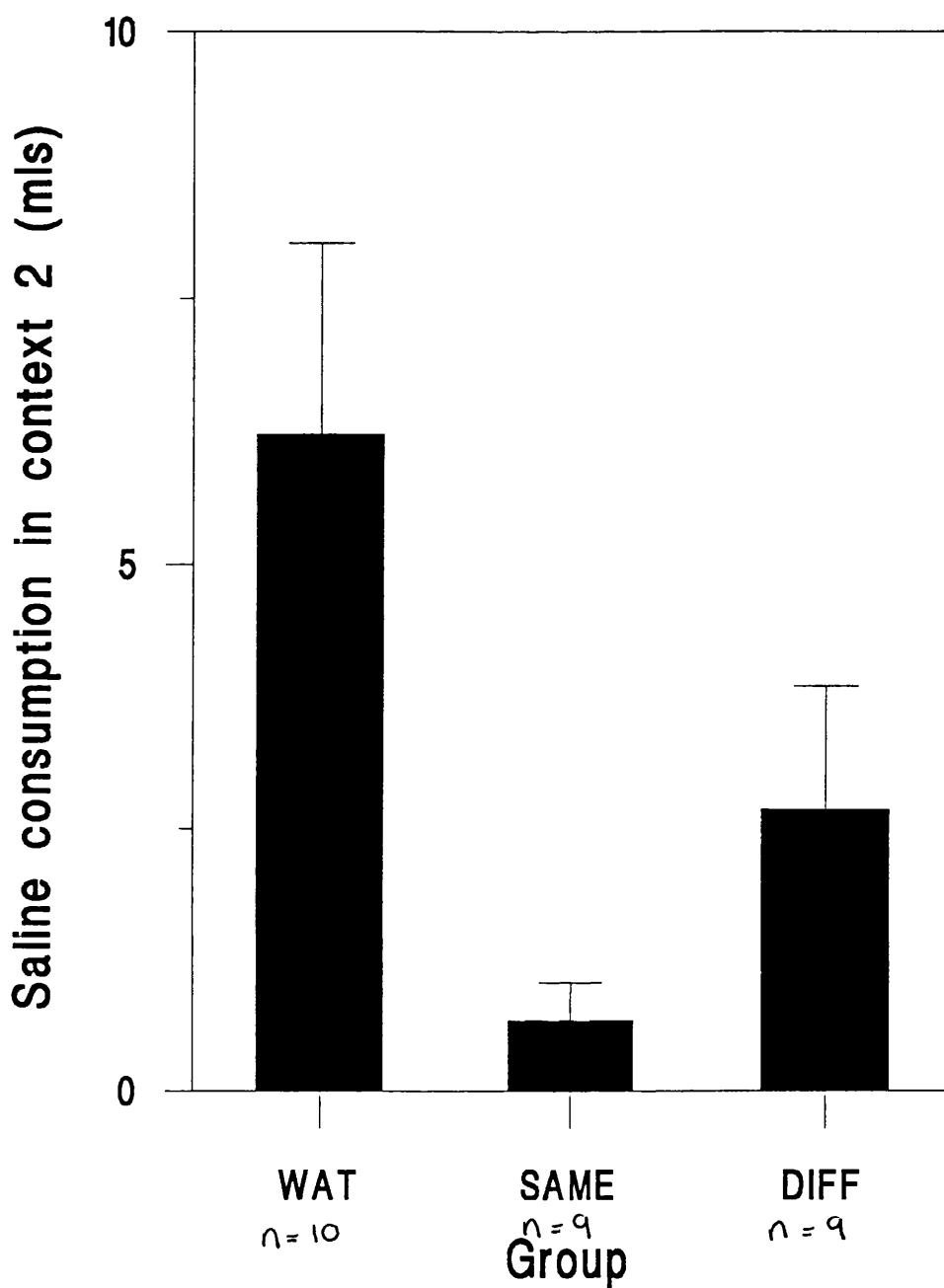
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Figure 7.3. Mean consumption of saline in context 2 on test in Experiment 7C. Context 2 was paired with LiCl in the presence of water (WAT), the same flavour on each trial (SAME), or a different flavour on each trial (DIFF). Error bars indicate SEMs.

on which generalization might take place. Thus, on the second and third conditioning trials, the novel flavours which were presented to this group may have been, to some extent, aversive as a result of generalization from an aversion towards the previously presented flavours. The aversiveness of these flavours on the second and third trials may be crucial for potentiation to occur, perhaps because of stimulus salience.

Alternatively, it may be the case that re-exposure to the flavours is necessary for a taste aversion to occur, and, therefore, for potentiation to take place. It is not clear, at this stage, whether potentiation will take place when a different novel flavour is presented on each conditioning trial. It would seem that, if it does, demonstration of such an effect may require more than three conditioning trials.

## 7.3: General Discussion

In Experiments 7A and 7B, an attempt was made to demonstrate one-trial potentiation of a context aversion. When the aversion towards the context was assessed by consumption of a familiar flavour in that context, no evidence for potentiation following one conditioning trial was found (Experiment 7A). Similarly, when the aversion towards the context was measured by its ability to block a taste aversion, no potentiation was observed (Experiment 7B).

Finally, Experiment 7C examined the ability of the presentation of a different flavour on each conditioning trial, using a three-trial procedure such as that used in Experiment 6A, to potentiate a context aversion. When the same flavour was presented in the context on three successive conditioning trials (Group SAME), the aversion towards that context was enhanced, while the results from a group which received a different flavour on each conditioning trial (Group DIFF) were ambiguous. The consumption of familiar saline in the context, of a group which received a different flavour on each trial (Group DIFF), was suppressed compared to the controls, but not reliably. In addition Group DIFF did not consume reliably more saline on test than Group SAME. It is, therefore, unclear as to what effect the presentation of a different flavour on each conditioning trial had on the context aversion.

In Experiment 7C, some suppression of consumption of familiar saline solution on test was noted in Group DIFF (animals which received a different flavour on each conditioning trial), suggesting the formation of a stronger context aversion than that which occurred in Group WAT (the control

group which received water in the context during training). However, this difference was not reliable. In the introduction to this chapter, the possibility was raised that the presentation of a different flavour on each conditioning trial might lead to the overshadowing of a context aversion, but this would seem not to have occurred. It might be argued that, since there was no control group to detect conditioning of the context aversion, Group WAT did not show suppression of consumption of saline on test and therefore, it would not be possible to detect overshadowing. However, Group WAT received identical treatment to Group WAT-LI in Experiment 6A which showed a reliable aversion towards the context compared to Group WAT-SAL (an unpoisoned group). It would seem then, that multiple-trial multiple-flavour compound conditioning of a context aversion does not give rise to an attenuation in that aversion.

The remaining question regards the context potentiation resulting from this multi-flavour procedure. It is not at all clear whether the presentation of a novel flavour in a novel context during toxicosis conditioning, if that flavour is varied across conditioning trials, will lead to potentiation of a context aversion. In Experiment 7C, some suppression of consumption of saline on test was observed, but this was not reliable and, were it to be found to be reliable following further studies, may be due to generalization between the flavours presented in the context. The failure to find reliable potentiation in Group DIFF as compared to Group SAME is consistent with all three hypotheses outlined in the introduction (Durlach & Rescorla 1980; Galef & Osborne, 1978; Rusiniak et al, 1979) if it is assumed that an aversive flavour is more salient than a non-aversive flavour.

It is clear that Group DIFF in Experiment 7C did not develop a stronger aversion towards the context than did Group SAME. It was argued in the introduction to Experiment 7C that the model proposed by Durlach and Rescorla (1980) might predict greater potentiation of a context aversion if the flavours presented were different across conditioning trials, this was not found to be the case. Since there were other reasons why varying the flavour across trials may not have led to an an increase in the level of potentiation (associations of the context with flavours presented on early trials both extinguishing on later trials, and interfering with the development of associations with the flavours presented on later trials), this cannot be seen as evidence against the Durlach and Rescorla model. Both Galef and Osborne (1978) and Rusiniak et al (1979) would predict greater potentiation in Group DIFF if a novel flavour on each trial were more salient than one which previously had been paired with illness. The relative salience of a novel and a previously paired flavour is not known, and therefore, it is not possible to distinguish between the associative and

non-associative models of potentiation on the basis of the data from Experiment 7C.

In Experiment 7C, there was some suggestion that the presentation of a different flavour on each conditioning trial led to a suppression of consumption of saline in the test context on test. It was suggested in Experiment 7B that a blocking procedure might be more sensitive than a consumption test for detecting differences in context aversions. Thus, it might be informative to use such a test following a multiple-trial multiple-flavour conditioning procedure. However, there is no evidence from Experiment 7B that the blocking test is more sensitive than the consumption test (as used in Experiment 7A), since neither test revealed a potentiation effect in Experiments 7A and 7B.

It might be argued that the blocking test used in Experiment 7B was not sensitive to any difference in the strength of the context aversions, not because a blocking test would not, in general, be more sensitive, but because the specific procedure used in this experiment was not appropriate. There are reasons why blocking might not be expected using the procedure of Experiment 7B. First, only one compound conditioning trial was used. It is clear that one-trial blocking is not as reliable as multi-trial blocking (for failures to observe one-trial blocking see Mackintosh, 1975b; Mackintosh, Dickinson & Cotton, 1980). However, it has been suggested that failures to observe blocking in one trial may be due to second order conditioning. Although the target stimulus may not become associated directly with the US as a result of blocking, it becomes associated instead with the blocking stimulus and therefore acquires higher order associative strength (Dickinson, Nicholas & Mackintosh, 1983). The conditions in Experiment 7B do not seem to be those in which significant second order conditioning would be expected to occur; the blocking stimulus (the context) was both temporally and spatially separated from the target (the vinegar solution), being presented in a different experimental room and three hours after the target stimulus was presented.

Second, the relative salience of the stimuli presented in compound in a blocking procedure are thought to be important in determining when blocking will occur (Pearce, 1987). In particular, it has been suggested that a stimulus of low salience is less likely to block conditioning to a highly salient stimulus than vice versa (Hall, Mackintosh, Goodall & Dal Martello, 1977). Conversely, a stimulus of low salience is more likely to be subject to potentiation in a toxicosis conditioning procedure (Bouton et al, 1987). In combination, it would seem that a stimulus which has been shown to be potentiated by the presence of a flavour in toxicosis conditioning is unlikely to be able to block a taste aversion. However, the salience of the context might be expected to increase

as a result of the presence of the taste during pre-training (Galef & Osborne, 1978), and therefore become able to block an aversion towards a second taste. In addition, Willner (1978) observed one-trial blocking of an aversion towards saccharin solution by contextual cues that had been paired with illness in the absence of a distinctive flavour. Thus, there would seem to be no a priori reason to reject the possibility that one-trial blocking of a taste aversion might occur using the procedure of Experiment 7B and, in addition, that this blocking might be more complete if the context has been pre-trained in the presence of a flavour. It would seem, therefore, that the failure to observe enhanced blocking of a taste aversion by a flavour-potentiated context aversion, compared to that by a context in which water was present during training, was due to the absence of any reliable difference in the aversiveness of these two contexts.

In conclusion, the potentiation effect found in Experiment 6A was replicated using different flavours from those used in Experiment 6A. No effect of sucrose presentation in a novel context before toxicosis conditioning was found when only one conditioning trial took place, and no potentiation resulted from a procedure in which the flavour was varied across three conditioning trials. Therefore, it is possible that the presentation of novel flavoured foods in the chemotherapy clinic, to overshadow aversions towards previously consumed normal dietary items, may not lead to the potentiation of the aversion towards the clinical context if the overshadowing flavour is varied from one treatment session to another.

# Chapter Eight

# Conclusions and Future Directions

## 8.1: Introduction

The aim of the experiments presented here was to investigate, using a rat model, two side effects of cancer chemotherapy: anticipatory nausea and vomiting (ANV), and taste aversions. Previous clinical and animal studies have addressed one, or the other of these problems, but not both. Psychological and pharmacological interventions have been tested for their efficacy in controlling conditioned taste aversions and ANV with some success. It is argued in this final chapter that an intervention used to combat taste aversions and ANV requires an understanding, not only of each of these problems in isolation, but the possible interactions which may occur between the two.

It was argued in Chapter 1 that both ANV and taste aversions in cancer chemotherapy patients might result from either associative or non-associative mechanisms. ANV has been considered to be either directly induced through the high levels of anxiety experienced by patients in anticipation of receiving treatment, or through the association of stimuli present in the clinic with the nausea and vomiting induced by the drugs which the patients receive. Two explanations for the development of taste aversions were also presented: rejection of specific flavours by cancer chemotherapy patients may be the result of a treatment-induced heightened sensitivity to gastric stimulation, which would lead to certain foods (strong gastric stimulants) becoming aversive, or from a learned association between the foods consumed before treatment and the illness which the patients subsequently experience (a conditioned taste aversion (CTA)).

The evidence presented in Chapter 1 suggested that both ANV and taste aversions in chemotherapy patients are conditioning phenomena. In the case of ANV, the anxiety hypothesis failed to account for the results of studies on bone marrow aspiration patients, who are thought to experience a very high level of anxiety, but who experience relatively low levels of ANV. ANV is similar to the phenomenon of context aversion, which has been demonstrated in rats. For example, in an experiment by Boakes et al (1992) rats developed an aversion towards a novel context as a result of its pairing with lithium chloride.

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The best evidence that taste aversions in chemotherapy patients are the result of a conditioning process comes from the finding that the presentation of a novel flavour before treatment results in attenuation of these aversions (Broberg & Bernstein, 1987). This finding could not be easily explained if the taste aversions were thought to result from a non-associative mechanism. However, this finding is consistent with the very robust conditioning phenomenon of overshadowing (Mackintosh, 1976; Pavlov, 1927; Revusky, 1971), in which the presentation of a second cue during conditioning leads to less conditioning of the target cue. In the light of this evidence, taste aversions resulting from chemotherapy have been referred to as conditioned taste aversions (CTA).

In this chapter, the results of the experiments presented in Chapters 3 to 7 will be discussed in light of the literature reviewed in Chapters 1 and 2. Following this, the validity of the animal models used in these experiments will be examined, and directions for further research will be outlined.

## 8.2: Pharmacological Interventions

To the extent that both CTA and ANV are the result of the illness induced by the administration of the cytotoxic chemotherapy drugs, one would expect the reduction of such illness to reduce CTA and ANV. However, evidence was presented in Chapter 2 from which it was concluded that the reduction in the level of nausea and vomiting resulting from the administration of an antiemetic would not necessarily be associated with a reduction in CTA (Grant, 1987). Since no firm prediction as to the effects of particular antiemetics on CTA could be made, it was considered worthwhile to test two antiemetic drugs which have recently been found highly effective in the control of chemotherapy-induced nausea, but which have not been tested for their efficacy in the attenuation of CTA.

### 8.2.1: 5-HT$_3$ receptor antagonists

The first of these drugs was the 5-HT$_3$ receptor antagonist ondansetron. Ondansetron is thought to block cisplatin-induced vomiting through activity at 5-HT$_3$ receptors on the vagus nerve. Detection of cisplatin by the vagus nerve is thought to give rise to the vomiting reflex by stimulating the vomiting centre located in the nucleus of the solitary tract (NST) (Andrews et al, 1990). The route of action of cisplatin in the induction of CTA is not known. However, from

experiments monitoring cisplatin-induced *c-fos* expression (Reynolds et al, 1991), it is clear that cisplatin gives rise to neuronal activity in both the vagus nerve and the chemoreceptive trigger zone, located in the area postrema (AP). In the same study, it was shown that treatment with the 5-HT$_3$ receptor antagonist granisetron led to the blockade of the vagus nerve but not the AP. These results allow the possibility that cisplatin induces CTA through either the vagus nerve, the AP, or both of these routes. The effect of ondansetron on cisplatin-induced CTA will depend on whether the vagus nerve or the AP is responsible for this response.

The results presented in Chapters 3 and 4 did not support unequivocally the hypothesis that ondansetron will attenuate cisplatin-induced CTA. A number of experiments (Experiments 3D, 3E and A(i)) provided evidence that 0.3 mg/kg ondansetron delivered on three separate occasions following administration of 0.3 mg/kg cisplatin, attenuated CTA to a sucrose solution consumed before drug administration. However, this effect was not replicated in a number of further studies. It was suggested that the fragility of the effect may have been due to the short half life of ondansetron, which, given the protracted effects of cisplatin, may have led to incomplete blockade of cisplatin-induced nausea across the 24 hours following drug administration. Incomplete blockade might allow learning of a flavour-illness association since CTA learning can occur across a period of many hours (Smith & Roll, 1967).

Three experiments (Experiments 4A, 4B and 4C) presented in Chapter 4 attempted to demonstrate an effect of ondansetron on cisplatin-induced CTA when ondansetron was administered throughout the 24 hours following cisplatin administration from subcutaneously implanted osmotic minipumps. The implantation procedure, requiring the use of anaesthetic, led to a disruption of the cisplatin-induced CTA, and it was found necessary to increase the cisplatin dose to 0.6 mg/kg in order to demonstrate CTA using this procedure. At this dose of cisplatin, no attenuation of CTA by ondansetron was detected. In all of the experiments in which 0.3 mg/kg cisplatin was used to induce CTA, although the CTA was not reliable, the consumption of sucrose solution was numerically greater in the animals that received the highest dose of ondansetron (0.5 mg/kg/hour across 24 hours) than in those which received cisplatin alone. This suggests that ondansetron at 0.5mg/kg/hour across 24 hours may be effective in attenuating 0.3 mg/kg cisplatin-induced CTA, but further experiments, possibly using a different form of anaethetic (perhaps using an injected anaesthetic rather than an anaesthetic chamber in order to eliminate interference by the odour), would be required to reach a firm conclusion.

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The data from Experiment 4C suggested that ondansetron, even when administered across a period of 24 hours, does not attenuate CTA induced by 0.6 mg/kg cisplatin. Without further experiments, it is not possible to establish whether the effect of ondansetron on cisplatin-induced CTA is dependent on the dose of cisplatin. However, indirect evidence that this may be the case comes from studies suggesting that radiation may gives rise to vomiting in a similar manner to cisplatin (Andrews et al, 1988) and that high doses of radiation are detected by the AP, while low doses are not (Andrews et al, 1988; Carpenter et al 1988).

At this stage it would be unwise to rely heavily on a parallel between radiation-induced vomiting and cisplatin-induced CTA. However, one can speculate that high dose cisplatin is detected by the AP to a greater extent than is low dose cisplatin, but that both are detected by the vagus nerve. If this were the case, then ondansetron, which blocks the vagus nerve, would be expected to attenuate low dose. but not high dose, cisplatin-induced CTA. This hypothesis is testable using a procedure in which the vagus nerve is lesioned, and the effect of this intervention is tested in cisplatin-induced CTA in which the dose of cisplatin is varied. If it were the case that low dose cisplatin leads to CTA through the vagus nerve but that high dose cisplatin is also detected in the AP, then vagotomy. and therefore a 5-HT$_3$ receptor antagonist, should lead to greater attenuation of CTA induced by low dose than by high dose cisplatin.

## 8.2.2: NK$_1$ receptor antagonists

In a further attempt to find an antiemetic likely to attenuate CTA, the experiments in Chapter 5 tested two NK$_1$ receptor antagonists in a procedure similar to that used to test the efficacy of ondansetron in cisplatin-induced CTA. Evidence that NK$_1$ receptor antagonists block rather than delay vomiting in ferrets suggested that the half life of these antiemetics would not present as great a problem as did the half life of ondansetron. CP-99,994 and L-742,694, both selective NK$_1$ receptor antagonists. were tested.

It was pointed out in Chapter 5 that a rat model of CTA might not be ideal to assess the effect of these compounds on CTA since both CP-99,994 and L-742,694 are selective antagonists for the human, guinea pig and ferret NK$_1$ receptor, but not the rat NK$_1$ receptor. However, an effect of a very high dose of L-742,694 was found in Experiment 5C: 100 mg/kg L-742,694 attenuated 0.3 mg/kg cisplatin-induced CTA. A number of further experiments were suggested in order to establish both that the effect demonstrated in Experiment 5C was a result of NK$_1$ receptor

antagonism, and why the effect of L-742,694 on cisplatin-induced CTA was very small. Clearly, the most important experiment would attempt to demonstrate the effect using either an $NK_1$ receptor antagonist with affinity for the rat receptor, in the rat model used here, or the current compound in a guinea pig or ferret model. In the meantime, the results of Experiment 5C are a very promising indicator that $NK_1$ receptor antagonism might attenuate cisplatin-induced CTA.

## 8.2.3: The use of antiemetics in the control of CTA

In Chapter 2 it was argued that the effectiveness of a compound in the control of vomiting could not be taken as evidence that the compound would be effective in attenuating CTA. It might seem, then, that antiemetics are no more likely to be effective in attenuating CTA than any other drug, and therefore that testing of such compounds is not justified. There are three reasons why, in the search for an effective anti-CTA drug, those which have already been established as having antiemetic properties are good candidates. The first and most obvious of these is that effective antiemetics are likely to be given to chemotherapy patients regardless of their effectiveness in attenuating CTA. Thus, treatment of CTA could be achieved through the selection of an appropriate antiemetic. Since, antiemetics form part of the chemotherapy patients routine treatment, control of CTA would require no further medication. Second, although the relationship between CTA and vomiting apparently is not as strong as Garcia et al (1974) claimed (see Chapter 2), certain neural substrates of CTA are known to mediate the vomiting response (the NST, AP and vagus nerve). Thus, if the suppression of the activation of such areas reduces vomiting, and a compound is found to reduce vomiting, there is a possibility that this compound will also attenuate CTA. Finally, Revusky and Martin (1988) provided some evidence that gut-related distress is the US or reinforcer in CTA learning and, if this is true, then any drug which reduces gut-related distress in the clinic would be expected to reduce CTA.

Although it was not their principle purpose, the experiments presented here (Experiments 3A to 5C) provide data pertinent to the testing of Revusky and Martin's hypothesis. Ondansetron has been shown to control gut-related distress in the clinic (Jones et al, 1991), and therefore Revusky and Martin's hypothesis predicts that this compound will lead to an attenuation of CTA. This prediction did not receive strong support from the data presented in Chapters 3 and 4. Ondansetron was not effective in attenuating cisplatin-induced CTA in these experiments, although such an effect might be demonstrated using low doses of cisplatin and a long duration of ondansetron infusion,

Experiment 5C showed that L-742,694 reduces CTA in the rat and therefore Revusky and Martin's hypothesis would predict that L-742,694 will be effective in reducing gut-related distress in the clinic. The prediction that $NK_1$ receptor antagonists will alleviate gut-related distress in the clinic has yet to be tested. Such a demonstration would constitute strong evidence that gut-related distress is the reinforcer in CTA formation, and that testing the efficacy of compounds in the attenuation of CTA might serve as a screening method in the development of anti-nausea agents.

## 8.3: Psychological Interventions

### 8.3.1: Potentiation in the chemotherapy clinic

It is possible that no pharmacological intervention will lead to complete blockade of CTA in cancer chemotherapy patients. Certainly, taken at face value, the evidence presented in Chapters 3, 4 and 5 suggests that the attenuation of CTA by the administration of antiemetic drugs is, at best, likely to attenuate CTA only marginally. If this were the case, then the development of a psychological intervention based on established learning theoretic principles may provide a more effective means of controlling the conditioned side effects of cancer chemotherapy. The experiments presented in Chapters 6 and 7 were designed to test the efficacy of one particular intervention for the control of chemotherapy-induced CTA, that of overshadowing. In addition, it was anticipated that such experiments would promote understanding of the likely interactions between the development of aversions towards foods which are consumed before therapy, and those towards the context in which the therapy is given.

It was found in Chapter 6 that, in a rat model of ANV and CTA, the presentation of a novel flavour in a novel context was effective in attenuating an aversion towards a previously presented flavour, but was also effective in potentiating an aversion towards the context in which it was presented (Experiments 6A and 6B). The results of Experiments 6C(i) and (ii) indicated that the observed potentiation effect is very unlikely to have resulted from generalization between the flavour presented during conditioning (sucrose) and the test fluid (familiar saline). It was concluded that the 'scapegoat' intervention proposed by Broberg and Bernstein (1987) may lead to an increase in the level of ANV in patients who receive such treatment.

In Chapter 7, three experiments sought to establish whether the potentiation effect observed in Experiment 6A would occur in the absence of repeated exposure to the flavour cue in the context during conditioning. Two experiments failed to demonstrate one-trial potentiation (Experiment 7A and 7B), while a third experiment (Experiment 7C) showed no reliable evidence that potentiation would result from the same procedure as that used in Experiment 6A, if the flavour presented in the context was varied across the conditioning trials. It was suggested that if a 'scapegoat' intervention were to be used, then the novel overshadowing flavour would have to be varied from one treatment session to the next. The presentation of an aversive flavour to patients undergoing chemotherapy is not likely to lead to a reduction in the general level of distress experienced by these patients; Bovbjerg et al (1993) found that a flavour which had been paired with chemotherapy on a number of occasions was able to elicit nausea on subsequent exposure.

It is not clear from the experiments presented in Chapter 7 whether the presentation of a range of different flavours across successive treatment sessions will lead to an enhancement in the aversiveness of the context in which the flavours are presented. In Experiment 7C marginal effects of these flavours were detected in the potentiation of an aversion to the context. Although the group of animals which received a different flavour in the context on each conditioning trial (Group DIFF) did not consume any less saline on test than animals in Group WAT (the control group), neither did they consume reliably more saline on test than Group SAME (the group which received the same flavour in the context during conditioning). Thus, the results of Experiment 7C leave the issue open as to whether the presentation of different flavours in the context across conditioning trials (a procedure which would be used in the clinic) will lead to potentiation.

It is clear that further studies are required in order to establish the effect of varying the flavour component across conditioning trials in a potentiation procedure. There is some suggestion that this procedure may lead to potentiation, since Group DIFF consumed numerically less saline on test than Group WAT. Further experiments with more conditioning trials may reveal just such an effect. However, as discussed in Chapter 7, different flavour presentation during conditioning may lead to potentiation as a result of generalisation between these flavours on the basis of novelty (Honey, 1990). Thus, even if potentiation were observed using this procedure, it is not clear how the results could be interpreted without a thorough examination of the similarity of the flavours used.

It might be argued that, for a model of ANV, it is not important whether or not the potentiation observed in the rat model is due to stimulus generalisation, since such generalisation may be expected to occur in the clinic. However, it may be possible to present human cancer patients with very distinct flavours which will not interact with the development of an aversion towards the clinic. The difference between humans and rats in this respect is that humans have extensive experience of a wide range of flavours throughout their lives, while laboratory rats are typically exposed only to the flavours of water and laboratory chow during their lifetimes. The experience of many different flavours might allow these flavours to become highly distinct as a result of perceptual learning (Hall, 1991). The processes underlying perceptual learning are not fully understood. These flavours may acquire distinctiveness through being associated with different outcomes (e.g. Honey & Hall, 1989), elements which the stimuli have in common may become highly latently inhibited (e.g. McLaren, Kaye & Mackintosh, 1989), or some non-associative process may occur (e.g. Gibson & Walk, 1956). This possibility suggests the use of an animal model ANV in which rats with a history of dietary variety are used.

## 8.3.2: Avoiding potentiation in the chemotherapy clinic

Overshadowing requires a novel and salient stimulus to be presented in addition to the target stimulus in order for a large attenuation in the conditioning of the target stimulus to be demonstrated (Mackintosh. 1976). Thus, it is necessary to present a highly salient and novel food before treatment in order to attenuate the CTA which might develop towards previously consumed dietary items. Such a stimulus is likely to give rise to an increase in the level of the aversion towards the context in which it is presented. One obvious way to alleviate this problem might be to present the overshadowing stimulus outside of the clinical context. However, there are two reasons why such a stimulus should be presented in the chemotherapy clinic.

First, it is known that CS-US contiguity is one determinant of the level of conditioning which occurs towards a stimulus. It is also thought that the degree to which an overshadowing stimulus attenuates the conditioning of the target stimulus is related to the level of conditioning of the overshadowing stimulus. Thus, the temporal interval between the overshadowing stimulus and the reinforcer is likely to determine the effectiveness of that stimulus in the attenuation of the conditioning to the target and. in practice, exposure to the overshadowing stimulus outside the clinical context is likely to reduce contiguity.

Second, there is the potential for such a novel flavour to potentiate any context in which it is presented. Thus, if such a stimulus were presented in the home context, an aversion may develop towards that context. It might be thought that, due to latent inhibition (Lubow, 1973), presentation of a novel flavour in the home environment would not lead to an aversion to that context. However, Best and Meachum (1986) found that preexposure to a context in which a novel flavour was presented did not protect this context from becoming as aversive through toxicosis conditioning as a novel context in which a novel flavour was presented. The adverse effects on quality of life of an aversion towards the chemotherapy clinic are likely to be less severe than those of an aversion to the home environment.

The study by Best and Meachum (1986) may not be entirely reliable in making predictions for what might happen in the human case, since they used a procedure in which the animals were preexposed to the context without access to liquid, while, during conditioning and testing, liquid was available. Latent inhibition is known to be context specific (e.g. Hall & Channell, 1986). Thus, it is possible that the availability of liquid during conditioning represented a context change from that in which preexposure of the target cue took place, leading to an attenuation in the latent inhibition towards the target cue (the context). It is being argued here that the liquid formed the context for the conditioning of the context CS. If the preexposure of the context CS was ineffective due to a change in the context in which this cue was presented, from preexposure to conditioning, then it is possible that the home environment will be immune to the effects of toxicosis conditioning through chemotherapy, and the presentation of flavours in that context.

## 8.3.3 Conclusion

Experiment 6B provides some preliminary evidence that interactions between CTA and ANV may occur in cancer chemotherapy. Additional indirect evidence has come from a study by Nerenz et al (1986), who found that flavours experienced in the mouth as a direct result of drug treatment are correlated with the development of ANV (see Chapter 1). It may be the case that ANV and flavours in the mouth are both symptoms of a high sensitivity to the effects of the chemotherapeutic drugs. Instead, or in addition, flavours in the mouth may be a direct cause of ANV. Just as the presence of sucrose in a novel context in Experiment 6A led to potentiation of an aversion towards the context, the drug-induced flavours in the mouth may have led to an increase in the aversion towards the context in Nerenz et al's subjects, which resulted in a higher level of ANV.

If flavours in the mouth occur as a result of drug administration in chemotherapy patients, and this leads to the potentiation of a context aversion, and therefore an increase in ANV, it is possible that the presentation of novel flavours in the clinical context might lead to a reduction in ANV. If patients were to consume a novel flavour during drug infusion, this flavour might overshadow the drug-induced flavour. If a different flavour were presented on each visit to the clinic, simultaneous experience of both the presented flavour and the drug-induced flavour may lead to the perception of a novel configural stimulus (Rescorla, 1973) which would be different on each visit to the clinic. The evidence from Experiment 7C suggests that multiple flavour presentations do not give rise to potentiation to the same extent as does the presentation of the same flavour on each context-flavour conditioning trial. Since the drug-induced flavour component of the configural stimulus would be the same across trials, generalization would be expected between the different flavours. However, if the added cue were highly distinctive and salient, this generalization could be kept to a minimum. The salience of the drug-induced flavours (Nerenz et al, 1986) is not known, and therefore it is not possible, on the basis of the available data, to predict what sort of administered flavours would be effective in changing the perception of these endogenous flavours.

It is possible then, that the presentation of a series of novel flavours across treatment sessions might lead to a reduction in the aversion which develops towards the context and therefore reduce ANV. In addition, this intervention might lead to an attenuation in the number and intensity of CTAs which develop in these patients. It would be necessary to monitor the development of ANV and its relationship with the experience of drug-induced flavours, while controlling for the emetogenicity of the therapy, to establish fully whether this is a viable intervention for the control of ANV.

In conclusion, it is possible that the development of ANV both leads to a reduction in the number of CTAs which are learned through the blocking of these aversions after a number of context-illness pairings. In addition, flavours which are present in the clinic, including those which are a direct result of drug-infusion, may potentiate aversions to the context, and therefore lead to an increase in the level of ANV experienced by these patients. This has implications for the development of interventions for CTA and ANV based on learning theoretic principles as outlined above; the use of overshadowing stimuli in the clinic may give rise to potentiation, or attenuation of ANV, depending on the importance of the role played by the drug-induced flavours in the development of ANV.

If such a close relationship exists between ANV and CTA as is suggested above, that is, if ANV is largely the result of a flavour-potentiated context aversion, this has implications for the work carried out on the effectiveness of $5-HT_3$ and $NK_1$ receptor antagonists in the attenuation of CTA. If flavours which are present in the clinical context become aversive, and as a result potentiate aversions to the context, then the control of CTA will, as a matter of course, lead to a control of ANV. A stronger prediction can be made; drugs which attenuate CTA will attenuate ANV, regardless of their effect on drug-induced vomiting. It is possible that ondansetron is effective in controlling vomiting but not CTA induced by high dose cisplatin. If this is the case, then ondansetron will not alleviate ANV. A failure of ondansetron in the control of CTA and therefore ANV, would lead to the counter-intuitive prediction that patients receiving ondansetron as an antiemetic agent during chemotherapy may experience vomiting before, but not after treatment. Morrow (1992) has claimed that despite recent developments in antiemetic medication, specifically $5-HT_3$ receptor antagonists, ANV remains refractory to such interventions.

Since L-742,694 led to an attenuation of CTA in Experiment 5C, it is possible that this compound will lead to a reduction in CTA, ANV, vomiting and gut-related distress in cancer patients. If the reason for the limited effect of this compound in the attenuation of cisplatin-induced CTA in Experiment 5C is that the drug has low affinity for the rat $NK_1$ receptor, then this drug may significantly alleviate all the side effects of cancer chemotherapy, rendering the development of psychological interventions for the control of these effects superfluous. Clearly, this prediction needs to be tested in the clinic.

## 8.4: The Clinical Validity of the Animal Model

Issues, relating to the clinical validity of the rat model used in the present experiments, will be discussed: use of the rat, a species that does not vomit, to investigate CTA and ANV; use of LiCl, not a chemotherapy drug, to investigate interactions between flavours and contexts in toxicosis conditioning (Chapters 6 and 7); use of low doses of i.p. cisplatin to investigate antiemetics which, in the clinic, are used to control the vomiting induced by higher doses of i.v. cisplatin.

### 8.4.1: the use of a non-vomiting species in a model of ANV

Since the experiments presented in Chapters 6 and 7 aimed to model, among other things, anticipatory vomiting, clearly, the most important problem with respect to the use of a rat model

in the investigation of the conditioned side effects of chemotherapy is that rats do not vomit. . It could be argued that the results of Experiment 6A suggest that a novel flavour presented in a context before illness may lead to an increase in the aversiveness of that context, but that it could not be concluded that this aversiveness would necessarily lead to vomiting. One response to this suggestion might be to argue that an attempt to understand and control conditioned aversions towards the chemotherapy clinic would be, in itself, beneficial to cancer patients, but that the issue of anticipatory vomiting will require further research using a species which has the vomiting reflex.

Studies of a vomiting species would be desirable. However, if ANV (specifically anticipatory vomiting (AV)) results from classical conditioning, or an association between contextual cues and illness (see Chapter 1), then the nature of the response elicited by the aversiveness of the context would seem to be a secondary issue to the nature of the associative representations which give rise to this response. It is assumed, both in contextual conditioning in the rat, and in aversions to the clinic in human cancer patients, that the contextual cues which are paired with illness later come to re-elicit a representation of illness. This context-evoked representation of illness then gives rise to a reduction in consumption of a familiar flavour in that context in rats, and ANV in humans.

It would seem that the difference between rat and human in this respect is in one particular response to the experience of illness. It has been suggested that rats have a functionally equivalent mechanism to vomiting, that of pica, the consumption of large quantities of detritus (such as sawdust bedding) in order to absorb toxic substances in the gut. The administration of cisplatin to a human leads to the response of vomiting. This vomiting can be blocked by the administration of a 5-HT$_3$ receptor antagonist. In rats, the response to the administration of cisplatin is pica, and cisplatin-induced pica in rats is also blocked by the administration of a 5-HT$_3$ receptor antagonist (Takeda, Hasegawa, Masahiro & Matsunaga, 1993). It would certainly be interesting to investigate the conditioning of vomiting or pica in an animal model of AV (see section 8.5). However, if both AV and the context aversions observed in rats (Experiment 6A) result from the re-elicitation of a representation of illness by the contextual cues, as argued in Chapter 1, then it may not be essential in order to make valid inferences regarding ANV from animal studies.

The clinical literature contains many indications that ANV results from the same associative processes that mediate the behaviour of rats, and other non-human animals in conditioning experiments. In addition to those reviewed in Chapter 1, a strong correlation has been found

between the occurrence of ANV and that of post-treatment nausea and vomiting (PNV); patients who suffer from ANV are those who suffer from PNV. In addition, the *level* of ANV suffered by patients is strongly correlated with the degree of PNV experienced during prior treatment episodes (see Carey & Burish, 1988 for review). These findings support the view that ANV is the result of the association between the clinical context and illness; patients who develop ANV have suffered from PNV on previous visits to the clinic, and the severity of ANV is related to the severity of PNV.

### 8.4.2: The use of LiCl as the nausea-inducing agent

A related issue concerns the use of lithium chloride as the reinforcing agent. It might seem more appropriate to have used cisplatin in the experiments discussed in Chapters 6 and 7; there is ample evidence that different toxins affect the emetic system in very different ways (see Chapter 2), and therefore it may not be possible to generalise from data gathered from work using LiCl as a the reinforcer to conditioning in which cisplatin serves as the US. However, in the experiments reported in Chapters 6 and 7, it was assumed that the rats experienced illness of some sort, induced through some route, but that the specific route of induction and specific quality of this experience would not affect the ability of the animal to learn a relationship between that illness and the other stimuli presented. There is strong evidence that learning phenomena investigated in rats, using LiCl as the reinforcer, can be informative in the development of interventions for the control of conditioned side effects of cancer chemotherapy. For example, overshadowing in rats using LiCl as the reinforcer (e.g. Revusky, 1971) formed the basis of the development of the 'scapegoat' intervention found to be effective by Broberg and Bernstein (1987). Thus, there is some suggestion that the investigation of the associative mechanism underlying CTA and ANV using stimuli which do not necessarily model directly those used in the clinic, may lead to a better understanding of these phenomena, and allow the development of effective interventions for their control.

### 8.4.3: The use of low dose i.p. cisplatin

In chemotherapy, cisplatin is typically administered at a relatively high dose i.v., while a relatively low dose i.p. was used in Chapters 3 to 5. It has been suggested that radiation-induced and cisplatin-induced emetic responses are alike (Andrews et al, 1988), and that high dose radiation behaves in a different manner to low dose radiation. Thus, it is possible that the low doses of

cisplatin used here may lead to CTA which can be blocked by the antiemetics tested, but that, in the clinical context, no effect of the antiemetics on cisplatin-induced CTA would be evident. Specifically, it has been suggested that low dose cisplatin may be detected by the vagus nerve which can be blocked by antagonism of 5-$HT_3$ receptors, but that high dose cisplatin might be detected in the AP which is not affected by 5-$HT_3$ receptor antagonists (see Chapter 2 for discussion). This issue would have been more salient if a reliable effect of ondansetron on cisplatin-induced CTA had been demonstrated. This possible dose-dependent behaviour of cisplatin would not be expected to affect the ability of an $NK_1$ receptor antagonist to attenuate CTA; the $NK_1$ receptor antagonists, are likely to be active in the NST and PBN (Maeno et al, 1993) and thus the route of detection (through the vagus nerve or AP) would not affect the ability of these compounds to attenuate CTA.

A similar problem might arise as a result of the route of administration. In the experiments carried out here, cisplatin was administered i.p. while, in the clinic, cisplatin is infused i.v., and there is some evidence that the route of administration might affect which components of the emetic system detect the toxin (e.g. Andrews et al, 1990). In particular, i.v. cisplatin administration in the ferret induces vomiting which is blocked by vagotomy, whereas i.p. cisplatin administration appears to be detected through the AP; vagotomy does not completely block i.p. cisplatin-induced vomiting (Andrews et al, 1990). The possibility that i.p. cisplatin is detected by the AP, as well as in the gut by the vagus nerve, suggests that administration of ondansetron may attenuate responses to i.v. but not i.p. cisplatin. This may be a reason why ondansetron was found not to attenuate cisplatin-induced CTA reliably in the experiments presented in Chapters 3 and 4. Again, the route of detection of cisplatin should not affect any attenuative effect an $NK_1$ receptor antagonist may have, and this may be the reason that L-742,694 was found to be effective against cisplatin-induced CTA in Experiment 5C.

It would seem that the route of administration and dose of cisplatin may have worked against the possibility of detecting an effect of ondansetron, but not L-742,694, on cisplatin-induced CTA. This suggests that the rat model used in Experiments 3A to 4C provided a very conservative test of the hypothesis that ondansetron will attenuate cisplatin-induced CTA, but not that the data should be treated with suspicion were an effect of ondansetron to be found.

## 8.5: Future directions

A number of areas for further research have been suggested during the discussion of the particular findings from the experiments presented here. In this section, some more general possibilities will be presented. Reflecting the dual foci of the present work, some potential pharmacological investigations will be considered, followed by some suggestions for further psychological research.

## 8.5.1: Pharmacological studies

It was suggested in section 8.4.1 that pica in rats is functionally equivalent to vomiting in humans. If AV is a conditioning phenomenon, then conditioned pica in rats may have potential as a model for the investigation of AV. Takeda et al (1993) found that rats which have been administered cisplatin will reliably consume non-nutritious solids to a greater extent than those given an injection of saline (pica). If a context were paired with illness on a number of occasions, placement in that context in the absence of the toxin on test, may, similarly, lead to pica as a result of the re-elicitation of the illness by that context (conditioned pica).

It was pointed out in section 8.4.1 that the development of a procedure for the demonstration of conditioned pica may not be necessary for the investigation of the psychological processes which underlie ANV. If the nausea and vomiting that occurs in the clinic before treatment is the result of the re-elicitation of a representation of illness, then it is likely that the same process is responsible for ANV and the suppression of consumption of a familiar flavour in an aversive context in rats (Experiment 6A). However, although conditioned pica may not be necessary for the investigation of the associative processes which give rise to ANV, there are interesting pharmacological questions which could be addressed using such a procedure. Specifically, the prediction was made earlier that ondansetron might attenuate vomiting but not CTA and therefore not ANV (if ANV is largely the result of potentiation of a context aversion by a novel flavour). This prediction could be tested directly using the model of conditioned pica. It would be predicted that toxin-induced pica would be attenuated by ondansetron but that context-induced pica would not. Furthermore, it could be predicted that no such dissociation would be apparent if L-742,694 were used as the antiemetic.

Experiments are required to investigate further the nature of the 5-HT$_3$ and NK$_1$ receptor antagonists in their attenuation of cisplatin-induced CTA. It has been suggested that the effect of

ondansetron on low dose cisplatin-induced CTA be investigated using a procedure in which ondansetron is administered across the 24 hours following cisplatin administration. This was not successfully carried out here, and probably requires the use of a different anaesthetic to that used in Experiments 4A, 4B and 4C (isoflorane). Further investigation of this issue might involve the testing of the effects of vagotomy on cisplatin-induced CTA across a range of cisplatin doses. It is possible that vagotomy will block low but not high-dose cisplatin-induced CTA. This would indicate that, similar to the evidence from the vomiting response, low dose cisplatin is detected by the vagus nerve in the gut, but that high dose cisplatin is also detected elsewhere, for instance in the AP.

### 8.5.2: Psychological studies

A non-associative explanation for the occurrence of both CTA and ANV was presented in Chapter 1. The non-associative explanation of CTA is testable in animals. It was hypothesised that cisplatin and other chemotherapeutic drugs might lower the threshold for nausea across a long period following treatment. This might allow foods which are gastric stimulators (such as coffee and chocolate) to induce nausea on consumption. The possibility that this might occur is easily tested. An experiment in which animals are injected with cisplatin and then presented with a number of food items, some gastric stimulators and some not would test such a notion. If the consumption on first exposure of these gastric stimulants were suppressed in animals which had been treated with cisplatin compared to those which had been treated with saline, or which had been given a non-gastric stimulant foodstuff, then a non-associative interaction between cisplatin and gastric stimulants would be implicated. Such a study would require further control groups in order to establish that the salience of the flavours had been equated.

As well as the non-associative explanation for CTA. a non-associative explanation for ANV was also presented (Chapter 1). It was suggested that ANV might result from an increase in anxiety preceding therapy which directly induces nausea. However, data were presented indicating that subjects undergoing bone-marrow aspiration suffer form equivalent anxiety, but a lower prevalence of ANV (Katz et al, 1980). Such a finding requires replication and direct comparison with the effects of chemotherapy; it is necessary to control for measures of anxiety and measures of ANV in these two patient groups before a strong conclusion is drawn as to the relative effects of the two treatment types on ANV and anxiety.

If it were found that none of the antiemetics which are presently available give rise to a reduction in CTA and ANV, and that both of these effects are associative, then the development of new interventions based on learning theoretic principles would be of considerable value. Three interventions for the attenuation of ANV are possible which will be presented below: overshadowing of the clinic context by a second context in which a novel flavour is presented; overshadowing of the clinic context by an novel odour; latent inhibition of the clinic context.

The overshadowing interventions would be similar; they would differ only with respect to the stimuli presented in order to overshadow the aversion towards the context. Either a second context, which is experienced before entering the treatment clinic, or a novel odour in the clinic, might be used to reduce ANV. Taukulis and St George (1982) observed that the presentation of a novel odour in a novel context during toxicosis conditioning led to an attenuation in the aversion which developed towards the context. It is possible that an enhancement of this effect may result from the presentation of a novel flavour in compound with this odour. If the efficacy of a stimulus in overshadowing a target stimulus is dependant on its associability (Mackintosh, 1976), and a cue's associability can be potentiated by the presence of a novel flavour in compound with that cue, then the presence of that flavour might be expected to enhance the ability of the overshadowing stimulus to attenuate the aversion which develops toward the target cue, i.e. the context.

Latent inhibition of a context has been found to have little effect on the conditioning of that context when a novel flavour is presented in compound with the context on test (Best & Meachum, 1986). However, it is possible that the effect of preexposure could be enhanced by the presence of a novel flavour in the context during preexposure in much the same way as conditioning is enhanced in procedures which demonstrate potentiation. If latent inhibition showed cue-to-consequence specificity in the same way as does conditioning (Domjan & Wilson, 1972), then the presence of a novel flavour in the context during preexposure would be expected to enhance the level of latent inhibition which accrues to this stimulus. Such a procedure might allow the context to become immune to conditioning with the toxic reinforcer during treatment.

The theories of potentiation, presented in Chapter 6, allow that such a process may occur. The presence of a novel flavour may increase vigilance in attending to the context during preexposure, and thus increase the level of latent inhibition (Galef & Osborne, 1978). Alternatively, the flavour may gate the context into the feeding system during preexposure, and thus the latent inhibition

which accrues to the contextual cues may be feeding-specific, thus retarding the association between the context and illness (see Rusiniak et al, 1979 for this account of potentiation). Finally, the context might become associated with the flavour which is present during preexposure, and this association might interfere with the development of an association between the context and illness during treatment, or between the context and some other flavour present during treatment which might lead to potentiation through within-compound associations.

## 8.6: Conclusions

The available data suggest that both ANV and CTA in cancer chemotherapy patients arise as the result of classical conditioning (see Chapter 1). It has been suggested that the presentation of a novel flavour before treatment may lead to the overshadowing of CTA towards normal dietary items, but that this attenuation may be accompanied by an increase in ANV as a result of a flavour-potentiated context aversion (see Chapter 6). However, whether potentiation will occur when a different overshadowing flavour is presented at each treatment session remains to be established (see Chapter 7).

The antiemetics tested in Chapters 3 to 5 did not effect complete blockade of CTA; L-742,694, but not CP-99,994 or ondansetron, reliably attenuated, but did not block, cisplatin-induced CTA. Previous research (e.g. Revusky & Martin, 1988) also suggest that the CTA mechanism may be so sensitive to the presence of toxins that, although antiemetics may reduce CTA, they will not lead to a complete abolition of this response. It is possible that flavour potentiation of a context aversion might underlie all ANV, possibly as a result of the experience of drug-induced flavours in the mouth. If this were the case then one would not expect antiemetics to lead to complete control of ANV, unless they lead to complete blockade of CTA. Thus a psychological, learning theoretic, approach to the development of interventions for CTA and ANV, involving, for example, overshadowing (Broberg & Bernstein, 1987), has several potential advantages. First, in isolation, or in combination with antiemetic drugs, a psychological intervention may effect the abolition of CTA and ANV. Second, a psychological intervention may limit the range and number of drugs necessary to control cancer chemotherapy side effects. Finally, the application of learning theory in the process of developing psychological interventions may contribute to a better understanding of the ways in which cues interact in the course of associative learning.

# Appendix

**Experiment A(i) Cisplatin and ondansetron as the US in CTA**

(T. Cripps, unpublished third year undergraduate project)

In the present experiment, saline, cisplatin and ondansetron were administered either immediately following or 24 hours after consumption of 3% sucrose solution. Male Sprague Dawley rats were used (300-450 g), which were housed, fed and watered as in Experiment 3A. The procedure was also similar to that of Experiment 3A. Thus, animals were taken off water on Day 1, and presented given 20 minutes drinking training in the experimental cages on Days 2 and 3. In addition, 1 hr and 10 minutes access to water was given in the home cages 4 hours following drinking training.

Conditioning took place over Days 4 and 5. The animals were assigned to six groups, and equated for water consumption in the experimental cages on Days 2 and 3. On Day 4, all animals received either water or sucrose solution in the experimental cages as detailed below. On Day 5, the animals again consumed water or sucrose in the experimental cages on Day 5 and were given three injections: one immediately following consumption, and two further injections 45 and 90 minutes later. Two Groups received three injections of saline solution (Groups SAL-P and SAL-UP), two received 0.3 mg/kg cisplatin on the first injection, and saline in the following two injections (Groups CIS-P and CIS-UP), and two received three administrations of 1.0 mg/kg ondansetron (ONS-P and ONS-UP).

On Day 4, the animals in Groups SAL-P, CIS-P and ONS-P consumed water in the experimental cages, while those in Groups SAL-UP, CIS-UP and ONS-UP received sucrose solution. On Day 5, the solutions were reversed: Groups SAL-P, CIS-P and ONS-P received sucrose in the experimental cages and the remaining groups received water. The first term in the group name (SAL, CIS and ONS) refers to the compound administered by injection on Day 5, while the second term refers to whether sucrose was consumed immediately before injection (paired with injection - P) on Day 5, or 24 hours prior to injection on Day 4 (unpaired - UP). Thus, animals received sucrose solution (the CS) and either saline, cisplatin or ondansetron (the US). In addition, the CS and US were either paired or unpaired in their presentation. Day 6 was a rest day which was followed by testing on Day 7. On test, animals were presented with sucrose solution for 10 minutes in the experimental cages.
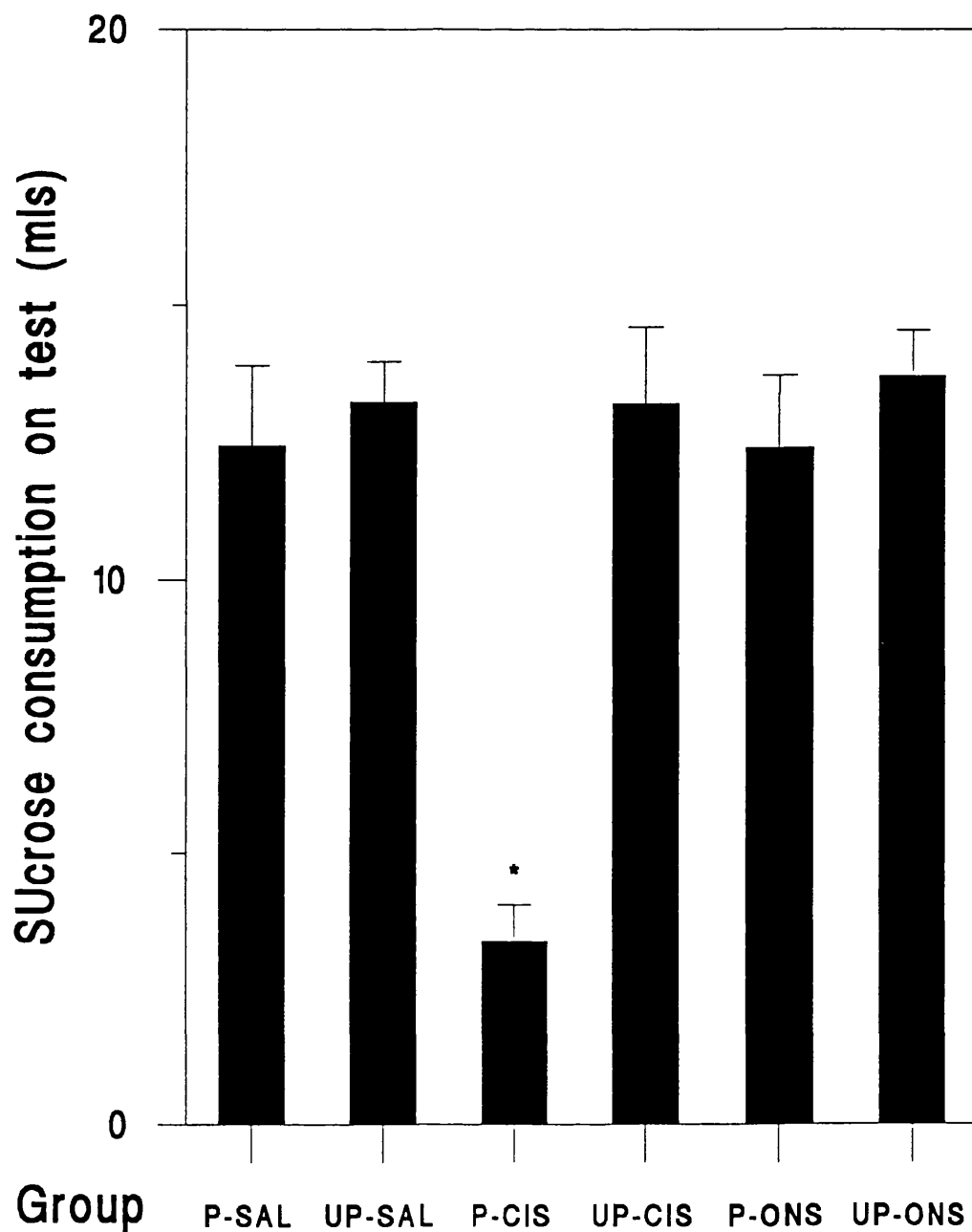
Figure A.1. Mean consumption of sucrose solution on test in Experiment A(i). The CS (sucrose) and the US (saline, cisplatin or ondansetron) were presented contiguously (paired) or on separate days (unpaired). Error bars=SEMs. * Different from other Groups.

**Results**

Figure A.1 shows the sucrose consumption of all groups on test. It would appear that the animals in Group CIS-P drank less sucrose on test than all other groups. A two-way ANOVA, with pairing (paired and unpaired) and drug treatment (saline, cisplatin and ondansetron) as factors, revealed an effect of pairing ($F_{(1,42)}$ = 18.67), an effect of drug treatment ($F_{(2,42)}$ = 11.47) and an interaction between these factors ($F_{(2,42)}$ = 10.22). A Tukeys post hoc analysis revealed a difference between Group CIS-P and each of the other groups. No other differences were significant. This suggests that cisplatin, when paired with sucrose, led to a taste aversion towards that flavour which was not due to any direct effects of cisplatin. Further more, it suggests that ondansetron (1.0 mg/kg administered three times) does not induce CTA.

**Experiment A(ii) Ondansetron in the attenuation of cisplatin-induced CTA**

The present experiment was a replication of Experiment 3D. The subjects were male Sprague Dawley rats (200-250g) which were housed, fed and watered as in Experiment 3D. The procedure was the same as Experiment 3D except that only five groups were used, the doses of ondansetron use were 0.03, 0.1 and 0.3 mg/kg (administered three times, as in Experiment 3D). Thus Group 1 consumed sucrose solution followed by administration of water. Group 2 received 0.3 mg/kg cisplatin (i.p.) following sucrose consumption, while Groups 3-5 received 0.3 mg/kg cisplatin (i.p.) and ondansetron (0.03-0.3 mg/kg s.c.).

**Results**

Figure A.2 shows the consumption of sucrose solution on test for all groups. It can be seen that animals in Group 2 (treated with cisplatin only) showed suppressed consumption of sucrose solution compared to Group 1 (treated with vehicle). There would appear to be an attenuation in the cisplatin-induced suppression of consumption of sucrose in Group 5 (those animals which received cisplatin and 0.3 mg/kg ondansetron). A one way ANOVA revealed an effect of treatment ($F_{(4,35)}$ = 6.7). A Dunnets post hoc analysis, with Group 2 as the control, showed that both Group 1 and Group 5 differed reliably from Group 2. This suggests that 0.3 mg/kg ondansetron administered on three occasions attenuated a cisplatin-induced CTA.
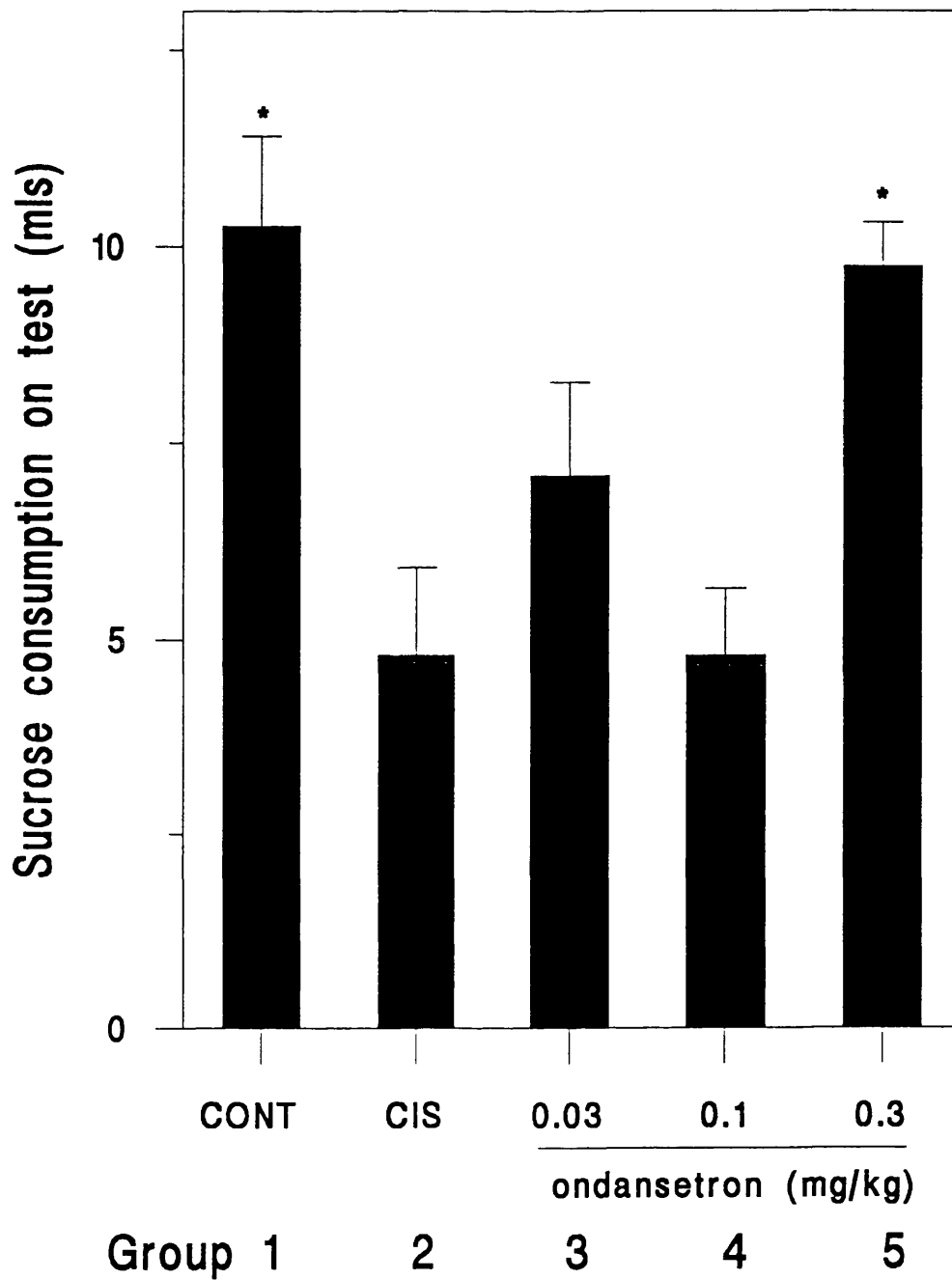
Figure A.2. Mean consumption of sucrose solution on test in Experiment A(ii). Sucrose was paired with water (CONT), cisplatin (0.3 mg/kg) or cisplatin and ondansetron. Error bars indicate SEMs. * Significantly different from cisplatin group (Group 2).

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