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Taste aversion after ingestion of lithium chloride: An associative analysis

Ignacio Loy
University of Oviedo, Oviedo, Spain
Geoffrey Hall
University of York, York, UK

In five experiments with rats we examined the aversion established by consumption of a solution of lithium chloride (LiCl). Experiment 1 showed that consumption of LiCl established an aversion to saline (NaCl). Experiment 2 showed that the size of the aversion was reduced in rats given pre-exposure to saline (a latent inhibition effect). Experiment 3 showed that experience of a sucrose–saline compound prior to consumption of LiCl generated an aversion to sucrose (a sensory preconditioning effect). Experiments 4 and 5 examined the effects produced by consumption of a sucrose–LiCl compound and demonstrated reciprocal overshadowing between the two tastes. These results confirm that consumption of LiCl establishes an aversion to the taste of this substance. Their implications for the use of orally consumed LiCl as a technique for the control of predatory behaviour are discussed.

Studies of flavour aversion learning have made much use of lithium chloride (LiCl) as the unconditioned stimulus (US), usually in the form of an intraperitoneal injection and usually with the intention of establishing an aversion to some arbitrarily chosen flavour, presented prior to the injection. It has been noted (e.g., Nachman, 1963) that most toxic substances have unpleasant tastes, but that LiCl appears to be one of the exceptions to this rule. Nachman reported that thirsty rats given access to a 0.12 M solution of LiCl drank it readily, at least for the first 5 min of its presentation, during which period they drank about 8 ml. Thereafter the rate of drinking declined, and when tested 3 days later the rats refused to drink LiCl, although they readily drank plain water presented in the same way. Similar results have been reported by Simbayi (1987, Experiments 1 and 4). Nachman suggested that this effect was the consequence of an associative learning process in which the taste of the LiCl had become associated with the aversive consequences (i.e., the nausea) induced by its initial consumption. In support of this conclusion he reported that the rats also refused to drink a sodium chloride (NaCl) solution, which appears (to humans) to have the same (salty) taste as LiCl. Furthermore,

Requests for reprints should be sent to G. Hall, Department of Psychology, University of York, York, YO10 5DD, UK. Email: GH1@york.ac.uk

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recording from single fibres in the chorda tympani in rats has shown the pattern of neural responsiveness produced by LiCl to be the same as that produced by NaCl (Fishman, 1957).

The experiments reported here were designed to test the hypothesis that simply consuming a solution of LiCl engages a conditioning mechanism that establishes an association between the salty taste of LiCl and its aversive effects and to show that the principles governing this form of learning are analogous to those determined for more orthodox forms of conditioning in which separate events are used as the conditioned stimulus (CS) and the US. Experiment 1 investigated the acquisition and extinction of an aversion to the taste of salt. Experiment 2 looked for a latent inhibition effect in this training procedure, and Experiment 3 for a sensory preconditioning effect. Experiments 4 and 5 investigated the effects produced by presenting the LiCl in compound with another flavour. In Experiment 4 we asked whether the presence of this other flavour would overshadow the development of an aversion to the salty taste of LiCl; in Experiment 5 we sought evidence for the proposal that the taste of LiCl might overshadow acquisition of an aversion to the added flavour. In the General Discussion we consider the implications of our findings for theoretical interpretations of aversion learning generally and for the application of aversion conditioning techniques in practical situations.

EXPERIMENT 1

Our first step in investigating the suggestion that drinking LiCl establishes an association between its salty taste and nausea was to attempt to replicate the finding that rats given this treatment develop an aversion to NaCl. Accordingly we gave rats in group O (for oral) access to about 5 ml of a LiCl solution (this being an amount that they will drink fully according to Nachman's, 1963, observations). They were then given a series of tests with a salty but nontoxic solution (NaCl). We anticipated that they would initially refuse the NaCl, but that, if their aversion depended on an associative learning process, repeated presentation of NaCl would allow extinction to occur so that consumption would recover. For purposes of comparison, we also included a group given an orthodox flavour aversion conditioning procedure. Subjects in this group (group I, for intraperitoneal) were given access to 5 ml of NaCl followed by an injection of LiCl before the test trials with NaCl. In order to equate the groups in other respects, group O was given a saline injection immediately following the trial on which LiCl was consumed. The design of the experiment is summarized in Table 1.

Method

Subjects and apparatus

The subjects were 16 experimentally naive male Wistar rats, 55 days old (weighing 214–319 g) at the start of the experiment. They were housed individually in standard plastic cages (27 cm long × 27 cm wide × 15 cm high) with unlimited access to food. Their access to fluids was restricted as described later. The experiment was conducted in the home cages at the same time each day during the light portion of a 12:12-hr light:dark cycle. Inverted 50-ml centrifuge tubes equipped with stainless steel, ball-bearing-tipped spouts were used to present measured amounts of fluid. The fluids used were (approximately isotonic) solutions of LiCl (0.15 M) and NaCl (0.15 M). Fluid consumption was measured, by weighing, to the nearest 0.5 ml.

TABLE 1
Experimental designs

Experiment	*Group*	*Pre-exposure*	*Conditioning*	*Test*	*Test 1*	*Test 2*
1	Group O		LiCl → NaCl (ip)	6 NaCl		
	Group I		NaCl → LiCl (ip)	6 NaCl		
2	Group Pre	5 NaCl	LiCl	3 NaCl		
	Group W	5 Water	LiCl	3 NaCl		
	Group Suc	5 Sucrose	LiCl	3 NaCl		
3	Group Com-Li	4 Suc + NaCl	LiCl		2 Suc	2 NaCl
	Group Ele-Li	4 Suc / 4 NaCl	LiCl		2 Suc	2 NaCl
	Group Com-Na	4 Suc + NaCl	NaCl		2 Suc	2 NaCl
	Group Ele-Na	4 Suc / 4 NaCl	NaCl		2 Suc	2 NaCl
4	Group Com		Suc + LiCl	2 NaCl, 2 Suc		
	Group Ele		Suc / LiCl	2 NaCl, 2 Suc		
5	Group P-C	4 NaCl	Suc + LiCl		6 Suc	NaCl
	Group N-C	—	Suc + LiCl		6 Suc	NaCl
	Group P-E	4 NaCl	Suc / LiCl		6 Suc	NaCl
	Group N-E	—	Suc / LiCl		6 Suc	NaCl

Note: All substances were ingested unless ip (intraperitoneal injection) is indicated. Number of trials of a given type is indicated (if more than one). Suc: sucrose.

Procedure

Throughout the experiment the rats received access to fluids twice each day: The experimental sessions (in which flavoured solution were presented) occurred at 0900, and recovery sessions (in which water was presented for 10 min) occurred at 1300. The rats were first adapted to a water deprivation schedule over the course of 5 days. On Day 1 (at 1300) the standard water bottles were removed from the home cages. On Day 2 the rats received access to tap water for 30 min at 0900. On Day 3 they received water for 15 min at 0900. On Days 4 and 5, the rats were given water for two daily 10-min periods at 0900 and 1300. By the end of this phase the rats had achieved a steady baseline of water consumption. The animals were then assigned to the two groups. On the next day the animals in group I received a conditioning trial in which they were given access to a bottle containing 7 ml of NaCl for 5 min. (Typically with the bottles we used, a small amount of fluid remains in the tube when the animal has finished drinking; offering 7 ml ensures that a minimum of 5 ml will be easily available to the animal.) This was followed immediately by an intraperitoneal injection of 5 ml of 0.15 M LiCl. For group O, the conditioning trial consisted of access for 5 min to a bottle containing 7 ml of the LiCl solution. These animals then received an intraperitoneal injection of 5 ml of 0.15 M saline.

On the following day the test phase began. It consisted of the presentation of free access to NaCl for both groups for 30 min at 0900. There were six trials.

Results and discussion

On the conditioning trial, both groups drank almost all the fluid that was made available (and thus slightly more than the 5 ml anticipated). There was no difference between the groups in the amount consumed: Group O drank 6.2 ml of LiCl, and group I drank 6.3 ml of NaCl ($F < 1$). Group mean consumption scores for the test phase are shown in Figure 1. When tested with

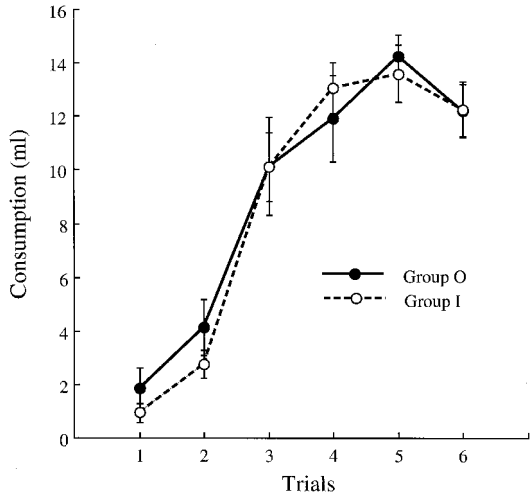

Figure 1. Experiment 1: Group mean consumption on the test trials with NaCl. Group O drank LiCl on the conditioning trial; group I drank NaCl followed by an intraperitoneal injection of LiCl. Vertical bars represent standard errors of the mean.

NaCl, group I, which had received the LiCl injection after consuming NaCl on the conditioning trial, not surprisingly initially showed a profound aversion. Over successive extinction trials, consumption returned to near-normal levels. Group O, which merely drank LiCl on the conditioning trial, showed an identical pattern of consumption on the NaCl tests. An analysis of variance was carried out on the test data, with group and trial as the variables. This showed there to be a significant effect of trial, $F(5, 80) = 72.01$ (in this and subsequent analyses, $p < .05$ is taken as significant), but no reliable difference between the groups, and no interaction (Fs < 1).

The results for group O are just what would be expected if drinking LiCl endows its salty taste with aversive properties. Consumption of the toxic LiCl established an aversion to the innocuous saline solution and this aversion waned with repeated presentations of saline, an effect consistent with the occurrence of extinction. This pattern of results exactly matches that reported for rats allowed to drink a lactose solution (Simbayi, Boakes, & Burton, 1986). Lactose has a sweet taste and will, initially, be consumed readily by rats. However, the inability of adult animals to metabolize lactose results in their suffering lower gastrointestinal distress after consuming it. Rats given lactose have been found to develop an aversion to another sweet-tasting substance (sucrose), an aversion that was lost over the course of repeated presentations of sucrose. This result was interpreted by Simbayi et al. as reflecting the extinction of an acquired aversion between the sweet taste of lactose and its aversive consequences.

It is interesting that group I in the present experiment, which was given orthodox taste aversion conditioning, with the substance presented on the test being the conditioned stimulus, showed exactly the same pattern of results as did group O. This suggests that the effectiveness as a US of a given amount and concentration of LiCl is not much influenced by the route of administration (oral vs. intraperitoneal). It also suggests that the generalization from the salty taste of LiCl to that of NaCl was complete for group O in this situation. Previous work

(Kiefer, 1978) has shown that in optimal conditions (in particular when a two-bottle test is used), rats may be capable of discriminating between these two solutions. Our results (perhaps because of the use of a single-bottle test) give no indication of such discrimination.

EXPERIMENT 2

Assuming that the results for group O of Experiment 1 reflect the formation of an association between the salty taste of LiCl and the nausea it produces, then it should be possible to modulate the strength of this association in predictable ways by manipulating the variables that are effective in standard conditioning paradigms. In this experiment we sought evidence that the postulated association was sensitive to latent inhibition. To do this, it is necessary to give animals experience of the salty taste alone before giving access to the salt–nausea combination produced by drinking LiCl. This can be achieved by giving pre-exposure to NaCl. If latent inhibition occurs when the conditioning trial consists of the oral consumption of LiCl, then animals given pre-exposure to NaCl should show only a weak aversion when subsequently tested with the salty taste (i.e., with NaCl). The performance of subjects given this treatment (group Pre) was compared with that shown by two control groups, one given only water in the pre-exposure phase (group W) and one given pre-exposure to a novel, but presumably irrelevant, flavour (sucrose, group Suc). Table 1 present a summary of the experimental design.

Method

Subjects and procedure

The subjects were 24 male Wistar rats with an age of 60 days at the start of the experiment and a mean weight of 271 g (range 222–328 g). After the water deprivation schedule had been established the rats were assigned to three equal-sized groups. Animals in group Pre received a 10-min period of access to 8 ml of NaCl on each of the 5 days of the pre-exposure phase. Animals in group W were given equivalent access to water on these sessions; those in group Suc received equivalent access to a 0.15 M sucrose solution. On the day following pre-exposure all the animals received 5 min of access to 7 ml of LiCl. On each of the three sessions of the test phase, all animals were given free access to NaCl for 10 min. Procedural details not specified here were identical to those described for Experiment 1.

Results and discussion

Figure 2 shows the group mean consumption scores for each phase of the experiment. In the pre-exposure phase, all three groups consumed almost all of the fluid offered on each trial, and there were thus no differences among the groups. An analysis of variance conducted on the pre-exposure data summarized in the figure, with day and group as the variables, revealed no significant effects: For the effect of group, $F < 1$, for the effect of day, $F(1, 21) = 2.42$, and for the interaction, $F(2, 21) = 1.51$.

On the conditioning day, all groups drank at least 5 ml of LiCl, but group Pre drank rather more than group Suc, which in turn drank more than group W. A one-way analysis of variance showed the difference among the groups to be significant, $F(2, 21) = 5.11$. Subsequent tests showed that group W differed from each of the other groups; for the comparison with group Pre, $t(14) = 3.05$; for the comparison with group Suc, $t(14) = 1.93$, $p < .1$. The difference between groups Suc and Pre was not reliable, $t(14) = 1.36$. The relatively low level of

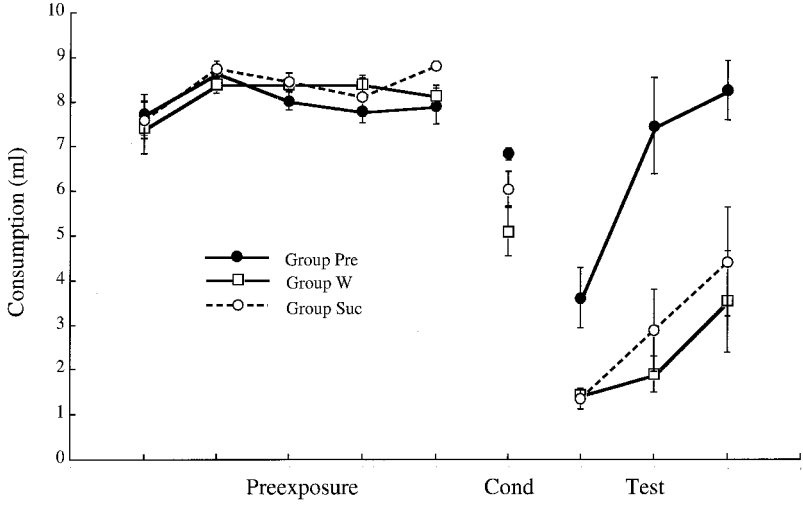

Figure 2. Experiment 2: Group means for consumption during pre-exposure, conditioning, and test. All groups received LiCl on the conditioning trial (Cond) and NaCl on the test trials. During pre-exposure group Pre received NaCl, group W received water, and group Suc received sucrose solution. Vertical bars represent standard errors of the mean.

consumption in group W presumably reflects a neophobic reaction to the novel salty taste of LiCl, a reaction that was attenuated to some extent in subjects given prior exposure to another distinctive taste (i.e., in group Suc).

The results of central interest come from the test trials with NaCl. As Figure 2 shows, all three groups showed an initial suppression of consumption that recovered over the course of testing, but this suppression was less profound, and the return to a higher level of consumption more rapid, in group Pre than in the other groups. An analysis of variance with group and day as the variables revealed significant effects of day, $F(2, 42) = 21.50$, and of group, $F(2, 21) = 12.67$; the interaction between the variables was not significant, $F(2, 42) = 6.46$. Comparison of the individual groups showed that group Pre differed both from group W, $t(14) = 5.34$, and from group Suc, $t(14) = 3.67$. The two control groups did not differ from one another, $t(14) = 0.64$.

The results obtained here are just what would be expected according to the hypothesis that pre-exposure to the salty taste of NaCl produces a latent inhibition effect that attenuates the effectiveness of the salt–nausea pairing produced by consumption of LiCl. It may be noted that this latent inhibition effect was evident in spite of the fact that group Pre drank rather more LiCl on the conditioning trials than did the control groups, a circumstance that might be expected to facilitate conditioning in group Pre.

EXPERIMENT 3

Demonstrations of sensory preconditioning in flavour aversion learning have made use of a procedure in which rats are first permitted to consume a compound flavour solution (e.g., sucrose plus salt). An aversion is established to one of the elements of the compound (say salt),

and it is found that the other (in this case, sucrose) elicits an aversion (e.g., Rescorla & Cunningham, 1978; Rescorla & Freberg, 1978). It has usually been assumed that this effect reflects the formation of a within-compound association during the first phase of training, although an interpretation in terms of generalization (between the pre-exposed configure generated by the two flavours and the flavour presented on test) is also possible (see Rescorla & Durlach, 1981). The present experiment was intended to demonstrate sensory preconditioning with the lithium (Li) consumption procedure. In order to achieve this, animals in the experimental condition were given initial exposure to a compound of sucrose plus NaCl before being allowed to consume LiCl. We expected that the association between the salty taste of LiCl and nausea would endow sucrose with aversive properties.

The full experimental design is given in Table 1, where the experimental group is designated Com-Li (for compound pre-exposure; Li consumption). There were three control groups. Group Ele-Li was also allowed to consume Li but was given pre-exposure to elements of the compound, saline and sucrose, on separate trials. Group Com-Na received pre-exposure to the compound but drank saline rather than LiCl on the "conditioning" trial. Group Ele-Na was exposed to the elements and received saline on the conditioning trial.

Method

Subjects, apparatus, and procedure

The subjects were 32 male Wistar rats, 90 days old at the start of the experiment, and with a mean weight of 412 g (range: 344–505 g). The apparatus and fluids were the same as those in Experiment 1; compound solutions were made up so as to maintain a molarity of 0.15 M for each of the individual elements. The pre-exposure phase lasted 8 days. On each day the animals were given access to fluid for 10 min in the morning session. Subjects in the Com groups received four presentations of the sucrose–saline compound, alternating with four presentations of water. Subjects in the Ele groups received alternating presentations of sucrose and of saline, four of each. For half the animals, sucrose was presented on the first trial of the sequence; for the remainder saline was presented on this trial. On the day following pre-exposure, one Com group and one Ele group were given access to 7 ml of LiCl; the other two groups received saline. The next two days constituted the test phase; on each of these, all animals were given free access to sucrose in he morning drinking sessions. Finally, all animals were given two further test sessions, this time with NaCl. Any procedural details not specified here were the same as those described for previous experiments.

Results and discussion

During the pre-exposure phase, all groups drank a mean of about 10 ml of the fluid offered on each day. Thus, on the final pre-exposure session with the sucrose–saline compound, group Com-Li drank a mean of 10.8 ml and group Com-Na a mean of 11.1 ml; these scores did not differ reliably ($F < 1$). Group Ele-Li drank 11.3 ml on the last pre-exposure trial with sucrose and 11.6 ml on the last trial with saline; the equivalent scores for group Ele-Na were 10.1 and 10.2 ml. An analysis of variance with group and flavour as the variables yielded no significant effects either of group ($F < 1$) or of flavour, $F(1, 14) = 2.61$, and no significant interaction, $F(1, 14) = 2.00$. Figure 3 shows the amount consumed by each group on the conditioning trial; there was no significant difference among the groups on this score ($F < 1$).

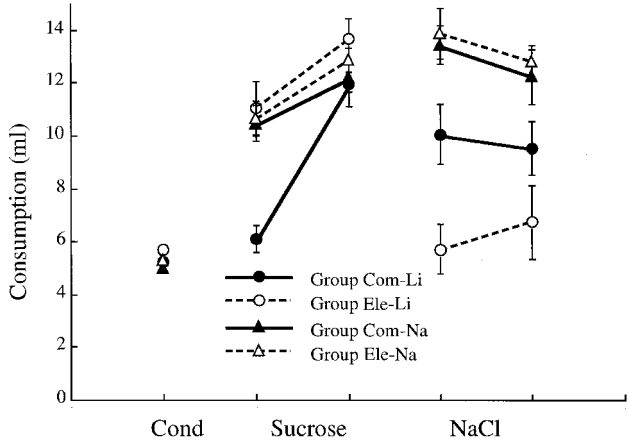

Figure 3. Experiment 3: Group means for consumption for the conditioning and test phases of the experiment. During pre-exposure the Com groups had received experience of a compound of NaCl and sucrose; the Ele groups received exposure to sucrose and to saline on separate trials. On the conditioning trial groups Com-Li and Ele-Li received LiCl; groups Com-Na and Ele-Na received NaCl on this trial. The conditioning trial was followed by two test trials with sucrose and two test trials with NaCl. Vertical bars represent standard errors of the mean.

The results of central interest come from the test trials with sucrose. As Figure 3 shows, all groups drank more on the second trial than on the first; critically, however, group Com-Li drank less on both these trials, particularly the first, than did the other three groups, which did not differ one from another. An analysis of variance with group and trial as the variables revealed a significant main effect of trial, $F(1, 28) = 45.45$, of group, $F(3, 28) = 6.96$, and of the interaction between the variables, $F(3, 28) = 4.12$. A one-way analysis of variance conducted on the data for each test trial revealed a significant difference among the groups on the first, $F(3, 31) = 9.69$, but not on the second, $F(3, 31) = 1.22$. Pairwise comparisons of individual groups on the first test trial showed that group Com-Li differed from each of the other groups, smallest $t(14) = 4.33$. It appears that pre-exposure to the saline–sucrose compound followed by consumption of LiCl endows sucrose with aversive properties that are not evident in subjects that do not experience the sucrose and saline together or do not consume LiCl (or both of these). This result is consistent with the hypothesis that the treatment given to group Com-Li generates a sensory preconditioning effect that depends on the formation of an association between the tastes of salt and of sucrose in the pre-exposure phase and the establishment of an association between the taste of salt (produced by LiCl in this case) and nausea in the conditioning phase.

This interpretation requires that consumption of LiCl in group Com-Li should establish an aversion to the taste of salt (group Ele-Li might also be expected to show such an aversion). The results of the final test trials with NaCl were that these two groups consumed less than the other two groups. An analysis of variance conducted on the NaCl consumption data summarized in Figure 3 showed there to be a significant effect of group, $F(3, 28) = 13.01$; there was no significant effect of trial, $F(1, 28) = 1.10$, nor of the interaction between these variables ($F < 1$). Comparing the scores for the individual groups, pooled over both test trials, showed that each of the LiCl groups differed significantly from each of the NaCl groups, smallest $t(14) = 2.37$.

The difference between groups Com-Li and Ele-Li was also significant, $t(14) = 2.41$. That both of the groups allowed to consume LiCl should show an aversion to NaCl accords with the results of our previous experiments. It is not obvious, however, why the aversion should be stronger in group Ele-Li than in group Com-Li. A possible explanation of this result is that it reflects a mediated extinction effect. Holland and Forbes (1982) have shown that a CS can apparently lose associative strength as a consequence of extinction trials given to another CS with which it has been associated. If this process operates in the present experiment, then the salt–sucrose association formed during pre-exposure in group Com-Li would allow the non-reinforced presentations of sucrose given on the first two test trials to mediate extinction of the aversion to salt. No such mediated extinction effect could be expected in group Ele-Li, however, as these subjects would not have formed the critical salt-sucrose association.

EXPERIMENT 4

The aim of this experiment was to seek an overshadowing effect in the Li consumption conditioning procedure. Our hypothesis is that drinking LiCl allows the formation of an association between the salty taste of the solution and its aversive consequences. Overshadowing would be demonstrated if it could be shown that the addition of another flavour to the solution detracted from the acquisition of the aversion to salt. Accordingly, in this experiment one group of rats (group Com for compound) was allowed to consume a compound solution consisting of LiCl plus sucrose and then received a test with saline (see Table 1). If overshadowing occurs, these animals should show less of an aversion to saline than should rats in the control condition (group Ele for elements), which received experience of the LiCl and sucrose solutions on separate trials during training.

Method

Subjects, apparatus, and procedure

The subjects were 16 male hooded Lister rats with a mean weight of 436 g (range: 375–470 g) at the start of the experiment. They were housed individually in opaque plastic cages measuring 38 cm long, by 26 cm wide, and 19 cm high. In all other respects the housing arrangements were the same as those described for the previous experiments. After the water deprivation schedule had been established, the animals were divided into two equal-sized groups. Over the next two morning sessions, rats in group Com received access to 5 ml of the sucrose–LiCl compound on one day and 5 ml of water on the other; half received the compound on the first of these sessions and half on the second. Rats in group Ele received presentations of the elements: 5 ml of sucrose on one day and 5 ml of LiCl on the other. Again the order of presentation was counterbalanced within the group. The test phase followed a recovery day, on which water was presented in both morning and afternoon sessions. Over the course of two test days, both saline and sucrose were presented. Half the animals in each group received saline on Test 1 and sucrose on Test 2; the remainder received the reverse sequence. Procedural details not specified here were the same as those described for the previous experiments.

Results and discussion

During the first stage of the experiment, both groups drank almost all the fluid made available. Group Com drank a mean of 4.4 ml of the sucrose–LiCl compound; group Ele drank 4.4 ml of

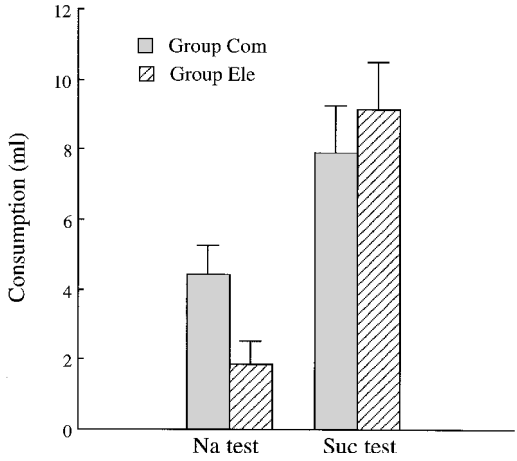

Figure 4. Experiment 4: Group means for consumption of NaCl and sucrose on the test trials. Group Com had previously consumed a compound of sucrose and LiCl; group Ele has experienced LiCl and sucrose on separate trials. Vertical bars represent standard errors of the mean.

sucrose and 4.5 ml of LiCl. Figure 4 shows group means for consumption of saline and sucrose in the test phase (the scores being pooled over both of the trials given with a particular substance). It appears that the presence of sucrose on the conditioning trials overshadowed learning about the salty taste of the compound, as group Com showed less of an aversion to saline than did group Ele, $t(14) = 2.68$. There was some indication that overshadowing between the elements of the compound might be mutual in that group Com drank sucrose a little less readily on the test than did group Ele. This difference between the groups was not, however, statistically significant, $t(14) = 0.52$. Like all demonstrations of overshadowing, the effect obtained on the saline test in this experiment is open to a number of explanations (e.g., in terms of generalization decrement effects, or in terms of the cue-competition effects postulated by formal models of conditioning such as that proposed by Rescorla & Wagner, 1972). None the less, the effect seen here exactly parallels that observed using more orthodox conditioning procedures.

EXPERIMENT 5

Experiment 4 was designed to show that presenting another flavour (sucrose) in compound with LiCl would restrict the development of an aversion to the salty taste of LiCl (and thus to saline on the test). In the present experiment we sought evidence of a reciprocal effect, asking whether the acquisition of the aversion to the salty taste of the lithium solution might act to restrict the development of an aversion to sucrose when LiCl and sucrose are experienced together. In order to achieve this we made use of the latent inhibition effect demonstrated in Experiment 2—the finding that pre-exposure to saline retarded the acquisition of the aversion to saltiness generated by subsequent consumption of LiCl. Rats in the critical experimental condition (group P-C) received pre-exposure (P) to saline before the conditioning trial in which LiCl and sucrose were presented as a compound (C). We anticipated that the pre-

exposure phase would limit the development of the aversion to the salty taste of the compound and thus might allow the development of a strong aversion to sucrose. Such an effect (a reduction in the overshadowing effect as a consequence of prior exposure to the overshadowing element of a compound stimulus) has been demonstrated for an orthodox conditioning procedure (conditioned suppression) by Carr (1974).

The performance of group P-C was compared with that shown by rats given the compound conditioning trial but no pre-exposure to saline (group N-C). In this group, conditioning to the salty taste should proceed normally, and, if this is capable of overshadowing learning about sucrose, these rats should show a less marked aversion to sucrose than should those in group P-C. Two further control groups were included, groups P-E and N-E (see Table 1). These matched the two groups just described except that instead of receiving a conditioning trial on which the sucrose–LiCl compound was presented, they received presentations of the elements (E) of the compound (i.e., of LiCl and of sucrose) on separate trials. In neither of these groups is an aversion to sucrose to be expected.

Finally, the aversion governed by saline was tested for all subjects. This permitted a further investigation of the overshadowing effect demonstrated in Experiment 4. Overshadowing would be evident if animals in the C groups, which drank the LiCl in compound with sucrose, showed less of an aversion to saline than did those in the E groups, which drank the simple LiCl solution. This test also allowed an assessment of the interaction between the latent inhibition and overshadowing effects. Rats in group P-C received both pre-exposure to NaCl and conditioning with a compound stimulus. If the two effects summate, then this group should show a less of an aversion than should any of the other groups.

Method

Subjects and procedure

The subjects were 32 male hooded Lister rats with a mean weight of 327 g (range: 300–350 g) at the start of the experiment. After the water deprivation schedule had been established as in the previous experiments, the rats were divided into four equal-sized groups. Over the next four morning sessions, rats in the P groups received access to 10 ml of the NaCl solution; rats in the N groups received 10 ml of water on these sessions. Over the next two days the C groups received 5 ml of the sucrose–LiCl compound on one day and 5 ml of water on the other; half of each group received the compound on the first of these sessions and half on the second. Rats in the E groups received presentations of the elements: 5 ml of sucrose on one day and 5 ml of LiCl on the other. After a recovery day, on which water was presented in both morning and afternoon sessions, all animals received a series of six test sessions in which they were given free access to the sucrose solution for 30 min each morning. Finally, all subjects received a single test session in which they were given free access to the saline solution for 30 min. Any details not specified here were the same as those described for Experiment 4.

Results and discussion

During the pre-exposure phase the P groups drank all the saline that was made available. On the conditioning trial group P-C drank a mean of 4.6 ml of the sucrose–LiCl compound and group N-C a mean of 4.4 ml. The E groups both drank the full 5 ml of sucrose and of LiCl.

Figure 5 shows group means for consumption of sucrose during the six trials of the test phase. It is evident that experience of the compound was effective in establishing an aversion to sucrose in the C groups. Thus, on the first trial of the test no animal in the C groups drank more than 3.6 ml, whereas the lowest consumption recorded for an animal in the E groups was 7.5 ml. Animals in the E groups drank somewhat less on the first test trial than on later trials, but thereafter a high level of consumption was maintained, and there was no consistent difference between those pre-exposed to saline and those not given pre-exposure. An analysis of variance conducted on the test scores from groups P-E and N-E, with group and trial as the variables, revealed only a significant effect of trial, $F(5, 70) = 2.80$; other Fs > 1. For the C groups, consumption increased dramatically over the course of the test, presumable reflecting extinction of the aversion to sucrose. As levels of consumption rose, a difference emerged between the groups, with group N-C drinking more than group P-C. An analysis of variance conducted on these scores showed there to be a significant effect of session, $F(1, 14) = 78.22$. The main effect of group fell short of significance, $F(5, 70) = 4.07$, $p > .1$, but the interaction between the variables was statistically reliable, $F(5, 70) = 11.09$. Analysis of simple main effects showed that the groups differed on Sessions 3, 4, and 5; Fs(1, 63) = 6.33, 6.38, and 4.93, respectively. This difference between the C groups accords with the suggestion that the salty taste of LiCl, experienced in compound with the sucrose on the conditioning trial, will overshadow learning about the sucrose, but that prior exposure to saline can limit the size of this overshadowing effect.

Group mean scores for the test trial with NaCl are given in Figure 6. As the figure shows, suppression of consumption was more marked in the E groups than in the C groups, confirming that the presence of sucrose during consumption of the LiCl solution will overshadow acquisition of the aversion to the taste of salt (see also Experiment 4). The effect

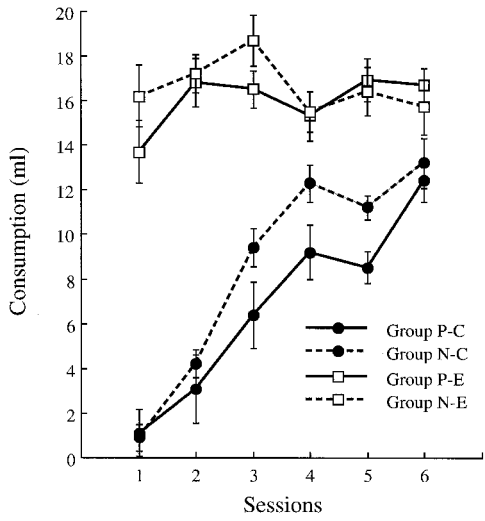

Figure 5. Experiment 5: Group means for consumption of sucrose over the six trials of Test 1. P indicates pre-exposure to NaCl; N, no such pre-exposure. C groups consumed the sucrose–LiCl compound; E groups experienced the elements of the compound on separate trials. Vertical bars represent standard errors of the mean.

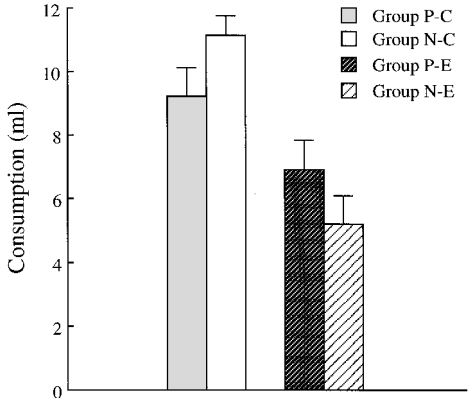

Figure 6. Experiment 5: Group means for consumption of saline on Test 2. P indicates pre-exposure to NaCl; N, no such pre-exposure. C groups consumed the sucrose–LiCl compound; E groups experienced the elements of the compound on separate trials. Vertical bars represent standard errors of the mean.

of pre-exposure to saline differed between the E groups and the C groups. For the E groups, the aversion was somewhat less in subjects given pre-exposure (i.e., group P-E drank more than group N-E) whereas the position was reversed for the C groups (with group P-C drinking slightly less than group N-C). Statistical analysis gave support to these impressions. An analysis of variance was conducted on the data summarized in Figure 6, with pre-exposure condition (P or N) and training condition (C or E) as the variables. There was no significant main effect of pre-exposure ($F < 1$), but there was a significant effect of training condition, $F(1, 28) = 21.5$, confirming the reliability of the overshadowing effect. The interaction between the two variables was significant, $F(1, 28) = 4.22$, although subsequent analysis of simple main effects failed to reveal a significant difference either between the two C groups, $F(1, 28) = 2.35$, or between the two E groups, $F(1, 28) = 1.89$.

In the light of the outcome of this last statistical analysis, conclusions must be tentative, but the pattern of interaction between the latent inhibition and overshadowing effects suggested by these results is worthy of comment. In the E groups, where overshadowing will not operate, the effect of pre-exposure was to reduce the size of the aversion displayed by group P-E; that is, a normal latent inhibition effect was observed. It might be expected that this pre-exposure effect would summate with the effect of overshadowing in group P-C and that the aversion shown by this group would be particularly weak. Such a summation effect has been obtained for the standard taste aversion learning procedure by Nakajima, Ka, and Imada (1999). In the present experiment, however, the aversion shown by group P-C was somewhat stronger than that shown by group N-C, suggesting that the latent inhibition effect might counteract overshadowing rather than summate with it. A similar result has recently been reported (from experiments using the conditioned suppression procedure) by Blaisdell, Bristol, Gunther, and Miller (1998) who offer an explanation in terms of a comparator hypothesis. The explanation they offer could be applied to the results reported here, but it would remain to explain why the comparator process should operate in the present study but apparently not in the closely similar experiment of Nakajima et al. Speculation on this matter would be premature at this stage.

GENERAL DISCUSSION

Early observations of the phenomenon of "bait shyness" (in which wild rats that become ill after consuming a less-than-lethal dose of a poisoned bait will not longer eat that bait) were interpreted as reflecting the operation of some learning process (see, e.g., Chitty, 1954; Richter, 1953). Direct study of related effects under laboratory conditions (e.g., Garcia, Kimeldorf, & Koelling, 1955; Revusky & Bedarf, 1967) established the reality of flavour aversion learning and the validity of an interpretation of this effect in terms of standard principles of Pavlovian conditioning (e.g., Domjan, 1980; but see Garcia, Brett, & Rusiniak, 1989). In the majority of analytic studies of flavour aversion conditioning the state of illness has been generated by something other than the ingested foodstuff itself (i.e., separate events have been used for the CS and the US); and in a large number of studies the US has been provided by the intraperitoneal injection of LiCl. The present experiments come round full circle—although they use LiCl as the illness-inducing agent, by presenting this substance orally they allow investigation of the development of an aversion to the taste of the substance that is directly responsible for producing the illness.

The results obtained with this procedure are all consistent with the proposal that simply consuming a solution of LiCl engages a conditioning mechanism that results in the formation of an association between the salty taste of the solution and its aversive consequences (the illness that it induces). Having consumed LiCl, rats will also reject an otherwise palatable NaCl solution, although with prolonged exposure to this solution the tendency to consume it will return, an effect that we have interpreted as indicating extinction of the aversion to the salty taste (Experiment 1). Pre-exposure to saline prior to ingestion of the LiCl solution restricts the development of an aversion to the taste of salt (Experiment 2), suggesting that the learning that occurs as a consequence of ingestion of LiCl is subject to latent inhibition. Experience of another flavour in compound with NaCl, prior to ingestion of LiCl, results in that flavour acquiring aversive properties (Experiment 3), parallelling the sensory preconditioning effect obtained with orthodox conditioning procedures. Finally, another flavour present during consumption of LiCl will acquire aversive properties and restrict the extent to which the taste of salt becomes aversive (the overshadowing effect of Experiment 4). However, as Experiment 5 showed, the degree of overshadowing will depend on the readiness with which the salty taste is itself learned about—pre-exposure to saline reduces learning about saltiness and allows more learning to occur to the flavour presented in compound with the LiCl.

Apart from the intrinsic interest of the phenomenon, an important reason for studying the effects of ingestion of LiCl was the hope of developing a model system that would allow the study of procedures that might be employed in modifying food preferences (and controlling predation) in wild species. Several attempts have been made to establish aversions in predator species to certain types of prey by allowing the predator to eat examples of the prey that have been laced with sufficient poison to produce illness, but not death, in the predator (e.g., Brett, Hankins, & Garcia, 1976; Gustavson, Garcia, Hankins, & Rusiniak, 1974; Nicolaus, Cassel, Carlson, & Gustavson, 1983). In several of these procedures LiCl has been used as the illness-producing agent. It has proved to be effective and has certain advantages over other substances that have been tried for this purpose (see Cowan, Reynolds, & Gill, 2000). It is stable (unlike apomorphine, which rapidly oxidizes losing its emetic effect); it is unlikely to be lethal (unlike carbachol, for which there is only a limited margin between the effective and lethal dose); it is

palatable, at least initially (thiabendazole and cinnamamide have been found to be rejected from the outset by some species); and it is ecologically acceptable for baits containing LiCl to be introduced into the natural environment (potentially a problem for the otherwise effective synthetic oestrogenic hormone ethinyloestradiol).

The problem that remains, as Cowan et al. (2000) point out, is that LiCl has a distinctive salty taste making it possible that the predator will acquire its aversion to this taste rather than to the characteristic taste or odour of the prey species (the outcome that is intended). The experiments reported here confirm the validity of this concern. They show, indeed, that consuming LiCl results in the acquisition of an aversion to the taste of the lithium itself, also that the formation of this aversion detracts from the strength of the aversion formed to another taste (sucrose in our experiments) to which the LiCl has been added. One suggested solution to this problem is to present the LiCl as pellets or capsules so as to obscure its flavour; unfortunately, however, some predators will reject such capsules, and it is difficult to use this method for some types of bait (Nicolaus, Farmer, Gustavson, & Gustavson, 1989). Our experiments point to a possible alternative. We have found (Experiment 5) that exposure to a salty taste prior to consumption of a flavoured LiCl solution reduces the extent to which conditioning to the salty taste overshadows learning about the added flavour. The implication is that latent inhibition training, in which the target species is pre-exposed to bait treated with NaCl before being given bait containing LiCl, might promote the formation of an aversion to the flavour of the bait. Whether this procedure will be effective, given that, for any predator, the distinctive flavour of its usual prey will itself presumably have undergone extensive latent inhibition training, will need to be determined by further experiment.

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