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# The role of habituation of the response to LiCl in the US-preexposure effect

ISABEL DE BRUGADA, FELISA GONZÁLEZ, and MARTA GIL
*University of Granada, Granada, Spain*

and

GEOFFREY HALL
*University of York, York, England*

In two experiments, rats received preexposure consisting of six intraperitoneal injections of lithium chloride (LiCl). This treatment reduced the magnitude of the unconditioned response (UR; suppressed consumption of a novel flavor) evoked by an additional injection (Experiment 1) or by oral consumption (Experiment 2) of LiCl. In both experiments, preexposure also attenuated the acquisition of a conditioned aversion with an LiCl injection as the unconditioned stimulus (US) but had no effect on the aversion produced when the US was oral consumption of LiCl (Experiment 2). These results are consistent with the view that the reduced ability of the preexposed US to serve as a reinforcer depends on blocking by injection-related cues and is independent of habituation of the UR recorded in the present study. Possible interpretations of this dissociation are discussed.

Acquisition of a conditioned response (CR) is retarded in animals given prior exposure to the event to be used as the unconditioned stimulus (US); this is referred to as the *US-preexposure effect*. Two (not necessarily mutually exclusive) explanations have been offered for this effect (Randich & LoLordo, 1979). One possibility is that habituation occurs during preexposure and that this influences not only the ability of the US to evoke an unconditioned response (UR)—the usual index of habituation—but also its effectiveness as a reinforcer. The second possibility is that preexposure allows the acquisition of associative strength by various contextual cues and that the latter then act to block the acquisition of strength by the experimenter's conditioned stimulus (CS) in the formal conditioning stage of the procedure. In a recent report, de Brugada, Hall, and Symonds (2004) examined these explanations for the US-preexposure effect seen in flavor aversion conditioning in rats. Their results were entirely consistent with the blocking account and gave no support to the habituation hypothesis.

In all of the experiments reported by de Brugada et al. (2004), the preexposure procedure consisted of giving rats three intraperitoneal injections of lithium chloride (LiCl). When such an injection was subsequently used as the US in flavor aversion conditioning, acquisition of the aversion was found to be retarded (i.e., the US-preexposure effect was observed). This effect was obtained despite the fact that the magnitude of the UR evoked by the LiCl injection appeared to be uninfluenced by the preexposure procedure. After an injection of LiCl, rats show a reduced willingness to consume a novel, but normally palatable, flavored solution (Domjan, 1977; see also Symonds & Hall, 2002). This UR was as marked in rats given preexposure as in those that experienced LiCl for the first time prior to the consumption test. By this measure, therefore, there was no evidence of habituation of the response to the LiCl injection. Evidence for the blocking interpretation came from experiments in which the US consisted of the oral consumption of an LiCl solution. The aversion produced by this procedure was not attenuated by prior experience of (injections of) LiCl, suggesting that cues associated with the injection procedure itself might be responsible for the blocking of acquisition when the US was administered by injection. In the absence of such cues, aversion conditioning proceeded normally. Support for this analysis came from the observation that introducing such cues (by way of a saline injection) prior to oral consumption of LiCl restored the US-preexposure effect.

Although these results lend no support to the habituation hypothesis, they do not prove that habituation plays no role in the US-preexposure effect. What we have provided is evidence of the US-preexposure effect in the absence of evidence of habituation; however, this may simply mean that our measure of habituation (the consumption test) was a less sensitive measure of the effectiveness of LiCl than was the conditioning procedure. What is needed to prove the point is a procedure that is capable of producing habituation but nonetheless fails to generate the US-

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preexposure effect. The present experiments were designed to meet this need.

In our previous experiments (de Brugada et al., 2004), no habituation effect was found in animals given preexposure consisting of three injections of LiCl. We suspected, however, that habituation might be obtained with more extensive preexposure. In Experiment 1, therefore, we examined the effects of giving six injections in preexposure. As we anticipated, we found that this preexposure regimen resulted in a clear habituation effect (as measured by the consumption test). That it also generated a US-preexposure effect does not mean that this example of the effect was a consequence of habituation; because the effect was demonstrated in a standard conditioning procedure in which the US was administered by injection, it could have been a result of blocking by injection-related cues. Therefore, in Experiment 2 we examined the effects of extensive preexposure on the conditioning produced by oral consumption of LiCl (i.e., in the absence of injection cues). If habituation to the US is capable of producing the US-preexposure effect, then the effect should be evident in this case too.

## EXPERIMENT 1

The design of this experiment is outlined in Table 1. There were three groups of subjects. In the first phase of training, the experimental group (Group E) received preexposure consisting of six injections of LiCl. The control subjects (those in Groups C-1 and C-2) received saline injections in this phase. The effect of this preexposure on the UR evoked by an injection of LiCl was assessed in the second phase. All of the animals were given access to a novel flavored solution (A in the table), and consumption was monitored. Prior to this test, the rats in Groups E and C-1 received an injection of LiCl; those in Group C-2 received an injection of saline. Our previous work (de Brugada et al., 2004; see also de Brugada, González, & Cándido, 2003, and Domjan, 1977) has shown that after an injection of LiCl, rats are reluctant to consume a novel flavored solution. We expected, therefore, that Group C-1 would drink less on this test than would Group C-2. The question of interest was whether or not this tendency of the LiCl injection to suppress consumption would be attenuated in Group E. Such a result would indicate that the preexposure to LiCl given to Group E had resulted in habituation.

The remaining phases of the experiment were designed to aid in the search for evidence of the US-preexposure effect. All of the animals received standard flavor-aversion conditioning with a new flavor (B in the table) as the CS and an injection of LiCl as the US. We expected that Group E (given six preexposures in the first phase) would show less of an aversion than Group C-1, which had experienced LiCl only once before, or Group C-2, which had not experienced it at all.

### Method

**Subjects and Apparatus**. The subjects were 24 experimentally naive female Wistar rats with a mean ad lib weight of 208 g at the start of the experiment. They were housed in individual home cages with continuous access to food throughout the experiment and were maintained on a water deprivation schedule as detailed below. The home cages measured 50 × 26 × 14.5 cm and were kept in a colony room under a 12:12-h light:dark illumination cycle, with the lights coming on at 8:00 a.m. All experimental treatments were given during the light period of the illumination cycle. The walls and floors of the cages were made of translucent plastic and the roof of wire mesh; a layer of wood shavings covered the floor. Fluids were administered at room temperature from a 50-ml plastic centrifuge tube with a rubber stopper fitted with a stainless steel ball-bearing tipped spout. Fluid consumption was measured by weighing the tubes before and after fluid presentation. The fluids used were solutions of 0.1% sodium saccharine and 2% cider vinegar.

**Procedure**. Before the start of training, the animals were subjected for 3 days to a water-deprivation schedule consisting of a daily 30-min period of free access to water presented in the centrifuge tubes. The drinking period took place at 11:00 a.m. In subsequent phases of the experiment, either water or a flavored solution was presented at that time. The rats were also given access to water for 30 min starting at 5:00 p.m. on recovery days (see below) during the preexposure phase and on each day during the subsequent phases of the experiment. All experimental treatments were given in the home cages.

For the preexposure phase of the experiment, the subjects were allocated to three equal-sized groups. On each of the 6 preexposure days, all of the subjects in Group E were given a 4-ml intraperitoneal injection of 0.15-M LiCl 1 h before the morning drinking session. The subjects in Groups C-1 and C-2 were given a 4-ml injection of 0.15-M NaCl at that time. Each preexposure day was followed by a recovery day on which the animals received access to water for 30 min in both the morning and the afternoon drinking sessions. The UR test followed the last of these recovery days. The subjects in Groups E and C-1 received an injection of LiCl; those in Group C-2 were injected with saline. Thirty minutes after the injections, all of the animals were given access to 30 ml of Solution A for 1 h (for half of the subjects in each group, Solution A was the saccharin solution; for the rest, it was the vinegar solution). The drinking tubes were removed and weighed after 30 min and again at the end of the session, allowing consumption to be assessed over two 30-min bins. On the next day, the animals were given a further test with access to Flavor A for 30 min in the morning drinking session. In the conditioning session, all of the subjects received access to 10 ml of Solution B (saccharin for those animals that had had vinegar as Flavor A, and vinegar for

**Table 1**
**Design of Experiment 1**

| Group | Preexposure | UR Test | A Test | Cond | CR Test |
|---|---|---|---|---|---|
| E | 6 Li inj | inj Li–A | A | B–Li inj | B |
| C-1 | 6 sal inj | inj Li–A | A | B–Li inj | B |
| C-2 | 6 sal inj | inj sal–A | A | B–Li inj | B |

Note— Cond, conditioning trial; Li, lithium chloride; sal, saline; inj, injection. A and B were solutions of vinegar and of saccharin (counterbalanced).

those that had had saccharin as A) for 30 min, followed by an injection of 4 ml of LiCl. After 1 day of recovery, all the animals were given 3 test days, during which they received access to 30 ml of Flavor B for 30 min in the morning drinking session.

## Results and Discussion

No data were recorded during the preexposure phase. Group means for consumption of Flavor A after an injection of LiCl (Group C-1) or of saline (Group C-2) in the UR test are shown in Figure 1. It is evident that Group C-2 drank readily, and somewhat more so in the first half of the test than in the second, presumably as a result of a reduction in thirst over the course of the test. Consumption in Group C-1, which received its first LiCl injection immediately prior to the test, was suppressed throughout the test. This difference replicates previous findings. The new results are for Group E, which had received preexposure to LiCl. These animals showed suppression of consumption during the first half of the test but a recovery during the second half, in which they drank as much as Group C-2. Statistical analysis confirmed this observation. The data summarized in Figure 1 were subjected to an ANOVA with group and bin as the variables. There was no significant main effect of bin ($F < 1$), but there was a significant main effect of group [$F(2,21) = 12.68$, $p < .05$]. The interaction between these variables yielded $F(2,21) = 3.38$ ($p = .05$). Analysis of simple main effects showed a significant difference ($p < .05$) among the groups on both Bin 1 [$F(2,42) = 10.81$] and Bin 2 [$F(2,42) = 6.09$]. Pairwise comparisons using Tukey's test showed that Group E and Group C-1 did not differ on Bin 1, but that both differed from Group C-2. On Bin 2, Groups E and C-2 did not differ but both differed significantly from Group C-1. The results of the second test with Flavor A (given after 1 recovery day) confirmed that the suppression of consumption shown by Groups E and C-1 was indeed an immediate response to the injection of LiCl. On the second test, Group E drank 9.4 ml, Group C-1 drank 6.8 ml, and Group C-2 drank 6.9 ml. These scores did not differ significantly [$F(2,21) = 1.48$].

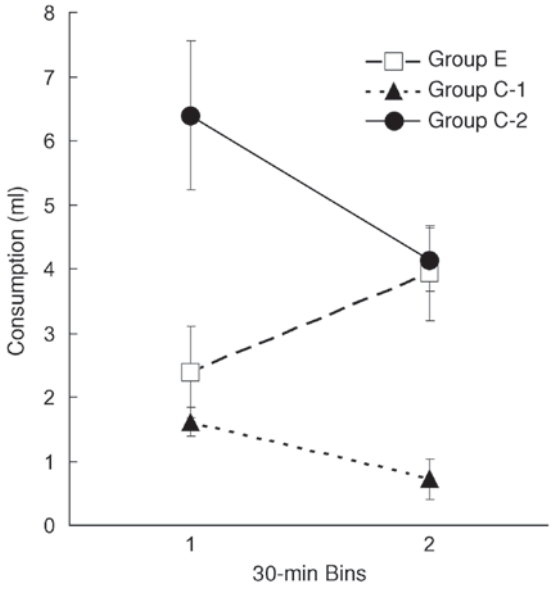


**Figure 1. Experiment 1: Group mean consumption scores for the test with Flavor A (the UR test of Table 1) given after animals in Groups E and C-1 had received an injection of LiCl. Animals in Group C-2 received an injection of saline prior to the test. Animals in Group E had received preexposure consisting of six injections of LiCl. Vertical bars represent standard errors of the means.**

In line with previous findings, the results presented in Figure 1 demonstrate that the UR to a first injection of LiCl is the suppression of consumption—Group C-1 drank little during the test, whereas Group C-2 (injected with saline) drank readily. The results for Group E provided evidence that six preexposures to LiCl produced a measure of habituation. Initial consumption (Bin 1) was low, but as the test progressed consumption resumed to the level shown by Group C-2. It seems, therefore, that the initial impact of the LiCl injection is the same in preexposed animals as in controls but that the effects of the injection are less long-lived in the former. It should be noted that de Brugada et al. (2004), who found no effects of three preexposures to LiCl on the suppression of consumption produced by an additional injection, used a test-trial duration of 30 min. Nevertheless, the failure of that experiment to generate a habituation effect does not seem to be a consequence of the shorter duration—in a parallel study of the effects of three preexposures, de Brugada et al. (2003) assessed suppression of consumption over a full 60-min test and found no sign of a difference between preexposed subjects and controls.

There were no differences among the groups in the amount of Flavor B drunk on the conditioning trial. The group mean scores were 6.7 ml for Group E, 7.0 ml for Group C-1, and 6.9 ml for Group C-2 ($F < 1$). The results for the test trials with Flavor B are shown in Figure 2. Both of the control groups showed a profound initial aversion that extinguished to some extent over the course of the test. Group E showed less of an aversion—an example of the US-preexposure effect. An ANOVA conducted on the data summarized in the figure yielded significant main effects of group [$F(2,21) = 7.93$] and of trial [$F(2,21) = 126.14$] and a significant interaction between these variables [$F(4,42) = 6.77$]. Analysis of simple main effects revealed a difference among the groups on each test trial [$F$s(2,63) = 13.33, 3.35, and 3.21 for Trials 1, 2, and 3, respectively]. Tukey's test showed that Group E differed from each of the other two groups on Test 1 and from Group C-2 on Test 2.

The results of this experiment can be summarized simply: Preexposure consisting of six injections of LiCl produces both habituation of the UR evoked by such an injection and a reduction in the effectiveness of the injection as a reinforcer in aversion conditioning. Interpretation is less straightforward. From previous work in which three preexposure trials were used, we know that the US-preexposure effect can be obtained when there is no evidence of ha-

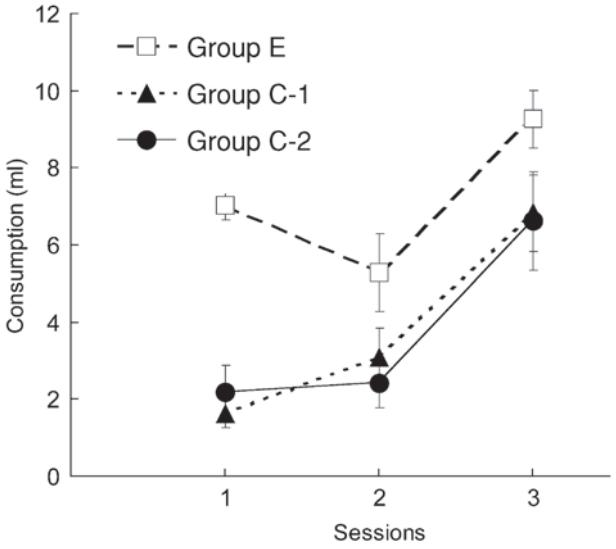


**Figure 2. Experiment 1: Group mean consumption scores for the test with Flavor B (the CR test of Table 1) given after aversion conditioning with B as the conditioned stimulus. Animals in Group E had received preexposure consisting of six injections of LiCl. Vertical bars represent standard errors of the means.**

bituation and that the effect obtained in these conditions can be fully explained in terms of blocking by injection-related cues (de Brugada et al., 2004). We assume that the blocking mechanism will also be operating in the present experiment, but we cannot tell from the present results whether the habituation process also plays a role. One obvious possibility is that the US-preexposure effect obtained after extensive preexposure is a consequence both of blocking and of a habituation-induced reduction in the effectiveness of the US. However, it is also possible that the result of habituation reflects a change in processing that is quite unrelated to the effectiveness of LiCl as a US, and that this instance of the US-preexposure effect is again solely dependent on the blocking mechanism. In order to resolve this issue, it is necessary to investigate the effect of habituation on conditioning in a procedure in which blocking by injection-related cues is not possible. We attempted to accomplish this in our next experiment.

## EXPERIMENT 2

With some exceptions, the design of this experiment (see Table 2) followed that of Experiment 1. The major exception was that after the preexposure phase had been completed the rats in Groups E and C-1 were given a trial on which they were permitted to drink 4 ml of a solution of LiCl. (The rats in Group C-2 drank a similar quantity of saline.) Our previous work (de Brugada et al., 2004; Loy & Hall, 2002; see also Nachman, 1963; Smith & Balagura, 1969) has shown that drinking LiCl will establish an aversion to saltiness, which reveals itself in a reluctance to drink a saline (NaCl) solution. We anticipated, therefore, that in the saline test (see Table 2) Group C-1 would be less willing to drink than Group C-2. The question of interest was whether or not the aversion would be attenuated in Group E (i.e., whether a US-preexposure effect would be obtained). With this conditioning procedure, there is no possibility of blocking by injection-related cues and the effect, if observed, could thus be attributed to habituation produced by preexposure. To confirm that habituation had indeed occurred, we included a UR test of the sort used in Experiment 1. After the animals in Groups E and C-1 had drunk the LiCl and those in Group C-2 had drunk saline, they were given access to Flavor A. Habituation to the effects of LiCl (orally consumed in this case) would be reflected in a higher level of consumption in Group E than in Group C-1.

The final phase of the experiment was designed to aid in the search for the conventional US-preexposure effect. All the animals were given Flavor B followed by an injection of LiCl. In accordance with Experiment 1, we expected to see a strong aversion in Groups C-1 and C-2 and a lesser aversion in Group E.

### Method

**Subjects and Procedure**. The subjects were 24 experimentally naive female Wistar rats with a mean ad lib weight of 192 g at the start of the experiment. They were divided into three equal-sized groups as in Experiment 1 and received preexposure just as the subjects in that experiment had. In the morning session of the day following the last preexposure day, the subjects in Groups E and C-1 received a conditioning trial in which they were given access to 4 ml of the LiCl solution for a period of 5 min; the subjects in Group C-2 were given 4 ml of an NaCl solution. After a 10-min interval, all of the subjects were given 30 min access of 30 ml of Solution A. (The test was restricted to 30 min, as opposed to 1 h as in Experiment 1, because the animals in this experiment had already had the opportunity to drink up to 4 ml of fluid immediately prior.) After 1 day of recovery, all of the subjects were given a 30-min presentation of 30 ml of saline, and on the next day they were given a 30-min presentation of 30 ml of Solution A. In the final conditioning session, all the subjects received access to 10 ml of Solution B for 30 min followed by an injection of 4 ml of LiCl. After 1 day of recovery, the rats were given three test sessions on each of which they received access to 30 ml of Flavor B for 30 min in the morning drinking session. Any details not specified here were the same as those described in Experiment 1.

**Table 2**
**Design of Experiment 2**

| Group | Preexposure | Cond & UR Test | Test Sal | A Test | Cond | CR Test |
|---|---|---|---|---|---|---|
| E | 6 Li inj | drink Li–A | sal | A | B–Li inj | B |
| C-1 | 6 sal inj | drink Li–A | sal | A | B–Li inj | B |
| C-2 | 6 sal inj | drink sal–A | sal | A | B–Li inj | B |

Note— Cond, conditioning trial; Li, lithium chloride; sal, saline; inj, injection. A and B were solutions of vinegar and of saccharin (counterbalanced).

**Results and Discussion**

No data were recorded during the preexposure phase. On the first conditioning trial, the groups given LiCl to drink consumed less than that given NaCl: Group means were 3.4 ml for Group E, 4.2 ml for Group C-1, and 4.6 ml for Group C-2. An ANOVA showed a significant difference among the groups [$F(2,21) = 5.73$]; Tukey's test showed that Group E differed significantly from Group C-2. The source of this difference is unclear; in our previous experiments with this procedure, we found that rats drank LiCl as readily as NaCl during an initial brief presentation. It is important to note, however, that there was no reliable difference in consumption between Group E and Group C-1 (i.e., the two groups given LiCl).

The conditioning produced by this treatment was assessed in the saline test given after the recovery day (the results of the UR test immediately following the conditioning trial will be discussed shortly). Group means for saline consumption are shown on the right side of Figure 3. It is evident that Group C-2 drank saline readily but that oral LiCl consumption successfully established an aversion in both Group E and Group C-1. An ANOVA conducted on the saline test scores showed a significant difference among the groups [$F(2,21) = 29.92$]; pairwise comparisons using Tukey's test showed that Group C-2 differed from each of the other groups, which did not differ between themselves. Thus, there was no US-preexposure effect, since the suppression of saline consumption was nearly as marked in preexposed Group E as in nonpreexposed Group C-1. Although the preexposure regimen used here is known to produce habituation to the effects of LiCl (Experiment 1), it does not result in a retardation of subsequent conditioning when the US is administered orally rather than by injection.

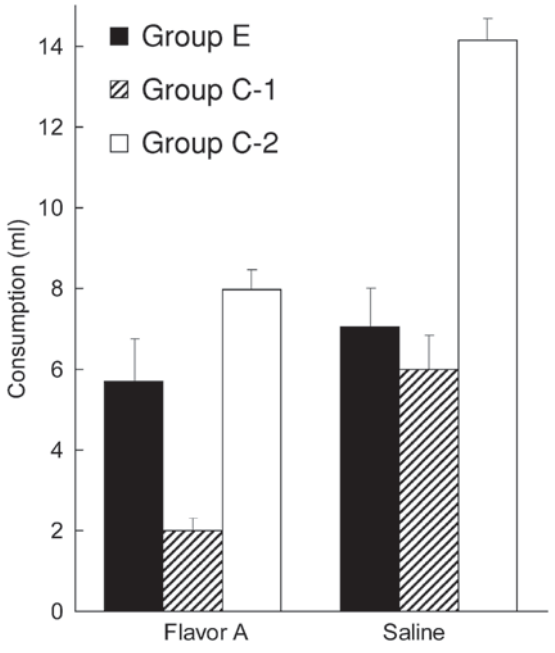


**Figure 3. Experiment 2: Group mean consumption scores for the UR test given immediately after consumption of LiCl, and for the test with saline, given 1 day after animals in Groups E and C-1 had drunk a solution of LiCl. Animals in Group C-2 drank saline rather than LiCl. Animals in Group E had received preexposure consisting of six injections of LiCl. Vertical bars represent standard errors of the means.**

The other results presented in Figure 3 confirm that habituation occurred in this experiment. Group means for consumption of Flavor A in the UR test are shown on the left side of the figure. As in Experiment 1, Group C-2 drank Flavor A readily but Group C-1, which had just received LiCl for the first time, consumed very little. Group E, by contrast, consumed almost as much as did Group C-2, indicating that preexposure to LiCl had produced habituation of this UR for these subjects. An ANOVA confirmed the existence of a reliable difference among the groups [$F(2,21) = 17.94$]. Pairwise comparisons (Tukey's test) showed that Group C-1 differed from each of the other groups and that there was no reliable difference between the Group E and Group C-2. It can be noted that the habituation effect (a higher level of consumption in Group E than in Group C-1) was evident in this 30-min test, but it appeared only in the second half of the 60-min test used in Experiment 1. We cannot easily explain why this should have been so. It seems unlikely that it reflects a difference in the efficacy of LiCl determined by the route of administration; the study by Loy and Hall (2002, Experiment 1) included a direct test of the flavor aversions produced by injection and by consumption of the same amount of LiCl and found no sign of a difference.

Given that the critical finding of this experiment is that the tests with saline and Flavor A produced different patterns of results, we thought it worthwhile to confirm the reliability of this difference by subjecting all the scores presented in Figure 3 to a common analysis. An ANOVA with UR test with Flavor A and CR test with saline as the variables showed significant main effects of group [$F(2,21) = 41.79$] and of test [$F(1,21) = 39.44$] and a significant interaction between the variables [$F(2,21) = 5.25$]. Analysis of simple main effects showed significant differences among the groups both on the test with Flavor A [$F(2,24) = 15.31$] and on the test with saline [$F(2,42) = 34.77$]. Pairwise comparisons using Tukey's test confirmed that on the test with Flavor A Group C-1 differed significantly from each of the other two groups, which did not differ reliably from each other. On the test with saline, there was no difference between Groups E and C-1, but both differed significantly from Group C-2.

Group means for the second test with Flavor A, conducted on the day after the saline test, were 5.9 ml for Group E, 4.3 ml for Group C-1, and 7.8 ml for Group C-2. An ANOVA revealed a significant difference among these scores [$F(2,21) = 3.78$], with Group C-2 showing a higher level of consumption than the other two groups, which did not differ reliably from each other. No such differences were seen in the equivalent test in Experiment 1, and we can only speculate as to the origins of the difference that we found in the present experiment. One possibility is that it reflects a generalization of the aversion acquired to saline in Groups E and C-1 as a result of the first conditioning

trial. There was no evidence of differential generalization, however, in consumption of Flavor B on the conditioning trial on which B was presented as the CS. Group E consumed 7.1 ml on this trial, Group C-1 6.9 ml, and Group C-2 7.5 ml ($F < 1$).

The results of the test trials with Flavor B are shown in Figure 4. They reveal a clear US-preexposure effect. All three groups showed an initial suppression of consumption that declined over the course of the test; however, the two control groups showed levels of aversion that were consistently and substantially greater than that shown by Group E. There was no difference between the control groups. An ANOVA with group and trial as the variables yielded significant main effects of both [for group, $F(2,21) = 4.27$; for trial, $F(2,21) = 53.21$]. Although the interaction between the variables was not significant [$F(4,42) = 1.42$], analysis of simple main effects showed a significant difference among the groups on Trial 1 [$F(2,63) = 5.2$] and on Trial 2 [$F(2,63) = 4.66$], but not on Trial 3 ($F < 2$). Tukey's test showed that Group E differed from each of the control groups on Trials 1 and 2; no other between-groups difference was significant.

The finding that preexposure to injections of LiCl attenuates subsequent conditioning when the LiCl US is administered by injection but not when it is consumed orally replicates the results reported by de Brugada et al. (2004) and is consistent with the conclusion that the US-preexposure effect obtained in this paradigm is a consequence of blocking by injection-related cues. The absence of an effect following oral consumption even though the preexposure effect yields evidence of habituation of one of the URs evoked by LiCl suggests that habituation does not, in itself, imply that the US will be less effective as a reinforcer in subsequent conditioning.

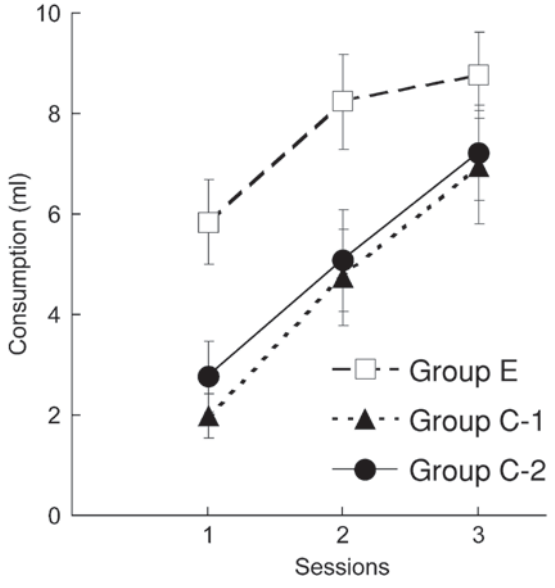


**Figure 4. Experiment 2: Group mean consumption scores for the test with Flavor B (the CR test of Table 2) given after aversion conditioning with B as the conditioned stimulus. Animals in Group E had received preexposure consisting of six injections of LiCl. Vertical bars represent standard errors of the means.**

## GENERAL DISCUSSION

Previous studies of the effects of preexposure to LiCl have usually failed to detect any habituation of the various URs it elicits. Thus, Batson (1983) found no effect of preexposure on the hypoactivity induced by an injection of LiCl (but see Cain & Baenninger, 1977) or on the hypothermic response that such an injection evokes. De Brugada et al. (2003) found no effect of preexposure on the suppression of consumption of a novel flavor that follows an LiCl injection. Both these studies also included a test of the efficacy of LiCl as the reinforcer in flavor aversion conditioning, and both gave evidence acquisition of the aversion was retarded by preexposure. Although habituation (or the development of tolerance) may play a role in the US-preexposure effect observed with other pharmacological agents (see, e.g., Dacanay & Riley, 1982, for a discussion of the case of morphine), it does not appear to do so for LiCl. With LiCl, the effect is a consequence of blocking by contextual cues—specifically, for the training procedures used in the experiments reported here, of blocking by injection-related cues (de Brugada et al., 2004).

Clearly, when habituation does not occur it cannot play a part in the US-preexposure effect. However, the experiments described for the first time in the present work succeeded in obtaining evidence of habituation: When rats were given six preexposure trials rather than the three used by de Brugada et al. (2003), the suppression of consumption produced by a further presentation of LiCl was much attenuated. This was true both when the test involved an injection of LiCl and when the LiCl was consumed orally (and thus in the absence of the cues that are critical for the US-preexposure effect). Given this finding, we can ask whether or not habituation contributes to the US preexposure effect that was also observed in these experiments. The answer appears to be no. In Experiment 2, we made use of a conditioning procedure in which injection-related cues were eliminated and the resulting aversion was as substantial in the preexposed subjects as in the nonpreexposed controls. In summary, preexposure to the US in these experiments appears to establish an association between injection-related cues and the US, which is responsible for the retardation of subsequent conditioning with LiCl as the reinforcer. Preexposure may also result in habituation, but the effect is independent of this association and plays no part in the retardation of conditioning.

This pattern of results presents an explanatory challenge for current theories. It is particularly problematic for Wagner's (1981) influential theory, perhaps because it is one of the few to provide a coherent account of both habituation and the effects of US-preexposure on subsequent conditioning. This theory holds that when the interstimulus interval is long (as it is in the experiments reported here) contextual cues (which could be injection related) will become

associated with the US and acquire the ability to induce a secondary state of activation (A2) in its representational node. When a node is in the A2 state, presentation of the relevant stimulus is unable to engender the state of primary activation (A1) that it would otherwise evoke. The A1 state is necessary for excitatory conditioning to occur—hence the US-preexposure effect. The problem arises because the same mechanism is offered as an explanation of habituation. Specifically, it is assumed that a full UR requires that the stimulus evoke the A1 state, so that the decline in the UR is again attributed to the ability of contextual cues to produce the A2 state. (In addition, for some USs the A2 state may even activate an opponent response, which would also contribute to the attenuation of the observed UR.) It follows that if the contextual cues are capable of producing blocking, they should also be capable of producing habituation of the UR—not the result obtained by Batson (1983) or by de Brugada et al. (2003). Moreover, if the habituation effect depends on an association controlled by injection-related cues, it should not have been found when (as in Experiment 2 of the present study) these cues were omitted on the test.

This analysis makes it clear that we need an account that allows that a signaled US will be less effective as a reinforcer (thus explaining the US-preexposure effect in terms of blocking) yet attributes habituation to an independent process that reduces the ability of the US to evoke its UR but does not reduce its reinforcing properties. One widely supported theory of habituation (Groves & Thompson, 1970) attributes the effect to a decline in transmission along the direct US-to-UR pathway. According to this interpretation, habituation will occur simply as a consequence of repeated presentation of the stimulus and will not depend on associations between the contextual cue and the US. Furthermore, since the habituation process occurs in mechanisms that follow activation of the US node, it is reasonable to assume that this node is fully activated and thus capable of supporting normal associative learning, as was seen in the first conditioning phase of Experiment 2. What is lacking, however, is any explanation of why the effectiveness of the presence of cues signaling the occurrence of the US should be reduced as a reinforcer, thus producing the US-preexposure effect observed in Experiment 1 and in the second conditioning phase of Experiment 2.

One possibility emerges if we acknowledge the complexity of the event used as the US in these experiments and accept that it may be inappropriate to suppose that an injection of LiCl activates a single representational node. We would need to assume the existence of at least two nodes: one susceptible to habituation by repeated US presentation and responsible for the UR measured in these experiments, and one responsible for conditioned suppression of consumption and susceptible to associative modulation (and thus to blocking effects). Support for this interpretation can be sought in the distinction recently proposed by Parker (2003) between *taste aversion* and *taste avoidance*. According to Parker's analysis, a substance such as LiCl has two major effects on rats: It not only produces a state of nausea but also (like drugs that do not produce nausea) produces a novel change in the physiological state that signals danger. Both of these effects can support conditioning. A taste associated with nausea will acquire a conditioned aversion that will be evident in modified consummatory behavior when rats are subsequently exposed to that taste. This effect, however, is not held to be responsible for the suppression of intake observed in the standard consumption test. The latter effect, which is a modification of appetitive behavior, is attributed to taste avoidance—conditioning (akin to fear conditioning) supported by an association between the taste and the dangerous change of physiological state.

The application of this analysis to our own results suggests the following. The UR measured in these experiments is taken to reflect the state of nausea induced by injection or consumption of LiCl. With repeated administration, the ability of LiCl to induce this state is reduced and the magnitude of the UR declines. This habituation process, however, does not influence conditioning as measured by the consumption test, which depends on the taste-avoidance learning process. Assuming that LiCl continues to elicit a change in physiological state even when it does not evoke nausea, avoidance learning could still occur, provided it is not blocked by the presence of pretrained contextual cues.

We must admit that this account is speculative and rests on the perhaps questionable assumption that one of the consequences of LiCl administration will show habituation whereas the other will not. After all, if nausea can habituate, then why can't the change of state responsible for avoidance learning? Nevertheless, this account has testable implications. In particular, it implies that habituation to LiCl might well be capable of producing a US-preexposure effect when the CR is assessed by a measure that is sensitive to nausea-induced conditioning. The taste reactivity test (pioneered by Grill & Norgren, 1978) has been put forward as just such a measure (Parker, 2003) and could be usefully employed in future research. Although the specifics of this account may be debatable and in need of further experimental verification, we should acknowledge that the dissociation between habituation and the US-preexposure effect demonstrated in these experiments is hard to explain without an assumption of this general sort.

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