UNCLASSIFIED
SECURITY CLASSIFICATION

AD-A207 916

DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION: UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): SR89-11	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Armed Forces Radiobiology Research Institute; 6b. OFFICE SYMBOL (If applicable): AFRRI	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Defense Nuclear Agency, Bethesda, Maryland 20814-5145	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: Defense Nuclear Agency; 8b. OFFICE SYMBOL (If applicable): DNA	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code): Washington, DC 20305	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. NWED QAXM; PROJECT NO.; TASK NO.; WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Attenuation and cross-attenuation in taste aversion learning in the rat: Studies with ionizing radiation, lithium chloride and ethanol

12. PERSONAL AUTHOR(S)
Rabin et al.

13a. TYPE OF REPORT	13b. TIME COVERED FROM ____ TO ____	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Reprints		1989 January	10

16. SUPPLEMENTARY NOTATION

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Gloria Ruggiero	(301)295-3536	ISDP

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

Pharmacology Biochemistry & Behavior, Vol. 31, pp. 909–918. Pergamon Press plc. 1989. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Attenuation and Cross-Attenuation in Taste Aversion Learning in the Rat: Studies With Ionizing Radiation, Lithium Chloride and Ethanol

BERNARD M. RABIN,*†[1] WALTER A. HUNT* AND JACK LEE*

**Behavioral Sciences Department, Armed Forces Radiobiology Research Institute Bethesda, MD 20814-5145*
and †Department of Psychology, University of Maryland Baltimore County Baltimore, MD 21228

Received 20 November 1987

RABIN, B. M., W. A. HUNT AND J. LEE. *Attenuation and cross-attenuation in taste aversion learning in the rat: Studies with ionizing radiation, lithium chloride and ethanol.* PHARMACOL BIOCHEM BEHAV 31(4) 909–918, 1988.—The preexposure paradigm was utilized to evaluate the similarity of ionizing radiation, lithium chloride and ethanol as unconditioned stimuli for the acquisition of a conditioned taste aversion. Three unpaired preexposures to lithium chloride (3.0 mEq/kg, IP) blocked the acquisition of a taste aversion when a novel sucrose solution was paired with either the injection of the same dose of lithium chloride or exposure to ionizing radiation (100 rad). Similar pretreatment with radiation blocked the acquisition of a radiation-induced aversion, but had no effect on taste aversions produced by lithium chloride (3.0 or 1.5 mEq/kg). Preexposure to ethanol (4 g/kg, PO) disrupted the acquisition of an ethanol-induced taste aversion, but not radiation- or lithium chloride-induced aversions. In contast, preexposure to either radiation or lithium chloride attenuated an ethanol-induced taste aversion in intact rats, but not in rats with lesions of the area postrema. The results are discussed in terms of relationships between these three unconditioned stimuli and in terms of implications of these results for understanding the nature of the proximal unconditioned stimulus in taste aversion learning.

Conditioned taste aversion Attenuation Cross-attenuation Ionizing radiation Lithium chloride Ethanol Area postrema

A conditioned taste aversion (CTA) is produced when ingestion of a novel tasting solution is paired with a novel unconditioned stimulus (UCS), such that the organism will avoid ingestion of that solution at a subsequent presentation. This avoidance behavior can be produced by pairing a novel saccharin or sucrose solution with a wide variety of stimuli, including treatment with lithium chloride (LiCl), ethanol, or exposure to ionizing radiation (13,33).

Rabin and Hunt (25) have proposed that exposure to ionizing radiation or injection of LiCl causes the release of the same endogenous humoral factor which mediates the acquisition of a CTA following treatment with these stimuli. However, the support for this hypothesis is based, for the most part, on the indirect evidence provided by the observation that lesions of the area postrema (AP) disrupt the acquisition of both LiCl- and radiation-induced taste aversions (19, 24, 27, 30).

A more direct test of this hypothesis may be provided by pairing different toxins in a UCS preexposure paradigm. In this design, the subject is exposed to a UCS before it is paired with the conditioned stimulus. When this UCS is later paired with ingestion of a novel solution, a CTA does not develop (3, 5, 7). While the mechanisms underlying the UCS preexposure effect vary depending upon the nature of the drug UCS (2, 4, 6, 7, 10), the previous experience with the drug-induced effects disrupts the CTA learning. The attenuation of the CTA may be due to the development of physiological tolerance, as with morphine (8, 9, 34), or to the associative effects of prior experience with the UCS, as with LiCl (2–6, 10). While tolerance produces a broad change in the sensitivity of the central nervous system resulting in a reduced capacity to respond to a variety of stimuli, preexposure that does not cause tolerance has a more limited effect because it is restricted to learned generalization gradients. The more similar the effects of the unconditioned stimuli, the greater the generalization and the greater the preexposure

[1]Requests for reprints should be addressed to Bernard M. Rabin, Department of Psychology, University of Maryland Baltimore County, Baltimore, MD 21228.

effect, such that the greatest disruption of CTA learning by preexposure would be expected from the use of the same UCS in both the conditioning and test phases of the experiment.

In addition to single-drug effects, the preexposure paradigm can also be utilized to assess the similarity of different unconditioned stimuli [e.g., (1,22)]. If preexposure to one UCS disrupts the acquisition of a CTA to treatment with a second UCS, the clear implication is that the effects of treatment with the preexposure UCS have generalized to the conditioning UCS. This suggests that the two different stimuli must be similar in some way in order for the experience with one UCS to affect the novelty of the second UCS. Therefore, if taste aversions produced by radiation and LiCl, both of which are dependent upon the integrity of the AP, result from the action of a common endogenous factor, then it should be possible to disrupt the acquisition of a CTA produced by one UCS by preexposure to the other UCS. Conversely, since the acquisition of a CTA following treatment with ethanol does not depend upon the integrity of the AP (16) and may, therefore, involve different mechanisms, preexposure to one UCS should not affect the capcity of the other UCS to produce a CTA. These experiments were designed to evaluate these hypotheses.

GENERAL METHOD

Subjects

The subjects were male Sprague-Dawley-derived rats weighing 300–400 g at the start of the experiment. Rats were maintained in an AAALAC-accredited facility. Animal holding rooms were maintained at 21±1°C with 50±10% relative humidity. The rats were maintained on a 12-hr light:dark cycle. Food and water were continually available, except as required by the experimental protocol.

Procedure

The general procedure was to place the subjects on a 23.5-hr water deprivation schedule during which water was available for 30 min during the early light part of the diurnal cycle. During the preexposure phase, the rats were treated with one UCS or with a control treatment immediately following the drinking period on days 2, 5 and 8. On the conditioning day (day 10), they were presented with two calibrated drinking tubes, one containing tap water and the other containing a 10% sucrose solution. Immediately following the 30-min drinking period, the rats were treated with either the UCS that they received during the preexposure phase, with a second UCS, or with a control treatment. On the test day (day 12), all rats were again given a choice between the tap water and sucrose solution.

Data Analysis

For all experiments, the relative intake of water and sucrose solution was transformed into preference score: sucrose intake divided by total fluid intake. A preference score less than 0.50 indicates a greater intake of water than sucrose and, therefore, an aversion to the normally preferred sucrose solution. For statistical analyses, the arcsin transformation was used to normalize the distribution of preference scores, and initial data analyses were done with mixed analyses of variance. Where necessary, comparisons between relevant groups were made using planned comparisons with the Scheffe test to correct for familywise Type I error (18).

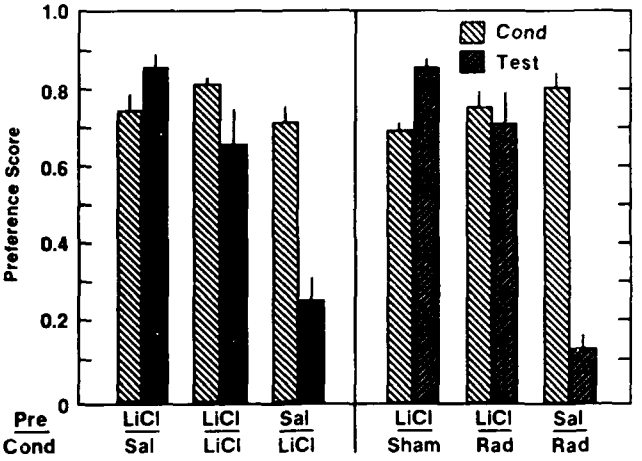


FIG. 1. Effects of pretreatment with lithium chloride (LiCl) or saline (Sal) on LiCl or radiation-induced (Rad) conditioned taste aversions, or sham-irradiated (Sham) rats. Pre: Preexposure UCS; Cond: Conditioning Day UCS. Error bars indicate the standard error of the mean.

EXPERIMENT 1

The first set of experiments was designed to evaluate the hypothesis that a similar factor mediates the acquisition of both radiation- and LiCl-induced taste aversions, but not ethanol-induced aversions, by determining whether or not preexposure to one UCS would disrupt the acquisition of a CTA following treatment with the other UCS.

Method

The subjects were 252 male rats divided into 24 groups of 9–13 rats each. Each UCS served as both preexposure and conditioning day treatment.

For irradiation, the rats were placed in ventilated plastic restraining boxes and carried to a ^{60}Co source. The radiation UCS for both preexposure and conditioning consisted of 100 rad at a dose rate of 40 rad/min. Dosimetry was accomplished using thermoluminescent detectors (LiF TLD 100s) and a 3.3-ml Victoreen chamber. The sham-irradiated rats were placed in plastic boxes and carried to the source, but not exposed. Two doses of LiCl were used. For the preexposure phase, all rats were given 3.0 mEq/kg, IP. On the conditioning day, rats were given either 3.0 or 1.5 mEq/kg, IP. The control animals were given equivalent volume injections of isotonic saline. Ethanol (4 g/kg) was administered intragastrically with an infant feeding tube in both preexposure and conditioning phases of the experiment. Control rats were intubated with an equivalent volume of water.

Results

Repeated treatment with radiation or LiCl produced no major effects on either conditioning day water or sucrose intake in comparison to the control treatments. Preexposure to LiCl disrupted the acquisition of a CTA to both LiCl and ionizing radiation (Fig. 1). For the LiCl-induced aversion, the analysis with planned comparisons showed that there were no differences in sucrose preference between the group preexposed to LiCl and given saline on the conditioning day

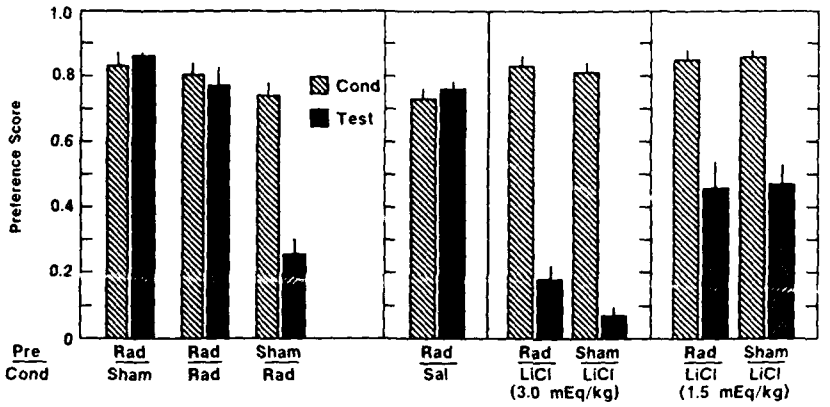


FIG. 2. Effects of preexposure to radiation on the acquisition of radiation- and LiCl-induced taste aversions. LiCl-induced aversions were produced by injection of either 3.0 mEq/kg or 1.5 mEq/kg, as indicated. Abbreviations as in Fig. 1.

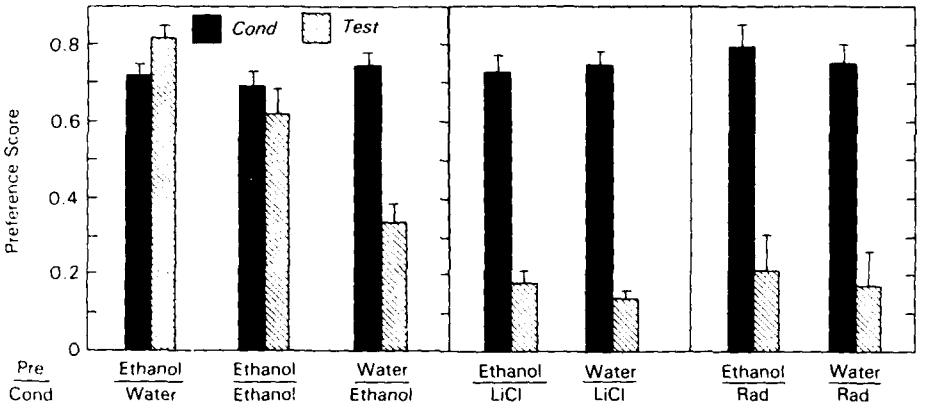


FIG. 3. Effect of preexposure to ethanol (4 g/kg, PO) on the acquisition of a CTA produced by intubation of ethanol or by injection of LiCl (3.0 mEq/kg) or exposure to ionizing radiation (100 R). Abbreviations as in Fig. 1.

and the group preexposed to LiCl and given LiCl on the conditioning day, $F(1,27)=7.45$, $p>0.05$. Both LiCl preexposure groups differed significantly from the group given saline during the preexposure phase and LiCl on the conditioning day, $F(1,27)=42.31$, $p<0.01$.

For the radiation-induced CTA following preexposure to LiCl, the planned comparisons indicated that the sucrose preference of the two groups given LiCl during the preexposure phase did not differ significantly from each other, $F(1,27)=4.33$, $p>0.05$. Both of these groups differed significantly from the group given saline injections during the preexposure phase and exposed to radiation on the conditioning day, $F(1,27)=150.06$, $p<0.001$.

As shown in the first panel of Fig. 2, preexposure to ionizing radiation prevented the acquisition of a radiation-induced CTA. Planned comparisons showed that the two radiation preexposure groups did not differ significantly from each other, $F(1,30)=1.25$, $p>0.05$, in test day sucrose preference even though one group was exposed to radiation on the conditioning day while the other group was given a sham exposure. Both of these groups did differ from the group that was subjected to sham irradiation procedures during the preexposure phase of the experiment and irradiated on the conditioning day, $F(1,30)=11.64$, $p<0.01$.

In contrast, preexposure to radiation had no effect on a CTA produced by treatment with either 3.0 or 1.5 mEq/kg LiCl (Fig. 2). Analysis of the data using a three-way analysis of variance with one repeated factor showed that the main effects for the dose, $F(1,40)=34.14$, $p<0.001$, and for day, $F(1,40)=280.99$, $p<0.001$, were significant. However, the observation that neither the main effect for treatment between the radiation- and sham-preexposed groups, $F(1,40)=1.14$, $p>0.05$, nor the dose by treatment, $F(1,40)=1.80$, $p>0.05$, nor day by treatment, $F(1,40)=0.69$, $p>0.05$, interactions were significant would indicate that there were no differences in sucrose preferences between the radiation- and sham-preexposed groups at either dose level.

Preexposing the organism to ethanol (4 g/kg, PO) blocks the acquisition of a CTA following intubation with the same dose of ethanol (Fig. 3). The analysis with planned comparisons showed that the group preexposed to ethanol and given ethanol on the conditioning day did not show a significant

test day decrease in sucrose preference, $F(1,24)=0.03$, $p>0.10$. In contrast, the controls given water intubation during the preexposure phase did show a significant decrease in sucrose preference on the test day, $F(1,24)=26.04$, $p<0.01$.

Preexposing rats to ethanol had no effect on the acquisition of either a LiCl- or radiation-induced CTA (Fig. 3). For the LiCl-induced CTA, the analysis of variance showed that the main effect for treatment for the comparison between the ethanol- or water-treated rats was not significant, $F(1,20)=0.20$, $p>0.10$, while the main effect for the comparison between conditioning and test day was highly significant, $F(1,20)=306.74$, $p<0.001$. The treatment by day interaction was not significant, $F(1,22)=0.58$, $p>0.10$, indicating that the rats developed an LiCl-induced aversion to the sucrose regardless of whether they received ethanol or water during the preexposure phase of the experiment.

Similar results were obtained with the analysis of the radiation-induced CTA. Neither the main effect for treatment, $F(1,16)=0.28$, $p>0.10$, nor the treatment by day interaction, $F(1,16)=0.005$, $p=1.00$, was significant. The main effect for day was significant, $F(1,16)=57.79$, $p>0.001$, indicating that preexposure to ethanol does not attenuate the development of a CTA when the rats are subsequently exposed to radiation on the conditioning day.

In contrast, pretreatment with LiCl blocks the acquisition of an ethanol-induced CTA (Fig. 4). The analysis using planned comparisons showed that there were no significant differences between the animals pretreated with LiCl and given ethanol on the conditioning day and those given water on the conditioning day, $F(1,28)=2.02$, $p>0.05$. However, the test day sucrose preference of these groups of animals did differ significantly from the preference of the animals pretreated with saline and given ethanol on the conditioning day, $F(1,28)=32.30$, $p<0.01$.

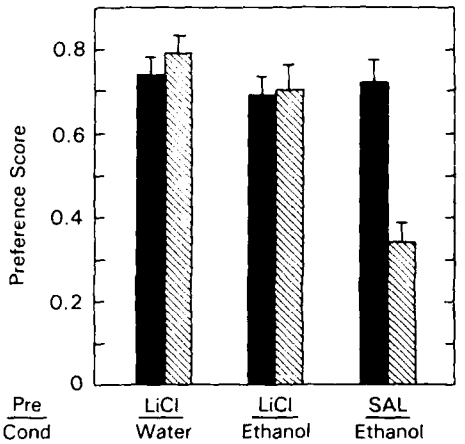


FIG. 4. Effects of preexposure to LiCl (3.0 mEq/kg) on the acquisition of ethanol-induced CTA. Abbreviations as in Fig. 1. Black bars: Cond; hatched bars: Test.

Discussion

These results only partially support the original hypothesis that radiation and LiCl utilize a common humoral factor to produce CTA learning. The observation of asymmetrical preexposure effects between LiCl and radiation suggests that these two stimuli do have some effects in common. However, the failure of radiation preexposure to attenuate a LiCl-induced CTA would indicate that preexposure to ionizing radiation did not produce effects sufficiently similar to those produced by LiCl to attenuate a CTA in response to treatment with LiCl on the conditioning day. This failure to attenuate an LiCl-induced CTA cannot be due to a general inability of the radiation UCS to produce a preexposure effect because preexposure did attenuate a radiation-induced CTA. Also, because attenuation of the LiCl-induced CTA was seen with neither the high nor the low dose of LiCl, it would not seem likely that the failure to observe a cross-attenuation between the radiation preexposure and LiCl could be due to inappropriate doses of either UCS.

The results with ethanol agree with previous research showing that preexposure to ethanol will block the acquisition of an ethanol-induced CTA (6). They are also consistent with the hypothesis that different mechanisms are involved in CTA learning with these different stimuli, so that exposure to radiation or LiCl on the conditioning day may be perceived as a novel UCS resulting in the acquisition of a CTA, despite the prior exposure to ethanol. Because there is the possibility that the failure of ethanol preexposure to attenuate a LiCl-induced CTA is dose-dependent, some additional animals were run using 1.5 mEq LiCl as the conditioning day UCS. At this dose, there was also no effect of ethanol preexposure on the LiCl-induced aversion.

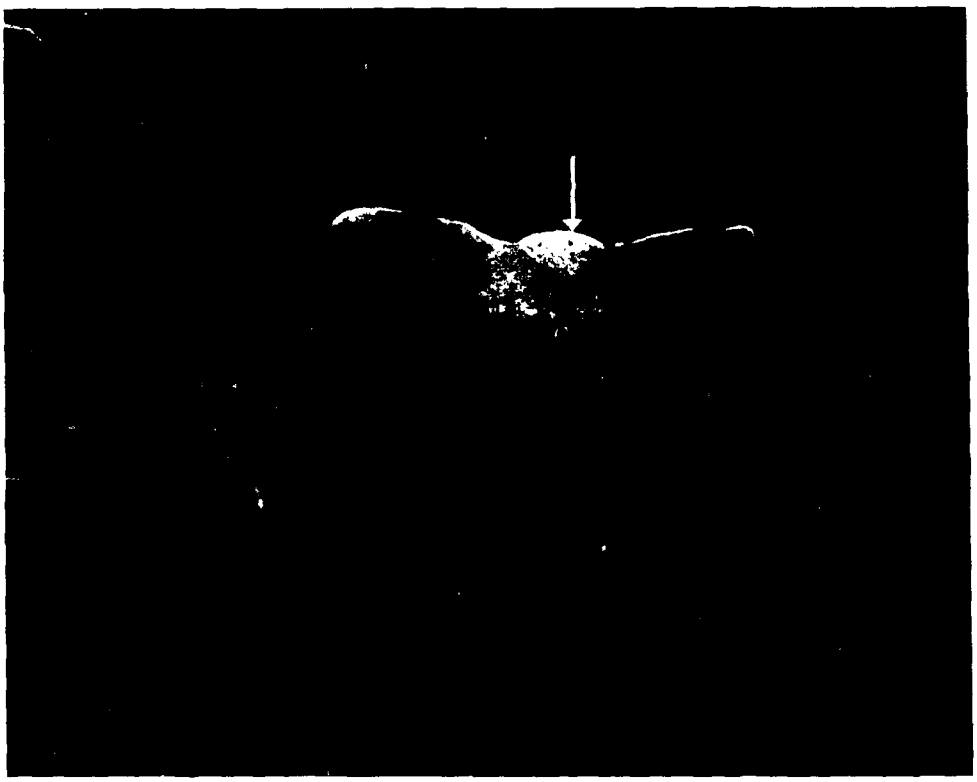
These results apparently differ from those of Cannon *et al.* (6) who reported that preexposure to ethanol could block a LiCl-induced CTA. However, they did not verify that their procedure of 4 consecutive daily intubations of 5 g/kg ethanol did not produce tolerance to ethanol. In contrast to the present procedures which have been shown not to produce tolerance, their procedure is very similar to a procedure which has been shown to produce ethanol tolerance as measured by sleep times (15). Ethanol tolerance, unlike simple preexposure to ethanol, does produce an attenuation of both radiation- and LiCl-induced taste aversions [(15); Rabin and Hunt, unpublished observations].

In contrast to the effects of ethanol preexposure on a LiCl-induced CTA, the observation that preexposing rats to LiCl will block the acquisition of an ethanol-induced CTA is in agreement with the results reported by Cannon *et al.* (6), and therefore not consistent with the present hypothesis. This asymmetrical cross-attenuation between LiCl preexposure and ethanol-induced CTA learning may derive from the fact that LiCl, which crosses the blood-brain barrier (21), does have central effects similar enough to those produced by ethanol to result in the attenuation of the ethanol-induced CTA following pretreatment with LiCl.

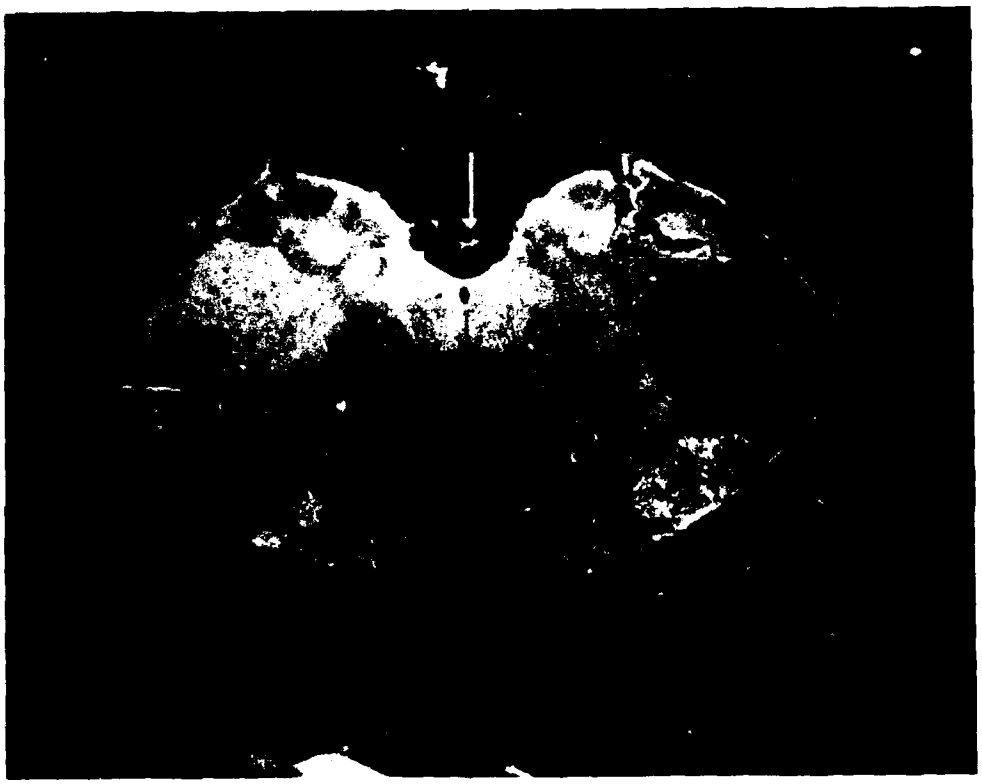
FACING PAGE

FIG. 5. Sample photomicrographs showing the area postrema (A, arrow) and a representative lesion (B, arrow). The lesion includes the area postrema and parts of the dorsal nucleus of the solitary tract.

A



B



EXPERIMENT 2

Although lesions of the AP will disrupt the acquisition of a LiCl-induced CTA (27,30), if it is the central actions of LiCl that are responsible for the attenuation of an ethanol-induced CTA by preexposure to LiCl, then destruction of the AP should have no effects on the cross-attenuation of the ethanol-induced CTA because LiCl should still be expected to cross the blood-brain barrier even in the absence of the AP. The present experiment was designed to evaluate the role of the AP in the LiCl-induced attenuation of an ethanol-induced CTA by preexposing rats with AP lesions to LiCl.

Method

The subjects were 21 rats with lesions of the AP. The behavioral methods were identical to those detailed above, except that only two conditions were run: LiCl and saline pretreatment groups. Both groups received ethanol on the conditioning day.

The surgical procedures have been detailed in previous reports (27–29). Briefly, all rats were anesthetized with sodium pentobarbital (35 mg/kg, IP). The AP was exposed and thermal lesions were made using a cautery probe under direct visual control. After surgery, the rats were given an injection of bicillin (60,000 units) and allowed to recover in their home cages for a period of 2–4 weeks before beginning behavioral testing.

At the conclusion of the testing, all rats were anesthetized with sodium pentobarbital (50 mg) and perfused intracardially with isotonic saline followed by 10% formalin saline. Sections were cut through the brainstem at the level of the AP at 50 μm and stained with thionin. Representative sections from an intact rat and one with AP lesions are presented in Fig. 5. Examination of the histological material indicated that for the most part the lesions were restricted to the AP, although they did occasionally affect the dorsal parts of the nucleus of the solitary tract.

Results

Pretreating rats with AP lesions with LiCl did not affect conditioning day fluid intake relative to rats that were given saline injections. In rats with AP lesions, preexposure to LiCl does not attenuate the acquisition of an ethanol-induced CTA (Fig. 6). The analysis of variance showed that neither the main effect for treatment, $F(1,19)=0.01$, $p>0.10$, nor the treatment by day interaction, $F(1,19)=0.02$, $p>0.10$, was significant. The main effect for day, however, $F(1,19)=38.41$, $p<0.001$, was significant, indicating that both the LiCl- and saline-preexposed rats showed identical aversions following conditioning day intubation with ethanol regardless of the nature of the preexposure treatment.

Discussion

These results do not support the hypothesis that the cross-attenuation of an ethanol-induced CTA by preexposure to LiCl in intact rats is due to the central actions of LiCl. Rather, the failure to observe a cross-attenuation in rats with lesions of the AP suggests that the AP is somehow involved in this effect. Because AP lesions do not disrupt the acquisition of an ethanol-induced CTA (16), the nature of the AP involvement in the cross-attenuation of an ethanol-induced CTA by LiCl is not certain. It may be that the effects of treatment with specific toxins on AP neurons is a sufficient condition for the cross-attenuation of a CTA produced by a UCS that does not require the mediation of the AP for the original learning.

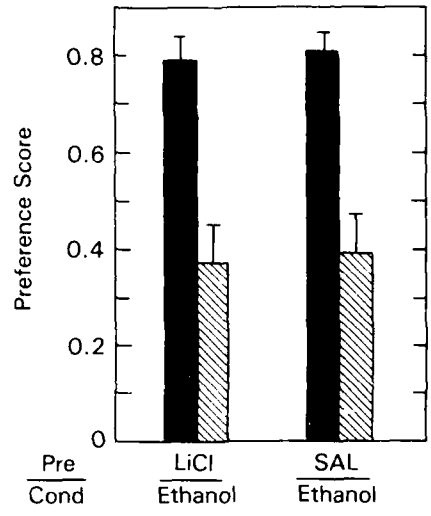


FIG. 6. Effects of preexposure to LiCl on the acquisition of an ethanol-induced CTA in rats with lesions of the area postrema. Abbreviations as in Fig. 1. Black bars: Cond; hatched bars: Test.

EXPERIMENT 3

If, as suggested above, the action of specific toxins on AP neurons is sufficient for the attenuation of an ethanol-induced CTA by LiCl, then preexposure to radiation, which also involves the AP, should result in a similar attenuation of an ethanol-induced CTA in intact rats, but not in rats with lesions of the AP. The present experiment was designed to evaluate this hypothesis.

Method

The first phase of the experiment utilized 18 intact rats divided into two groups of 9 rats each. The second phase of the experiment utilized 19 rats, all of which had histologically verified lesions of the AP and which were divided into two groups of 10 and 9 rats. The surgical and histological procedures were identical to those detailed above. In each phase, the first group of rats was given three preexposures to ionizing radiation (100 rad) while the second group was given sham exposures. On the conditioning day, all rats were treated with ethanol (4 g/kg, PO) immediately following sucrose ingestion.

Results

For the intact rats, preexposure to ionizing radiation resulted in the attenuation of an ethanol-induced CTA compared to the sham preexposed rats (Fig. 7). The analysis of variance indicated that the main effect for day, $F(1,16)=13.16$, $p<0.01$, and the preexposure by day interaction, $F(1,16)=11.62$, $p<0.01$, were both significant. Although the main effect for preexposure condition did not achieve significance, $F(1,16)=1.39$, $p>0.10$, the significant interaction would indicate that the test day sucrose preference of the rats preexposed to radiation was different than the preference of the groups given the sham preexposures.

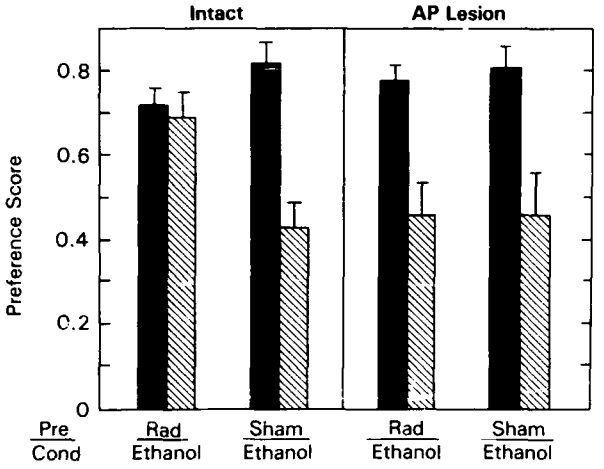


FIG. 7. Effects of preexposure to ionizing radiation on the acquisition of an ethanol-induced CTA in intact rats and in rats with lesions of the area postrema. Abbreviations as in Fig. 1. Black bars: Cond; hatched bars: Test.

In contrast, preexposing rats with lesions of the AP to radiation had no effect on an ethanol-induced CTA (Fig. 7). The analysis of variance showed that neither the main effect for preexposure, $F(1,17)=0.17$, $p>0.10$, nor the preexposure by day interaction, $F(1,17)=0.10$, $p>0.10$, were significant. Only the main effect for day, $F(1,17)=24.80$, $p<0.001$, was significant, indicating that equivalent reductions in test day sucrose preference were observed in both the radiation- and sham-preexposed rats with lesions of the AP.

Discussion

The observation that preexposure to ionizing radiation disrupts the acquisition of an ethanol-induced aversion in intact rats, but not in rats with lesions of the AP, is concordant with the hypothesis that activation of the AP by a UCS may be a sufficient condition for the UCS preexposure effect even though the conditioning day UCS itself does not require the mediation of the AP for CTA learning to occur.

GENERAL DISCUSSION

The initial experiments were designed to test two hypotheses about the nature of the proximal UCS in taste aversion learning and the relationships between ionizing radiation, LiCl and ethanol as unconditioned stimuli for CTA learning. First, that because radiation and LiCl may utilize a common mediator, preexposure to one UCS should block the acquisition of a CTA following treatment with the other UCS. Second, that ethanol, which does not require the mediation of the AP, may involve different mechanisms for CTA learning and, therefore, that preexposure to one UCS would not disrupt the acquisition of a CTA following treatment with another UCS. The results of the experiments, which show asymmetrical preexposure effects between radiation and LiCl and between ethanol and both radiation and LiCl, provide only partial support for these hypotheses.

The results of the first series of experiments, which showed that preexposure to LiCl blocks the acquisition of a radiation-induced CTA, would be consistent with the hypothesis that the stimulus effects produced by treatment with LiCl encompass those produced by exposure to radiation. The attenuation of a LiCl-induced CTA by preexposure to LiCl seems to be primarily an associative phenomenon because changing the environmental conditions between the two phases of the experiment disrupts the UCS preexposure effect (4,10). As such, the cross-attenuation between LiCl and radiation would seem to require generalization from the effects of the pretreatment UCS to those of the conditioning UCS. The greater the perceived similarity of the UCS effects by the organism across the two phases of the experiment, the greater the degree of cross-attenuation. Because the degree of LiCl attenuation of a radiation-induced CTA was nearly identical to the degree of LiCl attenuation of a LiCl-induced CTA, the stimulus effects of treatment with LiCl must be very similar to those of treatment with ionizing radiation. Otherwise, such strong stimulus generalization would not occur and, consequently, cross-attenuation would not occur.

Conversely, the failure to observe cross-attenuation of a LiCl-induced CTA by preexposure to radiation would suggest that the stimulus effects which result from exposing an organism to ionizing radiation are not similar enough to those produced by LiCl for generalization and cross-attenuation to occur. It may be that treating an organism with LiCl, which crosses the blood-brain barrier (21), produces a series of changes in neural functioning which are not produced by exposure to ionizing radiation at the dose levels used in the present experiment (28). Thus, for example, injection of LiCl has been reported to produce changes in neurohypophyseal functioning (23), in phospholipid metabolism (17), and in dopamine and serotonin receptor activity in the brain (20,35). Although these changes in neural functioning following treatment with LiCl do not constitute a sufficient condition for CTA learning (32), they form a part of the stimulus configuration when peripheral LiCl is used as a UCS for CTA learning. The failure of radiation to reproduce the complete constellation of stimulus events associated with LiCl treatment would mean that there is a weaker generalization gradient when radiation is used as the preexposure UCS and, consequently, preexposure to radiation does not produce cross-attenuation of a LiCl-induced CTA because LiCl is perceived as a novel discriminative UCS on the conditioning day.

Similar factors may be involved in the asymmetrical relationship between ethanol and radiation and LiCl. Preexposure to ethanol does not produce cross-attenuation of a radiation- or LiCl-induced CTA because ethanol, as a UCS for taste aversion learning, does not require the mediation of the AP (16). As a result, the pattern of neural activity produced by pretreatment with ethanol may not be similar enough to that produced by LiCl or radiation, particularly since it might not result in similar effects on the AP. Conversely, it is possible that anatomical connections of the AP are sufficiently broad that the stimulus pattern resulting from stimulation of the AP by pretreatment with a radiation or LiCl UCS will encompass the stimulus pattern produced by treatment with ethanol leading to the generalization from one UCS to the others and, therefore, to the cross-attenuation of an ethanol-induced CTA by preexposure to LiCl or radiation.

Similarly, an intact AP is necessary for preexposure to LiCl or radiation to produce an attenuation of an ethanol-induced CTA because the AP is necessary for CTA learning when either of these stimuli is used as the UCS (19, 24, 27, 30). It is only when the AP is intact that a pattern of neural

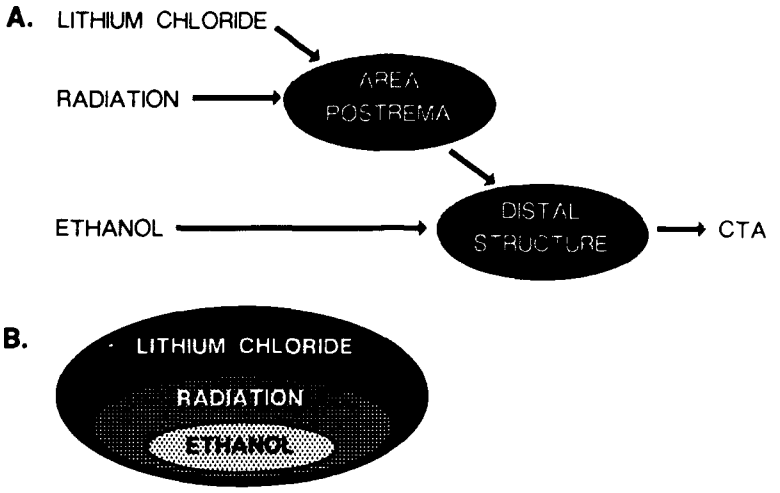


FIG. 8. (A) Diagrammatic representation of the neural pathways associated with the response of the organism to the UCS leading to the acquisition of a CTA following exposure to ionizing radiation or treatment with LiCl or ethanol. (B) Schematic illustration of the range of the stimulus configuration produced by exposure to ionizing radiation or by treatment with LiCl or ethanol. Each labelled oval represents the stimulus complex associated with the UCS. Preexposure to a UCS having a greater range of effects will disrupt the acquisition of a CTA produced by a UCS having a smaller range of stimulus effects. See text for details.

activity relevant to CTA learning with these stimuli can be initiated. In the absence of the AP, this pattern of neural activity is not produced by preexposure and, therefore, there is no generalization related to CTA learning from the preexposure stimuli to the conditioning day UCS. As a result, preexposure to radiation or LiCl cannot lead to the cross-attenuation of an ethanol-induced CTA in rats with lesions of the AP.

The proposed role of the AP in the UCS preexposure effect between radiation, LiCl and ethanol is shown schematically in Fig. 8A. Treatment with either ionizing radiation or LiCl produces changes in the activity of the AP which, in turn, affects the activity of some distal brain structure leading to the acquisition of a CTA (29). Although presently available data do not allow the specification of this intermediate structure, anatomical studies have shown that the AP sends projections to both the nucleus of the solitary tract and the parabrachial nuclei (31,36). Ethanol, which does not require the mediation of the AP for CTA learning, may affect this distal structure directly. Preexposure to LiCl or radiation produces an attenuation of an ethanol-induced CTA only when the AP is intact because treatment with these stimuli can affect the activity of this distal structure only through the mediation of the AP. In contrast, ethanol, which may affect this distal structure directly, cannot produce an attenuation of a radiation- or LiCl-induced CTA because the ethanol pretreatment does not affect the activity of the AP.

With regard to the determination of the proximal UCS in CTA learning, a number of different effects of treatment have been proposed. Because most stimuli that will lead to CTA learning also make the organism sick, Garcia (13) has proposed that the experience of a UCS-induced gastrointestinal illness is the proximal UCS for the acquisition of a CTA. In contrast, Gamzu (11,12) and Hunt and Amit (14), noting that a CTA will develop with nontoxic stimuli such as amphetamine that do not produce overt signs of illness, have suggested that any stimulus that produces a novel pattern of neural activity will also produce a CTA. Rabin and Rabin (26) obtained a CTA in anesthetized rats treated with LiCl or exposed to ionizing radiation. Since these rats could not have experienced a UCS-induced illness under the anesthesia, they suggested that any stimulus that excited the neural pathways associated with illness would lead to the acquisition of a CTA, independently of any possible experiential factor.

The present observation of an asymmetrical UCS preexposure effect between LiCl and ionizing radiation would be most consistent with the hypothesis that the proximal UCS for CTA learning involves activation of the neural pathways associated with illness (26). If an experienced gastrointestinal illness itself were the proximal UCS, then preexposure to the radiation-induced "illness" should have attenuated the CTA response to the LiCl-induced "illness" just as preexposure to the LiCl-induced "illness" attenuated the CTA response to the radiation-induced "illness." While it is possible that the cross-attenuation between radiation preexposure and LiCl might be dose-dependent, then cross-attenuation between radiation and LiCl should have been obtained when the conditioning day dose of LiCl was reduced if dose were a significant factor. The failure to observe such an effect (Fig. 2) does not support this possibility.

Rather, as discussed above and illustrated in Fig. 8B, the observation of asymmetrical preexposure effects would seem to derive from the total pattern of neural activity resulting from treatment with a specific UCS. Where the pattern of neural activity produced by the preexposure UCS fully encompasses the pattern of such activity produced by the conditioning day UCS, either because the same stimuli are used in both phases of the experiment or because the preexposure UCS produces all the neural stimulus effects of the condi-

tioning day UCS, then attenuation of the CTA will result. Since both LiCl and irradiation produce a CTA using an AP-dependent peripheral mediator, preexposure to LiCl attenuates a radiation-induced CTA. However, because LiCl apparently has effects in addition to those produced by the low dose of radiation, preexposure to radiation does not attenuate a LiCl-induced aversion. Similarly, preexposure to ethanol does not attenuate either a radiation- or LiCl-induced CTA because the ethanol-induced CTA, unlike one produced by radiation or LiCl, is independent of the AP and may not, therefore, produce an appropriate pattern of neural activity which involves the AP. Conversely, both LiCl and radiation may attenuate an ethanol-induced CTA, in rats with an intact AP, because the pattern of neural activity induced by these stimuli completely mimics the pattern of neural activity produced by intubation of ethanol.

In summary, preexposure to ionizing radiation or LiCl attenuates the CTA produced by conditioning day treatment with the same UCS and an asymmetrical cross-attenuation following treatment with the other UCS; such that LiCl attenuates a radiation-induced CTA, but radiation does not attenuate a LiCl-induced aversion. Because the UCS preexposure paradigm, in contrast to a tolerance paradigm, is based upon a generalization gradient from the preexposure UCS to the conditioning UCS, these results are consistent with the hypothesis that, as stimuli for CTA learning, both radiation and LiCl have common components, although they are not identical stimuli. Both LiCl and ionizing radiation attenuate an ethanol-induced CTA in intact rats, but not in rats with lesions of the AP. In contrast, preexposure to ethanol, which does not require the integrity of the AP for CTA learning, does not attenuate either radiation- or LiCl-induced taste aversions. These observations are consistent with the hypothesis that the critical stimulus for the acquisition of a CTA is the generation of a particular pattern of activity, perhaps related to the neural circuits associated with illness, in the central nervous system.

ACKNOWLEDGEMENTS

We wish to acknowledge the support of the Computer Science Center Facilities of the University of Maryland Baltimore County. This research was supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under work unit B4123. Views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency has been given or should be inferred. This research was conducted according to the principles described in the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Research, National Research Council. A preliminary report of some of the data was presented at the 17th Meeting of the Society for Neuroscience, New Orleans, LA, 1987.

REFERENCES

1. Aragon, C. M. G.; Abibtol, M.; Amit, Z. Acetaldehyde may mediate reinforcement and aversion by ethanol. An examination using a conditioned taste-aversion paradigm. Neuropharmacology 25:79–83; 1986.
2. Batson, J. D.; Best, P. J. Drug preexposure effects in flavor-aversion learning: Associative interference by conditioned environmental stimuli. J. Exp. Psychol. [Anim. Behav. Proc.] 5:273–283; 1979.
3. Braveman, N. S. Formation of taste aversions in rats following prior exposure to sickness. Learn. Motiv. 6:512–534; 1975.
4. Braveman, N. S. The role of blocking and compensatory conditioning in the treatment preexposure effect. Psychopharmacology (Berlin) 61:177–189; 1979.
5. Cain, N. W.; Baenninger, R. Habituation to illness: Effects of prior experience with US on the formation of learned taste aversions in rats. Anim. Learn Behav. 5:359–364; 1977.
6. Cannon, D. S.; Baker, T. B.; Berman, R. F. Taste aversion disruption by drug pretreatment: Dissociative and drug-specific affects. Pharmacol. Biochem. Behav. 6:93–100; 1977.
7. Cannon, D. S.; Berman, R. F.; Baker, T. B.; Atkinson, C. A. Effect of preconditioning unconditioned stimulus experience to learned taste aversions. J. Exp. Psychol. [Anim. Behav. Proc.] 104:270–284; 1975.
8. Cappell, H.; Le Blanc, A. E. Parametric investigations of the effects of prior exposure to amphetamine and morphine on conditioned gustatory aversion. Psychopharmacology (Berlin) 51:265–271; 1977.
9. Cappell, H.; Poulos, C. X. Associative factors in drug pretreatment effects on gustatory conditioning: Cross drug effects. Psychopharmacology (Berlin) 64:209–213; 1979.
10. Dacanay, R. J.; Riley, A. T. The UCS preexposure effect in taste aversion learning: Tolerance and blocking are drug specific. Anim. Learn. Behav. 10:91–96; 1982.
11. Gamzu, E. The multifaceted nature of taste-aversion-inducing agents: Is there a single common factor? In: Barker, L. M.; Best, M. R.; Domjam, M., eds. Learning mechanisms in food selection. Waco, TX: Baylor University Press; 1977:477–509.
12. Gamzu, E.; Vincent, G.; Boff, E. A pharmacological perspective of drugs used in establishing conditioned food aversions. Ann. NY Acad. Sci. 443:231–249; 1985.
13. Garcia, J.; Lasiter, P. A.; Bermudez-Ratoni, F.; Deems, D. A. A general theory of aversion learning. Ann. NY Acad. Sci. 443:8–21; 1985.
14. Hunt, T.; Amit, Z. Conditioned taste aversions induced by self-administered drugs: Paradox revisited. Neurosci. Biobehav. Rev. 11:107–130; 1987.
15. Hunt, W. A.; Rabin, B. M. Attenuation of a radiation-induced conditioned taste aversion after the development of ethanol tolerance. Life Sci. 43:59–66; 1988.
16. Hunt, W. A.; Rabin, B. M.; Lee, J. Ethanol-induced taste aversions: Lack of involvement of acetaldehyde and the area postrema. Alcohol 4:169–173; 1987.
17. Joseph, N. E.; Renshaw, P. F.; Leigh, J. S., Jr. Systemic lithium administration alters rat cerebral cortex phospholipids. Biol. Psychiatry 22:540–544; 1987.
18. Keppel, G. Design and analysis: A researcher's handbook. Englewood Cliffs, NJ: Prentice-Hall; 1973.
19. Ladowski, R. L.; Ossenkopp, K.-P. Conditioned taste aversions and changes in motor activity in lithium treated rats: Mediating role of the area postrema. Neuropharmacology 25:71–77; 1986.
20. McIntyre, I. M.; Kuhn, C.; Demitriou, S.; Flick, F. R.; Stanley, M. Modulating role of lithium on dopamine turnover, prolactin release, and behavioral supersensitivity following haloperidol and reserpine. Psychopharmacology (Berlin) 81:150–154; 1983.
21. Nelson, S. C.; Herman, M. M.; Bensch, K. G.; Barchas, J. D. Localization and quantitation of lithium in rat tissue following intraperitoneal injections of lithium chloride. II. Brain. J. Pharmacol. Exp. Ther. 212:11–15; 1980.
22. Ng Cheong Ton, J. M.; Amit, Z. Symmetrical effect of preexposure between alcohol and morphine on conditioned taste aversion. Life Sci. 33:665–670; 1983.

2)'Connor, E. F.; Cheng, S. W. T.; North, W. T. Effects of intraperitoneal injection of lithium chloride on neurohypophyseal activity: Implications for behavioral studies. Physiol. Behav. 40:91–95; 1987.

24. Ossenkopp, K.-P. Taste aversions conditioned with gamma radiation: Attenuation by area postrema lesions in rats. Behav. Brain Res. 7:295–305; 1983.
25. Rabin, B. M.; Hunt, W. A. Mechanisms of radiation-induced conditioned taste aversion learning. Neurosci. Biobehav. Rev. 10:55–65; 1986.
26. Rabin, B. M.; Rabin, J. S. Acquisition of radiation- and lithium chloride-induced conditioned taste aversions in anesthetized rats. Anim. Learn. Behav. 12:439–441; 1984.
27. Rabin, B. M.; Hunt, W. A.; Lee, J. Acquisition of radiation and drug-induced conditioned taste aversions following area postrema lesions in the rat. Radiat. Res. 93:388–394; 1983.
28. Rabin, B. M.; Hunt, W. A.; Lee, J. Effects of dose and of partial body ionizing radiation on taste aversion learning in rats with lesions of the area postrema. Physiol. Behav. 32:119–122; 1984.
29. Rabin, B. M.; Hunt, W. A.; Lee, J. Recall of a previously acquired conditioned taste aversion in rats following lesions of the area postrema. Physiol. Behav. 32:503–506; 1984.
30. Ritter, S.; McGlone, J. J.; Kelly, K. W. Absence of lithium-induced taste aversion after area postrema lesions. Brain Res. 201:501–506; 1980.
31. Shapiro, R. E.; Miselis, R. R. The central neural connections of the area postrema of the rat. J. Comp. Neurol. 234:344–364; 1985.
32. Smith, D. F. Central and peripheral effects of lithium on conditioned taste aversions in rats. Psychopharmacology (Berlin) 68:315–317; 1980.
33. Smith, J. C. Radiation: Its detection and its effects on taste preferences. In: Stellar, E.; Sprague, J. M., eds. Progress in physiological psychology, vol. 4. New York: Academic Press; 1971:53–117.
34. Stewart, J.; Eikelboom, R. Pre-exposure to morphine and the attenuation of conditioned taste aversion in rats. Pharmacol. Biochem. Behav. 9:639–645; 1978.
35. Tanimoto, K.; Maeda, K.; Terada, T. Inhibitory effect of lithium on neuroleptic and serotonin receptors in rat brain. Brain Res. 265:148–151; 1983.
36. van der Kooy, D.; Koda, L. Y. Organization of the projections of a circumventricular organ: The area postrema in the rat. J. Comp. Neurol. 219:328–338; 1983.