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Taste Aversion Learning Produced by Combined Treatment With Subthreshold Radiation and Lithium Chloride

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RABIN, B. M., W. A. HUNT AND J. LEE. *Taste aversion learning produced by combined treatment with subthreshold radiation and lithium chloride.* PHARMACOL BIOCHEM BEHAV 27(4) 671–675, 1987.—These experiments were designed to determine whether treatment with two subthreshold doses of radiation or lithium chloride, either alone or in combination, could lead to taste aversion learning. The first experiment determined the thresholds for a radiation-induced taste aversion at 15–20 rad and for lithium chloride at 0.30–0.45 mEq/kg. In the second experiment it was shown that exposing rats to two doses of 15 rad separated by up to 3 hr produced a taste aversion. Treatment with two injections of lithium chloride (0.30 mEq/kg) did not produce a significant reduction in preference. Combined treatment with radiation and lithium chloride did produce a taste aversion when the two treatments were administered within 1 hr of each other. The results are discussed in terms of the implications of these findings for understanding the nature of the unconditioned stimuli leading to the acquisition of a conditioned taste aversion.

Conditioned taste aversion   Radiation   Lithium chloride   Combined treatment

WHEN a novel tasting solution is paired with an unconditioned stimulus (UCS) such as exposure to ionizing radiation or injection of lithium chloride (LiCl), an organism will avoid ingestion of that solution at a subsequent presentation. This avoidance behavior, called a conditioned taste aversion (CTA), is typically acquired in a single pairing of the novel conditioned stimulus (CS) and the UCS.

Although both the radiation- and LiCl-induced CTA seem to share some mechanisms in common [9], they differ in terms of their capacity to produce a CTA utilizing a "backwards" conditioning procedure in which the UCS is presented before the CS. In the typical conditioning experiment the CS is followed by the presentation of the UCS. When the UCS is administered before the CS, the conditioned response is much weaker or may not occur at all. As a UCS for CTA learning, LiCl follows this pattern, such that a CTA is not acquired if the UCS is presented as little as 5 min before the CS [1]. With the radiation UCS, in contrast, CTA learning will occur even when the organism is irradiated up to 6–24 hr before the presentation of the CS [1, 2, 15]. These findings suggest that exposing an organism to ionizing radiation, in contrast to injection of LiCl, produces some change within the organism that remains active over an extended period of time, and that serves to produce the temporal contiguity between UCS and CS necessary for conditioning to occur.

If, as suggested above, exposing an organism to ionizing radiation does produce some long-lasting change within the organism, it may be possible that successive exposures will cumulate to the extent that normally ineffective irradiations will have an effect on the behavior of the organism. Conversely, the observation that LiCl does not produce a CTA when administered before the CS would suggest that LiCl does not produce a similar long-lasting change within the organism and therefore, that its effects would not cumulate over successive treatments.

In addition, because experimental manipulations that affect the acquisition of a radiation-induced CTA have similar effects on the acquisition of a LiCl-induced CTA, Rabin and Hunt [9] have proposed that similar mechanisms underlie the CTA learning produced by treatment with these apparently disparate stimuli. In general, the more similar different stimuli are, the greater the probability that the organism will

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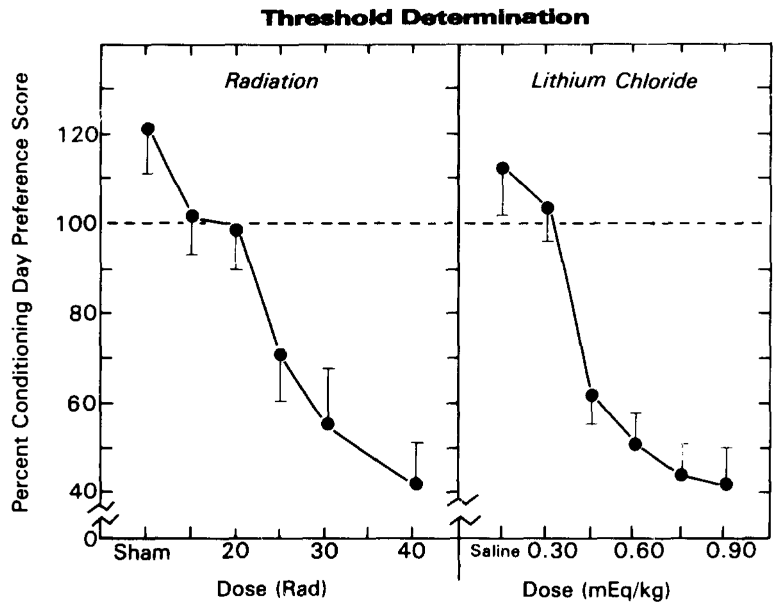

FIG. 1. Threshold determinations for producing a taste aversion following treatment with radiation or LiCl. The test day sucrose preferences are presented as the percentage of the conditioning day preference score. Error bars indicate the standard error of the mean.

make similar responses to them. Under these conditions, if similar mechanisms do mediate the acquisition of a CTA produced by irradiation and by LiCl treatment, then it should be possible to produce a CTA by combining subthreshold irradiation with injection of a subthreshold dose of LiCl.

The present experiments were designed to evaluate these hypotheses by examining the effects of repeated subthreshold exposures to ionizing radiation or LiCl, either separately or in combination, on the acquisition of a CTA.

GENERAL METHOD

Subjects

The subjects were male Sprague-Dawley-derived rats weighing 300–400 g at the start of the experiment. The rats were housed in individual cages in a room with a 12:12 light: dark cycle. Food and water were continually available except as required by the experimental protocol.

Taste Aversion Training

All taste aversions were produced using a two-bottle design. The rats were first placed on a 23.5 hr water deprivation schedule for 10 days during which water was available for 30 min a day during the early light phase of the diurnal cycle. On the conditioning day (day 10), the rats were presented with two calibrated drinking tubes for the 30 min drinking period, one tube containing tap water and the other containing a 10% sucrose solution, and the intake of each was recorded. In order to make certain that each rat sampled a sufficient quantity of the novel sucrose solution, any rat that did not show a greater sucrose than water intake was discarded from the experiment. Where possible, rats were assigned to the various experimental or control conditions following presentation of the CS to minimize the possibility that this procedure would selectively affect one treatment group.

TABLE 1

FLUID INTAKE (ml) AS A FUNCTION OF DOSE OF RADIATION OR LITHIUM CHLORIDE

	Conditioning Day		Test Day	
Dose	Water	Sucrose	Water	Sucrose
Radiation (Rad)				
Sham	6.25±1.37*	17.40±1.55	2.20±0.79	18.20±1.88
15	5.45±1.18	20.36±1.52	5.45±1.35	21.82±1.39
20	5.45±1.22	17.00±1.38	6.55±1.49	18.18±1.56
25	6.45±1.40	16.82±1.23	10.36±1.98	10.82±2.04
30	4.40±1.06	17.50±1.98	11.60±2.11	8.20±1.89
40	4.36±1.00	14.27±1.21	14.00±1.23	6.45±1.29
Lithium Chloride (mEq/kg)				
Sal	3.40±0.85	18.00±1.23	3.30±0.98	19.00±1.19
0.30	4.82±1.13	17.55±1.73	3.82±1.19	18.36±4.05
0.45	3.09±1.00	18.45±1.09	10.00±1.55	9.27±0.93
0.60	3.55±1.02	19.55±1.24	12.45±2.11	11.09±1.56
0.75	3.73±0.85	19.09±1.05	12.45±1.66	7.09±1.13
0.90	3.44±0.71	18.33±0.80	11.33±1.79	7.56±1.89

*Mean±standard error.

Over the course of the experiment, approximately 10% of the animals tested failed to show an initial preference for the sucrose CS. Immediately following the drinking period, the subjects were given the appropriate UCS. On the test day (day 11) the rats were again presented with the two calibrated drinking tubes and their intake of water and sucrose recorded.

Data Analysis

Water and sucrose intakes were transformed into preference scores: sucrose intake divided by total fluid intake. Test day preference is presented as the percentage of the conditioning day preference score. Statistical analyses were initially performed using two-way analyses of variance. Comparisons between specific groups were made using orthogonal comparisons, and the Scheffe correction applied to take into account the fact that the comparisons were made on a *post hoc* basis [6].

EXPERIMENT 1

The first experiment was designed to establish the thresholds for radiation- and LiCl-induced CTA learning under the specific conditions for the current experiments.

Method

For the determination of the radiation-induced CTA threshold, 64 rats were divided into 6 groups of 10–11 rats per group. One group of rats served as a sham irradiated control group and was placed in a clear plastic restraining box and then carried to the source, but not exposed. The remaining 5 groups of rats were exposed to one of a successively lower dose of radiation until a dose was reached that did not produce a test day decrease in sucrose preference. The tested doses, provided by a ^{60}Co source, were 40, 30, 25, 20, and 15 rad administered at a dose rate of 20 rad/min. Dosimetry was performed using thermoluminescent detectors (LiF TLD 100's) and a 3.3 ml Victoreen chamber.

For the determination of the threshold for an LiCl-induced CTA, 65 rats were divided into 6 groups of 10–11 rats per group. The sham-treated control group was given an IP injection of isotonic saline. The remaining 5 groups of rats were given an IP injection of one of successively lower doses of 0.3 M LiCl. The tested doses were 0.90, 0.75, 0.60, 0.45, and 0.30 mEq/kg.

Results and Discussion

The determination of the threshold doses for radiation- and LiCl-induced taste aversions is presented in Table 1 and Fig. 1. Increasing the dose of both stimuli produced non-linear increases in the intensity of the CTA. As shown in Table 1, the increasing intensity of the CTA is reflected as a test day increase in water intake combined with a decrease in sucrose intake, such that total fluid intake remained relatively constant over the range of LiCl and radiation treatments.

For ionizing radiation, exposure to a dose of approximately 25 rad produced a decrease in test day sucrose intake relative to conditioning day intake. Further increases in the dose of radiation to 40 rad produced corresponding increases in the strength of the aversion. This threshold level for the radiation-induced CTA is somewhat higher than that reported by Garcia *et al.* [4], although these variations may reflect differences in the quality of the radiation and in the conditions associated with the behavioral testing [13,14].

The threshold for a CTA induced by treatment with LiCl is between 0.30 and 0.45 mEq/kg. Further increases in the dose of LiCl produced a non-linear increase in the strength of the aversion. This figure is in general agreement with the threshold of 0.15 mEq/kg determined by other investigators (e.g., [3,4]). As with the radiation threshold, these differences probably reflect differences in the specific testing conditions.

Although treating rats with a 15-rad dose of radiation and a 0.30-mEq/kg dose of LiCl seems to limit the test day increase in sucrose preference observed with the control animals, the animals given these doses showed no test day decrease in sucrose preference relative to their conditioning day preference. Therefore, these doses were taken as the threshold doses for the following experiments using combined subthreshold treatments with radiation and LiCl, either alone or in combination.

EXPERIMENT 2

If, as suggested above, irradiation does produce a long-lasting change within the organism, then it should be possible to present two subthreshold doses of radiation, neither of which would by itself produce a CTA, such that the combined doses would result in the acquisition of a CTA following the single conditioning trial. For LiCl, on the contrary, combining subthreshold doses in a single conditioning trial should not have a similar additive effect leading to the acquisition of a CTA. In addition, if the mechanisms by which both LiCl and irradiation produce a CTA are similar [9], then combined subthreshold doses of LiCl and radiation should also lead to CTA learning.

The present experiment was designed to evaluate these hypotheses by examining the effects of successive treatments with radiation or LiCl alone or in combination using a single conditioning trial. In addition, a series of delay intervals between the presentation of the successive treatments was tested in order to determine the time course of these effects.

Method

The subjects were 312 rats divided into groups of 9–13 animals per group. For the subjects exposed to the successive radiation doses, independent groups of experimental and control animals were tested at delay intervals (time between successive exposures) of 0, 0.25, 0.50, 1.0, 1.5, 2, 3, 4 and 5 hr. The subjects given successive injections of LiCl or radiation exposure combined with a LiCl injection were tested at delay intervals of 0.25, 0.50, 1.5 and 2 hr.

The control subjects were treated identically to the experimental groups, except that they were not given the second subthreshold treatment. For the radiation controls this meant that the rats were kept in the exposure box for the delay interval or returned to it at the appropriate time and carried to the radiation source, but not exposed. For the LiCl groups or the combined radiation/LiCl controls, the rats were given a second injection of isotonic saline at the end of the appropriate delay interval. At the delay intervals of 1.0, 1.5 and 2.0 hr, the same animals were utilized as controls for both the dual radiation exposure and the combined radiation/LiCl groups by keeping the rats in the restraining box for the delay interval and giving them an injection of isotonic saline when they were removed from the box.

The general procedure was as follows. Immediately following ingestion of the sucrose solution on the conditioning day, the rats were treated with the appropriate UCS, either irradiation (15 rad at a dose rate of 20 rad/min) or LiCl (0.30 mEq/kg, IP). At the end of the delay interval, the subjects were given the second exposure to the UCS without further access to the sucrose solution. In all cases, the radiation UCS was administered before the LiCl UCS in the combined treatment groups.

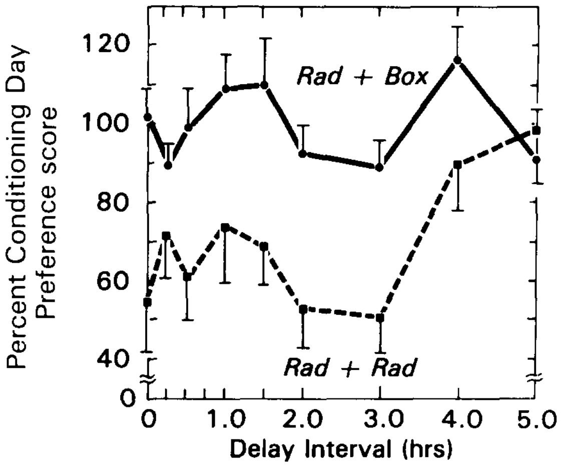

FIG. 2. Effect of treatment with two subthreshold (15 rad) exposures to radiation (dashed lines) or a single exposure and a sham treatment in which the rats were maintained in, or returned to, the exposure box after the appropriate delay interval (solid lines) on sucrose preference as a function of the delay interval between the two successive exposures. Test day preference is expressed as the percentage of the conditioning day sucrose preference. Error bars indicate the standard error of the mean.

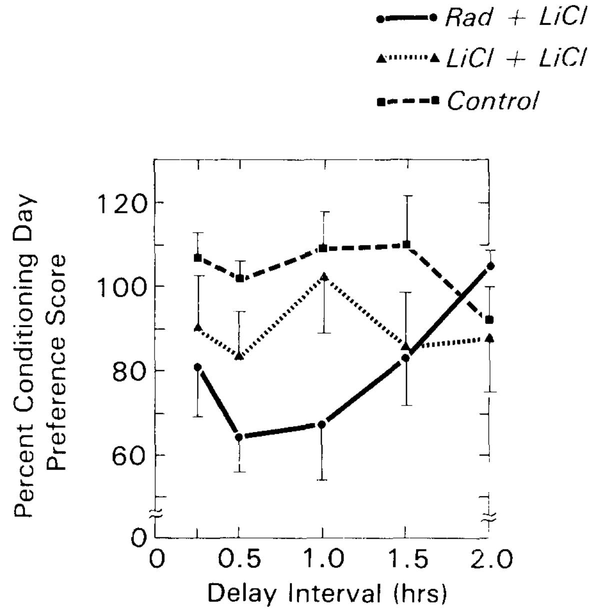

FIG. 3. Effect of treatment with subthreshold (0.3 mEq/kg) injections of LiCl (dashed lines) or with combined subthreshold radiation and LiCl (solid lines) on sucrose preference. Test day sucrose preference is expressed as the percentage of the conditioning day preference. Error bars indicate the standard error of the mean.

Results and Discussion

Conditioning day water intake across all groups showed a range of 3.00–7.50 ml while the range of sucrose intakes was 16.00 to 24.00 ml. As in the previous experiment (Table 1), the acquisition of a CTA was reflected in a change in the relative intakes of water and surcose while total fluid intake remained constant across the various treatment groups.

The effects of combining two subthreshold doses of radiation following a single presentation of the CS are summarized in Fig. 2. The analysis of variance showed that the main effect for condition for the comparison between the experimental and control subjects was highly significant, $F(1,174)=44.94$, $p<0.001$, as was the comparison for delay interval, $F(8,174)=2.61$, $p=0.01$. These results indicate that it is possible to combine two subthreshold doses of radiation, neither of which has an effect when administered separately, to produce a CTA. The effective time period for the combined effects is over the range of delay intervals from 0.25 to 3.0 hr. An inspection of Fig. 2 suggests that the two combined doses of 15 rad have an effect equivalent to that of a single dose of 30 rad (0 hr delay interval) over the entire range of effective delay intervals (0.25–3.0 hr). The observation that the interaction was not significant, $F(8,174)=1.51$, $p>0.10$, suggests that the general trends in both experimental and control subjects were similar, although the experimental animals, given the two radiation exposures, showed a consistently greater change in test day sucrose preference than the control animals, which had been given only the single exposure.

As shown in Fig. 3, combined subthreshold injections of LiCl were not effective in producing a CTA, whereas the combined treatment with radiation and LiCl was effective over delay intervals of 1 hr or less. The analysis of variance indicated that the main effect for the comparison between conditions was significant, $F(2,138)=6.76$, $p<0.01$. Neither the main effect for delay interval, $F(4,138)=0.54$, $p>0.10$, nor the condition by interval interaction, $F(8,138)=1.40$, $p>0.10$, was significant. Since the main effect for interval was not significant, the scores for each condition were combined across intervals and comparisons between the different treatment conditions were run using orthogonal comparisons with the Scheffe test [6]. These comparisons showed that the sucrose preference of the combined radiation/LiCl group was significantly different than that of the control group, $F(1,138)=13.18$, $p<0.05$, while the group given the two successive subthreshold injections of LiCl did not differ significantly from the controls, $F(1,138)=4.55$, $p>0.05$.

In general these results are concordant with the hypotheses proposed above. The observation that it is possible to combine two subthreshold doses of radiation to produce a CTA suggests that exposing the organism to ionizing radiation produces some change in the organism that remains active for the 3-hr delay interval over which the combined effects were obtained. In addition, the apparent similarity in the strength of the CTA between the single 30 rad dose and the two combined 15 rad doses suggests that the two subthreshold doses are combining additively over the interval to produce an effect on behavior. The differences in the time scale over which these effects were obtained in the present experiment and the longer intervals over which a radiation-induced CTA can be observed using the backwards conditioning paradigm [2,15], may be related to the dose of radiation used in the two experiments. In contrast to the marginal dose used in the present experiments (a total of 30 rad), the radiation doses used in the backwards conditioning experiments (100 rad and greater) are sufficient to produce a nearly maximal avoidance of the CS.

LiCl, in contrast, is a relatively short-acting agent as shown by its inability to produce a behavioral effect when used in a backwards conditioning design [1]. As such, it was proposed above that treatment with two subthreshold injections of LiCl would not combine to produce a CTA. The present results support this hypothesis. Although these results do suggest that the effects of combined LiCl injections may act to reduce CS intake compared to controls given only a single injection of LiCl, these differences were not significant.

Also, the present results, which show that combined treatment with a single subthreshold exposure to radiation and a single subthreshold injection of LiCl is sufficient to produce a CTA, are consistent with the hypothesis that similar mechanisms mediate taste aversions produced by radiation and LiCl. However, in contrast to the duration of the effects produced by two radiation exposures (approximately 3 hr), the time course of the combined radiation/LiCl interaction (approximately 1 hr) is much shorter. Although the present data do not allow a determination of the reasons for this difference in the effective interval of the combined effects, it may be possible that the longer action of the radiation-induced change permits a greater summation of effects when two doses of radiation are used in contrast to a lesser summation following the use of both radiation and the shorter-acting LiCl.

GENERAL DISCUSSION

Overall, the results are consistent with the hypotheses proposed above. Exposing the organism to ionizing radiation produces a relatively long-lasting change in functioning that serves to mediate both the acquisition of a CTA in a backwards conditioning paradigm [1, 2, 15] and when two subthreshold doses are given within a 3 hr interval. With LiCl, in contrast, two subthreshold doses cannot be combined to produce a CTA, which is concordant with the observation that LiCl does not produce a CTA when administered in a backwards conditioning design [1].

The observation that a subthreshold dose of LiCl can be combined with a subthreshold dose of radiation to produce a CTA is consistent with the hypothesis proposed by Rabin and Hunt [9] that similar mechanisms underlie the capacity of both radiation and LiCl to produce a taste aversion. At some level, either peripheral or central, both of these unconditioned stimuli must be producing similar effects on organismic functioning in order for the combined treatments to produce an effect. The finding that a subthreshold dose of radiation can be combined with a subthreshold dose of LiCl to produce a CTA may indicate that the organism fails to discriminate between these stimuli, but instead responds to them as if they were related stimuli. The present data, however, do not allow a determination of the mechanisms underlying the combined interaction of these apparently disparate stimuli. It may be that both radiation and LiCl chloride produce a common "malaise" [5] such that the combined treatment is sufficient to bring it above the threshold level for a behavioral response. Alternatively, it is possible that both radiation and LiCl similarly affect specific neural circuits [10], and that the combined treatment is capable of producing a sufficient change in neural activity to produce a corresponding change in behavior leading to the acquisition of a CTA. In the latter case, it seems likely that interaction involves the area postrema either directly or indirectly, because the integrity of the AP is necessary for CTA learning to occur following treatment with either radiation or LiCl [8, 11, 12].

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