Pharmacology Biochemistry & Behavior, Vol. 12, pp. 239–248. Printed in the U.S.A.

Suppression by Lithium of Voluntary Alcohol Intake in the Rat: Mechanism of Action

FREDERICK J. BOLAND

Department of Psychology, Queen's University, Kingston, Ontario, Canada K7L 3N6

AND

MURIEL H. STERN

Department of Psychology, McGill University, Montreal, Quebec, Canada

Received 2 February 1979

BOLAND, F. J. AND M. H. STERN. *Suppression by lithium of voluntary alcohol intake in the rat: Mechanism of action.* PHARMAC. BIOCHEM. BEHAV. **12**(2) 239–248, 1980.—Subjects were 70 Wistar rats showing either low preference for aversive alcohol solutions or a high preference induced by hypothalamic stimulation. Experiments 1 and 2 showed that a large lithium chloride injection (3 meq/kg) suppressed alcohol intake only if alcohol was tasted. Pairing lithium contiguously with water or intubed alcohol failed to reduce subsequent alcohol intake despite the concurrent presence of high serum lithium levels. In Experiments 3 and 4 a series of seven lithium injections increased rather than decreased alcohol intake if lithium was allowed to accumulate in the blood and brain during alcohol exposure while the transitory sickness associated with each injection was prevented from association with the taste of alcohol. When sickness was allowed to occur during alcohol exposure a suppression of intake resulted after two injections. Contrary to current interpretations these results suggest that the suppression of voluntary alcohol intake by acute and chronic lithium administration is due to a learned taste aversion rather than to a pharmacological mechanism specific to alcohol.

Lithium chloride Voluntary alcohol intake Conditioned taste aversion Biogenic amines
Chronic lithium treatment for alcoholics Lithium aversion treatment for alcoholics

IN recent years there has been increased use of lithium carbonate in the treatment of alcoholism. One double-blind study [23] involved chronic administration of lithium to alcoholics over periods of time ranging up to three years and reported a significant reduction in the number of rehospitalizations (for detoxification) in the lithium treated group. In another placebo controlled study [27] lithium was administered to alcoholics for 10 months and there was a reduction in days spent incapacitated by drinking in the lithium group. There have been several uncontrolled studies utilizing chronic lithium treatment with alcoholics. Two [10,53] reported no beneficial effects while the other [36] claimed that alcoholics on lithium tended to cease drinking before the onset of blackouts and binges and to experience a reduction in their normal "high" from alcohol.

Since lithium treatment has been shown to be effective with mood disorders [11,19] it was originally postulated that the mechanism by which it beneficially affected alcoholism was through relief of underlying depression. However, Kline *et al.* [23,24] found no difference in depressive mood between alcoholics on lithium and those on placebo. They postulated that lithium must have a beneficial effect on alcoholism through some direct, but as yet unknown pharmacological mechanism, probably of central origin.

The claim that lithium has a direct effect on alcoholism has generated a number of animal studies showing that acute [13] and chronic [14, 16, 17, 26, 46, 47, 49] lithium administration also attenuates voluntary alcohol intake in rats. In all of these studies, the authors suggest that lithium produced its suppression of voluntary alcohol intake by a central pharmacological mechanism specific to alcohol. There is evidence that acute and chronic alcohol intake produces changes in the metabolism of the catecholamines [18, 31, 38] and acetylcholine [30] and that interference with these substances through depletion or administration of neurotoxins can alter voluntary alcohol intake [31]. Since lithium is also known to interfere with aspects of acetylcholine [50] and amine metabolism [45] it appeared reasonable as a working hypothesis to attribute the suppression of voluntary alcohol intake by lithium to its effect on either acetylcholine [16] or the catecholamines [49]. The fact that lithium suppressed alcohol intake was in turn suggested as evidence that these substances were involved in the control of voluntary alcohol intake.

A second approach to the use of lithium with alcoholics, together with supporting animal studies, casts serious doubts on the hypothesis of a direct pharmacological mechanism. Lithium is considered the most effective drug for producing conditioned taste aversions (CTA) in animals [34]. In humans, a number of aversive side effects including nausea and vomiting commonly occur shortly after lithium is ingested [48]. In a recent study [5] these aversive side effects of acute

lithium administration were repeatedly paired with the taste of alcohol to produce aversion to alcohol in alcoholics. At six-month follow-up, the lithium-aversion group was superior in terms of abstinence to a group of alcoholics treated with citrated calcium carbimide.

That lithium administration can produce CTA's in animals to a wide variety of flavored solutions [41] including an inert saccharin solution from which one would not expect a pharmacological interaction with lithium [33], poses an interpretation problem for studies reporting a lithium-induced suppression of voluntary alcohol intake in rats. In all such studies [14, 16, 17, 26, 46, 47, 49] lithium was administered during alcohol exposure, a procedure allowing the taste of alcohol to become associated with the aversive side effects of the lithium administration and produce a CTA to alcohol. Because of the confounding, it is not possible to attribute the suppression of voluntary alcohol intake in rats by lithium to either a pharmacological mechanism specific to alcohol or to a non-specific CTA to alcohol. The present study was undertaken to assess the relative contribution each mechanism might make to the suppression of voluntary alcohol intake in the rat.

EXPERIMENT 1

The first study explores the effects of a single large injection of lithium chloride on voluntary alcohol intake. Two questions are of interest.

The first was whether lithium paired with alcohol in an intentional CTA paradigm would produce a suppression of voluntary alcohol intake in animals that were highly familiar (3 months) with alcohol solutions. Familiarity with the taste of a solution is known to reduce the strength of a CTA to that solution [28, 40, 42]. If the answer is not positive it could hardly be argued that the suppression of alcohol intake under similar conditions of familiarity [26,46] is due to the inadvertent production of a CTA.

The second question was whether lithium paired with water and given outside the conditioning interval with respect to alcohol, would produce any attenuation of subsequent voluntary alcohol intake. A positive answer would support the hypothesis of a direct pharmacological effect of lithium on alcohol intake.

The questions were investigated using animals showing high preference for alcohol over water and animals showing low preference for alcohol. The rationale for including high alcohol preferring rats was derived from finding that changes in neuroamines following alcohol intake are more likely to be associated with high alcohol preferring animals [1,15].

METHOD

Subjects

The subjects were 64 male albino rats of the Wistar strain weighing 275–300 g at the beginning of the experiment. The animals were housed individually in steel cages with wire mesh floors and fronts. Two 100 cc graduated Richter-type drinking tubes were located 1 inch above the floor at the front of each cage. Purina chow pellets were available, and with the exception of one 24 hr deprivation period, subjects had continuous access to tap water. A 12 hr light-dark cycle was maintained throughout. Alcohol solutions were mixed volume per volume from 95% ethanol and tap water. Lithium was administered in a 0.3 molar solution of lithium chloride in distilled water.

TABLE 1

THE BASIC CHARACTERISTICS OF THE SIX EXPERIMENTAL GROUPS

Group	Implanted	Stimulated	Reversed	Lithium pairing
SRHL (N=6)	Yes	Yes	Yes	Water
SREL (N=8)	Yes	Yes	Yes	Ethanol
SNREL (N=12)	Yes	Yes	No	Ethanol
IEL (N=7)	Yes	No	No	Ethanol
NIEL (N=13)	No	No	No	Ethanol
NIHL (N=11)	No	No	No	Water

S=implanted and stimulated; I=implanted but not stimulated; NI=not implanted; R=reversal of preference; NR=non-reversal of preference; E=alcohol; H=water; L=lithium chloride.

Procedure

The techniques used to establish low and high preference for alcohol have been described in detail elsewhere [2,3]. Thirty-eight of the animals were selected randomly for implantation of monopolar electrodes in the left lateral hypothalamus. After recovery, all rats were exposed to a daily choice between water and a solution of alcohol (3% v/v) which was increased in concentration each day until the animals completely rejected it. This initially rejected concentration (IRC), which in this experiment averaged 17% and ranged between 10–33%, was used for a particular animal throughout the experiment, insuring that each animal experienced an approximate equivalence in terms of aversive concentration of alcohol.

For 72 days following establishment of IRC all animals were placed on an alternate-day schedule of choice between alcohol and water on one day followed by only water on the next. The position of water and alcohol tubes was alternated to avoid position preference. For a group of 30 animals selected randomly a daily 30-min period of electrical stimulation was superimposed on the first 30 days of the alternate-day schedule. The stimulation was administered in smooth wooden boxes in the absence of alcohol or water at current levels below the aversive threshold (X=16 microamps). This resulted in the majority of stimulated animals developing a permanent reversal of preference for alcohol over water such that they consumed between 80–85% of their daily fluid intake from IRC alcohol solutions. The stimulated animals which did not reverse their preference for alcohol showed the same low preference (30–45% of daily fluid intake from IRC) as non-stimulated animals.

Throughout the experiment, attrition through death and loss of electrodes eliminated seven animals. On Day 72, the remaining animals were divided into six groups as shown in Table 1.

The non-implanted animals were assigned randomly to receive either lithium paired with water (NIHL) or lithium paired with alcohol (NIEL). Similarly, stimulated animals showing reversal of preference for alcohol over water were divided randomly into a lithium-water group (SRHL) or lithium-alcohol group (SREL). The fact that some stimulated animals did not show reversal of preference allowed a lithium-alcohol group (SNREL) which showed low preference for alcohol but was still equated with high preference

groups on the stimulation factor. The effect of the electrode implant on the lithium-alcohol pairing was investigated using an implanted but non-stimulated group (IEL).

On Day 73 all animals were deprived of fluid for a 24 hr period in order to increase their readiness to drink an upcoming stimulus solution within a prescribed time period. At the beginning of Day 74 (8 a.m.) and depending on group assignment, animals were offered either 3 ml of IRC alcohol solution or 3 ml of water. Pilot work showed this amount would be consumed within 10 min. After 10 min animals received an intraperitoneal (IP) injection of lithium (3 meq/kg body weight). Fifty min later, water tubes were placed on all cages, and for the remainder of Day 74 the animals were allowed to recouperate from the general malise and sluggishness which acute lithium sickness temporarily induces in animals. On Day 75 and for the next 18 days, the alternate-day free choice test between water and IRC alcohol solutions was reinstated.

RESULTS

Lithium suppressed alcohol intake when paired with alcohol in the CTA paradigm, but it had no effect on subsequent alcohol intake when paired with water.

Figure 1 shows the results at six-day intervals in terms of absolute alcohol per kg body weight (top) and percent of total daily fluid intake that was IRC alcohol solution (bottom). The data are shown starting at a point of stable baseline 60 days after the initiation of the alternate-day schedule and 30 days after the end of the stimulation period. The second measure was included because the absolute alcohol measure does not control for variation in alcohol intake due to increases or decreases in general fluid intake. Statistical analysis of both measures yielded identical results, and only analysis of the second measure is reported here.

A Groups × Trial Periods Analysis of Variance was carried out on the seven free-choice IRC alcohol versus water days shown in Fig. 1. The differences between Groups, $F(5,51)=35.38$, $p<0.0001$, and Trial Periods, $F(6,306)=108.06$, $p<0.0001$, were highly significant as was the Groups × Trial Periods interaction, $F(30,306)=21.25$, $p<0.0001$.

Analysis of simple effects [52] showed that the groups differed reliably in their mean percent alcohol of total fluid intake on each of the trial periods sampled: Day 60, $F(5,133)=16.26$, $p<0.001$; Day 66, $F(5,133)=15.44$, $p<0.001$; Day 72, $F(5,133)=18.39$, $p<0.001$; Day 75, $F(5,133)=47.80$, $p<0.001$; Day 81, $F(5,133)=45.55$, $p<0.001$; Day 87, $F(5,133)=31.56$, $p<0.001$; Day 93, $F(5,133)=32.84$, $p<0.001$.

Each of the four groups administered lithium paired with alcohol showed a significant difference in preference for alcohol across the seven trial periods: SREL, $F(6,306)=127.17$, $p<0.001$; SNREL, $F(6,306)=33.41$, $p<0.001$; IEL, $F(6,306)=28.95$, $p<0.001$; NIEL, $F(6,306)=29.32$, $p<0.001$. The two groups which had lithium paired with water showed a tendency to increase alcohol intake immediately following lithium administration, but the increase was not sufficient to produce significance across trial periods: SRHL, $F(6,306)=1.84$, $p>0.05$; NIHL, $F(6,306)=1.64$, $p>0.05$.

Further analysis utilized Tukey's honest significant difference (HSD) test [52] to make a posteriori comparisons between the mean percent alcohol of total fluid intake of individual groups within key trial periods (Days 72, 75, 93) and for individual groups across these trial periods.

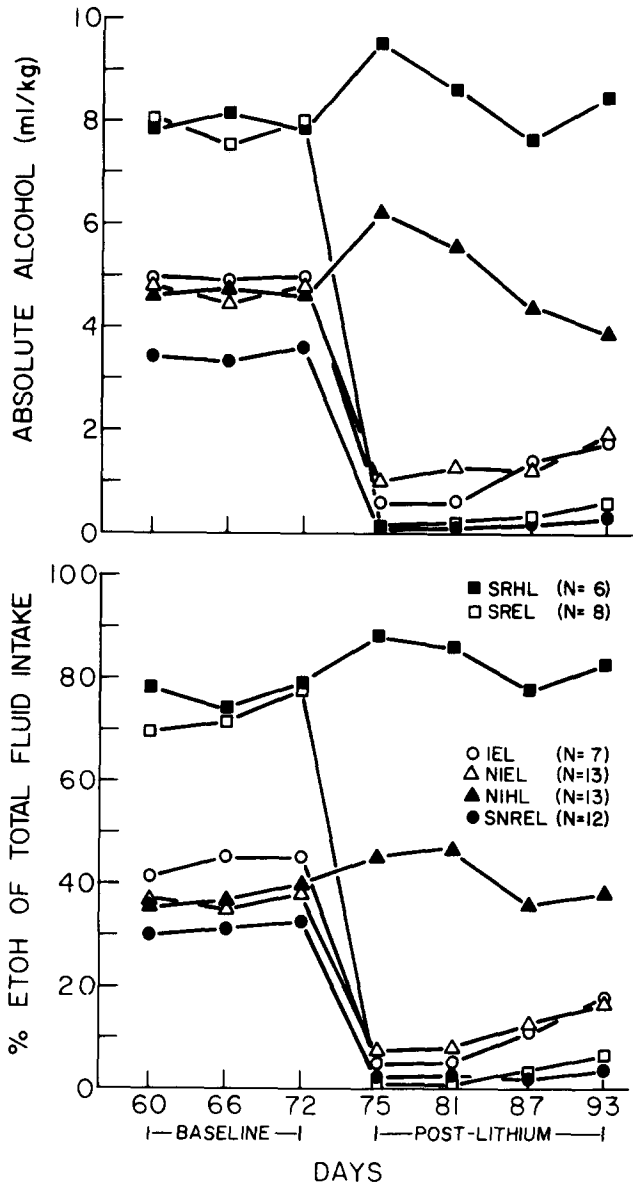

FIG. 1. Mean intake of absolute alcohol ml/kg (top) and percent ethanol solution of total fluid intake (bottom) at six-day intervals before and after lithium (3 meq/kg) administration for animals in Experiment 1.

During the last baseline day (72) there was no difference in percent alcohol of total fluid intake between the two high preference groups (SRHL, SREL), but these groups differed significantly ($p<0.01$) from the four low intake groups (SNREL, IEL, NIEL, NIHL) which did not differ among themselves.

During the immediate post-lithium period (Day 75) HSD test analysis showed that the two groups given lithium paired with water (SRHL and NIHL) maintained the differences ($p<0.01$) in preference for alcohol that were evident during baseline. The latter two groups showed significantly higher preference for alcohol ($p<0.01$) than the four groups given lithium paired with alcohol (SREL, SNREL, NIEL, IEL).

The latter four groups showed no differences, suggesting the presence of a floor effect.

The strength of the lithium-induced suppression is evident from the HSD test comparisons between individual groups on Day 93. The four groups having lithium paired with alcohol maintained the same attenuation of preference for alcohol ($p<0.01$) relative to the two groups having lithium paired with water, that was evident 18 days earlier.

The HSD test was also used to assess group changes in preference for alcohol between the last day of baseline (72), the first post-lithium day (75) and the last post-lithium day (93). Compared to baseline levels, each of the groups receiving the lithium-alcohol pairing (SREL, SNREL, IEL, NIEL) showed a significant ($p<0.05$) drop in preference for alcohol on the first post-lithium day and remained reliably different ($p<0.05$) from baseline 18 days later. Comparisons between Day 75 and Day 93 showed that none of the groups increased preference for alcohol significantly after the initial suppression. The two groups receiving lithium paired with water were not included in this analysis as they failed to show a significant trial effect during the prior analysis of simple effects.

It was of some interest to determine whether or not the tendency shown by SRHL and NIHL to increase alcohol intake during the immediate post-lithium period was due to the pairing of lithium with water. For example, it was possible that a CTA to water produced a compensatory increase in alcohol intake. However, a comparison of differences in water intake on the last water-only day (71) before lithium and the first water-only day (76) after lithium between SRHL (lithium-water) and SREL (lithium-alcohol) showed no difference in water intake, $t(1,12)=1.06$, $p>0.05$. Similarly, the comparison of NIHL with NIEL did not yield significance, $t(1,23)=1.15$, $p>0.05$.

Although not of direct concern to this investigation, histological examination using techniques described elsewhere [12] verified that electrode placement sites did not differ from those of other studies utilizing lateral hypothalamic stimulation to increase alcohol intake [24].

DISCUSSION

It is clear that acute lithium administration within the CTA paradigm produces a suppression of alcohol intake. No studies utilizing similar periods of pre-exposure to alcohol in an intentional CTA paradigm were available for comparison but the degree of suppression appeared very strong considering the known influence of familiarity in weakening the strength of a CTA [42]. However, it would be expected that the large lithium dosage [34] and close temporal contiguity [41] would work to strengthen the aversion, while the two-bottle choice test would be sensitive to its detection [9,13]. In addition, the use of aversive alcohol concentrations, which in this experiment were considerably higher than rats normally prefer [21,43], might be expected to increase the "salience" of the taste cues and to strengthen the aversion [22, 35, 51].

Lithium paired with water did not produce a reduction in subsequent alcohol intake. However, this does not exclude the possibility of a direct pharmacological effect on alcohol intake. For example, it may be that alcohol must be present in the system at the time of lithium administration before the pharmacological effect is produced. All experiments demonstrating a suppression of alcohol intake by lithium have allowed the presence of alcohol during lithium administration [14, 16, 17, 26, 46, 47, 49]. The problem has been whether to attribute the decrease to a direct pharmacological effect or to a CTA. Experiment 2 addresses this problem.

EXPERIMENT 2

The previous experiment did not eliminate the possibility that alcohol must be present in animals during lithium administration for a direct pharmacological effect on voluntary alcohol intake to be observed. To satisfy this requirement, animals in the present experiment were intubed with alcohol 10 min before lithium administration. Since a CTA is dependent on the availability of gustatory cues for association with sickness [41], the procedure should prevent the development of a CTA but not the possibility of a pharmacological effect.

METHOD

The method was identical to that of Experiment 1 except for the following changes. Sixteen male albino rats of the Wistar strain weighing 250–275 g at the start of the experiment were divided randomly into two groups. Six were chosen as a Non-stimulated group of low alcohol preferring animals, and 10 were implanted with electrodes and stimulated as in Experiment 1. Seven of the latter group reversed their preference for IRC alcohol over water, and these became the Stimulated group.

On Day 73 all animals were deprived of fluids for 24 hr. At the start of Day 74 (8 a.m.), all animals received gastric intubations of 3 ml of their IRC alcohol solution using an established intubation technique [4,6]. Ten min later all animals received an injection (IP) of lithium chloride at 3 meq/kg. Fifty min later, water tubes were returned, and the animals were allowed the remainder of Day 74 for recovery. On Day 75 the alternate-day free choice between water and IRC alcohol was reinstated for a period of 22 days. One animal from the Stimulated group was discarded because it inadvertently received the wrong lithium dosage.

Serum lithium. It was considered of value to have estimates of serum lithium during the period following the alcohol-lithium pairing. However, it was thought best to rule out any effect the stress of taking blood samples from the rat's tail might have on subsequent alcohol intake. Accordingly, a group of five Wistar rats of comparable weight and pre-exposure to the alternate-day alcohol schedule as animals in the experiment proper were used as a comparison group to acquire blood samples. These animals received 3 ml of IRC alcohol solution followed 10 min later by the 3 meq/kg lithium injection and 55 min later were overdosed with injections of Nembutal. Five min after the nembutal injection the chest-cavity of each rat was opened and a 2 ml blood sample drawn from the vena cava artery. The timing of the sample (one hr after lithium) was meant to give an estimate of the serum lithium peak. Samples were analyzed using atomic absorption spectrophotometry.

RESULTS

Figure 2 shows the results in terms of absolute alcohol (top) and percent alcohol of total fluid intake (bottom) for a period of seven IRC alcohol versus water choice days preceding lithium and for 11 such days following lithium administration. It is apparent that the procedure of pairing intubed

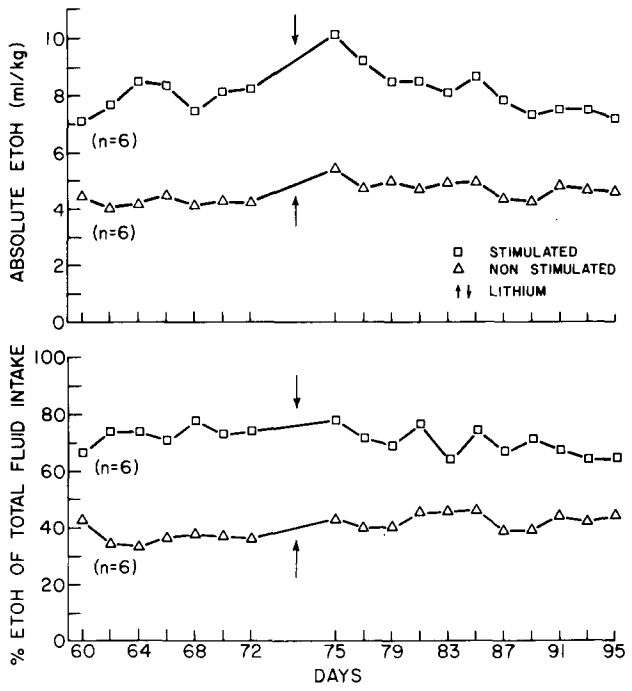

FIG. 2. Mean intake of absolute alcohol ml/kg (top) and percent ethanol solution of total fluid intake (bottom) on alcohol-water choice days before and after lithium (3 meq/kg) administration for animals in Experiment 2.

alcohol with lithium failed to induce a suppression of subsequent alcohol intake.

Analysis of Variance with repeated measures of the absolute alcohol measure for the 18 days shown in Fig. 2 showed the expected difference between Groups, $F(1,10)=19.40$, $p<0.01$, and a weaker but reliable Trials effect, $F(17,170)=2.09$, $p<0.05$. There was no Groups × Trials interaction, $F(17,170)=1.06$, $p>0.05$, indicating that both groups were affected similarly over trials. The Trials effect appeared to be due to the increase in alcohol intake occurring during the immediate post lithium period.

An identical analysis carried out on the percent alcohol of total fluid intake data again showed a Groups difference, $F(1,10)=25.62$, $p<0.001$, but the Trials effect did not reach significance, $F(17,170)=0.27$, $p>0.05$, suggesting that the increase observed in the analysis of absolute alcohol data was at least in part due to a general increase in total fluid intake. However, since there was still a weak but reliable Groups × Trials interaction, $F(17,170)=1.76$, $p<0.05$, the possibility existed that one of the groups increased preference for alcohol following lithium administration. This was assessed using the Dunnet Test [52] to compare the mean percent alcohol of total fluid intake on each of the first two alcohol-water choice days (75,77) following lithium with the last day (72) of baseline. In the Stimulated group neither Day 75, $t(3,10)=0.69$, $p>0.05$, nor Day 77, $t(3,10)=0.47$, $p>0.05$, differed from Day 72. Similarly, in the Non-stimulated group differences between Days 75 and 72, $t(3,10)=1.29$, $p>0.05$, and between Days 77 and 72, $t(3,10)=0.89$, $p>0.05$, failed to reach significance. This suggests that the shift in alcohol intake accounting for the Groups × Trials interaction was due to additional factors operating outside the immediate post-lithium period (possibly, as can be seen from Fig. 2, to the overall tendency of the Non-stimulated group to drift towards the Stimulated group following the lithium-alcohol pairing).

One hr after lithium administration the mean serum lithium levels of animals in the blood-sample comparison group was 2.61 milliequivalents per litre of blood (meq/l) with a standard deviation of 1.6 meq/l. The high variability of lithium levels was consistent with the high variability of lithium peak estimates reported in humans [39].

DISCUSSION

In Experiment 2 animals receiving a large lithium injection following intubations of alcohol showed the same lack of suppression of alcohol intake (and the same tendency to increase intake) as animals which had lithium paired with water in Experiment 1. It is clear that exposure to the gustatory cues of alcohol is essential to attenuation of alcohol intake by acute lithium administration.

A variety of lithium dosages ranging up from a minimum of 0.3 meq/kg have been sufficient to initiate a suppression of voluntary alcohol intake [13, 16, 46, 47], usually on the first day of administration, yet no suppression resulted in this study at lithium dosages ten times this minimum level. Furthermore, serum lithium estimates showed that animals would have had high serum lithium levels during the period of alcohol metabolism and one can assume that these conditions would favor the development of a pharmacological effect of lithium on alcohol intake. Taken together, the results of these two experiments suggest a CTA explanation of the suppression of voluntary alcohol intake by acute lithium administration.

The majority of studies reporting attenuation of alcohol intake by lithium utilize different parameters from those used here in that lithium is usually administered chronically at lower daily dosages. Also, in the present experiments, 3 ml of IRC alcohol were specifically and contigiously paired with a lithium injection in animals deprived of fluid. Other studies using chronic lithium administration used non-deprived animals, and no specific attempt was made to pair the ever-present alcohol with lithium administration. The possibility that a specific pharmacological effect of lithium on alcohol intake would become apparent under these conditions of chronic lithium administration was investigated in the next experiment.

EXPERIMENT 3

In the third experiment lithium was administered chronically to non-deprived rats in a manner that would favor the development of a pharmacological effect of lithium on voluntary alcohol intake while still preventing the occurrence of a CTA. The rationale for the procedure involves a consideration of the forward and backward conditioning curves for lithium in rats. CTA have been produced with flavor-lithium sickness intervals of up to at least four hr [32]. Also, since lithium sickness appears to be associated with the gradient of absorption into the serum [37], CTA are possible to flavored solutions introduced up to one hr after the start of lithium sickness but before the sickness reaches its peak [4,8]. In the present experiment animals were injected with lithium on seven consecutive water days six hr before the start of the seven respective free choice alcohol versus water days. This should have avoided any possibility

of forward or backward conditioning since there is an 18 hr period between each lithium injection and last exposure to alcohol and a six hr period between the injection and next alcohol exposure. Such temporal intervals are conservative, but it was essential to rule out the possibility of a CTA. However, since lithium has a 24 hr half-life, the procedure should still result in serum lithium levels during alcohol exposure which are well within the range of those reported to be associated with suppression of alcohol intake [16]. In addition, lithium levels in the brain are known to peak 8–18 hr after a lithium chloride injection [29], and since this period falls entirely within the alcohol exposure period, it can be argued that the development of a central pharmacological effect is still favored.

METHOD

The subjects were the Stimulated and Non-stimulated animals used in Experiment 2 with the addition of the one stimulated animal previously discarded because of an incorrect lithium dosage. The animals had continued the alternate-day schedule of alcohol exposure instituted in Experiment 2, and Day 117 (42 days since last lithium injection) marked the start of baseline measurements for Experiment 3. On Day 130 (water only) six hr before the start of Day 131 (free choice between alcohol and water), each animal was removed from its home cage long enough to be weighed and receive a lithium injection (IP) of 1.5 meq/l. On the next six consecutive water-only days (up to Day 142), the animals received identical lithium injections six hr prior to the start of the respective alcohol-water days up to Day 143. The alternate-day schedule was then continued to Day 159.

As in Experiment 2, a comparable group of 10 Wistar rats receiving a parallel procedure were used to obtain blood-lithium estimates six hr after the last lithium injection.

RESULTS

Chronic administration of lithium under the conditions of this experiment failed to suppress alcohol intake. In fact, animals tended to increase alcohol intake following lithium.

Figure 3 shows the results in terms of absolute alcohol intake (top) and percent IRC alcohol solution of total fluid intake (bottom) for seven alcohol days preceding lithium, seven alcohol days during lithium administration, and eight alcohol days after lithium had been discontinued. As total fluid intake was expected to increase with chronic lithium administration, the latter measure was chosen as the more conservative for statistical analysis.

Analysis of Variance with repeated measures over the 22 alcohol-water choice days indicated a reliable difference between Groups, $F(1,11)=7.42$, $p<0.05$, and a significant effect across Trials, $F(21,231)=3.79$, $p<0.01$. Both groups were affected similarly by the lithium as reflected in the lack of a Groups × Trials interaction, $F(21,231)=0.96$, $p>0.05$.

The effect across trials was apparently due to the increase in preference for alcohol occurring after the start of lithium administration. Since there was no interaction the mean percent alcohol of total fluid intake for the combined groups on the last baseline day (129) was compared with the mean preference on each of the alcohol-water days during the lithium period using the Dunnet Test. The groups did not differ from baseline levels on the first lithium day (131), $t(8,231)=0.78$, $p>0.05$, but did differ reliably on the second (133), $t(8,231)=2.35$, $p<0.05$, and the third lithium day (135), $t(8,231)=2.53$, $p<0.05$. On the last four lithium days the increase in mean preference for alcohol failed to reach significant levels over baseline; Day 137, $t(8,231)=1.94$, $p>0.05$; Day 139, $t(8,231)=1.44$, $p>0.05$; Day 141, $t(8,231)=2.10$, $p>0.05$; Day 143, $t(8,231)=0.93$, $p>0.05$.

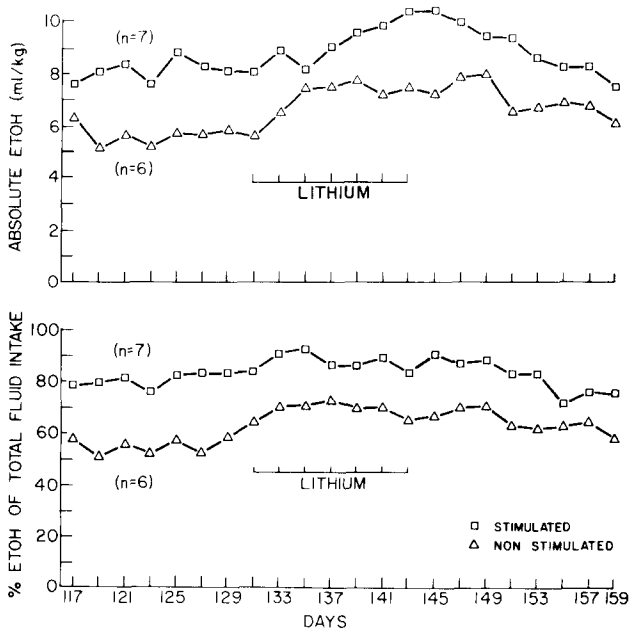

FIG. 3. Mean intake of absolute alcohol ml/kg (top) and percent ethanol solution of total fluid intake (bottom) on alcohol-water choice days before, during and after a series of seven injections (IP) of lithium (1.5 meq/kg) for animals of Experiment 3.

Repeated administration of lithium salts was expected to increase total fluid intake. However, while the two groups differed on intake of and preference for alcohol there should have been no differences in water intake on days when they received only water. Figure 4 shows water intake on water-only days throughout the experiment. Analysis of Variance with repeated measures over the last baseline water day and the seven water days occurring during lithium administration showed no differences between Groups F, $(1,11)=0.002$, $p>0.05$, and no Groups × Trials interaction, $F(7,77)=1.04$, $p>0.05$. As expected, there was a significant increase in water intake in both groups over Trials, $F(7,77)=2.80$, $p<0.05$.

Six hours after the last of seven lithium injections, the blood-sample comparison group yielded mean serum lithium levels of 0.81 meq/l with a standard deviation of 0.082 meq/l.

DISCUSSION

Chronic administration of lithium failed to produce a decrement in voluntary alcohol intake when the possibility of a CTA was eliminated. Chronic lithium levels greater than those found here have been associated with suppression of alcohol intake [47]. However, it is of interest that rats in the present study did not suppress alcohol intake despite serum lithium levels approximately four times those reported elsewhere [16,47] to be associated with suppression. Furthermore, most studies reporting a suppression of alcohol intake after chronic lithium report a suppression on the first

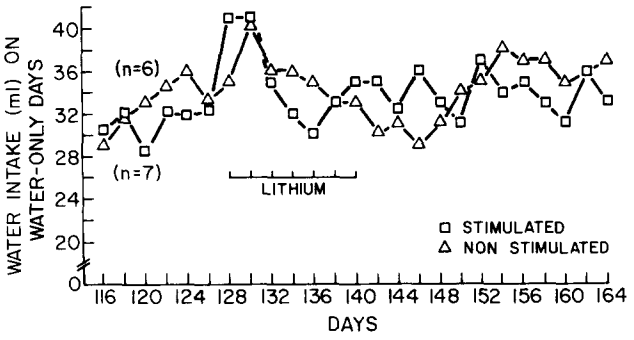

FIG. 4. Mean water intake (ml) on water-only days before, during and after lithium administration for animals of Experiment 3.

day of lithium administration [16, 46, 47]. In the present experiment, no suppression resulted even after seven consecutive administrations of lithium. A central pharmacological explanation of the suppression of alcohol intake by lithium would appear to be especially weakened since, after each lithium injection, brain lithium levels would peak during the alcohol exposure period [29].

Recall that the analysis of water intake in Experiment 1 did not support the hypothesis that a compensatory increase in alcohol intake following lithium occurred as a result of a CTA to water. A similar analysis could not be carried out here, but it is of interest to note that the increase in alcohol intake during the lithium period occurred mainly after the water intake had reverted to baseline levels.

EXPERIMENT 4

The results of Experiment 3 suggest, by default, that a CTA might be responsible for the suppression of alcohol intake noted in studies using chronic lithium administration. It follows, however, that an arrangement which would allow the essential pairing of alcohol cues with lithium sickness should produce a suppression of alcohol intake. To demonstrate this, conditions were arranged in the fourth experiment such that lithium injections were administered to animals 6 hr before the free-choice alcohol versus water day ended rather than 6 hr before it began as in Experiment 3.

METHOD

The subjects were those used in Experiment 3. All animals had continued their alternate day schedule of alcohol exposure, and Day 167 (24 days after their last lithium injection) marked the start of baseline measurement for Experiment 4. On Day 181 (free-choice alcohol versus water), 6 hr before the start of Day 182 (water-only), each animal was removed from its home cage long enough to be weighed and receive a lithium injection (IP) of 1.5 meq/kg. An identical procedure was repeated on the next three alcohol versus water days up to Day 187. The alternate-day schedule was maintained for a further 30 days, after which time the animals were killed for histological examination as in Experiment 1.

As before, a comparable group of 10 Wistar rats receiving a parallel procedure were used to obtain blood-lithium estimates 6 hr after the last lithium injection.

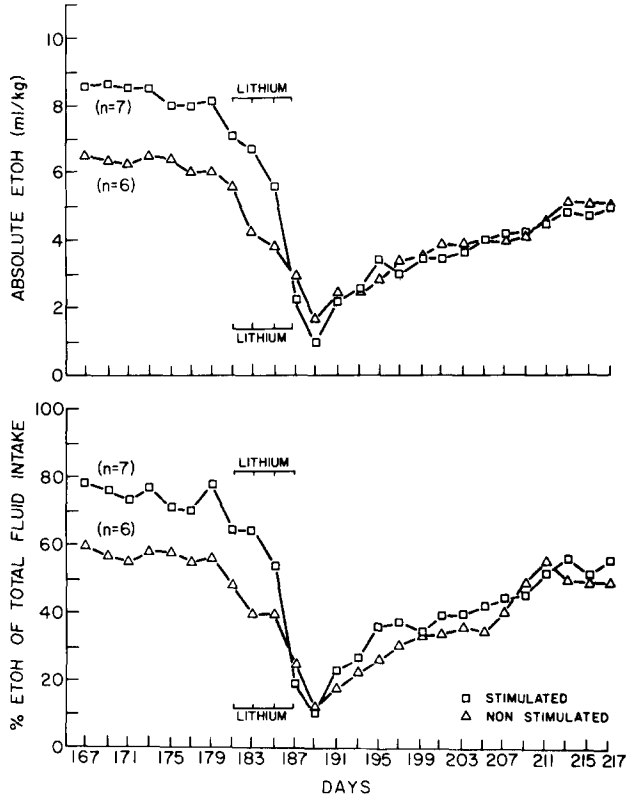

FIG. 5. Mean intake of absolute alcohol ml/kg (top) and percent ethanol solution of total fluid intake (bottom) on alcohol-water choice days before, during and after a series of four injections (IP) of lithium (1.5 meq/kg) for animals of Experiment 4.

RESULTS

Repeated administration of lithium within the conditioning interval for alcohol produced a suppression of alcohol intake in both groups which lasted beyond the period of lithium administration.

Figure 5 shows the results in terms of intake of absolute alcohol (top) and percent IRC alcohol of total fluid intake (bottom). As before, the latter measure was chosen for analysis.

Analysis of Variance with repeated measures over the full 26 days shown in Fig. 5 indicated no significant difference between Groups, $F(1,11)=1.56$, $p>0.05$, a reliable Trials effect, $F(25,175)=19.65$, $p<0.001$, and no Groups × Trials interaction, $F(25,275)=1.40$, $p>0.05$. A priori differences between Groups during baseline had been expected and were verified by a comparison between groups on the last baseline day (179), $t(1,275)=3.01$, $p<0.01$. Since both groups were affected similarly over trials their combined mean preference for alcohol was used to assess the decrease produced by lithium administration. Dunnet test comparisons with the last baseline day (179) showed no change on the first lithium day (181), $t(8,275)=1.86$, $p>0.05$, but a significant decrease in preference on the second (183), $t(8,275)=2.65$, $p<0.05$; third (185), $t(8,275)=4.05$, $p<0.01$; and fourth (187), $t(8,275)=8.93$, $p<0.01$ lithium day; the first post-lithium day (189), $t(8,275)=11.09$, $p<0.01$; the day (203) midway through the recovery period, $t(8,275)=5.80$, $p<0.01$; and the final recorded recovery day (217), $t(8,275)=2.81$, $p<0.05$.

Six hr after the last of four lithium injections, the blood sample comparison group yielded mean serum lithium levels of 0.79 meq/l with a standard deviation of 0.11 meq/l.

As in Experiment 1 histological examination of electrode sites showed no outstanding differences between these placements and those found in other studies using lateral hypothalamic stimulation [2,3].

DISCUSSION

The pattern of suppression of intake and preference for alcohol differed somewhat from that seen in Experiment 1. There animals showed a significant suppression after a single lithium injection. In the present experiment, the suppression did not reach statistical significance until the second lithium day. In the context of CTA theory the discrepancy might be expected. Some of the difference can be attributed to the higher lithium dosage, to the specific pairing of alcohol with lithium sickness, and to the conditions of close temporal contiguity in effect in Experiment 1 [34,41]. In addition, animals in Experiment 4 had not only over 100 days more familiarity with alcohol solutions than animals in Experiment 1 but also had received eight prior injections of lithium before the start of the experiment. Both factors have been shown to attenuate a CTA [42]. Finally, since animals had 18 hr of alcohol exposure prior to the first lithium injection, the effect of that injection on subsequent alcohol intake would be detected on the next lithium day.

Serum lithium levels drop by approximately one-half over a 24 hr period [5,29]. This would mean that serum lithium levels would be essentially negligible 30 days after the end of lithium administration, yet at this point in time, relative to baseline, animals in the present experiment were still showing a suppression of alcohol intake. It is not known how long a possible pharmacological effect of lithium would last, nor the amount of lithium necessary to produce it, but the long suppression of alcohol intake following cessation of lithium in this experiment would be consistent with animals forming a CTA to alcohol [41].

The serum lithium estimates in this experiment were similar to those found in Experiment 3 where rats showed no suppression of alcohol intake. The major difference appears to be that rats in Experiment 4 experienced the sickness associated with the post-injection absorption of lithium in to the serum during alcohol exposure allowing for the development of a CTA.

GENERAL DISCUSSION

Taken together, the results of the four experiments are consistent with the hypothesis that acute and chronic lithium administration reduces voluntary alcohol intake in rats through a CTA. Experiment 1 showed that a single lithium injection paired contiguously with a highly familiar alcohol solution suppressed alcohol intake. An identical lithium injection paired with water showed no signs of attenuating subsequent alcohol intake. Experiment 2 showed that the suppression of voluntary alcohol intake did not depend on the presence of alcohol in the system since a single lithium injection paired with intubed alcohol produced the same results as lithium paired with water. These two experiments indicate that the availability of gustatory cues is critically important for the suppression of voluntary alcohol intake by acute lithium administration.

Experiment 3 showed that seven consecutive lithium injections were not sufficient to suppress voluntary alcohol intake when the sickness reactions associated with these injections were arranged to occur outside of the conditioning interval for alcohol. When the sickness reactions were allowed to occur during alcohol exposure as in Experiment 4, a suppression of alcohol intake resulted after just two lithium injections. While these results with chronic lithium administration are predictable from the point of view of CTA learning they are not as conclusive in ruling out a pharmacological explanation as were the results of Experiments 1 and 2 using acute lithium. In the latter two, the temporal parameters were held constant and the taste variable manipulated, whereas in Experiment 3 and 4 the temporal parameters were manipulated. It is possible that a pharmacological interaction between lithium and alcohol might operate within the same temporal parameters as those of a CTA. Thus, arranging the temporal parameters to eliminate a CTA (as in Experiment 3) would also eliminate the possibility of a pharmacological interaction. However, two points can be made in favor of a CTA explanation applying to chronic as well as acute lithium-induced attenuation of voluntary alcohol intake.

First, although differences in parameters between the acute and chronic experiments of this investigation preclude using the results of one as direct support for the other, generalization appears to be at least suggestively enhanced by the observation that in many of the studies using chronic lithium, suppression of alcohol intake occurred after the first day of lithium administration [16, 46, 47].

Second, consideration of serum lithium estimates suggest that the lithium sickness rather than the actual serum lithium levels was the critical factor involved. In Experiment 3, no suppression of alcohol intake occurred despite the fact that serum lithium levels were estimated to be at least as high as in Experiment 4 (where suppression did occur) and more than three times the minimum level reported elsewhere to be associated with suppression of alcohol intake [16,47]. Also, brain lithium levels peak about 8–18 hr after lithium chloride injections in the rat [29]. In Experiment 3 (where no suppression of intake occurred) the lithium levels in the brain would have peaked well within the period of alcohol exposure. In Experiment 4, however, animals showed a clear suppression of alcohol intake even though brain lithium levels must have peaked well within the period when animals were exposed only to water.

The present investigation suggests that a CTA may have been responsible for the suppression of voluntary alcohol intake by lithium reported in other studies [14, 16, 17, 26, 46, 47, 49]. The fact that lithium both suppresses alcohol intake and interferes with central acetylcholine [16] and catecholamines [49] has been used as evidence for the role of these substances in the control of voluntary alcohol intake. While not discounting the latter possibility, the results of the present investigation question the further assumption that these neurochemical substances mediate the suppression of voluntary alcohol intake induced by lithium. A cautionary note is extended to other investigators using centrally active drugs to explore the neurochemical correlates of voluntary alcohol intake. Many of these drugs may have toxic properties capable of producing a CTA to alcohol [7,33] in addition to any possible pharmacological effect they may have on voluntary alcohol intake. The methodological strategy developed here may serve as one possible way to test these drugs without confounding by the ubiquitous CTA.

The present investigation does not imply that the bene-

ficial effect of chronic lithium treatment with alcoholics was due to the formation of inadvertent aversions to alcohol. The lithium dosage used in chronic treatment with alcoholics [23, 24, 27] was not high enough to produce satisfactory aversive reactions sufficient to create aversion to alcohol [5]. However, a tentative alternate hypothesis was suggested by the tendency of rats in Experiment 3 to increase alcohol intake during chronic lithium administration. It is possible that this is a valid pharmacological effect of lithium as the increase could not be explained readily in terms of a compensation due to a CTA to water.

One of the authors (FJB) is currently exploring the hypothesis that lithium increases tolerance for alcohol and/or to its aversive effects in rats. Human studies suggest that lithium administration antagonizes some of the intoxicating effects of alcohol [20,25], and reduces the severity of withdrawal symptoms [44]. Studies utilizing chronic lithium treatment with alcoholics have reported beneficial results despite the fact that alcoholics continued to drink [22, 23, 27, 36]. None of these studies monitored alcohol intake directly. Instead, the measure of treatment outcome was in terms of rate of rehospitalization for detoxification [23,24] or number of days spent incapacitated by alcohol [27]. If lithium increases tolerance for alcohol and/or reduces withdrawal symptoms, it seems reasonable to assume that this would positively effect these measures of treatment outcome.

In conclusion, the results of the present investigation suggest that the mechanism by which lithium attenuates voluntary alcohol intake in rats is a CTA. The hypothesis that lithium may increase tolerance for alcohol and/or to its aversive side effects has been suggested for further study.

ACKNOWLEDGEMENTS

The authors wish to thank Drs. A. Baker and D. Mewhort as well as Paul Berry, Bob Bialik, Sharon Flanagan and Ruth Shirley for assistance in the preparation of this manuscript. This investigation was carried out while the senior author was at McGill University.

REFERENCES

1. Ahtee, L. and K. Eriksson. 5-hydroxytryptamine and 5-hydroxyindolyacetic acid content in brain of rat strains selected for their alcohol intake. *Physiol. Behav.* **8:** 123–126, 1972.
2. Amit, Z., M. H. Stern and R. A. Wise. Alcohol preference in laboratory rat induced by hypothalamic stimulation. *Psychopharmacologia* **17:** 367–377, 1970.
3. Amit, Z. and M. E. Corcoran. Regulation of ethanol intake by rats with an induced preference for ethanol. *Neuropharmacology* **14:** 688–691, 1975.
4. Boland, F. J. Saccharin aversions induced by lithium chloride toxicosis in a backward conditioning paradigm. *Anim. Learn. Behav.* **1:** 3–4, 1973.
5. Boland, F. J., C. S. Mellor and S. H. Revusky. Chemical aversion treatment of alcoholism: Lithium as an aversive agent. *Behav. Res. Ther.* **16:** 401–409, 1978.
6. Braveman, N. and P. J. Capretta. The relative effectiveness of two experimental techniques for the modification of food preferences in rats. *Proc. 73rd a. Conv. Am. Psychol. Ass.* 129–130, 1965.
7. Cappell, H., A. E. LeBlanc and L. Endrenyi. Aversive conditioning by psychoactive drugs: Effects of morphine, alcohol and chlordiazipoxide. *Psychopharmacologia* **29:** 239–246, 1973.
8. Domjan, M. and B. Gregg. Long-delay backward taste aversion conditioning with lithium. *Physiol. Behav.* **18:** 59–62, 1977.
9. Dragoin, W., G. E. McCleary and P. A. McCleary. A comparison of two methods of measuring conditioned taste aversions. *Behav. Res. Meth. Instrum.* **3:** 309–310, 1971.
10. Fries, H. Experience with lithium carbonate treatment at a psychiatric department in the period 1964–1967. *Acta psychiat. scand.* **207:** 41–48, 1969.
11. Gattozzi, A. A. *Lithium in the Treatment of Mood Disorders.* National Clearinghouse for Mental Health Information Washington (Publication No. 5033), 1970.
12. Gilbert, J. R. and B. J. Nuttall. *Manual of techniques for neuropathology.* Montreal, Quebec: Montreal Neurological Institute, 1965.
13. Grote, F. W. and R. T. Brown. Conditioned taste aversions: Two-stimulus tests are more sensitive than one-stimulus tests. *Behav. Res. Meth. Instrum.* **3:** 311–312, 1971.
14. Ho, A. K. S. and B. Kissin. Evidence of a central cholinergic role in alcohol preference. In: *Alcohol Intoxication and Withdrawal II,* edited by M. M. Gross. New York: Plenum Press, 1974.
15. Ho, A. K. S., C. S. Tsai, R. C. A. Chen, H. Begleiter and B. Kissin. Experimental studies on alcoholism: Increase in alcohol preference by 5,6-dehydroxytryptamine and brain acetylcholine. *Psychopharmacologia* **40:** 101–105, 1974.
16. Ho, A. K. S. and C. S. Tsai. Lithium and ethanol preference. *J. Pharm. Pharmac.* **27:** 58–59, 1975.
17. Ho, A. K. S. and C. S. Tsai. Effects of lithium on alcohol preference and withdrawal. *Ann. N.Y. Acad. Sci.* **273:** 371–377, 1976.
18. Hunt, W. A. and E. Majchrowicz. Alterations in the turnover of brain norepinephrine and dopamine in alcohol-dependent rats. *J. Neurochem.* **23:** 549–552, 1974.
19. Johnson, F. N. *Lithium Research and Therapy.* New York: Academic Press, 1975.
20. Judd, L. L., R. B. Hubbard, L. Y. Huey, R. A. Attewell, D. S. Janowsky and K. I. Takahashi. Lithium carbonate and ethanol induced "highs" in normal subjects. *Arch. gen. Psychiat.* **34:** 463–467, 1977.
21. Kahn, M. and G. Stellar. Alcohol preference in normal and anosmic rats. *J. comp. physiol. Psychol.* **53:** 571–575, 1960.
22. Kalat, J. W. and P. Rozin. "Salience": A factor which can override temporal continuity in taste-aversion learning. *J. comp. physiol. Psychol.* **71:** 192–197, 1970.
23. Kline, N. S., J. C. Wren, T. B. Cooper, E. Varga and O. Canal. Evaluation of lithium therapy in chronic and periodic alcoholism. *Am. J. Med.* **268:** 15–22, 1974.
24. Kline, N. S. and T. B. Cooper. Evaluation of lithium therapy in alcoholism. *Finnish Foundation of Alcohol Studies,* edited by J. D. Sinclair and K. Kiianmaa. **24:** 1975.
25. Linnoila, M., I. Saario and M. Maki. Effect of treatment with diazepam or lithium and alcohol on psychomotor skills related to driving. *Eur. J. clin. Pharmac.* **7:** 337–342, 1974.
26. McCaughran, J. A. and M. E. Corcoran. Lithium reduces preference for ethanol induced by hypothalamic stimulation. *J. Pharm. Pharmac.* **29:** 120–121, 1977.
27. Merry, J., C. M. Reynolds, J. Bailey and A. Coppen. Prophylactic treatment of alcoholism by lithium carbonate: A controlled study. *Lancet* **7984:** 481–482, 1976.
28. Mikulka, P. J. and S. B. Klein. The effect of CS familiarization and extinction procedure on the resistance to extinction of a taste aversion. *Behav. Biol.* **19:** 518–522, 1977.
29. Morrison, J. M., H. D. Pritchard, M. C. Braude and W. D'Aguanno. Plasma and brain lithium levels after lithium carbonate and lithium chloride administration by different routes in the rat. *Proc. Soc. exp. Biol. Med.* **137:** 889–892, 1971.
30. Moss, J. M., R. D. Smyth, H. Beck and G. J. Martin. Ethanol impact on brain acetylcholine and its modification by cysteine. *Archs int. Pharmacodyn.* **168:** 235–238, 1967.
31. Myers, R. D. Psychopharmacology of alcohol. *A. Rev. Pharmac. Toxicol.* **18:** 125–144, 1978.

32. Nachman, M. Learned taste and temperature aversions due to lithium chloride sickness after temporal delays. *J. comp. physiol. Psychol.* **73:** 22–30, 1970.
33. Nachman, M., D. Lester and J. LeMagnen. Alcohol aversion in the rat: Behavioral assessment of noxious drug effect. *Science* **168:** 1244–1246, 1970.
34. Nachman, M. and J. H. Ashe. Learned taste aversions in rats as a function of dosage, concentration, and route of administration of lithium chloride. *Physiol. Behav.* **10:** 73–78, 1973.
35. Nowlis, G. H. Conditioned stimulus intensity and acquired alimentary aversions in the rat. *J. comp. physiol. Psychol.* **86:** 1173–1184, 1974.
36. Pendery, M. and L. Huey. Lithium and the alcoholic. *Highlights of the 19th Annual Conference, Veterans Administration Studies in Mental Health and Behavioral Sciences*, New Orleans, 1974.
37. Persson, G. Lithium side effects in relation to dose and to levels and gradients of lithium in plasma. *Acta psychol. scand.* **55:** 208–213, 1977.
38. Pohorecky, L. A. Effects of ethanol on central and peripheral noradrenergic neurons. *Pharmac. exp. Ther.* **189:** 380–391, 1974.
39. Prien, R. F., E. M. Chaffey and C. J. Klett. Lithium carbonate: A survey of the history and current status of lithium in treating mood disorders. *Dis. Nerv. Syst.* **32:** 521–531, 1971.
40. Revusky, S. H. and E. W. Bedarf. Association of illness with prior ingestion of novel foods. *Science* **155:** 219–220, 1967.
41. Revusky, S. H. and J. Garcia. Learned associations over long delays. In: *The Psychology of Learning and Motivation IV*, edited by G. H. Bower. New York: Academic Press, 1970.
42. Revusky, S. H. and H. Taukulis. Effects of alcohol and lithium habituation on the development of alcohol aversions through contingent lithium injection. *Behav. Res. Ther.* **13:** 163–166, 1975.
43. Richter, C. P. and K. H. Campbell. Alcohol taste thresholds and concentrations of solutions preferred by rats. *Science* **91:** 507–508, 1940.
44. Sellers, E. M., S. D. Cooper, D. H. Phil and C. Shanks. Lithium treatment during alcoholic withdrawal. *Clin. Pharmac. Ther.* **20:** 199–206, 1976.
45. Shaw, D. M. Lithium and amine metabolism. In: *Lithium Research and Therapy*, edited by F. M. Johnson. New York: Academic Press, 1975, pp. 411–424.
46. Sinclair, J. D. Lithium-induced suppression of alcohol drinking by rats. *Med. Biol.* **52:** 133–136, 1974.
47. Sinclair, J. D. The effects of lithium on voluntary alcohol consumption in rats. In: *The Finnish Foundation for Alcohol Studies*, edited by J. D. Sinclair and K. Kiianmaa. **24:** 1975.
48. Trautner, E. M., R. Morris, C. H. Noack and S. Gershon. The excretion and retention of ingested lithium and its effect on the ionic balance of man. *Med. J. Aust.* **42:** 280–291, 1955.
49. Truitt, E. B. and B. Vaughen. Effects of lithium on chronic ethanol consumption and behavior. *Fedn Proc.* **35:** 814, 1976.
50. Vizi, E. S. Lithium and acetylcholine metabolism. In: *Lithium Research and Therapy*, edited by F. N. Johnson. New York: Academic Press, 1975, pp. 391–410.
51. Wilcoxon, H. C., W. B. Dragoin and P. A. Kral. Illness reduced aversions in rat and quail: Relative salience of visual and gustatory cues. *Science* **171:** 826–828, 1971.
52. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1971.
53. Young, L. D. and M. H. Keeler. Sobering data on lithium in alcoholism. *Lancet* **1:** 144, 1977.