ABSTRACT

M.A. Psychology

Kenneth Frumkin

INTERACTION OF SPECIFIC HUNGRERS AND CONDITIONED AVERSION IN THE ADRENALECTOMIZED RAT

The effects of four combinations of the presence or absence of preoperative LiCl exposure on the reactions of groups of adrenalectomized and sham-adrenalectomized rats to a standard test exposure to LiCl were studied. Any taste experience with LiCl led sham-operated rats to consistently avoid NaCl. Adrenalectomized animals developed only a small and short-lived aversion immediately after the operation. Twenty-one adrenalectomized subjects died from the increased toxicity of LiCl for adrenalectomized rats, coupled with their increased tendency to drink it. No differences were found between groups of adrenalectomized rats which had tasted LiCl and groups which had had it loaded into their stomachs, indicating that these animals regulated their salt intake on the basis of need alone. The results support the large body of literature on the immodiability of the specific hunger for sodium. Conflicting data are discussed.
SPECIFIC HUNGERS AND CONDITIONED AVERSIONS
INTERACTION OF SPECIFIC HUNGER AND CONDITIONED
AVERSIONS IN THE ADRENALECTOMIZED RAT

by
Kenneth Frumkin

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Arts.

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July, 1970
ACKNOWLEDGEMENTS

I should like to acknowledge the help of Murray Gilman in collecting data in the early phases of this research.
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1.

INTRODUCTION

Adrenalectomy in the rat produces a rapid loss of sodium via the urine and a concomittant and dramatic increase in salt appetite (Bare, 1949; Richter, 1936). Richter (1947) concluded that when a physiological need arises for a particular dietary item an animal develops a "specific hunger" for the taste of that substance. A process of learned association between the taste of a needed food and its beneficial aftereffects has been found to be sufficient to account for such similar phenomena as thiamine-specific hunger, calcium hunger, magnesium aversion, and potassium-specific hunger (cf. Milner & Zucker, 1965; Rodgers, 1967). However, strong evidence exists for the unlearned nature of the specific preference of sodium deficient rats for the taste of sodium salts. The best evidence is that of Nachman (1962), who found that an increased preference for sodium chloride (NaCl) occurs in adrenalectomized rats within the first 15 sec. of exposure to salt.

Attempts to modify this innate preference for the taste of sodium have met with a singular lack of success. One exception was Harriman's (1955) experiment in which rats were allowed to establish a very strong preference for sucrose over salt solutions prior to adrenalectomy. His animals maintained their
preference postoperatively in spite of weight loss and even death for some subjects. However, the failure of several experimenters to find any effect of preoperatively established saccharin (Grimsley & Fisher, 1967) or sugar preferences (Grimsley & Cullen, 1968) on post-adrenalectomy salt-drinking or on bar-pressing for salt (Cullen & Scarborough, 1969), combined with Grimsley's (1970) recent failure to replicate Harriman's (1955) results, lead one to doubt the Harriman phenomenon.

One extremely powerful method of producing long-term changes in taste preferences consists of following a distinctive taste with gastro-intestinal upset produced by X-irradiation or injection of toxic substances. Taste aversions produced in this manner are extremely resistant to extinction, can often occur in one trial, and can be learned over very long CS-US intervals (cf. Garcia & Ervin, 1968, for a review). Cullen (1969) applied this technique in an attempt to create an aversion for NaCl in adrenalectomized rats. His subjects received X-irradiation on three daily sessions while drinking a NaCl solution, and were either adrenalectomized or sham-operated on the fourth day. This procedure was sufficient to produce, in the sham animals, a sodium chloride aversion which lasted for 20 days of post-operative testing. Adrenalectomized animals showed an initial
aversion; but it dissipated relatively quickly, so that "within seven days after adrenal extirpation these Ss were consuming enough salt to gain weight (p. 528)." Since his adrenalectomized animals suffered little weight loss and no deaths, demonstrating only a relatively short lived conditioned aversion, Cullen concluded that, up to that time, the Harriman (1955) data still stood as the "only nonadaptive behavior witnessed in the adrenalectomized rat (p. 529)."

Experimenters dealing with salt appetite have sometimes employed lithium chloride (LiCl), an extremely toxic salt, the taste of which, in solution, is indistinguishable from that of an equimolar concentration of NaCl. This similarity in taste has been attributed to the fact that equimolar concentrations of the two substances applied to the tongue of the rat produce similar intensities of neural discharge in the chorda tympani (Beidler, 1953; Beidler, Fishman, & Hardiman, 1955) and identical discharge patterns in single fibers of the same nerve (Fishman, 1957). Nachman, (1963a) found that normal rats could not discriminate between .12M concentrations of NaCl and LiCl in the first few minutes of repeated daily exposures. In that same experiment Nachman demonstrated that exposure to LiCl will produce a long-lasting learned aversion to that substance which generalizes completely to NaCl and less markedly to other salts.
Smith and Balagura (1969) also found that rats exposed to LiCl avoid both LiCl and NaCl in later single-bottle tests. Adrenalectomized rats will, in a 10 min. test, prefer NaCl and LiCl equally over water (Nachman, 1962); but they do not differentiate between the two (Nachman, 1963b). However, when presented with a 24-hour choice, adrenalectomized and normal rats can learn to avoid LiCl and other poisonous salts (Fregly, 1958; Nachman, 1963b). There is also evidence (Nachman, 1963a) that normal rats can make the difficult discrimination between LiCl and NaCl if given several 10-min. exposures. However, this learning is apparently due to the short time course of LiCl-induced illness which is associated with a particular container or location, rather than to the development of an ability to discriminate between the tastes of the two solutions, since in the first three minutes, even after several exposures, no differential responding occurs.

Recently a most striking instance of maladaptive behavior involving LiCl and adrenalectomized rats has been reported by Cullen (1970). He found that rats given LiCl for eight days before adrenalectomy drank even less salt postoperatively than sham controls, lost weight, and died. A pilot study in this laboratory in which five adrenalectomized rats being maintained on NaCl died after a brief (12 hr.) exposure to LiCl, gave rise to
the present research.

The study reported here was intended to investigate possible interactions of previous experience with the effects of LiCl poisoning on subsequent NaCl consumption in adrenalectized rats. Four separately conducted but closely related subexperiments were designed to investigate the effects of preoperative experience with LiCl and postoperative familiarity with NaCl on the outcome of a standard test exposure to LiCl. In two experiments (Experiments 1 and 2) rats had no preoperative salt-drinking experience; while in a second pair of experiments (3 and 4) a preoperative LiCl aversion was established. In addition, two of the studies (1 and 3) provided subjects with prolonged postoperative exposure to NaCl before testing with LiCl, while subjects in the remaining two experiments (2 and 4) had no postoperative NaCl prior to the test procedure. The variations in the four experiments are diagrammed in Table 1.

The effects of these four different combinations of pre- and postoperative experience were evaluated in each experiment by a standardized test procedure designed to produce a NaCl aversion. The three-day test phase consisted of two days on which a 12 hr. choice between NaCl and water was followed by a 12 hr. period in which LiCl alone was available, separated by 24 hr. of free access to NaCl and water. The effects of this
### TABLE 1

**VARIATIONS IN EXPERIMENTAL PROCEDURE AMONG THE FOUR EXPERIMENTS**

<table>
<thead>
<tr>
<th>Preoperative LiCl</th>
<th>Postoperative NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Yes Exper. 1</td>
</tr>
<tr>
<td>No</td>
<td>No Exper. 2</td>
</tr>
<tr>
<td>Yes</td>
<td>Yes Exper. 3</td>
</tr>
<tr>
<td>Yes</td>
<td>No Exper. 4</td>
</tr>
</tbody>
</table>
test procedure on NaCl preference was assessed in a 10 day two-choice extinction phase. This procedure was found in control experiments to produce long-lasting aversions to NaCl in normal rats.

Within each experiment there were three groups of subjects: adrenalectomized and sham-operated rats (the two experimental groups) and an Adrenalectomized Yoked Control (AC) group. Subjects in the AC group received the same treatment as experimental groups except that they did not drink LiCl during the two 12 hr. periods in the test phase. Instead, these subjects had LiCl tubed into their stomachs in quantities equal to those drunk by weight-matched adrenalectomized-experimental subjects. This group was included in order to assess the effects of LiCl poisoning alone on salt-seeking behavior, independent of any aversion to NaCl established on the basis of its taste. (Smith and Balagura (1969) have shown that rats receiving LiCl directly into their stomachs do not subsequently avoid either LiCl or NaCl.)

**GENERAL METHOD**

**Subjects and Apparatus**

Subjects were a total of 126 naive male hooded rats obtained at 75-85 days of age from Quebec Breeding Farms, Inc.,
St. Eustache, Quebec. Subjects were housed in individual plastic cages with metal tops and maintained ad. lib. on Purina Rat Chow for the duration of the experiment. (Purina Rat Chow contains approximately .42% sodium by weight.) Liquids were presented in half-pint glass bottles with straight metal drinking tubes projecting at an angle, 1\(\frac{1}{4}\)" into the cage from the top. When two bottles were presented their spouts were 3/4" apart. To control for position preferences the location of each bottle was alternated daily. Fluid consumption was calculated by subtracting the weight of the bottle at the end of a 24 hr. period from its weight at the beginning of that period. Spillage was calculated in control experiments to be a negligible .4 gm./day. Solutions used in all experiments were .15M NaCl, .15M LiCl, and distilled water.

Procedure

When each rat arrived in the laboratory it was given ad. lib. Purina Chow and distilled water and assigned randomly to one of the three treatment groups in each experiment: Group AX, adrenalectomized experimental; Group SX, sham experimental; or Group AC, adrenalectomized yoked-control.

Preoperative phase. Subjects in Experiments 3 and 4 received a six-day preoperative exposure to LiCl and water, while subjects in Experiments 1 and 2 were operated on immediately.
The preoperative procedure was identical for all groups in both experiments and was analogous to the test procedure for producing a LiCl-NaCl aversion. On the first day (P1) water intake was measured for 12 hr. (usually morning to evening). Then LiCl alone was presented for the next 12 hr. On the next day (P2) water alone was present, while on P3 the 12 hr. water, 12 hr. LiCl procedure was repeated. The effectiveness of this procedure was assessed on the next three days (P4-P6) during which LiCl and water were present at all times.

**Operation.** Groups AX and AC were bilaterally adrenalectomized under Nembutal anesthesia. Subjects in Group SX received sham operations.

**Postoperative phase.** Subjects in Experiments 1 and 3 received ad. lib. NaCl and water for 10 days postoperatively. In Experiments 2 and 4 only distilled water was available for five postoperative days. (Richter and Eckert, 1938, indicated that increased appetite for sodium appears in an average of 3.3 days after adrenalectomy, with a range of 1-9 days.)

**Test phase.** This three-day procedure was identical in all four experiments and began immediately after the postoperative phase. At the start of the first test day (T1) each subject was presented with a choice between NaCl and water for 12 hr. (usually 10 A.M. to 10 P.M.) At the end of this period the bottles were
removed and experimental subjects (Groups AX and SX) were exposed, for the next 12 hr., to a bottle containing .15M LiCl located in the same position the NaCl bottle had occupied. Approximately in the middle of the same 12 hr. period the subjects in the AC group were stomach-loaded, under light either anesthesia, with a quantity of LiCl equal (in cc.) to that amount (in g.) drunk by a subject in the AX group with which the AC subject had been matched by their previous day's body weight. (AC subjects in each experiment were run on a schedule one day behind that of their respective AX group.) One the second test day (T2) all groups were given 24 hr. access to NaCl and water. On test day three (T3) the T1 procedure was repeated, i.e. 12 hr. of NaCl and water followed by 12 hr. of LiCl for AX and SX subjects and stomach-loading with LiCl for AC groups.

**Extinction phase.** This was also the same in all experiments. Starting the day following T3 all groups were given ad. lib. access to NaCl and water for 10 days.

The complete experimental procedure is detailed in Table 2.

Body weights and fluid intake were recorded daily to the nearest gm. At the end of the Extinction Phase all surviving AX and AC subjects were placed on an ad. lib. diet of rice boiled in distilled water, with only distilled water to drink. Only those animals that died within 18 days after being placed on this
<table>
<thead>
<tr>
<th>Exper. No.</th>
<th>AX</th>
<th>SX</th>
<th>AC</th>
<th>Preoperative Phase (Days P1-P6)</th>
<th>Postoperative Phase</th>
<th>Test Phase (Days T1-T3)</th>
<th>Extinction Phase (10 Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>6</td>
<td>11</td>
<td>NONE</td>
<td>10 Days</td>
<td>T1</td>
<td>24 hr: NaCl + H₂O</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24 hr: NaCl + H₂O</td>
<td></td>
<td>12 hr: NaCl + H₂O</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>5 Days</td>
<td>5 Days</td>
<td>T2</td>
<td>24 hr: NaCl + H₂O</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24 hr: only H₂O</td>
<td></td>
<td>12 hr: NaCl + H₂O</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>11</td>
<td>9</td>
<td>10 Days</td>
<td>10 Days</td>
<td>T3</td>
<td>24 hr: NaCl + H₂O</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24 hr: NaCl + H₂O</td>
<td></td>
<td>12 hr: NaCl + H₂O</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>13</td>
<td>11</td>
<td>5 Days</td>
<td>5 Days</td>
<td></td>
<td>24 hr: only H₂O</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24 hr: only H₂O</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2**

**EXPERIMENTAL PROCEDURE**

- **Exper. No.**
- **Group (n)**
- **Preoperative Phase (Days P1-P6)**
- **Postoperative Phase**
- **Test Phase (Days T1-T3)**
- **Extinction Phase (10 Days)**
diet were considered to have been completely adrenalectomized and
and were included in the data analysis. Rats with complete adre­
nalectomy do not live longer than 18 days after removal of sup­
plemenmary NaCl (Gaunt, Tobin, & Gaunt, 1935). Adrenalectomized
experimental animals placed on this died died in a mean of 6.0
days, while no sham-operated rat ever died while on this diet.
Postmortem examinations performed on several supposedly adrena­
lectomized rats which did not die while on this diet revealed
supplementary adrenal tissue on one or both sides.

RESULTS

The results for each phase of the experimental procedure
are presented separately. All statistical comparisons were two­
tailed Mann-Whitney U-tests (Siegel, 1956) unless otherwise spec­
ified. In general water intake measures varied reciprocally with
NaCl consumption, and therefore are not reported.

Preoperative Phase

In Experiments 3 and 4 all groups were given a six-day
series of exposures to LiCl and water in order to produce a LiCl-
NaCl aversion. By the end of this period (Day P6) subjects in
these two experiments were drinking a mean of 2.3 gm. LiCl and
29.6 gm. of water per day (p<.002).
Postoperative Phase

Subjects in Experiments 1 and 3 were given a 10-day postoperative exposure to NaCl and water. The mean daily NaCl intake per group for each experiment is found in Fig. 1.

In both experiments Kruskal-Wallis one-way analyses of variance (Siegel, 1956) indicated significant differences in NaCl drinking (H=9.38 for Experiment 1, 23.14 for Experiment 3; df=2; p<.01 and .001 respectively). Salt intake for the SX subjects was always lower than for the other two groups (p's<.05). AX and AC groups were found not to differ in Experiment 1 (p>.10), but the AX group in Experiment 3 drank less NaCl than did the AC group (p<.05). Increases in NaCl consumption from Postoperative Day 1 to Day 10 were statistically reliable for the AX group in Experiment 1 (p<.05) and for both adrenalectomized groups in Experiment 3 (p<.002 in both cases).

Comparisons between Experiments 1 and 3 revealed that the effectiveness of Experiment 3's preoperative LiCl exposure in producing a NaCl aversion was greatly attenuated in adrenalectomized animals as compared with sham-operated subjects. SX subjects in Experiment 3 drank a mean of 1.3 gm. NaCl per day, significantly below the 11.3 gm. per day drunk by SX animals in Experiment 1 (p<.002). The effect of the preoperative phase on adrenalectomized rats can be seen by comparing the first day's
Figure 1. Mean number of grams of NaCl drunk per day during the Postoperative Phase by AX (triangles), AC (squares), and SX (circles) groups.
postoperative NaCl intake of combined AX and AC groups in the two experiments. In Experiment 1 the adrenalectomized groups drank a mean of 24.9 gm. NaCl, compared with 16.0 gm. for Experiment 3 (p<.03). Even though the adrenalectomized rats in Experiment 3 started out drinking less NaCl, they increased their drinking sharply over days so that when a comparison was made between adrenalectomized subjects in the two experiments on mean daily NaCl consumption over the entire 10-day period, the difference had disappeared (p>.11).

Two aspects of the data require further explanation. The first is a relative aversion to NaCl in the SX group of the first experiment. Groups 1AX and 1AC clearly preferred NaCl, ingesting a mean of 30.2 gm. daily, compared with 12.3 gm. per day of water. However, the sham-operated subjects in Experiment 1 drank significantly more water than NaCl (26.0 gm. of water versus 11.3 gm. NaCl daily; p=.002), even though the .15M NaCl concentration used is normally a highly preferred one. This water preference may have been due to association of postoperative malaise with the taste of the novel NaCl solution. The notion that rejection of NaCl by sham-operates was due to association with the operation is supported by the data from SX subjects in Experiment 2 which did not receive their first (12 hr.) exposure to NaCl until five days after the operation. These
animals significantly preferred NaCl to water (16.3 gm. versus 6.7 gm. in 12 hr.; p<.05).

Secondly, the difference in NaCl intake between the 3AX and 3AC subjects is puzzling, since these groups had received identical treatment. This variation may be accounted for by differences in the preoperative LiCl drinking experiences of the two groups. Although both 3AX and 3AC subjects drank the same total amount of LiCl when their two 12-hr. exposures to that substance alone (Days P1 and P3) were combined (a mean of 9.2 and 9.0 gm. respectively), the two groups did differ in their drinking pattern: AC animals drank significantly more LiCl than AX subjects on Day P1 (5.9 versus 4.0 gm.; p<.02), whereas the reverse was true on Day P3 (3.1 versus 5.2 gm.; p<.05). These small but significant fluctuations did effect the subsequent LiCl-water preference tests (Days P4-P6). For this period mean daily LiCl intake was 1.5 gm. for 3AX subjects and 3.6 gm. for those in 3AC (p<.002). In addition 3AX animals weighed on the average slightly less (283.2 gm.) than 3AC subjects (at 294.9 gm.) on the day after LiCl removal (Operation Day) (p<.05, one-tailed). No weight difference existed prior to LiCl exposure (Day P1). The drinking and weight data suggest that different LiCl drinking patterns in the two groups produced a stronger aversion to LiCl in the 3AX subjects, which, when combined with weight loss and
possible illness preoperatively, led to a temporary depression in NaCl drinking relative to 3AC subjects. The difference between the groups had disappeared by Postoperative Day 10 (p>.10).

**Test Phase**

The most striking result of the three-day Test Phase was the death of 21 subjects within a mean of 2.9 days after Test Day 1. Table 3 presents the number of deaths in each group of the four experiments, together with what percentage of their group the dead subjects represented. Deaths occurred primarily in Experiments 1, 2, and 3. Only one death occurred in Experiment 4. A greater number of deaths occurred in the AC groups (12) than in AX groups (9). No sham-operated rats died.

LiCl intake during the test phase also varied both within and between the four experiments. The mean number of grams of LiCl drunk by AX and SX groups of each experiment during the two 12-hr. single-bottle exposures to that substance (on Days T1 and T3) is found in Table 4. These data include the drinking of subjects which subsequently died.

It can be seen from the table that the amount of LiCl drunk varied between experiments (columns). Kruskall-Wallis one-way analyses of variance indicated that this variation was significant across the AX groups on Day T1 (H=23.93, df=3, p<.001) and for SX subjects on T1 and T3 (H=29.44 and 22.16 respectively,
TABLE 3

NUMBER AND PERCENTAGE OF DEATHS
PER GROUP FOR EACH EXPERIMENT

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>AX</th>
<th>SX</th>
<th>AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>(30.0%)</td>
<td></td>
<td>(36.4%)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>(16.7%)</td>
<td></td>
<td>(41.7%)</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>(33.3%)</td>
<td></td>
<td>(22.2%)</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(9.1%)</td>
</tr>
</tbody>
</table>
df=3, $p<.001$ in both cases). On T1, AX subjects in Experiment 1 drank more LiCl than similar groups in any of the other three experiments ($p<.002$ in all cases). While Experiment 2's AX animals were found to drink significantly less than their counterparts in Experiment 4. No other differences for AX subjects were significant. Among the SX groups, for both T1 and T3, subjects in Experiment 1 drank the most LiCl, while those from Experiment 3 drank the least. In paired comparisons each of these two groups differed significantly from all other groups for the same day ($p$'s$<.02$).

Within a given experiment LiCl intake also differed between adrenalectomized and sham-operated animals (rows of Table 4). On Day T1, AX subjects in Experiments 1, 3, and 4 drank significantly more LiCl than corresponding SX groups ($p$'s$<.05$). In Experiment 2 there was no such difference ($p>.10$). On T3, AX subjects in Experiment 3 continued to drink more LiCl than SX subjects ($p<.002$) while a reversal occurred for Experiment 1 with SX animals drinking more LiCl than AX subjects ($p=.05$). In every experiment except number 3, both AX and SX groups significantly reduced their intake of LiCl from Day T1 to T3 (all $p$'s$<.02$). In Experiment 3 T1 and T3 LiCl intake did not differ for either group ($p$'s$>.05$).
<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AX</td>
<td>8.51</td>
<td>3.65</td>
<td>4.78</td>
<td>4.96</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SX</td>
<td>6.42</td>
<td>4.69</td>
<td>1.53</td>
<td>3.96</td>
</tr>
<tr>
<td></td>
<td>AX</td>
<td>3.02</td>
<td>2.85</td>
<td>3.92</td>
<td>2.94</td>
</tr>
<tr>
<td>T3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SX</td>
<td>3.88</td>
<td>2.86</td>
<td>1.58</td>
<td>2.63</td>
</tr>
</tbody>
</table>
Extinction Phase

The results for the extinction phase include only those subjects which survived for the entire 10-day period. Fig. 2 contains the mean number of grams of NaCl drunk per day by each group in each of the four experiments. In all experiments NaCl intake of the SX group is very low (mean = 3.0 gm. per day). Adrenalectomized animals, on the other hand, drank an average 55.6 gm. NaCl daily. All comparisons between SX groups and AX and AC subjects within each experiment were highly significant (p's<.002), while no difference was found in any experiment between AX and AC groups (p's>.10). Not even the apparent AX-AC differences on Extinction Day 1 were significant (p's>.05). The elevation in fluid intake for adrenalectomized subjects on Day 1 was most likely due to the immediately preceding 12 hr. of exposure to LiCl alone, during which little liquid was consumed. SX animals showed a similar first day's increase, but in water intake (not shown). The apparent increases in NaCl consumption from Day 2 to Day 10 on Fig. 3 were significant for the AX subjects in Experiments 1, 3, and 4 (p's<.04) and for AC subjects in Experiments 2 and 4 (p's<.02). Salt-drinking by SX groups remained constant over this period (p's>.05).

Even though the pattern of NaCl drinking within experiments is essentially the same, the amount of NaCl drunk by the
Figure 2. Mean number of grams of NaCl drunk per day during the Extinction Phase by AX (triangles), AC (squares), and SX (circles) groups in each of the four experiments.
same group did vary among the different experiments. For example, the small differences in the NaCl drinking of sham-operated animals were highly reliable when compared across the four experiments (H=19.73, df=3, p<.001, Kruskal-Wallis). The mean daily NaCl consumption for these subjects and the results of comparisons between experiments are found in Table 5. It is evident that the sham-operated rats in Experiment 1 drank the most NaCl, significantly more than SX subjects in either Experiment 2 or Experiment 4 (p's<.02), while sham subjects in Experiment 4 drank the least, significantly less that comparable groups in either Experiment 1 or 3 (p's<.002).

NaCl drinking of AX groups also varied reliably between experiments (H=10.81, df=3, p<.02, Kruskal-Wallis), with AX animals in Experiment 2 drinking the least NaCl (43.6 gm. per day), significantly less that AX rats in Experiments 3 and 4 (p's<.05). No other paired comparisons were statistically significant (p's>.05).

Within the Extinction Phase no group of subjects showed significant loss of weight. Significant increases in weight (by two-tailed sign tests) from Extinction day 1 and 2 to Extinction Day 10 occurred for all SX subjects (p's<.04), for AX subjects in Experiments 1, 2, and 4 (p's<.04), and for AC animals in Experiment 4 only (p<.002).
<table>
<thead>
<tr>
<th>Experiment No. (mean)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(7.88)</td>
<td>(2.42)</td>
<td>(3.28)</td>
<td>(1.05)</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>&lt; .02</td>
<td>n.s.</td>
<td>&lt; .002</td>
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<tr>
<td>2</td>
<td>-</td>
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<td>n.s.</td>
<td>n.s.</td>
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<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt; .002</td>
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<tr>
<td>4</td>
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</table>
Although analyses of variance (Kruskal-Wallis) did not reveal any differences in body weight among the three groups for any of the four experiments on the day of operation (p's > .05), by the end of the Extinction Phase SX subjects in Experiments 2, 3, and 4 weighed significantly more than either AX or AC animals in the same experiment (p's < .02). In Experiment 1 AX subjects weighed more than the SX group (p = .022), while no other differences were significant.

DISCUSSION

Establishment of a preoperative LiCl aversion led to reliable and consistent postoperative avoidance of NaCl by sham-adrenalectomized rats. However, postoperative decreases in NaCl intake of adrenalectomized rats were small and short-lived. On the first Test Day adrenalectomized rats drank more LiCl than sham-operates in three of the four experiments, with the greatest amounts of LiCl being drunk by groups in Experiment 1. Twenty-one rats, all adrenalectomized, died as a result of this exposure to LiCl. AC subjects, with LiCl tubed into their stomachs, died in even greater numbers than did animals in AX groups. Following the Test Phase all sham-operated animals demonstrated a NaCl aversion, while adrenalectomized rats did not. AC and AX groups
did not differ in NaCl intake. No animals lost weight during the Extinction Phase, while two-thirds of the groups gained weight during this period.

The behavior of the sham-operated rats in all four experiments conforms to the results of previous studies: that experience with LiCl produces long-lasting aversions to NaCl in normal rats (Nachman, 1963a; Smith & Balagura, 1969).

In addition to the large differences in NaCl consumption found in each experiment between adrenalectomized and sham-adrenalectomized animals, significant variation also occurred between experiments, reflecting the different combinations of NaCl and LiCl experience. For example, the LiCl aversion established in Experiment 3 did lead to a transient NaCl aversion in AX and AC rats relative to the same groups in Experiment 1. However, these differences disappeared over the 10-day post-operative Phase, possibly with the development of an increasing sodium need.

During the Test Phase AX and SX groups of Experiment 1, which had had a combination of no previous LiCl experience with maximal exposure to the effects of NaCl, drank more LiCl than comparable groups in any of the other three experiments. Similarly, even though sham-operated subjects in all experiments avoided NaCl during the Extinction Phase, those with maximal NaCl exposure
and minimal LiCl experience (Experiment 1) drank the most NaCl, while those with maximal LiCl experience (Experiment 4) drank the least.

The deaths which occurred can be attributed solely to the greatly increased toxicity of LiCl for adrenalectomized rats (Smith, Balagura, & Lubran, 1970) combined with a greater tendency for AX subjects in this experiment to drink even more of this substance than SX groups. The deaths were not due to a fatal aversion for NaCl produced by experience with LiCl. This second alternative is eliminated by considering the results for the AC subjects. These animals died in numbers equal to or greater than AX subjects, even though they did not taste LiCl, and therefore could not have developed a salt aversion (Smith & Balagura, 1969). The slightly greater number of deaths in the AC groups may have been due to an increased toxic effect of the same amount of LiCl when it is stomach-loaded all at one time, rather than drunk over a 12 hr. period. This finding points up the necessity for including such a yoked-control group in any experiment in which the effects of ingestion of toxic substances on taste preferences is studied.

In addition, throughout the Extinction Phase in all four experiments, no drinking differences were found between AX and AC subjects, indicating that adrenalectomized animals were
regulating their NaCl intake solely on the basis of their sodium requirements, without regard to the Test Phase experience with the taste of NaCl.

Supporting the present results, Smith et al. (1970) have found that adrenalectomized rats will overcome a LiCl-induced aversion to NaCl within three hours of exposure to that substance in a single-bottle test. However, the results of a recent experiment by Cullen (1970) are in sharp contrast to these findings. He exposed rats to one of two concentrations of LiCl (.15 or .35M) paired with water or .15M NaCl for eight days. After a recovery period, half of his animals were adrenalectomized and half were sham-operated. All were then placed on a choice between .15M NaCl and .27M sucrose solutions. Postoperatively, sham-adrenalectomized animals drank more salt than the adrenalectomized rats, some of which lost weight and died. The drinking patterns of Cullen's sham animals (a gradual increase in NaCl consumption) and of his adrenalectomized rats (a decrease in NaCl) are in direct contrast with the results of the present experiment.

No certain explanation can be given for the differences between the two experiments. However it is possible to point out some differences in method and interpretation between the two studies.
Cullen's subjects were Dublin/DR albino rats around 195 gm. at the start of his experiment, compared with the 75-85 day old hooded rats (ranging from 225-300 gm.) used in the present research. Secondly, the Purina Rat Chow used in the studies reported here contained around 100 times the sodium and potassium found in Cullen's deficient diet. A third difference was that the postoperative drinking choice available in the Cullen study was between NaCl and sucrose, rather than NaCl versus water.

Differences in the effects of the preoperative LiCl exposures employed in the two studies may be important. Cullen's procedure was "more toxic", in that 12 of his 74 animals (16%) died during this exposure. None of the 62 rats died during the Preoperative Phase of this experiment. Despite the dramatic effect of this procedure preoperatively, sham rats in the Cullen experiment did not subsequently avoid NaCl. In fact they drank 14.6 ml. per 100 gm. of body weight per day (around 33 ml. per day by conservative calculation from Cullen's Fig. 1), while sham-operated subjects in the present experiment drank around 3 ml. per day.

It is also important to note that although Cullen's adrenalectomized subjects did drink less than sham animals, they were drinking around 21 ml. NaCl per day (by calculation from
his figures), enough to keep 27 out of 31 adrenalectomized rats (89%) alive for the 17-day postoperative period.

Cullen attributed the failure of his adrenalectomized rats to gain weight postoperatively to his LiCl poisoning procedure, when, in fact, weight loss after adrenalectomy is a common finding. Fregly (1958, Fig. 1C) found that adrenalectomized rats maintained on NaCl weighed consistently less than controls, while Richter (1936, Table 1) found that adrenalectomized rats show minimal weight gain or even weight loss up to 30 days postoperatively. Cullen and Scarborough (1969) reported that adrenalectomized rats bar-pressing for NaCl "lost about 20 per cent of their body weight within two weeks and then maintained this approximate level throughout the experiment (p. 417)."

AX and AC subjects in the present study weighed less at the end of the Extinction Phase than SX animals.

Although these comparisons suggest that the nature of the aversion to NaCl established in Cullen's adrenalectomized rats was not so extreme as to produce a large number of deaths (only 11%), or an unusual weight loss, they still do not reveal why Cullen's sham subjects did not avoid NaCl, nor why his adrenalectomized rats drank less than sham-operated animals. One possibility may be that strain and weight differences between the animals in the two experiments may have produced differences in...
the strength of the specific hunger for sodium after adrenalectomy, or in their reactions to toxic LiCl. The difference in the diet may have been a factor, since Grimsley (1970) has suggested that the relative concentrations of sodium and potassium in the diet may be important in shifts of established food preferences. It is also possible that the use of sucrose (rather than water) as an alternative to NaCl in the Cullen study may have had an effect, because Richter (1941) demonstrated a decrease in Dextrose consumption of adrenalectomized rats, indicating an inability to absorb carbohydrates.

In general, the results of the present study, combined with the recent observations of Smith et al. (1970) fall in line with the accepted immodiﬁability of the homeostatic sodium-specific hunger of the adrenalectomized rat, in that they show the failure of a LiCl aversion to generalize to NaCl in these animals.

Exclusive of Harriman's (1955) data recently rejected by Grimsley (1970), Cullen's (1970) results (with the qualifications discussed above) now stand as the only example of a long-lasting modification of the adrenalectomized rat's salt-seeking behavior.
REFERENCES


Cullen, J. W. Modification of NaCl appetite in the adrenalectomized rat consequent to extensive LiCl poisoning. Paper presented at the meeting of the Eastern Psychological Association, Atlantic City, April, 1970.


Fishman, I. Y. Single fiber gustatory impulses in rat and


Harriman, A. E. The effect of preoperative preference for sugar


Nachman, M. Learned aversion to the taste of lithium chloride and generalization to other salts. *Journal of Comparative and Physiological Psychology*, 1963a, 56, 343-349.


