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Effects of Nutritional Lithium Deficiency on Behavior in Rats

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ABSTRACT

To demonstrate whether nutritional lithium deficiency is associated with behavioral changes, male Sprague-Dawley rats were placed on a lithium-deficient diet (Li content <.01 ppm). A lithium-deprived group, receiving drinking water containing 31 μ*M* NaCl, were compared to a control group receiving drinking water containing 31 μ*M* LiCl. Growth and general appearance were the same in both groups. However, lithium-deficient animals demonstrated decreased aggression in social interactions with other rats and also in response to handling. The phase of wheel-running activity was delayed by 0.8 h and exhibited decreased amplitude ($p < .05$). Other behaviors, including acquisition and retention of a passive avoidance response, were unaffected by lithium deprivation.

Index Entries: Lithium; lithium deficiency; aggression; circadian rhythms; passive avoidance.

INTRODUCTION

It has recently been recognized that lithium functions as an essential trace element in several mammalian species, and it has also been suggested to have physiological roles in humans. In goats, for example, lithium deficiency was associated with a decreased life-span, reproductive abnormalities, such as decreased success of first insemination, a decreased conception rate, and a significantly increased abortion rate *(1)*. Lithium-deficient goats also gave birth to more female kids, and their

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birth weights were 9% lower than those of control goats. Lithium deficiency furthermore reduced the activity of enzymes of the citrate cycle, glycolysis, and nitrogen metabolism, and liver monoamine oxidase *(1)*. In rats, deleterious effects on reproduction and lactation of dams maintained on a low-lithium diet have been reported *(2,3)*. Based on this and similar evidence, lithium (0.1 mg/kg) has been added to the recommended formulation of the American Institute of Nutrition Rodent Diet, AIN-93 *(4)*.

The lithium content of human diets is variable. The element is normally present in trace amounts in both food and water, but the daily intake depends greatly on dietary sources and local water supplies. In one report, eggs contain 9.0 mg Li/kg dry matter, whereas beans contain <0.2 mg Li/kg *(1)*. Lithium in drinking water varies at least tenfold in the United States, and more in other countries *(5)*. Differences in bioavailability or individual metabolism may lead to further variation in lithium concentration between individuals within the same community.

Behavioral effects of lithium deficiency have been suggested by studies in humans and animals. Statistically significant inverse associations have been reported between lithium concentrations in drinking water and the rate of homicide, suicide, rape and other violent crimes, mental hospital admissions, and arrests for drug use *(6,7)*. In a recent study with former drug users, low supplementary doses of lithium (400 µg lithium/d) significantly improved scores in a mood scale test questionnaire compared to controls given placebo *(8)*. Experiments with mice showed that animals maintained on a diet deficient in lithium (6.6 ng Li/g) showed depressed lever-press active avoidance behavior compared to animals receiving a control diet *(9)*. These data support the idea that trace levels of lithium may influence brain and behavior.

In view of apparent effects of lithium deficiency on human and rodent behavior, we examined the effects of chronic lithium deprivation on specific behaviors in rats. Since the epidemiological data suggested that low lithium was associated with increased violence, we examined effects of chronic lithium deprivation on intraspecies aggression as well as response to handling. Passive avoidance responding was investigated to provide a test of learning and retention that would be relatively unaffected by alterations in alertness or motor stimulation. We also examined effects of lithium deprivation on biological rhythms of locomotor activity, since rhythms have been implicated in mood disorders, and pharmacological doses of lithium have dramatic effects on the pattern and timing of wheel-running behavior *(10)*.

MATERIALS AND METHODS

Male Sprague-Dawley rats initially weighing 200–225 g were purchased from Harlan, Inc. (San Diego, CA) and housed in shoe-box cages

in groups of three, with lights on from 6:00 AM to 6:00 PM. After 1 wk, 21 rats were started on a lithium-free diet (Rat Diet, AIN-76, Lithium Deficient #F2978, Bio-Serv, Frenchtown, NJ), which contained <1 nmol/g (<.01 ppm) lithium by assay. Nine of the rats were given a drinking solution consisting of deionized water with 1.3 mg LiCl added to each liter (31 μM Li) to approximate normal trace intake of lithium *(1,5)*. The remaining 12 rats were given deionized water containing 31 μmol NaCl/L. Rats continued to receive lithium-free diet and the same drinking solutions until the end of the experiment.

Passive Avoidance

Each rat was tested for acquisition and retention of a passive avoidance response. The apparatus consisted of an illuminated white Plexiglas chamber (10 × 4.5 × 7.25 in.) and a dark chamber (14 × 4.5 × 7.25 in.), separated by a black removable partition. In both chambers, the floor was made of stainless-steel rods about 1 cm apart. The rods on the black side only were connected to a shock generator and timer. For acquisition and retention trials, each rat was placed in the illuminated compartment facing the partition. When at least 15 s had passed and the rat was facing in the direction of the partition, the partition was removed and a timer was automatically started. As soon as the rat had completely entered the dark side (not necessarily including his tail), the partition was replaced and the timer stopped. An AC-scrambled shock (0.3 mA for 3 s) was delivered, and 15 s later, the rat was removed and replaced in his home cage. When the rat remained in the illuminated side for 300 s after the partition was removed, the rat was removed and no shock was administered. The rat was considered to have learned the avoidance response. Acquisition trials were run at weeks 4, 5, 6, and 7 of the experiment. Rats were tested for retention of the passive avoidance response using the same procedure, except that no shock was administered. Retention trials were carried out at weeks 12, 15, 18, 22, and 26 of the experiment.

Wheel-Running Activity and Resident–Intruder Test

During weeks 8–10, rats were moved from the vivarium to the laboratory and separated into three groups, each consisting of three animals receiving trace lithium in the water and four rats receiving trace sodium in water. Each week, one group of seven was tested in wheel-running cages. The other two groups were reserved for the resident–intruder aggression test.

Rats were placed in Lafayette Instruments (Lafayette, IN) Model 86041 wheel-running cages with a 14-in. diameter wheel and 10 × 6 × 5 in. rest cage. Wheel rotations were detected by a phototransistor and automatically recorded in 5-min bins on a computer system. Mesor (mean), amplitude, and acrophase of wheel-running activity were calcu-

lated using the cosinor procedure, which mathematically determines the best-fitting cosine curve with a period of 24 h. We also estimated the total power of this base cosine plus nine true harmonic frequencies, using regression techniques based on cosinor analysis *(11)*. Power is an estimate of the amplitude of the rhythm. Unlike cosinor amplitude, this measure takes into account the shape of the waveform.

Another group of seven rats ("residents") were housed individually in large (1 m^2) shoe-box cages, and the third group ("intruders") were housed individually in small (0.3 m^2) shoebox cages. After 1 wk, the entire resident cage was placed under a video camera. Five minutes later, one intruder rat from the same treatment group as the resident (i.e., both lithium-deficient or both lithium control) was introduced into the resident rat's cage. Behavior of the resident rat was videotaped for the next 300 s, and then both rats were moved to new cages. Behaviors were subsequently evaluated using a variation of the method of Sijbesma et al. *(12)* and a computer program designed for this purpose. For each second of the resident–intruder test, the rater was instructed to press a key corresponding to one of the following behavioral categories:

1. Offensive behavior (threatening or attacking);
2. Exploring (locomotion or rearing);
3. Social behavior (sniffing, grooming, or crawling on or next to partner);
4. Avoidance (moving away from partner);
5. Self-grooming; or
6. Defensive behaviors (resisting or lying on back during attack).

If animals were inactive, no keypress was required. Since it was often difficult to distinguish attack from defensive behaviors, these two categories were combined in the final analysis.

Response to Handling

During weeks 20–25, rats were housed individually in small shoebox cages. Once per week at 7:00 AM, each rat was observed and handled by an experienced animal technician who was blind to treatment. He was instructed to rate aggression on a scale of -3 to +3, where -3 indicated that a rat was unusually passive or fearful of the rater compared to naive group-housed rats, 0 signified a normal degree of curiosity without aggression, and +3 indicated that the rat appeared extremely aggressive toward the rater. Cages were randomly relocated each week so that the rater could not remember individuals by location.

Data are presented as mean ± SE and were analyzed for statistical significance by two-tailed unpaired *t*-test, unless otherwise specified.

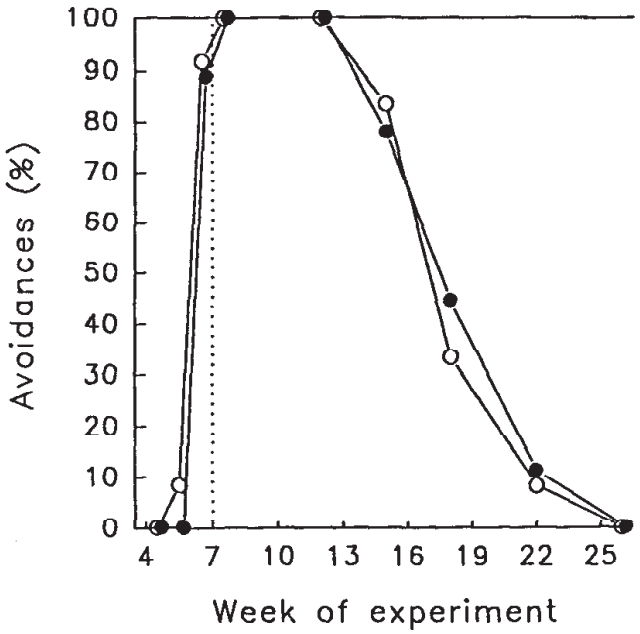

Fig. 1. Effects of lithium deprivation on passive avoidance. Rats fed lithium-deficient diet (○) or trace lithium (•) were tested for acquisition of passive avoidance at weekly intervals between weeks 4 and 7 of diet, and for retention of the learned response between weeks 12 and 26. The percentage of animals that did not enter the black (shocked) compartment within the 5-min observation interval is presented.

RESULTS

There was no difference in appearance between lithium-deficient rats and rats given trace lithium in the water. All rats gained weight consistently, and the mean body weight of lithium-deficient rats after 25 wk (480 ± 12 g) did not differ from controls (484 ± 8 g).

Passive Avoidance

All of the rats learned to avoid entering the dark chamber by the fourth trial and retained the avoidance response for at least 5 wk (Fig. 1). Over the following 14 wk, the percentage of rats entering the dark chamber gradually increased. Lithium deprivation affected neither the percent entering nor the latency to enter the dark side at any of the eight trials.

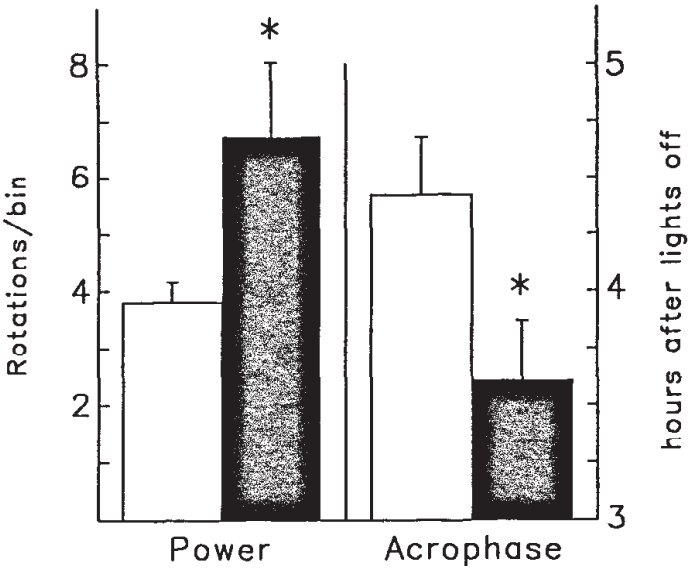

Fig. 2. Effects of lithium deprivation on wheel-running behavior. Acrophase (right side) was calculated from the best-fitting cosine curve, with frequency 1/24 h , using the cosinor technique. Power (left side) is the sum of the amplitudes of cosine curve with frequency 1/24 h plus the amplitudes of the first nine harmonics. Presented as mean ± SE; $*p < .05$ by unpaired two-tailed *t*-test.

Wheel Running

As shown in Fig. 2, the acrophase of the running rhythm was significantly delayed in the lithium-deficient rats compared to lithium controls (4.42 ± .26 vs 3.61 ± .20 h after lights off), and the harmonic amplitude (power) was decreased in the lithium-deficient group (2.3 ± 0.2 vs 4.1 ± 0.8 rotations/min). The mesor of wheel-running activity was also decreased, but not significantly, in lithium-deficient rats (1.0 ± 0.1 vs 1.3 ± 0.2 rotations/min in controls; $p = 0.16$).

Resident–Intruder Aggression Test

Figure 3 presents the behavior of the resident rat during the first 300 s after introduction of the intruder rat. Inactivity and nonthreatening social activity took up most of the first 200 s of the test interval. During the final 100 s, occasional attacks, rarely lasting more than a few seconds each, occurred with increasing frequency. As shown in Fig. 3, the lithium-deficient rat pairs exhibited diminished attack behavior compared to lithium-control animals.

Response to Handling

The subjective rating of the response to handling indicated that lithium-deficient rats were consistently less aggressive than control rats, reaching statistical significance during the last week (Fig. 4). Mean

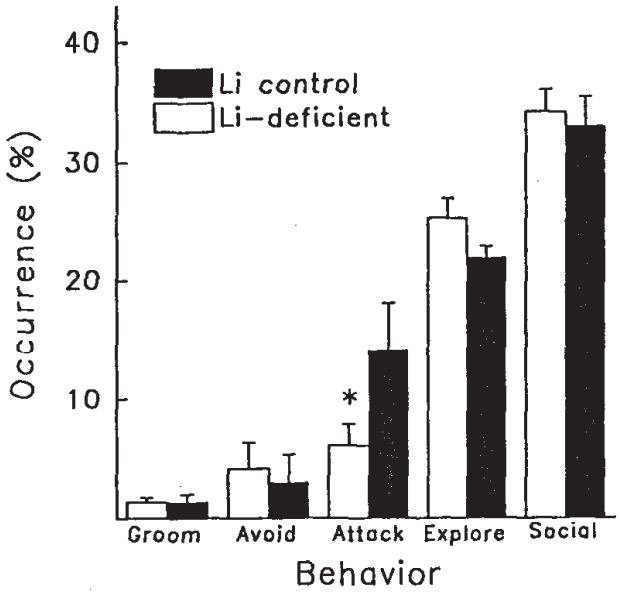

Fig. 3. Behavior of a resident rat during the first 5 min following exposure to an intruder rat. During each second of the exposure, behavior was classified as one of the five behaviors listed or as inactivity. The percentage of time that each behavior occurred (mean ± SE) is presented for each treatment group. *p <.05 by t-test.

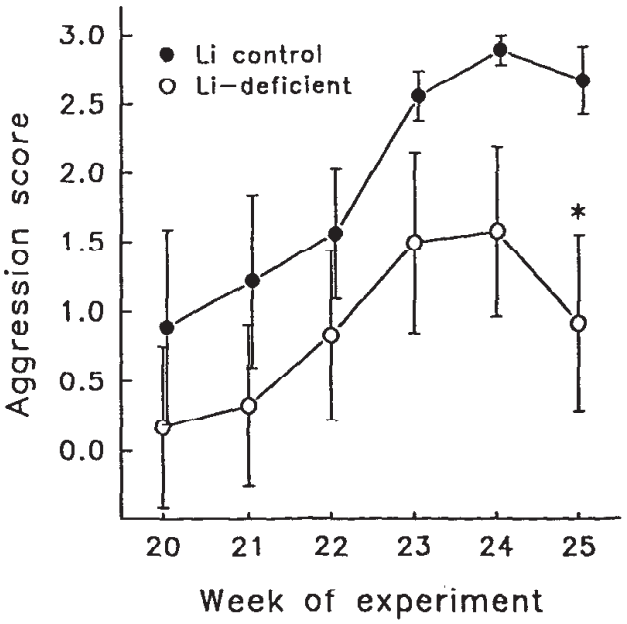

Fig. 4. Aggression exhibited toward the animal technician over the course of 5 wk of isolation. The night before the first trial, rats were housed singly. The technician handled each rat individually and assigned a rating of –3 (passive) to +3 (aggressive). Presented as mean ± SE; *p < .05 by Mann-Whitney rank-sum test.

aggression scores for both groups increased over the 6-wk test period while rats were housed separately, and this effect of time was statistically significant by repeated measures analysis of variance ($p < .05$).

DISCUSSION

The present results suggest that lithium, in the range normally ingested in food and water, may have specific central nervous system actions. Lithium deficiency appeared to reduce aggressive behaviors toward conspecifics, and deficient rats were more passive to manual handling. This increased passivity in lithium-deficient rats was not anticipated, since the epidemiological data in humans *(6,7)* could be interpreted to suggest that lithium-deficiency is associated with increased aggression.

Our results are also in apparent disagreement with previous experiments in mice, in which lithium deprivation resulted in decreased conditioned avoidance responding *(9)*. This contrast may be the result of species differences. Alternatively, the contrast may be explained by the fact that, in the mouse studies, animals were trained to press a key actively to avoid shock. This test should be more sensitive to alterations in motor activity or motivation than the passive avoidance procedure that we used. Under the conditions of the present study, increased passivity of lithium-deficient rats to intrusion and handling may be mirrored in a passive response to the avoidance test situation. In an active shock avoidance test, passivity would result in decreased escape responding and therefore more shocks, whereas in a passive avoidance test, passivity would not necessarily alter the rate of learning or of retention.

Pharmacological lithium treatment is well known to alter behavior in humans and animals, at daily intakes in the range of 24–40 mmol/d in human patients *(5,13)* and 0.4–0.8 mmol/d for rats *(10)*. At these doses, lithium prevents extremes of affect in patients with bipolar disorder *(13)*, decreases aggressive behavior in rats and humans *(13,14)*, and delays the phase of biological rhythms in rats and humans *(10)* compared to a standard diet. Lithium deprivation, like lithium treatment in rats, decreases aggressive behavior and delays the phase of the wheel-running acrophase. Some investigators have reported that lithium decreases amplitude of some biological rhythms (reviewed in ref. [*10*]), although the amplitude of the wheel-running rhythm in rats was increased by lithium treatment (Klemfuss and Kripke, in press).

CONCLUSIONS

The present results support the evidence that lithium may have central nervous system actions at trace levels. If rodent chow suppliers fol-

low the 1993 recommendations of the American Institute of Nutrition, rodent behavioral studies will have to take into account the possibility of changes in biological rhythms and aggressiveness owing to addition of trace lithium levels. Of greater significance, the wide variation in lithium in the water supply and in human diets may be related to the incidence of mental disorders and violence. There is a need for further study of the potential effects of trace lithium in treatment of individuals as well as in populations.

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