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Effects of Lithium Chloride Injections on Rank-related Fighting, Maternal Aggression and Locust-killing Responses in Naive and Experienced 'TO' Strain Mice

PAUL F. BRAIN AND SAMI AL-MALIKI

Zoology Department, University College of Swansea, Singleton Park, Swansea, SA2 8PP, Great Britain

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Anosmic opponents Lithium chloride Locusts Maternal aggression Mice Predation Rank-related aggression

IT HAS been argued that aggression is not a unitary concept [3, 4, 21, 31]. Some support for this view has been provided by the observation that simple experimental manipulations often have different influences on rank-related fighting (i.e. conflict between two intact non-habituated conspecifics) and locust-killing behavior (a form of predatory aggression?) in TO strain mice [5]. Effects of simple treatments have also been used to contrast maternal aggression (attack by a lactating female with litter on an intruding male) and locust-killing behavior in this strain of mouse [6]. These three models of aggression appear very differently motivated.

Lithium has been used clinically in the treatment of manic-depressive patients [14, 18, 19, 27, 28, 29, 30] because of its beneficial effects on the manic phase. As one might expect, human aggressiveness is said to be reduced by lithium administration [13, 25, 42, 43]. These clinical effects have generated much interest in the effects of lithium on a wide range of animal behaviors (ably reviewed by Smith [44]). A basic problem in analysing the behavioral effects of this ion is that it has two basic actions (see [23]) on different forms of attack. It:—(a) *Inhibits certain forms of aggression.* For example, oral administration of this compound reduces spontaneous fighting in a range of infrahuman species including Siamese fighting fish and small laboratory rodents [47]. Lithium injections also suppress foot shock-induced [15, 32, 40] and PCPA-induced fighting [41]. In contrast, lithium failed to significantly inhibit septal lesion-induced attack [33] or spontaneous muricidal behavior [15] in rats. (b) *Acts as a distasteful aversive toxic treatment* which may, by being made contingent with eating prey, discourage further consumption of prey. Clody and Vogel [11] have demonstrated that a number of toxic drugs can be used to condition an aversion to mouse killing in rats. Specifically, Rush and Mendels [39] reported that rats receiving lithium after killing mice which they did not consume, showed no detriment in this form of attack. Rats permitted to feed on the carcases continued, however, to kill but showed reduced eating of the prey after a single lithium injection [1]. Krames *et al.* [22] and O'Boyle *et al.* [34] confirmed that mouse killing in rats may be suppressed by using LiCl in conditioned aversion procedures. Similar phenomena have been demonstrated with respect to rabbit and lamb attack by coyotes [20] and cricket-killing by mice [23].

It consequently, appears important (especially in the case of predatory aggression) to differentiate between lithium's aversive properties and its (presumed) suppressive action on aggressive motivation. As the present studies were concerned with LiCl's effects on motivation rather than its utility as an aversive agent, attempts were made to minimise the effects of conditioning by giving small numbers of tests and

TABLE 1

EFFECTS OF THE LOWER DOSE OF LITHIUM CHLORIDE AND SALINE ON RANK-RELATED FIGHTING IN MALE MICE GIVEN STANDARD OPPONENT TESTS OVER 3 DAYS (MEDIANS WITH RANGES)

Daily Treatment	Proportion of animals fighting in at least 2/3 tests	Latency to attack (secs)	Accumulated attacking time (secs)	Number of attacks
Uninjected controls	11/12	288† (47–1564)	39.4† (16.7–71.6)	73† (30–130)
0.2 mEq saline	10/12	267 (50–1800)	38.5 (0–82.5)	72 (0–131)
0.2 mEq lithium	8/12	1204.5* (299–1800)	8.7* (0–50.6)	19* (0–84)

*Differs from saline control $p<0.05$ (Mann Whitney U Test)
†Differs from lithium injected category $p<0.02$ (Mann Whitney U Test)

administering lithium before behavior assessment.

It was hoped that lithium treatment might further differentiate between murine models of rank-related, maternal and predatory aggression. A series of studies investigating this cation's influences on attack responses by TO strain mice in a variety of situations are consequently reported.

METHOD

Animals

Tuck 'TO' strain albino mice (originally obtained from A. Tuck and Sons, Rayleigh, Essex) were used in this study. Husbandry was performed under highly controlled conditions in Animal Facilities of the University College of Swansea. Mice used here were the primiparous progeny of females, mated at 9 weeks of age, with identically-aged males. Litters were adjusted at birth to six and fathers remained with their offspring until 10–15 days after parturition. Pups then remained unhandled until 18–22 days of age when they were allocated to single-sexed groups of six or experimental categories. Lactating females were used six days after their second parturition. Such animals remained with their litters but the males (as above) were removed 10 days after the birth of the first litters (females remaining with males show little attack on intruders). White opaque, Makrolon cages (North Kent Plastics) measuring 30 × 12 × 11 cm with wire mesh tops were routinely used in this study. Food (Pilsbury's diet) and water were available ad lib. The animals were maintained on a reversed lighting schedule (white lights on 22.30–10.30 hr) but dim red lighting was used during the dark period for husbandry and behavioral testing. The ambient temperature was regulated between 18–22°C.

Lithium and Control Treatments

Daily IP injections of dilute aqueous lithium chloride (LiCl) solution were administered over specificied treatment periods. The treatment volume was always 0.1 ml and aqueous injections containing equivalent quantities of sodium chloride (NaCl) served as controls.

'Rank-related' Aggression Tests

These were modifications of procedures described in detail elsewhere [7,8]. Mice had 10 minute encounters in their cleaned home cages between 10.30 and 16.30 hr with standard opponents (adult anosmic male mice). Mice were rendered anosmic by nasal perfusion with 4% zinc sulfate solution. Such mice rarely threaten or attack experimental animals. Social activity recorded in such encounters has been described as 'rank-related aggression' by Brain [3]. Indices of attack recorded included:—

(a) The incidence of attack i.e. number or proportion of animals in each category showing biting attack on standard opponents.

(b) The latency (in seconds) from the opponent's introduction to the first biting attack on that animal. A maximal latency of 600 seconds was allocated to animals failing to attack within 10 min.

(c) The accumulated attacking time (AAT) i.e. the total time spent biting the standard opponent.

(d) The number of attacking bouts directed towards the standard opponent. Attacks were separated by periods of grooming, exploration, rearing, etc.

The reliability of the measures, as assessed by videotape analysis, is good and only one observer was involved here.

'Maternal Aggression' Tests

This test was a modification of that used by Gandelman [16] and Svare and Gandelman [45]. Mice were mated (as described in [7]), males being removed when the females became notably pregnant. At this time, shredded paper was provided to facilitate nest construction.

Litters were culled to six on the day of parturition to minimize differences in lactational activity. The intruder tests were conducted in the mother's home cage under dim red lighting in dark phase of light/dark cycle. At this time, an anosmic male standard opponent was introduced into the cage of the test animal for a 10 min period. Measures of attack used were identical to those employed in the rank-related aggression tests.

Locust-killing Tests

Examples of predatory aggression have been recently described in a variety of small laboratory rodents including *Onychomys* species [17,26], the Golden hamster [35, 36, 37, 38] and several strain of laboratory house mouse [10,46].

TABLE 2

EFFECT OF THE LOWER DOSE OF LITHIUM CHLORIDE AND SALINE ON LOCUST-KILLING BEHAVIOR IN MALE MICE

Daily Treatment	Number out of 12 of killing and consuming locust (in at least 2/3 tests) after the following time intervals									
	10 min		1 hr		2 hr		3 hr		6 hr	
	K	C	K	C	K	C	K	C	K	C
Uninjected controls	0	0	1	1	1	1	1	1	2	2
0.2 mEq saline	1	1	2	2	3	3	4	4	4	4
0.2 mEq lithium	0	0	1	1	2	2	2	2	2	2

N.B. There are no significant differences between these categories.
K=killed
C=consumed

TABLE 3

EFFECTS OF TWO DOSES OF LITHIUM CHLORIDE AND APPROPRIATE CONTROLS ON RANK-RELATED FIGHTING IN MALE MICE ON AN INITIAL TEST DAY (MEDIANS WITH RANGES)

Daily Treatment	Proportion of animals fighting	Latency to attack (secs)	Accumulated attacking time (secs)	Number of attacks
Uninjected controls	11/12*	72 (18–600)	14.53§	26.5§ (0–51)
0.2 mEq saline	11/12	195.5 (34–600)	10.7 (0–39)	21† (0–53)
0.4 mEq saline	11/12*	148.5 (32–600)	13§ (0–22.4)	26§ (0–39)
0.2 mEq lithium	8/12	322‡ (105–600)	0.55‡ (0–36.1)	1‡ (0–36)
0.4 mEq lithium	4/9	+600 (68–600)	0 (0–8.1)	0 (0–16)

*Differs from 0.4 m Equivalents of lithium treatment $p<0.05$ (Fisher's Test)
†Differs from 0.2 m Equivalents of lithium treatment $p<0.05$ (Mann Whitney U Test)
‡Differs from uninjected control of category $p<0.05$ (Mann Whitney U Test)
§Differs from 0.4 m Equivalents of lithium treatment $p<0.002$ (Mann Whitney U Test)

Cockroaches, crickets and locusts have been used as prey in most of these studies. It is interesting to note that feral mice are omnivorous and consume arthropods in their natural habitats [2]. The locust killing test for mice has been fully described elsewhere [5]—it was essentially derived from Butler's/Thomas' [10,46] and Polsky's [35, 36, 37, 38] earlier studies. Briefly, an adult locust (*Locusta migratoria*) was introduced into the cleaned home cage of the experimental mouse and the wire top was replaced by a perforated perspex lid. Detailed observations were carried out for 10 min, cages being subsequently checked 1, 2, 3 and 6 hr after the locust's introduction. Responses are more difficult to elicit in locust-killing tests than in intraspecific attack. One may also comment that non-experienced mice (as used in a majority of previous studies in our laboratory) generally take a long time to make their attack/kill in this situation and attacks have a low incidence in some experimental categories. Consequently, simple alive/killed data on the condition of the locust have been found to be the most appropriate indices [5] in this type of test. Comparable batches of mice do show some variability in the proportions of killers/non-killers but qualitative effects of treatments [5] have been found to be consistent. The measure of attack is, of course, unequivocal.

EXPERIMENT 1

EFFECTS OF LITHIUM CHLORIDE INJECTION ON NAIVE MALE MICE

Method

Sixty naive adult male mice were employed in this experiment. These animals were individually-housed at 18–22 days of age for three weeks before applying treatments. Mice were randomly allocated to the five experimental categories (N=12) listed below:—

1. untreated controls
2. a lower NaCl control ≡ 0.2 mEq/day
3. a higher NaCl control ≡ 0.4 mEq/day
4. a lower LiCl dose ≡ 0.2 mEq/day
5. a higher LiCl dose ≡ 0.4 mEq/day

First injections were given four days prior to behavioral tests and they were continued throughout the six consecutive days of testing. Animals received three encounters with standard opponents and three encounters with locusts in a balanced design.

Results

Summed data (over three tests) for rank-related fighting and locust-killing responses for animals injected with 0.2 mEq of LiCl (together with saline and uninjected controls) are presented in Tables 1 and 2 respectively. Animals injected with the higher dose of LiCl exhibited a high mortality during tests. Consequently only first test data is presented for this category and its saline control (Tables 3 and 4). First day data for the lower LiCl category, its saline control and uninjected counterparts are included for comparison. It was felt that first day data would be particularly easy to interpret as prior experience could not account for differences in responses. Tables include statistical comparisons between different treatment categories. Non-parametric tests were employed because of the non-continuous nature of the data. Tables of indices of rank-related fighting are consequently median values with ranges.

Mice injected with both doses of LiCl exhibited reduced rank-related fighting on Day 1 compared with appropriate saline and uninjected controls. Conversely, all experimental categories showed similar (low) incidences of locust-killing behavior.

Discussion

LiCl injection, at doses comparable with earlier studies (e.g. [40]), suppressed rank-related fighting in male mice. This action confirms Malick's [24] observations on male CF10 mice. It was not possible to demonstrate an action of LiCl on locust killing in this study because the incidence of this activity was low. The apparent depression in treatment categories (c.f. [5]) may be due to the stress of repeated injection.

This study did not fulfil the initial aim of demonstrating that predatory aggression and rank-related fighting in mice were dissimilar. Motivation for rank-related fighting was clearly markedly reduced by the LiCl treatment but the low levels of locust-killing (predatory aggression) were not conducive to showing a treatment effect in these naive mice. The incidence of locust-killing was low even in controls in these inexperienced mice. In addition, the high mortality obtained with the higher dose of LiCl suggested that non-specific debilitating effects could account for reduced rank-related attack in this study.

TABLE 4

EFFECTS OF TWO DOSES OF LITHIUM CHLORIDE ON LOCUST-KILLING BEHAVIOR IN MALE MICE IN AN INITIAL TEST

Treatment	Proportion killing and consuming locust after the following intervals									
	10 min		1 hr		2 hr		3 hr		6 hr	
	K	C	K	C	K	C	K	C	K	C
Uninjected controls	0/12	0/12	1/12	1/12	1/12	1/12	1/12	1/12	2/12	2/12
0.2 mEq saline	0/12	0/12	1/12	1/12	2/12	2/12	3/12	3/12	4/12	4/12
0.4 mEq saline	0/12	0/12	0/12	0/12	2/12	2/12	3/12	3/12	5/12	5/12
0.2 mEq lithium	0/12	0/12	0/12	0/12	0/12	0/12	2/12	2/12	2/12	2/12
0.4 mEq lithium	0/11	0/11	1/11	1/11	2/11	2/11	2/11	2/11	4/11	4/11

N.B. There are no significant differences between these categories.
K=killed
C=consumed

TABLE 5

MEDIAN (WITH RANGES) DATA FOR A SINGLE "RANK-RELATED AGGRESSION" TEST FOR EXPERIENCED "KILLER" MICE GIVEN LITHIUM CHLORIDE OR SALINE CONTROL INJECTIONS

Treatment	Proportion fighting	Latency of attack (sec)	AAT (sec)	Number of attacks
0.2 mEq Saline	10/10*	77.5 (35–390)	11.4† (3.1–23.5)	17.5† (5–39)
0.2 mEq Lithium	5/9	306 (53–600)	1.7 (0–10.6)	3 (0–18)

*Differs from the lithium treated category $p<0.05$ (Fisher's exact probability test)

†Differs from lithium treated category $p<0.05$ (Mann Whitney U test)

EXPERIMENT 2

EFFECTS OF LITHIUM CHLORIDE INJECTION ON EXPERIENCED MALE LOCUST-KILLERS

This experiment attempted to overcome the problems associated with Experiment 1. The modification involved assessing the effects of LiCl treatment on the locust-killing response in experienced "killer" mice where there is a maximal incidence of this behavior.

Method

Thirty-two male mice (comparable to those used in Experiment 1) were individually-housed for three weeks from 18–22 days of age. After this time, animals were initially given a six-hour locust-killing test each day for three consecutive days and animals failing to kill locusts in all three tests were discarded. Nineteen mice reached the required criterion and, immediately after the last screening locust-killing test, killer mice were randomly allocated to categories treated each day for four consecutive days with one of the following materials:—

1. 0.2 mEq NaCl (N=10)
2. 0.2 mEq LiCl (N=9)

Treatments were comparable to those used in Experiment 1. The day after the fourth injection, mice were given 6 hr tests for locust-killing. Two additional tests were administered over the next two days. A single standard opponent test was given the day after the last exposure to a locust and the injection program was continued throughout the encounters.

Results

Rank-related fighting data is presented in Table 5. Table 6 gives the proportions of animals killing locusts, together with the latency to attack these prey objects.

Most mice attacked locusts in the first 10 minutes of the test, a rapid response which is presumably a consequence of experience. It has been found [6] that prey killing experience increases the speed of subsequent reactions in 'killer' mice. Mice in both categories exhibited similar incidences of locust-killing. In contrast, LiCl-injected animals evidenced lower incidences and intensities of rank-related fighting than saline controls.

Discussion

The locust-killing data from Experiment 2 confirms that LiCl has little influence on predatory attack when given in this way. One may confidently claim that motivation for locust-killing by mice is not significantly altered by doses of LiCl that suppress rank-related fighting (as recorded in Experiments 1 and 2).

EXPERIMENT 3

EFFECTS OF LITHIUM CHLORIDE INJECTION ON NAIVE AND EXPERIENCED LOCUST KILLER FEMALES

Female mice that do not receive sex-steroids rarely show behavior comparable with rank-related fighting. However, as profound differences between locust-killing and maternal aggression have been shown for this sex [6], it was thought desirable to determine the effects of LiCl on these two forms of aggression.

Method

Forty-five lactating female mice (as described in the General Methods) were used in this study. Twenty-one animals

TABLE 6

EFFECTS OF LITHIUM CHLORIDE OR SALINE INJECTIONS ON LOCUST KILLING BEHAVIOR IN EXPERIENCED KILLER MALES

Treatment	Median Latency of attack (sec)	Proportion of mice killing locust in at least 2/3 tests after the following intervals									
		10 min		1 hr		2 hr		3 hr		6 hr	
		K	C	K	C	K	C	K	C	K	C
Saline	440.5 (79–1800)	8/10	0/10	8/10	8/10	9/10	9/10	9/10	9/10	9/10	9/10
Lithium	498 (56–1264)	7/9	0/9	8/9	8/9	8/9	8/9	8/9	8/9	8/9	8/9

were naive when tested but the remaining twenty-four were selected as being swift, efficient locust-killers on the basis of three 6 hr locust-killing tests. Mice were then randomly allocated to the following categories that were given one injection per day for four consecutive days:—

1. Naive mice given 0.2 mEq. NaCl (N=10)
2. Naive mice given 0.2 mEq. LiCl (N=11)
3. Experienced killers given 0.2 mEq. NaCl (N=12)
4. Experienced killers given 0.2 mEq. LiCl (N=12)

Mice were tested for locust killing in three daily 6 hr tests. Naive females were also given three daily encounters with an anosmic male intruder to assess maternal aggression. A balanced design was used and the injection program was continued throughout the tests.

Results

Maternal aggression data for the naive mice is presented in Table 7. The proportions in all four categories killing and consuming locusts, together with the latencies to attack these prey objects are given in Table 8.

Lithium chloride treatment had little influence on any index of maternal aggression used in this study—attack was intense in both LiCl and NaCl treated mice. Both naive and experienced females killed and consumed large numbers of locusts irrespective of whether they received treatment with LiCl or NaCl.

Discussion

LiCl does not appear to suppress maternal aggression in the same way as rank-related fighting. The results also confirm that this compound does not change locust-killing activity in this sex.

TABLE 7

SUMMATED MEDIAN (WITH RANGES) DATA FOR THREE "MATERNAL AGGRESSION" TESTS FOR NAIVE MICE GIVEN LITHIUM CHLORIDE OR SALINE CONTROL INJECTIONS

Treatment	Proportion attacking	Latency of attack (sec)	AAT (sec)	Number of attacks
0.2 mEq Saline	7/10	864 (3–1800)	55.2 (0–102.5)	93 (0–175)
0.2 mEq LiCl	8/11	607 (12–1800)	49.2 (0–92.1	86 (0–143)

N.B. No significant differences are evident.

Conclusions

These results provide further support for the contention that aggression is not a unitary phenomenon. Furthermore, they indicate that research workers should specify the type of model employed in their aggression research. One should not extrapolate too freely between different forms of aggression, as some forms of attack seem controlled by very different physiological features.

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TABLE 8

EFFECT OF LITHIUM CHLORIDE AND SALINE ON LOCUST-KILLING BEHAVIOR IN NAIVE AND EXPERIENCED LACTATING FEMALE MICE

Experience	Daily Treatment	Proportion of mice killing and consuming locusts after the following time intervals									
		10 min		1 hr		2 hr		3 hr		6 hr	
		K	C	K	C	K	C	K	C	K	C
Naive Animals	0.2 mEq Saline	0/10	0/10	4/10	4/10	8/10	8/10	8/10	8/10	9/10	9/10
	0.2 mEq LiCl	1/11	0/11	3/11	3/11	5/11	5/11	6/11	6/11	7/11	7/11
Experienced Killers	0.2 mEq Saline	12/12	0/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12
	0.2 mEq LiCl	11/12	0/12	11/12	11/12	11/12	11/12	11/12	11/12	11/12	11/12

N. B. There are no significant differences between LiCl-treated animals and appropriate controls.
K=killed
C=consumed

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