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Dose–response effects of chronic lithium regimens on spatial memory in the black molly fish

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Abstract

Lithium is widely used in the management of bipolar disorder, yet memory impairment is a serious side effect. To assess the effects of lithium on spatial working and reference memories, we have employed a plus maze utilizing spontaneous alternation (SA) and place-learning paradigms in two experiments with the black molly fish. Four treatment groups were gavaged with 20 μl of a 10, 100, or 1000 mM lithium chloride (LiCl) solution or ddH_2O vehicle every 12 h for 22 to 24 days. On Day 15, subjects began an 8-day SA task or a 10-day place-learning task. Results indicate that there is a significant difference in SA performance among the treatment groups for Days 1, 2, and 3. Results of the place-learning task indicate that the 1 M dose group needed significantly more trials to reach criterion and made significantly fewer correct first choices than the other dose groups. Capillary ion analysis determinations of plasma and brain lithium levels illustrate linear dose–response relationships to doses administered. Regression analyses indicate that there is a relationship between SA performance and plasma/brain lithium levels during the initial part of testing. Collectively, the results indicate that chronic lithium administration impairs spatial working and reference memories.

Keywords: Lithium chloride; Cognitive impairment; Working memory; Reference memory; *Poecilia latipinna*; Capillary electrophoresis

1. Introduction

Treatment of bipolar disease (BD) is limited not only by the scarcity of mood stabilizers available for pharmacological intervention but also by their pervasive side effects that promote treatment noncompliance. This is particularly evident with patients being prescribed lithium. Although lithium is efficacious in the management of BD, it is notorious for its long list of side effects. Cognitive impairment is one of the most disturbing of these side effects according to BD patients (Gitlin et al., 1989). Lithium has been reported to manifest a wide range of cognitive-related complications (Glue et al., 1987, Judd et al., 1977; Weingartner et al., 1985) including short-term and long-term memory (STM and LTM) deficits in BD patients and healthy volunteers (Karniol et al., 1978; Kusumo and Vaughan, 1977; Squire et al., 1980; Stip et al., 2000). Because these effects are seen in healthy volunteers, they likely do not characterize BD sequelae (Manji et al., 1993). Stoll et al. (1996) has reported that valproate substitutions alleviate much of the cognitive-related deficits experienced by their BD patients.

Fishes offer several advantages as animal models for studying the behavioral effects of lithium. Euryhaline fishes such as the black molly live naturally in a brackish ecosystem and thus have evolved to cope with rapidly changing ionic concentrations in their environments (Johnson, 1981). Because cyprinids are continuously active fishes with limited response repertoires, they acclimate well to a maze setting (Ingle, 1965) and provide uncomplicated observation for the experimenter. For example, fearful or apathetic fish are easily detected by a "freezing" behavior. Other specific advantages are beyond the scope of the present investigation

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yet are related to our laboratory's interests in neuroreceptor-mediated mechanisms for lithium action that may be confounded in experiments with mammals that are endowed with a limbic system. To our knowledge, only one study (Johnson, 1980) has investigated the effects of lithium on spontaneous alternation (SA) behavior in fishes. The purpose of the present study is to establish a dose–response effect of lithium on spatial working and reference memories in a fish animal model.

In two experiments with black molly fish we demonstrate that chronic lithium regimens disrupt both STM, as assessed by a standard SA paradigm, and LTM, measured with a typical place-learning task.

2. Experiment 1

SA behavior is considered an index of spatial working memory (Creson and Monaco, unpublished observations; Livesey et al., 1981), a form of STM that requires retention of locations visited within a trial session and their exclusion from memory during subsequent trial sessions (Olton, 1979). An SA session typically employs two discrete nonreinforced trials to measure a subject's innate propensity to choose alternate arms at a choice point in a T or Y maze. The motivation to alternate originates from a curiosity drive aroused by novel stimuli manifested as efficient exploration of an animal's environment (Dember and Fowler, 1958; Montgomery, 1951). As the intertrial interval (ITI) between two consecutive trials (T1 and T2) is increased, subjects' memory traces of T1 extramaze cues fade, thus decreasing the probability that they will alternate on T2. Over several trial sessions, performance generally exceeds that of chance-level alternation (50%) in the rat. Because SA is nonreinforced, subjects typically habituate to the task after about four trials, with alternation rates declining toward chance level. Habituation is avoided with a learned alternation paradigm in which subjects are rewarded with food for alternation after successive trials. Lower vertebrate organisms such as fishes commonly require a forced-choice condition with minimal ITI lengths to promote significant levels of SA (Richman et al., 1986/1987). In the forced-choice condition, one of the choice arms is blocked during T1, yet both arms are accessible during T2. Results of previous studies in this laboratory indicate that the black molly fish alternates significantly below chance level (perseverates) in a free-choice condition, yet alternates significantly above chance level in a forced-choice condition with ITIs of less than 10 min. Results from several studies with untreated fish are consistent with our forced-choice findings (Aderman and Dawson, 1970; Fidura and Leberer, 1974; Neiberg et al., 1970). Typically, various limbic system lesions and drug administrations induce perseveration in free-choice SA conditions in rats (Kokkinidis, 1989). Other than previous studies in our laboratory, the issue of perseveration in free-choice SA conditions in untreated fish has not been addressed. However, Bitterman (1965) has proposed a rat–fish dichotomy schema that recognizes phyletic differences in learning among a host of organisms.

2.1. Methods

2.1.1. Subjects

Black mollies (melanistic varieties of *Poecilia latipinna*), 4–6 cm in length, obtained from a local supplier, were utilized in three consecutive repetitions (Rs) of an 8-day SA task ($N = 128$). Subjects were randomly yet evenly assigned to one of four dose groups based on gender and size only: ddH_2O control ($n = 34$), 10 mM lithium chloride (LiCl) ($n = 31$), 100 mM LiCl ($n = 30$), and 1 M LiCl ($n = 33$). Each dose group was maintained in a 14-l glass tank with conditioned ddH_2O water (Start Right and Fungus Guard, Jungle Laboratories, Cibolo, TX), which was aerated and temperature controlled for at least 1 week before each of the repetitions of the experiment. A 30-l container with identically treated water was available for maintenance of water levels in the tanks, the maze, and the holding tank. Thereafter, all water that the fish inhabited was monitored chemically for excess ammonia and nitrite levels, general and carbonate hardness, and for proper pH of 7.2 (Master Test Kit, Aquarium Pharmaceuticals, Chalfont, PA). Each tank was cleaned and replenished with conditioned and aerated water after each repetition. A 12:12-h light–dark cycle was automatically clocked by timed overhead fluorescent lights. The fish were fed once a day (Tetra Min flake food, RamFab, Oak Ridge TN). On trial days, fish were fed after completion of trials.

The rather large number of subjects used in the study was justified by the following sample size determination equation: $N > (\theta)(P_1Q_1 + P_2Q_2)/(P_1 - P_2)^2$ where N represents the minimum number of subjects needed for statistical difference in an experiment with four groups. The θ value reflects α ($P < .05$) and B (90% power) values. P and Q represent proportional values obtained from pilot study data. Some mortality was expected during the experiment; therefore, a few more fish per group were included to insure a reliable subject number. Fortunately, most of the mortality occurred before trial days. Only three subjects (in the 100 mM group of R1) died during the experiment and were excluded from data analyses. No animals were run if they appeared sickly. The experimental protocol (Project No. P010204) was reviewed and approved by the East Tennessee State University Committee on Animal Care, Division of Laboratory Animal Resources for the black molly fish.

2.1.2. Apparatus

The modifiable plus maze (Fig. 1) was made out of clear glass allowing for the incorporation of four spatially distinct T mazes without its reorientation. No intramaze cues were added other than the partitions. The intention was to keep the intramaze environment as uniform as possible. The side of the holding tank facing the maze was visually obscured.

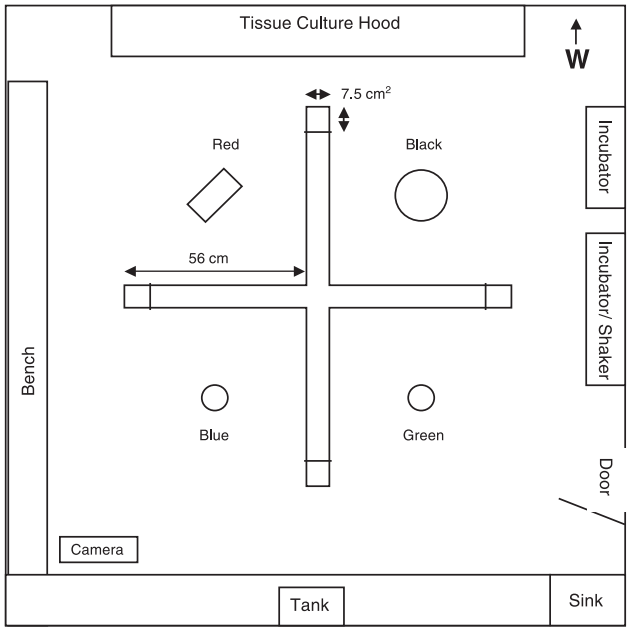

Fig. 1. Maze schematic. The maze was mounted on an optically neutral platform atop a table (not shown) electrically equipped to aerate and heat the water, maintained at a depth of 12.5 cm. The maze walls were 15 cm high. A layer of gravel (1 cm) covered the entire floor of the maze. The central area included a 1.25-cm circular opening for drainage and slots at the entrances of each arm for placement of glass partitions to selectively block arm entries. Start boxes were delimited at the distal ends of the arms by partitions extending 5 cm from the gravel surface. Red, black, blue, and green denote solid colors of 3-D objects, atop the platform, serving as immediate extramaze cues. The sizes and locations of these objects are drawn to scale relative to that of the maze. The maze and room are drawn in relative scale. The maze room (5×3.5×3 m) was amply illuminated. The tank at the east end of the room served to collectively hold subjects of each dose group during their trials. A video camera, mounted on a tripod, was connected to a TV located just outside the maze room where behaviors were monitored.

The ddH_2O in the holding tank was conditioned in the same manner as the maze and home tank water.

2.2. Procedure

2.2.1. Dosing regimen

One week after commencement of acclimation to their home tanks, subjects began a 2-week dosing regimen. Every 12 h, subjects were gavaged via micropipette with 20 μl of one of three doses of LiCl (Purified LiCl, Fisher Scientific, Fair Lawn, NJ) dissolved in ddH_2O as vehicle. The control group was dosed with vehicle only. For each dosing session, with gloved hands, the fish were individually handled while a micropipette tip was inserted past the throat and the solution dispensed. Dosing times were 9 a.m. and 9 p.m. at the beginning and end of the subjects' light period.

2.2.2. SA test

On Day 15, subjects were given two successive trials per day for eight consecutive days in a 0-min ITI/forced-choice SA task. Beginning each day's testing, the subjects of each dose group were collectively transferred from their home tanks to the small holding tank in the maze room. A trial began when the subject was gently lowered into a designated start area of the maze with a small scoop. The experimenter immediately left the room to monitor trial performance. Direction and latency to choice were recorded once the animal was fully inside one of the two choice arms. The subject was allowed to remain in the choice arm approximately 30 s before being gently removed. Subjects were given 10 min to make a choice. Subjects were eliminated from the study if they froze in the start arm for over 2 min. Each start area, designated E, N, W, and S was used once for all of the subjects for Days 1–4 and Days 5–8, respectively. After completion of T1, the subject was returned to the start area to begin T2 and returned to its home tank after completion of T2. All subjects were run during their light cycle.

The arm opposite the start arm, as well as one of the other choice arms, was blocked during T1. Only the arm opposite the start arm was blocked during T2. The arm opposite the start arm was always blocked with a glass partition made opaque by white plastic inserted behind the glass partition to create a T maze. The choice arm blocked on T1 was blocked with a glass partition. During T1, half of the subjects of each group were forced to the left arm while the other half were forced to the right arm. The blocked choice arm of T1 was alternated for all subjects for each consecutive testing day to control for potential laterality preferences. Testing order of dose groups and their subjects were preserved for each testing day so that each subject would be run at approximately the same time of day. These measures also prevented any potential exposure of the control group to any LiCl excreted in the holding tank or maze by the other groups. Water in the holding tank and maze were exchanged daily for freshly conditioned and aerated ddH_2O.

2.2.3. Lithium measurement

The last dose was administered the following morning after SA testing was completed. Two hours after the last dose was given, half of the subjects from each dose group were sacrificed for plasma and brain lithium levels. Subjects were euthanized in a 0.1% methanesulfonate salt solution (MS222, 3-aminobenzoic acid ethyl ester, Sigma, St Louis, MO). After 2 min in the solution, when opercular movements had ceased, cardiac punctures were performed for blood collection and whole brains were extracted, weighed, and frozen at -80 °C until the time of processing for lithium measurement. The blood was immediately spun for plasma and frozen with the brains. Plasma and brain lithium levels were determined using a capillary ion analysis technique specifically developed for measurement of cations in the black molly fish (Creson et al., 1998).

2.2.4. Data analyses

Contingency table analyses were utilized to compare numbers of left and right T2 turns for determinations of

SA levels of performance within dose groups and to compare numbers of alternations vs. perseverations among dose groups. Dose group comparisons of day block SA percentages were analyzed using the Kruskall–Wallis test. Latencies to choice were analyzed using a three-factor, repeated-measures ANOVA. Plasma and brain lithium levels were compared among groups using a one-factor ANOVA. Regression analyses were conducted to determine whether a relationship existed between plasma and brain lithium levels and to infer linear relationships between doses administered and lithium levels obtained. Regression analyses were also conducted to determine whether relationships existed between SA performances and lithium levels.

2.3. Results

Contingency table analyses (Table 1) indicate that chronic LiCl regimens impair SA performance. Contingency table analyses of left vs. right T2 turns, contingent upon forced T1 turns, for each day of testing indicate that the controls alternated significantly above chance level for Days 1, 2, and 3 (χ^2s = 7.556, 6.103, and 5.765 and *P*s=.0060, .0135, and .0164, respectively), but not for any day thereafter. Although the 100 mM LiCl group significantly perseverated for Days 1–4, no significant values were achieved for individual days (χ^2s = 0.002, 2.010, 2.143, and 1.094; *P*s=.9607, .1563, .1432, .2956, respectively, for Days 1, 2, 3, and 4). However, a trend toward perseveration is indicated for Days 2 and 3. The control groups of each of the three Rs alternated significantly above chance level for Days 1–4 (χ^2s = 4.912, 7.056, and 7.056; *P*s=.0267, .0079, and .0079, for R1, R2, and R3, respectively). However, the 100 mM group perseverated during Days 1–4 only in repetition 2 (χ^2s = 0.444, 6.481, and 0.100; *P*s=.5050, .0109, and .7515, for R1, R2, and R3, respectively). Higher mortality rates of the R1 100 mM group ($n = 3$) during acclimation to home tanks may justify results seen in R1 but not in R3. Significance in the R2 100 mM group is likely due to the larger group size ($n = 19$) employed to compensate for increased pretest mortality rate of the R1 100 mM group. All other dose group sizes per repetition conformed to $n = 10 \pm 2$. Only one significant difference in the numbers of left vs. right turns within any of the repetitions was indicated for any dose group for Days 5–8. R2 controls alternated significantly above chance level ($\chi^2 = 4.148$; *P*=.0417). All subjects were forced to the left and right during T1 an equal number of times for Days 1–4 and Days 5–8 to justify 50% SA as chance level.

Table 1
Degree of spontaneous alternation or perseveration within each dose group for testing day blocks 1–4 and 5–8

Day block/ dose group	T1 turn	T2 turns		χ^2 test		SA vs. P
		Left	Right	χ^2	*P*	
1–4/Control	Left	21	47	18.386	<.0001	SA>>P
	Right	46	22			
1–4/10 mM	Left	27	35	0.033	.8560	SA ~ P
	Right	26	36			
1–4/100 mM	Left	33	27	4.062	.0439	P>SA
	Right	22	38			
1–4/1 M	Left	38	28	3.030	.0817	P>SA
	Right	28	38			
5–8/Control	Left	39	29	0.000	>.9999	SA ~ P
	Right	39	29			
5–8/10 mM	Left	29	33	0.525	.4688	SA ~ P
	Right	25	37			
5–8/100 mM	Left	30	30	0.301	.5834	SA ~ P
	Right	33	27			
5–8/1 M	Left	33	33	0.030	.8618	SA ~ P
	Right	32	34			

Trial 1 turns are fixed by the experimenter. T1 = trial 1; T2 = trial 2; SA = spontaneous alternation; P = perseveration.
> greater than; >> much greater than; ~ approximately equal.

Significant differences among dose groups in numbers of alternations and perseverations within Days 1, 2, and 3 (Table 2) are generally attributed to superior SA performance by the controls as indicated by contingency table analyses for all six possible combinations of groups within each of these days. Values for control vs. 10 mM groups for Days 1, 2, and 3 are χ^2s = 1.111, 4.317, and 5.429; *P*s=.2919, .0377, and .0198. For control vs. 100 mM groups, χ^2s = 3.765, 7.401, and 7.401; *P*s=.0523, .0065, and .0065, for Days 1, 2, and 3, respectively. For control vs. 1 M groups, χ^2s = 7.949, 4.347, and 3.401; *P*s=.0048, .0370 .0652, for Days 1, 2, and 3, respectively. None of the other possible group combinations (10 vs. 100 mM, 10 mM vs. 1 M, or 100 mM vs. 1 M) yielded remotely significant contingency table values for any of the 3 days except 10 mM vs. 1 M groups on Day 1 ($\chi^2 = 3.065$; *P*=.0800). Contingency table analyses revealed no significant differences among the three Rs for numbers of alternations within any of the dose groups for Days 1–4 or Days 5–8 (*P*s>.05).

Fig. 2 depicts dose group mean percent alternations for each day of testing. Standard errors are not indicated because the data are represented categorically. That is, subjects either alternated or perseverated during a trial session. The Kruskall–Wallis test indicates a significant difference in alternation percentages among the dose groups for Days 1–4 ($H = 9.044$; *P*=.0287) but not for Days 5–8 ($H = 1.301$; *P*=.7288). Mann–Whitney tests comparing SA percentages between the six different dose group combinations again reflect superior SA performance by the controls during Days 1–4 (controls vs. 10 mM: $U = 1$; *P*=.0433, controls vs. 100 mM and 1 M: *U*s = 0; *P*s=.0209, respectively, 10 vs. 100 mM: $U = 3$; *P*=.1489, 10 mM vs. 1 M: $U = 5$; *P*=.3865, and 100 mM vs. 1 M: $U = 8$; *P*>.9999). No significant differences in SA percentages were indicated between any combinations of groups for Days 5–8 (*P*>.05). Contingency table analyses specify no significant differences in SA percentages among the three repetitions for Days 1–4 ($\chi^2 = 13.970$; *P*=.9944) nor for Days 5–8 ($\chi^2 = 30.611$; *P*=.4347).

Fig. 3 depicts T1 and T2 latencies to choice for each testing day. A three-factor, repeated-measures ANOVA

Table 2
Dose group comparisons of numbers of alternations and perseverations

Testing day(s)	χ^2	P
1	8.731	.0331
2	8.461	.0374
3	8.735	.0330
4	3.989	.2627
5	0.802	.8489
6	1.621	.6546
7	1.910	.5912
8	0.457	.9283
1–4	25.411	<.0001
5–8	0.815	.8458

revealed significant main effects for latency [$F(1,1031) = 11.663$; $P = .0007$], testing day [$F(7,1025) = 5.357$; $P < .0001$], and repetition [$F(2,1030) = 4.149$; $P = .0161$], but not for dose group [$F(3,127) = 0.731$; $P = .5335$]. There were no significant interactive effects between dose group and testing day [$F(21,1001) = .680$; $P = .8555$], testing day and repetition number [$F(14,1009) = 1.348$; $P = .1725$], nor dose group, testing day, and repetition number [$F(42,937) = 0.961$; $P = .5439$]. There was a significant interaction between dose group and repetition number [$F(6,1021) = 2.311$; $P = .0320$]. There were no significant interactions between latency and any of the other factors or combinations of factors (Ps>.05).

Fig. 4 illustrates near linear representations of plasma and brain lithium levels relative to administered lithium doses. One-factor ANOVAs indicate significant differences in plasma lithium levels among the four dose groups [$F(3,55) = 6.0485$; $P = .0012$] but not for brain lithium levels [$F(3,59) = 1.4232$; $P = .2450$]. Fisher's PLSD tests indicate significant plasma lithium level differences between the control and 100 mM groups ($P = .0224$), the control and 1 M groups ($P = .0002$), and the 10 mM and 1 M groups ($P = .0029$). Regression analysis verifies a significant linear relationship between plasma and brain lithium levels among dose group mean values [$R^2 = .959$; $F = 47.351$; $P = .0205$]. Regression analyses indicate significant relationships between each of the plasma and brain mean lithium levels, respectively, and mean SA percentages for Days 1 and 4, respectively [R^2s=.986 and .989; Fs = 141.865 and 187.424; Ps=.0070 and .0053, for Day 1, and R^2s=.921 and .991; Fs = 23.224 and 213.926; Ps=.0405 and .0046, for Day 4]. These relationships failed to reach significance for Days 2 or 3, yet tended toward significance for Days 1–4 for plasma and brain levels, respectively [R^2s=.710 and .857; Fs = 4.895 and 12.001; Ps=.1574 and .0742].

2.4. Discussion

Results of this study indicate that chronic lithium regimens impair STM in the black molly as indexed by SA, a spatial working memory task. Lower doses decreased alternation rate to around chance level while higher doses further decreased alternation rates to perseveration levels. The overall effect of lithium on SA was not linear throughout the range of administered lithium concentrations; however, there is a clear pattern of SA performance decrement from controls to the 100 mM group for Days 1–4. Control group performance in this experiment is consistent with that of forced-choice/0-min ITI group performances in previous experiments in this laboratory (Creson and Monaco, unpublished observations). Also, controls in both studies habituated to the SA task by Day 4. Although the LiCl dosages were relatively high compared with those of humans, according to CIA results, plasma and lithium levels were at, or below, therapeutic levels. Interspecies comparisons may have little bearing on what constitutes therapeutic indices for lithium in fish; however, black molly plasma and brain lithium levels reflect linear relationships to the doses administered.

Granted the lithium dosages were relatively high, the insignificant main effect in latencies to choice for dose groups suggests that neither motivation to perform the task nor locomotor ability were compromised by the lithium treatments. Although not statistically relevant, T1 and T2 latency values are consistent with those of the forced-choice/0-min groups of previous experiments in our lab (Creson and Monaco, unpublished observations). T2 latencies were generally shorter in duration than T1 latencies, and this, too, is consistent with earlier work in our lab. The significant findings tied with the repetition factor are likely due to the unequal subjects' numbers of the 100 mM groups of R1 and R2 discussed previously.

A number of interpretations have been presented to account for lithium's effects on exploratory-based behaviors but none that directly address the mechanism by which lithium affects STM. Johnson has conducted several studies examining different aspects of lithium action in rats and goldfish. His hypothesis states that the effects of lithium on animal behavior may result from an impairment of central analysis of sensory input such that treated animals become less responsive to their surroundings (Johnson, 1983). In one of these studies, Johnson (1980) demonstrated that

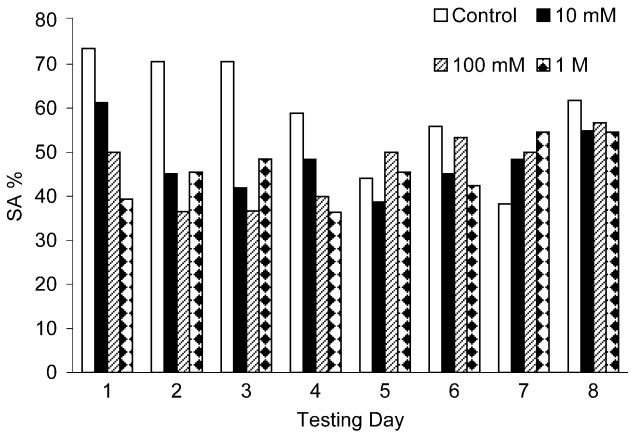

Fig. 2. SA percentage means for each dose group during each testing day. Standard error bars are not indicated because results reflect tallies of categorical data.

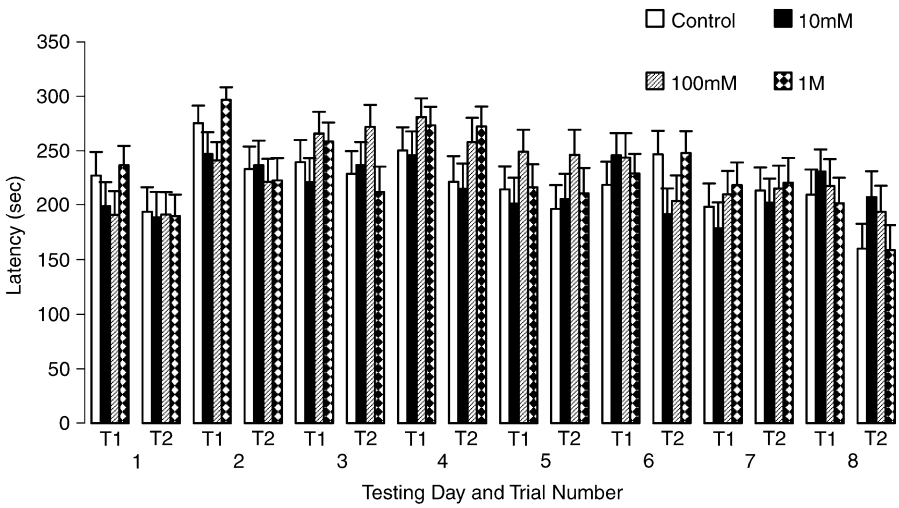

Fig. 3. T1 and T2 dose group mean latencies to choice (+S.E.) for each testing day of the SA task.

goldfish, kept in a solution of 10 mM LiCl for 2 days before testing in a forced-choice SA condition, alternated significantly above chance level, yet alternated significantly less than the 10 mM NaCl controls. Johnson's theory is consistent with clinical results suggesting that lithium may induce slowing of information processing (Glue et al., 1987; Judd et al., 1977; Squire et al., 1980). This line of reasoning may explain the results of the 10 mM group in the present experiment, which alternated at chance level, yet may not be consistent with the perseverative tendencies of the higher dose groups. The present experiment utilizes a chronic dosing regimen throughout the testing phase, which is consistent with human therapeutic intervention. The present study also incorporates a wider range of dosages with a larger sample number than Johnson's (1980) study. The discrete trial procedure used in the present study may also provide a more suitable measure of STM than the continuous trial procedure used in the Johnson study.

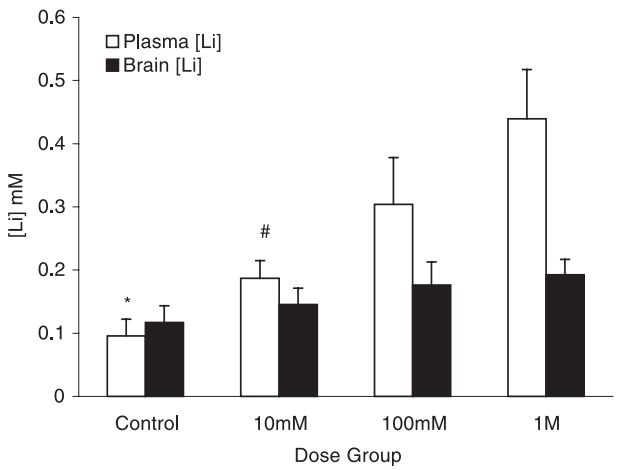

Fig. 4. Dose group mean plasma and brain lithium levels (+S.E.) from chronically dosed black molly fish in the spontaneous alternation task. Plasma and brain samples were analyzed for lithium levels via a capillary ion analysis (CIA) technique using the Waters Quanta 4000 Capillary Electrophoresis System (Milford, MA). CIA was performed using a 75-μm internal diameter × 60-cm length fused silica capillary and a run electrolyte of 67.7-mg hydroxybutyric acid, 53.8-mg 18-crown-6-ether, and 64-μl UV-CAT reagent (4-methylbenzylamine) in a volume of 100 ml ddH_2O (18 MΩ with a voltage of 20 kV using indirect UV absorption detection at 214 nm. *$P<.05$. Fisher's PLSD for control vs. 100 mM and control vs. 1 M groups. #$P<.05$. Fisher's PLSD for 10 mM vs. 1 M groups.

Perseveration in SA behavior is a hallmark result of limbic system damage (Roberts et al., 1962; Thomas, 1972) and drug-induced, i.e., amphetamine, toxicosis in rats (Kokkinidis, 1989). Because, at least, the hippocampus receives input from all sensory modalities, manipulations of this system will affect cue salience and thus performances in spatial tasks. Although the debate is long running, there is considerable evidence that hippocampal activity may be important in the regulation of spatial working memory (Olton et al., 1979) as well. Disputing claims that SA impairments after hippocampectomy are due to loss of internal inhibition, Isseroff (1979) demonstrated that, although operated rats were able to perform as well as controls in an SA task with minimal ITI delay, operated rats were significantly impaired relative to controls when a 10-s ITI was introduced. Using ITIs ranging from 50 s to 5 h, Livesey et al. (1981) demonstrated that, in untreated rats, SA rates were indirectly proportional to ITI lengths. Dalland (1976) has demonstrated that hippocampal-damaged rats perseverate body turns in a two-trial, free-choice SA condition but alternate like controls in a forced-choice modification of the task. This phenomenon is precisely what our lab has demonstrated in the untreated black molly (Creson and Monaco, unpublished observations). The black molly alternates significantly above chance level in a forced-choice SA condition but perseverates in a free-choice condition. Dalland (1976) suggested that hippocampal-damaged rats are unable to shift to another response once a turn has been made. These animals appear to abandon normal use of

relevant extramaze cues for navigation and to be impaired in their ability to inhibit certain types of responses related to choice. So the argument could be made that higher lithium dosages may not just interfere with hippocampal function but turn its function off more completely, such that the subject is forced to rely on more primitive forms of navigation such as proprioceptive feedback proposed for insects (Lester, 1968) or, similarly, Hughes' (1985) bilaterally asymmetrical leg movements (BALM)-induced compensatory mechanism for woodlice that corrects for previously forced turns. Nevertheless, Wilson and Fowler (1976) have developed a compelling experimental arrangement utilizing a forced-choice SA paradigm to measure STM in the cockroach. Alternatively, if animals perseverate on the basis of stimulus factors as opposed to response feedback (Kokkinidis and Anisman, 1976), at least for lower LiCl dosages, then Johnson's (1979) stimulus significance hypothesis of lithium action is favored. That is, lithium may impair stimulus processing by reducing the significance with which the animal attaches to incoming stimuli. Cappeliez and Moore's (1988) hypothesis similarly states that lithium narrows the breadth of attention onto stimuli of high salience at the expense of the processing of stimuli of low salience. These altered processes, in turn, compromise the animal's STM of stimulus placement.

3. Experiment 2

Place learning is a type of discrimination learning in which subjects associate distinctive exteroceptive stimuli with a particular spatial location (Olton and Samuelson, 1976). Typically, a subject is rewarded for remembering a fixed goal location over several trials dispersed over time, a process that invokes the use of a long-term reference memory process. Rats are excellent place learners whose rate of learning depends directly on the proportion of relevant, usable cues in the total set available (Restle, 1957). Restle (1957) further qualifies the proposal stating that the main factor in determining the outcome of a place-learning task is the amount of extramaze visual stimulation that differentiates a goal area from another area. Similarly, Warburton (1990) demonstrated that goldfish learned food-patch placements more quickly and with greater accuracy when visually distinct landmarks were positioned about the food patches than when they were absent. Although fishes perform qualitatively differently than higher vertebrates in discriminative learning paradigms (Bitterman, 1975), they are capable of solving maze-learning tasks that involve visual discrimination capacities requiring spatial memory (Churchill, 1916; Hughes and Blight, 1999, 2000; Ingle and Sahagian, 1973; Rodriguez et al., 1994; Roitblat et al., 1982). From an ecological standpoint it is critical to the survival of various fishes that they can utilize landmark memories within a spatial guidance framework for foraging and migratory purposes (Dodson, 1988).

3.1. Methods

3.1.1. Subjects

Black mollies (melanistic varieties of *P. latipinna*), 4–6 cm in length, weighing 1.00–3.30 g, obtained from a local supplier, were utilized in four consecutive replications (Rs) of a four-trial per day, 10-day place-learning task ($N = 140$). Subjects were randomly yet evenly assigned to one of four dose groups based on gender and size only: ddH_2O control ($n = 31$), 10 mM LiCl ($n = 37$), 100 mM LiCl ($n = 34$), and 1 M LiCl ($n = 38$). Subject numbers for R1, R2, R3, and R4 were 29, 30, 40, and 41, respectively. Each dose group was maintained in the same housing and water conditions as in Experiment 1. The fish were fed once a day (Tetra Min flake food, RamFab). On trial days, fish were fed half their usual amount after completion of trials.

As in Experiment 1, most of the mortality occurred before commencement of the dosing regimen. Six subjects (two controls, two 100 mM, two 1 M) died during the experiment and were not included in data analyses. No animals were run if they appeared sickly. Animals that froze in the maze for more than 2 min at the initiation of testing were eliminated from the experiment. The experimental protocol (Project No. P010204) was reviewed and approved by the East Tennessee State University Committee on Animal Care, Division of Laboratory Animal Resources for the black molly fish.

3.1.2. Apparatus

The same modifiable plus maze (Fig. 1) was used as in Experiment 1.

3.2. Procedure

3.2.1. Dosing regimen

The dosing regimen followed that of Experiment 1, except that dose administrations were carried out for two more days through the end of the 24-day experiment.

3.2.2. Place-learning test

Because this task involved food reinforcement, subjects were conditioned to food procurement in the maze 15 min per day for three successive days before commencement of the task. On the first pretraining day, food flakes (normal staple) were sprinkled onto the water surface of the entire maze. Subjects of each dose group were collectively transferred from their home tanks to the choice point and allowed to feed in and explore all four arms of the maze. During the second and third pretraining days, food was placed only at the distal ends of the arms behind partitions now set into place. No other food was provided thereafter. On Day 15, subjects began a 10-day task, consisting of four trials per day, separated by approximately 30-min ITIs, conducted on an individual basis. The arm opposite the start arm was always closed with a white opaque partition while the two other arms were always open. A single large food flake was

located in only one goal area for each subject throughout the task. Each dose group was subdivided equally for designated goal areas. Start positions were alternated 180° after T1 and T3 for each day of testing. T1 start positions were switched for each subsequent day of testing. These measures controlled for any turn preferences. Subjects that had not obtained food within 10 trials of testing were eliminated from the experiment.

Several measures of performance were analyzed to evaluate reference memory assessed by the place-learning task as detailed in the following list:

Number of trials to criterion. Subjects that swam to their designated goal areas before any others and obtained food in five out of six consecutive trials were deemed to have reached criterion or acquisitioned the task.

Percent correct first choices. The percent of correct first choices to reward within each 10-trial block were recorded.

Number of errors. Subjects made errors when they fully entered undesignated goal arms. An error was not made if the subject swam in its designated goal arm but did not obtain food.

Number of omissions. One omission was recorded when subjects failed to obtain food within the 5-min framework allowed for each trial.

Latencies to reward. The time taken between introduction to the start area and obtaining the food reward was recorded to assess lithium's effects on motivation and locomotion.

Left turn preference. Because percent correct first choices were lower than anticipated, we wanted to know if this was due to turn preferences.

3.2.3. Lithium measurement

Plasma and brain processing and lithium level measurements were conducted in the same manner as in Experiment 1.

3.2.4. Data analyses

A series of two-factor ANOVAs and post hoc Fisher's PLSD tests were conducted where appropriate to evaluate each of the different levels of performance measures as well as plasma and brain lithium levels.

3.3. Results

The high-dose group (1 M LiCl) took significantly longer to learn the task than the rest of the groups. Fig. 5 illustrates the mean number of trials to criterion for each dose group. A two-factor ANOVA specified no significant main effects for dose group [$F(3,136)=2.222$; $P=.0890$] or replication number [$F(3,136)=0.316$; $P=.8140$], or the interactive effect between these two factors [$F(9,123)=1.877$; $P=.0615$]. However, Fisher's PLSD tests indicated significant differences between the 10 mM and 1 M groups ($P=.0360$) and the 100 mM and 1 M groups ($P=.0376$) and a virtually significant difference between the controls and 1 M groups ($P=.0541$). No other post hoc test differences between other dose group or replication number pairings were designated ($P>.05$).

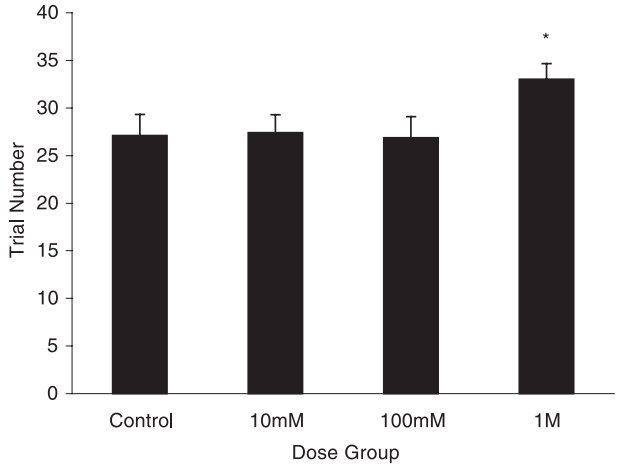

Fig. 5. Dose group mean (+S.E.) trials to criterion. *$P<.05$: 1 M group vs. 10 and 100 mM groups.

In addition, the high-dose group firstly chose their designated goals significantly fewer times during T11–20 than the other dose groups. T11–20 appears to be a period of intense learning. Other than the controls, all other groups maximized correct first-choice performance during T21–30. Fig. 6 depicts the mean percentages of correct first choices to reward within each trial block for each dose group. A two-factor ANOVA indicated no significant main effects during T1–10 for dose group [$F(3,136)=1.285$; $P=.2826$], replication number [$F(3,136)=1.357$; $P=.2590$], or the factors' interactive effects [$F(9,123)=1.507$; $P=.1527$]. A significant main effect is indicated for dose group during T11–20 [$F(3,136)=3.160$; $P=.0271$] but not for replication number [$F(3,136)=0.601$; $P=.6156$] or their interaction [$F(9,123)=1.168$; $P=.3212$]. Fisher's PLSD tests revealed significant differences between the 10 mM and 1 M groups ($P=.0360$), and between the 100 mM and 1 M groups ($P=.0066$), as well as a near-significant difference between controls and the 1 M group ($P=.0560$) during T11–20. No other significant between-group differences were designated with these post hoc tests during T11–20. Two-factor ANOVAs computed for T21–30 and T31–40, respectively, disclose no significant main effects for dose group [Fs(3,136)=1.175 and 1.178; Ps=.3222 and .3211], replication number [Fs(3,136)=2.158 and 0.568; Ps=.0964 and .6369], or their interaction [Fs(9,123)=0.180 and 0.648; Ps=.9958 and .7539].

Fig. 7 illustrates mean dose group number of errors committed before making a correct choice for each dose group during each of the four trial blocks. Again the 1 M LiCl group appears to perform more poorly than the other groups during T11–20. A two-factor ANOVA for T1–10 indicates no main effect for dose group [$F(3,136)=1.063$;

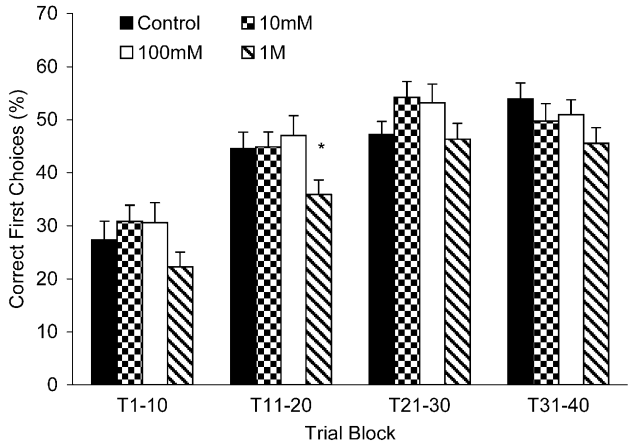

Fig. 6. Dose group mean (+S.E.) correct first-choice percentages during each of four trial blocks. * $P<.05$: 1 M group vs. 10 and 100 mM groups.

P=.3673], yet a significant main effect for replication number [$F(3,136)=3.254$; P=.0241]. No significant interactive effect was shown [$F(3,136)=1.355$; P=.2158]. Fisher's PLSD tests indicate one significant difference between replication pairings, R2 and R4 (P=.0021). No significant main or interactive effects were noted for T11–20: dose group [$F(3,136)=2.223$; P=.0888], replication number [$F(3,136)=1.009$; P=.3915], interaction [$F(9,123)=1.003$; P=.4411] or T31–40: dose group [$F(3,136)=1.135$; P=.3376], replication number [$F(3,136)=1.155$; P=.3300], interaction [$F(9,123)=1.115$; P=.3569]. Nevertheless, Fig. 7 illustrates an obvious difference in the number of errors committed by the high-dose group during T11–20. Yet, only the 100 mM group significantly differs from the 1 M group (P=.0130). Post hoc tests also reveal a significant difference in errors committed during T31–40 between the controls and the 1 M group. During T21–30 there was no significant main effect for dose group [$F(3,136)=0.236$; P=.8712] but there was for replication number[$F(3.136)=2.839$; P=.0408]. No significant interaction was seen for T21–30 [$F(9,123)=0.393$; P=.9365]. Significant T21–30 differences in errors between R2 and R3 (P=.0098) and between R2 and R4 (P=.0233) are denoted by Fisher's PLSD tests.

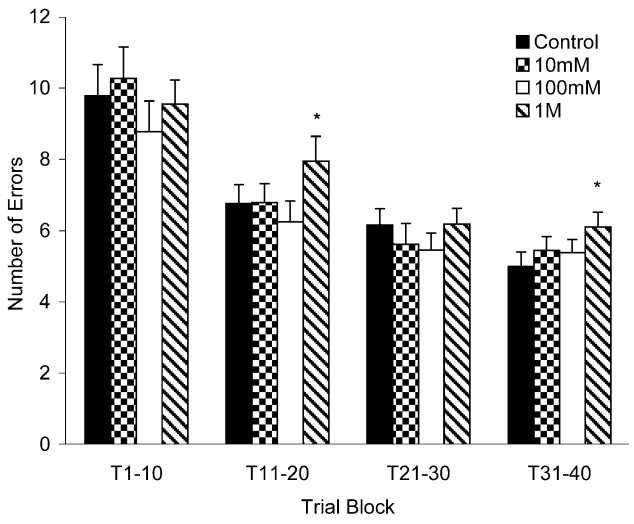

Fig. 7. Dose group mean (+S.E.) number of errors committed before acquiring reward during each of four trial blocks. * $P<.05$: 1 M group vs. 100 mM group during T11–20; 1 M vs. controls during T31–40.

Evaluation of the omission number parameter illustrated in Fig. 8 plainly indicates inferior place-learning performance for the 1 M group for each of the trial blocks. A two-factor ANOVA for T1–10 revealed no significant main effect for dose group [$F(3,136)=2.280$; P=.0827], yet a significant main effect for replication number [$F(3,136)=5.070$; P=.0024], and a significant interactive effect [$F(9,123)=2.196$; P=.0266] was found. However, Fisher's PLSD tests indicated significant T1–10 dose group differences between the 10 mM and 1 M groups (P=.0096) and a closely significant difference between the 10 and 100 mM groups (P=.0606). Post hoc tests also revealed significant differences during T1–10 between R1 and R3 (P=.0020), R1 and R4 (P=.0284), and R2 and R3 (P=.0058). Two-factor ANOVAs for the other three trial blocks specified no other significant main or interactive effects for dose group and replication number: T11–20, T21–30, and T31–40 dose groups [Fs(3,136)=1.686, 0.474, and 1.033; Ps=.1735, .7012, and .3804], T11–20, T21–30, and T31–40 replications [Fs(3,136)=1.705, 1.342, and 2.253; Ps=.1695, .2638, and .0855], T11–20, T21–30, and T31–40 interactions [Fs(3,136)=1.733, 1.658, and 1.001; Ps=.7163, .4050, and .2349].

Generally, the wide range of lithium dosages used in this investigation did not impair the subjects' motivation to perform the task nor did it compromise their locomotor abilities. Fig. 9 designates mean latencies to reward for each dose group during each trial block. A two-factor ANOVA indicates no significant dose group main effect for latency during T1–10 [$F(3,136)=1.057$; P=.3701], yet a significant main effect for replication was found [$F(3,136)=7.297$;

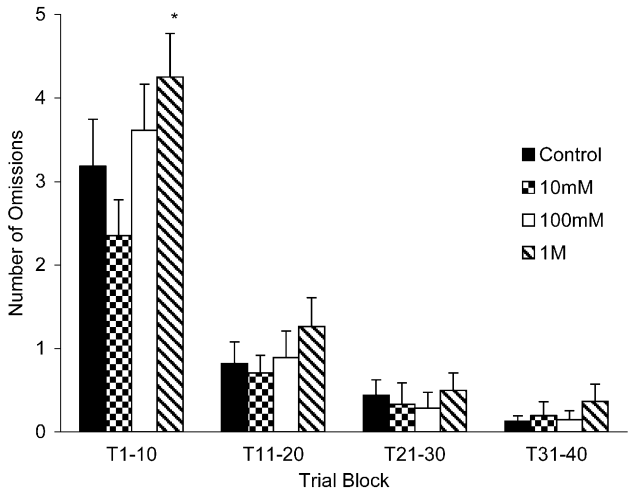

Fig. 8. Dose group mean (+S.E.) number of omissions during each of four trial blocks. * $P<.01$: 1 M vs. 10 mM groups.

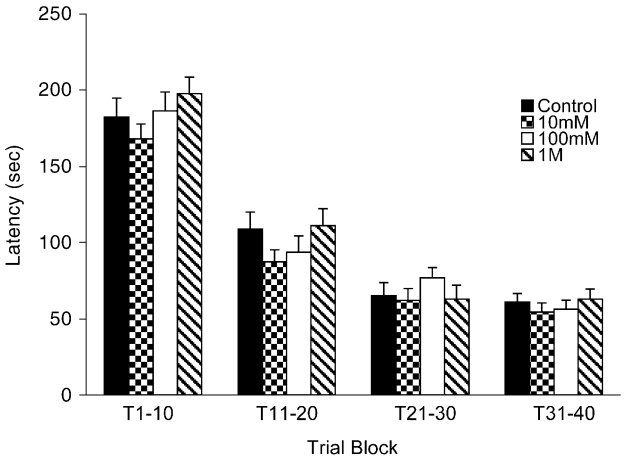

Fig. 9. Dose group mean (+S.E.) latencies to reward for each of four trial blocks. No significant differences between dose group pairings in latencies to reward were found within any of the four trial blocks suggesting that the lithium dosages utilized did not interfere with subject motivation to perform the task nor did they compromise subject locomotor ability.

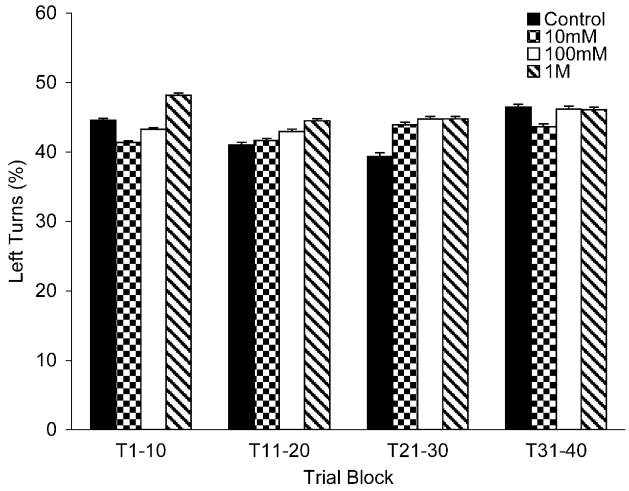

Fig. 10. Dose group mean (+S.E.) percentages of left turns committed at the choice area. Analyses indicate that, collectively, none of the dose groups exhibited a turn preference during the experiment. No significant differences between any dose group pairings within any of the trial blocks were found.

P=.0002]. No significant interactive effect is noted during T1–10 [$F(9,123)=1.401$; P=.1949]. Fisher's PLSD tests for the replication number factor indicate significant differences between R1 and R3 (P=.0002), R1 and R4 (P=.0158), R2 and R3 (P=.0006), and between R2 and R4 (P=.0297). A two-factor ANOVA indicates significant main effects for dose group [$F(3,136)=2.932$; P=.0362] and replication number [$F(3,136)=4.349$; P=.0060] during T11–20. However, Fisher's PLSD reveal no significant differences between any of the possible dose group pairings (P>.05). Significant replication number differences were detected between R1 and R3 (P=.0019), R2 and R3 (P=.0248), and R3 and R4 (P=.0440). No significant interactive effect was detected for T11-20 [$F(9,123)=1.442$; P=.1773]. No significant main or interactive effects were designated for T21-30 and T31-40, respectively: dose group [Fs(3,136)=1.164 and 0.896; P=.3263 and .4455], replication number [Fs(3,136)= 1.465 and 1.618; Ps=.2274 and .185], and interaction [Fs(9,123)=1.201 and 1.530; Ps=.3006 and .1445].

Because percentages of first correct choices were lower than expected, we were interested in whether the subjects were exhibiting turn preferences. Left turns were arbitrarily chosen for analysis. Results indicate the subjects did not exhibit turn preferences. Fig. 10 illustrates mean percent left turns negotiated at the central choice area of the maze for each dose group during each trial block as a measure of directional turn preference, i.e., left or right turns from the central area. Results of these analyses indicate none of the dose groups exhibited a turn preference for any of the trial blocks. For T1–10, T11–20, T21–30, and T31–40, respectively, no significant differences among dose groups [Fs(3,136)=1.364, 0.182, 0.642, and 0.049; Ps=.2570, .9083, .5896, and .9857], replication numbers [Fs (3,136)=0.880, 0.486, 1.206, and 1.631; Ps=.4534, .6927, .3104, and .1856], or their interactions [Fs(9,123)=1.051, 0.812, 0.832, and 0.541; Ps=.4040, .6061, .5882, and .8422].

Both plasma and brain lithium levels assume linear dose–response effects for the doses administered. Further analyses indicated neither body weight nor gender influenced plasma or brain levels; therefore, these factors were not accounted for during performance measure analyses. Fig. 11 depicts the linear relationship between plasma and brain lithium concentrations and dosages used in this investigation. Two-way ANOVAs of plasma lithium levels designate significant main effect differences for dose group [$F(3,136)=15.538$; $P<.0001$] but not for replication number [$F(3,136)=2.189$; P=.0953]. No interactive effect is

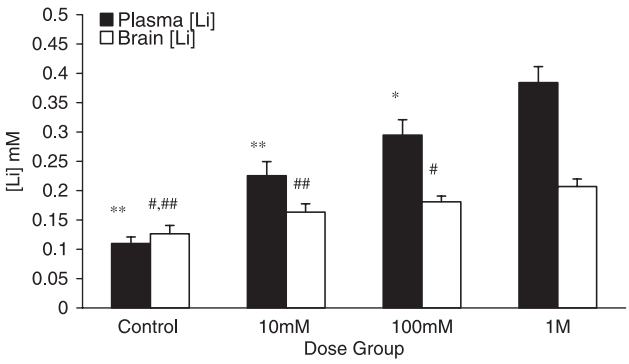

Fig. 11. Dose group mean (+S.E.) plasma and brain lithium concentrations representing a chronic (25-day) lithium regimen. Plasma and brain samples were analyzed for lithium levels via the capillary ion analysis (CIA) technique exactly as in Fig. 4 of the SA task. *$P<.05$: 100 mM vs. 1 M groups. **$P<.001$: controls vs. 10, 100 mM, and 1 M groups; 10 mM vs. 1 M groups. #$P<.05$: control vs. 10 mM groups; 100 mM vs. 1 M groups. ##$P<.005$: control vs. 100 mM and 1 M groups; 10 mM vs. 1 M groups.

indicated [$F(9,123) = 0.667$; $P = .7366$]. Fisher's PLSD tests show significant differences between controls and the 10 mM group ($P = .0005$), controls vs. the 100 mM and 1 M groups, and the 10 mM vs. the 1 M groups ($P < .0001$). There was also a significant difference between the 100 mM and 1 M groups ($P = .0238$) but not between the 10 and 100 mM groups $P = .0896$). Two-way ANOVAs conducted for brain lithium levels indicate significant main effect differences for dose group [$F(3,136) = 6.550$; $P = .0005$] and replication number [$F(3,136) = 9.138$; $P < .0001$] and a significant interactive effect [$F(9,123) = 2.566$; $P = .0116$]. Fisher's PLSD tests indicate significant differences between the controls vs. 1 M group ($P = .0022$), controls vs. 100 mM group ($P = .0022$), controls vs. 1 M group ($P < .0001$), 10 vs. 100 mM groups ($P = .0026$), and 100 mM vs. 1 M groups ($P = .0454$) but, as with the plasma levels, no significant difference between the 10 and 100 mM groups. Post hoc tests for replications indicate significant differences between R1 and R2, R3, and R4 ($P < .0005$) and between R2 and R3 ($P = .0196$).

Subjects used in this investigation had a relatively wide range of body weights (1.00–3.30 g; mean = 1.747 g). Results indicate that a uniform dose quantity (20 μl) was appropriate for all subjects. A two-way ANOVA comparing plasma lithium levels among three different body weight groups (1.00–1.50 g, 1.51–2.00 g, and >2.00 g) according to dose group indicated no significant main effect for body weight group [$F(2,137) = 0.343$; $P = .7102$] or an interactive effect between dose group and body weight group [$F(6,118) = 0.931$; $P = .4756$]. For brain lithium level comparisons, the two-factor ANOVA revealed a significant main effect for body weight group [$F(2,137) = 3.160$; $P = .0470$] and an interactive effect [$F(6,94) = 2.407$; $P = .0330$]. However, according to Fisher's PLSD tests, there were no significant differences between the body weight group pairings within respective dose groups ($P > .05$).

Two-factor ANOVAs comparing lithium levels between genders (male and female) of each dose group reveal no significant main gender effects for plasma [$F(1,138) = 0.154$; $P = .6950$] or brain [$F(1,138) = 0.004$; $P = .9522$] or the interactive effects between dose group and gender for plasma [$F(3,122) = 0.241$; $P = .8677$] or brain [$F(3,122) = 1.057$; $P = .3711$].

3.4. Discussion

Performance trends measured in this investigation suggest that a high dosage of lithium impairs LTM. Results from Experiment 1 suggest a more robust linear dose–response effect of lithium dosage on STM as assessed by the forced-choice SA task. The general trend of performance for the high-dose group indicated slower acquisition of the task and lower performance levels as indicated by fewer correct first choices and greater numbers of errors and omissions. This trend is generally significant within the T11–20 block when a heightened level of task learning may have occurred, yet is evident in the other trial blocks. Generally, subjects take some time to learn a task, i.e., as with T1–10, after which they acquire the task as with T11–20, reflected as heightened or increased rates of performance compared with those while learning the task. Because mean latency values within each of the trial blocks were similar among the four dose groups, neither motivation to perform the task nor locomotor abilities of the subjects were compromised by the lithium doses administered. Therefore, we are confident that our dosage range was appropriate for these euryhaline fish that can live in fresh and salt water. Capillary ion analysis results indicated that the mean plasma lithium level for the high-dosage group was just under the range of plasma lithium levels considered to be within the human therapeutic index for treatment of BD (0.5–1.0 mM). Fish and mammalian plasma lithium levels may not be comparable given the differences in ionic pump systems with which the two animals are equipped. However, group mean plasma and lithium levels for this investigation were highly consistent with those of Experiment 1 utilizing the same dosing regimen.

4. General discussion

Regardless of the mechanism proposed for solving allocentric reference frame problems, damage to the limbic system of higher vertebrates, particularly to the hippocampal formation, is generally considered to impair place-learning tasks, which require encoding of relationships among multiple environmental features (Hollup et al., 2001; Jarrard, 1993; Morris et al., 1982; O'Keefe et al., 1975; Olton et al., 1979; Rodriguez et al., 2002; Sutherland and Rudy, 1989). Fishes are not equipped with a limbic system like that of amniotes. However, several investigators contend there are structural homologies between various forebrain regions of ray-finned fishes and limbic system components of amniotes (Braford, 1995; Butler, 2000; Echteler and Saidel, 1981; Northcutt and Braford, 1980). Ohnishi (1997) has shown that telencephalic-ablated goldfish are impaired relative to controls in an STM task utilizing a Y maze training paradigm. Salas et al. (1996) has demonstrated that telencephalic-ablated goldfish are impaired in place-learning strategies. The same group (Lopez et al., 2000; Rodriguez et al., 2002) has recently shown that goldfish with lateral, but not medial or dorsal, telencephalic ablations are impaired in similar place-learning strategies. Neurohistochemical work in our laboratory confirms that the black molly possesses a central nervous system representative of the ray-finned fishes.

Our working hypothesis with the black molly model suggests that chronic lithium administration down-regulates 5-HT_{1A} receptors leaving serotonergic neuronal firing unchecked in caudal midbrain raphe nuclei (Friedman and Wang, 1988; Goodwin, 1989; Goodwin et al., 1986; Hotta

and Yamawaki, 1986; Hotta et al., 1986; Odagaki et al., 1990; Price et al., 1990). In turn, forebrain terminal areas are bombarded with excess 5-HT release, which is thought to impair cognition. There is ample evidence demonstrating a role for the 5-HT_{1A} receptor in memory (Bertrand et al., 2000; Buhot, 1997, 2000; Meneses and Hong, 1997; Ohno et al., 1993; Sirvio et al., 1994; Warburton et al., 1997; Winter and Petti, 1987). Considerable debate has been generated whether lithium and other mood stabilizers adversely affect cognition through either a presynaptic or postsynaptic 5-HT_{1A} receptor down-regulation mechanism. Our animal model may offer an advantage in addressing this question because teleosts are not equipped with a postsynaptic 5-HT_{1A} receptor system typically found in limbic systems and frontal cortical regions of the mammal. Future studies will compare the effects of lithium and combinations of 5-HT_{1A} agonists and antagonists on 5-HT_{1A} receptor numbers in the black molly brain and their relation to STM and LTM impairments.

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