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Research report

Enhancing effects of chronic lithium on memory in the rat

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Abstract

Background: In spite of recent enrichment of neurochemical and behavioural data establishing a neuroprotective role for lithium, its primary effects on cognitive functioning remain ambiguous. This study examines chronic lithium effects on spatial working memory and long-term retention.
Methods: In three discrete experiments, rats subjected to 30 daily intraperitoneal injections (2 mmol/kg) of lithium (lithium groups: serum lithium = 0.5 ± 0.4 mEq/l, 12 h post-injection) or saline (controls) were trained in 0-s delay T-maze alternation and then tested in 30-, 45- and 60-s delay alternation (Experiments 1, 2, 3, respectively). Animals from Experiment 1 were further tested in one-trial step-through passive avoidance under mild shock parameters (0.5 mA, 1 s). Retention was assessed 6 h later. Daily lithium or saline injections continued throughout behavioural testing.
Results: Lithium animals were indistinguishable from controls during 0-delay alternation baseline (Experiments 1–3, accuracy > 88%) but showed significantly higher accuracy than controls at 30- and 45-s delays (93% versus 85% and 92% versus 82%, Experiments 1 and 2, respectively). At 60-s delay (Experiment 3) this beneficial effect of lithium was no longer apparent (lithium and control accuracy = 78%). In Experiment 4, the shock used did not support 6-h passive avoidance retention in controls, whereas lithium animals showed significant step-through latency increases.
Conclusions: Chronic lithium enhanced spatial working memory and promoted long-term retention of a weak aversive contingency. The results suggest that lithium may have potential as a cognitive enhancer.

Keywords: Lithium; Working memory; Passive avoidance retention; T-maze alternation; Rat

1. Introduction

The mood-stabilizing agent lithium is the drug of choice in the treatment of and prophylaxis against both mania and depression in bipolar disorder [1–5]. In addition to its established role as a mood stabiliser, a plethora of recent findings attribute a neuroprotective [6–11] and an antiapoptotic [12,13] role to lithium.

The neuroprotective effect of lithium, which has been associated with long-term administration of therapeutic levels of the substance [9] raises expectations regarding its potential as a prophylactic agent against cognitive decline. However, early clinical reports linked lithium treatment to cognitive blurring and memory deficits (for review see [14]). More recent

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neuropsychological testing has yielded ambiguous results, associating lithium treatment with subjective complaints but not with actual impairments in terms of performance accuracy [15]. There have been several studies which tested the effects of lithium treatment on different types of memory, in psychiatric (mainly bipolar) patients [16–21], in bipolar patients compared to healthy volunteers [22–25] and in normal subjects [26–29]. The latter studies have the advantage of isolating the neuropsychological effects of lithium from those of bipolar disease processes.

In terms of lithium effects on short-term verbal memory performance, several studies report that lithium treatment caused deficits in immediate recall [16–18,20,22–25,28,29] while only three studies failed to replicate this finding [19,21,26].

In most studies investigating lithium effects on long-term verbal memory lithium was found to have a negative impact on delayed recall tasks [16,17,20,23–25,28]. Nevertheless, according to Pachet and Wisniewski [15] who reviewed these studies, the trend toward verbal memory impairment in the lithium-treated population was relatively weak and replications are needed to confirm it.

In contrast to its reported effects on verbal memory, no significant effects of lithium emerge in the area of visual memory. The immediate form of visual memory tasks was reported unimpaired by lithium administration in most relevant studies [18,21,23,25]. A single study [16] reported transient impairments in visual memory which dissipated following discontinuation of lithium. With respect to delayed recall in visual memory tasks, one study [18] reported no lithium-associated decrease in performance, whereas a later study [16] found reversible lithium-induced deficits.

The animal literature on the cognitive effects of lithium presents similar inconsistencies. Older studies suggest that lithium induces cognitive deficits, which they attribute to a narrowing of attentional filtering onto high salience stimuli. Hines and Poling [30] reported hindered acquisition of passive avoidance with no effect on active avoidance after lithium pretreatment. Hines [31,32] observed deficits in the acquisition of position discrimination and compromised shock-induced activity suppression in the open field. Cappeliez and Moore [33] and Cappeliez et al. [34] reported increased attention to high salience cues and impaired latent inhibition in lithium-treated rats.

Recent animal studies on the effects of lithium in various memory tasks are sparse. A transient deficit in spatial reference but not working memory was observed in the hole board task after chronic lithium [35]. The authors attributed this deficit to the suppressive effect of lithium on the basal expression of the immediate-early gene Nurr1 (implicated in neuronal plasticity) in the hippocampus. However, Vasconcellos et al. [36], investigating the effects of chronic lithium treatment on reference and working memory in a chronic stress model, reported no effect of lithium on reference memory in the water maze. Furthermore, they showed that the reference memory deficit induced by chronic stress was attenuated by lithium treatment. In the same study, neither stress nor lithium had any effect on working memory. The only recent study suggesting that chronic lithium impairs spatial working and reference memory used spontaneous alternation in a plus maze in black molly fish [37].

In summary, recent human studies suggest that chronic lithium causes subtle negative effects on psychomotor speed and verbal memory but no impairment on visuospatial skills, attention or concentration [15]. Animal studies suggest some behavioural deficits in active avoidance and visually cued discrimination learning, while spatial learning and memory appear to be transiently if at all impaired, or even protected by lithium pretreatment [36]. These recent findings are more in line than older studies with the biochemical literature which establishes a neuroprotective role for chronic lithium treatment. They are also in line with some early findings of our laboratory, which suggested a beneficial effect of chronic lithium on working memory in the rat [38], thus raising the possibility that lithium may act as a cognitive enhancer. This hypothesis was further investigated in the series of experiments reported here. These experiments assessed the effects of chronic lithium, at doses sustaining lithium plasma levels within the human clinical range, on spatial working memory and long-term retention.

2. Materials and methods

Experiments 1 and 4 were carried out in the Experimental Psychology laboratory, Institute of Psychiatry, University of London [38] while Experiments 2 and 3 were conducted in the Experimental Psychology laboratory, Department of Psychiatry, University of Athens Medical School. For this reason Experiments 1–3, all of which involve delayed alternation differing in delay length only (30-, 45- and 60-s, respectively) were carried out as discrete procedures rather than as a repeated-measures alternation procedure with varying delays. For the same reason the reader will note differences in rat strains and lithium suppliers used. Given the consistency of our findings, these procedural differences, as well as the fact that Experiment 2 constitutes a replication of our basic finding reported in Experiment 1, strengthen our results.

2.1. Subjects

Rat strains and group *n*'s for Experiments 1–3 are shown in Table 1. Experiment 1 included 14 naïve adult male Sprague–Dawley rats (Harlan Olac Ltd., Bicester). These same animals were then tested in Experiment 4. They were housed four per cage under a 14–10 h light cycle (lights on at 06.00), at 22–24 °C and maintained on Grain Harvesters Ltd. Rat and Mouse Diet. Experiments 2 and 3 each included 23 naïve adult male Wistar rats (Pasteur Institute of Athens). A separate group of 24 animals (from the litters of Experiments 2 and 3) were used for biochemical assessment of serum lithium levels after acute and chronic lithium administration. The total of 70 animals was housed in triads under a 12 h light cycle (lights on at 07.00), at 24–260 °C and maintained on Mucedola s.r.l 4RF18 Standard Diet.

All subjects were habituated to their respective animal rooms for at least 3 weeks, on ad libitum food. Ad libitum water was available throughout the course of the experiments. A week before the onset of behavioural training animals were placed on a 23-h food deprivation schedule, feeding for an hour after behavioural training. Having noted some weight loss in lithium chloride (LiCl)-treated animals compared to saline controls during LiCl pre-treatment we resorted in housing LiCl animals together, monitoring their weight daily and allowing extra feeding time to cages showing undue weight loss. As a result LiCl and saline controls' weights were comparable at the onset of behavioural training, ranging between 260 and 320 g (Experiments 1 and 4), 280–320 g (Experiment 2) and 250–310 g (Experiment 3). Physiological saline was available ad libitum in all home cages, so LiCl animals could replenish NaCl stores. Under these conditions animals remained in good health throughout the experiments.

Table 1
Drug groups (lithium chloride: LiCl; saline: Sal) and rat strain (SD: Sprague–Dawley, W: Wistar) used in the T-maze alternation experiment series (Experiments 1–3) and in the accompanying biochemical procedure

T-maze experiment (delays used)	Drug group	Group *n*'s (rat strain)	0 s delay baseline				Delay phase		
			Trials to criterion (mean)	Phase trials	% of animals to reach criterion	Mean accuracy (%)	Phase trials	% of animals to reach criterion	Mean accuracy (%)
1 (0 s, 30 s)	LiCl	8 (SD)	95	152	100	86	120	100	93
	Sal	6 (SD)	65		100	88		50	85
2 (0 s, 45 s)	LiCl	12 (W)	72	104	100	91	136	100	92
	Sal	11 (W)	62		100	92		54	82
3 (0 s, 60 s)	LiCl	11 (W)	87	168	100	89	128	18	78
	Sal	12 (W)	102		100	83		16	78
Biochemical procedure	LiCl (acute vs. chronic)	24 (W)	(no behavioural training)						

The delays used in each experiment along with mean accuracy rates and group percentages that achieved the accuracy criterion (5 days, accuracy > 85%) in each delay phase are shown.

2.2. Apparatus

2.2.1. Experiments 1–3

Identical wooden, flat grey T-mazes placed in a fixed position in the experimental rooms were used for Experiments 1 and 2–3. The experimental rooms were illuminated by fluorescent ceiling lights. The mazes stood 120 cm above floor surface. Their stem measured 90 cm long × 10 cm wide. The first 20 cm of the stem acted as the start area, being separated from the main maze by a guillotine Plexiglas door. The cross arm measured 140 cm long × 10 cm wide and had two reward cups fixed on the floor 2 cm from each end. The reward cups were opaque, 2 cm in diameter and 0.75 cm deep so that visual detection of reward from a distance was not possible. The maze was wiped clean with alcohol after each run.

2.2.2. Experiment 4

A standard 2-compartment Campden Instruments step-through shuttle box (each compartment measuring 22 cm wide × 24 cm long × 20 cm high) was used. Foot shock (0.5 mA, 1 s) was administered through the grid floor by a Campden Instruments shock source (CI 521C) and scrambler (CI 521S). One compartment was darkened by black cardboard coating, the other was amply illuminated (60 W overhead bulb).

2.3. Pharmacological procedure

In Experiments 1–3 (30-, 45- and 60-s delayed alternation, respectively) animals received daily i.p. injections of either physiological saline or LiCl dissolved in saline for 30 days before the onset of behavioural training. Daily injections continued throughout behavioural training and were administered 30 min before training onset. Therefore animals in Experiment 4 (which had previously participated in Experiment 1) had already received 70 daily injections of either saline or LiCl by the onset of their behavioural training. The daily dose of LiCl was 84.7 mg/kg (2 mmol/kg). All injection volumes were 1 ml/kg. For Experiments 1 and 4 (LiCl group $n = 8$, saline $n = 6$) LiCl was purchased from SIGMA (L4408), in Experiments 2 and 3 it was obtained from PANREAC (Montplet & Esteban, Barcelona, Spain, 141392) (Experiment 2: LiCl $n = 12$, Saline $n = 11$; Experiment 3: LiCl $n = 11$, Saline $n = 12$). The 24 animals which were used for the assessment of serum lithium levels also received 30 daily saline or lithium injections (as described below) and did not participate in the behavioural experiments.

2.4. Biochemical procedure

The purpose of examining LiCl serum levels after acute and chronic treatment was to ensure that our administration regime did not result in accumulation of the substance, which might cause increased serum levels over days and, possibly, deterioration of animals' health. The 24 animals included in this procedure received either 30 daily lithium injections (chronic administration) or 29 saline injections followed by a single LiCl injection (acute administration). At the end of the injection course they were sacrificed by cervical fracture, decapitated, and blood samples were collected from the trunk. Samples from acutely ($n = 14$) and chronically ($n = 10$) treated animals were obtained either 1 h ($n = 7$ and 3, respectively), 12 ($n = 4$ in both cases) or 24 h ($n = 3$ in both cases) after the last LiCl injection. LiCl serum levels were determined by ion selective electrodes (ISE direct, Bayer 600 series).

2.5. Behavioural procedure

2.5.1. Experiments 1–3

2.5.1.1. The behavioural task. The task was delayed alternation in the T-maze, a non-matching to place procedure commonly used in the study of working memory [39-41].

2.5.1.2. Habituation. All animals had received daily handling for a week before entering the 30-day injection course. After the 30th injection day they were given 5 daily T-maze habituation sessions: the maze was initially loaded throughout with reward (cereal Coco Pops), which was gradually restricted to the reward cups.

2.5.1.3. Acquisition of 0-s delay alternation. Task acquisition began the next day. For the first 2 days each animal received 2 daily alternation trials, incremented to 4 trials for the next 3 days and to 8 daily trials thereafter. Each trial consisted of two stages: an "information" run and a "test" run. Both reward cups of the maze were baited before the onset of a trial. In the information run, one arm of the maze was blocked with a wooden barrier (according to a daily pseudorandom sequence: four left and four right information runs with a maximum of two consecutive ones in the same direction). The rat was placed in the start area, back towards the closed guillotine door. The experimenter would then raise the door and the rat was allowed to run to the end of the accessible arm. As soon as the animal reached the end and consumed the reward the experimenter moved it back to the start area, removed the wooden barrier and raised the guillotine door to allow the rat to run to the choice point for the test run. The delay between the end of the information run and the beginning of the test run was approximately 5 s. On the test run both arms were open, and the rat was allowed a free choice. The test run was completed when all paws of the animal were in one of the lateral arms. Thereafter, change was prevented with the wooden barrier. If a correct choice was made, i.e. if the rat entered the arm opposite to that visited on the information run, the rat was allowed to eat the reward before being removed from the T-maze. If an incorrect choice was made the rat was confined to the arm without food reward for 10 s before being removed from the T-maze. Each daily session consisted of eight trials, and rats were tested in groups of three (for Experiment 1) or four (for Experiments 2 and 3) with each rat having one trial in turn. The resulting intertrial interval was approximately 2 min. Between trials

rats were kept singly in a wooden, compartmentalised holding box. Alternation training continued until every rat reached a criterion of 7/8 trials correct per day for 5 consecutive days. In Experiment 1, once an animal reached criterion it was excluded from the daily training until all others also reached it, in order to avoid overtraining. A 3-day 0-s delay 'reminder' phase was given to all animals before the next (30-s delay) phase. Nevertheless, the break in training experienced by the animals which reached criterion quickly was not experienced by the 'slower' learners. To assess possible effects of this break, in Experiment 2 all rats kept running until the last one reached criterion and the next phase (45 s delay) started immediately after. The consistency of results across Experiments 1 and 2 was taken as evidence that overtraining or the break in training between 0-s delay and delayed alternation phases did not influence subsequent performance. The less laborious procedure of Experiment 1 was therefore adopted for Experiment 3. It took 152, 104 and 168 trials for all animals of Experiments 1, 2 and 3, respectively, to reach the 5-day criterion. One rat from Experiment 2 and one from Experiment 3 showed preservation, could not reach criterion and were excluded.

2.5.1.4. Training in delayed alternation. This was identical to 0-delay training, apart from the interposition of a delay between information and test runs. Timing of the delays with a stop-watch began with reward consumption in the information trial, upon which animals were returned to the holding box, to be replaced on the start point 5 s before delay completion. The guillotine door opened for the test run at the end of the interval exactly. Delay intervals were 30-, 45- and 60-s for Experiments 1–3, respectively. The delayed alternation phase continued until all LiCl animals included in the experiment had reached the 5-day criterion, i.e. for 120 trials in Experiment 1 (30-s delay) and 136 trials for Experiment 2 (45 s delay). In Experiment 3 (60-s delay) where both groups were clearly unable to attain criterion, training stopped after 128 trials.

2.5.2. Experiment 4

2.5.2.1. The behavioural task. The task was single trial step-through passive avoidance in the shuttle box. This procedure utilizes the natural preference of rats for dark spaces, which drives them to move from a well-lit compartment of a shuttle box to the darker one with a low (baseline) latency. The procedure lends itself to the study of long-term retention, which is assessed in terms of increases in step-through latency over baseline several hours after a single pairing of the step-through response with foot shock [42]. Accordingly, the experiment had three stages:

- *Baseline latency recording*. LiCl and saline animals were each placed in the lit compartment of the shuttle box facing the wall opposite the passage to the dark compartment. Their latency to cross over to the dark compartment was recorded.
- *Conditioning*. Three seconds after entry to the dark compartment with all four paws each animal was given a single, mild shock (0.5 mA, 1 s). It was then returned to the home cage and the shuttle box was cleaned with alcohol to remove odours before testing the next subject.
- *Retention Test*. Six hours after the conditioning phase each animal was replaced in the lit shuttle box compartment and its step-through latency was again recorded. If an animal failed to step through for a criterion latency of 180 s it was removed from the lit compartment and returned to the home cage.

2.6. Statistical analysis

Analyses were carried out using the STATISTICA for Windows statistical package (1999, version 5.5, Statsoft Inc., Tulsa, Oklahoma).

2.6.1. Biochemical data analysis

Biochemical data, expressed as Li+ mEq/l, were analysed by a 2-way ANOVA (pre-treatment length: acute versus chronic LiCl; post-injection time: 1, 12 and 24 h).

2.6.2. Behavioural data analysis

Data were collected as follows: in 0-s delay phases, we recorded total errors for each rat and calculated individual % success rates. Additionally, we recorded total number of responses to 0-delay criterion (5 days at 7/8 correct responses).

In delayed alternation phases, % success rates were calculated on the basis of errors made per phase response opportunities (120, 136 and 128 trials for Experiments 1, 2 and 3, respectively).

Since both rewarded alternation and step-through passive avoidance involved cut-off criteria (7/8 correct responses for 5 days and 180 s in the bright shuttle box compartment, respectively), non-parametric analysis was used on the behavioural data [43]. Accordingly, the data are depicted as medians and confidence intervals in the associated graphs. Where Mann–Whitney *U*-tests were employed, *Z*-values given are adjusted for unequal *n*'s and the probabilities quoted are exact probabilities computed for small samples.

2.6.3. Experiments 1–3

Each of these experiments was analysed in phases corresponding to the delays used [Phase 1: 0-delay alternation and Phase 2: delayed alternation for Experiments 1 (30-s delay) and 2 (45 s delay)]. In Experiment 3, where rats could not reach Phase 2 (60-s delay) criterion, an additional 0-delay reminder phase (Phase 3) was added at the end to ensure that alternation performance had not deteriorated for some extraneous reason beyond the use of a long delay in Phase 2. Mann–Whitney *U*-tests were used to compare lithium chloride-treated (LiCl) to saline groups in each delay condition of individual experiments.

An additional analysis was carried out on the measure of Trials to 0'delay Criterion, on the combined data of Experiments 1–3 (2-way ANOVA, drug treatment × experiment).

2.6.4. Experiment 4

Step-through baseline and retention test latencies (in s) were recorded. Baseline and retention latencies of LiCl and saline groups were compared through separate Mann–Whitney *U*-tests. Repeated measures within pharmacological group were compared through sign-tests.

3. Results

3.1. Serum lithium levels following acute or chronic LiCl treatment

Serum lithium levels sustained by a daily dose of 2 mmol/kg i.p. are shown in Fig. 1. There was no evidence of lithium accumulation after chronic treatment, as indicated by the lack of difference in serum lithium levels produced by a single LiCl injection preceded either by 29 saline or by 29 LiCl daily injections (factor of pre-treatment length = NS). Plasma levels did change significantly over post-injection time (factor of post-injection time: $F(2, 18) = 34.15$, $p < 0.001$). The dose of 2 mmol/kg produced peak serum levels of 1.7 ± 0.4 mEq/l within the first hour. Lithium concentration had fallen to a mean of 0.5 ± 0.4 mEq/l 12 h later and to a minimum of 0.4 ± 0.4 mEq/l 24 h post-administration.

3.2. Effects of chronic lithium administration on spatial alternation

3.2.1. 0-s delay alternation

In Experiments 1–3 (Figs. 2a, 3a and 4a, respectively) animals chronically treated with LiCl did not differ from saline controls in 0-s delay alternation acquisition in terms of overall success rates (Mann–Whitney *U*-tests: Experiment 1: $U = 21.000$, Z adjusted $= -0.38901$, LiCl $n = 8$, saline $n = 6$; Experiment 2: $U = 46$, Z adjusted $= -0.636661$, n's $= 12, 11$; Experiment 3: $U = 36.5$, n's $= 11, 12$, Z adjusted $= 1.817396$). Furthermore,

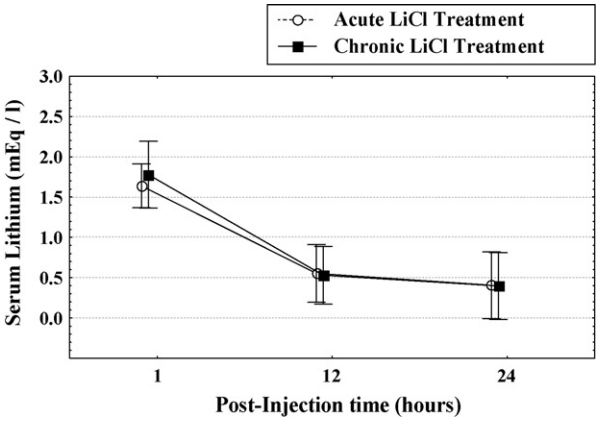

Fig. 1. Group means and standard errors of serum lithium levels after acute and chronic lithium chloride treatment (LiCl: 2 mmol/kg, intraperitoneally). Acute treatment consisted of 29 daily saline injections followed by a single LiCl injection ($n = 14$), chronic of 30 daily LiCl injections ($n = 10$). Samples were obtained 1, 12 or 24 h after the last injection ($n = 10$, 8 and 6, respectively). The factor post-injection time was significant [$F(2, 18) = 34.15$; $p < 0.001$]. The observed decline of serum lithium concentrations over time is in agreement with human pharmacokinetic studies and the serum concentrations noted were within the clinical therapeutic window of lithium. There were no differences between acute and chronic LiCl treatment conditions.

there was no difference in the number of trials taken by LiCl-treated and control animals to reach the 0-delay 5-day 7/8 correct criterion [drug treatment main effect: $F(1, 52) = 0.987$; drug treatment × experiment: $F(2, 52) = 1.637$, NS: Table 1].

3.2.2. Delayed alternation

In Experiments 1 and 2 (30- and 45-s delayed alternation respectively; Figs. 2b and 3b) the LiCl groups were significantly more accurate than saline controls (Mann–Whitney U-tests: Experiment 1: $U = 0.00$, LiCl $n = 8$, saline $n = 6$, Z adjusted = 3.0984, 2 × 1 sided exact $p = 0.0017$; Experiment 2: $U = 5.50$, n's = 12, 11, Z adjusted = 3.494774, 2 × 1 sided exact $p = 0.000108$).

In contrast, in Experiment 3 (60-s delayed alternation; Fig. 4b) there was no differentiation between LiCl and saline groups (Mann–Whitney U-test, $U = 57$, LiCl $n = 11$, saline $n = 12$, Z adjusted = 0.555423, NS). In this experiment, a brief return to 0-s delay baseline conditions (6 days, 48 trials) resulted in full recovery of response accuracy in both pharmacological groups (Fig. 4c).

3.3. Effects of chronic lithium on one trial step-through passive avoidance

Chronic LiCl and saline groups had comparable baseline step-through latencies (Mann–Whitney U-test: $U = 17.5$, Z adjusted = 0.840997, n's = 8, 6, NS; Fig. 5a). However the LiCl group showed significantly higher latencies than those of saline controls in the retention test given 6 h later (Mann–Whitney U-test: LiCl $n = 8$ and saline $n = 6$; Fig. 5b). A sign test comparing baseline saline control latencies to their latencies during the retention test showed that the small latency increase they demonstrated was not significant (sign test: no. of non-ties = 6, $\%v < V = 33.333$, $Z = 0.408248$, $p = 0.68$). In contrast, the latency increase between baseline and retention test observed in the LiCl animals was significant (sign test: no. of non-ties = 8, $\%v < V = 100.0$, $Z = 2.4749$, $p = 0.0133$).

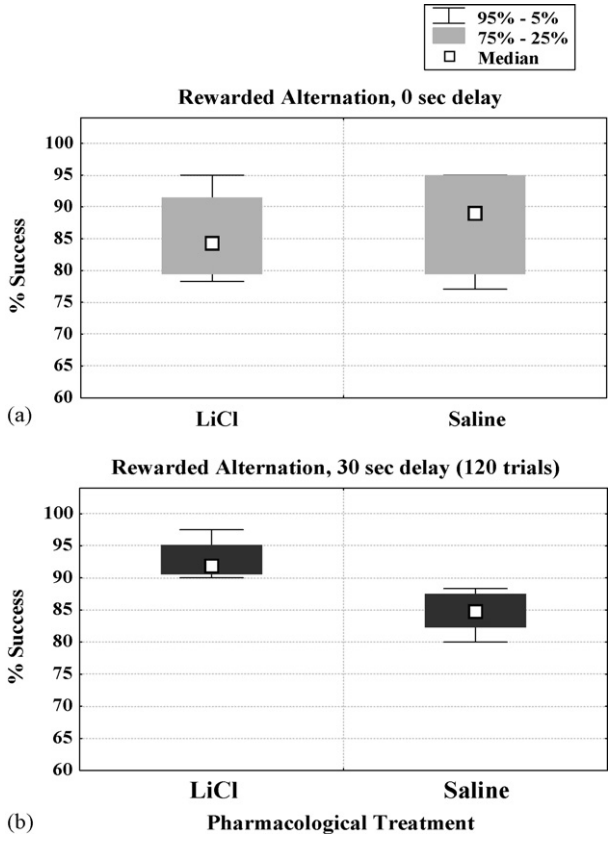

Fig. 2. Effects of chronic lithium chloride treatment (LiCl: 2 mmol/kg daily, intraperitoneally) on working memory (Experiment 1). Data are shown as group medians and percentiles. (a) Success rates of the LiCl group ($n = 8$) and saline controls ($n = 6$) were comparable during 0-s delay alternation in the T-maze [$U(8, 6) = 21$, Z adjusted = −0.38901, NS]. (b) The significant difference of the pharmacological groups in the subsequent, 30-s delayed alternation phase [$U(8, 6) = 0.00$, Z adjusted = 3.0984, 2 × 1 sided exact $p = 0.00067$] reflects superior accuracy of the LiCl-treated group upon increased demand from the working memory system.

4. Discussion

This study addressed the controversy emerging from human and animal studies with respect to the effects of lithium on cognitive functioning, memory in particular. We examined the effect of chronic lithium on basic memory processes, taking special care to use doses which would sustain serum lithium levels within the clinical therapeutic window and would not lead to tissue accumulation of the substance after prolonged administration, with possible detriment to animals' general state of health. As seen in Fig. 1, both these requirements were met: chronic treatment with 2 mmol/kg i.p. daily sustained stable serum lithium levels after one single or the last of 30 consecutive daily administrations, with no evidence of lithium accumulation. These levels were within clinical therapeutic window values [44].

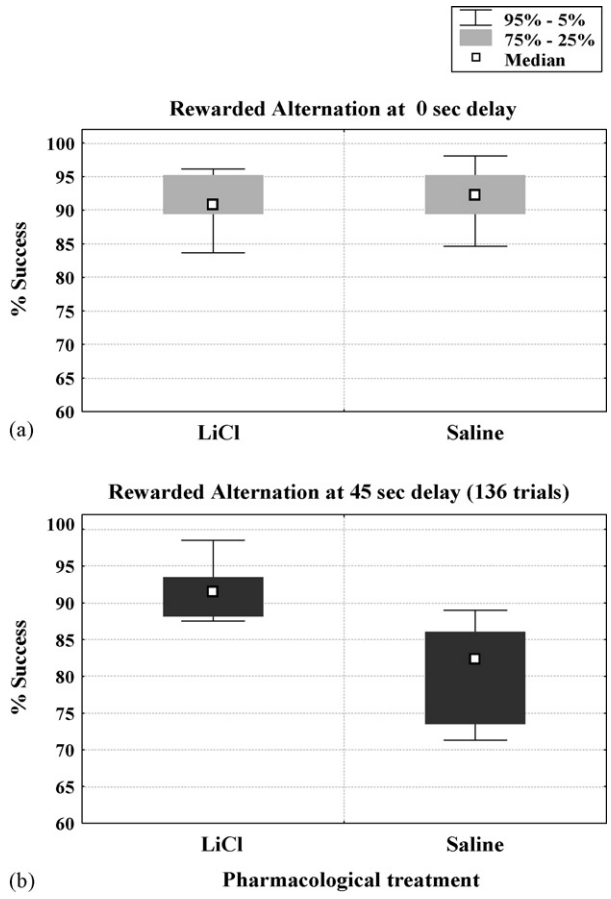

Fig. 3. Effects of chronic lithium chloride treatment (LiCl: 2 mmol/kg, intraperitoneally) on working memory (Experiment 2). Data are shown as group medians and percentiles. (a) Success rates of the LiCl ($n = 12$) and the saline control group ($n = 11$) were comparable during 0-s delay alternation [$U(12, 11) = 46$, Z adjusted = −0.636661, NS]. (b) At 45-s delay, LiCl animals demonstrated significantly higher success rates than saline controls, due to deterioration in the performance of the latter group [$U(12, 11) = 5.50$, Z adjusted = 3.494774, 2×1 sided exact $p = 0.000108$]. The finding replicates that of Experiment 1, at 30-s delay.

At this point, the issue of lithium's well-documented effects as a nausea and taste-aversion inducing agent must be addressed, since it could have directly affected the appetitively motivated T-maze task; while in the step-through passive avoidance procedure, if the rats were experiencing sickness during the test, then the aversive properties of lithium may have sensitised them to the aversive properties of the shuttle box, resulting in enhanced passive avoidance. It is unlikely, however, that our daily dose of 84.7 mg/kg i.p., which is much lower than the dose usually employed in taste aversion studies ([45]: 127 mg/kg i.p.) produced sickness. Our dose sustained Li+ levels in the viscinity of 0.5 mEq/l 12 h post-injection, comparable to those used in another recent behavioural study [36]: as reported there, our animals also were healthy and normally active during lithium administration, showing none of the distress symptoms associated with the taste aversion paradigm [46]. The claim that our LiCl dose did not induce sickness and taste aversion is further supported by our finding that LiCl did not affect 0-delay alternation acquisition, as shown by the lack of difference between LiCl-treated and control animals in the number of trials taken to reach the stringent 5-day, 7/8 correct criterion used to signal full acquisition of 0-delay alternation.

4.1. Effects of chronic LiCl on 0-s delay spatial alternation

We have shown that chronic lithium treatment does not affect the acquisition of 0-s delay alternation. Given that this finding was replicated three times and in two different rat strains (Experiments 1–3, Figs. 2a, 3a and 4a, respectively) it constitutes strong evidence that chronic lithium at clinical doses does not affect

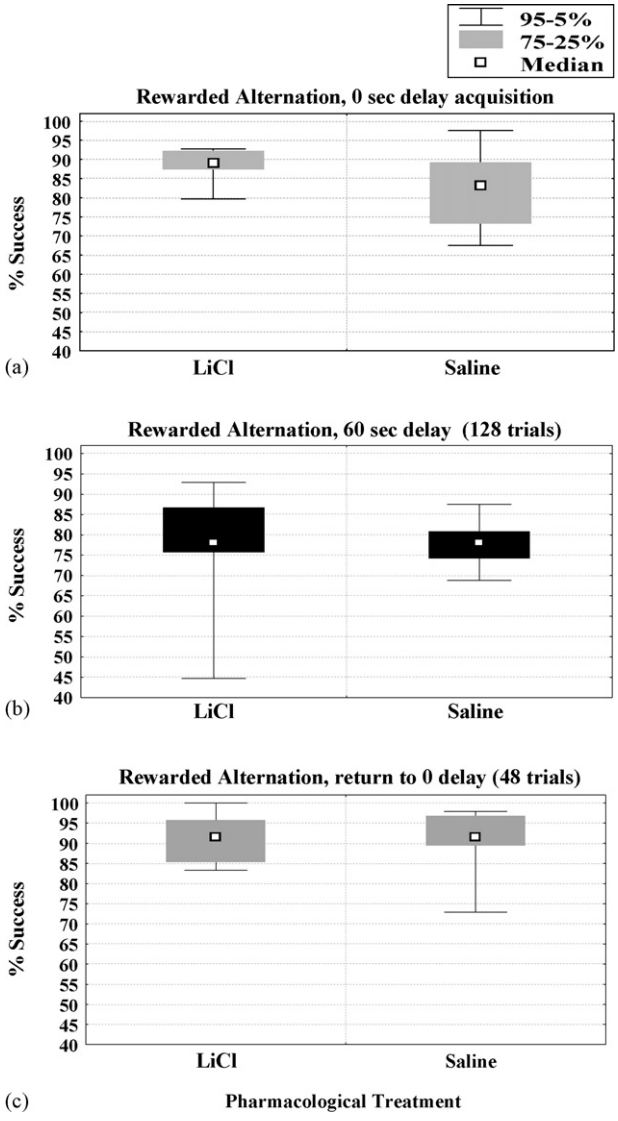

Fig. 4. Effects of chronic lithium chloride treatment (LiCl: 2 mmol/kg, intraperitoneally) on working memory (Experiment 3). Data are shown as group medians and percentiles. (a) LiCl and saline treated groups did not differ in success rates during 0 s delay alternation baseline [$U(11, 12) = 36.5$, Z adjusted = 1.817396, NS]. (b) At 60 s delay, the LiCl group did not show the superior to control accuracy [$U(11, 12) = 57$, Z adjusted = 0.555423, NS] observed in Experiments 1 and 2 (30 and 45 s delay, respectively). (c) Upon return to 0 delay, both LiCl and saline group success rates returned to baseline levels [$U(11, 12) = 66$, Z adjusted = 0000, NS].

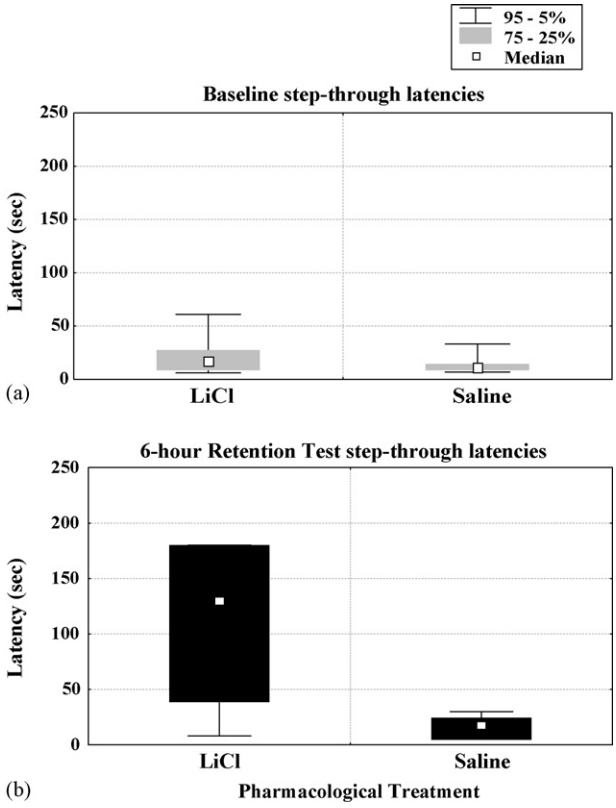

Fig. 5. Effects of chronic lithium chloride treatment (LiCl: 2 mmol/kg, >70 days) on step-through passive avoidance retention (Experiment 4). Data are shown as group medians and percentiles. (a) LiCl animals ($n = 8$) were indistinguishable from saline controls ($n = 6$) on baseline step-through latencies in the shuttle-box [Mann–Whitney U-test: $U(8, 6) = 17.5$, Z adjusted = 0.840997, NS]. (b) 6 h after a single, mild shock (0.5 mA, 1 s) paired with the baseline step-through response, saline controls did not show passive avoidance, sustaining their baseline step-through latencies. The LiCl group differed significantly [$U(8, 6) = 5$, Z adjusted = 2.48309, 2×1 sided exact $p = 0.013$], showing good retention of the aversive association.

acquisition of a non-matching to place rule. It also suggests that chronic lithium treatment does not affect reference memory. Reference memory is operationally defined as memory for features which remain invariant for at least several trials, while working memory is memory for trial-unique stimuli or events [47]. While one could argue that T-maze alternation is not the ideal task for differentiating reference from working memory, it nevertheless incorporates a reference memory component, namely the acquisition and retention of a non-matching to place rule during the 0-s delay training phase of our experiments. Therefore, the normal acquisition of that rule can be taken to reflect intact reference memory processes [40]. This finding is consistent with that reported by Vasconcellos et al. [36] who observed no effects of lithium on reference memory, using a more rigorous test of this process (Morris water maze). The two studies considered together suggest that the transient reference memory deficit reported by Al Banchaabouchi et al. [35] may be specific to the task used by the latter authors (spatial memory formation in the hole-board) or attributable to the shorter lithium pre-treatment used by them (20 days versus 40 days in Vasconcellos et al. [36], and >30 days in our experiments).

4.2. Effects of chronic LiCl on delayed spatial alternation

During delayed alternation, the performance of LiCl animals was significantly more accurate than that of saline controls in Experiments 1 and 2 (30- and 45-s delay, respectively). As seen in Figs. 2b and 3b, this differentiation between the drug groups arose as a result of deterioration of saline control performance. It is noteworthy that the accuracy of saline controls systematically declined across experiments, responding to the corresponding augmentation of delay intervals (Table 1, "Delay Phase-Mean accuracy (%)": 85, 82 and 78% for delays of 30, 45 and 60 s, respectively). In contrast, LiCl-treated animals sustained 0-delay baseline latencies for the first two delays and deteriorated only in the 60 s delay (93, 92 and 78% for delays of 30, 45 and 60 s, respectively). These results indicate that chronic lithium treatment has a beneficial effect on spatial working memory, provided that this memory function is not stressed beyond a certain point: the beneficial effect of lithium was not observed when the alternation delay was incremented to 60-s (Experiment 3, Fig. 4b). Under this delay, choice accuracy declined significantly in both LiCl and saline animals, compared to their respective 0-s delay baseline. This global deterioration was due to the length of the delay used rather than to extraneous factors affecting both drug groups, as shown by the complete restoration of baseline accuracy performance in both drug groups (Fig. 4c) when the animals underwent a brief 0-s delay reminder phase.

The robustness of the (provisional) beneficial effect of lithium on working memory is highlighted by the fact that all LiCl-treated animals reached the stringent 5-day 7/8 correct criterion in the delay phases of Experiments 1–2 (30 and 45 s delay, respectively, Table 1, "% of animals to reach criterion") whereas only about 50% of saline rats achieved it. This difference was no longer evident in Experiment 3 (60-s delay) where only 18% and 16% of LiCl-treated and control animals, respectively, were able to reach criterion.

4.3. Chronic lithium treatment and spatial memory

Chronic lithium had no effect on the acquisition of a non-matching to place alternation rule, which suggests that it does not affect spatial reference memory. With respect to spatial working memory, the delays used in our experiments placed systematically varying 'levels of demand' on the working memory function. As can be seen in Table 1, these levels of demand can be operationally defined on the basis of (a) percentage of control animals capable of reaching criterion and (b) performance accuracy of control animals, at each level. Thus 0 s delay can be said to represent minimum demand (100% criterion achievement, accuracy ≃90% in controls); 30–45 s delays to place moderate demand (≃50% criterion achievement, accuracy <85% in controls); while 60 s delay represents severe demand on working memory (<20% criterion achievement, accuracy <80% in controls). With this gradation in mind, our findings suggest a parametric effect on spatial working memory: lithium had no effect on working memory while minimum demand was placed on that function (even though the controls' accuracy of approximately 90% makes the operation of a ceiling effect unlikely at

this level). It also failed to provide memory enhancement when the capacity of working memory was stretched beyond a certain limit which, in our procedure, was met at the 60-s delay interval. However, it had a significant enhancing effect on working memory under moderate demand conditions.

To our knowledge, this is the first report of a significant enhancing effect of chronic lithium on memory, although there have been previous reports that lithium protected from compromise of the mnemonic function by stress [36] or by excitotoxic lesion [48]. Therefore our finding alone must be treated with caution as it may be limited to the alternation task used here, or to working memory only. Thus, in order to evaluate the range of lithium's memory enhancing potential, we also tested chronic lithium effects on long term recall within an aversively motivated behavioural phenomenon, retention of passive avoidance.

4.4. Effects of chronic LiCl on step-through passive avoidance

Chronic lithium treatment did not affect baseline step-through latencies in the shuttle box (Fig. 5a, Experiment 4). This suggests that lithium does not change the perceived aversiveness of the intense illumination used to motivate transition to the dark compartment of the shuttle box, nor does it significantly affect motor ability. This observation is in line with recent studies using chronic lithium, which report normal open field locomotion [36].

The conditioning parameters used here were chosen with the following rationale: lithium treatment had no perceptible effect in 0-delay spatial alternation performance, when control animals performed well within their working memory capacity. Its beneficial effect emerged once control performance started deteriorating in response to increased demand on the working memory function. Accordingly, we attempted to use shock parameters which would minimise the possibility of perfect performance in the control group (mild shock, single conditioning trial). Indeed, these parameters did not support passive avoidance retention in saline controls 6 h post-conditioning (Fig. 5b). This may reflect either complete failure of conditioning or, more likely, a weak conditioning trace which did not bridge the 6-h retention gap. In contrast, LiCl-treated animals showed good retention under the same conditioning parameters, with significant latency increases in the 6-h retention test compared to their own and to saline control baseline scores. The above suggest that chronic LiCl treatment either potentiates long-term retention of a weak conditioning trace, or facilitates aversive conditioning, both exciting possibilities in terms of cognitive enhancement. Given that the working memory enhancement we noted in the spatial alternation experiments was not accompanied by any evidence of enhancement of appetitively motivated learning (LiCl-treated and control animals were indistinguishable in the number of trials to criterion and in performance accuracy in 0″ delay acquisition: Table 1), the memory improvement explanation of the passive avoidance result is more parsimonious. However, direct tests of the effects of lithium on learning are necessary.

Another interpretation of the finding would be that it is caused by increased shock sensitivity in lithium-treated animals. This less interesting possibility is unlikely, however, since previous studies report decreased reaction to shock delivery and attenuation of shock-induced suppression of open-field activity [30,32].

Finally, the effect of lithium on passive avoidance which we examined under a single conditioning parameter (mild shock, single conditioning trial) requires further investigation, which is currently under way.

In conclusion, our results demonstrate that chronic lithium enhances spatial working memory, provided that moderate demand is placed on that function, and it also enhances long-term retention of weak conditioning traces. Clearly, there is a need for new animal experiments examining the effects of lithium on different types of behavioural tasks and task parameters, in different species. There is also a need for re-evaluating previous animal literature results in the light of the present findings. For example, a recent study reporting beneficial effects of lithium on stress-induced memory deficits [36] shows data strongly suggestive of lithium-induced memory enhancement in the Morris water-maze (increased number of crossings over the initial position of the escape platform by the LiCl-treated, unstressed group, compared to unstressed controls, Fig. 3). However this evidence, being at odds with earlier views on lithium effects on memory, and embedded as it was in an experiment focussed on stress-induced memory failure, received no notice in that publication.

In terms of cognitive enhancement potential, these results are in line with a study by Gallo et al. [49], reporting that chronic lithium produces a significant increase in a learning ability index, as well as in the cortex/subcortex weight ratio in young rats, effects which were also produced by enriched environmental stimulation. The exact site and mechanism through which lithium may exert these beneficial effects remains, as of yet, unknown. However, several candidate loci of action emerge from neurochemical evidence which links chronic lithium action to cellular components involved in neuronal processes underlying cognitive functioning [9,50–52]. Future experiments must investigate the neuronal mechanisms of lithium's actions by means of central or pharmacological (peripheral co-administration of pharmacologically specific compounds), electrophysiological and possibly genetic approaches.

On the clinical level, our results raise exciting possibilities regarding the use of lithium as a cognitive enhancer in psychiatric conditions where cognitive deficits play a major role in producing adverse symptoms, such as depression. In fact, the possibility that the substantial efficacy of lithium augmentation of antidepressant action [53] may involve cognitive enhancement deserves to be explored in patient populations.

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