

# Effects of Chronic and Acute Lithium Treatment on the Long-term Potentiation and Spatial Memory in Adult Rats

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**Objective:** Although, accumulating evidence is delineating a neuroprotective and neurotrophic role for lithium (Li), inconsistent findings have also been reported in human studies especially. Moreover, the effects of Li infusion into the hippocampus are still unknown. The aims of this work were (a) to assess whether basal synaptic activity and long-term potentiation (LTP) in the hippocampus are different in regard to intrahippocampal Li infusion; (b) to assess spatial learning and memory in rats chronically treated with LiCO<sub>3</sub> in the Morris water maze.

**Methods:** Field potentials were recorded from the dentate gyrus, stimulating perforant pathways, in rats chronically (20 mg/kg for 40 days) or acutely treated with LiCO<sub>3</sub> and their corresponding control rats. In addition, performance of rats in a Morris water maze was measured to link behaviour of rats to electrophysiological findings.

**Results:** LiCO<sub>3</sub> infusion into the hippocampus resulted in enhanced LTP, especially in the late phases, but attenuated LTP was observed in rats chronically treated with Li as compared to controls. Li-treated rats equally performed a spatial learning task, but did spend less time in target quadrant than saline-treated rats in Morris water maze.

**Conclusion:** Despite most data suggest that Li always yields neuroprotective effects against neuropathological conditions; we concluded that a 40-day treatment of Li disrupts hippocampal synaptic plasticity underlying memory processes, and that these effects of prolonged treatment are not associated with its direct chemical effect, but are likely to be associated with the molecular actions of Li at genetic levels, because its short-term effect preserves synaptic plasticity.

**KEY WORDS:** Lithium; Hippocampus; Learning; Memory; Long-term potentiation.

## INTRODUCTION

The hippocampal formation is involved in a circuit that is required for several types of memory in both humans<sup>1)</sup> and rodents.<sup>2)</sup> At the physiological/cellular level, memory processes generally are believed to involve long-term potentiation (LTP) of synaptic function.<sup>3)</sup> Lithium (Li) salts like lithium carbonate (Li<sub>2</sub>CO<sub>3</sub>) is a neuroprotector that improves memory and learning in preclinical models of aging,<sup>4)</sup> traumatic brain injury,<sup>5)</sup> drug addiction,<sup>6)</sup> glutamate neurotoxicity,<sup>7)</sup> and Alzheimer's disease.<sup>8)</sup> However, inconsistent findings have been reported in human studies. Li treatment inhibited learning, memory, and

speed of information processing in patients with bipolar disorder and to some extent in control subjects<sup>9-12)</sup> and many patients administered Li<sub>2</sub>CO<sub>3</sub> complained of memory impairment.<sup>9,13)</sup> So far, only a limited attempt has been made to study the effect of Li treatment on the LTP, which is believed to underlie learning and memory.

Although it has been previously demonstrated that the LTP in the dentate gyrus (DG) of hippocampal formation is enhanced in rats treated intraperitoneally with Li for 2 to 28 days, compared to control rats,<sup>4,14,15)</sup> neither effect of more prolonged exposure nor acute effect have not been studied up to date. The objective of this study was to determine, in normal adult rats, whether Li treatment for 40 days produces deficits in spatial learning in the Morris water maze and synaptic plasticity in the DG of the hippocampal formation *in vivo*. To further investigate the contribution of direct chemical effect of Li to its prolonged effect on synaptic plasticity, we also present the results of Li infusion into the DG.

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## METHODS

### Animals and Treatment with Lithium

The experiments were performed on adult male Wistar rats between the ages of 4 and 6 months in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) regarding the protection of animals used for experimental purposes and with the guiding principles for the care and use of laboratory animals approved by the Erciyes University.

In experiments investigating the outcome of long-term administration,  $\text{Li}_2\text{CO}_3$  (Sigma Chemical Co., St. Louis, MO, USA) was administered intraperitoneally (1 mEq/kg/day) for 40 days. This treatment protocol resulted in serum Li concentrations of  $1.18 \pm 0.54$  mM/L (mean  $\pm$  standard error of mean) which are also close to the range of 0.30–1.30 mM/L, concentrations that are achieved and maintained during Li therapy in human subjects.<sup>16)</sup> Saline was provided ad libitum to the Li-treated rats to reduce potential toxicity. In experiments investigating the outcome of short-term Li administration,  $\text{Li}_2\text{CO}_3$  (10 mM) was infused at the rate of 4.0  $\mu\text{l}/\text{min}$  for 20  $\mu\text{l}$  before LTP induction.

### Morris Water Maze

Performance in the Morris water maze of 22 saline-treated and 19 Li-treated rats was evaluated for measurement of spatial memory in a circular, galvanized steel maze (2 m in diameter and 75 cm in depth), which was filled with water to a depth of 50 cm and kept at 22°C. The water was made dark blue with non-toxic dye. The maze was located in a large quiet test room, surrounded by many visual cues external to the maze, which were visible from within the pool and could be used by the rats for spatial orientation. Locations of the cues were unchanged throughout the period of testing. There were four equally divided quadrants in the pool. In one of the quadrants, a hidden platform (10 cm in diameter, 2 cm below the surface) was located centrally and fixed in a position and was kept constant throughout the acquisition trials. The platform was removed on probe phase. The rats performed four trials per day for four consecutive days (16 trials). In the swimming trials, each individual rat was released gently into the water in a randomly chosen quadrant other than the target one that contained the platform for facing an extra maze cue. During an acquisition period, rats learned how to find the platform within 120 seconds.

After reaching it, the rat was allowed to stay on the platform for 15 seconds and was then put back into its cage. The rats were placed on the platform by hand for 15 seconds if they could not escape to the platform within 120 seconds by themselves. During the inter-trial intervals, animals were kept in a dry home cage for 60 seconds. The probe trial was performed 24 hours after the training period. Latency of finding the platform (escape latency, EL), distance moved (DM), swimming speed, and the time in seconds spent in the quadrant occupied by the platform was measured for individual animals in each trial by the EthoVision video tracking system (Noldus, Wageningen, the Netherlands). The EL of rats who could not escape to the platform within 120 seconds was accepted as 120 seconds. The experimenter always stood at the same position. All the trials were completed between 10.00 to 12.00 hour.

### Electrophysiology

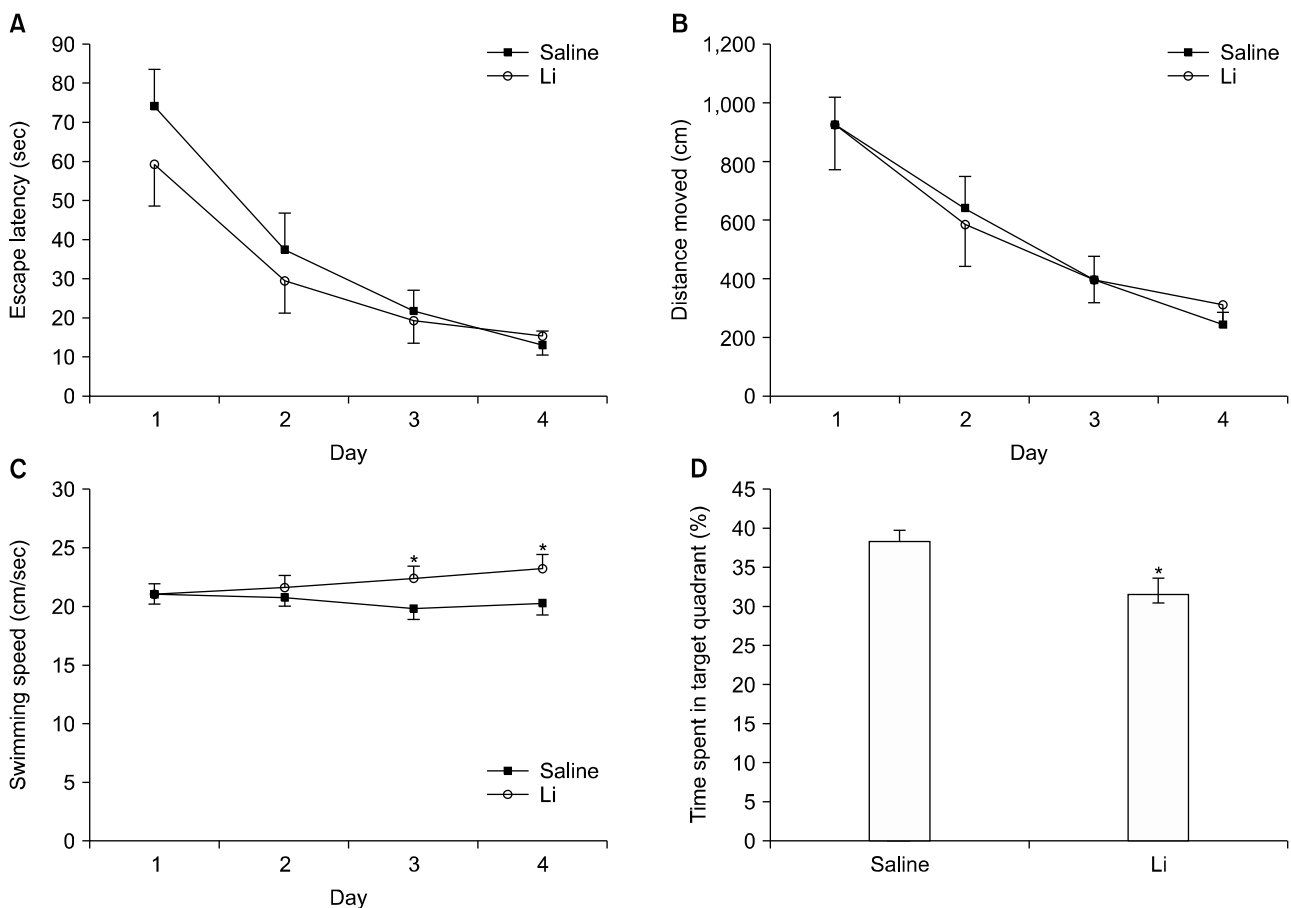
Details of the protocols used for electrophysiological experiments are described elsewhere.<sup>17)</sup> Briefly, after rats were anesthetized with intraperitoneally injected urethane (1.2 g/kg), a bipolar tungsten electrode (stainless steel, Teflon-coated, 127  $\mu\text{m}$  in diameter, insulated except at its tips) was used to stimulate the medial perforant path (PP; from bregma; anteroposterior:  $-8.0$  mm, mediolateral: 4.2 mm, dorsoventral: 2–2.5 mm below the dura) of the right hemisphere. A two-barrel micropipette (Borosilicate, outer diameter 1.5 mm, length 10 cm length; World Precision Instruments, Sarasota, FL, USA) was inserted into the granule cell layer of the DG in the right hemisphere (from bregma; anteroposterior:  $-3.5$  mm, mediolateral: 2.15 mm, dorsoventral: 2.5–3 mm below the dura) to record the field potential. The depth of recording and stimulating electrodes (dorsoventral coordinate) was adjusted to obtain a large positive excitatory postsynaptic potential (EPSP) followed by a negative-going population spike (PS) in response to PP stimulation. These positions of both electrodes were previously verified to be in the granule cell layer of the DG and in the PP.<sup>18,19)</sup> One of the barrels was filled with 3 M NaCl (tip resistance: 2–10 M $\Omega$ ) for recording of field potentials. The other was filled with  $\text{Li}_2\text{CO}_3$  in experiments investigating the outcome of short-term administration, or saline in control rats and rats chronically treated with  $\text{Li}_2\text{CO}_3$ , and connected to a Hamilton syringe (25  $\mu\text{l}$ ) driven by a syringe pump

(Stoelting Co., Wood Dale, IL, USA). Five minutes prior to LTP induction, a 20  $\mu$ l volume was infused into the DG at a rate of 4.0  $\mu$ l/minute.

After a stable EPSP was obtained, the PP was stimulated by pulses at an intensity that ranged from 0.1 to 1.5 mA at 0.05 Hz three times and by increasing the intensities from a 0.1 mA to a 1.5 mA step by 0.2 mA per step to create an input-output (I/O) curve, which was stored for off-line analysis. The stimulus intensity produced by half of the maximum PS amplitude was determined (test stimulus) and then used throughout the experiment. LTP was induced using high-frequency stimulation (HFS; 100 Hz, 1 second, 4 times). Test stimulus intensities were  $0.81 \pm 0.17$  and  $0.90 \pm 0.16$  mA in control and Li groups, respectively. Following the delivery of HFS, the test stimulus was repeated every 30 seconds for up to the end of recording.

### Data Analysis and Statistics

Two components of each field potential, the EPSP and PS, were used for statistical purposes. The slope of the EPSP was calculated as the amplitude change at 20% to 80% of the voltage difference between the start and the peak of the waveform. The PS amplitude was calculated as the average of the two potential differences of the negative spike peak to the preceding and following positive peaks. Raw values for the EPSP slopes and PS amplitudes obtained during the I/O experiment were analyzed separately using repeated measures ANOVA, with the drug (saline vs. Li) used as between-subjects variables and stimulus intensity (8 levels of intensity) used as a within-subjects factor. The mean value of the EPSP slope and the PS amplitude during baseline recording was chosen to represent 100%, and each EPSP and PS was expressed as



**Fig. 1.** Effects of lithium (Li) treatment on acquisition and memory performance in the water maze. All rats improved their ability to find the hidden platform as shown by decreasing escape latency (A) and distance moved (B) over training days. Swimming speed (C) did change as a function of day and was affected by Li treatment. Li-treated rats ( $n=19$ ) spent less time in target quadrant than control rats ( $n=22$ ) (D). Data are expressed as mean  $\pm$  standard error of mean. \*vs. control rats. Note that Li-treated rats performed spatial learning task as control rats however underperformed retrieval task.

a percentage of this value. The average slope or amplitude was calculated from ten sweeps at the end of recording as a measure of LTP. Unpaired Student's *t* test was used to compare groups' means. Significance was set at  $p < 0.05$  (two-tailed). All statistical analyses were performed using IBM SPSS Statistics (ver. 22.0; IBM Corp., Armonk, NY, USA).

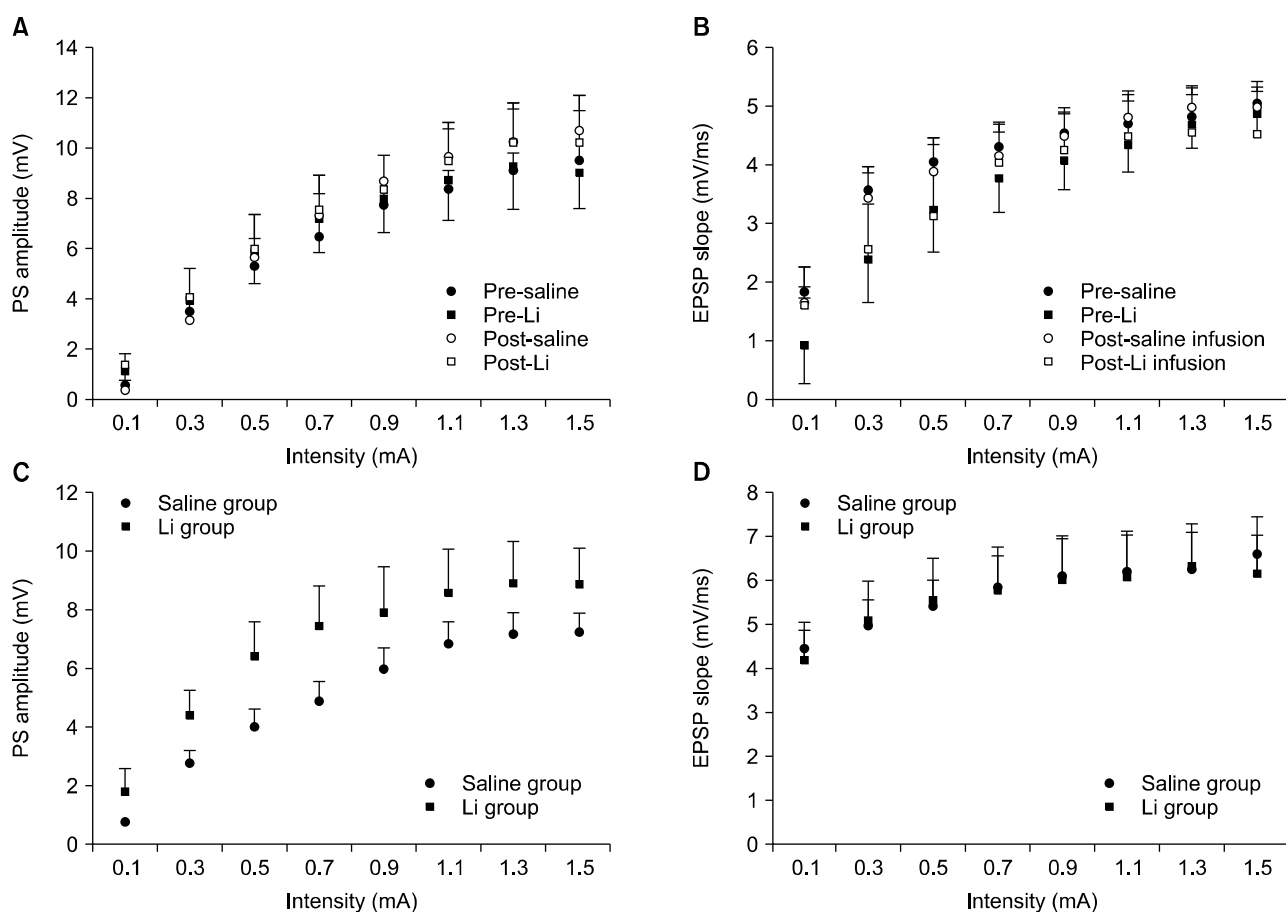
## RESULTS

### Learning Behavior of Rats Chronically Treated with Lithium Carbonate

To address the question of whether Li treatment results in altered learning capabilities, we performed the Morris water maze test. During acquisition trials, Li-treated rats

as well as saline-treated rats improved their ability to find the hidden platform as showed by decreasing EL ( $F_{3,510} = 85.356$ ,  $p < 0.001$ ) and DM ( $F_{3,510} = 71.263$ ,  $p < 0.001$ ) as a result of training (Fig. 1). Between-subjects effect was not significant for EL ( $F_{1,170} = 3.833$ ,  $p = 0.052$ ) and DM ( $F_{1,170} = 0.192$ ,  $p > 0.05$ ). Interaction effects group $\times$ EL and group $\times$ DM were not significant ( $F_{3,519} = 1.955$  and  $0.405$ ,  $p > 0.05$ ). Repeated measures ANOVA showed that speed did not change as a function of day ( $F_{3,510} = 1.974$ ,  $p > 0.05$ ) in control group, however was affected by Li treatment (group effect:  $F_{1,170} = 14.084$ ,  $p < 0.001$ ; interaction:  $F_{3,510} = 4.245$ ,  $p = 0.006$ ). Li-treated rats swam faster than control group at the 3rd day ( $F_{1,170} = 19.165$ ,  $p < 0.010$ ) and at the 4th day ( $F_{1,170} = 9.733$ ,  $p = 0.002$ ).

Twenty-four hours after the last trial session a probe tri-



**Fig. 2.** Effect of  $\text{Li}_2\text{CO}_3$  on baseline synaptic transmission in the dentate gyrus of hippocampal formation. The input-output curves were generated by plotting population spike (PS) amplitude and excitatory postsynaptic potential (EPSP) slope across a range of stimulation intensities (0.1-1.5 mA). Input-output (I/O) curves in A and B were taken prior to and after  $\text{Li}_2\text{CO}_3$  (or saline) infusion from the same animal ( $n=5/\text{group}$ ). I/O curves in C and D were prior to long-term potentiation induction in the lithium (Li) group and control group ( $n=7/\text{group}$ ). PS and EPSP at each intensity level are mean of three consecutive responses in 5 rats for each group. Data are expressed as mean $\pm$ standard error of mean. Note that the PS amplitude and EPSP slope were not different across the eight stimulus intensities as indicated by no significant interactions between intensity and drug.

al, in which the platform had been removed, was given in order to test the rats' spatial accuracy and their actual ability to remember the position of the hidden platform. Under normal conditions in such a probe trial, it is expected that animals spend more time than the chance level (25%) in the quadrant where the platform had been located. The time spent in target quadrant was significantly above the 25% chance level for control ( $38.22 \pm 1.62\%$ ;  $df=21$ ;  $t=8.182$ ,  $p<0.001$ ; one-sample  $t$  test) and Li-treated rats ( $31.44 \pm 2.15\%$ ; degree of freedom [ $df$ ]=18;  $t=3.046$ ;  $p=0.007$ ). However, Li-treated rats spent significantly less time in the target quadrant than did control rats in probe trial ( $df=39$ ;  $t=2.584$ ;  $p=0.014$ ) (Fig. 1D). These results showed that Li-treated rats underperformed retrieval task in spite of similar spatial learning performance to control.

### Electrophysiology

To establish that transmission at PP-DG synapses was altered by  $\text{Li}_2\text{CO}_3$  administration, I/O curves were constructed using eight stimulus levels. The PS amplitude and EPSP slope were not different, neither between prior to and after infusions in cases where I/O recordings were taken prior to and after  $\text{Li}_2\text{CO}_3$  (or saline) infusion from the same animal nor between Li group and control group in case where I/O recordings were taken prior to LTP induction across the eight stimulus intensities as indicated by no significant interactions between intensity and drug (Fig. 2). These data indicate no effectiveness of  $\text{Li}_2\text{CO}_3$  on transmission at PP-DG synapses, and no gross differences in baseline function between the control and Li groups that complicate with measure of synaptic plasticity.

### Effects of Intrahippocampal $\text{Li}_2\text{CO}_3$ on the LTP in the Dentate Gyrus

The potentiation of the field EPSP (fEPSP) is associated with the formation and enlargement of dendritic spines, whereas the PS potentiation reflects the somatic response, the amplitude of which is dependent on the number of granule cells that discharge in synchrony. The product of the synaptic component of LTP (fEPSP) together with the non-synaptic component (PS) has a much stronger effect on the neuronal output in comparison to cases when only one of these two components is potentiated. As shown in Figure 3, four tetanus trains of 100 pulses at 100 Hz induced LTP of both PS (Fig. 3A) and fEPSP (Fig. 3B) in the

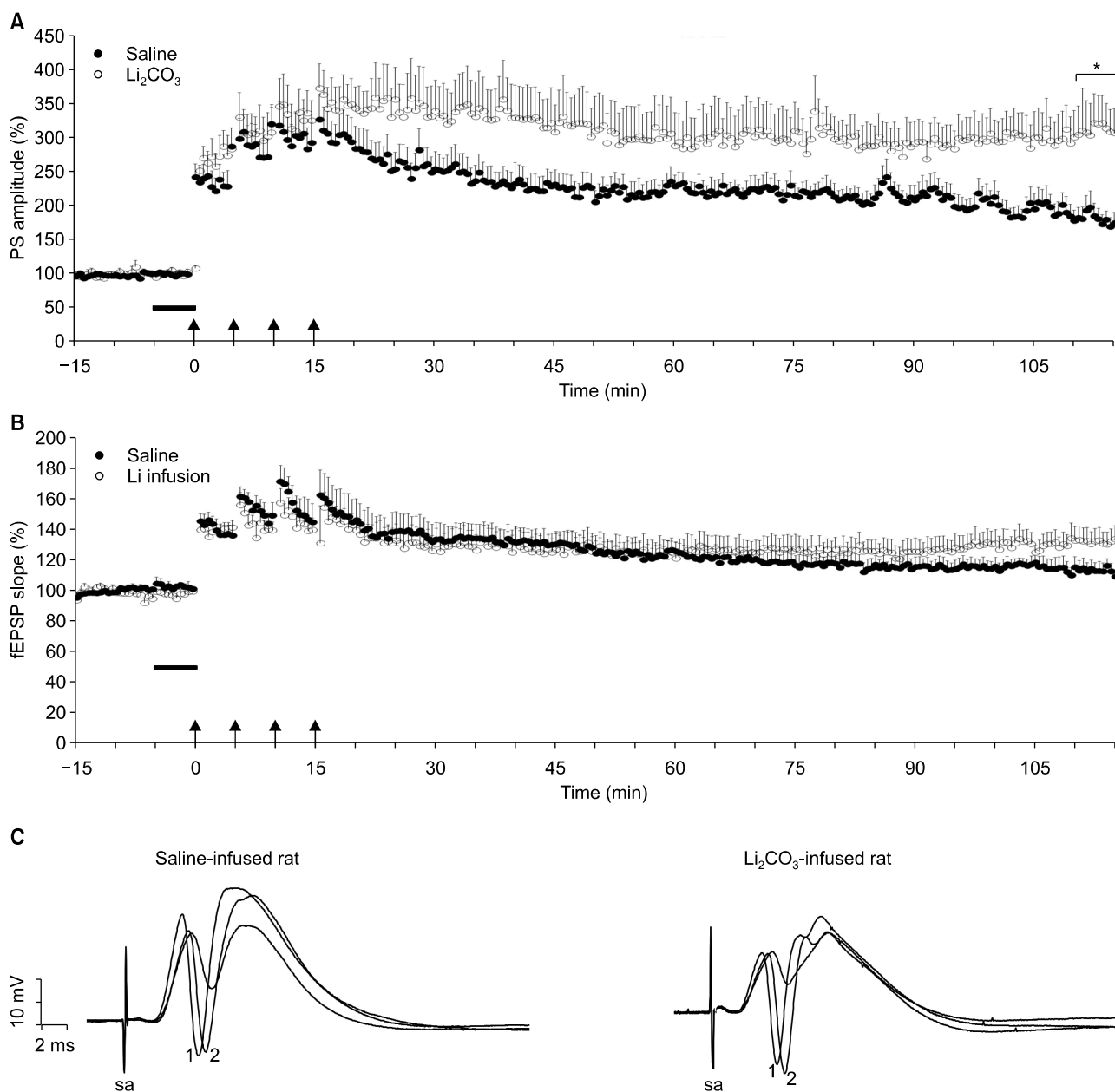
DG neurons of both groups. Independent samples  $t$  test failed to show a significant difference between saline- and Li-infused rats in the magnitude of the short-term potentiation measured at 1 to 5 minutes after tetanus as the PS amplitude ( $304 \pm 32\%$  vs.  $352 \pm 34\%$  of baseline, respectively;  $p>0.10$ ) and the EPSP slope ( $153 \pm 15\%$  vs.  $144 \pm 9\%$  of baseline;  $p>0.10$ ). Nevertheless, induced LTP of the PS, but not of fEPSP, measured at 100 minutes after the last HFS was greatly enhanced by pre-HFS application of  $\text{Li}_2\text{CO}_3$  (10 mM, 5 minutes;  $312 \pm 42\%$  vs.  $182 \pm 16\%$  of baseline,  $p=0.012$ ). The difference between LTP magnitudes of fEPSP remained a trend level for significance ( $132 \pm 8\%$  vs.  $113 \pm 5\%$  of baseline,  $p=0.055$ ). These data indicates that induction of LTP at the DG synapses was enhanced by infusion of  $\text{Li}_2\text{CO}_3$ , by a mechanism so-called non-synaptic plasticity such as increased somatic and dendritic excitability or increases in the membrane potential.<sup>20</sup>

### Effects of Chronic $\text{Li}_2\text{CO}_3$ Treatment on the LTP in the Dentate Gyrus

Independent samples  $t$  test failed to show a significant difference between control and Li groups in the magnitude of the short-term potentiation measured at 1 to 5 minutes after tetanus ( $349 \pm 36\%$  vs.  $285 \pm 10\%$  of baseline, respectively;  $p>0.10$ ) and the LTP measured at 75 to 80 minutes after tetanus ( $256 \pm 20\%$  vs.  $227 \pm 16\%$  of baseline, respectively;  $p>0.10$ ). Nevertheless, induced LTP of fEPSP, measured at 75 to 80 minutes after the last HFS was significantly attenuated by chronic treatment of  $\text{Li}_2\text{CO}_3$  (1 mEq/kg/day, 40 day;  $107 \pm 1.1\%$  vs.  $135 \pm 11\%$  of baseline,  $p=0.038$ ). The difference between short-term potentiation of fEPSP also reached significance ( $144 \pm 4\%$  vs.  $165 \pm 8\%$  of baseline,  $p=0.004$ ). These data indicates that induction of LTP at the DG synapses was attenuated by  $\text{Li}_2\text{CO}_3$  treatment.

## DISCUSSION

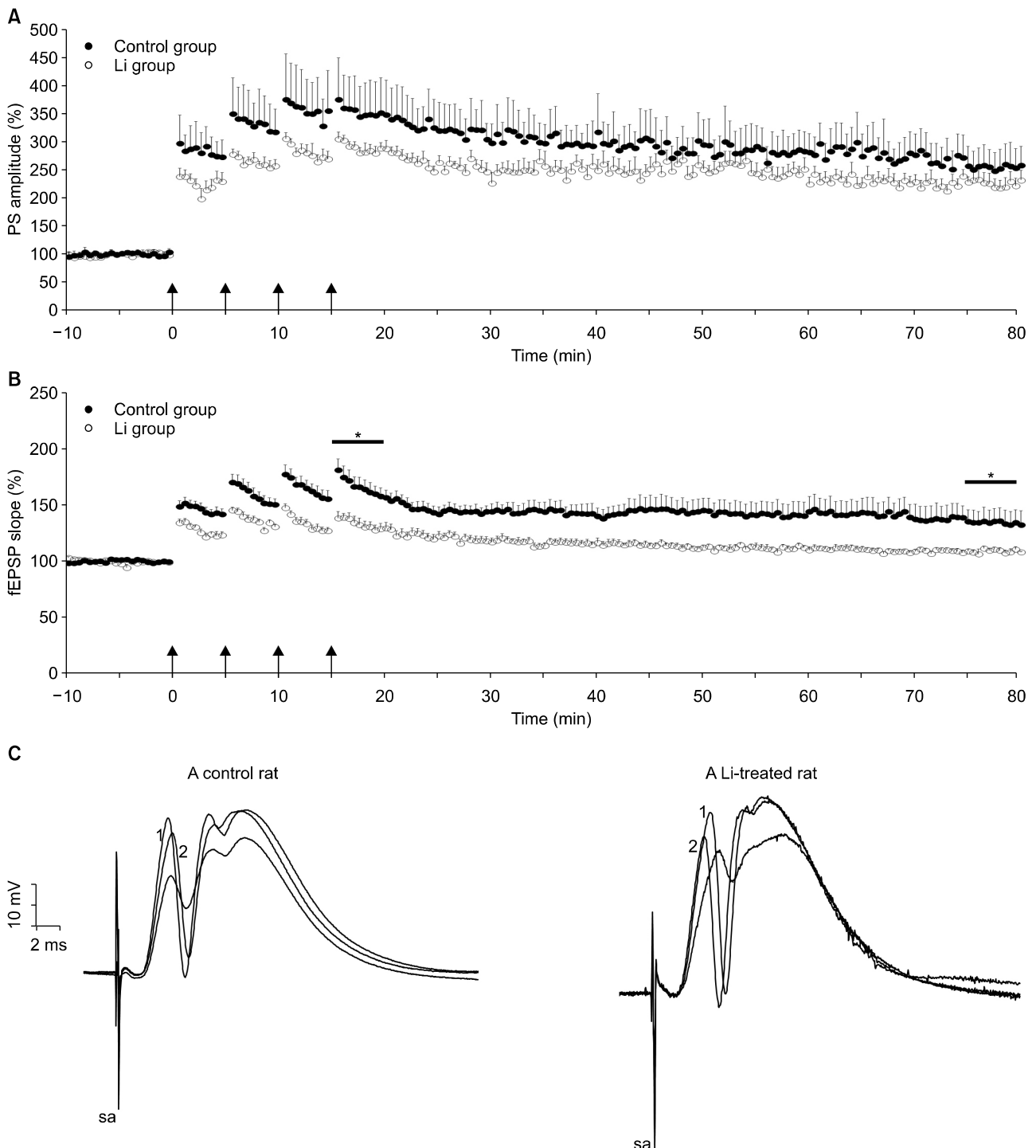
Although it has been previously reported that prolonged Li treatment promptly increased the LTP,<sup>14,15</sup> to our best knowledge, no study has addressed the Morris water maze performance of Li-treated rats, as well as *in-vivo* effects of Li infusion on the DG-LTP. The major result of this study is that infusion of Li magnifies the potentiation of PS amplitude (Fig. 3A) whereas chronic treat-



**Fig. 3.** A 5-minute infusion intrahippocampal  $\text{Li}_2\text{CO}_3$  infusion enhances the long-term potentiation (LTP) of population spike (PS) amplitude in the dentate gyrus of hippocampal formation. After a 10-minute baseline recording, saline or  $\text{Li}_2\text{CO}_3$  was infused into the dentate gyrus (horizontal black bar) and LTP was induced by means of high-frequency stimulation (HFS) (arrows; 100 Hz, 1 second, 4 times), which was applied beginning at time 0. This protocol induced LTP of both PS (A) and field excitatory postsynaptic potential (fEPSP, B) in the dentate gyrus neurons of both group. The magnitudes of the short-term potentiation of both fEPSP and PS measured at 1 to 5 minutes after tetanus are comparable between saline-infused rats and  $\text{Li}_2\text{CO}_3$ -infused rats. PS amplitude was more potentiated between 110 and 115 minutes in lithium-infused (Li) group compared to saline-infused group (\*). Error bars denote the standard errors of the means.  $n=7$  for each group. (C) Traces are representative of field potential recordings made immediately before (time point,  $-1$  minute; not depicted) and after HFS (time point, 15 minutes; depicted with "1") and at the end of the experiment (time point, 75 minutes; depicted with "2"). Note the higher ratio of PS (the down-going deflection) amplitude in trace depicted with "2" to that in trace not depicted (the magnitude of LTP) in  $\text{Li}_2\text{CO}_3$ -infused rat. sa, stimulus artifact.

ment for 40 days impairs hippocampus-dependent spatial memory without affecting platform learning task and attenuates the potentiation of EPSP slope (Fig. 4B) in the DG

of hippocampal formation compared with control rats, without enhancing baseline synaptic transmission (Fig. 2). The differences in the LTP between Li-infused and



**Fig. 4.** Time course of population spike (PS)-long-term potentiation (LTP) (A) and excitatory postsynaptic potential (EPSP)-LTP (B) in the dentate gyrus from the control group (filled) and the lithium group (empty circle, a 40-day treatment). After a 10-minute baseline recording, LTP was induced by means of high-frequency stimulation (arrows; 100 Hz, 1 second, 4 times), which was applied beginning at time 0 (arrows). This protocol induced LTP of both PS (A) and field EPSP (fEPSP) (B) in the dentate gyrus neurons of both group. The fEPSP slope was less potentiated between 15-20 minutes (short-term potentiation) and between 75-80 minutes (LTP) in lithium-infused (Li) group compared to saline-infused group (\*). (C) Traces are representative of field potential recordings made immediately before (time point, -1 minute; not depicted) and after high-frequency stimulation (time point, 15 minutes; depicted with "1") and at the end of the experiment (time point, 75 minutes; depicted with "2"). Note the higher ratio of fEPSP slope in trace depicted with "2" to that in trace not depicted (the magnitude of LTP) in a control rat. sa, stimulus artifact.

Li-treated rats suggest that effect of prolonged Li treatment may not be associated with its direct chemical effect, but is likely to be associated with the molecular actions of Li at genetic levels. Moreover, two components of LTP, which is previously well defined,<sup>21)</sup> can be differentially affected by Li so that synaptic component is attenuated when Li administered chronically, whereas magnification of non-synaptic component is observed when given acutely.

Non-synaptic plasticity is a form of neuroplasticity that involves modification of ion channel function in the axon, dendrites, and affects synaptic integration, sub-threshold propagation, spike generation, and other fundamental mechanisms of neurons at the cellular level.<sup>20,22)</sup> At doses used in this study, Li infusion led to a marked increase in PS-LTP without affecting on EPSP-LTP, indicating an effect on a non-synaptic rather than synaptic plasticity. Previously reported effects of injection of Li confirm EPSP-spike (E-S) potentiation, namely a larger PS amplitude for a given EPSP size. A microdialysis study in rat prefrontal cortex showed that acute application of Li increased the concentration of  $\gamma$ -aminobutyric acid (GABA) at doses of 4 meq/kg,<sup>23)</sup> and the activation of GABA<sub>A</sub> receptors increased the E-S potentiation.<sup>24)</sup>

Our findings suggest that long-term Li treatment attenuates synaptic plasticity in the hippocampus. Nevertheless, it is generally reported that enhanced synaptic plasticity in the DG is a functional consequence of long-term treatment with Li. Previous electrophysiological works showed that sub-chronic (1 mEq/kg/day for 2 weeks) and chronic treatments (4 mEq/kg/day for 4 weeks) of Li increased the LTP in the granule cells in the DG of the hippocampus.<sup>14,15)</sup> There are discrepancies in the methodology of studies. For instance, we obtained the field potentials from the principle cell layer which is made up largely of densely packed granule cells, whereas Son *et al.*<sup>14)</sup> from the molecular layer which is occupied by, among other things, the dendrites of the dentate granule cells. Rats were exposed to Li chloride for shorter time (14 days) relative to our study in Shim *et al.*'s study.<sup>15)</sup> Yu *et al.*<sup>4)</sup> studied the 20-month-old aged rats following chronic Li treatment.

The enhanced LTP, which has been reported by other authors, is best explained by the inhibitory action of Li on glycogen synthase kinase 3 (GSK-3 $\beta$ ).<sup>25,26)</sup> Unlike most protein kinases involved in signaling, GSK-3 $\beta$  is active in

unstimulated, resting cells and degrades  $\beta$ -catenin, blocking reach to nucleus. Therefore, inhibition of GSK-3 $\beta$  activates Wnt signaling pathway, promoting expression of neuroplastic factors involved in synaptic plasticity<sup>27)</sup> and this leads to favoring LTP. It was shown that induction of LTP resulted in deactivation of GSK-3 $\beta$  through phosphorylation<sup>28,29)</sup> and after 15 minutes' HFS, through one hour, the inhibitory phosphorylation of GSK-3 $\beta$  was increased in the DG where LTP had been potentiated relative to uninduced regions in the ipsilateral hippocampus, to the contralateral hippocampus or to native control animals.<sup>29)</sup> Deficient LTP in GSK-3 $\beta$  overexpressing mice could be rescued by Li.<sup>29,30)</sup> The other possible effect such as upregulation of brain-derived neurotrophic factor,<sup>31)</sup> Bcl-2 and CREB<sup>32)</sup> may underline increase in LTP by Li. However, contradictory data concerning an attenuation of LTP can be expected because Li has effects on multiple cellular processes in regard to synaptic proteins, depending on duration and dose of Li. Some effects of Li can negatively affect the cell signaling involved in synaptic trafficking, such as inhibition of N-methyl-D-aspartate receptor activity,<sup>7,33,34)</sup> down regulation of the gene for the glutamate/alpha-amino-3-hydroxy-5-methyl-4-isoxazolpropionate (AMPA) receptor,<sup>35)</sup> reduction in the expression of the protein kinase C and myristoylated alanine rich C kinase substrate.<sup>36)</sup> Although molecular studies are not covered in the current study, we have thought that forty days of Li treatment is long enough to produce inhibitor actions through the gene expression of proteins involved in synaptic plasticity.

The Morris water maze is a test of hippocampus-dependent spatial memory,<sup>37)</sup> the closest parallel to episodic memory in humans.<sup>38)</sup> The testing procedure used during the 4 days of locating the hidden platform provides a measure of spatial reference memory, while the probe trial is a measure of the strength of spatial learning.<sup>38)</sup> In the present experiments, lower efficacy of LTP, which is thought to be the major neurophysiological basis for learning and memory,<sup>39)</sup> was correlated with impaired spatial memory behavior in Li-treated rats. In line with the present study, intra-CA1 administration of Li after training on a one-trial step-down inhibitory avoidance task impaired memory when retrieval was tested 24 hours later.<sup>40)</sup> In humans, higher Li levels were found to be correlated with impairment of memory performance in psychotic patients treated with Li.<sup>41)</sup> Some investigators have



suggested that Li treatment inhibited learning, memory, and speed of information processing in patients with bipolar disorder and to some extent in control subjects.<sup>9-12)</sup> Li therapy did not improve deficit in episodic memory in bipolar patients.<sup>42)</sup> Some investigators, however, have revealed inconsistent findings with those obtained from studies mentioned above. Chronic Li treatment enhanced spatial working memory in rats<sup>43)</sup> and improved spatial learning and memory ability after transient global cerebral ischemia<sup>44)</sup> and cranial irradiation<sup>45)</sup> in Morris water maze. Li treated rats outperformed control rats in the training trails at dose higher than used in this study.<sup>46)</sup> In addition, as distinct from Morris water maze, Li increased learning in a hole-board arena, a conditioned place-preference box and a T-maze in rats fed with Li carbonate.<sup>47)</sup> Nevertheless, Li-treated rats shown upper motor ability in terms of swimming velocity (23.66±0.56 cm/sec vs. 21.47±0.43 cm/sec;  $F_{1,40}=9.430$ ,  $p=0.004$ ) and DM (2,830±68 cm vs. 2,575±51 cm;  $F_{1,40}=8.825$ ;  $p=0.005$ ) in the probe trial. Therefore this anxiety-like behavior in Li-treated rats can also be responsible from worse performance in the probe trial.<sup>48)</sup>

As a result, although explanation for the present results apparently needs further investigations, we concluded that a 40-day treatment of Li disrupts hippocampal synaptic plasticity underlying memory processes, and that these effects of prolonged treatment are not associated with its direct chemical effect, but are likely to be associated with the molecular actions of Li at genetic levels, because its short-term effect preserves synaptic plasticity. However, it may be suspected that the data of these articles were contaminated by the neuronal toxicity of Li, because of contradictory data concerning an improvement of hippocampal plasticity that has been previously reported.

#### ■ Acknowledgments

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