Circadian activation of the hypothalamic-pituitary-adrenal axis may affect central, but not peripheral, effect of lithium in conditioned taste aversion learning in rats.

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- ABSTRACT -

Circadian activation of the hypothalamic-pituitary-adrenal axis may affect central, but not peripheral, effect of lithium in conditioned taste aversion learning in rats.

Activation of the hypothalamic-pituitary-adrenal (HPA) axis has been implicated in conditioned taste aversion (CTA) learning induced by lithium chloride. This study investigated if circadian activation of the HPA axis affects the lithium-induced CTA formation. The pairing of conditioned stimulus (sucrose) and unconditioned stimulus (lithium chloride) was performed at night (shortly after light-off) when the HPA activity shows its circadian increase. Intraperitoenal injection of lithium chloride (0.15 M, 3 ml/kg or 12 ml/kg) at night induced CTA formation and the HPA axis activation and increased c-Fos expression in both the parabrachial nucleus (PBN) and the nucleus tractus of solitarius (NTS) in a dose dependent manner. However, intracerebroventricular lithium (0.6 M, 5 μl) at night failed to induce CTA or the HPA axis activation, although it increased c-Fos expression in the PBN and NTS. Results suggest that circadian activation of the HPA axis may affect central, but not peripheral, effect of lithium in CTA formation, and the lithium-induced c-Fos expression in brain regions may not be effective to induce CTA unless it is coupled with

the HPA axis activation. It is concluded that the HPA axis activation may play an important role mediating not only peripheral but also central effect of lithium in CTA

Keywords: Hypothalamic–pituitary–adrenal axis, Learning, Lithium chloride, Conditioned taste aversion, Circadian rhythm

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I. INTRODUCTION

Conditioned taste aversion (CTA) is a robust form of associative learning, in which a single pairing of a novel taste with a toxic substance produces a strong and persistent avoidance of substances containing that taste (Garcia et al., 1974, Garcia 1955). Lithium chloride is a toxic substance that produces gastrointestinal distress; hence it has been commonly used to formulate CTA (Domjan and Gillan, 1976 and Nachman and Ashe, 1973). Intraperitoneal (ip) injection with lithium chloride at large doses induces c-Fos expression in the brain regions, such as the hypothalamic paraventricular nucleus (PVN), the nucleus tractus of solitarius (NTS) and the parabrachial nucleus (PBN), and c-Fos expression in these brain regions is well correlated with CTA learning (Houpt et al., 1994, Jahng et al., 2004a, Lamprecht and Dudai, 1995, Sakai and Yamamoto, 1997, Schafe and Bernstein, 1996 and Yamamoto et al., 1992). Since PVN is considered as the center of the hypothalamic-pituitary-adrenal (HPA) axis, c-Fos expression in the PVN refers the activation of the HPA axis by stressful stimuli (Briski and Gillen, 2001 and Figueiredo et al., 2003); thus, c-Fos expression in the PVN by an ip lithium chloride suggests lithium-induced activation of the HPA axis (Figueiredo et al., 2003, Jahng et al., 2004a and Kim et al., 2014). In addition,

administration of lithium chloride by ip route induces adrenocorticotrophic hormone (ACTH) release (Sugawara et al., 1988), activates the HPA axis (Hennessy et al., 1980) and increases the plasma glucocorticoids (Jahng et al., 2004a and Spencer et al., 2005). It has been reported that the pattern of lithium-induced CTA learning is modulated by adrenalectomy or treatments with ACTH or glucocorticoids (Hennessy et al., 1980, Kim et al., 2014, Peeters and Broekkamp, 1994 and Revusky and Martin, 1988), which strongly suggests that HPA axis activation may mediate the lithium-induced CTA learning.

Administration of lithium chloride at sufficient doses to induce CTA by ip route increased gene expression of inducible cyclic adenosine monophosphate early repressor (ICER) in the rat adrenal cortex in a dose dependent manner (Jahng et al., 2004b). ICER is an inducible member of cAMP response element modulator (Sassone-Corsi, 1998) and is proposed to be responsible for terminating c-Fos transcription (Foulkes et al., 1991 and Mao et al., 1998). Induction of ICER mRNA expression in the adrenal gland is coupled to the HPA axis activation (Della Fazia et al., 1998 and Spencer et al., 2005). Spencer et al. (2005) have demonstrated a linear relationship between the adrenocortical ICER expression and the plasma corticosterone level following ip lithium chloride. Pharmacological suppression of the HPA axis activity with dexamethasone pretreatment

blunted not only the formation of lithium-induced CTA learning (Hennessy et al., 1980, Kim et al., 2014 and Smotherman, 1985) but also the adrenocortical ICER expression (Spencer et al., 2005), supporting its implication in lithium-induced CTA. Interestingly, a diurnal variation was also observed in the adrenocortical ICER expression (Spencer et al., 2005). Other study showed spontaneous increase of ICER expression in the adrenal cortex of rats at night time when the plasma corticosterone level shows its circadian increase (Atkinson and Waddell, 1997).

In this study, we have examined whether the circadian increase of plasma corticosterone and/or the adrenocortical ICER expression affects the lithium-induced CTA formation. We have also investigated whether intracerebroventricular (icv) injection of lithium chloride at night time induces CTA formation with increased ICER expression in the adrenal cortex. Previous studies performed at day time demonstrated that icv lithium chloride at a specific dose (0.6 M LiCl, 5 μl/rat) induces CTA formation (Barranco et al., 2001) and the adrenocortical ICER expression in rats (Spencer et al., 2005).

II. MATERIALS AND METHODS

A. Animals

Male Sprague-Dawley rats were purchased (200-250 g, Samtako Bio Osan, Republic of Korea) and maintained in a specific pathogen-free barrier zone with the constantly-controlled temperature (22±1 ° C) and humidity (55%) on a 12 h light/12 h dark cycle (lights on at 07:00 h) in the Seoul National University Animal Facility Breeding Colony. Rats had free access to Purina rodent chow (Purina Co., Seoul, South Korea) and water, and were habituated in the animal colony for a week prior to treatment to minimize handling stress. Animals were cared for according to The Guide for Animal Experiments, 2000, edited by the Korean Academy of Medical Sciences, which is consistent with the NIH Guideline Guide for the Care and Use of Laboratory Animals, 1996 revised. All experimental animal protocols were approved by the Committee for the Care and Use of Laboratory Animals at Seoul National University.

B. Intracerebroventricular cannulation

Under chloral hydrate (153 mg/kg) and pentobarbital (35 mg/kg) anesthesia, rats were stereotaxically implanted with a 22-gauge, stainless-steel guide cannula (Plastics One, Roanoke,VA) aimed toward the lateral ventricle (1.2 mm caudal to bregma, 1.5 mm lateral to the midline, and 4 mm below the skull surface). Guide cannulae were held in place with dental acrylic bonded to stainless-steel screws anchored to the skull. An obdurator was inserted into each guide cannula and remained in place except during injections, when it was removed and replaced with an injector that extended 1.0 mm beyond the tip of the guide cannula. After 1 week of post-operational recovery, patency and placement of the cannulae were verified by injection of 100 ng human angiotensin II (Sigma Chemical Co., St. Louis, MO, USA) dissolved in 5 μl of 0.15 M NaCl; rats with cannulae projecting into the lateral ventricle responded to the angiotensin injection by vigorously licking the water bottle within 2 min, whereas rats that failed to drink were dropped from the study (Jahng et al., 2004a, Lee et al., 2010 and Spencer et al., 2005). Cannula placements were also verified postmortem by sectioning through the brain.

C. Conditioning procedure

Rats (n=6 in each group; total 18 rats in ip groups and 12 rats in icv groups) had free access to chow pellets, but had only 4 h of access to water daily (19:00–23:00 h) as the only source of fluid for 5 days as training period. On the conditioning day (day 6), rats were allowed to drink 5% sucrose as the only source of fluid for 30 min, and then immediately after sucrose, they received an ip or icv injection of lithium chloride (0.15 M LiCl, 3 ml/kg or 12 ml/kg for ip; 0.6 M LiCl, 5 μl/rat for icv) at 19:30 h. Control groups received sodium chloride (0.15 M NaCl, 12 ml/kg for ip; 0.6 M NaCl, 5 μl/rat for icv) instead of lithium chloride. Rats were allowed to recover from the operation of icv cannulation for one week before the conditioning. The volume of icv injection was delivered over 30 s with a hand-held 50 μl syringe (Hamilton Co., Reno, NV, USA), and the injector was left in place for 30 s after solution delivery. Water was supplied immediately after the conditioning until 23:00 h. After 1 day of recovery with 5 h of water supply, rats had access to 5% sucrose for 30 min daily at 19:00 h and water was offered right after sucrose until 23:00 h. The weight of sucrose solution consumed was recorded and used to quantify the CTA.

D. Measurement procedures

1. c-Fos immunohistochemistry

One hour after the drug injections (at 20:00 h), rats (n=6 in each group; total 30 rats) were anesthetized with over doses of sodium pentobarbital (Hanllym Pharmaceutical Co., Seoul. Korea), and transcardiac perfusions were performed first with haparinized isotonic saline (0.9% NaCl, 0.5% NaNO2) followed by ice-cold fixative (4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2; Sigma Co., MO, USA). Brains and the adrenal glands were immediately dissected out, post-fixed for 3 h, and transferred into 30% sucrose (Sigma Co., MO, USA) for cryoprotection. Forty-micron coronal sections were cut on a freezing, sliding microtome (HM440E, Microm Co., Germany), and the brain sections were used for c-Fos immunohistochemistry and the adrenal sections for ICER in situ hybridization. Alternate sections were collected from rostro-caudal extend of the PBN (between bregma -8.64 mm and -9.60 mm) and the NTS (between bregma -13.2 mm and -14.3 mm). The coordinates were based on Paxinos and Watson (2005). Immunohistochemistry was performed with standard DAB reaction using commercial ABC kit (Vectastain Elite Kit, Vector Laboratories, CA, USA) as described previously (Jahng et al., 2004a). Polyclonal rabbit anti-c-Fos peptide antibodies (1:20,000 dilutions, Oncogene Sciences, CA, USA) were used as primary antibodies,

and biotinylated anti-rabbit IgG (1:200 dilution, Vector laboratories, CA, USA) as secondary. Immuno-stained sections were mounted in an anatomical order onto gelatin-coated slides from 0.05 M phosphate buffer, air-dried, dehydrated through a graded ethanol to xylene, and cover-slipped with Permount.

2. ICER in situ hybridization

Forty-micron sections of the adrenal glands were collected into 20-ml glass scintillation vials containing ice-cold 2X SSC (0.3 M sodium chloride, 0.03 M sodium citrate). The SSC was pipetted off, and the sections were suspended in 1 ml of prehybridization buffer (50% formamide, 10% dextran sulfate, 2X SSC, 1X Denhardt's solution, 50 mM dithiothreitol, and 0.5 mg/ml denatured herring sperm DNA) and incubated for 2 h at 48 ° C. In situ hybridization was performed with radioactively labeled ICER cDNA probes (166-bp restriction fragment comprising the ICER-specific portion of CREM cDNA; Stehle et al., 1993) as described previously (Choi et al., 2003). The tissue sections were then mounted on gelatin-subbed slides, air-dried, and apposed to Kodak BioMax film (Eastman Kodak Co., NY, USA) at 4 ° C. All adrenal sections from each experiment were exposed to the same piece of film at the same time, allowing simple comparison within each experiment. Exposure times varied to obtain autoradiographic images within a linear range of optical density

after development in Kodak D-19 developer. In situ hybridization was carried out on the representative members of each experimental group at the same time under identical conditions, allowing direct comparison of mRNA expression.

3. Plasma corticosterone assay

Cardiac blood was collected into heparinized glass tubes immediately after exposing the heart for perfusion, and centrifuged at 2000 rpm for 20 min. The plasma was transferred into eppendorf tubes, frozen in liquid nitrogen, and stored at −80 ° C until used. Plasma levels of corticosterone were determined by radioimmunoassay using 125I-labeled Coat-A-Count kit (Siemens, CA, USA). The sensitivity of the assay was 5.7 ng/ml. The intra-assay coefficient of variation was 4-12.2%.

4. Quantitative analysis

c-Fos immuno-positive nuclei in each brain region were hand-counted blind after digitizing 720×540 μm2 images using an Olympus BX-50 microscope (Olympus Co., Tokyo, Japan) and a Leica image analysis system (DFC290, Leica Microsystems, Wetzler, Germany). Only distinct brown dots were counted as c-Fos positive nuclei. The number of c-Fos positive nuclei in three sections from the PBN region (closest sections to bregma −9.10 mm) from each brain was averaged. The NTS was divided

into two subregions: caudal (ventral and caudal to the area postrema), and intermediate (abutting the forth ventricle). Each of these two subregions was represented by four sections of the NTS sections collected from each rat. Nuclei counts for all sections within each region of each rat were averaged per section, and the individual mean counts for each region averaged across rats by region within experimental groups.

Images on the autoradiographic films were digitized with the Olympus BX-50 microscope (Olympus Co., Tokyo, Japan) attached to the Leica digital camera system (DFC290, Leica Microsystems GmbH, Wetzlar, Germany). Quantification of the hybridized signals on X-ray films was analyzed with Multi Gauge V3.0 (FUJIFILM, Japan) software. mRNA expression level was determined by quantifying the mean relative optical density of pixels with densities of at least 2 S.D. above the mean density of the image background (mRNA pixels). For each section, the mean background value was subtracted from the mean mRNA pixel value. The mRNA pixel values were averaged across three sections from each individual rat, and the average mRNA values of each rat were then averaged across all rats within each experimental group. The average mRNA values of each experimental group were then converted to relative values to the control groups.

E. Statistical analyses

Data were analyzed by one-way analysis of variance and preplanned comparisons with the controls were performed by post-hoc PLSD when necessary, using SPSS 19.0 (IBM, Armonk, NY, USA). The level of significance was set at $P<0.05$, and all values were presented by means±S.E.M.

III. RESULTS

A. CTA learning by a systemic lithium chloride at night

For the pairing of conditioned stimulus (CS) and unconditioned stimulus (US), rats had 5% sucrose access as CS at 19:00 h for 30 min and then received an ip injection of lithium chloride (0.15 M, 3 ml/kg or 12 ml/kg) as US at 19:30 h. Control rats received the same injection volume of sterile saline instead of lithium chloride. On the test day 1, sucrose intake was significantly decreased both in low dose (3 ml/kg) and high dose (12 ml/kg) lithium groups compared with each group on the conditioning day (Fig. 1). Sucrose intake of the low dose group reached to the base line by the test day 2, while the high dose group still showed a significant reduction in sucrose intake compared with its conditioning day. Sucrose intake of the high dose lithium group reached to its base line by the test day 3.

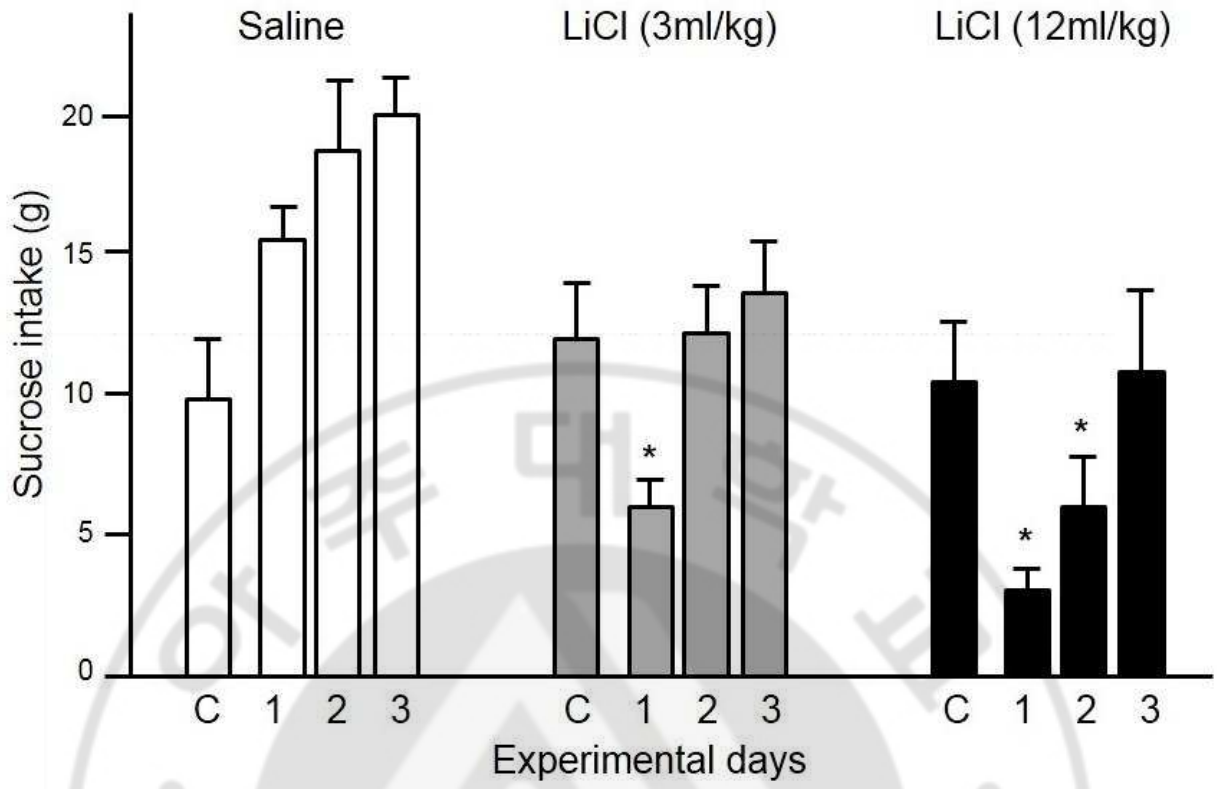

Fig. 1. Sucrose intake at the CS-US pairing (conditioning day; C) and each test day (1-3). For the CS-US pairing, rats were allowed to drink 5% sucrose as the only source of fluid for 30 min, and then received an intraperitoneal injection of lithium chloride (0.15 M LiCl, 3 ml/kg or 12 ml/kg) or saline (0.15 M NaCl, 12 ml/kg) at 19:30 h. n=6, *P<0.05 vs. the conditioning day in each group. Data are presented by means±S.E.M.

One hour after the ip lithium chloride (0.15 M, 3 ml/kg or 12 ml/kg) or saline at 19:30 h, rats were sacrificed to examine c-Fos expression in the brain regions, ICER expression in the adrenal cortex, and the plasma corticosterone levels. The ip lithium chloride markedly induced c-Fos

expression in the PBN and the NTS (Fig. 2A), and the quantificational analysis revealed a dose-dependent increase of c-Fos immuno-positive nuclei by ip lithium in all 3 regions examined (Fig. 2B). ICER mRNA expression in the adrenal cortex appeared to be increased by an ip lithium chloride at night in a dose-dependent manner (Fig. 3A and B). However, a significant increase in the plasma corticosterone level at 1 h after the ip lithium chloride at night was observed only in the high dose (0.15 M LiCl, 12 ml/kg) group, but not in the low dose (0.15 M LiCl, 3 ml/kg) group (Fig. 3C).

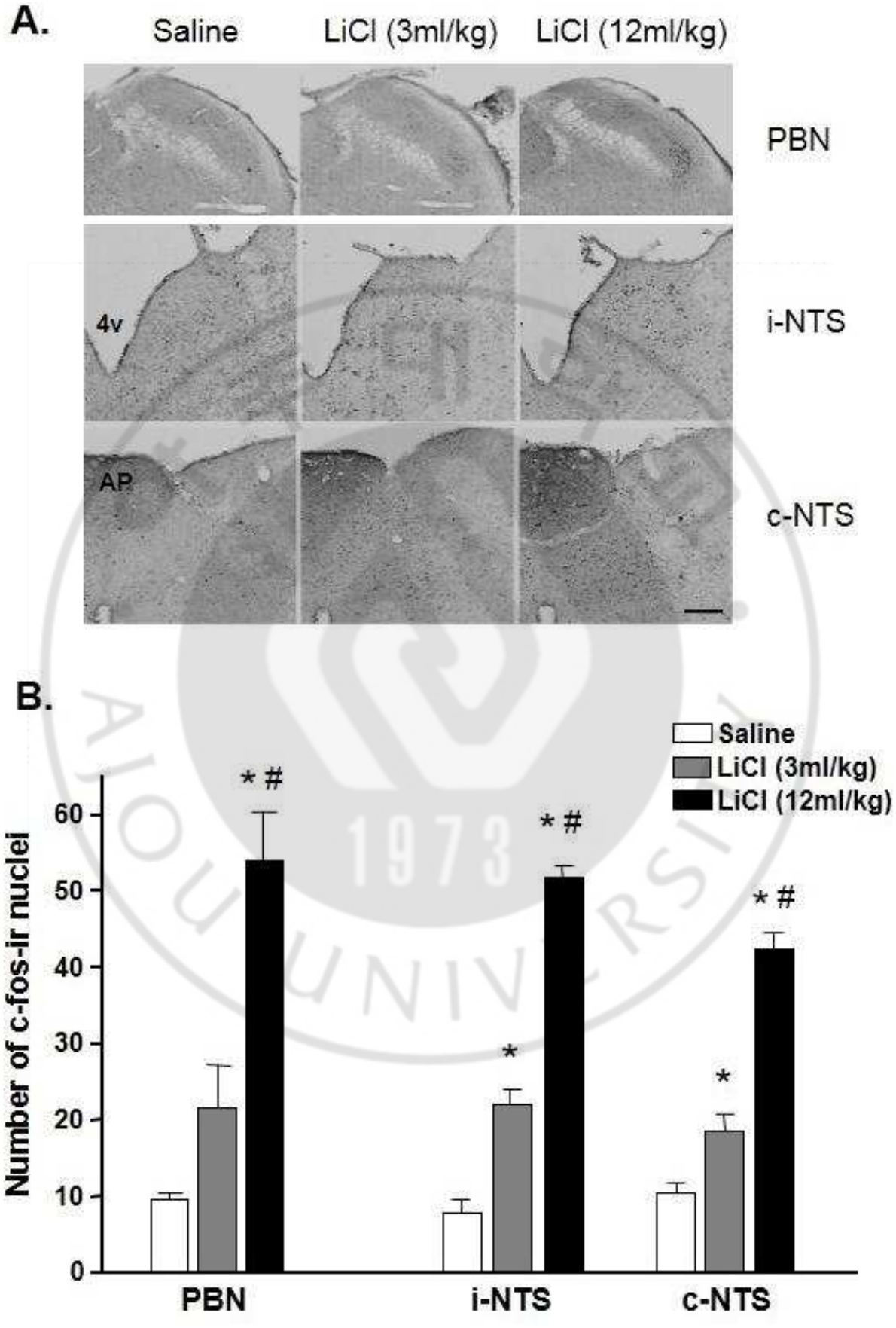
A.
Saline
LiCl (3ml/kg)
LiCl (12ml/kg)
PBN
4v
i-NTS
AP
c-NTS
B.
Saline
LiCl (3ml/kg)
LiCl (12ml/kg)
Number of c-fos-ir nuclei
0
10
20
30
40
50
60
* #
*
PBN
i-NTS
c-NTS

Fig. 2. c-Fos immunohistochemistry in the parabrachial nucleus (PBN) and the nucleus of solitary tract (NTS). Representative photographs of immuno-stained tissue sections (A) and the quatitative analysis of c-Fos immuno-reactive nuclei in each brain region (B). One hour after an intraperitoneal injection of lithium chloride (0.15 M LiCl, 3 ml/kg or 12 ml/kg) or saline (0.15 M NaCl, 12 ml/kg) given at 19:00 h, rats were transcardially perfused with 4% paraformaldehyde, and then the brain tissues were processed for c-Fos immunohistochemistry with standard DAB reaction using commercial ABC kit. 4v; 4th ventricle, AP; area postrema, i-NTS; intermediate NTS, c-NTS; caudal NTS, Scale bars: 200 μm, n=6, *P<0.05 vs. saline, #P<0.05 vs. LiCl (3 ml/kg). Data are presented by means±S.E.M.

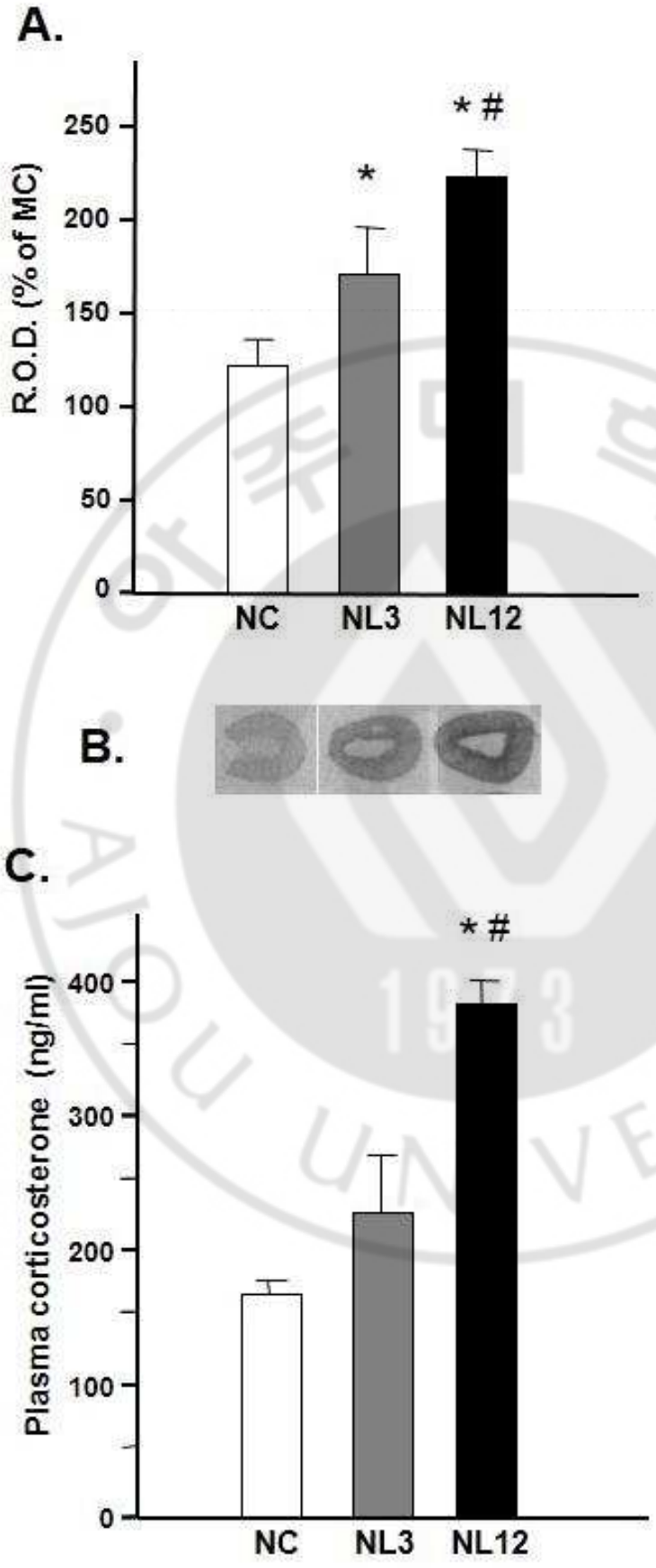
A.
R.O.D. (% of MC)
250
200
150
100
50
0
*
*#
NC
NL3
NL12
B.
C.
Plasma corticosterone (ng/ml)
400
300
200
100
0
*#
NC
NL3
NL12

Fig. 3. Quantitative analysis (A) and the representative autoradiography (B) of ICER in situ hybridization in the adrenal cortex, and the plasma corticosterone levels (C). Cardiac bloods were collected immediately after exposing the heart for perfusion at 1 h after an intraperitoneal injection of lithium chloride (0.15 M LiCl, 3 ml/kg or 12 ml/kg) or saline (0.15 M NaCl, 12 ml/kg) given at 19:00 h, and the plasma corticosterone levels were analyzed by radioimmunoassay. Adrenal glands were dissected out after the perfusion with 4% paraformaldehyde and then processed for ICER in situ hybridization with radioactively labeled cDNA probes. NC; night control (0.15 M NaCl, 12 ml/kg), NL3; night lithium 3 ml/kg, NL12; night lithium 12 ml/kg, R.O.D.; relative optical density, n=6, *P<0.05 vs. NC, #P<0.05 vs. NL3. Data are presented by means±S.E.M.

B. CTA learning by a central lithium chloride at night

For the CS–US pairing, rats had 5% sucrose access as CS at 19:00 h for 30 min and then received an icv injection of lithium chloride (0.6 M, 5 μl) as US at 19:30 h. Control rats received icv administration of 5 μl of 0.6 M sodium chloride instead of lithium. The icv lithium chloride as US at the dose used did not suppress CS intake during the test days (Fig. 4).

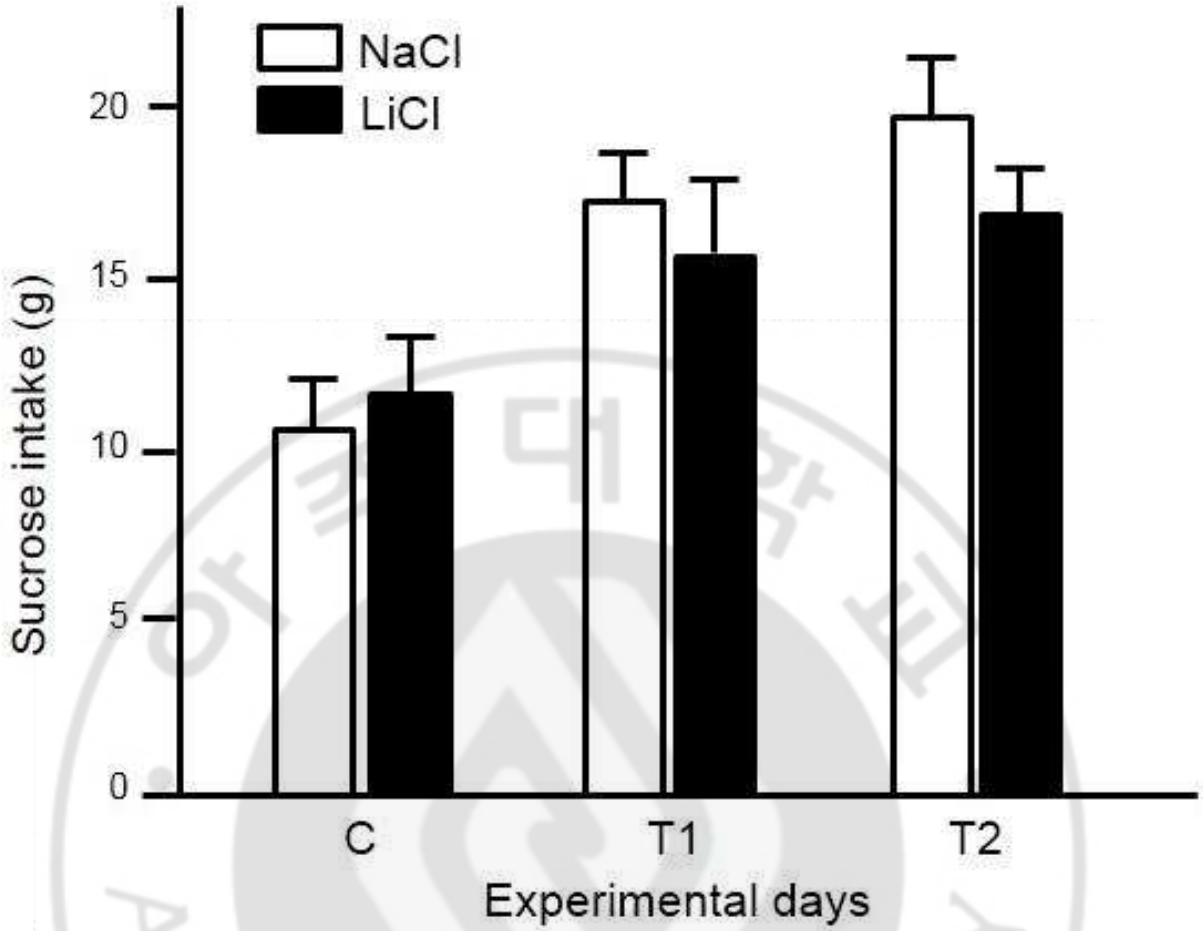

Fig. 4. Sucrose intake at the CS-US pairing (conditioning day; C) and each test day (T1, T2). For the CS-US pairing, rats were allowed to drink 5% sucrose as the only source of fluid for 30 min, and then received an intracerebroventricle injection of lithium chloride (0.6 M LiCl, 5 ㎕) or sodium chloride (0.6 M NaCl, 5 ㎕) at 19:30 h, n=6. Data are presented by means±S.E.M.

One hour after the icv lithium or sodium chloride (0.6 M, 5 ㎕, respectively) at 19:30 h, rats were sacrificed to examine c-Fos expression

in the PBN and the NTS, and ICER expression in the adrenal cortex. Interestingly, icv lithium at the dose used significantly increased c-Fos immuno-positive nuclei in all three brain regions examined (Fig. 5), but not ICER mRNA level in the adrenal cortex (Fig. 6A and B). The plasma corticosterone levels measured at 1 h after the icv injections did not differ between lithium and sodium injected rats (Fig. 6C).

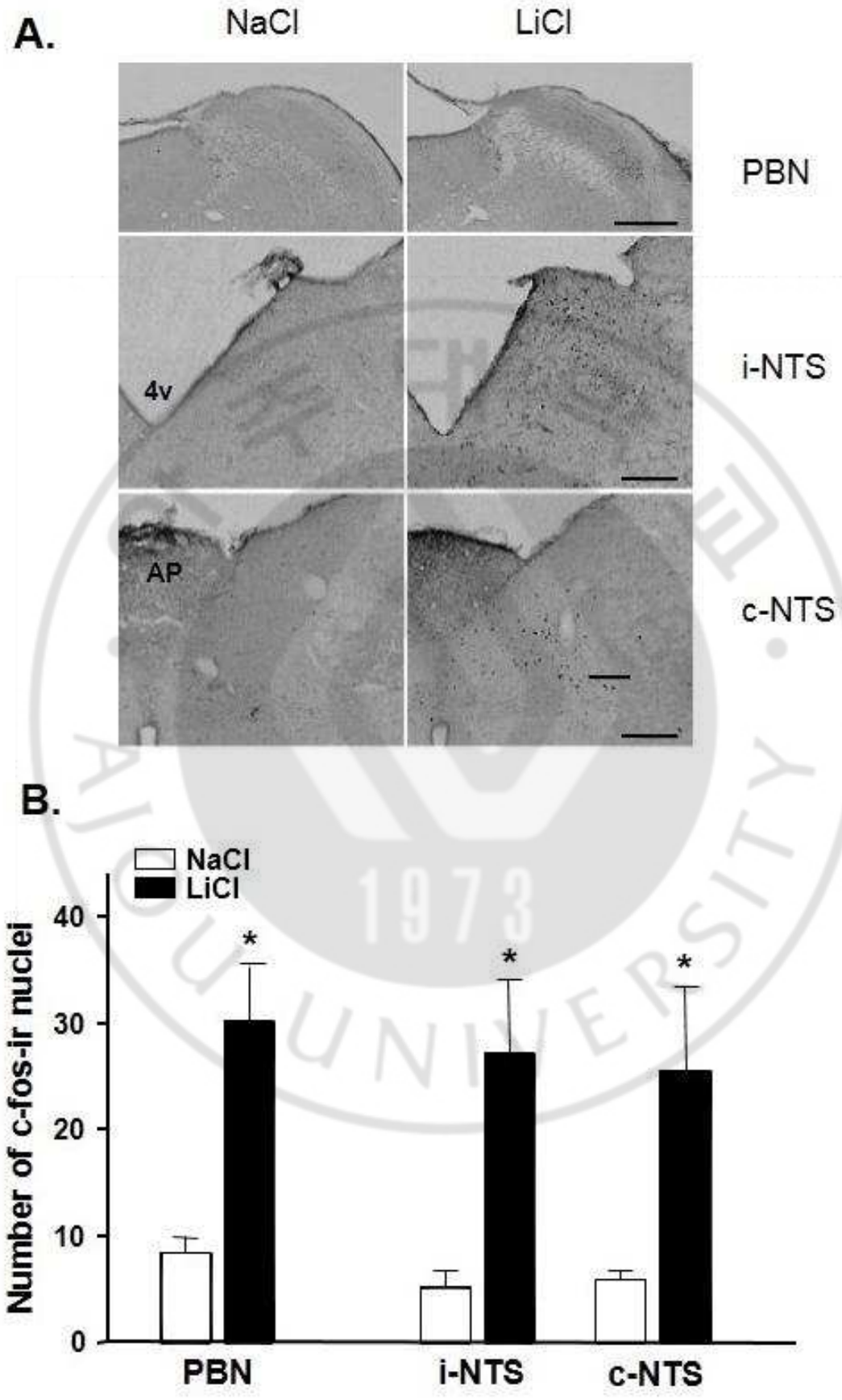
A.
NaCl
LiCl
PBN
i-NTS
c-NTS
4v
AP
B.
NaCl
LiCl
Number of c-fos-ir nuclei
40
30
20
10
0
*
*
*
PBN
i-NTS
c-NTS

Fig. 5. c-Fos immunohistochemistry in the parabrachial nucleus (PBN) and the nucleus of solitary tract (NTS). Representative photographs of immuno-stained tissue sections (A) and the quatitative analysis of c-Fos immuno-reactive nuclei in each brain region (B). One hour after an intracerebroventricle injection of lithium chloride (0.6 M LiCl, 5 μl) or sodium chloride (0.6 M NaCl, 5 μl) given at 19:00 h, rats were transcardially perfused with 4% paraformaldehyde, and then the brain tissues were processed for c-Fos immunohistochemistry with standard DAB reaction using commercial ABC kit. 4v; 4th ventricle, AP; area postrema, i-NTS; intermediate NTS, c-NTS; caudal NTS, Scale bars: 200 μm, n=6, *P<0.05 vs. NaCl in each group. Data are presented by means±S.E.M.

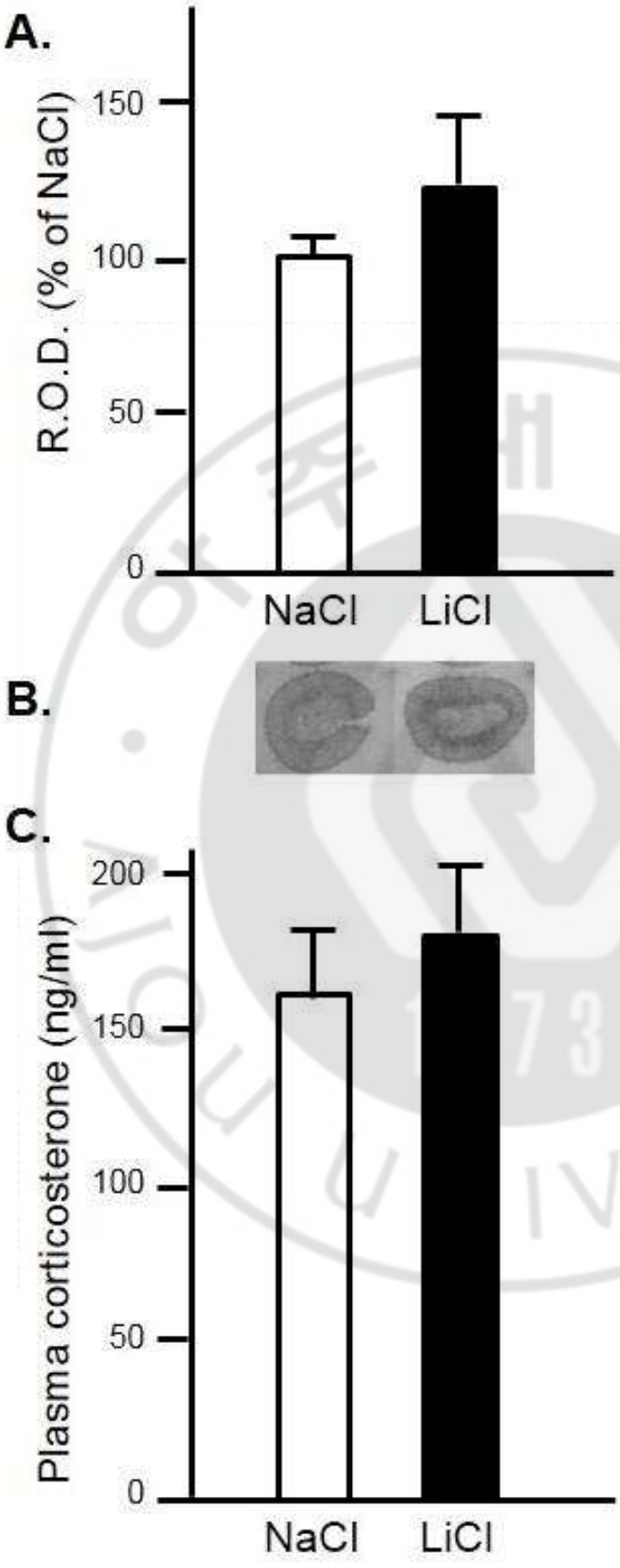
A.
R.O.D. (% of NaCl)
150
100
50
0
NaCl
LiCl
B.
C.
Plasma corticosterone (ng/ml)
200
150
100
50
0
NaCl
LiCl

Fig. 6. Quantitative analysis (A) and the representative autoradiography (B) of ICER in situ hybridization in the adrenal cortex, and the plasma corticosterone levels (C). One hour after an intracerebroventricle injection of lithium chloride (0.6 M LiCl, 5 μl) or sodium chloride (0.6 M NaCl, 5 μl) given at 19:00 h, rats were transcardially perfused with 4% paraformaldehyde, and then the adrenal glands were processed for ICER in situ hybridization with radioactively labeled cDNA probes. Cardiac bloods were collected immediately after exposing the heart for perfusion and the plasma corticosterone levels were analyzed by radioimmunoassay. R.O.D.; relative optical density, n=6. Data are presented by means±S.E.M.

IV. DISCUSSION

Previous studies have suggested that ip injection of lithium chloride activates the HPA axis with increased activity of the hypothalamic PVN neurons (Figueiredo et al., 2003, Hennessy et al., 1980, Jahng et al., 2004a, Kim et al., 2014, Spencer et al., 2005 and Sugawara et al., 1988), and the HPA axis activation may mediate the lithium-induced CTA learning (Hennessy et al., 1980, Kim et al., 2014, Peeters and Broekkamp, 1994 and Revusky and Martin, 1988). This study examined whether or not the circadian activation of the HPA axis affects the lithium-induced CTA acquisition, since the HPA axis activity shows diurnal variation and most of rodent studies reported have been performed during day time when their HPA axis maintains the base line activity. In this study, an ip lithium chloride at night increased the adrenocortical ICER expression in a dose dependent manner, and also induced CTA formation as US. Previous studies performed at day time demonstrated that ip lithium increases ICER mRNA expression in the adrenal cortex (Jahng et al., 2004b and Spencer et al., 2005) and the adrenocortical ICER expression can be a good marker of the HPA axis activation (Spencer et al., 2005). Thus the present results reveal that ip lithium chloride can further activate the HPA axis and induce CTA formation even at night when the HPA axis shows the circadian activation.

Interestingly, the plasma corticosterone levels that measured at 1 h after the ip lithium showed a significant increase only by the high dose lithium (0.15 M, 12 ml/kg), but not by the low dose (0.15 M, 3 ml/kg), as Jahng et al. (2004a) previously reported in a day time study, while the adrenal ICER expression showed a dose-dependent increase. It is plausible that the peak increase of corticosterone following the low dose lithium might have missed in both studies since the analysis was done only at one time point. Otherwise, it is suggested that the adrenocorticoal ICER expression may be a more sensitive marker of the lithium-induced HPA axis activation than the plasma corticosterone levels. Previous studies performed during day time have demonstrated that ip lithium chloride at large enough doses to induce CTA formation increases c-Fos expression in the brain regions including the PBN and NTS, and c-Fos expression in these brain regions is correlated with CTA learning (Houpt et al., 1994, Jahng et al., 2004a, Lamprecht and Dudai, 1995, Sakai and Yamamoto, 1997, Schafe and Bernstein, 1996 and Yamamoto et al., 1992). In this study, a dose-dependent effect of a night time ip lithium chloride was observed not only in CTA formation but also in c-Fos expression in the PBN and the NTS, suggesting that lithium-induced c-Fos expression in the brain regions may contribute to CTA formation regardless of the circadian activation of the HPA axis. Taken all together, it is concluded that ip lithium chloride increases c-Fos expression in the brain regions, activates the HPA axis

and induces CTA formation even at night when the HPA axis shows the circadian activation.

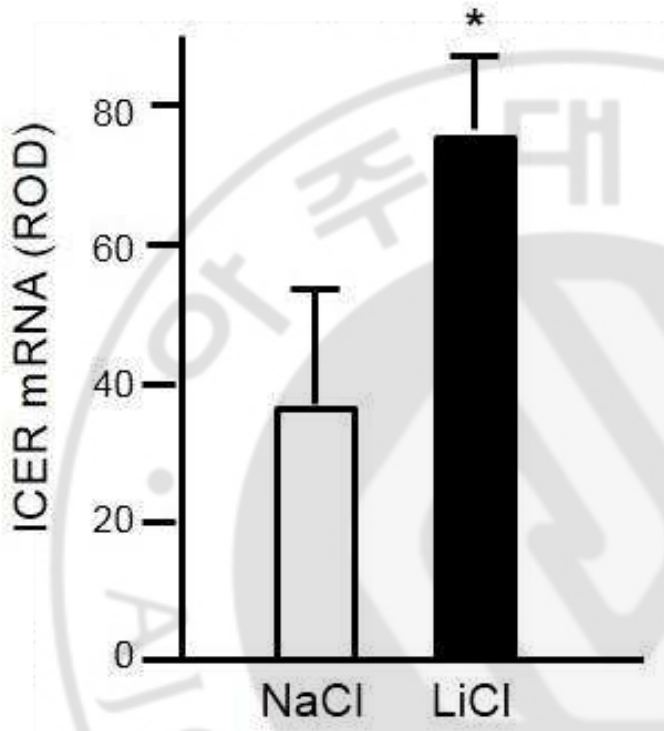

Fig. 7. ICER mRNA expression in the adrenal gland 1 hr after an intracerebroventricular LiCl (0.6 M) or isoosmotic NaCl (0.6 M). Central LiCl significantly induced ICER mRNA expression in the adrenal cortex. Data represent means 6 SEM. * P=0.016 vs. NaCl group. (Spencer, et al., 2005)

Smith (1980) proposed that lithium-induced CTA formation is mediated by peripheral effect of lithium such as gastrointestinal distress, since ip injection of iso-osmotic lithium chloride (0.15 M) induced CTA and icv injection of it failed to do so in his study. Later on, it was demonstrated that icv administration of hyper-osmotic lithium chloride (0.6 M) induces CTA formation with discrete inductions of c-Fos expression in multiple brain regions (Barranco et al., 2001). Also, ICER mRNA expression in the adrenal cortex was significantly increased at 1 h after an icv administration of 0.6 M lithium chloride (Fig 7; Spencer et al., 2005), suggesting the HPA axis activation by icv lithium. These reports support the idea that lithium-induced CTA formation can be mediated by central effect of lithium inducing c-Fos expression in the brain regions and the HPA axis activation, even without the gastrointestinal distress by peripheral effect of lithium. Gastrointestinal information reached to the NTS is principally relayed to the gustatory cortex via the PBN, but also targets to the other brain area including the hypothalamic PVN (Norgren, 2004). That is, although the gastrointestinal distress by peripheral effect of lithium can contribute to the CTA formation with neuronal activation in those brain regions followed by the HPA axis activation, central effect of lithium was proven to be sufficient to do so (Barranco et al., 2001 and Spencer et al., 2005). However, in this study, 0.6 M icv lithium chloride given at night failed to induce either CTA formation or the adrenocortical ICER expression, despite it significantly increased c-Fos expression in the PBN and the NTS. These results reveal

that the icv lithium at night was sufficient to increase c-Fos expression in the brain regions regardless of the circadian activation of the HPA axis, however, not sufficient to further activate the HPA axis overshooting its circadian activation and failed to induce CTA. As previously reported, it was confirmed that a day time 0.6 M icv lithium chloride induces CTA formation (Barranco et al., 2001).

Many studies have reported that ip lithium-induced c-Fos expression in the brain regions such as the PBN and NTS is correlated with CTA learning (Houpt et al., 1994, Jahng et al., 2004a, Lamprecht and Dudai, 1995, Sakai and Yamamoto, 1997 and Yamamoto et al., 1992). The brain c-Fos expression by central effect of lithium was also suggested to be correlated with CTA formation (Barranco et al., 2001). However, the present study suggests that the brain c-Fos expressions by lithium may not be effective to induce CTA formation unless it is coupled with the HPA axis activation. It has been reported that clinical doses of lithium as mood stabilizer have the potential to augment the HPA measures (Jacobson, 2014); suggesting that the central effect of lithium activating the HPA axis is important also for its therapeutic efficacy. Further studies are under consideration to examine if pharmacologic suppression of the HPA axis activity affects CTA formation by day time icv lithium. Also, it should be examined if the c-Fos expressing neurons in the PBN and NTS following

icv lithium are identical with ones following ip lithium. Lastly, the interpretation of present study may have a limitation because the current study did not directly compare the night time effects of ip/icv lithium with the day time effects of them in this study.

V. CONCLUSION

Administration of lithium chloride by ip route at night induces CTA formation with increased c-Fos expression in the brain regions and the HPA axis activity. Although icv lithium chloride at night increased c-Fos expression in the brain regions, it failed to induce the HPA axis activation and CTA formation. It is concluded that the HPA axis activation may play an important role mediating not only peripheral but also central effect of lithium in CTA formation.

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– 국문요약 –

백서에서 일주기에 따라 시상하부–뇌하수체–부신 축이 활성화되면 조건화 미각 혐오 학습에 대한 중추에서의 리튬 영향이 약화될 수 있다.

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시상하부–뇌하수체–부신 (HPA) 축의 활성화는 염화 리튬에 의한 조건화 미각 혐오 (CTA) 학습과 연관되어 있음이 보고되어 왔다. 본 연구에서는 일주기에 따른 HPA 축의 활성화가 리튬 유도 CTA 형성에 어떠한 영향을 미치는지 연구하였다. 백서에서 일주기성 HPA 축 활성화가 이루어지는 밤시간(조명 끈 직후)에 조건화 자극(설탕)과 비조건화 자극(염화 리튬)으로 조건화 과정을 시행하였다. 밤 시간의 염화 리튬(0.15M, 3ml/kg or 12 ml/kg)을 복강내에 투여하면 용량에 비례하여 CTA 형성이 유도되고, HPA 축이 활성화 되었으며, 뇌의 parabrachial nucleus(PBN)와 nucleus tractus of solitaries(NTS) 부위의 c–Fos 발현이 증가하였다. 그러나 밤 시간에

리튬(0.6M, 5㎕)을 뇌실내로 주입하게 되면, CTA 가 유도되지 않았으며, HPA 축이 활성화되지 않았다. 그러나 뇌의 PBN 과 NTS 부위의 c-Fos 는 발현되었다. 이러한 결과는 일주기에 의한 HPA 축의 활성화가 CTA 형성을 위한 중추에서의 리튬 효과에 영향을 미쳤음을 보여준다고 하겠다. 그러나 여전히 말초성 효과는 동일하였다. 또한, 뇌내 구역에서의 c-Fos 발현이 늘어나도 HPA 축 활성화로 이어지지 않는다면 CTA 는 유도되지 않음을 확인할 수 있었다. 결론적으로, HPA 축 활성화는 CTA 형성에 있어 리튬의 말초성과 중추성 역할 모두를 중개하는 중요한 역할을 하는 것으로 보인다.

핵심어: 시상하부-뇌하수체-부신 축, 학습, 리튬, 조건화 미각 혐오, 일주기 리듬