A New Immunohistochemical Method to Evaluate the Development of Vestibular Compensation after Unilateral Labyrinthectomy in Rats.

Kazunori Matsuda[a]* Tadashi Kitahara[b], Taeko Ito[b], Munehisa Fukushima[c], Junya Fukuda[a], Go Sato[a], Yoshiaki Kitamura[a], Koji Abe[a], Atsuhiko Uno[d], Koichi Tomita[e], Hiromi Sakata-Haga[f], Yoshihiro Fukui[g], Noriaki Takeda[a]

a) Department of Otolaryngology, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima 770-8503, Japan

b) Department of Otolaryngology-Head and Neck Surgery, Nara Medical University, Kashihara, Nara 634-8521, Japan

c) Department of Otolaryngology and Head and Neck Surgery, Kansai Rosai Hospital, Hyogo 660-8511, Japan

d) Department of Otolaryngology and Head and Neck Surgery, Osaka General Medical Center, 3-1-56 Bandai-Higashi, Sumiyoshi-ku, Osaka, 558-8558, Japan

e) Department of Anatomy and Developmental Neurobiology, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima 770-8503, Japan

f) Department of Anatomy, Kanazawa Medical University, Ishikawa 920-0293, Japan

g) Department of Human Care, Nagoya University of Arts and Sciences, Aichi 470-0196, Japan

*Correspondence to: Kazunori Matsuda, M.D.

Department of Otolaryngology, Institute of Biomedical Sciences, University of Tokushima Graduate School, 3-18-15 Kuramoto, Tokushima 770-8503, Japan.

Tel: +81-88-633-7168 Fax: +81-88-633-7170.

E-mail: matsudakazunori1024@gmail.com

Abstract

Background: Unilateral labyrinthectomy (UL) causes disappearance of ipsilateral medial vestibular nuclear (ipsi-MVe) activity and induces spontaneous nystagmus (SN), which disappears during the initial process of vestibular compensation (VC). Ipsi-MVe-activity restores in the late process of VC.

Objectives: We evaluated the late process of VC after UL in rats and examined effects of thioperamide (H3 antagonist) on VC.

Material and Methods: MK801 (NMDA antagonist)-induced Fos-like immunoreactive (-LIR) neurons in contra-MVe, which had been suppressed by NMDA-mediated cerebellar inhibition in UL-rats was used as an index.

Results: The number of MK801-induced Fos-LIR neurons in contra-MVe gradually decreased to the same level as that of sham-operated rats 14 days after UL. Thioperamide moved the disappearance of the MK801-induced Fos-LIR neurons 2 days earlier. The number of the MK801-induced Fos-LIR neurons in thioperamide-treated rats was significantly decreased, compared with that of vehicle-rats on days 7 and 12 after UL. But, thioperamide did not influence the decline of SN frequency in UL-rats.

Conclusion: There findings suggested that the number of MK801-induced Fos-LIR neurons in contra-MVe was decreased in concordance with the restoration of ipsi-MVe-

activity during the late process of VC after UL and that thioperamide accelerated the late, but not initial process of VC.

Keywords: vestibular compensation; medial vestibular nucleus; spontaneous nystagmus; Fos; N-methyl-D-aspartate receptor; thioperamide

Introduction

Unilateral labyrinthectomy (UL) causes disappearance of ipsilateral vestibular nuclear (ipsi-MVe) activity in the brainstem. UL-induced asymmetry between bilateral vestibular nuclear activities produces spontaneous nystagmus (SN) and postural deviation [1]. However, the asymmetry disappears over time with the development of vestibular compensation (VC) in the brain [1.2]. It was shown that the N-methyl-D-aspartate (NMDA) receptor, a subtype of glutamate receptor plays an important role in the development of VC [3]. Our previous study showed that NMDA receptor-mediated cerebellar inhibition on the contralateral vestibular nucleus (contra-MVe) was the initial process of the development of VC in unilaterally labyrinthectomized rats [4]. The initial process that rebalances the intervestibular nuclear activities was evaluated with the decline of SN frequency after UL [2.5].

Restoration of ipsilateral vestibular nuclear activity was reported to be the essential and late process of VC to reinforce symmetrical neural activities between bilateral vestibular nuclei after UL [6.7]. It was also reported that in unilaterally labyrinthectomized rats, MK801, an NMDA receptor antagonist, temporarily induced reappearance of both SN and postural deviation that is called decompensation and appearance of Fos-like immunoreactive (-LIR) neurons in the contra-MVe [8].

Because Fos-positive neurons in the contra-MVe had been suppressed by NMDA receptor-mediated cerebellar inhibition on the contra-MVe, we hypothesize that the number of MK801-induced Fos-LIR neurons in the contra-MVe was decreased in concordance with the restoration of ipsi-MVe neural activity during the late process of VC after UL.

In the present study, an attempt was made to develop a new method to evaluate the late process of VC after UL in rats. For this purpose, we examined if MK801-induced Fos-LIR neurons in the contra-MVe can be used as an index. It was reported that thioperamide, a histamine H3 receptor antagonist, facilitates VC in cats [9]. We then used this new evaluation method and examined the effects of thioperamide on the development of VC in unilaterally labyrinthectomized rats.

Materials and Methods

Experimental animals

Adult male Wistar rats (Japan SLC, Inc., Japan) weighing 150-200g were used in this study. Rats housed individually in polycarbonate cages with wood-chip bedding at an environmental temperature of 20-22°C with a 12-hours light/12-hours dark cycle (lights

on at 08:00, off at 20:00) and were fed with solid rat chow and water freely. This study was performed in accordance with the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of Ministry of Education, Culture, Sports, Science and Technology. All procedures were approved by the Division for Animal Research Resources and Genetic Engineering Support Center for Advanced Medical Sciences, Institute of Biomedical Sciences, Tokushima University Graduate School.

Unilateral Labyrinthectomy

Experimental rats were anesthetized with isoflurane (Wako Pure Chemical Industries, Ltd, Japan) and subjected to UL of the right ear as previously described [8]. Briefly, after the tympanic bulla was opened, the tympanic membrane, malleus and incus were removed by the postauricular approach under an operating microscope. The stapes crura were fractured and the foot plate was removed to open the oval window. The membranous labyrinth was destroyed chemically by injection of 100% ethanol. At the end of surgery, an antibiotic (Ofloxacin) cream was applied to the opened labyrinth to prevent infection. Finally, the operative wound was sutured and the animals were allowed to recover in the light. UL was confirmed by the appearance of SN and postural

asymmetry after recovery from anesthesia. Sham-operated rats were subjected to a sham-operation consisting of removal of the tympanic membrane and malleus handle of the right ear only.

Drug Administration

MK801 (Santa Cruz Biotechnology, Inc., CA, USA) was dissolved in 0.9% saline to a volume of 1.0 ml/kg and injected intraperitoneally at a dose of 1.0 mg/kg in rats 6 h, 12 h, 1 day, 2 days, 3 days, 5 days, 7 days, 10days, 12 days and 14 days. The dose of 1.0 mg/kg was decided, based on previous studies that showed it induced full decompensation in unilaterally labyrinthectomized rats [4].

Thioperamide (Santa Cruz Biotechnology, Inc., CA, USA) was dissolved in 0.9% saline and infused intraperitoneally (0.5 μl/hour) at a dose of 3.5 mg/kg/day via osmotic minipump (Alzet, Palo Alto, CA, USA) just after UL. Osmotic minipump was filled with thioperamide or saline and implanted intraperitoneally until day 14 after UL. The dose of 3.5 mg/kg/day was decided, because it was shown to accelerate the disappearance of SN and recovery of posture and locomotor balance in unilaterally neurectomized cats [9.10].

Behavioral investigation

Eye movements were videotaped using a Sony HDR-CX500V video camera with a zoom lens, and the frequency of SN was measured as the number of quick phase beats for 15 seconds in unilaterally labyrinthyectomized rats. The video images were replayed using SD Memory Card and a liquid-crystal display, and the frequency of SN was measured 3 times for each animal and the mean was obtained. These measurements were made at 0.5, 1, 2, 3, 6, 12, 18, 24, 30, 36 and 42 hours after UL.

Tissue preparation and Immunohistochemical staining

Two hours after intraperitoneal administration of MK801, rats were deeply anesthetized with isoflurane and perfused transcardially with 100ml of 4 °C saline, and then with 250ml of 4% paraformaldehyde dissolved in 0.1mol/L phosphate buffer (PB). The rat brain was immediately removed after perfusion, post-fixed in the same fixative solution at 4°C for 1-2 days. The fixed brains were immersed in 30% sucrose-PB at 4°C for 2-3 days. The tissue was then sectioned to a thickness of 30 μm on a cryostat (Leica CM1850, Germany), and the peroxidase-anti-peroxidase (PAP) method was used to visualize the immunohistochemical reaction. Free-floating sections were pretreated with 0.1%H2O2 in 0.3% Triton X-100 in phosphate-buffered saline (PBS) for 30 minutes,

and incubated in 5% normal goat serum (NGS) in PBS containing 0.3% Triton-X (PBST). The sections were, then incubated with rabbit polyclonal anti-Fos antibody (Santa Crus Biotechnology, CA, USA) diluted 1:5000 in PBST containing 1% NGS for 2 days at 4 °C. After incubation with the primary antibody and a brief wash, the sections were incubated with anti-rabbit IgG (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan) diluted 1:1000 in PBST for an hour at room temperature. The Fos-LIR neurons were visualized by incubating sections with DAB Substrate Kit (Vector Laboratories, CA, USA).

Cell counting

Transverse 30 μm-thick sections of the brainstem were examined under bright-field microscopy at ×40 and ×100 magnification to detect Fos-LIR cells in contra-MVe. Only cells that had significant levels of DAB reaction product in their nucleus above tissue background levels were counted with a digital image analysis system (Image-J software)

Experimental protocol

Experimental rats were divided into four groups: 1) control rats with UL only, 2)

thioperamide rats receives thioperamide after UL, 3) vehicle rats received saline after UL and 4) sham-operated rats. Control rats (n=35) were subjected to UL of the right ear and intraperitoneally administrated with MK801 6 h (n=2), 12 h (n=2) h, 1 day (n=5), 2 days (n=4), 3 days (n=2), 5 days (n=2), 7 days (n=5), 10 days (n=5), 12 days (n=5) and 14 days (n=3) after UL. Thioperamide rats (n=33) were subjected to UL of the right ear and received intraperitoneal injections of thioperamide via osmotic minipump. They were then intraperitoneally administrated MK801 6 h (n=2), 12 h (n=2) h, 1 day (n=2), 2 days (n=2), 3 days (n=4), 5 days (n=2), 7 days (n=6), 10 days (n=5), 12 days (n=5) and 14 days (n=3) after UL. Vehicle rats (n=10) were subjected to right UL and received intraperitoneal injections of saline via osmotic minipump. They were then intraperitoneally administrated MK801 7 days (n=5), 12 days (n=5) after UL.

Statistical analysis

Statistical analyzes were performed by two-factorial analysis of variance and Student's t-test. $p<0.05$ was considered significant.

Results

The development of vestibular compensation after UL in rats

After right UL, SN beating to the left was induced in rats. The frequency of SN rapidly decreased from the maximum of 30.1 ± 5.0 (mean ± SD, 25-35) beats/15 seconds at 30 minutes and disappeared within 42 hours of UL (control in Fig. 1). Six hours after UL, a substantial number of Fos-LIR neurons appeared in the right ipsi-MVe but not in the contra-MVe (Fig. 2A). Fos-LIR neurons in the right ipsi-MVe disappeared almost entirely within 5 days after UL (Fig. 2B). On day 5 after UL, 2 hours after intraperitoneal (i.p.) administration of 1.0 mg/kg of MK801, SN beating to the left temporarily reappeared (data not shown) and Fos-LIR neurons in the contra-MVe also temporarily appeared (Fig. 2C) in unilaterally labyrinthectomized rats. The number of MK801-induced Fos-LIR neurons in the contra-MVe reached a maximum on day 1 and gradually decreased thereafter (Fig. 3). On day 14 after UL, MK801 induced no SN (data not shown) and a few Fos-LIR neurons in the contra-MVe (Fig. 2D), of which number was almost the same as that in sham-operated rats (Fig. 3).

Effects of thioperamide on the development of vestibular compensation after UL in rats

Intraperitoneal infusion of 3.5 mg/kg/day thioperamide did not change the decline of SN frequency in unilaterally labyrinthectomized rats (thioperamide in Fig. 1). The number

of MK801-induced Fos-LIR neurons in the contra-MVe in control rats that did not received anything equaled that in sham-operated rats on day 14 after UL; whereas, the number of MK801-induced Fos-LIR neurons in the contra-MVe in thioperamide-treated rats equaled that in sham-operated and control rats on day 12 after UL (Fig. 3). The number of MK801-induced Fos-LIR neurons in the contra-MVe in thioperamide-treated rats on day 7 and 12 after UL was significantly decreased, compared with that of vehicle rats with saline infusion that received saline via osmotic minipump (Fig. 4).

Discussion

In the present study, Fos-LIR neurons temporarily appeared in the ipsi-MVe just after UL, as reported in our previous study [8], because Fos is the protein product of an immediate early gene, c-fos has been used as a marker of neural activity [11]. The finding suggests that some neurons were activated in the ipsi-MVe after UL, although electrophysiological studies reported that the spontaneous neural activity of neurons in the ispi-MVe disappeared just after UL [6.12]. Our previous study also showed that the activated neurons in ipsi-MVe drove NMDA-mediate cerebellar inhibition on the contra-MVe in unilaterally labyrinthectomized rats [13]. The inhibitory neural circuit was shown to

rebalance the intervestibular nuclear activities after UL, resulting in the decline of SN frequency in the initial process of the development of VC [4]. In the present study, the administration of MK801, an antagonist of NMDA receptor induced a temporal decompensation including reappearance of SN [13] and appearance of Fos-LIR neurons in the contra-MVe, as reported by Kitahara [8] in unilaterally labyrinthectomized rats. Our previous study also showed that the Fos-LIR neurons in the contra-MVe had been suppressed by NMDA receptor-mediated cerebellar inhibition on the contra-MVe, and Fos was induced in the neurons by the disinhibition due to MK801 in unilaterally labyrinthectomized rats [13].

In the present study, the number of MK801-induced Fos-LIR neurons in the contra-MVe was gradually decreased after UL in rats. Electrophysiological studies have shown that the spontaneous neural activity of ispi-MVe was gradually restored to reinforce symmetrical neural activities of bilateral MVe after UL in the late process of VC [6.7]. The finding suggests that the number of MK801-induced Fos-LIR neurons in the contra-MVe was decreased in concorance with the restoration of ipsi-MVe nuclear activity. It is also suggested that the disappearance of MK801-induced Fos-LIR neurons in the contra-MVe can be used as an index of the late process of VC after UL in rats. The number of MK801-induced Fos-LIR neurons in the contra-MVe in control rats equaled

that in sham-operated rats on day 14 after UL. This finding suggests that the late process of VC was completed in 14 days in unilaterally labyrinthectomized rat. In addition, the number of MK801-induced Fos-LIR neurons in the contra-MVe in thioperamide-treated rats equaled that in sham-operated rats on day 12 after UL. These findings suggest that thioperamide moved the VC completion 2 days earlier. Intraperitoneal infusion of thioperamide significantly decreased the number of MK801-induced Fos-LIR neurons in the contra-MVe on day 7 and day 12 after UL in rats. These findings suggest that thioperamide facilitate the restoration of ipsi-MVe nuclear activity, resulting in the acceleration of the late process of VC in rats.

In the present study, there were no significant differences in the frequency of UL-induced SN between the thioperamide and control rats. Although it was reported that thioperamide accelerates the disappearance of UL-induced SN in cats [9], the present finding suggests that thioperamide did not affect the initial process of VC in experimental rats.

Histamine plays an important role in the processing of sensory information in the vestibular nuclei, the control of vestibular functions and the recovery process following vestibular lesion [14]. In rats, both histamine H1 and H2 receptors are expressed in the vestibular nucleus [15]. It was reported that H2 and/or H1 receptors mediates the

excitatory effect of histamine on the medial vestibular nucleus neurons both in vivo and in vitro [16]. It was also reported that Na+-Ca2+ exchangers coupled to H1 receptors and hyperpolarization-activated cyclic nucleotide-gated channels linked to H2 receptors, co-mediate the postsynaptic excitatory action of histamine on MVe [17]. H3 receptors are located at presynaptic sites on histamine afferent fibers reaching the vestibular nuclei [18]. Blockade of presynaptic H3 receptors with their antagonist increases the release of histamine, which in turn increased the neural activities in-vestibular nucleus [19]. It is suggested that thioperamide-induced release of histamine increased vestibular nucleus activity to restore the spontaneous activity of the ipsi-MVe, resulting in the acceleration of the late process of VC. In cats, it was also reported that thioperamide accelerates the development of VC, suggesting the involvement of thioperamide-induced long-term changes in the expression of histidine decarboxylase (HDC) mRNA in the tuberomammillary nucleus (TMN) [9]. Moreover, blockade of the histamine H3 receptors may additionally release other neurotransmitters, such as glutamate, acetylcholine and GABA, which play a role in VC [20].

In conclusion, we used immunohistochemical technique and developed the new evaluation method of VC in unilateral labyrinthectomized rats. VC consists of two processes: the inhibition of the contra-MVe activities in the initial process, and the

restoration of the ipsi-MVe neural activities in the late process after UL. The initial process of VC was evaluated by the decline of SN frequency after UL, and the late process of VC considered to be the decline of MK801-induced Fos-LIR neurons number in the contra-MVe after UL in rats. We then used this evaluation method and showed that thioperamide, an H3 receptor antagonist, accelerated the late but not initial process of VC after UL in rats.

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Declaration of interest statement

No potential conflict of interest was reported by the authors.

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References

[1] Lacour M, Helmchen C, Vidal PP. Vestibular compensation: the neuro-otologist's best friend. J. Neurol. 2016;263:54-64.

[2] Dutia MB. Mechanisms of vestibular compensation: recent advances. Curr. Opin. Otolaryngol. Head Neck Surg. 2010;18:420–424.

[3] Vidal PP, Babalian A, de Waele C, Serafin M, et al. NMDA receptors of the vestibular nuclei neurons. Brain Res. Bull. 1996;40:347–352.

[4] Kitahara T, Takeda N, Kiyama H, et al. Molecular mechanisms of vestibular compensation in the central vestibular system—review. Acta Otolaryngol. Suppl. 1998;539:19–27.

[5] Smith PF, Darlington CL. Neurochemical mechanisms of recovery from peripheral vestibular lesions (vestibular compensation). Brain Res. Rev. 1991;16:117–133

[6] Ris L, Capron B, Vibert N, et al. Modification of the pacemaker activity of vestibular neurons in brainstem slices during vestibular compensation in the guinea pig. European Journal of Neuroscience. 2001;13:2234-2240.

[7] Beraneck M, Hachemaoui M, Idoux E, et al. Long-term plasticity of ipsilesional

medial vestibular nucleus neurons after unilateral labyrinthectomy. J. Neurophysiol. 2003;90:184-203.

[8] Kitahara T, Takeda N, Saika T. et al. Effects of MK801 on Fos expression in the rat brainstem after unilateral labyrinthectomy. Brain Res. 1995;700:182–190.

[9] Tighilet B, Trottier S, Mourre C. et al. Changes in the histaminergic system during vestibular compensation in the cat. J Physiol. 2006;573: 723–739.

[10] Tighilet B, Mourre C, Trottier S. et al. Histaminergic ligands improve vestibular compensation in the cat: behavioural, neurochemical and molecular evidence. Eur. J. Pharmacol. 2007;568:149-163.

[11] Cirelli C, Pompeiano M, D'Ascanio P. et al. C-fos expression in the rat brain after unilateral labyrinthectomy and its relation to the uncompensated and compensated stages. Neuroscience. 1996;70:515-546.

[12] Darlington CL, Dutia MB, Smith PF. The contribution of the intrinsic excitability of vestibular nucleus neurons to recovery from vestibular damage. Eur. J. Neurosci. 2002;15:1719-1727.

[13] Kitahara T, Fukushima M, Takeda N. et al. Effects of pre-flocculectomy on Fos expression and NMDA receptor-mediated neural circuits in the central vestibular system after unilateral labyriontyectomy. Acta Otolaryngol. 2000;120:866-871.

[14] Lacour M, Tighilet B. Vestibular compensation in cat: the role of the histaminergic system. Acta Otolaryngol. Suppl. 2000;544:15–18.

[15] Zhou L, Zhou W, Zhang S. et al. Changes in histamine receptors (H1, H2, and H3) expression in rat medial vestibular nucleus and flocculus after unilateral labyrinthectomy: histamine receptors in vestibular compensation. PLoS One. 2013;19: doi: 10.1371/journal.pone.0066684

[16] Wang JJ, Dutia MB. Effects of histamine and betahistine on rat medial vestibular nucleus neurones: possible mechanism of action of anti-histaminergic drugs in vertigo and motion sickness. Exp. Brain Res. 1995;105:18–24.

[17] Zhang XY, Yu L, Zhuang QX. et al. Postsynaptic mechanisms underlying the excitatory action of histamine on medial vestibular nucleus neurons in rats. Br. J. Pharmacol. 2013;170:156-69.

[18] Pillot C, Heron A, Cochois V. et al. A detailed mapping of the histamine H3 receptor and its gene transcripts in rat brain. Neuroscience 2002;114:173–193.

[19] Soto E, Vega R, Seseña E. Neuropharmacological basis of vestibular system disorder treatment. J. Vestib. Res. 2013;23:119-37.

[20] Bergquist F, Ruthven A, Ludwig M. et al. Histaminergic and glycinergic modulation of GABA release in the vestibular nuclei of normal and labyrinthectomised

rats. J. Physiol. 2006;577:857–868.

Figures Legends

Figure 1

Changes over time in the frequency of spontaneous nystagmus after unilateral labyrinthectomy in rats and effects of thioperamide on the decline of spontaneous nystagmus. Each point represents the mean number of quick-phase eye movements in 15 seconds (ordinates) for 5 animals per group (three repeated measures per animal per sampling). Error bars represent the standard errors of the mean (S.E.M.). Control: control rats without treatment as open circles with dotted line. Thioperamide: thioperamide-treated rats with intraperitoneal infusion at a dose of 3.5 mg/kg/day as filled circles with solid line.

Figure 2

Bright-field photomicrographs of Fos-like immunoreactive(-LIR) neurons in the medial vestibular nucleus (MVe) after unilateral labyrinthectomy (UL) in rats. A: Bright-field photomicrographs of Fos-LIR neurons in the MVe on ipsilateral side to UL 6 hours after UL. B: Bright-field photomicrographs of Fos-LIR neurons in the MVe on ipsilateral side to UL 5 days after UL. C: Bright-field photomicrographs of Fos-LIR neurons in

the MVe on contralateral side to UL 2 hours after injection of MK801 on day 5 after UL. D: Bright-field photomicrographs of Fos-LIR neurons in the MVe on contralateral side to UL 2 hours after injection of MK801 on day 14 after UL. Bar: 200μm. IPSI: ipsilateral side to UL. CONTRA: contralateral side to UL.

Figure 3

Changes over time in the number of Fos-like immunoreactive (-LIR) neurons in the medial vestibular nucleus on contralateral side (contra-MVe) to unilateral labyrinthectomy (UL) 2 hours after injection of MK801 in rats and effects of thioperamide on the disappearance of MK801-induced Fos-LIR neurons. Values are expressed as mean number ± S.E.M (standard errors of the mean) of Fos-LIR neurons in the contra-MVe. Control: non-treated control rats are shown as open circles with dotted line. Thioperamide: thioperamide-treated rats with intraperitoneal infusion at a dose of 3.5 mg/kg/day are represented by filled circles with solid line. Sham ope: sham-operated rats are shown as open diamond.

Figure 4

Effects of thioperamide on the number of Fos-like immunoreactive neurons in the medial

vestibular nucleus on contralateral side (contra-MVe) to unilateral labyrinthectomy (UL) 2 hours after injection of MK801 in rats. Columns and bars are expressed as mean number ± S.E.M (standard errors of the mean) of Fos-LIR neurons. Vehicle: vehicle rats with intraperitoneal infusion with saline are shown as dotted column. Thioperamide: thioperamide rats with intraperitoneal infusion with thioperamide at a dose of 3.5 mg/kg/day are shown as filled column. POD7 means 7 days after UL. POD12 means 12 days after UL. *$p < 0.05$

Figure 1

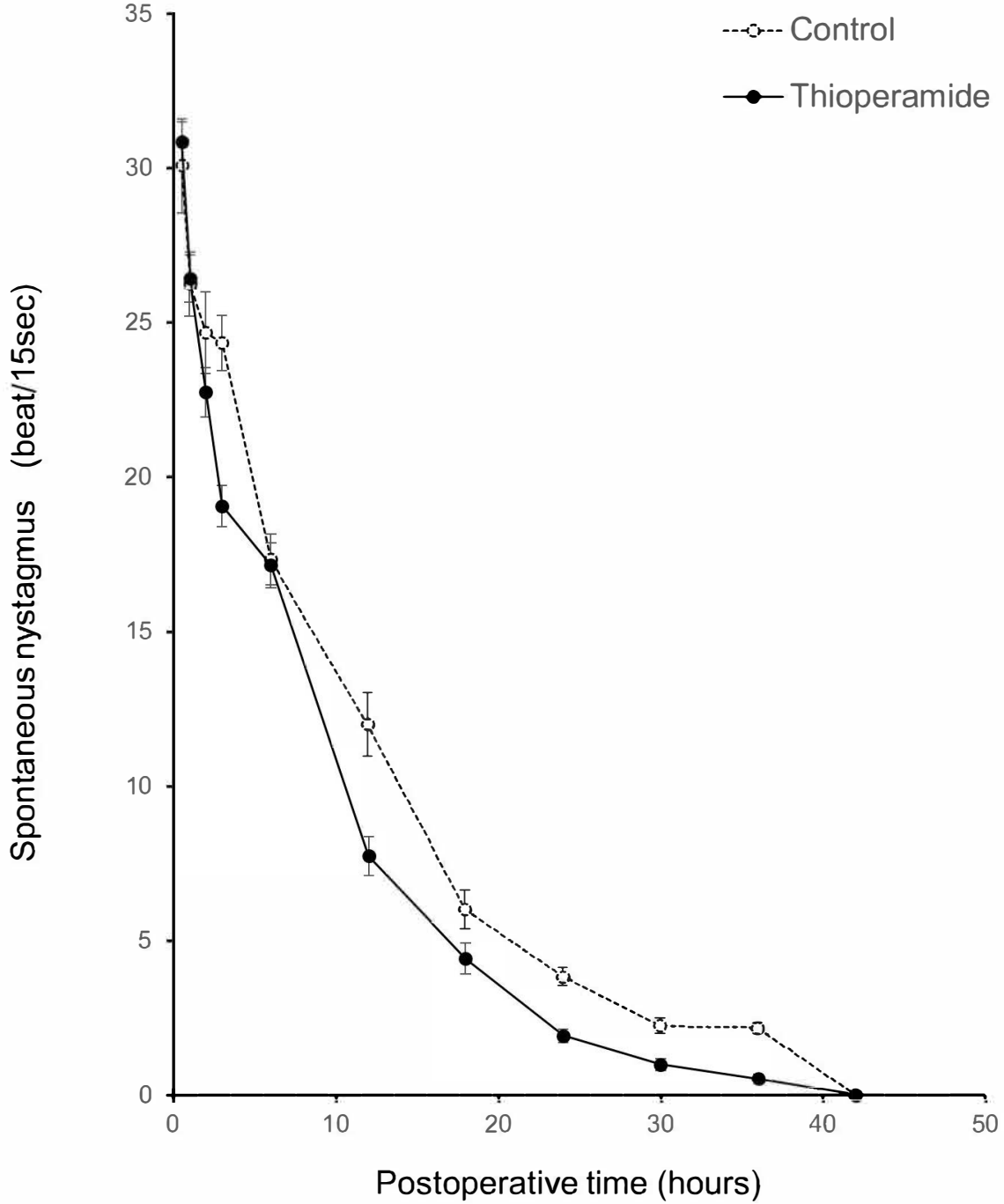
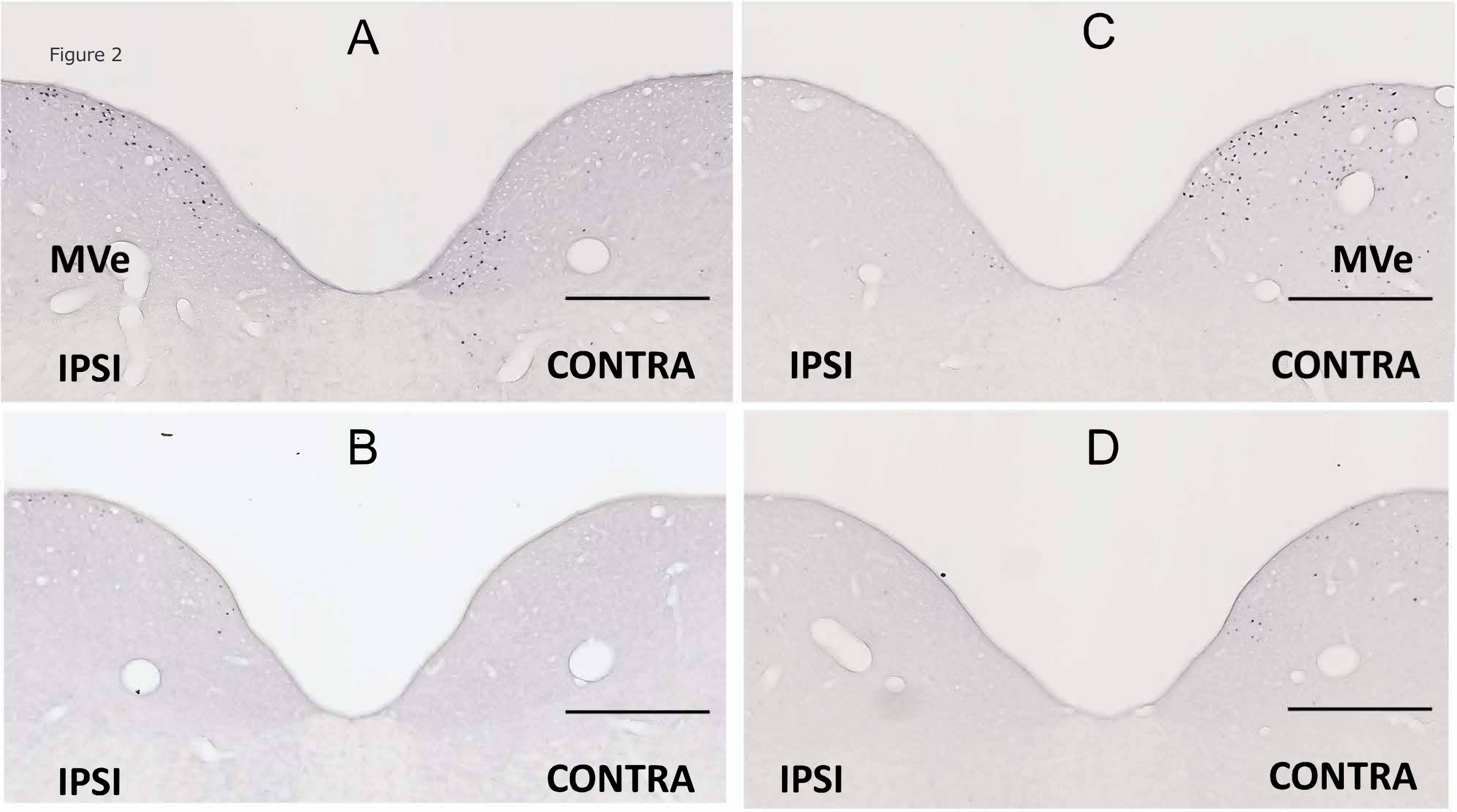

Control
Thioperamide
Spontaneous nystagmus (beat/15sec)
Postoperative time (hours)
0
5
10
15
20
25
30
35
0
10
20
30
40
50

Figure 2


A
MVe
IPSI
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C
MVe
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CONTRA
B
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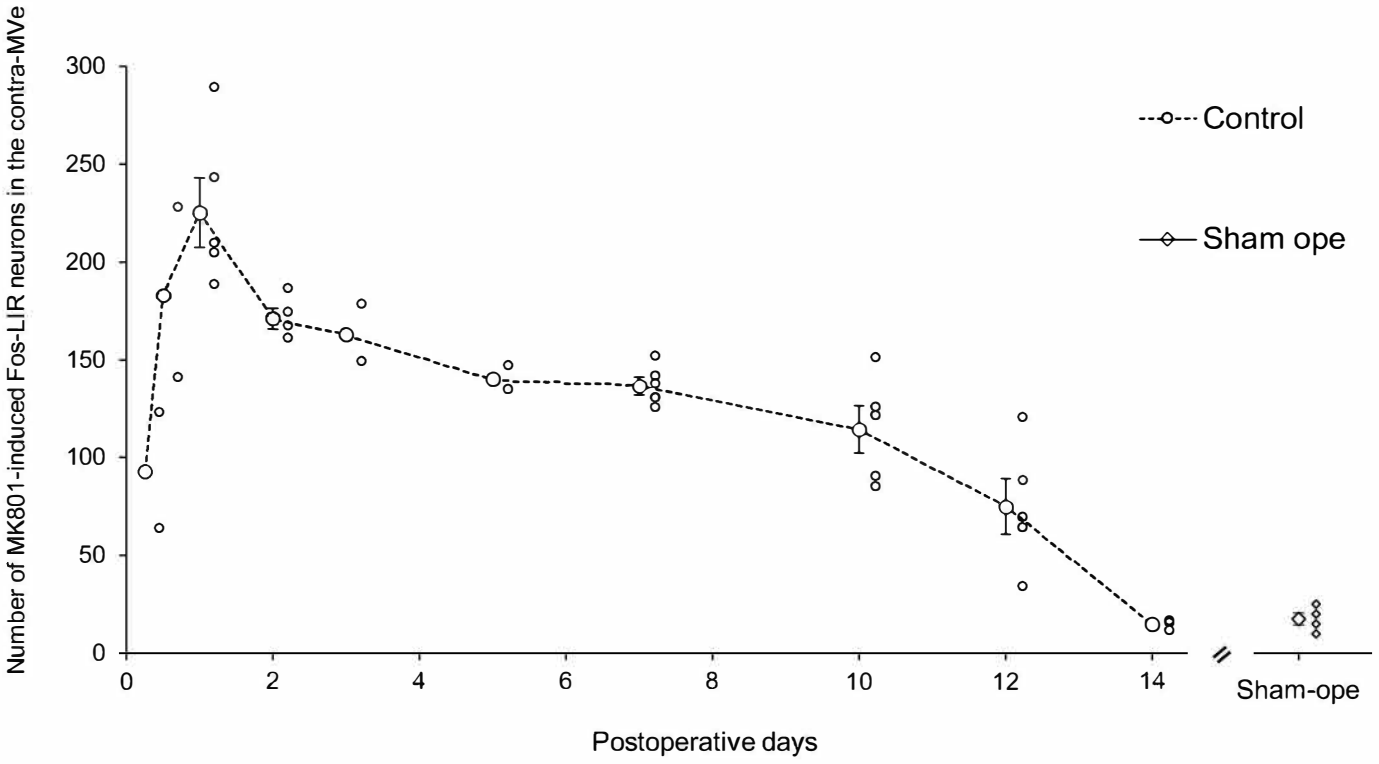
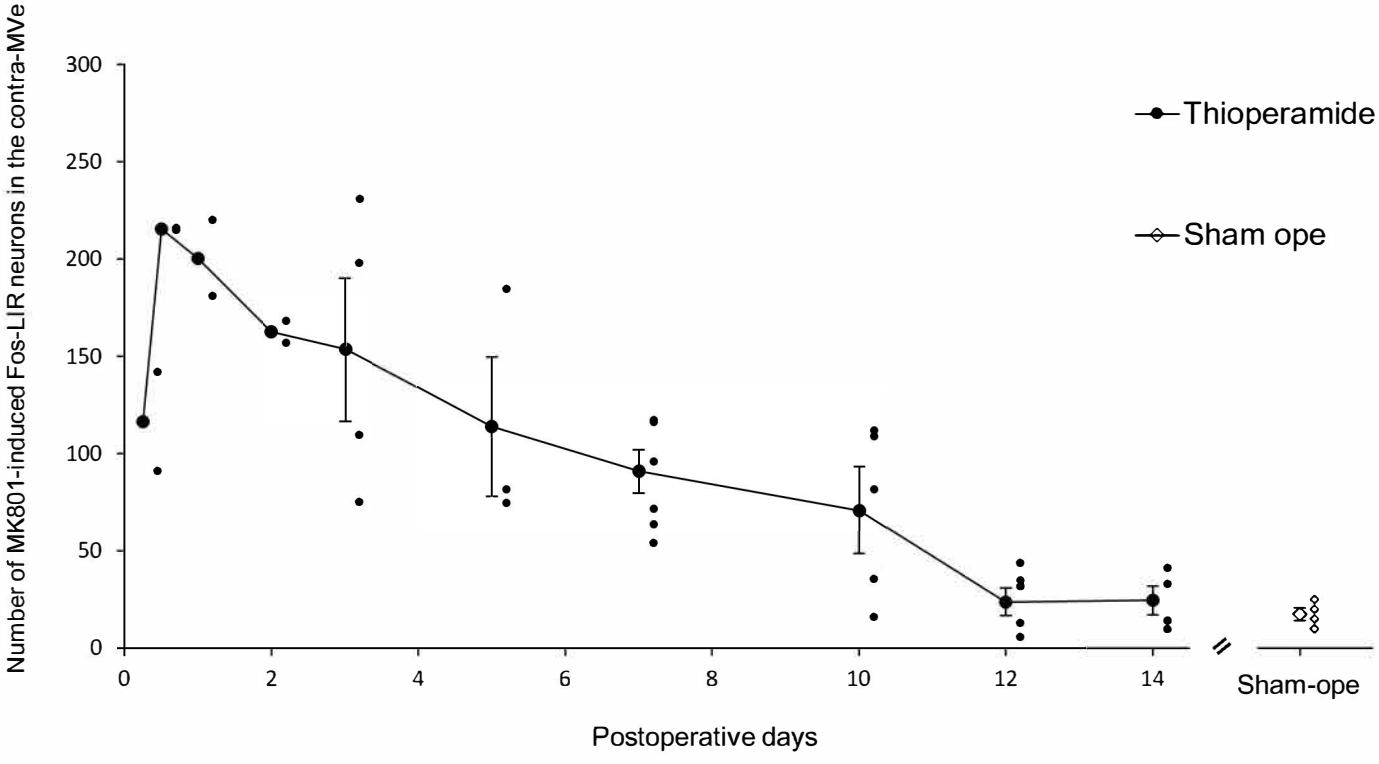
D
IPSI
CONTRA

Figure 3


Number of MK801-induced Fos-LIR neurons in the contra-MVe
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
Sham-ope
Postoperative days
Control
Sham ope


Number of MK801-induced Fos-LIR neurons in the contra-MVe
300
250
200
150
100
50
0
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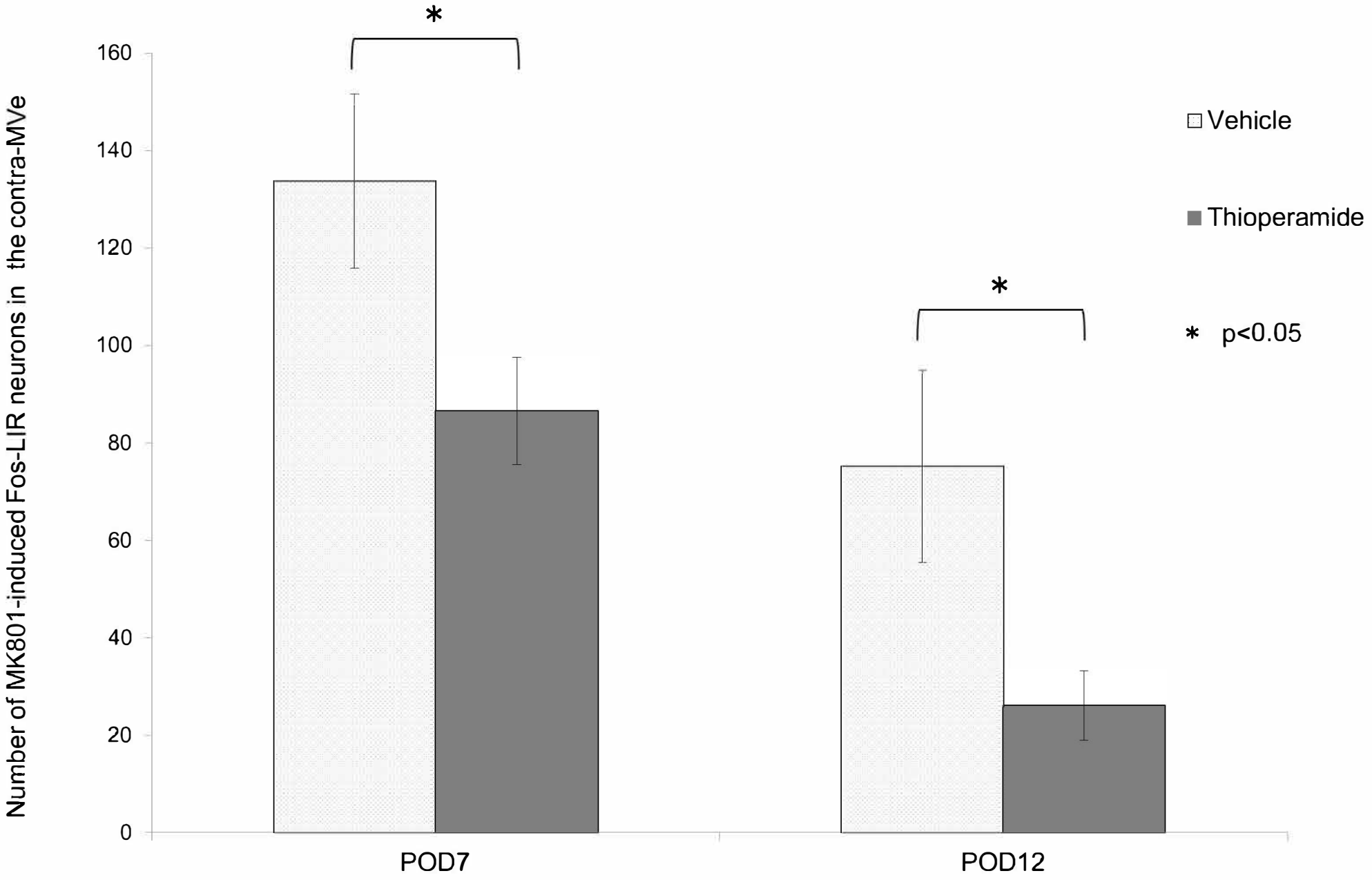
2
4
6
8
10
12
14
Sham-ope
Postoperative days
Thioperamide
Sham ope

Figure 4


Number of MK801-induced Fos-LIR neurons in the contra-MVe
160
140
120
100
80
60
40
20
0
POD7
POD12
*
*
Vehicle
Thioperamide
* p<0.05