Acta Med. Okayama, 2011
Vol. 65, No. 4, pp. 269–277

Acta Medica Okayama

http://escholarship.lib.okayama-u.ac.jp/amo/

Original Article

Inhibitory Effects of Valproate on Impairment of Y-maze Alternation Behavior Induced by Repeated Electroconvulsive Seizures and c-Fos Protein Levels in Rat Brains

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We previously showed that inhibition of repeated electroconvulsive shock (ECS)-induced seizures through 7-day administration of anti-epileptic drugs suppressed the impairment of spontaneous alternation behavior in the Y-maze test in rats. To clarify the precise mechanism(s), we investigated the effect of valproate on such impairment and examined the levels of brain-derived neurotrophic factor (BDNF) and c-Fos protein in the prefrontal cortex and the hippocampus 24 h after the last administration of ECS. Seven-day intraperitoneal (i.p.) administration of valproate (400 mg/kg) suppressed the impairment of spontaneous alternation behavior. Repeated ECS increased the BDNF protein levels in the hippocampus and prefrontal cortex in the presence or absence of valproate, indicating that the increase in BDNF protein levels resulted from electrical stimulation. c-Fos protein levels were significantly decreased in the hippocampal dentate gyrus after repeated ECS, but valproate had no significant effect on decreased c-Fos protein levels. Valproate + ECS significantly increased the c-Fos protein levels of the prefrontal cortex compared with the ECS group. These findings suggest that the inhibitory effect of valproate on repeated ECS-induced impairment of spontaneous alternation behavior may be linked to the prefrontal cortex.

Key words: electroconvulsive shock-induced seizure, spontaneous alternation behavior, valproate, brain-derived neurotrophic factor, c-Fos

Epilepsy is the most common neurologic disorder of the brain. It is characterized by recurrent seizures resulting from uncontrolled excess activity of a part or all of the central nervous system. Recurrent seizures frequently induce psychiatric disorders, including cognitive deficits, behavioral abnormalities and emotional impairment [1, 2].

In studies using experimental animals, we have shown that repeated electroconvulsive shock (ECS) decreased spontaneous alternation behavior in the Y-maze test (which assesses short-term memory) and increased the locomotor activity of rats in an open-field test [3]. Furthermore, inhibition of repeated ECS-induced seizures by pretreatment with the anti-epileptic drugs phenytoin (120 mg/kg, i.p.) and valproate (400 mg/kg, i.p.) abolished these behavioral impairments. These findings indicate that the inhibition of ECS-induced seizures suppresses the impairment of spontaneous alternation and the development of locomotor hyperactivity. Several investigators have shown that repeated administration of ECS affects the dopaminergic system and that this effect is related to

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locomotor hyperactivity induced by repeated administration of ECS in rats and mice [4–6]. However, little is known about the mechanism of the deficit of spontaneous alternation induced by repeated ECS.

The hippocampus [7] and prefrontal cortex [8] have been shown to play important parts in learning and memory. Brain-derived neurotrophic factor (BDNF), which is a member of the neurotrophin family, may have an important role in the brain. Studies have reported that ECS administration increases the level of BDNF mRNA [4–6] and BDNF protein in rats [6, 9, 10]. In this study, to clarify the detailed mechanisms for the protective effect of valproate, we investigated the effect of valproate (250–500 mg/kg (i.p.)) on the impairment of spontaneous alternation behavior in the Y-maze test and locomotor hyperactivity in the open-field test induced by repeated administration of ECS in rats. We then investigated whether cerebral BDNF is associated with the inhibitory effect of valproate on behavioral impairments. It is well known that c-Fos (the protein product of the immediate early gene *c-fos*) is rapidly induced by various stimuli and is used widely as a marker of neuronal activation [11]. To analyze the site of action of valproate in the brain, c-Fos protein levels were examined after repeated administration of ECS and valproate.

Materials and Methods

Approval of the study protocol. Animal experiments were carried out in compliance with the Guidelines for Animal Experimentation. The study protocol was approved by the Committee of Animal Experimentation of Ehime University School of Medicine (Ehime, Japan).

Animals. Male Wistar rats (age, 6 weeks) were obtained from Charles River Japan, Incorporated (Yokohama, Japan). Rats were housed 2 per cage (42 cm × 26 cm × 15 cm) at 24 ± 2℃ with a 12-h light period (7 am to 7 pm). Food and water were available *ad libitum*.

Drugs. Sodium valproate was purchased from Wako Pure Chemical Industries, Limited (Osaka, Japan). Valproate was dissolved in physiological saline (0.9% sodium chloride). Drugs were injected at a volume of 1 ml/kg body weight.

ECS-Induced seizures. ECS (100 V, 50 mA, 60 Hz, 0.2 sec) was administered via corneal electrodes once daily for 7 days. Rats that received ECS exhibited generalized tonic-clonic seizures lasting ～5–10 sec. Sham rats in the control group were handled and treated in a similar manner to the ECS group except for electroshock application.

Spontaneous alternation behavior in the Y-maze test. We examined continuous spontaneous alternation behavior using the Y-maze apparatus. The Y-maze apparatus was made of black plastic with three arms (40 cm × 15 cm × 35 cm) extending from a central platform at 120°. Each rat was placed at the end of one arm and allowed to move freely through the maze during a session lasting 8 min. Arm entry was defined as the entry of 4 paws into one arm. The sequence of arm entries was recorded visually. Alternation was defined as multiple entries into the 3 arms (A, B or C) on overlapping triplet sets. The percentage of spontaneous alternation was calculated as the ratio of the actual-to-possible alternations (defined as the total number of arm entries minus 2), multiplied by 100: as shown in the following equation:

$$\text{Alternation (\%)} = [(\text{number of alternations})/(\text{total arm entries} - 2)] \times 100$$

Locomotor activity in the open-field test. Rats were individually placed in an acrylic apparatus (69 cm × 28 cm) with a gray floor divided into 19 squares. Locomotor activity (defined as grid line crossings by all 4 paws) was measured during an 8-min period by a digital video camera.

Tissue preparation and measurement of BDNF protein by enzyme-linked immunosorbent assay (ELISA). Rats were killed by decapitation 24 h after the last administration of ECS. Brains were quickly removed and dissected on ice into the hippocampus and prefrontal cortex. Samples were frozen at 80℃ before homogenization. Sections were homogenized in lysis buffer (137 mM NaCl, 20 mM Tris, 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 0.5 mM sodium vanadate). Homogenates were centrifuged at 10,000 × g for 20 min. The supernatants were collected and processed for quantification of total free BDNF with a BDNF Emax Immuno Assay system kit (Promega, Madison, WI, USA). ELISAs were carried out using the BDNF Emax Immuno Assay System kit according to the manufacturer's instructions [12]. Nunc Maxisorp 96-well

immunoplates were coated with 100 μl/well of anti-BDNF monoclonal antibody (mAb) and incubated overnight at 4℃. Plates were incubated in a block and sample buffer at room temperature for 1 h. Samples were then added to coated wells (100 μl) and shaken for 2 h at room temperature. The antigen was then incubated with an anti-human BDNF polyclonal antibody (pAb) for 2 h at room temperature with shaking, followed by incubation with an anti-IgY antibody conjugated to horseradish peroxidase (HP) for 1 h at room temperature. Finally, plates were incubated with tetramethylbenzidine solution for 15 min, and 1 M hydrochloride acid was added to the wells. The colorimetric reaction product was measured at 450 nm. Standard curves were plotted for each plate. BDNF concentrations were determined from the regression line for the BDNF standard from 7.8 pg/ml to 500 pg/ml for each plate.

Immunohistochemistry of c-Fos. Rats were acclimated to handling every day for 7 days. The number of rats in each group was 3–4. Rats were deeply anesthetized with sodium pentobarbital. They were perfused transcardially with 200 ml of 0.9% sodium chloride followed by 300 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed and post-fixed in the same fixative. Coronal slices (40 μm) were cut consecutively through the target brain regions with a vibrating microtome. The immunohistochemistry of c-Fos was determined as described by Hamamura *et al.* [13]. Sections from different treatment groups were processed in parallel to minimize the variations in immunohistochemical labeling. Free-floating sections were rinsed in 0.05 M phosphate-buffered saline (PBS; pH 7.4). They were then incubated with 0.6% hydrogen peroxide in PBS to remove endogenous peroxidase activity. After rinsing again in PBS, sections were incubated with primary antibody (1 : 3000 dilution in PBS containing 0.3% Triton X-100, 0.05% sodium azide and 2% normal goat serum) for 72 h at 4℃. The c-Fos antibody (sc-52; Santa Cruz Biotechnology, Santa Cruz, CA, USA) is a rabbit polyclonal antibody raised against a peptide mapping at the amino terminus of human c-Fos p62. Sections were then rinsed and incubated with a secondary antibody (biotinylated goat anti-rabbit IgG [1 : 400 dilution; Vector Laboratories, Burlingame, CA, USA] in PBS with 0.3% Triton X-100) for 75 min at room temperature. After rinsing, sections were transferred into PBS containing 0.4% avidin-biotinylated horseradish peroxidase complex (Vector Laboratories) for a further 75 min. After successive washes in PBS and 0.2M sodium acetate buffer (pH 6.0), the reaction product was visualized using a glucose oxidase-diaminobenzidine-nickel method [14]. The reaction was terminated by washing the sections in sodium acetate buffer. Thereafter, the sections were mounted onto chrome/alum/gelatin-coated slides from 0.05 M PBS. After air-drying, sections were counterstained with neutral red, dehydrated through a graded alcohol series, cleared in xylene, and cover-slipped. Cells from the motor 1 (M1), motor 2 (M2), cingulate 1 (Cg1), prelimbic (PrL) and infralimbic (IL) areas of the prefrontal cortex and the CA1, CA2, CA3 and the dentate gyrus of the hippocampus (Fig. 1) were examined under a magnification of $\times$ 200. Counting of positive cells was carried out bilaterally on a minimum of three representative sections (400 μm $\times$ 400 μm) per region in each rat. These counts were averaged into a single score for each region of each rat, and the group mean $\pm$ S.E.M calculated.

Experimental procedures. ECS was administered for 7 consecutive days. The Y-maze and open-field tests were carried out 24 h after the last administration of ECS. Valproate (250–500 mg/kg, i.p.) or saline (i.p.) was injected 30 min before the daily administration of ECS or sham administration for 7 days. Levels of BDNF and c-Fos protein were measured 24 h after the last administration of ECS.

Statistical analyses. The prevalence of tonic-clonic seizures was assessed for significance by the chi-squared test for independence. If more than 2 groups were compared, the significance of the differences among groups was evaluated through a one-way analysis of variance (ANOVA). Two-way ANOVA, with ECS administration as the between-subjects factor and valproate treatment as the within-subject factor, was used. If significant differences were obtained, post-hoc comparisons within logical sets of means were carried out using Bonferroni's test. Significance was defined as $p < 0.05$.

Results

The Effect of valproate on the prevalence of ECS-induced tonic-clonic seizures. The effect

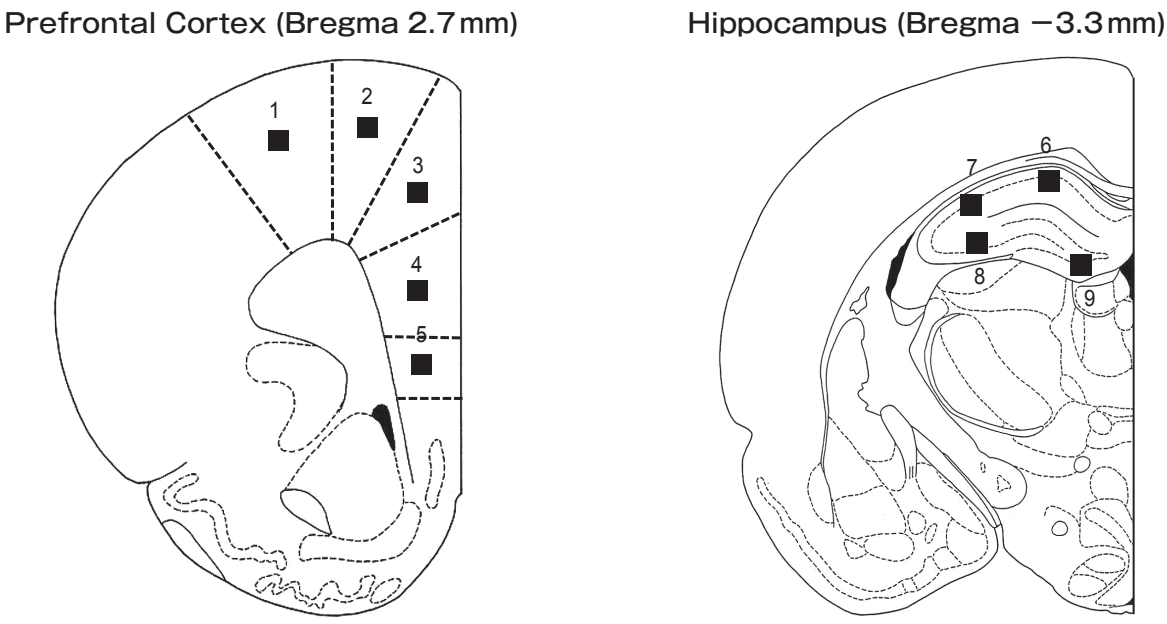

Fig. 1 Drawings of representative sections used for the quantification of expression of c-fos protein. Nine areas of the brain were selected for counting c-fos protein expression: the M1 (1), M2 (2), Cg1 (3), PrL (4) and IL (5) of the prefrontal cortex, and the CA1 (6), CA2 (7), CA3 (8) and dentate gyrus (9) of the hippocampus. The numbers above each brain section represent the distance (mm) from the bregma as detailed in the Atlas of Paxinos and Watson (2007).

of valproate (250–500 mg/kg) on the prevalence of ECS-induced tonic-clonic seizures in rats is shown in Fig. 2. Tonic-clonic seizures occurred in all rats of the saline-treated group that received ECS. Pretreatment with valproate (250–500 mg/kg) decreased the prevalence of tonic-clonic seizures in a dose-dependent manner; 500 mg/kg of valproate completely abolished ECS-induced seizures ($p<0.001$, chi-squared test).

Effect of repeated administration of valproate on spontaneous alternation behavior. The effect of repeated administration of valproate (350–500 mg/kg) on spontaneous alternation behavior in rats is shown in Fig. 3. A one-way ANOVA revealed that 7-day administration of valproate (500 mg/kg) had a significant effect of increasing the total number of arm entries [$F(3, 27)=4.188$, $p<0.05$], but had no significant effect on spontaneous alternation behavior [$F(3, 27)=0.756$, $p>0.05$] or locomotor activity [$F(3, 27)=0.653$, $p>0.05$]. The post-hoc comparison using Bonferroni's test revealed that 500 mg/kg of valproate significantly increased the number of total arm entries in comparison with those in saline-treated control rats ($p<0.05$).

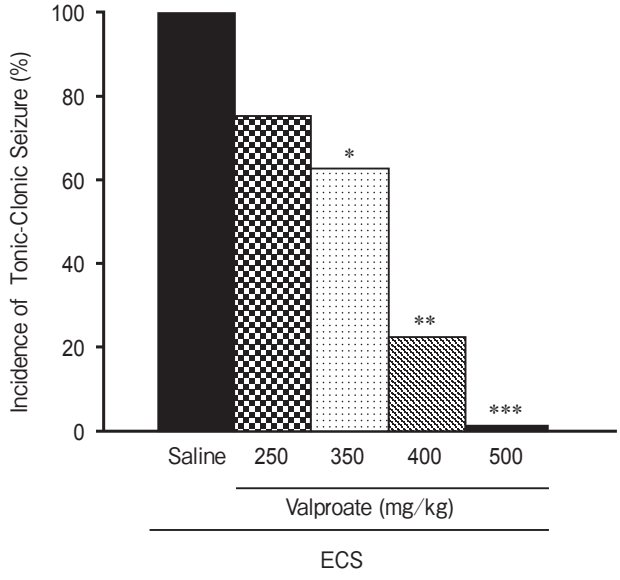

Fig. 2 Effect of valproate on ECS-induced tonic-clonic seizures in rats. Valproate (250–500 mg/kg, i.p.) was injected 30 min before ECS administration. The columns represent the percentage of tonic-clonic seizures (n = 8–9); $*p<0.05$, $**p<0.01$, $***p<0.001$ in comparison with ECS-administered saline-treated rats (chi-squared test).

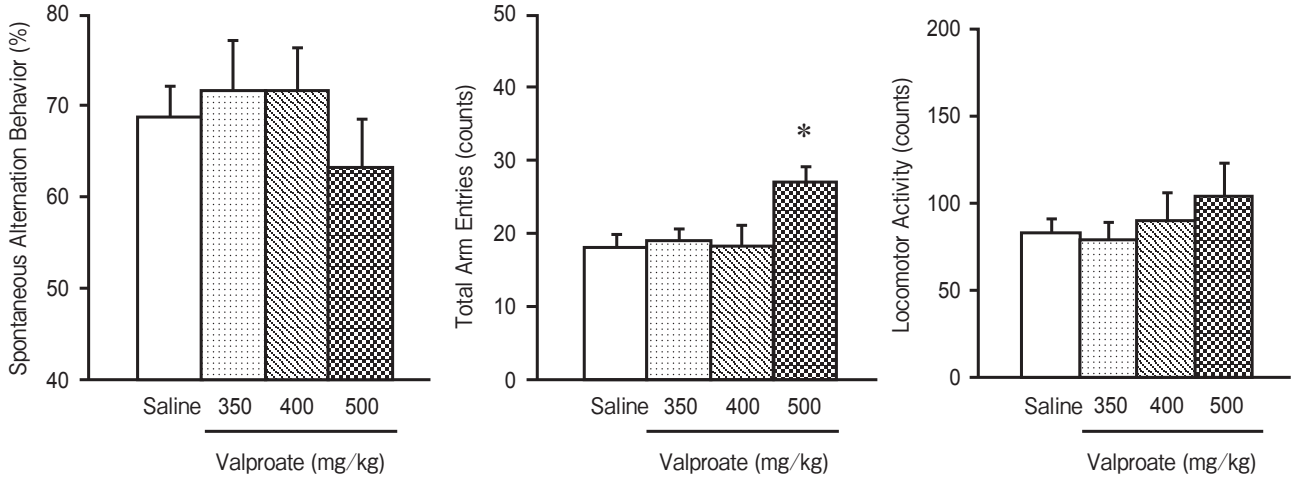

Fig. 3 Effects of valproate on spontaneous alternation behavior in the Y-maze test and locomotor activity in the open-field test. Rats were injected with valproate (350–500 mg/kg, i.p.) once daily for 7 days. The columns represent means ± S.E.M (n = 6–9). $^{*}p<0.05$ in comparison with saline-treated rats (one-way ANOVA followed by Bonferroni's test).

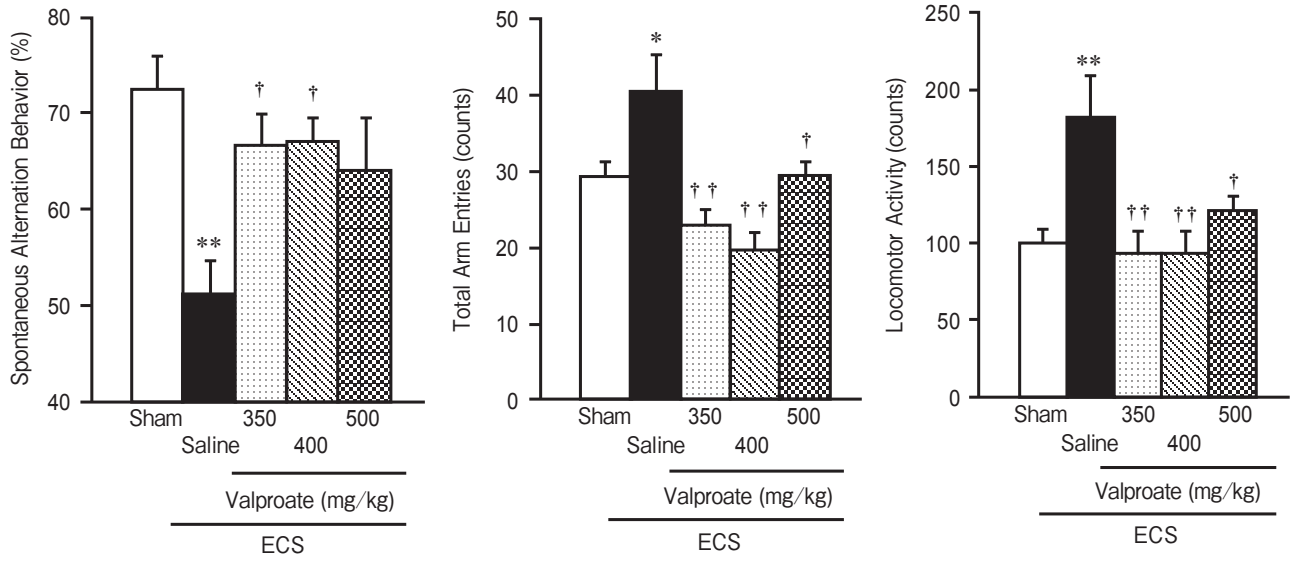

Fig. 4 Effects of valproate on the impairment of spontaneous alternation behavior and locomotor hyperactivity induced by repeated administration of ECS in Rats. Valproate (350–500 mg/kg, i.p.) was injected 30 min before the daily administration of ECS for 7 days. The columns represent means ± S.E.M. (n = 7). $^{*}p<0.05$, $^{**}p<0.01$ in comparison with sham-administered rats, $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ in comparison with repeated ECS-administered rats (one-way ANOVA followed by Bonferroni's test).

Seven-day administrations of ECS produced a significant impairment in spontaneous alternation [$F(4, 30) = 5.451$, $p<0.01$] and significantly increased the total number of arm entries [$F(4, 30) = 9.680$, $p<0.001$] and locomotor hyperactivity [$F(4, 30) = 5.881$, $p<0.01$]. Daily pretreatment with valproate (350 and 400 mg/kg) significantly reversed the impairment of spontaneous alternation behavior ($p<0.05$). All dosages of valproate significantly suppressed the increase in the total number of arm entries and locomotor hyperactivity ($p<0.05$ or $p<0.01$, Fig. 4). The effect of low-dose valproate (250 mg/kg) was also investigated. ECS-induced impairment of spontaneous alternation was not reversed by daily pretreatment

with 250 mg/kg of valproate [sham group, 64.9 ± 4.8%; ECS + saline group, 50.6 ± 5.3%; ECS + valproate group, 60.5 ± 3.1%; n = 7–8; significant difference ($p<0.05$) between sham and ECS + saline groups]. Similarly, the pretreatment of 250 mg/kg valproate had no significant effect on the locomotor hyperactivity induced by ECS [sham group, 106.7 ± 5.8; ECS + saline group, 155.0 ± 21.3; ECS + valproate group, 132.0 ± 8.1; n = 7–8; significant difference ($p<0.05$) between sham and ECS + saline groups].

Effects of repeated administration of ECS and valproate on levels of BDNF protein. The effects of repeated administration of ECS and valproate (400 mg/kg, i.p.) on BDNF protein levels in rat brains are shown in Fig. 5. Two-way ANOVA revealed a significant effect of ECS administration [hippocampus, $F(1, 36) = 62.264$, $p<0.001$; prefrontal cortex, $F(1, 36) = 18.046$, $p<0.001$] but no significant effect of valproate treatment [hippocampus, $F(1, 36) = 1.034$, NS; prefrontal cortex, $F(1, 36) = 3.650$, NS]. Bonferroni's test revealed that repeated administration of ECS significantly increased the BDNF protein levels of the prefrontal cortex ($p<0.05$) and hippocampus ($p<0.01$). Daily pretreatment with valproate had no significant effect on the increases in BDNF protein levels.

Effects of repeated administration of ECS and valproate on levels of c-Fos protein. The effects of repeated administration of ECS and valproate (400 mg/kg, i.p.) on c-Fos protein levels in rat brains are shown in Table 1. Repeated administration

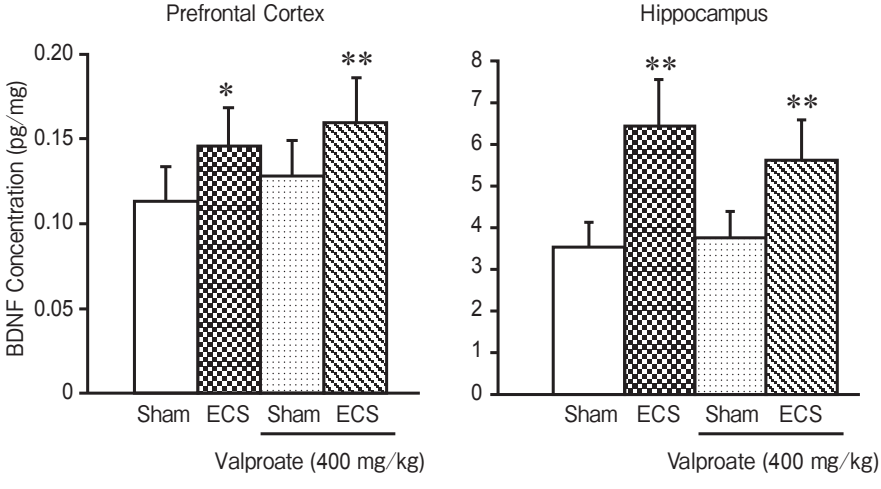

Fig. 5 Effects of valproate on the BDNF protein level in the hippocampus and the prefrontal cortex in rats. Valproate (400 mg/kg, i.p.) was injected 30 min before the daily administration of ECS for 7 days. The columns represent means ± S.E.M (n = 10). $^{*}p<0.05$, $^{**}p<0.01$ in comparison with sham-administered rats (two-way ANOVA followed by Bonferroni's test).

Table 1 Levels of c-Fos protein after the administration of ECS or valproate in rat brains

	Prefrontal Cortex					Hippocampus			
	M1	M2	Cg1	PrL	IL	CA1	CA2	CA3	Dentate Gyrus
Sham	8.5 ± 3.8	18.8 ± 6.4	58.3 ± 7.0	40.0 ± 6.4	58.1 ± 8.6	7.5 ± 1.2	10.3 ± 2.1	4.3 ± 1.1	10.2 ± 1.1
ECS	5.0 ± 3.1	16.3 ± 4.6	45.6 ± 7.8	64.3 ± 3.8	61.8 ± 5.6	9.0 ± 1.2	9.9 ± 1.7	6.4 ± 0.8	2.2 ± 0.4**
Valproate	5.0 ± 2.6	17.0 ± 4.2	40.8 ± 9.5	45.7 ± 6.1	95.8 ± 8.3	5.3 ± 1.6	3.4 ± 1.7	2.6 ± 1.0	21.8 ± 4.3**,††
ECS + Valproate	9.2 ± 1.6	34.0 ± 3.0*,†	80.6 ± 8.1$^{\dagger,\#}$	107.3 ± 7.6$^{**,\dagger\dagger,\#\#}$	89.4 ± 8.3	7.6 ± 1.5	8.5 ± 2.4	8.4 ± 1.6$^{*,\#\#}$	2.9 ± 0.5$^{**,\#\#}$

ECS was administered once daily for 7 d.
Valproate (400 mg/kg, i.p.) was injected 30 min before the daily ECS- or sham-administration rats for 7 d.
Values represent the mean ± S.E.M. (n = 4–8 rats in each group).
$^{*}p<0.05$, $^{**}p<0.01$ in comparison with sham-administered rats,
$^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ in comparison with repeated ECS-administered rats,
$^{\#}p<0.05$, $^{\#\#}p<0.01$ in comparison with repeated VPA-administered rats (two-way ANOVA followed by Bonferroni's test).

of ECS caused a specific reduction in the c-Fos protein level in the dentate gyrus of the hippocampus compared with the sham group ($p<0.01$, Bonferroni's test). Repeated administration of valproate produced a specific increase in the c-Fos protein level in the dentate gyrus of the hippocampus ($p<0.01$, Bonferroni's test). Daily pretreatment with valproate had no significant effect on the decrease in c-Fos protein level induced by ECS. In the prefrontal cortex, valproate + ECS significantly increased the c-Fos protein levels of M2, Cg1 and PrL compared with the ECS group ($p<0.05$ or $p<0.01$, Bonferroni's test).

Discussion

Our previous study indicated that repeated administration of ECS produced an impairment of spontaneous alternation behavior in a Y-maze test and the development of locomotor hyperactivity in an open-field test [3]. In the present study, we investigated in detail the inhibitory effect of valproate on these behavioral changes. In studies using naïve rats, valproate (350 and 400 mg/kg) had no effect on spontaneous alternation behavior and locomotor activity, but 500 mg/kg valproate slightly impaired spontaneous alternation behavior and significantly ($p<0.05$) increased the total number of arm entries in the Y-maze test. These results supported the notion that high doses of valproate produced behavioral impairments, in accordance with previous studies [15–18].

Valproate (250–500 mg/kg) dose-dependently inhibited ECS-induced tonic-clonic seizures, and 500 mg/kg valproate fully prevented seizures. Inhibition of ECS-induced seizures by pretreatment with valproate (350–500 mg/kg) significantly suppressed locomotor hyperactivity. Moreover, impairment of spontaneous alternation was improved by pretreatment with valproate (350 and 400 mg/kg). However, 500 mg/kg of valproate had no significant effect on the impairment in spontaneous alternation, suggesting that 500 mg/kg valproate may be an overdose. Based on these results, the minimum dose of valproate (400 mg/kg) was used to examine the mechanism of valproate against ECS-induced behavioral impairments.

Several studies have demonstrated that BDNF has important and varying roles in the development of learning or kindling. BDNF mutant mice had impaired spatial learning in the water maze test [20], and intraventricular infusion of an anti-BDNF antibody resulted in impairment of water-maze learning in rats [21]. Increases in the expression of BDNF have been reported in various experimental models of seizures such as pentylenetetrazole- or flurothyl-induced kindling [22–24] and recurrent seizures induced by ECS [5, 9]. However, its role remains controversial. Several data have suggested that BDNF may facilitate epileptogenesis [25, 26], while other studies support an inhibitory function [27]. Reibel *et al.*, reported that repeated intrahippocampal infusion of BDNF delays kindling epileptogenesis in rats [28]. On the other hand, electroconvulsive therapy and nonconvulsive electric-stimulation therapy (transcranial magnetic stimulation) are effective for depression, and these treatments have been reported to increase BDNF levels in the rat brain [29]. In the present study, repeated ECS-induced seizures significantly increased BDNF protein levels in the hippocampus and prefrontal cortex. Daily pretreatment with valproate inhibited the ECS-induced seizures and suppressed the impaired spontaneous alternation behavior induced by repeated administration of ECS [3], but valproate had no effect on the increase in BDNF protein levels. These findings suggest that electrical stimulation (but not seizure) increases BDNF protein levels, and that the increased BDNF levels may not be involved in repeated ECS-induced behavioral impairments in rats.

Previous studies have reported that 7-day pretreatment with valproate increased the hippocampal activator protein 1 (AP-1) activity in kainic acid-induced epilepsy rats [30]. Chronic treatment of valproate for 7 days increased c-Fos and AP-1 DNA binding activity in human neuroblastoma SH-SY5Y cells [31]. AP-1 forms a homodimeric or heterodimeric complex with 2 transcription factor families, c-Fos and c-Jun. Previous studies suggested that valproate may increase AP-1 DNA binding activity via protein kinase C (PKC)-mitogen activated protein kinase (MAPK) pathways [32] and promote neurogenesis [33]. In addition, valproate stimulates the proliferation of rat neural progenitor cells and increases neuronal differentiation associated with GABA expression [34, 35]. In the present study, a significant increase in the c-Fos protein level (a maker of neuronal activity) was shown in only the dentate gyrus of the hippocampus. Taken together, these results

suggest that the neurogenesis of valproate may be related with these phenomena. However, further investigation is required to evaluate the observation of neuronal proliferation by the bromodeoxyuridine (BrdU) labeling technique.

Lukoyanov *et al.* (2004) reported that six ECS administrations caused loss of cells in the hilus of the dentate gyrus [36]. In addition, ECS seizures produced atrophic changes in the dendritic arbors of dentate gyrus granule cells [37]. These findings suggest that repeated ECS-induced seizures can produce morphological changes in the brain. In the present study, the c-Fos protein level decreased in the dentate gyrus of the hippocampus after repeated administrations of ECS for 7 days. In addition, inhibition of ECS-induced seizures by pretreatment with valproate did not produce a significant ameliorating effect on the c-Fos level of the hippocampal dentate gyrus. On the other hand, valproate + ECS significantly increased the c-Fos protein levels of M2, Cg1 and PrL of the prefrontal cortex compared with the ECS group. The prefrontal cortex has been shown to have an important role in learning and memory [8]. Studies reported that lesions of PrL-IL impair the visual attention performance in the 3-choice serial reaction time task [38], and that Cg-lesioned rats disrupt the spatial alternation performance [39]. These results suggest that the inhibitory effect of valproate on repeated ECS-induced impairment of spontaneous alternation behavior may be linked to the prefrontal cortex. Our recent study showed that a serotonin-dopamine receptor antagonist, risperidone, improved the impairment of spontaneous alternation and the locomotor hyperactivity induced by ECS [40]. Therefore, further studies will be necessary to clarify the precise mechanisms of dopaminergic and serotonergic neurotransmissions in the prefrontal cortex.

In conclusion, the results of the current study indicate that pretreatment with valproate improved repeated ECS-induced impairment of spontaneous alternation behavior, and that the prefrontal cortex may be associated with these phenomena.

Acknowledgments. This work was supported by the Japanese Health Science Foundation and a Grant-in-Aid for Scientific Research (grant number 20927007) from the Ministry of Education, Science, Sports and Culture in Japan.

References

1. Thome-Souza S, Kuczynski E, Assumpção F Jr, Rzezak P, Fuentes D, Fiore L and Valente KD: Which factors may play a pivotal role on determining the type of psychiatric disorder in children and adolescents with epilepsy ? Epilepsy Behav (2004) 5: 988–994.
2. Vingerhoets G: Cognitive effects of seizures. Seizure (2006) 15: 221–226.
3. Hidaka N, Suemaru K, Li B and Araki H: Effects of repeated electroconvulsive seizures on spotaneous alternation behavior and locomotor activity in rats. Biol Pharm Bull (2008) 31: 1928–1932.
4. Nibuya M, Morinobu S and Duman RS: Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci (1995) 15: 7539–7547.
5. Zetterström TSC, Pei Q and Grahame-Smith DG: Repeated electroconvulsive shock extends the duration of enhanced gene expression for BDNF in rat brain compared with a single administration. Brain Res Mol Brain Res (1998) 57: 106–110.
6. Jacobsen JP and Mørk A: The effect of escitalopram, desipramine, electroconvulsive seizures and lithium on brain-derived neurotrophic factor mRNA and protein expression in the rat brain and the correlation to 5-HT and 5-HIAA levels. Brain Res (2005) 1024: 183–192.
7. Rolls ET and Kesner RP: A computational theory of hippocampal function, and empirical tests of the theory. Prog Neurobiol (2006) 79: 1–48.
8. Williams GV and Goldman-Rakic PS: Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature (1995) 376: 572–575.
9. Altar CA, Whitehead RE, Chen R, Wörtwein G and Madsen TM: Effects of electroconvulsive seizure and antidepressant drugs on brain-derived neurotrophic factor protein in rat brain. Biol Psychiatry (2003) 54: 703–709.
10. Li B, Suemaru K, Cui R, Kitamura Y, Gomita Y and Araki H: Repeated electroconvulsive stimuli increase brain-derived neurotrophic factor in ACTH-treated rats. Eur J Pharmacol (2006) 529: 114–121.
11. Sagar SM, Sharp FR and Curran T: Expression of *c-fos* protein in brain: metabolic mapping at the cellular level. Science (1988) 240: 1328–1331.
12. Schaaf MJ, de Jong J, de Kloet ER and Vreugdenhil E: Downregulation of BDNF mRNA and protein in the rat hippocampus by corticosterone. Brain Res (1998) 813: 112–120.
13. Hamamura T, Lee Y, Fujiwara Y and Kuroda S: Serotonin1A receptor agonists induce Fos protein expression in the locus coeruleus of the conscious rat. Brain Res (1997) 759: 156–159.
14. Shu SY, Ju G and Fan LZ: The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. Neurosci Lett (1988) 85: 169–171.
15. Meador KJ, Loring DW, Huh K, Gallagher BB and King DW: Comparative cognitive effects of anticonvulsants. Neurology (1990) 40: 391–394.
16. Meador KJ, Loring DW, Allen ME, Zamrini EY, Moore EE, Abney OL and King DW: Comparative cognitive effects of carbamazepine and phenytoin in healthy adults. Neurology (1991) 41: 1537–1540.
17. Meador KJ, Loring DW, Moore EE, Thompson WO, Nichols ME, Oberzan RE, Durkin MW, Gallaqher BB and King DW: Comparative cognitive effects of phenobarbital, phenyton, and valproate in healthy subjects. Neurology (1995) 45: 1494–1499.
18. Loring DW and Meador KJ: Cognitive side effects of antiepileptic

drugs in children. Neurology (2004) 62: 872–877.
19. Ortinski P and Meador KJ: Cognitive side effects of antiepileptic drugs. Epilepsy Behav (2004) Suppl 1: S60–65.
20. Linnarsson S, Bjorklund A and Ernfors P: Learning deficit in BDNF mutant mice. Eur J Neurosci (1997) 9: 2581–2587.
21. Mu JS, Li WP, Yao ZB and Zhou XF: Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. Brain Res (1999) 835: 259–265.
22. Reibel S, Larmet Y, Le BT, Carnahan J, Marescaux C and Depaulis A: Brain-derived neurotrophic factor delays hippocampal kindling in the rat. Neuroscience (2000) 100: 777–788.
23. Mhyre TR and Applegate CD: Persistent regional increases in brain-derived neurotrophic factor in the flurothyl model of epileptogenesis are dependent upon the kindling status of the animal. Neuroscience (2003) 121: 1031–1045.
24. Han D, Yamada K, Senzaki K, Xiong H, Nawa H and Nabeshima T: Involvement of nitric oxide in pentylenetetrazole-induced kindling in rats. J Neurochem (2000) 74: 792–798.
25. Lähteinen S, Pitkänen A, Saarelainen T, Nissinen J, Koponen E and Castrén E: Decreased BDNF signalling in transgenic mice reduces epileptogenesis. Eur J Neurosci (2002) 15: 721–734.
26. Scharfman HE, Goodman JH, Sollas AL and Croll SD: Spontaneous limbic seizures after intrahippocampal infusion of brain-derived neurotrophic factor. Exp Neurol (2002) 174: 201–214.
27. Reibel S, Depaulis A and Larmet Y: BDNF and epilepsy--the bad could turn out to be good. Trends Neurosci (2001) 24: 318–319.
28. Reibel S, Larmet Y, Lê BT, Carnahan J, Marescaux C and Depaulis A: Brain-derived neurotrophic factor delays hippocampal kindling in the rat. Neuroscience (2000) 100: 777–788.
29. Müller MB, Toschi N, Kresse AE, Post A and Keck ME: Long-term repetitive transcranial magnetic stimulation increases the expression of brain-derived neurotrophic factor and cholecystokinin mRNA, but not neuropeptide tyrosine mRNA in specific areas of rat brain. Neuropsychopharmacology (2000) 23: 205–215.
30. Hsieh CL, Lim JJ, Chiang SY, Su SY, Tang NY, Lin GG, LinIH, Liu CH, Hsiang CY, Chen JC and Ho TY: Gastrodia elata modulated activator protein 1 via c-Jun N-terminal kinase signaling pathway in kainic acid-induced epilepsy in rats. J Ethonopharmacol (2007) 109: 241–247.
31. Asghari V, Wang JF, Reiach JS and Young LT: Differential effects of mood stabilizers on Fos/Jun proteins and AP-1 DNA binding activity in human neuroblastoma SH-SY5Y cells. Mol Brain Res (1998) 58: 95–102.
32. Chen G, Yuan PX, Jiang YM, Huang LD and Manji HK: Valproate robustly enhances AP-1 mediated gene expression. Brain Res Mol Brain Res (1999) 64: 52–58.
33. Chen G and Manji HK: The extracellular signal-regulated kinase pathway: an emerging promising target for mood stabilizers. Curr Opin Neurobiol (2006) 19: 313–323.
34. Laeng P, Pitts RL, Lemire AL, Drabik CE, Weiner A, Tang H, Thyagarajan R, Mallon BS and Altar CA: The mood stabilizer valproic acid stimulates GABA neurogenesis from rat forebrain stem cells. J Neurochem (2004) 91: 238–251.
35. Yu IT, Park JY, Kim SH, Lee JS, Kim YS and Son H: Valproic acid promotes neuronal differentiation by induction of proneural factors in association with H4 acetylation. Neuropharmacology (2009) 56: 473–480.
36. Lukoyanov NV, Sá MJ, Madeira MD and Paula-Barbosa MM: Selective loss of hilar neurons and impairment of initial learning in rats after repeated administration of electroconvulsive shock seizures. Exp Brain Res (2004) 154: 192–200.
37. Cardoso A, Assunção M, Andrade JP, Pereira PA, Madeira MD, Paula-Barbosa MM and Lukoyanov NV: Loss of synapses in the entorhinal-dentate gyrus pathway following repeated induction of electroshock seizures in the rat. J Neurosci Res (2008) 86: 71–83.
38. Broersen LM and Uylings HBM: Visual attention task performance in Wistar and Lister Hooded rats: response inhibition deficits after medial prefrontal cortex lesions. Neurosci (1999) 94: 47–57.
39. Delatour B and Gisquet-Verrier P: Involvement of the dorsal anterior cingulate cortex in temporal behavioral sequencing: subregional analysis of the medial prefrontal cortex in rat. Behav Brain Res (2001) 126: 105–114.
40. Hidaka N, Suemaru K and Araki H: Serotonin-dopamine antagonism ameliorates impairments of spontaneous alternation and locomotor hyperactivity induced by repeated electroconvulsive seizures in rats. Epilepsy Res (2010) 90: 221–227.