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Chronic administration of valproic acid reduces brain NMDA signaling *via* arachidonic acid in unanesthetized rats

Mireille Basselin*, **Lisa Chang**, **Mei Chen**, **Jane M. Bell**, and **Stanley I. Rapoport**
Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA

Abstract

Evidence that brain glutamatergic activity is pathologically elevated in bipolar disorder suggests that mood stabilizers are therapeutic in the disease in part by downregulating glutamatergic activity. Such activity can involve the second messenger, arachidonic acid (AA, 20:4*n*-6). We tested this hypothesis with regard to valproic acid (VPA), when stimulating glutamatergic N-methyl-D-aspartate (NMDA) receptors in rat brain and measuring AA and related responses. An acute subconvulsant dose of NMDA (25 mg/kg i.p.) or saline was administered to unanesthetized rats that had been treated i.p. daily with VPA (200 mg/kg) or vehicle for 30 days. Quantitative autoradiography following intravenous [1-^{14}C]AA infusion was used to image regional brain AA incorporation coefficients k*, markers of AA signaling. In chronic vehicle-pretreated rats, NMDA compared with saline significantly increased k* in 41 of 82 examined brain regions, many of which have high NMDA receptor densities, and also increased brain concentrations of the AA metabolites, prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2). VPA pretreatment reduced baseline concentrations of PGE_2 and TXB_2, and blocked the NMDA induced increases in k* and in eicosanoid concentrations. These results, taken with evidence that carbamazepine and lithium also block k* responses to NMDA in rat brain, suggest that mood stabilizers act in bipolar disorder in part by downregulating glutamatergic signaling involving AA.

Keywords

valproic acid; NMDA; arachidonic acid; bipolar disorder; phospholipase A_2; prostaglandin E_2

Introduction

Valproic acid (VPA, 2-propylpentaenoic acid) is approved as a mood stabilizer for treating bipolar disorder, particularly its manic phase [17,39,62]. Although inhibition of GABAergic neurotransmission is considered its major pharmacological action, VPA has many other central effects [43]. It can inhibit histone deacetylase [45] and brain microsomal long-chain fatty acyl-CoA synthethase [12], increase brain levels of the neuroprotective proteins bcl-2 and brain derived neurotrophic factor [23,36], and alter transcription in brain of many genes [13].

VPA also has been reported to block excitatory responses induced by N-methyl-D-aspartate (NMDA) *in vivo* and *in vitro*, NMDA-induced convulsions *in vivo* [37,38,47,57,91,96], and other aspects of brain glutamatergic activity [50,78,89,92]. In view of evidence of upregulated or otherwise disturbed brain glutamatergic neurotransmission in patients with bipolar disorder

*Address Correspondence to: Mireille Basselin Ph.D., National Institutes of Health, National Institute on Aging, Brain Physiology and Metabolism Section, Bldg 9, Room 1S126, MSC 0947, 9 Memorial Drive, Bethesda, MD 20892, USA. Tel.: 301 594 5522; Fax: 301 402 0074; e-mail: mirvasln@mail.nih.gov.

[41,50,59,90], we thought it of interest to see whether VPA could interfere with NMDA receptor initiated signaling involving the second messenger, arachidonic acid (AA, 20:4*n*-6). AA and its metabolites have multiple effects, including regulation of neuronal activity, gene transcription, apoptosis, sleep and cerebral blood flow [49,58,81].

Binding of glutamate or of NMDA to NMDA receptors will increase intracellular Ca^{2+}, thereby activating Ca^{2+}-dependent enzymes including AA-selective cytosolic phospholipase A_2 ($cPLA_2$). $cPLA_2$ activation releases unesterified AA from the stereospecifically numbered-2 position of membrane phospholipid, which leads to increased formation *via* cyclooxygenase (COX) enzymes of eicosanoids such as prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2) [33,46,52,79,87,93]. We have developed an *in vivo* method to image this activation process, and decided to use the method to image the effect of chronic VPA in rats on the NMDA receptor-mediated AA signal [5]. Prior reports indicate that other mood-stabilizers, lithium and carbamazepine, when given chronically to rats block this NMDA-mediated AA signal [5,9].

The method to measure the AA signal involves infusing radiolabeled [1-^{14}C]AA intravenously following administration of drug or vehicle, imaging regional brain radioactivity after 15 min with quantitative autoradiography, and converting these images into regional AA incorporation coefficients k*. k* represents the AA that has been released during the signal and metabolized to eicosanoids and other products and is independent of changes in cerebral blood flow [28, 74–76].

In this study, we measured k* for AA in 82 brain regions of unanesthetized rats that had been injected with VPA (200 mg/kg i.p.) or vehicle (saline) daily for 30 days as described [22]. These rats were injected i.p. acutely with a subconvulsant dose (25 mg/kg) of NMDA [5,65] or with saline. Whole brain concentrations of PGE_2 and TXB_2 also were measured.

Experimental Procedures

Animals and Diets

Experiments were conducted following the “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health Publication No. 86-23) and were approved by the Animal Care and Use Committee of the National Institute of Child Health and Development. Two-month-old male Fischer CDF (F-344)/CrlBR rats (Charles River Laboratories, Wilmington, MA, USA) were acclimatized for 1 week in an animal facility with regulated temperature, humidity and light cycle, and had *ad libitum* access to food (NIH-31 diet, Zeigler, Gardners, PA, USA) and water. The diet contained (as percent of total fatty acids) 20.1% saturated, 22.5% monounsaturated, 47.9% linoleic, 5.1% α-linolenic, 0.02% AA, 2.0% eicosapentaenoic, and 2.3% docosahexaenoic acid.

Drugs and tracers

[1-^{14}C]AA in ethanol (53 mCi/mmol, >98% pure, Moravek Biochemicals, Brea, CA, USA) was evaporated and resuspended in HEPES buffer, pH 7.4, containing 50 mg/ml fatty acid-free bovine serum albumin (Sigma-Aldrich, St Louis, MO, USA). NMDA (25 mg/kg, Sigma-Aldrich) or saline was administered i.p.. The NMDA dose has been reported to produce paroxysmal spikes and spike trains but not status epilepticus in rats [65] and to significantly increase k* for AA [5,9]. VPA-treated rats received 200 mg/kg i.p. VPA (sodium salt; Sigma-Aldrich) in saline once daily for 30 days, as previously described [12,14,22,73]. Three hours after the last VPA injection, the plasma VPA concentration equals 31 ± 6 (mean ± SD) μg/ml [22], slightly below the therapeutic concentration range (45–150 μg/ml) recommended for bipolar disorder [35]. A control group received the same volume of saline (*vehicle*) under parallel conditions.

Surgical Procedures and Tracer Infusion

On the morning following the 30th VPA or vehicle injection, a rat was anesthetized with 2–3% halothane in O_2, and PE 50 polyethylene catheters were inserted into the right femoral artery and vein as described previously [5]. The wound was closed with surgical clips and the rat was wrapped loosely, with its upper body remaining free, in a fast-setting plaster cast (DePuy Inc., Raynam, MA, USA) that was taped to a wooden block. Surgery lasted 20–25 min. The rat was allowed to recover from anesthesia for 3 h in a quiet environment maintained at 25°C. Body temperature was maintained at 36.4–37.1°C using a feedback heating device and rectal thermometer. Arterial blood pressure and heart rate were measured with a blood pressure recorder (CyQ 103/302; Cybersense, Inc., Nicholasville, KY, USA). Arterial blood pH, pO_2 and pCO_2 were measured with a blood gas analyzer (Rapidlab 248, Bayer Health Care Diagnostics Division, Norwood MA, USA).

Ten minutes after injecting NMDA or saline, 2 ml [1-^{14}C]AA (170 pCi/kg) was infused into the femoral vein for 5 min at a rate of 400 pl/min using an infusion pump (Harvard Apparatus Model 22, Natick, MA, USA). Twenty minutes after starting the infusion, the rat was euthanized with an overdose of Nembutal® (90 mg/kg, i.v.) and decapitated. The brain was removed in less than 30 s, frozen in 2-methylbutane maintained at −40°C with dry ice, and stored at −80°C until sectioned. Thus, brains in the present study were sampled within 4 hours after the last daily VPA injection.

Chemical Analysis

Thirteen arterial blood samples collected before, during and after [1-^{14}C]AA infusion were centrifuged immediately (30 s at 18,000 g). Total lipids were extracted from 30 pl of plasma with 3 ml chloroform:methanol (2:1, by vol) and 1.5 ml 0.1 M KCl using Folch procedure [34]. Radioactivity was determined in 100 pl of the lower organic phase by liquid scintillation counting. As reported [26], greater than 97% of plasma radioactivity at 5 min following [1-^{14}C]AA infusion was radiolabeled AA, and brain phospholipids accounted for greater than 81% of brain lipid radioactivity over 2 hours, whereas aqueous metabolites of AA account for 10% at 5 min and decrease with time.

Quantitative Autoradiography

Frozen brains were cut in serial 20-pm thick coronal sections in a cryostat at −20°C. The sections were placed for 5 weeks together with calibrated [^{14}C]methylmethacrylate standards on Kodak Ektascan C/RA film (Eastman Kodak Company, Rochester, NY, USA). Brain regions from autoradiographs were identified from a stereotaxic rat brain atlas [67], and were sampled in both hemispheres. The average of bilateral measurements for each region from three consecutive brain sections was used to calculate regional radioactivity (nCi/g of brain) by digital quantitative densitometry, using a Macintosh computer and the public domain NIH Image program 1.62 (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Regional incorporation coefficients k* (ml plasma/s/g brain) of AA were calculated as [76],

$$k^* = \frac{c^*_{brain}(20\ \text{min})}{\int_0^{20} c^*_{plasma}\,dt} \qquad \text{(Eq. 1)}$$

c^*_{plasma} equals plasma radioactivity determined by scintillation counting (nCi/ml), c^*_{brain} equals brain radioactivity (nCi/g brain), and t equals time after starting [1-^{14}C]AA infusion.

Brain prostaglandin E_2 and thromboxane B_2 concentrations

In separate experiments, 3 h and 45 min after the last of 30 daily injections of VPA or vehicle, a rat was injected i.p. with NMDA (25 mg/kg) or saline. Ten minutes later, it was anesthetized with Nembutal® (45 mg/kg, i.p.) and immediately subjected to head-focused microwave irradiation (5.5 kW, 3.8 s; Cober Electronics, Stamford, CT, USA) to stop brain lipid metabolism [15,30]. Half-brains were weighed, homogenized with 18 volumes of hexane:isopropanol (3:2, by vol) using a glass Tenbroeck homogenizer and the homogenate was centrifuged for 5 min at 800 g. Tissue residues were rinsed with 3 × 2 volumes of the same solvent. The resultant lipid extract was concentrated to dryness under nitrogen and resuspended in the enzyme immunoassay buffer provided with the polyclonal PGE_2 and TXB_2 kits (Oxford Biochemical Research, Oxford, MI, USA).

Statistical analyses

An unpaired two-tailed *t*-test was used to compare mean physiological parameters in chronic VPA- and vehicle-treated rats, using GraphPad Prism version 4.0b (GraphPad Software, San Diego, CA, USA, www.graphpad.com). A standard two-way analysis of variance (ANOVA) was performed with SPSS 11.0 (SPSS Inc., Chicago, IL, USA, http://www.spss.com), to compare chronic VPA-versus-vehicle with acute NMDA-versus-saline with regard to arterial plasma radioactivity input functions, brain PGE_2 and TXB_2 concentrations and regional values of k*. Where interactions between VPA and NMDA were statistically insignificant, probabilities of effects of VPA and NMDA were reported. Where the interactions were significant, probabilities of main effects of VPA and NMDA were not reported [85]. Instead, unpaired two-tailed *t*-tests were used to compare NMDA and saline responses between chronic VPA- and vehicle-treated rats as well as saline responses in VPA- compared with vehicle-treated rats. Other comparisons were not considered relevant. A post-hoc test was not used to avoid a correction for multiple comparisons. Data are reported as the mean ± SD, with statistical significance taken as $p \leq 0.05$.

Results

Physiology, behavior and arterial plasma radioactivity

Rats injected daily with VPA for 30 days weighed significantly less than vehicle-treated rats (Table 1). In chronic vehicle-treated rats, acute NMDA (25 mg/kg) produced repeated cycles of activity (head weaving and body movements) lasting on average 4 s, following by a “calm” period averaging 9 s, with the net cycling period lasting a mean of 95 s (Table 1). The mean durations of the behavioral parameters were not significantly different in chronic VPA-treated rats. Furthermore, compared with acute saline, NMDA did not significantly affect arterial pH, pCO_2, pO_2 or blood pressure, but significantly decreased heart rate by 21–23% in both chronic vehicle and VPA-treated groups (Table 1), as previously reported [5].

Neither chronic VPA nor acute NMDA modified the time-course of arterial plasma radioactivity (Eq. 1) following intravenous [1-^{14}C]AA infusion. The mean integral of radioactivity in the plasma organic fraction, (nCi × s)/ml (n = 6), did not differ significantly between groups: chronic vehicle plus saline, 180,783 ± 24,145; chronic vehicle plus NMDA, 170,249 ± 16,855; VPA plus saline, 162,752 ± 18,879; VPA plus NMDA, 149,555 ± 21,089.

Regional brain AA incorporation coefficients, k*

Figure 1 presents coronal autoradiographs of brains from rats given saline or NMDA after chronic vehicle or VPA. Values of k* for AA, calculated by Eq. 1, are color-coded. The figure shows no apparent difference in regional values of k* in response to saline between the animals treated chronically with VPA compared with vehicle (both given acute saline). Acute NMDA

increased k* in many gray matter regions of the chronic vehicle-treated rat, but had no evident effect on k* in the VPA-treated rat. Data obtained from such autoradiographs are summarized in Table 2 and Fig. 2.

Effects of NMDA in chronic vehicle-treated rats—Mean values of k* in each of 82 brain regions were subjected to a two-way ANOVA, as illustrated in Table 2. Statistically significant interactions between VPA and NMDA were found in 41, in which unpaired *t*-tests then showed that NMDA compared with saline significantly increased k* by 19–61% in chronic vehicle-treated rats. Affected regions included prefrontal (38–41%), frontal (34–45%), primary olfactory (28%), anterior cingulate (61%), motor (35–46%), somatosensory (31–35%), auditory layer I (40%) and visual cortical areas (40–55%), hippocampus [CA1, CA2, CA3, dentate gyrus, stratum lacunosum-molecular] (19–38%), nucleus accumbens (22%), caudate-putamen (26–34%), lateral geniculate nucleus dorsal (27%), thalamus [paratenial, anteroventral and parafascicular nuclei] (25–37%), interpeduncular nucleus (28%), substantia nigra (40%), inferior colliculus (23%), and cerebellar gray matter (23–30%). The overall pattern of differences due to NMDA compared with saline in chronic vehicle-treated rats is illustrated in Fig. 2a.

Effects of chronic valproic acid at baseline—In the 41 regions in which VPA × NMDA interactions were statistically significant, chronic VPA compared with chronic vehicle significantly changed mean baseline (post-saline) k* in 3 of them (Table 2) -- frontal cortex (10) layer IV (16%), motor cortex layer V (19%) and interpeduncular nucleus (-15%). In the other 41 regions in which VPA × NMDA interactions were statistically insignificant, chronic VPA had a main effect in 5 of them, but reduced k* only in the olfactory tubercle. Thus, chronic VPA altered baseline k* in 4 of the 82 brain regions studied. The overall pattern of differences due to chronic VPA is illustrated in Fig. 2b.

Effects of acute NMDA in valproic acid-treated rats—NMDA compared with saline changed k* significantly (-12%) in only one of the 41 regions in which VPA × NMDA interactions were statistically significant, frontal cortex (10) layer IV (Table 2). Acute NMDA did not significantly affect k* in any of the 41 regions in which VPA × NMDA interactions were statistically insignificant. In none of these latter regions did NMDA have a main effect on k*. The complete lack of a significant NMDA effect in animals pretreated with VPA is illustrated in Fig. 2c.

Brain PGE_2 and TXB_2 concentrations

A two-way ANOVA demonstrated statistically significant interactions between VPA and NMDA with regard to brain PGE_2 and TXB_2 concentrations (Table 3). Consequent *t*-tests showed that chronic VPA alone significantly decreased basal concentrations of PGE_2 by 66% and of TXB_2 by 45%. Acute NMDA increased PGE_2 and TXB_2 concentrations in chronic vehicle-treated rats, but did not significantly affect either concentration in chronic VPA-treated rats.

Discussion

Consistent with reports that VPA interferes with glutamatergic function and NMDA receptor signaling (see “Introduction”), daily administration of VPA to rats for 30 days, at a dose that produces a plasma VPA concentration relevant to bipolar disorder, prevented the statistically significant increases in AA incorporation coefficients k*, and in whole brain PGE_2 and TXB_2 concentrations, that were caused by a subconvulsant acute dose of NMDA in chronic vehicle-treated rats. To the extent that glutamatergic signaling *via* NMDA receptors is pathologically upregulated in bipolar disorder patients, for which evidence exists (see

"Introduction") [41,50,59,90], these results suggest that VPA's efficacy in the disease is due in part to its ability to dampen upregulated NMDA signaling involving AA and its downstream metabolites. Chronic administration to rats of lithium or carbamazepine, resulting in therapeutic relevant plasma concentrations, also dampen NMDA-induced elevations in k* for AA and in brain eicosanoid [5,9]. Thus, reduced NMDA signaling involving AA and its metabolites may be common to the therapeutic action of mood-stabilizers in bipolar disorder.

Evidence that cholinomimetics [19] as well as drugs that interfere with dopaminergic [24,60, 68,71] or glutamatergic [2,61,88] signaling ameliorate bipolar disorder symptoms, and of defective serotonergic signaling [56], has suggested that bipolar symptoms reflect reduced cholinergic, altered serotonergic, and increased dopaminergic and glutamatergic neurotransmission. Our studies in rats now suggest that chronic VPA, lithium and carbamazepine as a group can correct this imbalance, and that the imbalance involves AA as a second messenger [4–9,21,77].

Our values of k* in this study agree with published values [5,9]. NMDA increased k* significantly in 41 of 82 brain structures with high densities of NMDA receptors [66], including the cerebral cortex, caudate-putamen, globus pallidus, hippocampus, thalamus, hypothalamus, colliculus, substantia nigra. Our measured PGE_2 and TXB_2 concentrations agree with other studies showing elevated brain concentrations of these eicosanoids following acute NMDA [9,64,69] and reduced concentrations following chronic VPA [14,84]. Concentrations of PGD_2 and $PGF_{2\alpha}$ also are increased after NMDA [51,54] but are decreased by chronic VPA [84].

VPA's ability to suppress NMDA-induced increases in k* for AA and to reduce PGE_2 and TXB_2 concentrations in rat brain could have been due to its ability to reduce COX-1 and COX-2 expression or interfere directly with the NMDA receptor [14,73]. When COX enzymes are pharmacologically inhibited or knocked out in rodent brain, k* responses to drugs acting at $cPLA_2$-coupled neuroreceptors are reduced or lost, as are the increases in brain PGE_2 and/or TXB_2 concentrations [10,11]. VPA can inhibit cyclic AMP-dependent protein kinases A and C by VPA, both of which can phosphorylate the receptor [32,53,94]. VPA also can reduce expression of two NMDA receptor-interacting proteins in rat brain, postsynaptic density protein PSD-95, which is altered in bipolar disorder [90], and type II Ca^{2+}/calmodulin-dependent protein kinase beta subunit [13]. It inhibits histone deacetylase, which acetylates the NMDA receptor transcription factor, specificity protein-1 (Sp1) [3,70], and can reduce methylation of the *reelin* gene, which encodes a protein that regulates NMDA receptor surface trafficking and synaptic subunit composition [29,31,40]. VPA blocks induction of Fos and of activator protein-1 DNA binding activity, both of which modulate transcription of the NMDA receptor subunit, NR2B [72,80]. It regulates expression and traffic of NMDA receptors in hippocampal neurons [20,36], and decreases basal glutamate release and increases glutamate uptake in brain [42,89,92]. VPA also may modulate neurotransmission involving $cPLA_2$ and AA coupled to glutamatergic alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors [25,32,38,48,93].

The behavioral effects of NMDA (Table 1) were not altered qualitatively by chronic VPA, lithium [5] or carbamazepine [9]. Thus, AA signaling *via* NMDA receptors likely does not contribute to these effects. In agreement, neither VPA, lithium or carbamazepine modified the seizure threshold to NMDA in rodents [65,82], and VPA did not reduce NMDA-induced running/jumping fits, clonic-tonic seizure or mortality in rats [44]. NMDA can promote also synaptic release of acetylcholine, adenosine, serotonin and γ-aminobutyric acid [27,86,93, 95].

Since mood-stabilization of bipolar patients appear only after 10 days of oral VPA [16], we as have others [5,9,12,13,18,22,42,55] studied effects only of chronic VPA in rats. An acute injection of VPA (300 mg/kg) in rats did not alter basal or stimulated extracellular glutamate in the hippocampus, whereas chronic VPA decreased whole brain glutamate concentration [1,63]. Chronic but not acute VPA administration changed corticotropin releasing factor [83] and AMPA glutamate receptors [32] in rat brain.

In conclusion, chronic VPA pretreatment prevented the statistically significant increases in k* for AA and in PGE_2 and TXB_2 concentrations that were observed in response to NMDA in chronic vehicle-treated rats. These and observations in rats administered chronic lithium or carbamazepine support the hypothesis that mood stabilizers commonly downregulate brain AA signaling *via* NMDA receptors.

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Abbreviations

AA
: arachidonic acid (20,4n-6)

PLA_2
: phospholipase A_2

cPLA_2
: cytosolic PLA_2

NMDA
: N-methyl-D-aspartic acid

PGE_2
: prostaglandin E_2

TXB_2
: thromboxane B_2

VPA
: valproic acid

COX
: cyclooxygenase

AMPA
: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

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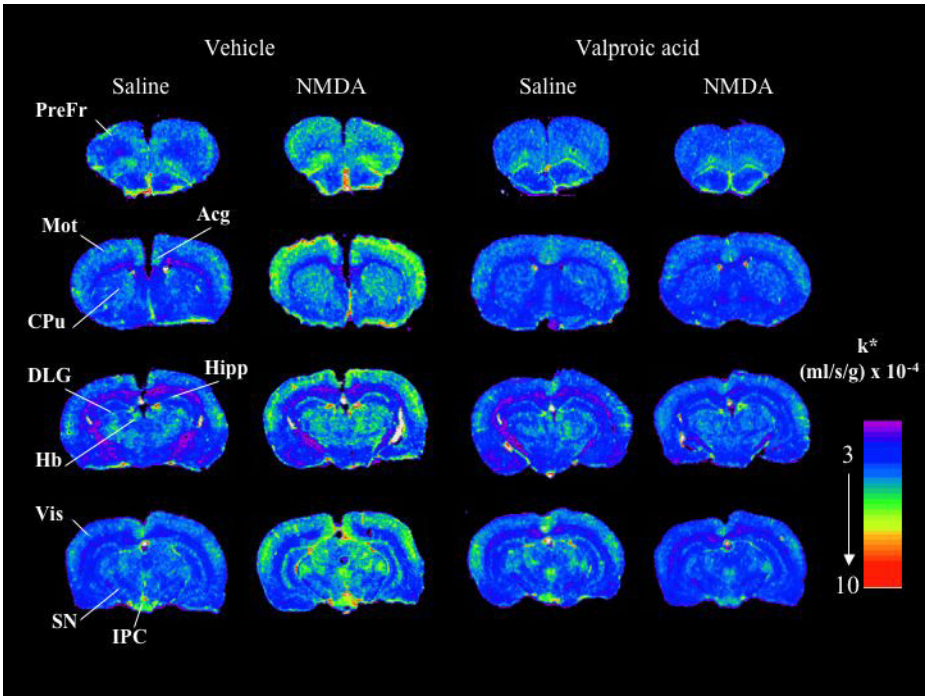

Fig 1. Coronal autoradiographs showing effects of NMDA and valproic acid on brain regional AA incorporation coefficients k* in rats

Values of k* (ml/s/g brain × 10^{-4}) are given on a color scale. Abbreviations: Acg, anterior cingulate cortex; CPu, caudate-putamen; DLG, dorsal lateral geniculate nucleus; Hb, habenular nucleus; Hipp, hippocampus; IPC, interpeduncular nucleus; Mot, motor cortex; PreFr, prefrontal cortex; SN, substantia nigra; Vis, visual cortex.

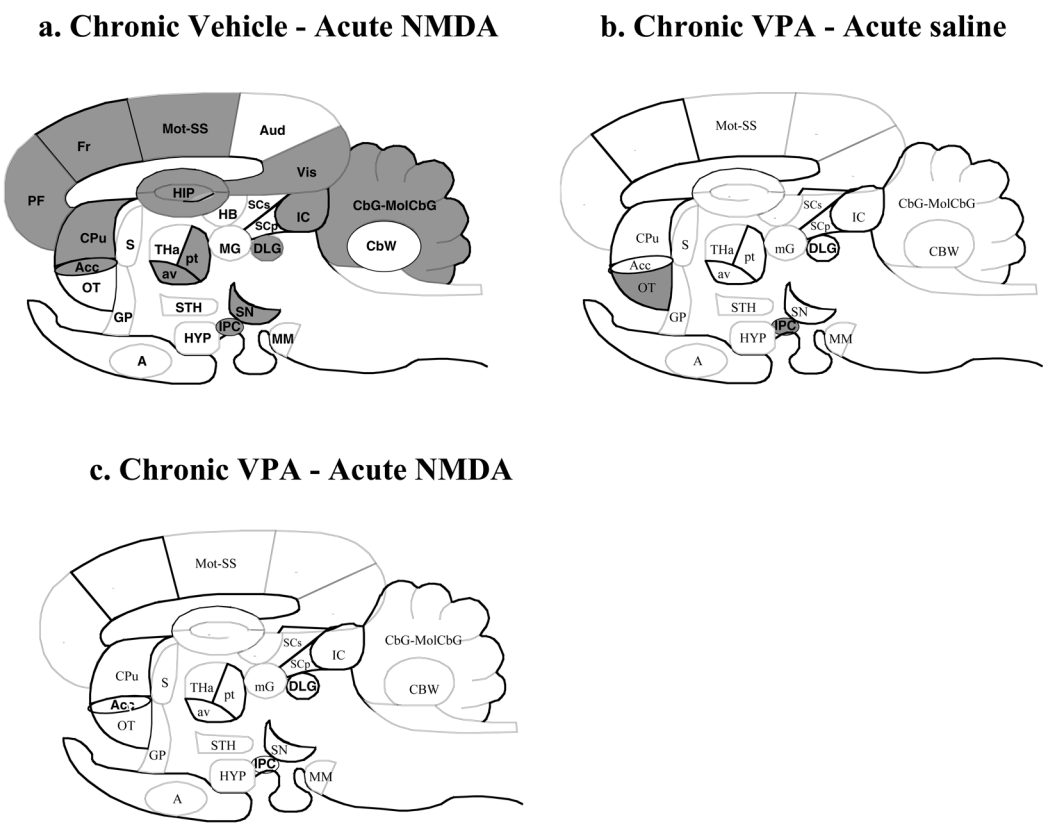

Fig 2. Difference patterns of k* responses to NMDA and valproic acid in sagittal representation of rat brain

Regions in which k* was increased significantly ($p < 0.05$) are solid black, regions in which k* was decreased significantly are hatched. List of regions: A, amygdala; Acc, nucleus accumbens; Aud, auditory cortex; av, anteroventral thalamic nucleus; CbG, cerebellar gray matter; CBW, cerebellar white matter; CPu, caudate putamen; DLG, dorsal lateral geniculate nucleus; Fr, frontal cortex; GP, globus pallidus; HB, habenular complex; HIP, hippocampus; HYP, hypothalamus; IC, inferior colliculus; IPC, interpeduncular nucleus; MM, mammillary nucleus; mG, medial geniculate nucleus; MolCBG, molecular layer of cerebellar gray matter; Mot, motor cortex; OT, olfactory tubercle; PF, prefrontal cortex; pt, paratenial thalamic nucleus; SN, substantia nigra; S, septum; SS, somatosensory cortex; SCp, deep layer of superior colliculus; SCs, superficial layer of superior colliculus; STH, subthalamic nucleus; THa, thalamus; Vis, visual cortex.

Table 1

Effects of chronic valproate and acute NMDA on physiological parameters

	Chronic vehicle				Chronic valproate			
	Saline		NMDA		Saline		NMDA	
	Before	After	Before	After	Before	After	Before	After
Body weight (g)	276 ± 15	-	265 ± 10	-	248 ± 19*	-	249 ± 16*	-
Body temperature (°C)	36.4 ± 0.2	36.6 ± 0.3	36.4 ± 0.3	36.5 ± 0.2	36.6 ± 0.2	36.7 ± 0.3	36.4 ± 0.3	36.6 ± 0.3
Heart rate (beats/min)	413 ± 31	427 ± 39	443 ± 32	342 ± 35***	448 ± 23	451 ± 22	458 ± 21	361 ± 12***
Arterial blood pressure (mmHg)								
Systolic	145 ± 18	144 ± 20	150 ± 15	154 ± 18	132 ± 19	128 ± 19	134 ± 16	134 ± 15
Diastolic	102 ± 7	106 ± 9	105 ± 10	103 ± 8	100 ± 6	102 ± 7	98 ± 7	99 ± 8
pH	7.442 ± 0.029	7.466 ± 0.019	7.446 ± 0.024	7.472 ± 0.023	7.447 ± 0.029	7.447 ± 0.012	7.462 ± 0.026	7.474 ± 0.008
pO_2 (mmHg)	94.9 ± 7.5	97.3 ± 17.5	95.9 ± 6.9	93.8 ± 8.3	98.4 ± 6.3	97.7 ± 10.9	97.4 ± 6.5	98.8 ± 17.5
pCO_2 (mmHg)	33.2 ± 2.5	35.6 ± 7.4	33.2 ± 2.8	35.6 ± 1.9	38.9 ± 2.3	37.8 ± 3.8	31.9 ± 0.7	34.1 ± 1.6
Behavior duration (s)								
Cycle								
Activity				4 ± 1				5 ± 1
Calm				9 ± 2				10 ± 1
Net cycling				95 ± 15				105 ± 10

Values are the means ± SD (n = 4–6) measured before [1- C]AA infusion.

*** $p < 0.001$ effect of 25 mg/kg, i.p. NMDA in chronic vehicle- or valproate-treated rats; -, not measured

Table 2

Effect of chronic valproic acid on NMDA-induced regional AA incorporation coefficients, k* in rat brain

	Chronic Saline		Chronic Valproate		Valproate × NMDA interaction	Valproate effect	NMDA effect
Brain region	**Saline (n = 5)**	**NMDA (n = 6)**	**Saline (n = 6)**	**NMDA (n = 6)**	***P*-value**	***P*-value**	***P*-value**
Prefrontal cortex layer I	4.03 ± 0.20	5.55 ± 0.61***	4.39 ± 0.43	4.24 ± 0.41	< 0.001		
Prefrontal cortex layer IV	4.46 ± 0.37	6.31 ± 0.82**	4.90 ± 0.54	4.55 ± 0.36	< 0.001		
Primary olfactory cortex	3.87 ± 0.58	4.96 ± 0.68*	3.92 ± 0.59	3.89 ± 0.50	0.003		
Frontal cortex (10)							
Layer I	4.17 ± 0.45	5.57 ± 0.53**	4.56 ± 0.31	4.12 ± 0.66	< 0.001		
Layer IV	4.49 ± 0.52	6.49 ± 0.92**	5.20 ± 0.49*	4.57 ± 0.21[a]	< 0.001		
Frontal cortex (8)							
Layer I	4.21 ± 0.40	6.07 ± 1.02**	4.36 ± 0.25	4.46 ± 0.44	< 0.001		
Layer IV	4.83 ± 0.62	6.63 ± 0.97**	4.56 ± 0.45	4.76 ± 0.51	0.011		
Pyriform cortex	3.39 ± 0.55	3.88 ± 0.55	3.61 ± 0.53	3.53 ± 0.54	0.219	0.777	0.387
Anterior cingulate cortex	4.68 ± 0.47	7.53 ± 1.5**	4.78 ± 0.54	5.09 ± 0.31	0.002		
Motor cortex							
Layer I	4.23 ± 0.54	6.07 ± 0.89**	4.60 ± 0.59	4.62 ± 0.57	0.004		
Layer II –III	4.40 ± 0.65	6.12 ± 1.02*	4.78 ± 0.51	4.57 ± 0.71	0.006		
Layer IV	4.67 ± 0.71	6.31 ± 0.92**	5.25 ± 0.39	5.18 ± 0.25	0.004		
Layer V	3.47 ± 0.35	5.05 ± 0.73**	4.14 ± 0.26**	3.75 ± 0.56	< 0.001		
Layer VI	3.35 ± 0.42	4.82 ± 0.84**	3.68 ± 0.54	3.84 ± 0.56	0.010		
Somatosensory cortex							
Layer I	4.23 ± 0.49	5.62 ± 0.55**	4.66 ± 0.42	4.25 ± 0.53	< 0.001		
Layer II – III	4.39 ± 0.52	5.89 ± 0.67**	5.09 ± 0.22	4.34 ± 0.54	< 0.001		
Layer IV	4.81 ± 0.55	6.51 ± 1.08*	5.27 ± 0.37	4.31 ± 0.48	< 0.001		
Layer V	4.25 ± 0.37	5.65 ± 0.67**	4.47 ± 0.36	4.50 ± 0.41	0.003		
Layer VI	4.19 ± 0.47	5.47 ± 0.69**	4.28 ± 0.35	4.38 ± 0.46	0.012		
Auditory cortex							
Layer I	5.37 ± 2.03	7.50 ± 1.00*	5.35 ± 0.66	4.88 ± 0.92	0.019		
Layer IV	6.12 ± 1.48	7.54 ± 1.55	4.98 ± 0.54	5.29 ± 0.50	0.373	0.011	0.168
Layer VI	5.21 ± 1.68	6.53 ± 1.17	4.71 ± 0.38	4.78 ± 1.16	0.214	0.032	0.171
Visual cortex							
Layer I	4.16 ± 0.51	6.43 ± 0.33***	4.52± 0.25	4.24 ± 0.30	< 0.001		
Layer IV	4.47 ± 0.38	6.26 ± 0.94***	4.54 ± 0.22	4.83 ± 1.04	0.001		
Layer VI	4.29 ± 0.62	6.37 ± 0.64***	4.49 ± 0.29	4.64 ± 0.34	0.025		
Preoptic area (LPO/MPO)	3.54 ± 0.45	3.96 ± 0.35	3.81 ± 0.60	3.82 ± 0.49	0.326	0.762	0.297
Suprachiasmatic nu	3.29 ± 0.62	3.96 ± 0.61	3.72 ± 0.67	3.78 ± 0.72	0.283	0.656	0.208
Globus pallidus	3.37 ± 0.39	3.65 ± 0.22	3.34 ± 0.24	3.52 ± 0.39	0.728	0.546	0.093
Bed nu stria terminalis	3.46 ± 0.60	4.00 ± 0.63	3.46 ± 0.22	3.25 ± 0.22	0.060	0.064	0.392
Olfactory tubercle	4.33 ± 0.44	4.91 ± 0.57	3.60 ± 0.38	3.59 ± 0.38	0.133	< 0.001	0.144
Diagonal band Dorsal	4.02 ± 0.32	4.21 ± 0.56	3.72 ± 0.45	3.89 ± 0.52	0.949	0.138	0.377
Ventral	3.79 ± 0.35	4.19 ± 0.38	3.76 ± 0.19	3.71 ± 0.21	0.079	0.051	0.179
Amygdala basolateral/medial	4.19 ± 0.64	4.60 ± 0.82	3.67 ± 0.31	3.87 ± 0.14	0.653	0.013	0.199
Hippocampus							
CA1	3.66 ± 0.35	4.37 ± 0.56**	3.47 ± 0.34	3.45 ± 0.35	0.048		
CA2	3.99 ± 0.67	4.85 ± 0.30*	3.76 ± 0.25	3.61 ± 0.27	0.006		
CA3	3.99 ± 0.65	5.10 ± 0.64*	3.70 ± 0.20	3.74 ± 0.44	0.022		
Dentate gyrus	3.63 ± 0.31	5.01 ± 0.67**	3.79 ± 0.27	3.56 ± 0.20	< 0.001		
SLM	4.03 ± 0.53	5.02 ± 0.69*	4.02 ± 0.14	4.13 ± 0.39	0.040		

	Chronic Saline		Chronic Valproate		Valproate × NMDA interaction	Valproate effect	NMDA effect
Brain region	**Saline (n = 5)**	**NMDA (n = 6)**	**Saline (n = 6)**	**NMDA (n = 6)**	***P*-value**	***P*-value**	***P*-value**
Accumbens nucleus	3.94 ± 0.55	4.79 ± 0.50*	4.07 ± 0.37	4.00 ± 0.17	0.016		
Caudate putamen							
Dorsal	3.98 ± 0.35	5.14 ± 0.18***	3.96 ± 0.34	4.10 ± 0.40	0.002		
Ventral	4.00 ± 0.36	5.02 ± 0.30***	3.97 ± 0.42	3.74 ± 0.17	0.001		
Lateral	3.96 ± 0.39	5.30 ± 0.49***	4.11 ± 0.53	3.80 ± 0.27	< 0.001		
Medial	3.93 ± 0.36	5.06 ± 0.42**	3.35 ± 0.33	3.55 ± 0.48	0.013		
Septal nu lateral	3.43 ± 0.49	4.17 ± 0.18	3.35 ± 0.23	3.54 ± 0.48	0.107	0.061	0.069
Septal nu medial	3.97 ± 0.45	4.10 ± 0.50	3.33 ± 0.33	3.35 ± 0.34	0.436	0.070	0.081
Habenular nu lateral	6.21 ± 1.12	7.07 ± 0.19	6.51 ± 1.19	6.51 ± 0.94	0.290	0.746	0.290
Habenular nu medial	6.46 ± 1.33	7.16 ± 0.30	6.13 ± 0.23	6.27 ± 0.92	0.405	0.083	0.218
Lateral geniculate nu dorsal	4.49 ± 0.46	5.72 ± 0.42**	4.59 ± 0.50	4.58 ± 0.54	0.007		
Medial geniculate nu	5.01 ± 0.88	6.09 ± 0.64	4.88 ± 0.33	4.89 ± 0.57	0.053	0.019	0.074
Thalamus							
Ventroposterior lateral nu	4.11 ± 0.33	4.48 ± 0.56	4.18 ± 0.37	4.44 ± 0.56	0.798	0.921	0.126
Ventroposterior medial nu	4.20 ± 0.39	4.64 ± 0.59	4.25 ± 0.33	4.38 ± 0.31	0.685	0.829	0.142
Paratenial nu	4.14 ± 0.26	5.17 ± 0.57**	4.08 ± 0.37	4.26 ± 0.32	0.022		
Anteroventral nu	5.66 ± 0.67	7.15 ± 0.70**	5.77 ± 0.62	5.54 ± 0.61	0.005		
Anteromedial nu	4.51 ± 0.37	4.98 ± 0.58	4.50 ± 0.39	4.58 ± 0.37	0.301	0.277	0.153
Reticular nu	4.42 ± 0.39	5.50 ± 0.45	4.18 ± 0.41	4.30 ± 0.38	0.072	0.245	0.125
Paraventricular nu	4.28 ± 0.47	4.25 ± 0.35	4.06 ± 0.45	4.38 ± 0.49	0.093	0.745	0.158
Parafascicular nu	4.17 ± 0.76	5.73 ± 0.51**	4.19 ± 0.28	4.12 ± 0.52	0.002		
Subthalamic nu	5.05 ± 0.83	5.91 ± 0.37	5.23 ± 0.38	5.30 ± 0.37	0.078	0.332	0.061
Hypothalamus							
Supraoptic nu	4.27 ± 0.81	3.30 ± 0.57	4.00 ± 0.22	3.80 ± 0.79	0.178	0.689	0.189
Lateral	3.36 ± 0.61	3.98 ± .22	3.54 ± 0.47	3.75 ± 0.40	0.274	0.888	0.088
Anterior	3.68 ± 0.35	3.82 ± 0.49	3.04 ± 0.35	3.72 ± 0.78	0.238	0.116	0.079
Periventricular	3.01 ± 0.43	3.12 ± 0.32	3.09 ± 0.35	3.39 ± 0.54	0.587	0.335	0.260
Arcuate	3.51 ± 0.67	3.38 ± 0.47	3.26 ± 0.41	3.19 ± 0.48	0.875	0.307	0.630
Ventromedial	3.87 ± 0.38	3.77 ± 0.60	3.72 ± 0.16	3.65 ± 0.76	0.941	0.547	0.689
Posterior	3.37 ± 0.19	3.32 ± 0.20	3.67 ± 0.25	3.81 ± 0.63	0.135	0.452	0.226
Mammillary nu	3.74 ± 0.89	3.57 ± 0.42	3.76 ± 0.14	3.52 ± 0.31	0.870	0.919	0.335
Interpeduncular nu	6.30 ± 0.19	8.06 ± 0.66***	5.33 ± 0.33***	5.70 ± 0.59	0.003		
Substantia nigra	3.97 ± 0.16	5.54 ± 1.02**	4.17 ± 0.39	4.17 ± 0.43	0.006		
Pretectal area	4.13 ± 0.55	4.02 ± 0.36	3.89 ± 0.27	3.71 ± 0.59	0.324	0.075	0.126
Grey layer Superior colliculus	4.00 ± 0.43	4.44 ± 0.56	4.00 ± 0.27	0.92 ± 0.32	0.144	0.160	0.314
Superior colliculus	4.14 ± 0.33	4.93 ± 0.90	3.89 ± 0.24	4.26 ± 0.88	0.335	0.051	0.074
Inferior colliculus	5.91 ± 1.20	7.24 ± 0.55*	6.25 ± 0.84	5.80 ± 1.10	0.036		
Flocculus	4.52 ± 0.68	4.63 ± 0.70	4.45 ± 0.48	4.75 ± 0.18	0.696	0.907	0.374
Cerebellar gray matter	4.33 ± 0.34	5.64 ± 0.84*	4.25 ± 0.34	4.29 ± 0.39	0.010		
Molecular layer cerebellar gray	6.02 ± 0.76	7.41 ± 0.90*	6.17 ± 0.53	6.04 ± 0.68	0.022		
White matter							
Corpus callosum	3.34 ± 0.60	3.68 ± 0.60	3.20 ± 0.36	3.13 ± 0.33	0.320	0.101	0.506
Zona incerta	3.47 ± 0.27	3.57 ± 0.38	3.40 ± 0.33	3.39 ± 0.31	0.726	0.414	0.726
Internal capsule	2.83 ± 0.51	2.85 ± 0.39	2.84 ± 0.47	2.89 ± 0.51	0.947	0.894	0.858
Cerebellar white matter	3.32 ± 0.35	3.06 ± 0.53	3.30 ± 0.29	2.93 ± 0.35	0.725	0.651	0.071
Non-blood-brain barrier regions							
Subfornical organ	4.50 ± 0.93	3.75 ± 0.72	3.90 ± 0.99	4.06 ± 0.99	0.249	0.706	0.453
Median eminence	3.58 ± 0.57	3.75 ± 0.31	3.76 ± 0.46	3.56 ± 0.50	0.347	0.988	0.958
Choroid plexus	21.5 ± 4.44	22.1 ± 3.09	22.4 ± 2.52	23.8 ± 1.15	0.737	0.309	0.417

Abbreviations: nu, nucleus; k* = (ml/s/g) × 10^{-4}, each k* value is a mean ± S.D

Main effects are not reported if statistically significant VPA × NMDA interaction when unpaired *t*-tests were performed.

* $p < 0.05$;

** $p < 0.01$;

*** $p < 0.001$; Chronic saline plus NMDA versus chronic saline plus saline, valproate plus saline versus chronic saline plus saline, and valproate plus NMDA versus valproate plus saline.

Table 3

Effect of NMDA on brain PGE_2 and TXB_2 concentrations in chronic vehicle- and valproate-treated rats

	Chronic vehicle		Chronic valproate		Valproate × NMDA interaction
	Saline	NMDA	Saline	NMDA	*P*-value
PGE_2 (ng/g brain)	7.6 ± 1.5	16.4 ± 4.4**	2.6 ± 0.4***	2.8 ± 0.7	0.003
TXB_2 (pg/g brain)	41.4 ± 2.8	88.5 ± 8.9***	22.8 ± 3.7***	23.1 ± 0.7	< 0.001

Each value is a mean ± SD (n = 4).

** P < 0.01,

*** P < 0.001; vehicle plus NMDA versus vehicle plus saline, valproate plus saline versus vehicle plus saline, valproate plus NMDA versus valproate plus saline