

## RESEARCH ARTICLES

### Effects of Valproyl Urea on Neurons of The Cerebral Cortex and Cerebellar Purkinje Cells in Rats

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#### Abstract

Preliminary studies in various animal models have established a greater anticonvulsant activity as well as broader margin of safety with less unwanted effects of N-(2-propylpentanoyl) urea (VPU) than its parent compound, valproic acid. We present herein the effects of VPU on neurons of the cerebral cortex and cerebellar Purkinje cells of anesthetized rats assessed by microiontophoretic technique and micropressure ejection. Similar to micropressure ejection of valproic acid, locally applied VPU depressed spontaneous firing of both neurons of the cerebral cortex and cerebellar Purkinje cells in a dose-dependent manner. Depressant effect on spontaneously firing Purkinje cells of VPU but not that of valproic acid was abolished in the presence of bicuculline given microiontophoretically. However, neither effect of VPU nor valproic acid was affected by strychnine. Further studies to probe interaction between VPU and other well established excitatory amino acid neurotransmitters of the brain given microiontophoretically were carried out on Purkinje cells. VPU exhibited different profile of responses from those of valproic acid which reversibly depressed excitant effect of glutamate and aspartate while had no effect on depressant effect evoked by either GABA or glycine. Virtually no effect of VPU was observed in corresponding environment. In conclusion, the present study demonstrated that VPU *per se* was able to exert anticonvulsant activity by different mechanisms than those exhibited by its parent compound, valproic acid. Interaction with GABA<sub>A</sub> receptor may at least, in part, involved in the anticonvulsant activity of VPU. More experiments are needed to identify other possible mechanisms of action of this compound.

**Key words :** valproyl urea, valproic acid, microiontophoretic technique, neurons of the cerebral cortex, cerebellar Purkinje cells.

## ผลของวาลโปอิลยูเรียต่อเซลล์ประสาทในเปลือกสมองใหญ่และเซลล์ประสาทเปอร์กินเจในเปลือกสมองน้อยของหนูแรท

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### บทคัดย่อ

การศึกษาฤทธิ์เบื้องต้นในการต้านชักในสัตว์ทดลอง พบว่าเอ็น-(2-โพรพิลเพนทาโนอิล)ยูเรีย (วีฟียู) มีประสิทธิภาพในการต้านชักสูงกว่าและมีฤทธิ์ที่ไม่พึงประสงค์น้อยกว่ากรทวาลโปรอิก (วีพีเอ) ซึ่งเป็นสารต้นแบบ การวิจัยนี้มีวัตถุประสงค์ที่จะใช้เทคนิคไมโครโอออนโดฟอเรซีส์ร่วมกับการใช้ความดันในการผลักรากออกจากปลายอิเล็กโทรด ในการศึกษาผลของวีฟียูต่อเซลล์ประสาทในเปลือกสมองใหญ่และเซลล์ประสาทเปอร์กินเจในเปลือกสมองน้อยของหนูแรทที่ทำให้สลบ วีฟียูและวีพีเอสามารถออกฤทธิ์ลดอัตราการปลดปล่อยกระแสประสาทที่เกิดขึ้นเองของทั้งเซลล์ประสาทที่เปลือกสมองใหญ่และเซลล์ประสาทเปอร์กินเจในเปลือกสมองน้อย ในลักษณะที่แปรตามขนาดของสารที่ได้รับ ฤทธิ์ในการกดเซลล์ประสาทเปอร์กินเจดังกล่าวของวีฟียูจะไม่เปลี่ยนแปลงแต่อย่างใดเมื่อให้ร่วมกับสารสตริควินแต่ถูกปิดกั้นได้อย่างสมบูรณ์โดยสารโบคุคูลินซึ่งเป็นสารที่มีฤทธิ์ปิดกั้นโดยจำเพาะที่ตำแหน่งจับของกาบาเอ ไม่พบว่าสตริควินหรือโบคุคูลินมีผลต่อฤทธิ์ในการกดการส่งกระแสประสาทของวีพีเอ เมื่อศึกษาผลของวีฟียูและวีพีเอที่มีต่อการออกฤทธิ์ของสารสื่อประสาทอื่นๆ ต่อเซลล์ประสาทเปอร์กินเจ พบว่าวีฟียูไม่มีผลต่อการออกฤทธิ์ของสารสื่อประสาทชนิดกระตุ้น (กลูตาเมตและแอสพาร์เทต) และยับยั้ง (กาบาและกลัยซีน) ซึ่งต่างจากผลของวีพีเอที่มีผลลดการออกฤทธิ์ของสารสื่อประสาทชนิดกระตุ้น (กลูตาเมตและแอสพาร์เทต) แต่ไม่มีผลต่อสารสื่อประสาทชนิดยับยั้ง (กาบาและกลัยซีน) การศึกษานี้แสดงให้เห็นว่าวีฟียูเป็นสารที่สามารถออกฤทธิ์ต้านชักด้วยตัวเองและด้วยกลไกการออกฤทธิ์ที่แตกต่างจากวีพีเอ ซึ่งส่วนหนึ่งของกลไกการออกฤทธิ์ต้านชักดังกล่าวจะมีความเกี่ยวข้องกับตัวรับของกาบาเอ และควรมีการศึกษาต่อไปถึงกลไกอื่นๆ นอกเหนือจากที่รายงานไว้ในงานวิจัยนี้

คำสำคัญ : วาลโปอิลยูเรีย, กรทวาลโปรอิก, เทคนิคไมโครโอออนโดฟอเรซีส์, เซลล์ประสาทในเปลือกสมองใหญ่, เซลล์ประสาทเปอร์กินเจในเปลือกสมองน้อย

## Introduction

N-(2-propylpentanoyl)urea or valproyl urea (VPU), (Fig.1) is a valproic acid analogue which has been reported in various animal models, to possess a higher anticonvulsant activity in parallel with a greater relative margin of safety while offering less unwanted effect than its parent compound, valproic acid or VPA<sup>1</sup>. No effect of VPU and VPA has been observed on total cytochrome P450 or CYP1A1, 1A2 or 2E1 activities of rats. However, VPU has been found to significantly increase CYP 2B1 and 2B2 activities<sup>2</sup>. Preliminary studies of pharmacokinetic as well as embryotoxic of this compound have been reported<sup>3,4</sup>. Many of the conventional antiepileptic drugs as well as those which just have been released into the market or newly discovered have been noted for their depressant effect on either the spontaneously or stimulus-driven firing neurons of the experimental animal's brain and thus may account for the anticonvulsant effect observed<sup>5,6</sup>. In the present study, we used microiontophoretic technique in combination with micropressure application to investigate the effect of VPU, in comparison with VPA, on neurons of the cerebral cortex and Purkinje cells of cerebellum of rats anesthetized with urethane. Interactions between VPU and other well established amino acid neurotransmitters or compounds with well defined mechanism of action were also observed.

## Materials and methods

### Animals

Male Wistar rats weighing 200-240 g obtained from the National Laboratory Animal Center, Mahidol University, Thailand, were used in this experiment. The rats were housed in groups of four to five rats and maintained on a standard light-dark cycle with a constant humidity and controlled temperature (28-32 °C). The animals had free access to food and tap water ad libitum.

### Experimental protocol

Wistar rats were anesthetized by urethane (1.6 g/kg B.W. i.p.) and placed onto

a stereotaxic apparatus (Narishige). The skull covering area of the frontal cortex and cerebellum were then removed. Recording of extracellular spike potentials was made from neurons of the sensorimotor cortex (AP=0-3 mm, L=1-2 mm, in relation to Bregma and V=0.8-2 mm from the pial surface<sup>7</sup>) and cerebellar Purkinje cells through the central microelectrode filled with a 4 M NaCl solution, of 3-5 barreled microelectrode tip diameter 6-9 µm.

The outer barrels of the electrode contain either  $\gamma$ -aminobutyric acid (GABA, 0.2 M, pH 3.5), glycine HCl (GLY, 0.2 M, pH 3.5), L-glutamate (GLU, 0.5 M, pH 7.5), L-aspartate (ASP, 0.5 M, pH 7.5), strychnine sulfate (STRY, 0.005 M in NaCl, pH 7.0), bicuculline methochloride (BMC, 0.005 M in NaCl, pH 3.5), VPA (0.025 M in 0.1 M hydroxypropyl- $\beta$ -cyclodextrin) and VPU (0.005 M in 0.1 M hydroxypropyl- $\beta$ -cyclodextrin). All standard compounds were injected to the vicinity of neurons using standard microiontophoretic method (Medical System) while the test substances, VPA and VPU, were delivered to neurons by means of micropressure application (Medical System FFM-2). Spike potentials were amplified by a standard set of high input impedance amplifier and monitored on a digital memory oscilloscope (Nikon Kodhen VC10) and were selected by a window slicer. The selected pulses were counted by digital rate meter and were displayed on the McIntosh computer (LC630) with a digital-to-analog converter (MacLab) and software (chart V 3.4.3, MacLab). The records were subsequently converted to neuronal firing rate for data presentation. The neuronal activity before, during and after microiontophoretic or micropressure application of various substances were compared and analysed. In the present study, two groups of neurons which generated spontaneously firing discharge were used. They were neurons in the sensory motor area of the frontal cortex and cerebellar Purkinje cells. The latter were identified by their high firing rates and by the characteristic pattern of simple and complex spike discharges<sup>8</sup> and the neurons were randomly recorded in the vermis of the cerebellum.

## Results

### Effects of VPA and VPU on spontaneously firing neurons of the cerebral cortex and Purkinje cells of the cerebellar cortex

Micropressure application of 0.1 M hydroxypropyl- $\beta$ -cyclodextrin, the solvent used to dissolve VPU or VPA had no effect on neuronal discharge of either neurons of the cerebral cortex ( $n=10$ ) or Purkinje cells of the cerebellum ( $n=20$ ). Micropressure application of VPU ( $n=12$ ) or VPA ( $n=10$ ) induced a dose-dependent and reversible reduction of the spontaneous firing of all neurons tested in the cerebral cortex (Fig. 2A) and cerebellum (Fig. 2B).

### Effects of bicuculline methochloride and strychnine sulfate on the depressant effect of VPU and VPA

As demonstrated in Fig. 3, microiontophoretic application of a convulsant alkaloid, bicuculline (BMC, 20-50 nA), in the amount that produced no excitation *per se* diminished the depressant effect elicited by either GABA or VPU (15-30 psi,  $n=12$ ) on Purkinje cells. On the contrary, inhibition of neuronal discharge resulting from micropressured application of VPA (20-40 psi,  $n=5$ ) was not at all altered by BMC.

Neither the depressant effect, on the neuronal discharge of Purkinje cells, of VPU (20-40 psi,  $n=10$ ) nor VPA (10-30 psi,  $n=5$ ) was altered by microiontophoretic application of STYR (30-60 nA) (Fig. 4) which clearly being able to antagonize the depressant effect of glycine.

### Effects of VPA and VPU on responses evoked by glutamate and aspartate on Purkinje cells

Discrepancy between the depressant effect elicited by VPU and VPA was noted when they were applied on Purkinje cells driven by either glutamate or aspartate. As illustrated in Fig. 5, glutamate (10-40 nA,  $n=15$ ) and aspartate (5-50 nA,  $n=12$ ) consistently increased firing rates of the neuronal discharge which were not affected by concurrent administration of VPU while

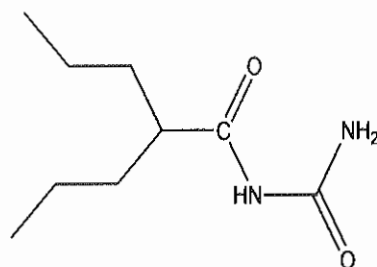
VPA apparently depressed glutamate or aspartate-evoked responses in 10 out of 12 Purkinje cells.

### Effects of VPA and VPU on responses evoked by GABA and glycine on Purkinje cells

Microiontophoretic application of GABA (1-60 nA) and glycine (20-100 nA) consistently depressed neuronal discharge of all Purkinje cells tested ( $n=30$ ) and such depressant effects were not affected by concomitant micropressured application of either VPU (10-35 psi) or VPA (5-30 psi). Typical responses of Purkinje cells under the influence of VPU or VPA to GABA and glycine are illustrated in Fig. 6.

## Discussion and Conclusion

The observation that the parent compound of VPU, VPA, demonstrated depressant effect on both the spontaneously firing neurons of the cerebral cortex and Purkinje cells is in accordance with previous finding of Chapman et al (1982)<sup>9</sup>. Furthermore, VPA, while had no effect on depressant effect elicited by microiontophoretically applied GABA or glycine, antagonized excitant effect induced by the excitatory neurotransmitters, glutamate and aspartate. A body of conflicting results on the effect of VPA that it may potentiate<sup>10,11,12</sup> or have no effect<sup>13,14</sup> on responses to GABA do exist. Differences in methodology as well as region of investigation possibly account for dissociation of the results observed, nevertheless, our observation confines to the latter. Inconsistent effect of microiontophoretically applied VPA on response to glycine and glutamate has been previously reported<sup>9</sup>. However, a suppression of responses to glutamate by VPA has been demonstrated in rat neocortex<sup>15</sup>. Similar result was obtained in our study and it is in line with the finding that VPA is able to reduce NMDA-stimulated  $\text{Ca}^{2+}$ -influx<sup>16</sup>. The finding that lack of interaction between VPA and bicuculline as well as strychnine rules out involvement of GABA<sub>A</sub> and strychnine-sensitive glycine receptors as possible site of action of VPA, respectively. Furthermore,



**Figure 1** Structure of N-(2-propylpentanoyl) urea

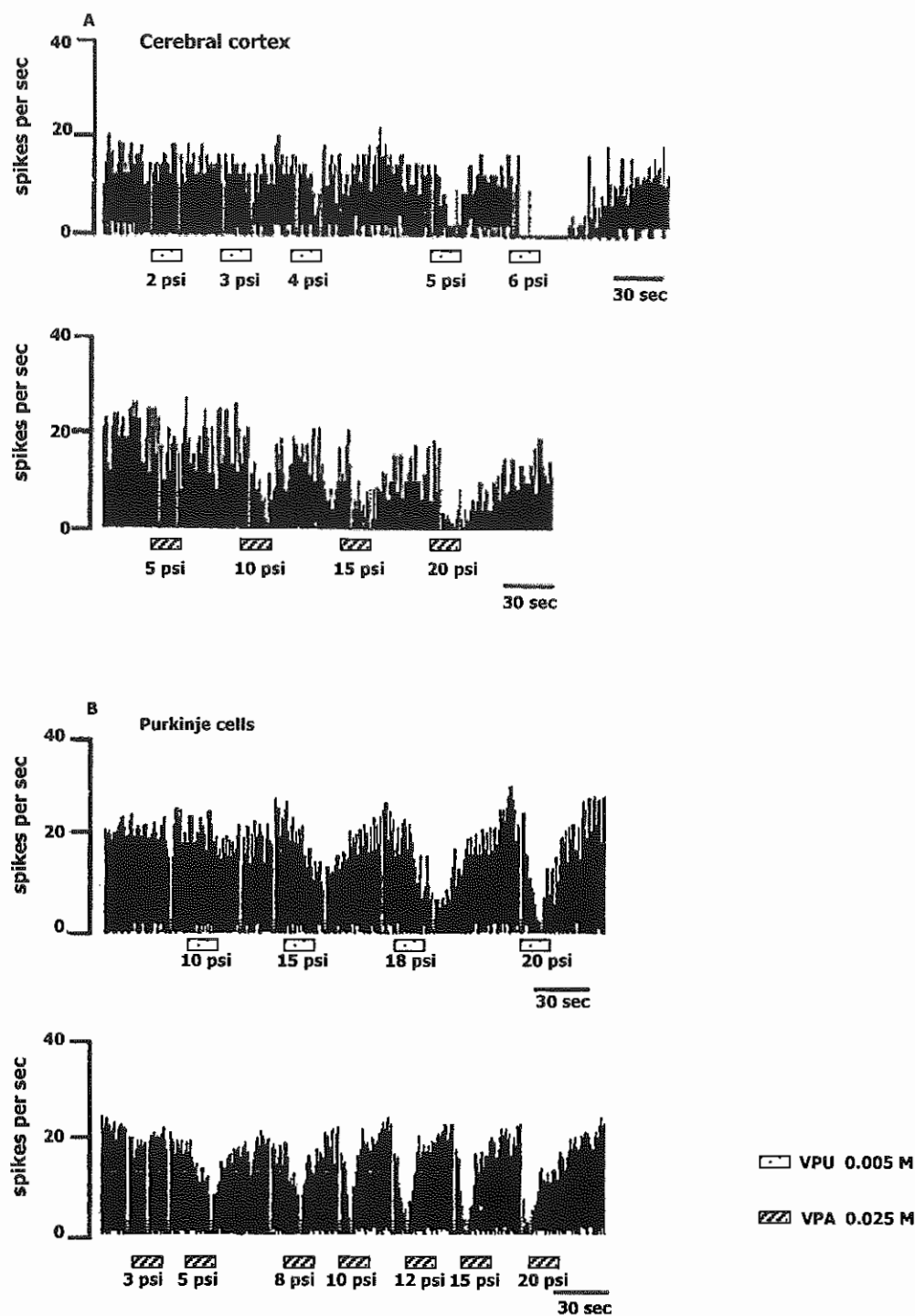
our results demonstrated the ability of locally applied VPA to suppress neuronal discharge of spontaneously firing neurons as well as those evoked by excitatory amino acid neurotransmitter, glutamate and aspartate. These observations could be considered as part of a spectrum of mechanisms underlying anticonvulsant activity of VPA, a broad spectrum of mechanisms known to act through a combination of several mechanisms<sup>17</sup>.

The result that VPU exerted dose-dependent depressant effect on both spontaneously firing neurons of cerebral cortex and Purkinje cells of cerebellum did suggest that VPU *per se* is an active anticonvulsant exerting protection against convulsions observed in various animal models<sup>1</sup>. Alternatively, metabolites of VPU other than being degraded to VPA may be responsible for anticonvulsant effect of VPU. This proposal is further strengthened by dissimilarity in profile of responses between VPU and VPA. Apparently, VPU did not modulate effects of either inhibitory GABA and glycine, or excitatory amino acid neurotransmitters, glutamate and aspartate, on spontaneously firing Purkinje cells. However, the effect of VPU was clearly antagonized by excitant effect of bicuculline, a specific GABA<sub>A</sub> receptor antagonist on 12

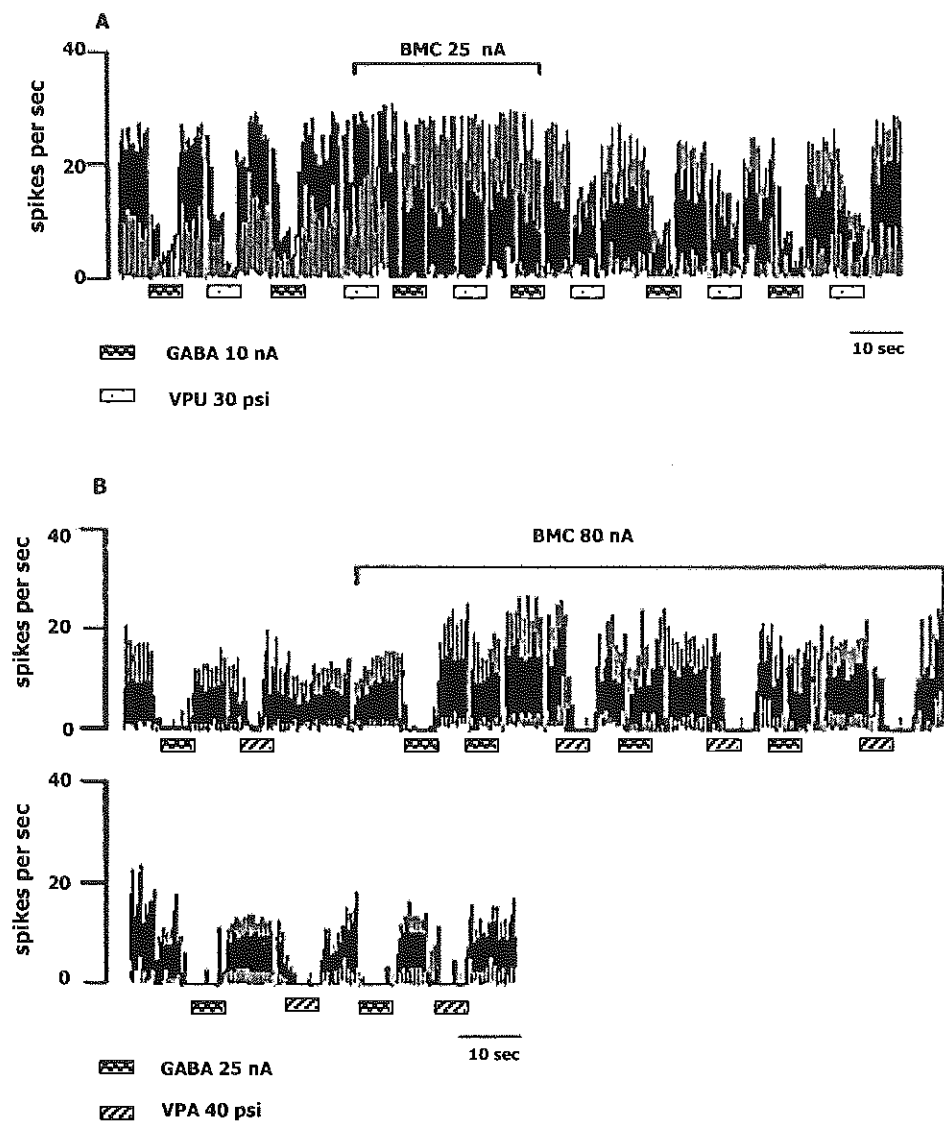
Purkinje cells indicating a participation of GABA<sub>A</sub> receptor in response to VPU.

However, when taken into consideration that protective effect of VPU against bicuculline induced convulsion was rather weak<sup>1</sup>, it is highly likely that the anticonvulsant activity of VPU through modulation of GABA<sub>A</sub> receptor should be one among other mechanisms that remain to be elucidated. Like VPA, VPU may possess a wide spectrum of anticonvulsant activity that act in concert to provide protection against convulsions in many animal models<sup>3</sup>.

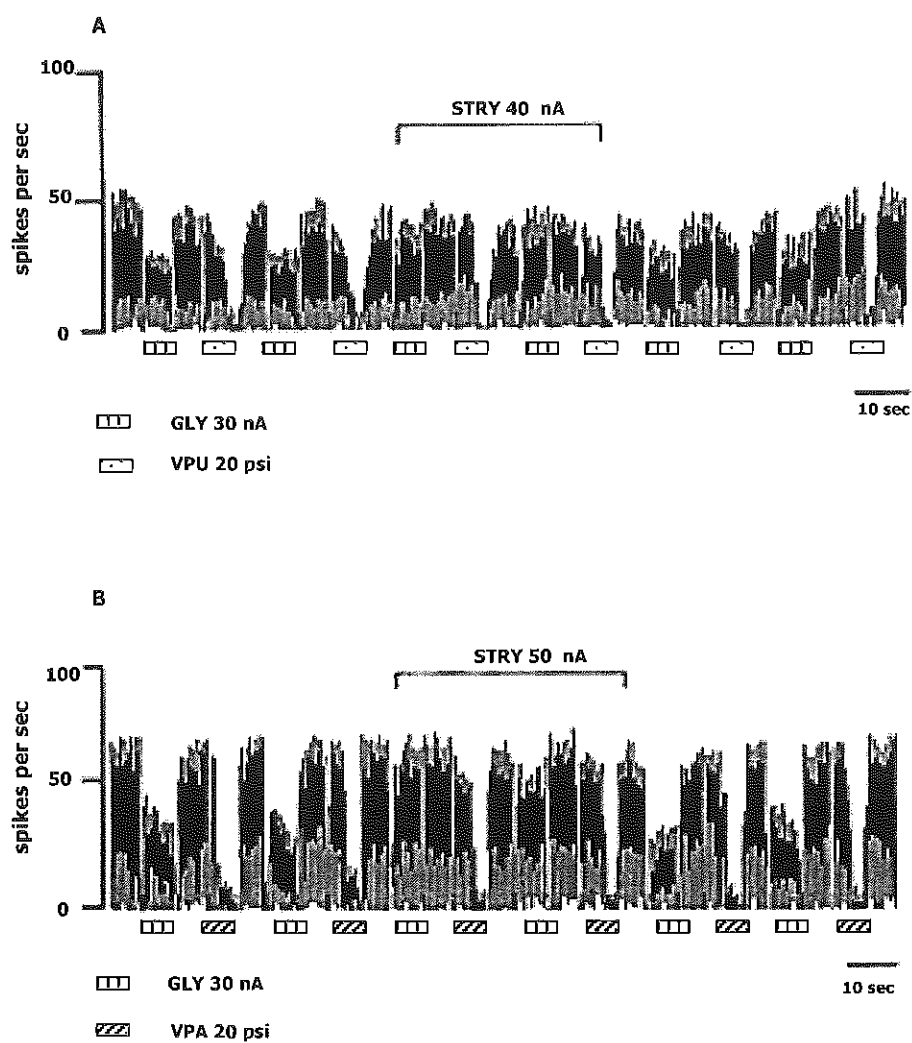
In conclusion, the present study demonstrated a dose-dependent and reversible depressant effects of locally applied VPU on spontaneously firing neurons. It is thus suggestive that VPU *per se* and/or any metabolites other than VPA is responsible for anticonvulsant activity previously reported in whole animal model. Like VPA, VPU may possess a wide spectrum of anticonvulsant activity, however, VPU seemed to act differently from VPA in terms of interaction with glutamate, aspartate and bicuculline. Questions regarding to the principal anticonvulsant mechanisms of this compound is a subject for further investigation.



**Figure 2** Effects of VPU (0.005 M) and VPA (0.025 M) in 0.1 M hydroxypropyl- $\beta$  cyclodextrin, being injected by different micropressure (psi) on spontaneously firing rate of neurons of cerebral cortex (A) and cerebellar Purkinje cells (B).

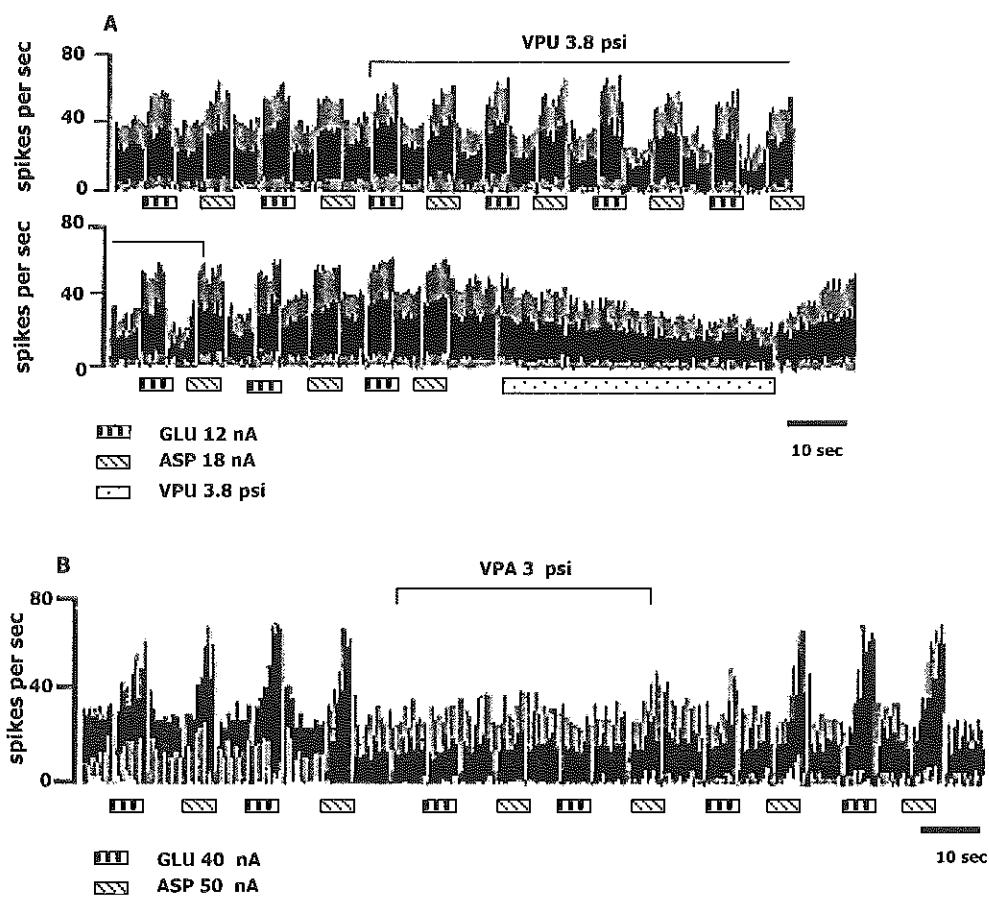


**Figure 3** Effect of continuously microiontophoretic application of bicuculline (BMC) superimposed on the response of Purkinje cells to micropressure application of VPU (0.005 M) (A) and VPA (0.025 M) (B) compared to microiontophoretic application of  $\gamma$ -aminobutyric acid (GABA).

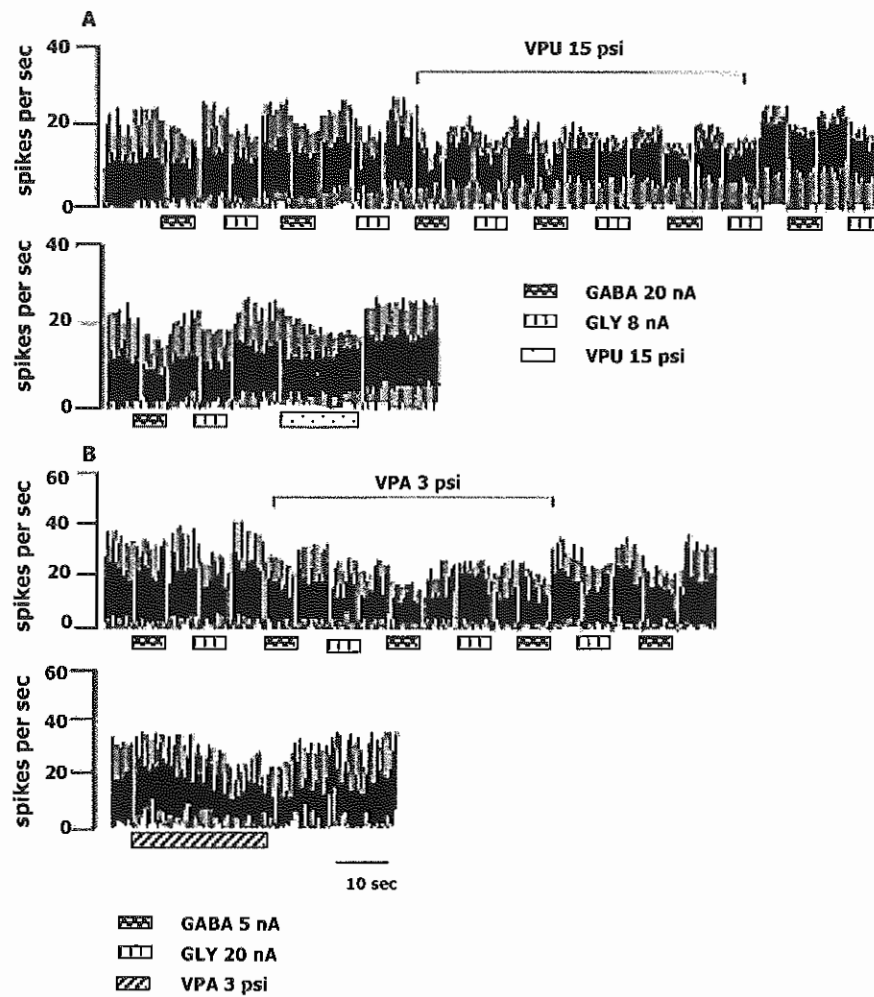


**Figure 4** Effect of continuously microiontophoretic application of strychnine sulphate (STRY) superimposed on the response of Purkinje cells to micropressure application of VPU (0.005 M) (A) and VPA (0.025 M) (B) compared to microiontophoretic application of glycine (GLY).





**Figure 5** Effect of continuously micropressure application of VPU (0.005 M) (A) and VPA (0.025 M) (B) on excitant action of microiontophoretic application of glutamate (GLU) and aspartate (ASP) on neuronal firing of Purkinje cells.



**Figure 6** Effect of continuously micropressure application of VPU (0.005 M) (A) and VPA (0.025 M) (B) on depressant action of microiontophoretic application of  $\gamma$ -aminobutyric acid (GABA) and glycine (GLY) on neuronal firing of Purkinje cells.

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