Chapter III

Results

Table 2 shows summary of microiontophoretic study of DCR on cortical neurones of the cerebellum.

Response of Purkinje Neurones to Dioscorine Application

Satisfactory unitary recordings were obtained from 75 cerebellar cortical neurones which were identified as Purkinje cell, sampled randomly in the cerebellar vermis. In all 75 vermian Purkinje cells, the effects of microiontophoretic application of DCR (5–25 nA, positive current) were consistent excitation of the spontaneous discharge in a dose–dependence manner. The responses were rapid in onset, and immediate recovery was observed upon cessation of DCR ejection. These excitatory effects were noticable from microiontophoretic current as low as 5 nA. For example, Figure 6 shows a progressive development of excitation of neuronal firing of a Purkinje cell during the application of DCR 5 nA, 10 nA, 15 nA respectively.

Table 2 Summary of microiontophoretic study of DCR on cortical neurones of the cerebellum

	Number of neurones	
	Tested	Responsive
A. Excitant action of DCR		
Purkinje cells of vermis	75	75
B. Abolition of action by DCR		
(1) Depressants		
GABA	70	70
glycine	25	25
taurine	20	20
noradrenaline	10	10
5–HT	5	5
(2) Excitants		
glutamate	10	0
aspartate	10	0

Effect of DCR on the responses of microiontophoretically applied depressant amino acid neurotransmitters (GABA, glycine, and taurine).

Pusatile cationic microiontophoretic applications (10 sec (on) duration and at 12 sec (off) regular intervals) of putative inhibitory amino acid neurotransmitters (GABA, glycine and taurine) were studied on Purkinje cells. GABA (15–16 nA; n=45), GLY (20–80 nA; n=25) and TAU (25–100 nA; n=20) produced consistent depression of all Purkinje cells tested. Continuous application of DCR (30nA) abolished the depressant action of all these three inhibitory amino acids. Example of these results were shown in Figure 7 and 8 respectively, recovery to the control level of responses was observed in each case after cessation of DCR administration.

Effect on the responses to microiontophoretically applied depressant monoamine neurotranamitters (NA and 5–HT)

Pulsatile cationic microiontophoretic applications (on 10 sec and off 12 sec) of putative inhibitory monoamine neurotransmitters were studied on the firing rate of Purkinje cells. NA(70–90 nA; n=10), 5-HT (80–120 nA; n=5) produced consistent depression of all Purkinje cells tested. When continuous microiontophoretic application of DCR (30 nA) were superimposed on the depressant pulses of putative neurotransmitters, in all cells tested, the depression of Purkinje cell activity caused by NA and 5–

HT were antagonized during microiontophoretic application of DCR. Figure 9 and 10 show a clear example for this effect, which also illustrates the antagonism of GABA action.

Effect on the responses to microiontophoretically applied excitant amino acid neurotransmitters (glutamate and aspartate).

Tests were also advanced further to see whether there was any DCR–induced modification of excitatory responses of Purkinje cells to putative excitatory neurotransmitters (GLU and ASP). Pulsatile anionic microiontophoretic application (on 10 sec and off 12 sec) of GLU (10–60 nA; n=10) and ASP (15–70 nA; n=10) produced consistent excitation in all Purkinje cells tested. When continuous microiontophoretic application of DCR (30 nA) were superimposed on the excitant pulses, the excitation of Purkinje cell activity caused by GLU and ASP remained unaffected. Example of these results were shown in Figure 11. On this cell, GABA was also tested. When continuous microiontophoretic application of DCR (30 nA) were superimposed on these pulses, only the depressant action of GABA was antagonized. Example of these results were shown in Figure 12.

Effect of Microiontophoretic Application of GABA–Antagonist, Bicuculline

The convulsant alkaloid bicuculline, a presumptive GABA–antagonist was selected as a tool to investigate and compare the possibility of the antagonistic action of DCR. The methochloride derivative of the alkaloid was chosen because it was more resistant to hydrolysis than the bicuculline base under physiological condition (Olsen et al., 1975).

Effect of DCR and BMC on neuronal response to some putative neurotransmitters (GABA and GLY).

Pulsatile microiontophoretic application (on 10 sec and off 12 sec) of GABA (10–30 nA; n=7) and GLY (30–70 nA; n=7) produced consistent depression of Purkinje cells tested. When continuous microiontophoretic application of BMC (30 nA, positive current) were superimposed on the depressant pulses, BMC antagonized only the depression effect of GABA, while the depression of neuronal activity caused by the control agonist GLY remained unaffected. When continuous microiontophoretic application of DCR (30 nA) were superimposed on the depressant pulses in the same cells (n=7), the depressant response to both GABA and GLY were antagonized in all cells tested. Example of these results were shown in Figure 13.

Effect of Microiontophoretic Application of Glycine–Antagonist, Strychnine

The convulsant alkaloid strychnine, a competitive antagonist of GLY, an amino acid presumable released as an inhibitory neurotransmitter on spinal motoneurons (Curtis, Duggan, and Johnston, 1971), was selected as a tool to investigate and compare the possibility of the antagonistic action of DCR.

Effect of DCR and STRY on neuronal response to some putative neurotransmitters (GABA and GLY).

Pulsatile microiontophoretic application (on 10 sec and off 12 sec) of GABA (5–20 nA; n=5) and GLY (50–70 nA;n=5) produced consistent depression of Purkinje cells tested. When continuous microiontophoretic application of strychnine sulfate (30 nA, positive current) were superimposed on the depressant pulses, STRY antagonized only the depression effect of GLY, while the depression of neuronal activity caused by the control agonist GABA remained unaffected. When continuous microiontophoretic application of DCR (30 nA) were superimposed on the depressant pulses in the same cells (n=5), the depressant response to both GABA and GLY were antagonized in all cells tested . Example of these results were shown in Figure 14.

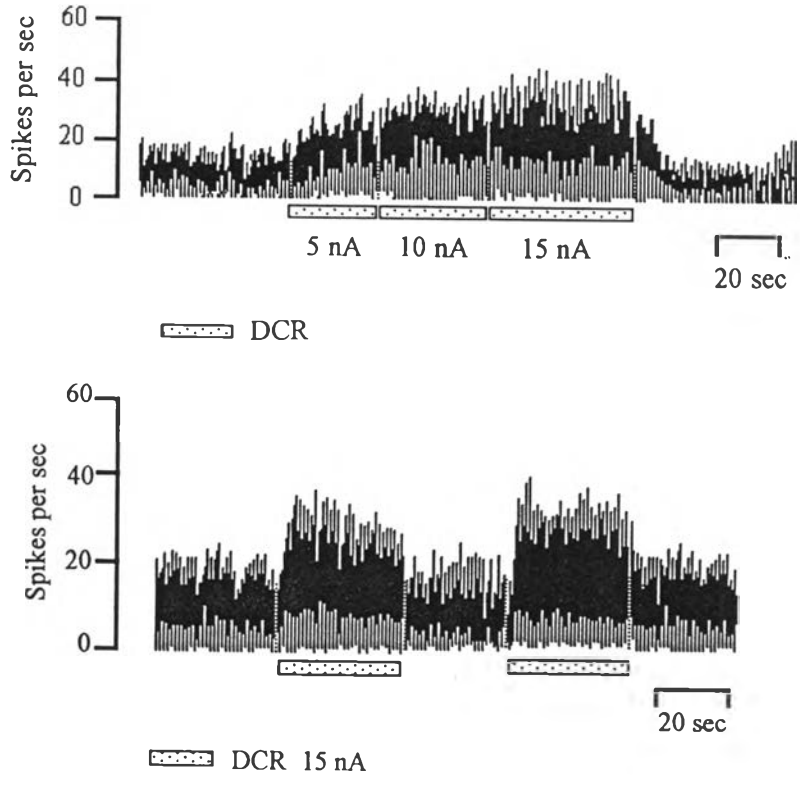

Figure 6. Effect of microiontophoretic application of DCR in dose dependent manner on spontaneous firing rate of Purkinje cells (upper). DCR produced excitation of neuronal firing of Purkinje cells, the apperance of excitation had rapid onset when ejected and immediately recovered to normal firing after the termination of DCR ejection (lower).

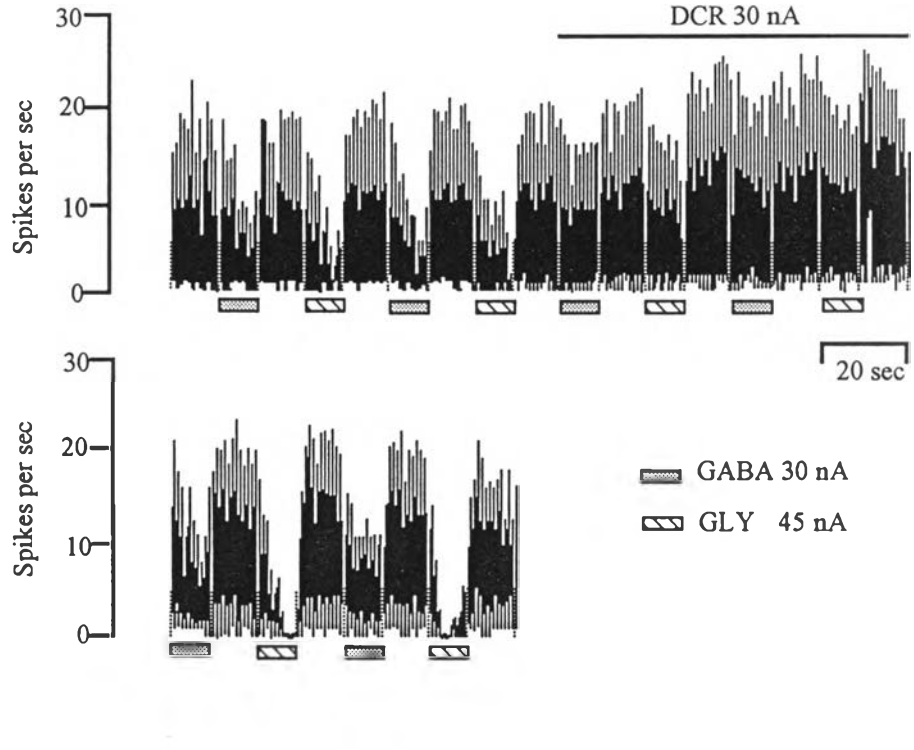

Figure 7. Effect of continuous microiontophoretic application of DCR in current dose (DCR; 30 nA) superimposed on the depressant actions of GABA (30 nA) and GLY (45 nA) on neuronal firing of Purkinje cells. The depressant actions of GABA and GLY were antagonized during microiontophoretic application of DCR.

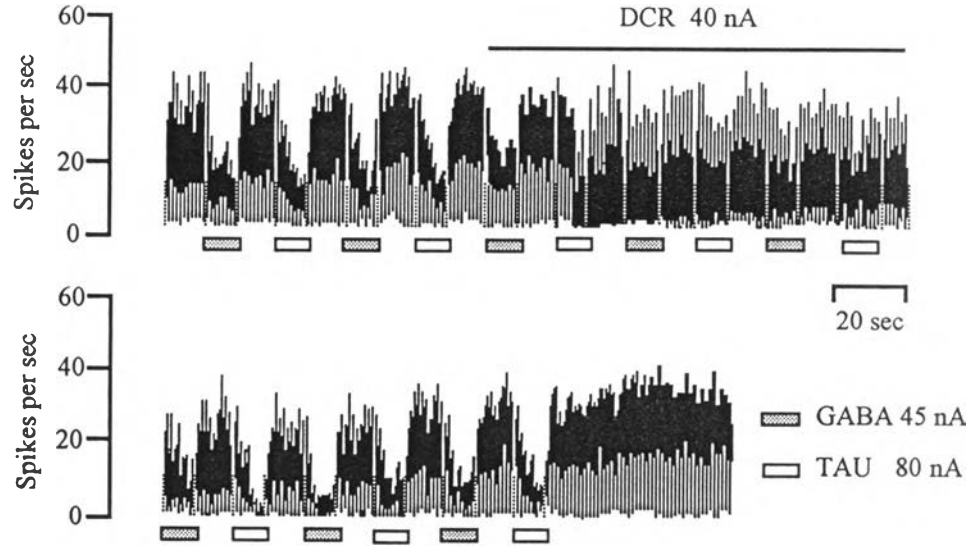

Figure 8. Effect of continuous microiontophoretic application of DCR in current dose (DCR; 40 nA) superimposed on the depressant actions of GABA (45 nA) and TAU (80 nA) on neuronal firing of Purkinje cells. The depressant actions of GABA and TAU were antagonized during microiontophoretic application of DCR.

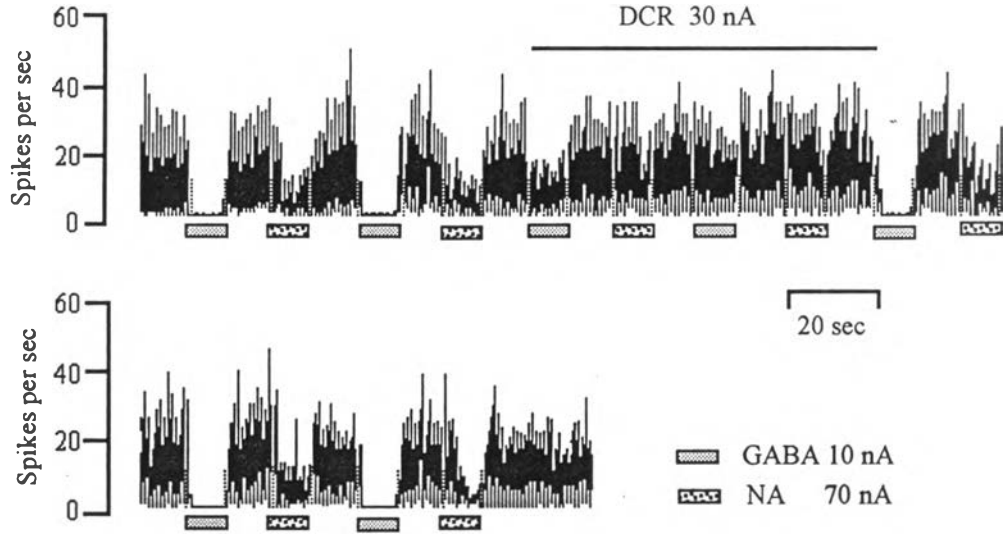

Figure 9. Effect of continuous microiontophoretic application of DCR in current dose (DCR; 30nA) superimposed on the depressant actions of GABA (10nA) and NA (70 nA) on neuronal firing of Purkinje cells. The depressant actions of GABA and NA were antagonized during microiontophoretic application of DCR.

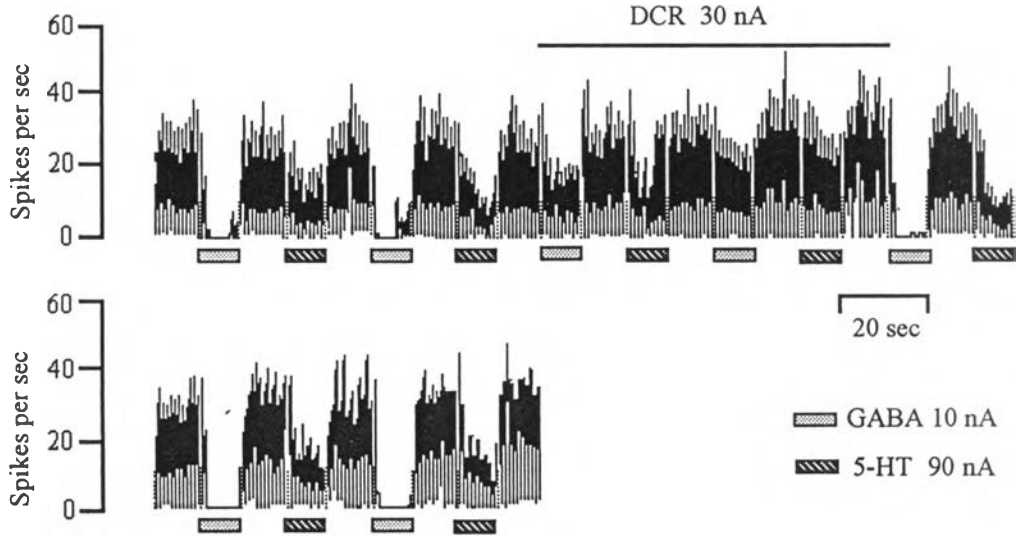

Figure 10. Effect of continuous microiontophoretic application of DCR in current dose (DCR; 30nA) superimposed on the depressant actions of GABA (10nA) and 5–HT (90 nA) on neuronal firing of Purkinje cells. The depressant actions of GABA and 5–HT were antagonized during microiontophoretic application of DCR.

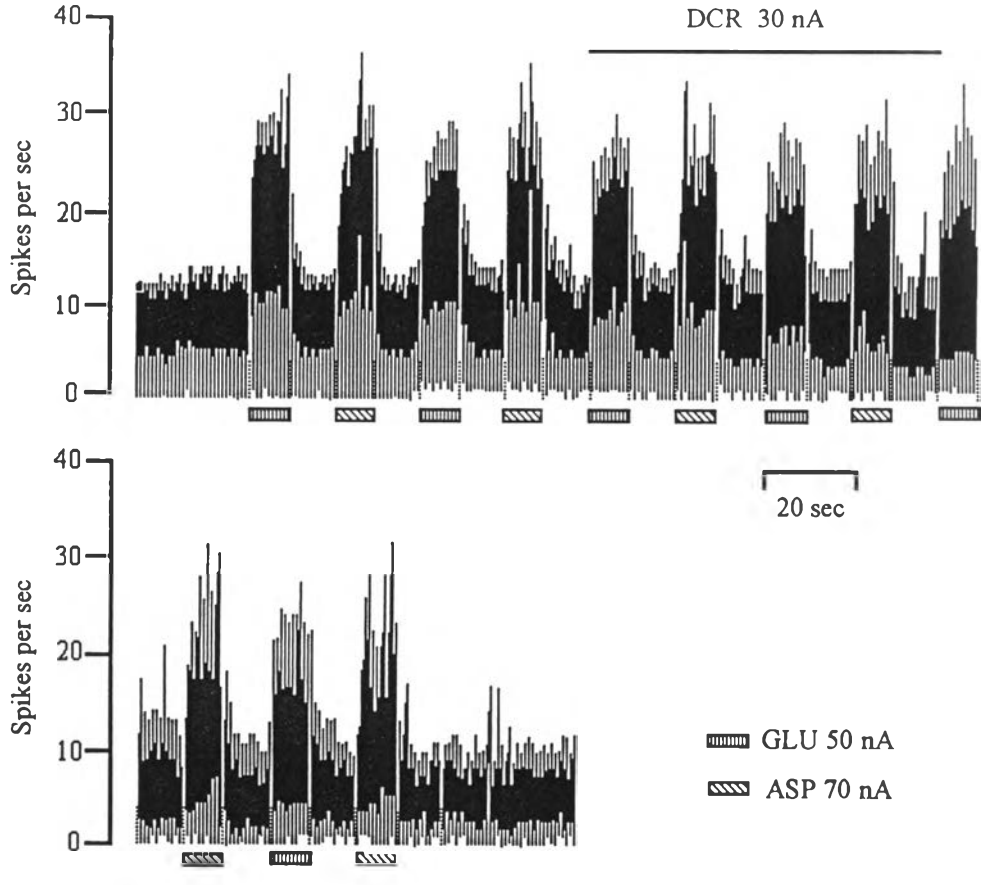

Figure 11. Effect of continuous microiontophoretic application of DCR in current dose (DCR; 30nA) superimposed on the excitation actions of GLU (50 nA) and ASP (70 nA) on neuronal firing of Purkinje cells. The excitant actions of GLU and ASP represented non appreciable effects on the action of these excitants.

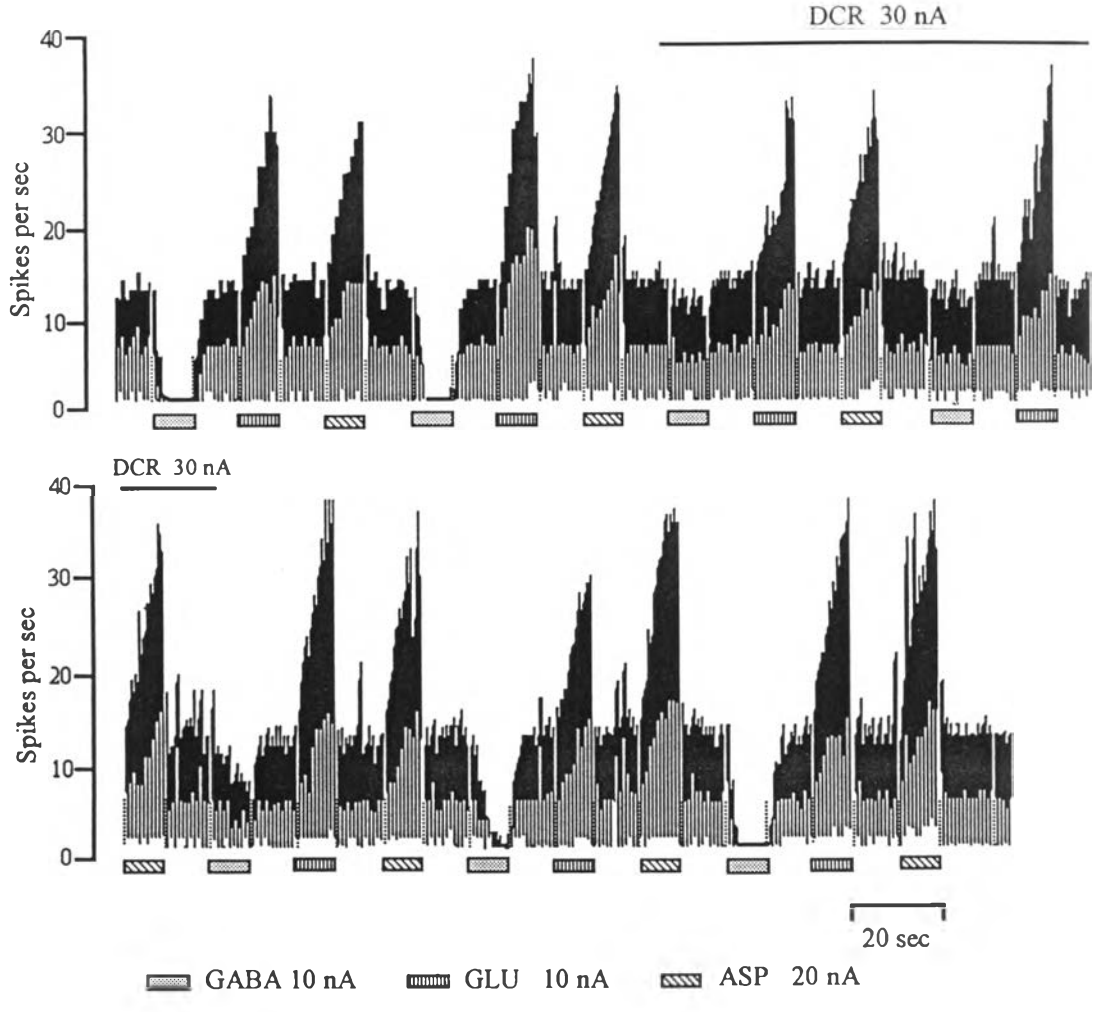

Figure 12. Effect of continuous microiontophoretic application of DCR in current dose (DCR; 30nA) superimposed on the depressant actions of GABA (10 nA) and the excitant actions of GLU (10 nA) and ASP (20 nA) on neuronal firing of Purkinje cells. Only the depressant actions of GABA were antagonized by DCR.

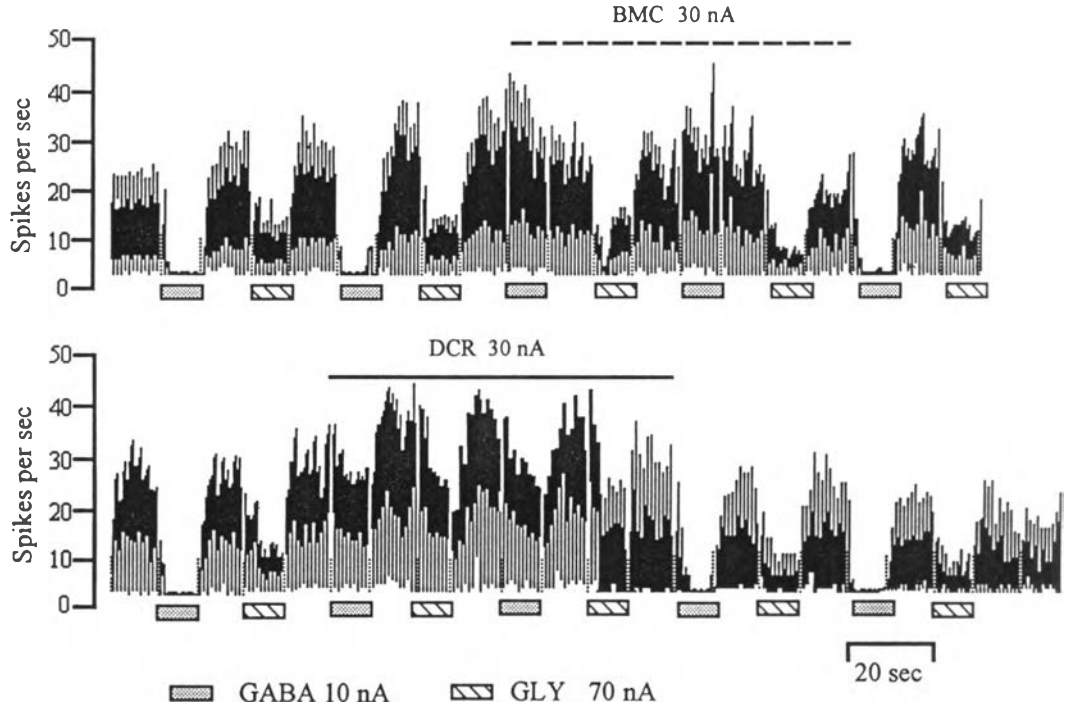

Figure 13. Effect of continuous microiontophoretic application of BMC (30 nA) and DCR (30 nA)) superimposed on the depressant actions of GABA (10 nA) and GLY (70 nA) on neuronal firing of Purkinje cells. The depressant actions of GABA were antagonized by both BMC and DCR application, while the depressant actions of GLY were only antagonized by DCR.

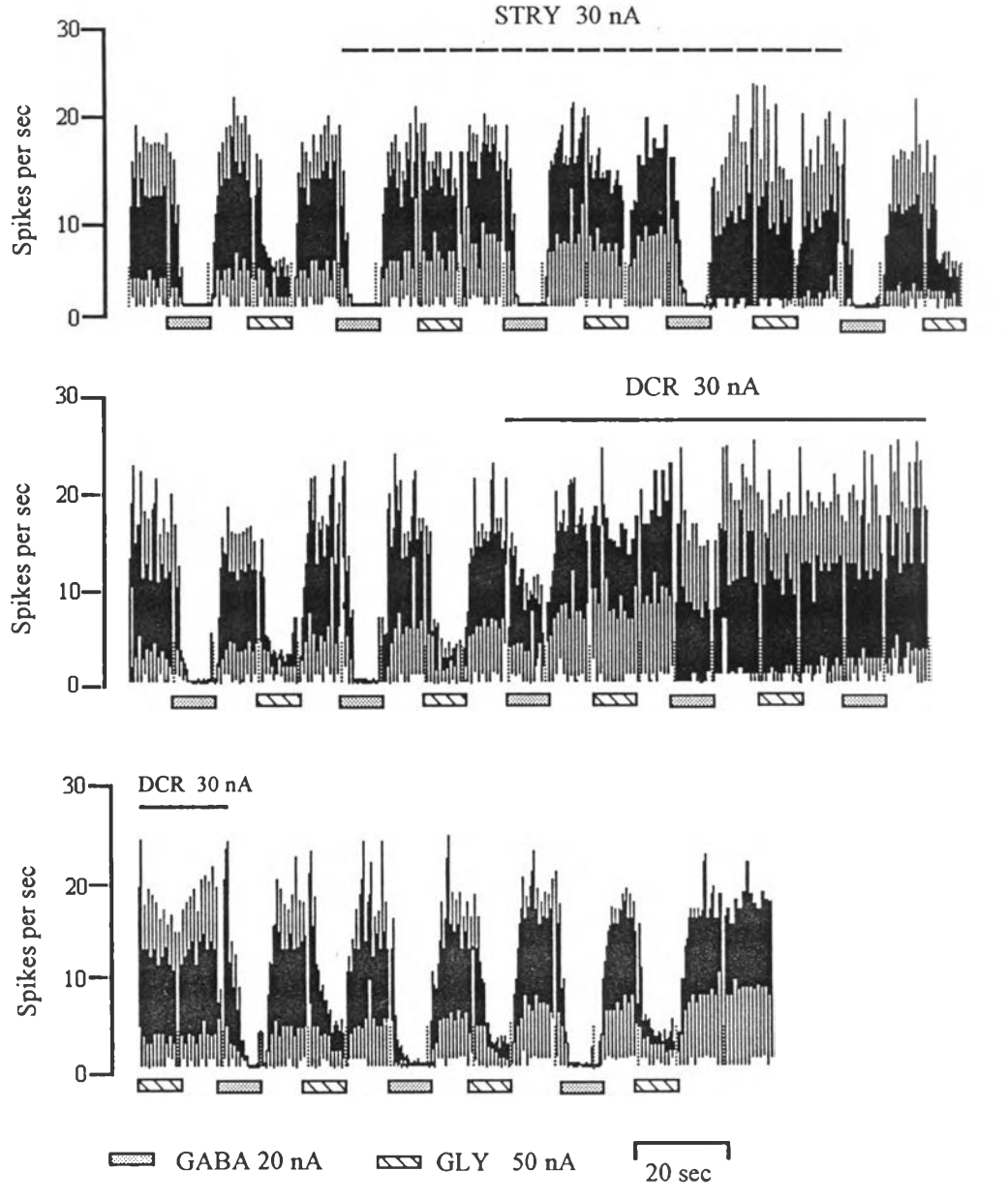

Figure 14. Effect of continuous microiontophoretic application of STRY (30 nA) and DCR (30 nA)) superimposed on the depressant actions of GABA (20 nA) and GLY (50 nA) on neuronal firing of Purkinje cells. The depressant actions of GLY were antagonized by both STRY and DCR application, while the depressant actions of GABA were only antagonized by DCR.