CHAPTER 3

RESULTS

1. General Observation.

In comparison of the yield of intact slices in this experiment, it was found that the use of Oscillotome produced perfect slices in greater number than that produced by hand-slicing. This is because the slice prepared by manual method varied considerably with regard the thickness occasionally more than 400 um. In addition, the results summarized in table 4 also confirmed that the use of the Oscillotome technique produced superior results than the manual slicing when comparing with the incidence of tissue survival obtained from the use of old animals (Osc. : H. = 67% : 0%). However, in the young group both methods produced comparable results (Osc. : H. = 75% : 70%).

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In addition, slices prepared by the machine also displayed uniform thickness while hand slicing frequently produced wedge shape in their longitudinal sectioned appearance. The sample of beautiful vermal slice obtained from Oscillotome technique is shown in Fig. 12 A and the good structure of its folium is also shown in Fig. 12 B.

Table 4. General observation of cerebellar slices obtained by Oscillotome technique and Hand-Slicing technique.

	Oscillotome technique (Osc.)	Hand-Slicing technique (H.)
1. thickness (um)	> 150	> 400
2. regular in thickness	YES	NO
3. no. of slices per 1 vermis block	4 - 6	2 - 3
4. time preparing (min.)	5 - 8	5
5. no. of tissue viability		
5.1 in young rats (15:15)	12 (75%)	11 (70%)
5.2 in old rats (15:15)	10 (67%)	0 (0%)

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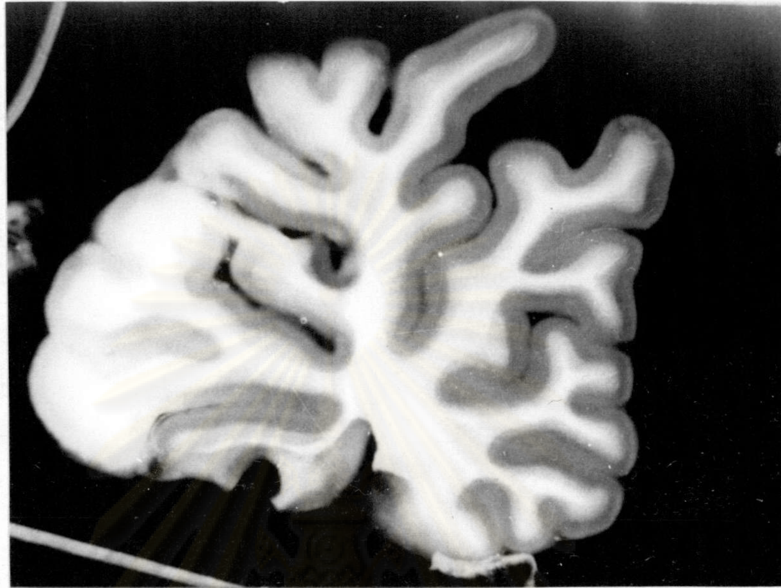
(A)



(B)

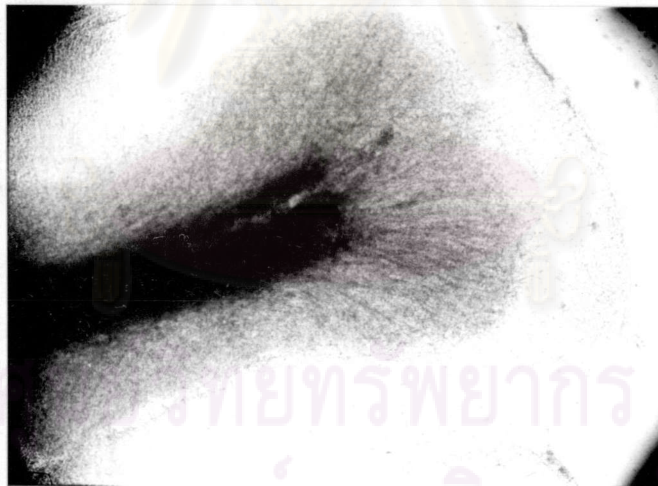


Fig. 12. Photographs of a 300 um cerebellar vermis slice (A) prepared with using the Oscillotome and magnified part of cerebellar folium (B).

The major disadvantage of the Oscillotome arose from number of steps required to complete the process and thus longer time was needed as compared with that required in the hand slicing method. However, utilization of the machine reduced the needs for skill practice which was usually required in case of manual method.

2. Spontaneous activity of the cortical neurons.

Extracellular recording obtained from neurons located within the Purkinje cell layer and adjacent areas (72 cells from 33 slices). In this experiment, spontaneously activity could be recorded from 2-5 cells in one cerebellar slice. Two types of spontaneous discharge were encountered : a continuous discharge in 49 cells (68%) (including activity of Purkinje cells and non-Purkinje cells) and a periodic pattern of firing in the rests (including bursty and phasic patterns). Continuous discharge was usually encountered when the recording microelectrode was in the area between Purkinje cell layer and the granular layer. The discharge presumably belonged to the Purkinje neurons was observed in 11 cells (15%), which occurred as a regular discharge of simple spikes interrupted occasionally by large biphasic positive-negative spike (0.4-0.6 mV), occurring at a frequency between 1 and 30 /sec (Fig. 13). Another type of

continuous action potentials characterized with a small amplitude (0.15 to 0.2 mV) and frequently found in this study (38 cells, 52%) was presumably generated by inhibitory interneuron known as a Golgi cell, see Fig. 14 A, B and C. These spikes displayed either a positive-negative or negative-positive biphasic spike with rather regular discharge at the rates between 20 and 40 /sec.

The bursty discharges were sometimes observed in some experiments (17 cells, 24%), see Fig. 15. Such pattern was similar to those previously reported in chronically isolated cerebellar folia <u>in vivo</u> (Snider, Teramoto and Ban, 1967), in culture preparations (Schlapfer Mamoon and Tobias, 1972; Gahwiler et al., 1972), and in cerebellar slice preparations (Okamoto and Quastel, 1973; Yamamoto, 1973; and Okamoto et al., 1976). In some case, this activity occurred in trains of bursts, each of which containing 3 to 10 spikes and following with silent periods for several milliseconds (Fig. 15 A) or sometimes, a few seconds (Fig. 15 B).

In rarely cases (6 cells, 8%), a phasic pattern of firing was also observed which was composed of a long period of continuous discharges and interrupted with a long silent period of about 2 to 5 seconds, as shown in Fig. 16.

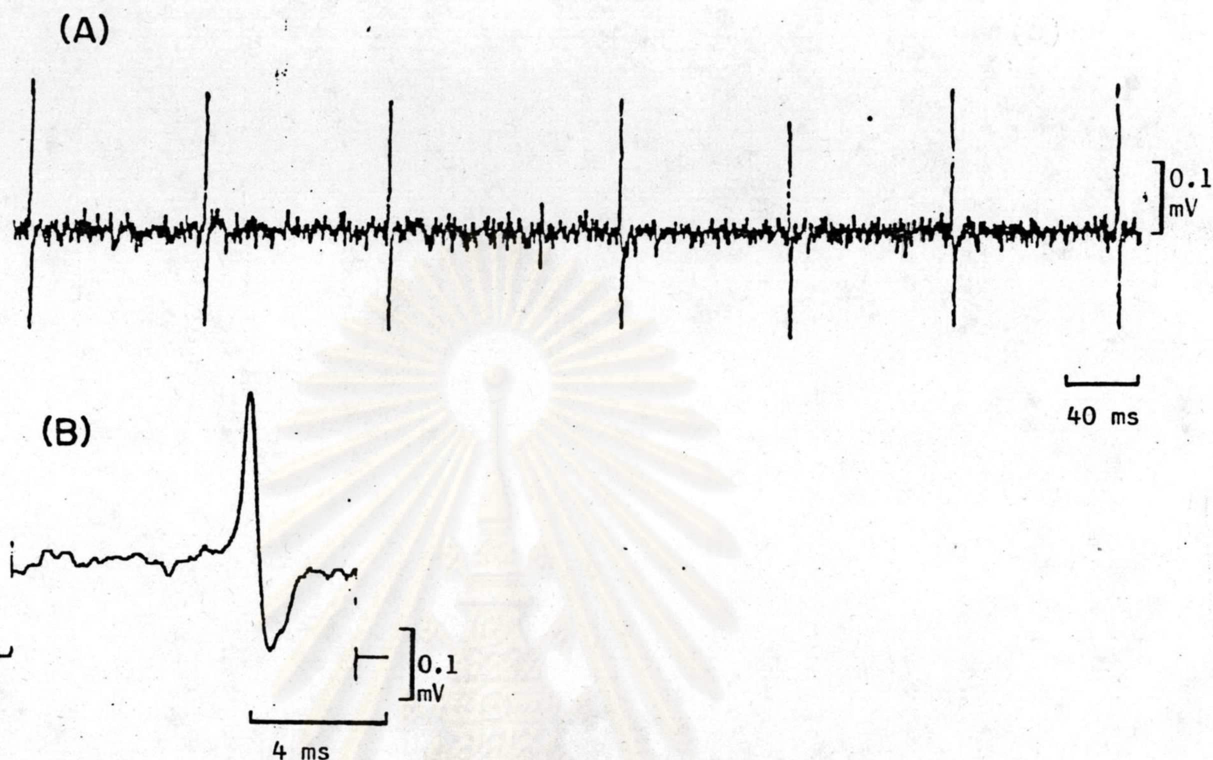


Fig. 13. Action potentials recorded extracellulary from a spontaneously active Purkinje cell in the in vitro rat cerebellar slice showing the typical biphasic spikes (0.38 mV). A : observed with using a low timebase (40 ms). B : time-base expansion of the signals from A. In this and subsequent figures, osscillograghic records were obtained on a pen recorder by retreiving the signal stored in the memory of a digital storage oscilloscope.

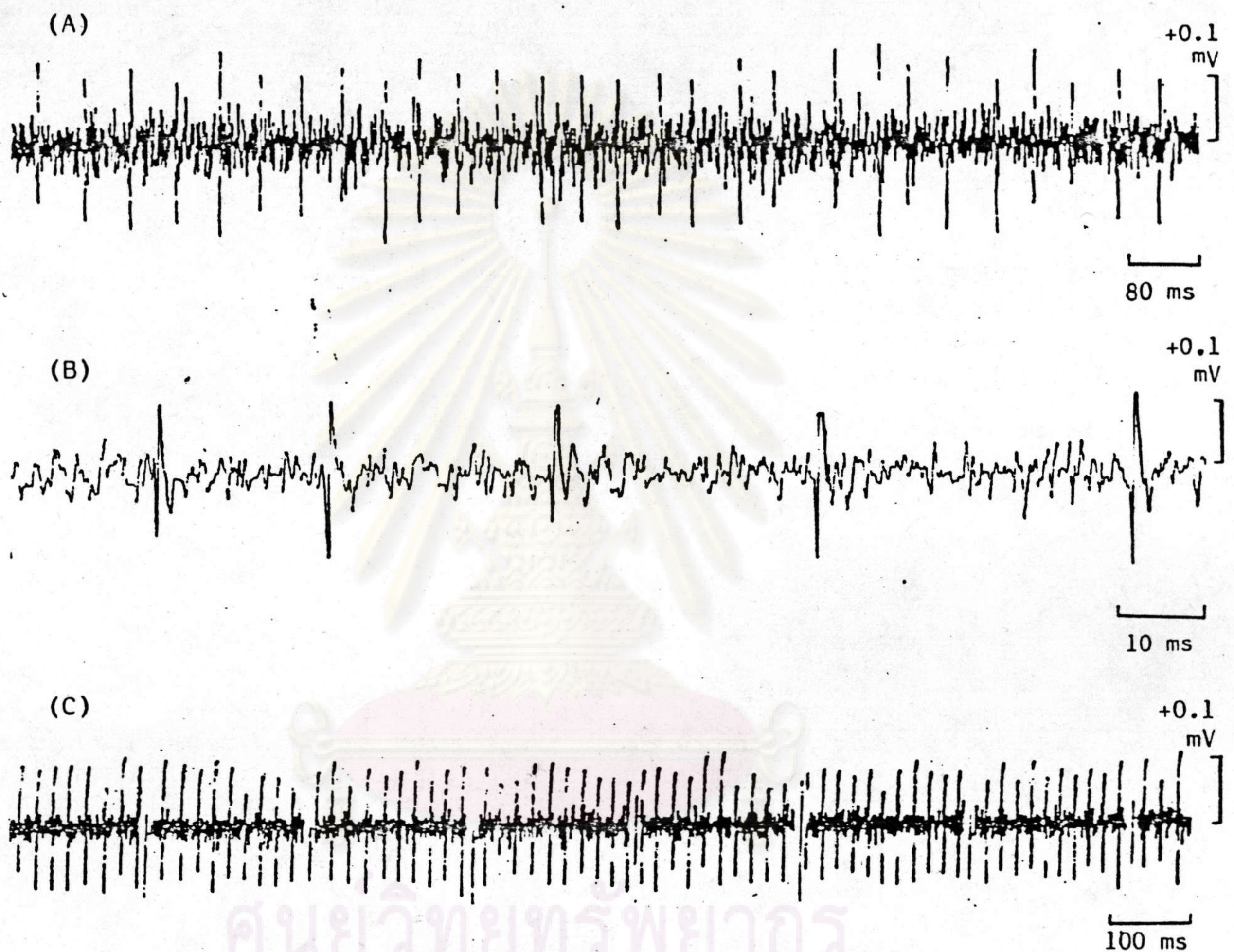


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Fig. 14. Spontaneous activity of inhibitory interneurons recorded at the granular layer which occured at relatively high rates of 35 /sec (A and B) and 50 /sec (C), and was usually found with a regular pattern of discharge.

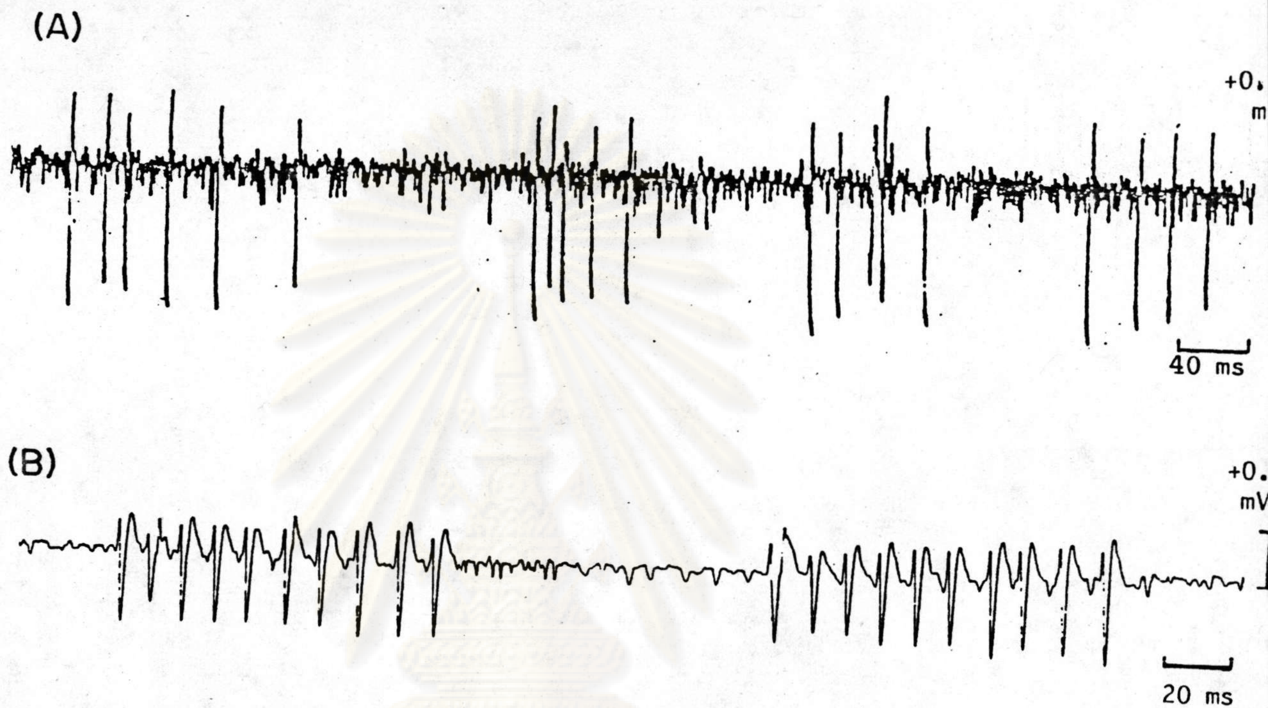

Fig. 15. Bursty discharges of the neurons recorded in vicinity of the Purkinje cell layer showing typical train of pulses composed of 4-5 biphasic spikes (A) or 10 triphasic pulses (B).

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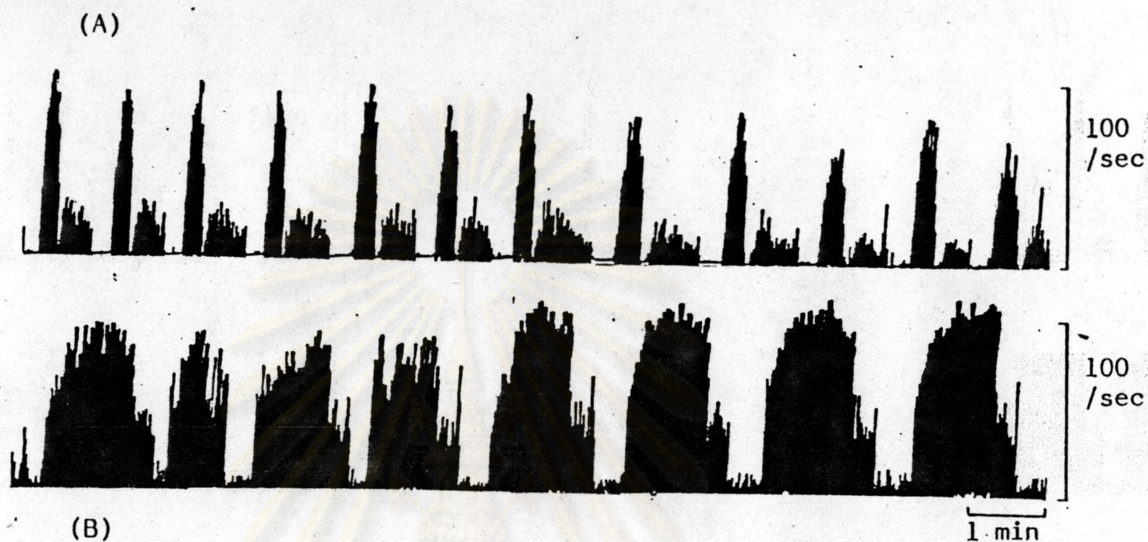

Fig. 16. Ratemeter records of the phasic discharge of two different bioelectric activities (A and B). The discharge usually composed of two different rates of activity which beginning with a high frequency pattern and interrupted with a low frequency pattern (B).

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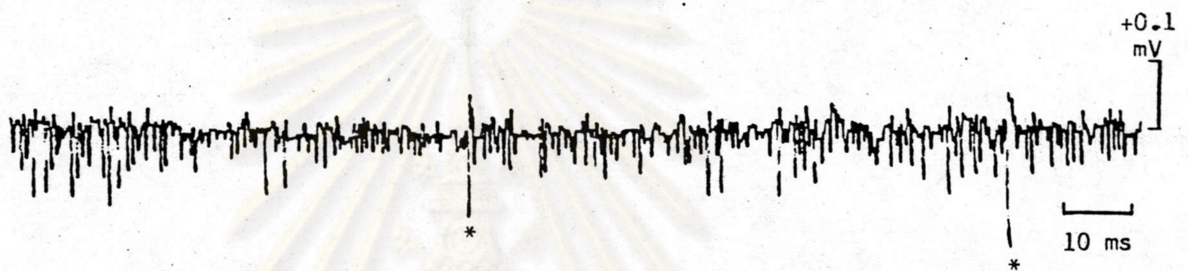

Fig. 17. Background noise recorded in granular layer showing the spontaneous activity of granule cells and some random discharges of a another types of interneuron (*).

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Recording in the granular layer sometimes showed some background activity which might belong to the granule cell. This activity was characterized mainly by negative spike with amplitude of 0.1 mV or less. This spontaneous activity was similar to that described in the previous study by Eccles et al. (1967). This activity usually occurred together with some random discharges of other inhibitory interneuron (Fig. 17).

3. Effects of Temperature.

The effects of temperature change were tested on 10 neurons which demonstrated stable firing rates. During superfusion with cold medium the incubating temperature was continuously monitored at the bubble trapping well (BTW temp.) which situated in close apposition to the incubating chamber. In 8 cells tested, the superfusion with cold ACSF (10°C) depressed spontaneous discharges. While in 2 cases biphasic responses were observed which displayed both an increase at the beginning (when BTW temp. was between 30° and 34°C) and followed by a decrease in firing frequencies (when BTW temp. was about 20°C), see Fig. 18 A. Recovery of discharges was also observed after the original medium was reapplied. The recovery time depended on periods of administration of the cold medium. In Fig. 18 A, when the administration periods

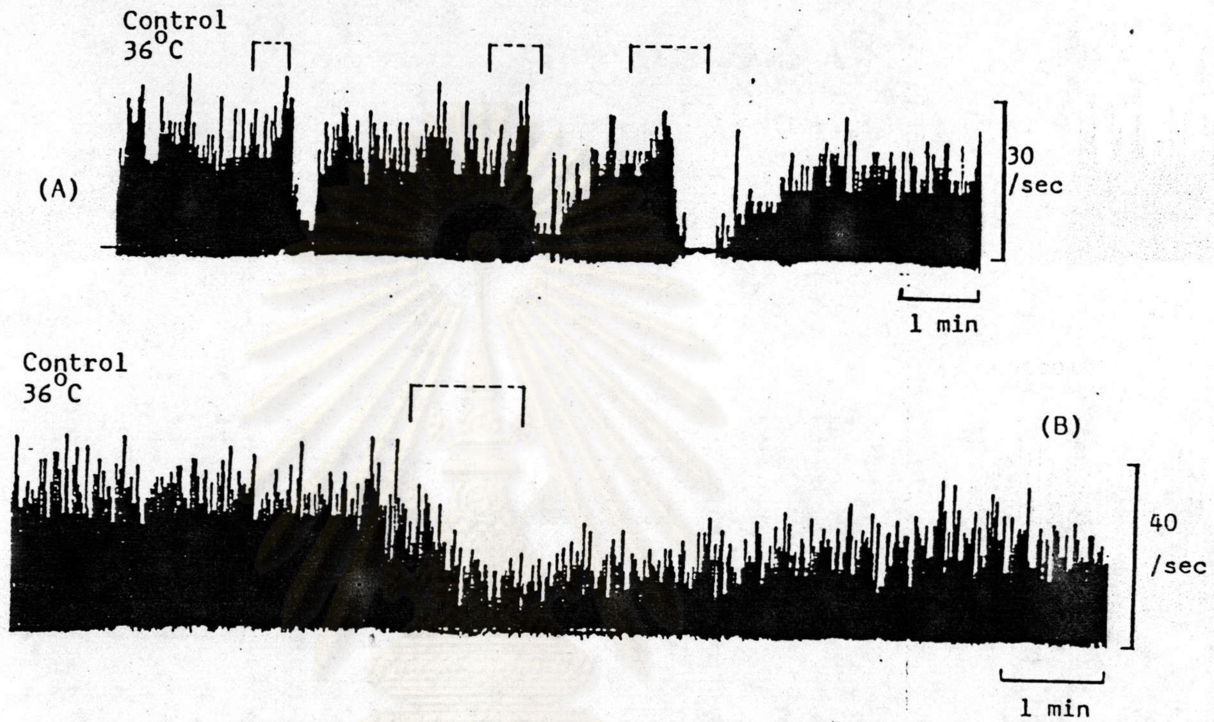

Fig. 18. Ratemeter records of Purkinje neuron responding to the cold ACSF ($10^{o}C$). Upper tracing (A) shows an increase in firing rate at the begining which was followed by a prolonged period of depression. Lower tracing (B) shows the decrease in firing rate in other cell in responding to cold ACSF. (---- = period of cold administration).

was extended from 30 to 40 and 60 sec, longer periods of recovery were observed (10, 30 and 70 sec, respectively). However, in some case as shown in Fig. 18 B, the neuron still actived, though its activity was depressed about 50%, during prolong exposure to cold ACSF applied as long as 90 sec.

4. Effects of Glutamate.

Excitatory action of glutamate on the mammalian Purkinje neurons has been well elucidated since the prior in vivo study by Crawford, Curtis, Voorhoeve and Wilson (1966), and later by Stone (1979). This putative amino acid was also used in investigation of in vitro brain slice. The results showed both excitatory (Okamoto and Quastel, 1973, 1976; Yamamoto, 1973; and Crepel et al., 1982) and inhibitory responses (Yamamoto, Yamashita and Chujo, 1976) according to various types of cerebellar cortical neurons.

In this experiment, glutamate was tested on 21 cortical cells which displayed spontaneous activity in the continuous patterns, as those illustrated in Fig. 13 (n=11) and Fig. 14 (n=10). All neurons which generated the large biphasic spikes (LBS)(Fig. 13; n=11) responded to the glutamate by increasing their firing rates following application by both superfusion (n=6) and microiontophoresis (n=5), whereas the presumptive inhibitory interneurons showed either no

change (Fig. 21; n=8) or decrease (Fig. 22; n=2) in firing rates.

In the perfusion technique, 0.2 mM sodium glutamate incorporated in superfusion medium was employed instead of the control medium, Within 15 to 17 sec after administration, the rate of spontaneous activity of the responsive LBS neurons markedly increased. Subsequently, the firing rates then rapidly dropped to the zero, as shown in Fig. 19 A and 19 B. Such results might be due to the desensitization of glutamate receptors that over prolong exposure to a large amount of glutamate in the superfusate. The neuron become non-responsive through this silent period (about 4.5 min). After replace with control medium, the cell recovered by regaining its spontaneous activity to the same degree as the control level (Fig. 19 A). An overshoot was sometime observed (Fig. 19 B). This biphasic response can be found in all of the LBS cells tested (n=6).

Responses of the same nature on LBS cells could also be demonstrated consistently when glutamate was applied microiontophoretically using negative current less than 30 nA. However, both onset and termination of the responses were relatively shorter and no desensitization was observed. The magnitude of responses was dose-dependent, i.e. the percentage of

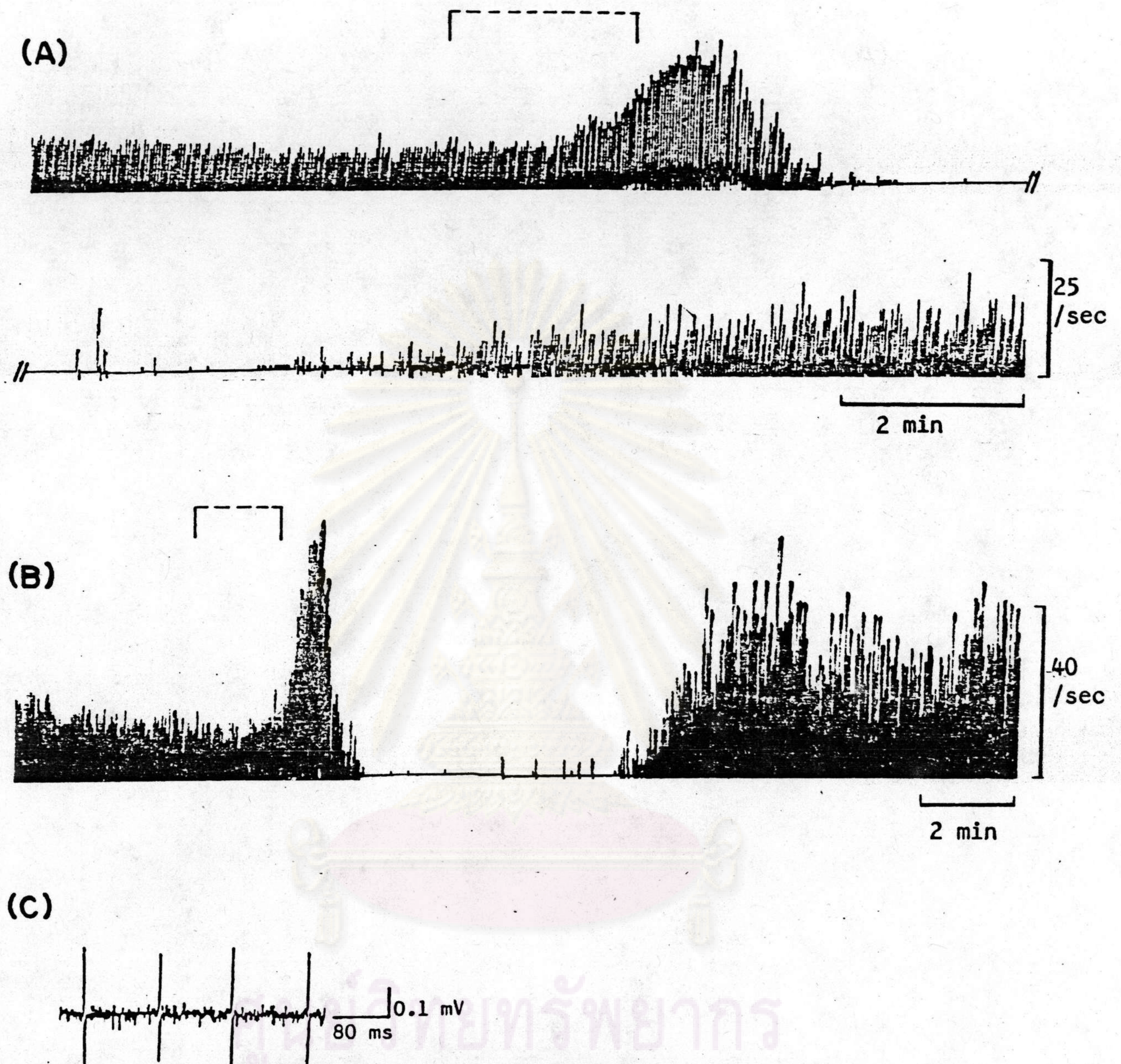

Fig. 19. Ratemeter records (A and B) showing the biphasic responses of the Purkinje cells to bath application of glutamate (0.2 mM two min. superfusion). In C, oscillographic record of the same cell from which the rate meter records were tacken.

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increase in firing rates varied with the magnitude of current applied (Fig 20 B and 20 C). The minimum effective current was between 11 and 13 nA. However, it was found that some cortical cells (n=8) did not respond to the putative glutamate administrated by either superfusion or iontophoresis, as shown in Fig. 21 A and 21 B. In addition, in some neurons (n=2), a single dose of glutamate (20 nA) induced a long period of depression of their discharges, as shown in Fig. 22. The general characteristic of spontaneous activity and glutamate induced responses of these neurons were similar to the prior studies reported by Yamamoto, Yamashita and Chujo (1976).

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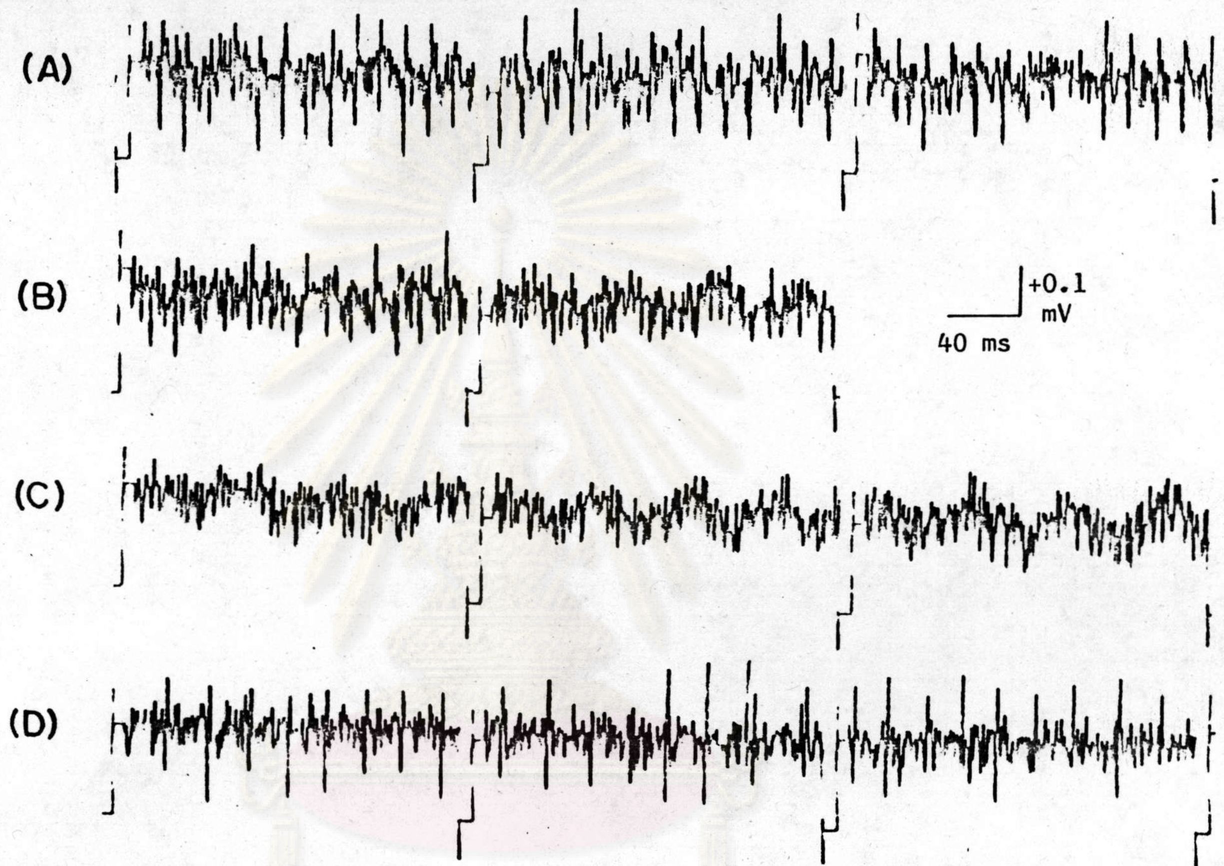

Fig. 22. Oscillograph records showing effects of glutamate induced inhibition of neuronal firing. A : control record. B : during ejection of glutamate (-20 nA). C : 10, 20 and 30 sec after ejection, respectively. D : 1 min after ejection.

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