CHAPTER 4

DISCUSSION

Preliminary description of the design and construction of Oscillotome, as well as viability of the brain slice prepared by this technique has been reported (Taepavarapruk and Tongroach, 1988). The present study describes in addition to investigate the application of brain slice in physiological and pharmacological studies.

General observation on both quantitative and qualitative aspects in the slices cut with either Oscillotome or hand-slicing technique clearly suggested that the former produced slices with superior quality. Of the three common methods for slice preparation, including hand-slicing, chopping and slicing with a vibrating blade, the use of vibrating microtome seems to be most suitable for preparing cerebellar slices (Dingledine et al., 1980; Richards, 1981; Hatton, 1983; Alger et al., 1984). Degree of tissue damage in brain slices prepared by different methods seem to vary considerably. Thus, hippocampal slices cut with the Vibratome showed the damaged area up to about 30 um from the cut edges (Bingmann and Klode, 1982). On the other hand, cerebellar slices cut with the McIlwain tissue chopper showed a damaged region extending up to

80 um from the cut edge of the slice (Garthwaite et al., 1979, 1980), but it seemed to be lower in slices of medulla and pons (less than 50 um) by chopping with the Sorvall chopper (Hinrichsen, 1980). Moreover, with hand-slicing technique, the affected area can be seen within the depth less than 40 um in cerebellar slices (Garthwaite et al., 1979, 1980), and approximately 50 um in neostriatal slices (Bak et al., 1980).

The test for tissue viability in the living slices could be performed by the measuring the occurrence of bioelectric potentials. Spontaneous discharges of the Purkinje cells in slice preparation were rather typical and easy to identify. In such case, the action potential usually has only simple biphasic spike with large amplitude (Okamoto and Quastel, 1973, 1976; Yamamoto, 1973) and continuously generated at firing rates between 10 and 40 /sec (Schlapfer et al., 1972; Okamoto and Sakai. 1981; Basile and Dunwiddie, 1984) which are relatively slower than those reported in <u>in vivo</u> (Eccles et al., 1967; Ito, Sakurai and Tongroach, 1982; Tongroach et al., 1984; Saguanrungsirikul, 1983; Sutthisunk, 1987). Spontaneous activities recorded in the areas not related to Purkinje cells are also observed in this experiment. This belong to other cortical interneurons, e.g., Golgi and granule cells. These spikes, especially those of the Golgi cell, sometimes

looked similar to those generated by Purkinje cell in both the pattern of activity, and regular firing frequency (6 to 30 /sec) (Eccles et al., 1967; Basile and Dunwiddie, 1984). However, they can be easily discriminated from the Purkinje neurons by their triphasic wave form of action potential and extremely regular firing discharges (Basile and Dunwiddie, 1984). Nevertheless, spike potentials of the interneurons in slice preparation may also be found with the simple biphasic pattern.

There were another patterns of discharge encountered in this study, i.e., the bursty and the phasic patterns. The bursty discharge was commonly found in the in vitro preparation (Gahwiler et al., 1972; Schlapfer et al., 1972; Okamoto and Quastel, 1973; Okamoto et al., 1976; Yamamoto, 1973). This discharge pattern in the cerebellum has been previously described by Snider et al. (1967) in the study of isolated cerebellar folia in situ. He has also reported that this intermittent discharge composed of less than thirty spikes in a burst, and was generated by basket cells. The phasic activity, unlike the burst, has longer period of discharge. In this experiment this group of activity was sometimes found in various different patterns according to different individual neurons. This type of activity was not found in any report on cerebellum, but there were some evidence

reported in in vitro study of the hypothalamus, such as that of the supraoptic nucleus slice and the paraventicular nucleus slice (Pittman et al., 1981; Hatton, 1984).

The incubating temperature is one of the most important factor in maintaining tissue metabolism. Most of the cortical neurons stop their activity when the ACSF temperature was lower to 20 ^{o}C, whereas some neurons still remain active. This finding was similar to that of the cerebellar culture preparation by Gahwiler et al. (1972) which has reported the same effects when temperature was lowered between 20^{o} to 10^{o}C. They also found that the lowest level which any discharge was hardly recorded at 5 ^{o}C. The useful application related to this finding concerns the use of low temperature medium to reduce metabolisms of the isolated brain. Many investigators have pointed out in slicing method using the Vibratome that the best slices are obtained from the medium with temperature between 2^{o} and 4^{o}C (Smith, 1970; Clouser, 1977; Cuello and Carson, 1983; and Alger et al., 1984). However, other reports suggested instead that mostly viable tissues are obtained if the temperature is between 4^{o} and 8^{o}C (Alger et al., 1984).

The electrophysiological actions of glutamate have been extensively studied on the cerebellar

Purkinje neurons *in vivo* (Krnjevic and Phillis, 1963; Curtis and Johnson, 1974; Stone, 1979; Ito et al, 1982; Saguanrungsirikul, 1983; Sutthisunk, 1988) and also *in vitro* (Okamoto and Quastel, 1973; Yamamoto, 1973; Crepel et al., 1982). Detailed description has been covered by Ito (1984). This study, therefore, has been aimed at reinvestigating the responses of Purkinje cells in the slice to the glutamate administrated with two different techniques, i.e., microiontophoresis and superfusion.

In general, effect of glutamate applied with both techniques produced the increase in firing rates of Purkinje cell. However, onset time as well as time-to-peak of the cellular responses to the drug was more prolonged when applied by superfusion. Additionally, following prolonged exposure to large amount of the glutamate, desensitization effect of the presumed glutamate receptors occurred as characterized by cessation of the cell firing. Another possible explanation is that the silence was due to membrane hyperpolarization caused by an electrogenic sodium-pump which excreted Na^+ accumulated in the cell under the action of glutamate (Yamamoto, 1973). This effect, however, did not occur when the drug was administrated by iontophoretic method.

In comparison, iontophoresis is clearly superior to superfusion method in its precision of temporal and spatial control over the drug application. The amount of drug released is controlled as a rectangular pulse of ejected current. However, the amount of the drug released must be estimated from strength of the current. In addition, the drug can be applied locally, as a spot, to the target neuron (Tongroach, 1988).

Moreover, the pharmacological test can also discriminated other types of cerebellar cortical neurons that did not show the positive response to glutamate. These are glutamate non-sensitive cell and glutamate inhibited cell (GI). Unfortunately, the former type has not been reported on either study in vivo or in vitro. However, there was an evidence of the GI cell in the guinea-pig cerebellar slice has been presented by Yamamoto, Yamashita and Chujo (1976). They also claimed that the GI neuron should be as large as Golgi cell because it locates in the granular layer and its spike amplitude was about 2 mV. In addition, this cell probably be the Golgi epithelial cell as has been described by Ito (1984).

In conclusion, this study attempts to develop cerebellar slice preparation by using materials available locally and some from discarded instruments. The developmental set-up including the brain slicer machine namely "Oscillotome" and the perfusion system has been proved its validation on electrophysiological as well as pharmacological studies of cerebellar cortical neurons. This indicates that the simple set-up should be useful for the other neurobiological research laboratories and can also be used in comparison with the intact experiment.

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