Chapter I

Introduction

Since the seventeenth century, there have been numerous neurophysiological investigation on the roles which the cerebellum plays in the nervous system functions. Evidence has been accumulating which indicated that cerebellum is an important neuronal machinery responsible for motor activity, motor learning and co-ordination of movement. An animal with cerebellar damage may still initiate and execute movement, but only in a clumsy manner. This clumsiness arises from difficulties in co-ordinating contractions of numerous muscles of various bodily components.

The most important neuron in the cerebellum is the Purkinje cell (P-cells). P-cell receives information through two main distinct afferent channels, the mossy and the climbing fibers whose morphological and functional organization has been extensively investigated.

However, the functional role of the climbing fibers is still far from being understood. The climbing

fiber afferents are a structure unique to the cerebellar cortex; they originate, presumably solely, from the inferior olive (IO), a compact collection of nerve cells alongside the medulla oblongata (Szentagothai and Rajkovits,1959). The climbing fibers formed synapses with the smooth surface of the P-cells dendrites and make an extensive excitatory synaptic contact with them (Eccles, Ito, and Szentagothai, 1967). The importance of the climbing fiber afferents in the cerebellar functions has been emphasised in connection with the learning process which may occur in the cerebellar cortex, and also in connection with the development and maintenance of normal dendritic structure of P-cells (Marr, 1969).

The mossy fiber afferents arise widely from the vestibular system, the reticular formation, cerebal cortex and spinal cord. It ultimately excites many P-cells, but through only a few contacts which each of them. It does not terminate directly on P-cells, as the climbing fibers do, but on small interneurons, the granule cells, which lie immediately under the Purkinje cell layer. Synaptic terminals of the mossy fibers structure, called "rosettes", are commonly found in the granular layer of cerebellar cortex. The granule cells serve as intermediaries, greatly increasing the

number of P-cells stimulated by a single afferent fiber. The axon of the granule cell projects upward, passes the Purkinje cell layer and into the molecular layer. There it splits, the two branches taking diametrically opposite directions, so that the axon assumes the form of a capital T. Fibers representing the horizontal portion of the T occupy all levels of the molecular layer. They are called parallel fibers. The parallel fibers come in contact with the P-cells through spines that emerge in enormous numbers from the terminal regions of the P-cell dendrites, regions called "spiny branchlets". The effects of stimulating the mossy fibers is excitatory (Fig.1 and Fig.2).

Proposal Roles of the Climbing and Mossy Fibers

Marr's hypothesis (Marr, 1969) proposed that the sole function of the climbing fiber synapse on P-cells is to modify the properties of the parallel fiber - Purkinje cell synapse and that this modification is the basis for motor learning in the cerebellum.

Miller and Oscarsson (1970) have suggested the comparator hypothesis of the inferior olive. The digram in Fig.3 is centered around the functional unit, consisting of a sagittal zone with its olivary region

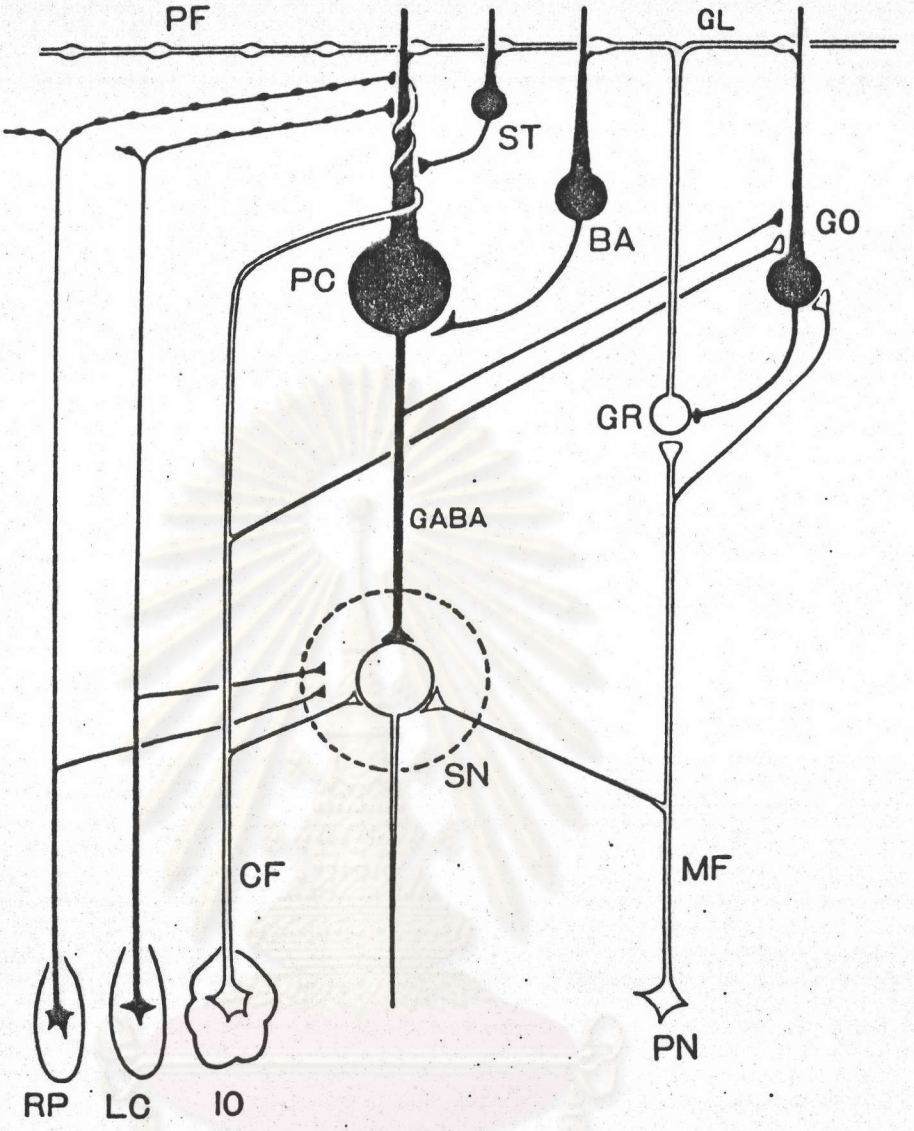

Figure 1. Basic neuronal circuitry and putative neurotransmitters in the cerebellum. PC, Purkinje cell; GO, Golgi cell; BA, basket cell; ST, stellate cell; GR, granule cell; PF, parallel fiber; MF, mossy fiber; CF, climbing fiber; SN, vestibular or cerebellar nucleur cell; PN, precerebellar neuron which issues mossy fiber; IO, Inferior olive; LC, locus coeruleus; RP, raphe nuclei. Inhibitory neurons and synapse are in black, and excitatory ones have been left unfilled , (From Ito, 1984) .

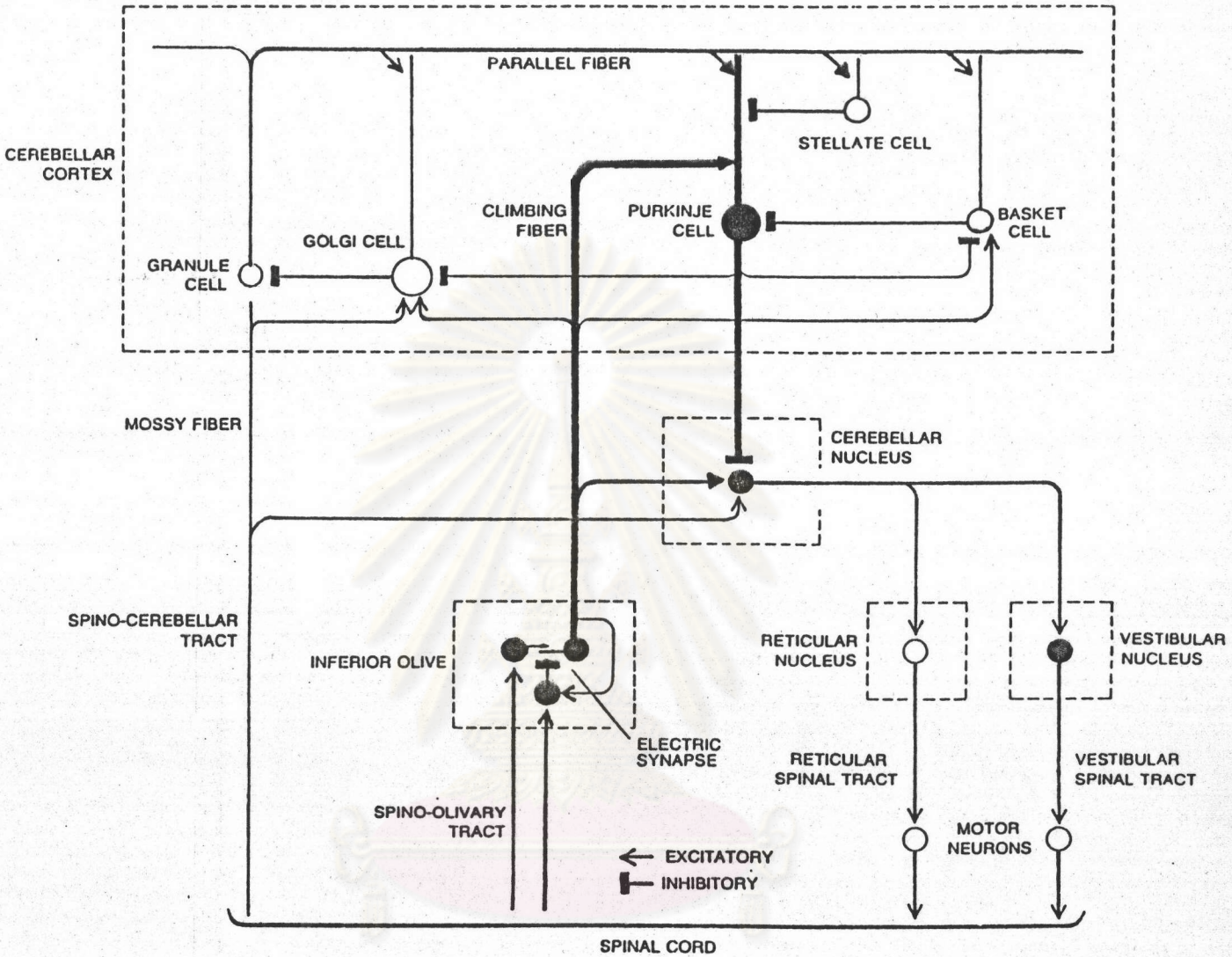

Figure 2. Wiring Diagram of the cerebellar cortex and the brain centers with which it communicates relates the structure of the nerve-cell circuits to their function ,(From Llinas, 1975) .

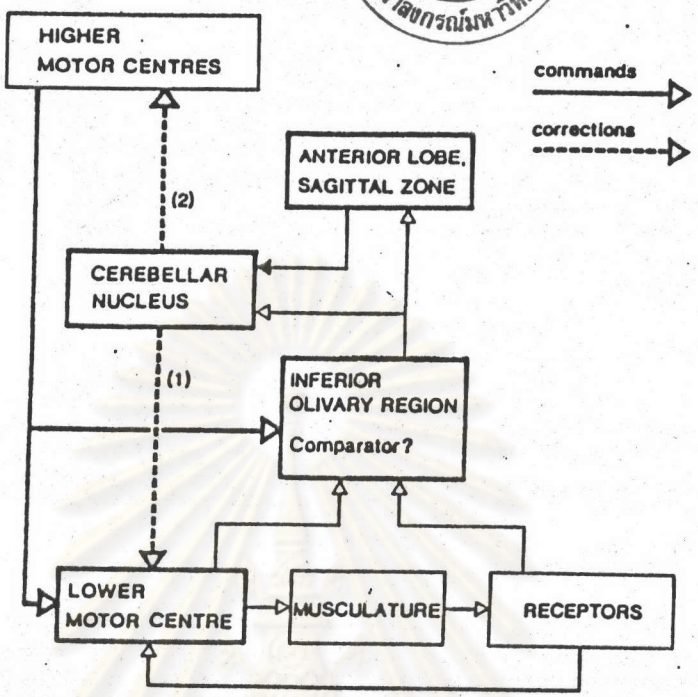

Figure 3. Comparator hypothesis of the inferior olive, (Proposed by Miller and Oscarsson, 1970). The diagram is centered around the functional unit consisting of a sagittal zone with its olivary region and cerebellar nucleus and the lower motor center controlled by this unit (thick outlines). It is assumed that the olivary region monitors commands from higher motor centers, the activity these commands evoke in the lower motor center, and the resulting movement. By comparing information from these sources, the olive would detect perturbations of the commands introduced in the lower center by reflex activity and perturbations in the evolving movement due to unexpected changes in load or resistance. Information about these perturbations might be used by the sagittal zone to send signals of correction either directly to the lower center (path 1) or to the higher centers (path 2). See text.

and cerebellar nucleus, and the motor mechanism controlled by this unit. The motor mechanism would consist of interneurons in the spinal cord or brainstem, in the diagram denoted "lower motor center", which could be a reflex arc, a link in descending motor path, or a collection of neurons responsible for such motor patterns as stepping or scratching. More likely, these interneurons combines all these functions. It is assumed that the olive monitors commands from higher motor center, the activity these commands evoke in the lower center and the resulting movement. By comparing the various pieces of information, the olive might detect perturbation of the command introduced in the lower center by reflex activity and perturbation of the evolving movement due to unexpected changes in load or resistance.

In addition to surgical and electrolytic destruction, 3-acetylpyridine intoxication is now widely used for climbing fiber deafferentation of the cerebellum. 3-Acetylpyridine has a structure much resembling that of nicotinamide. After administration, 3-acetylpyridine adinine dinucleotide is formed because of a lack of specificity of the enzyme for nucleotide synthesis, and this causes destruction of certain nervous tissue (Herken,1968). Treatment of rats

with 3-acetylpyridine results in the total death of the inferior olive neurons (Desclin and Escubi,1973). In order to enhance the selectivesness of the olivary destruction, harmaline may be combined to activate olivary neurons, while a large dose of nicotinamide may protect structure other than the inferior olive (Llinas et al.,1975)

Hamori (1973) proposed that destruction of the inferior olive leads to loss of dendritic spines of P-cells, but Sotelo et al. (1975) maintain that dendritic spines are instead increased in number. Normal expansion of dendritic trees of P-cells is important seriously when the inferior olive is destroyed during the early postnatal stage.

Llinas et al. (1975) have tested Marr's hypothesis. Specific chemical lesion of the rat inferior olive by intraperitoneal administration of 3-acetylpyridine prevents recuperation from motor abnormalities generated by unilateral labyrinthine lesion. Moreover, in animal that have recuperated from the labyrinthine lesion, 3-acetylpyridine produces a reversal of symptoms within 2 hours of administration. These results indicate that the integrity of the olivo-cerebellar system is necessary for the acquisition and

retention of this form of motor learning, but that the cerebellum itself is not the seat of such learning.

Dufosse, Ito and Miyashita (1978), however, reported a rather unexpected form of synaptic action of P-cells on their target neurons in vestibular nuclei. Application of electric pulse to the flocculus normally produces eye movement, presumably by activation of P-cells innervating relay cells for the vestibuloocular reflex. After destruction of the dorsal cap of the inferior olive - the source of climbing fiber to the flocculus - flocculus stimulation no longer evoke eye movement. This observation is difficult to explain only by the loss of climbing fibers and suggests that the inhibitory action of flocculus Purkinje cells is impaired after destruction of the inferior olive.

Subsequent experiments on rat Deiters neurons by Ito, Orlov and Shimoyama (1979) reveal that when the inferior olive is destroyed by administration of 3-acetylpyridine , inhibitory action of P-cells of vermis on Deiters neurons is lost rather rapidly. One day after olivary destruction, inhibitory postsynaptic potentials (IPSPs) induced in Deiters neurons become smaller in amplitude. More than 3 days after destruction, and up to 3 months, the rate of occurence

of IPSPs is substantially reduced. This observation indicates significant reduction in the occurence of vermal inhibition, associated with the prolongation of the IPSP latency in poisoned rats. Montarolo, Raschi and Strata (1981) observed on the contrary that there was no significant change in the occurence of vermal inhibition of extracellular spike discharge of Deiters neurons. A likely explanation for this is that slightly different stimulus parameters were employed by the two groups of investigators.

Ito, Nisimaru and Shibuki (1979) have studied in rabbit Deiters neurons. Reflex discharges in vestibulospinal tract cells were evoked by electrical stimulation of the labyrinth and recorded from the second cervical segment of spinal cord. These vestibulospinal reflex discharge are effectively inhibited by stimulation of vermal Purkinje cells of band B. After electrolytic destruction of the caudal part of the dorsal accessory olive, there is a drastic fall in the cerebellar inhibition. Within 5 hours after an olivary lesion has been made, the reflex inhibition is virtually abolished. Lesions avoiding the caudal part of the dorsal accessory olive do not cause such an effect, indicating that the effect is specific to the olivary area projection to the P- cells concerned

Another control experiment was performed by blocking impulse activity in the inferior olive with tetrodotoxin. Local application of tetrodotoxin in the vicinity of the inferior olive produces only a transient decline in the cerebellar inhibitory action. This indicates that the effect of olivary lesion is not due to blockade of olivocerebellar impules.

According to Ito, Nisimaru and Shibuki (1979), destruction of the inferior olive impairs neither the electrical excitability of P-cell axons nor impulse conducting along them. It appears that the effect of destruction of the inferior olive is mediated by a nonimpulse process in climbing fiber afferents and Purkinje cell axons, presumably by a fast axonal flow. Fast axonal flows travel at a velocity of 400 mm/day, fast enough to transfer the effect of olivary destruction to Deiters neurons via P-cells in 1 hour.

What is affected in synapse which P-cell axons supply to Deiters neurons? More than 5 hours after destruction of the inferior olive, the amplitude of IPSPs evoked in Deiters neurons by cerebellar stimulation is reduced to about half and their latent period increased. The fact that even though the impulse

conduction along P-cell axons is not affected, the latency of IPSPs is prolonged strongly points to the possibility that a presynaptic event in P-cell axon terminals is involved in the reduction of the cerebellar inhibition.

Early pharmacological investigations in Deiters neurons indicated that gamma-aminobutyric acid (GABA) is a neurotransmitter of P-cells. Release of GABA to the fourth ventricle was demonstrated after electrical stimulation of cerebellar cortex, representing liberation of GABA from P-cell axon terminals (Obata and Takeda,1969). Iontophoresis application of GABA induces a membrane hyperpolarization in Deiters neurons (Obata et al.,1967). Both the IPSP and GABA potentials have the same reversal potential and accompany an increase in the membrane conductance caused by increased Cl^- permeability (Obata, Takeda, and Shinozaki,1970 ; ten-Bruggencate and Engberg,1971).

Neurochemical measurement revealed that Deiters neurons has a high GABA content, particularly in the dorsal region. The GABA content in the dorsal area of Deiters neurones of cats is reduced by as much as 71 % when the the cerebellar cortex has been removed

chronically (Fonnum and Walberg, 1973). In Purkinje cell degeneration mice, the GABA content in cerebellar nuclei falls by 50 % immediately after the major period of P-cells loss in the cerebellar cortex, whereas no change occured in the GABA content of the cerebellar cortex (Roffler - Tarlov et al.,1979). It may be suggested that a fast axonal flow in olivocerebellar fibers normally supports the mechanisms for producing and releasing GABA in P-cell axons.

Purposal made by electrophysiological investigation, therefore, strongly suggests a decrease in P-cells inhibitory action on their target neurons. Mechanism underlying their dramatic change is still unresolved. The purpose of the present study is therefore to elucidate any possible change in GABA, as well as other amino acids , released from P-cells terminals. A push pull canula has been employed as collection method, whereas a high- performance liquid chromatography (HPLC) with sensitive fluorimetric detection was used for quantitative assay of the amino acids.