# Functional principles and neural mechanisms of the brain studied in the cerebellum

Masao Ito

In each region of the brain, numerous neurons interconnect to form elaborate neuronal networks. Neuronal networks having various structures and functions are combined to form a neural system which is an essential component of brain function. A major methodology of modern neuroscience is to investigate the brain's functional principles and mechanisms through studies of neuronal networks.

The cerebellum is a unique part of the brain comprising about 10% of the total brain volume. Its neuronal network structure is highly uniform, but it is involved in brain's diverse functions. My intention has been to explain this diversity based on unified functional principles and mechanisms of cerebellar neuronal networks and systems.

Five types of neurons are distributed and interconnected in the cerebellar cortex in geometrical regularity as depicted earlier by Cajal (1911). Researchers in the 1960's identified the excitatory and inhibitory nature of synaptic connections and neurotransmitters utilized in the cerebellum. Working in the Department of Physiology, University of Tokyo, Yoshida and I (1964) and Yoshida, Obata and I (1964) found that Purkinje cells, large neurons known for their magnificent dendritic arbor and serving a sole output from the cerebellar cortex, directly supply inhibitory synapses to their target neurons in the medulla and deep interior of the cerebellum. This discovery implying the exclusive inhibitory nature of Purkinje cells was unexpected because it was generally thought at that time that the vast majority of neurons are excitatory with only some neurons extending short axons being specialized for inhibition as discovered by Eccles et al. (1952) in the spinal cord.

Eccles, Ito and Szentágothai summarized the advances in the 1960's in the monograph "The Cerebellum as a Neuronal Mechine" which drew the attention of theorists interested in elucidating the structure of the brain. Marr (1969) and Albus (1971) formulated thoeries of the cerebellar neuronal networks based on the assumption that Purkinje cells retain synaptic plasticity as a basis for memory and learning functions associated with the cerebellum although at that time this assumption lacked the support of experimental evidence. Synaptic plasticity implies that transmission efficacy at a synapse is modifiable due to its experienced activity. Purkinje cells receive two distinct inputs, one from climbing fibers and the other from parallel fibers. When the two inputs are received simultaneously and repeatedly, the transmission effeicacy from parallel fibers to Purkinje cells is assumed to continually change. Marr assumed that it increases, while Albus assumed that it decreases.

While the synaptic plasticity assumption failed to be supported by results of research conducted in many laboratories. I tried to establish an experimental system for testing memory/learning functions of the cerebellum in a clear-cut manner. Highstein, Fukuda and I (1970) found a direct projection of flocculus Purkinje cells to relay neurons of the vestibuloocular reflex (VOR). Maekawa and Simpson (1973) found a retinal climbing fiber pathway to the flocculus. Gonshor and Melvill-Jones (1976) demonstrated adaptability of the VOR. Based on these findings early in the 1970's. I proposed (1982) the "Flocculus Hypothesis of VOR Control" which holds that by synaptic plasticity driven by climbing fiber signals encoding retinal errors, Purkinje cells adaptively control the VOR. This hypothesis is supported by the abolition of VOR

adaptation after lesioning of the flocculus or its climbing fiber pathway, and by changes in discharge patterns of flocculus Purkinje cells in parallel with VOR adaptation.

Consistency of the flocculus hypothesis with the experimental data implies the existence of the type of synaptic plasticity postulated by Albus in the flocculus. Sakurai, Tongraoch and I (1982) discovered that parallel fiber-mediated responses of Purkinje cells to vestibular nerve stimulation are persistently depressed by conjunctive stimulation of climbing fibers and the vestibular nerve, the first evidence of long-term depression (LTD). We also found that the magnitude of responses of Purkinje cells to iontophoretically applied glutamate was reduced after conjunctive application of glutamate and climbing fiber stimulation, and concluded that LTD is due to persistent reduction of glutamate receptor sensitivity in Purkinje cells.

From around 1990, the laboratories of Crepel (France). Konnerth (Germany) and Linden (USA) also studied LTD, and our understanding of signal transduction processes underlying LTD advanced markedly. At RIKEN to which I moved in 1989, Karachot and I developed a chemical stimulation method to induce LTD in a cerebellar slice by applying substances related to induction of LTD. For example, combined application of cGMP and AMPA induces LTD-equivalent changes. This became a basic method in later studies of LTD. Although there is still much to learn before we understand in detail the roles of second messengers in induction of LTD, it seems certain that at the end of complex reactions involving many receptors and second messengers, phosphorylation causes sensitivity reduction of AMPA-selective glutamate receptors in parallel fiber synapses. Nakazawa et al. (1995) raised an antibody against a peptide indentical to part of the GluR2 subunit of the AMPA recptor covering the 696 serine residue which could be a phosphorylation site. 12P3 labels parallel fiber synapses postsynaptically in cGMP/AMPA-treated cerebellar slices. We are trying to label synapses with this antibody under more natural stimulus conditions, and expect that this method will enable us to visualize memory traces in the cerebellum.

In considering mechanisms of the cerebellum in light of adaptive control theories, I specified a functional unit, the corticonuclear microcomplex (CNMC) as an assembly of a cortical microzome (Oscasson 1979), a nuclear cell group, an inferior olive neuronal group supplying climbing fibers, and a precerebellar nuclear neuronal group supplying mossy fibers. A CNMC can be inserted into any system to afford it adaptability. In the monograph "The Cerebellum and Neural Control" (1984). I developed a concept in which a CNMC connected to a reflex or a compound reaction system acts as an adaptive controller which improves the system's performance by reducing its control errors.

CNMCs are also inserted into the cerebral cortical systems. I proposed in 1970 that a CNMC acts as a model mimicking dynamics of a skeletomuscular system and that the cerebral cortex accurately performs voluntary controls relying on internal feedback through a CNMC model, even without using external feedback. The idea that CNMC is inserted parallel to, and functionally replaces, the cerebral cortex was proposed by Kawato et al. (1987). In this case, a CNMC represents an inverse dynamics model of a skeletomuscular system. Kawano et al. (1995)

reported a case where in the cerebellar ventral paraflocculus involved in eye movement control, Purkinje cells represents inverse dynamics of eyeballs.

The cerebellum has been regarded as a motor center; however, the most lateral part of the human cerebellum evolved in conjunction with the cerebral association cortex. Leiner, Leiner and Dow (1986) proposed its involvement in mental function. I pointed (1993) out the close analogy between movement and thought as control system functions and proposed that the cerebellum works in conjunction with the cerebral cortex to enables us to automatically conduct a thought process which is repeatedly exercised.

Studies of neuronal networks and system should lead to clarification of brain mechanisms of thought and behavior. Further advances in this field require close integration of experimental and thoretical studies.