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Synapse VS. Function: A Central Theme in Physiology

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INTRODUCTION

A living body is composed of innumerable cells. Each cell by itself is already a complex entity displaying a number of essential functions at the microscopic level, but these cells assemble to constitute a complex system which displays a wide range of functions at the macroscopic level of a body. How these microscopic cellular functions and macroscopic bodily functions are interrelated is a question which physiologists have been trying to answer. While our knowledge of these two levels continues to advance, we face a profound question as to how we can integrate them. Since cells assemble with the aid of various interactive processes, it is obvious that these intercellular interactive processes play a key role in generating complex system functions. The central nervous system composed of numerous neurons is typical of a highly complex system. Sherrigton (1906) emphasized that synapses through which neurons are interconnected are the key structures of the central nervous system. Furthermore, Hebb (1949) proposed that memory and learning capabilities of the central nervous system emerge from reorganization of neuron assembly through activity-dependent modification of synaptic transmission. Recently, much effort has been devoted to the investigation of cellular and molecular processes in synapses, but at the same time, various new non-invasive techniques for measuring cognitive activities of human brain have been introduced. Another remarkable progress has been obtained in the field of computational approaches to complex neuronal networks and systems. Time seems ripe for physiologists to challenge to fill the gap between the two levels of synapses and nervous system functions in order to get insight into mechanisms of our brain. This article reviews the recent development in studies on synapse functions, especially long-term depression in the cerebellum and the effort to relate this to learning capabilities of the cerebellum.

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SYNAPSE FUNCTION

Synaptic transmission

A major theme of physiology in the 1950's was specification of a variety of synaptic action. While it was generally thought that the central nervous system contains solely excitatory synapses across which impulses travel, the presence of inhibitory synapses was recognized early in the 1950's (Brock, Coombs and Eccles, 1952). Around 1960, another type of synapses devoted to presynaptic inhibition was found (Frank and Fuortes, 1957; Eccles, Eccles and Magni, 1961; Dudel and Kuffler, 1961), but distribution of the presynaptic inhibition is limited mainly to the spinal cord and thalamus. It is apparent that synapses in

the cerebrum and cerebellum are either excitatory or inhibitory type, which share about equal populations.

Except for electrical transmission at limited sites of the central nervous system, synaptic transmission is mediated by chemical transmitters, which, after being released from presynaptic terminals, diffuse across synaptic clefts and act upon subsynaptic membrane. Since the 1960's, a number of substances in groups of amino acids, peptides and monoamines have been added to the list of putative transmitters in addition to the classic transmitters, i.e., acetylcholine and norepinephrine. The so-called Dale's principle that each neuron release one particular type of transmitters seems to be invalid because there is evidence that two or more different types of transmitters are released from certain synapses.

When acetylcholine is released from motor nerve terminals, a thousand of molecules are discharged in a packet (Fatt and Katz, 1952). This quantal transmission has been shown to apply to central synapses (see Redman, 1990). A presynaptic impulse releases a quantum with a probability of the order of 0.25, which when released, produces unitary excitatory postsynaptic potentials around 200 $f\hat{E}V$ size. It has recently been suggested that the quantal release follows rules of quantum mechanics, and that a mental activity such as volition could be effected by a sudden increase of the probability of quantal release, coherent in a large number of synaptic boutons (Beck and Eccles, 1992).

Postsynaptic receptors are of two major types: ionotropic type associated with ion channels and metabotropic type coupled with metabolic processes. Recently, molecular structures have been identified for a number of receptors, and a new concept has emerged that in the nervous system, one and the same transmitter displays multiple postsynaptic effects by reacting with receptor subtypes of molecular diversity, as typically demonstrated for glutamate receptors (Kutsuwada et al, 1992).

A synapse forms a specialized structure composed of a presynaptic synaptic knob containing synaptic vesicles, a synaptic cleft of 200Å, and a subsynaptic membrane. However, those fibers containing monoamines often do not face subsynaptic membrane. Transmitters released from these fibers diffuse through extracellular fluid of brain tissues to act upon numerous target neurons. This type of transmission is called "volume transmission" as contrasted to the classic synaptical "wiring transmission" (Fux and Agnati, 1991). Volume transmission would play roles in regulating activities of a volume of nervous tisssues, switching operational modes of neural net -works in it to match functional requirements, to repair damaged cells etc.

Synaptic modulation and plasticity

Transmission across a synapse contains a series of chemical steps for synthesis, storage, and release of transmitters, reaccumulation of them into presynaptic terminals by transporters (see Uhl, 1992), and reaction of them with specific postsynaptic receptors. Any cause affecting these steps would modulate the synaptic transmission. A modulator substance is defined as having no direct action to mediate synaptic transmission, yet interfering with transmission by a proper transmitter. A number of peptides and monoamines could act as a modulator rather than as a transmitter.

While modulation in a usual sense is transient and reversible, long-lasting modulation following brief activity is called synaptic plasticity. Activity-dependent synaptic plasticity can arise from various causes acting upon any step of the chemical steps for synaptic transmission. In the 1960's, when posttetanic potentiation lasting only for 10 min was the only known case of such long-long lasting modulation, Brindley (1967) theoretically suggested the presence of ten different types of synaptic plasticity in the central nervous system. Since then, three major types of synaptic plasticity have been discovered.

Sensitization prevails in molluscan ganglia (see Hawkins and Kandel, 1990; Byrne et al, 1991), but this form of synaptic plasticity does not occur in vertebrate nervous system. Long-term potentiation (LTP) was orginally found in the hippocampus (Bliss and Lomo, 1973), but later also in the neocortex (see Teyler et al, 1990; Tsumoto, 1992), and even in the basal ganglia (Calabresi et al, 1992b). LTP has been demonstrated in excitatory synapses, but recently also in inhibitory synapses (Kano et al, 1992; Komatsu and Iwakiri, 1993). Long-term depression (LTD) has been found in the cerebellar cortex (Ito, Sakurai and Tongroach, 1982), but later, another form of LTD has been reported to occur in the hippocampus (Stanton and Sejnowsky, 1989), neocortex (see Tsumoto, 1993; Singer and Artola, 1993), and basal ganglia (Calabresi et al, 1992a).

Synaptic plasticity, at least in its initial phase, is a functional change of transmission efficacy, accompanied by no morphological changes. Morphological changes would follow later, leading to expansion of junctional areas, sprouting of presynaptic terminals, or withdrawal of previously active synapses. Through these morphological changes, functional synaptic plasticity could be converted to a more permanent form.

Morphological changes are essential for the so-called developmental synaptic plasticity displayed during development or recovery from lesions. Developmental plasticity could be preceded by a process similar to the functional plasticity, and it may share common cellular and molecular processes with morphological changes that could follow functional plasticity. Hence, functional and developmental plasticity would overlap each other, but these need to be distinguished from each other, for confusion often arises from equating them.

Long-term potentiation and long-term depression: LTP/LTD

Hippocampal LTP: Pyramidal cells in the hippocampus receive excitatory inputs from numerous (some thousand) presynaptic fibers (Fig. 1A). When a bundle of presynaptic fibers is stimulated with high-frequency pulse trains (for example, bursts with 5 stimuli at 100/sec repeated at 200msec intervals for 2 seconds), long-term potentiation (LTP) takes place in the stimulated synapses (homo-synaptic LTP); the excitatory synaptic potentials generated by the tetanized synapses would be doubled in size. However, when the bundle is stimulated with a low-frequency repetition of single stimuli (5/sec), no LTP is induced. Nevertheless, when the low-frequency stimulation of a testing bundle (AFt in Fig. 1A) is paired with the high-frequency train stimulation of another conditioning bundle (AFc), heterosynaptic LTP takes place in the testing bundle in the manner of association.

Cerebellar LTD: In the cerebellar cortex each Purkinje cell receives two distinct types of excitatory synapses, one from numerous (some 10,000) parallel fibers, axons of granule cells in the cerebellar cortex, and the other from a (normally only one) climbing fiber, axon of an inferior olive neuron located in the of the medulla oblongata (Fig. 1B). LTD occurs when these two types of synapses are activated repeatedly in approximate synchrony, leading to an enduring decrease of synaptic strength of the parallel fibers. LTD is typically induced by conjunctive stimulation at 4/sec for 25 seconds (100 stimuli), which reduces the transmission efficacy by 30% in terms of the magnitude of synaptic potentials induced by stimulation of the parallel fibers (Sakurai, 1987) or by 50~90 percents in terms of the probablity of firing thereby evoked in a Purkinje cell (Ekerot and Kano, 1985). LTD so induced has been followed for 1 - 3 hours without sign of recovery. LTD is input-specific and associative; it occurs only in those parallel fiber synapses activated in conjunction with a climbing fiber impinging on the same Purkinje cell. Activation of parallel fibers alone induces potentiation instead of LTD, and activation of climbing

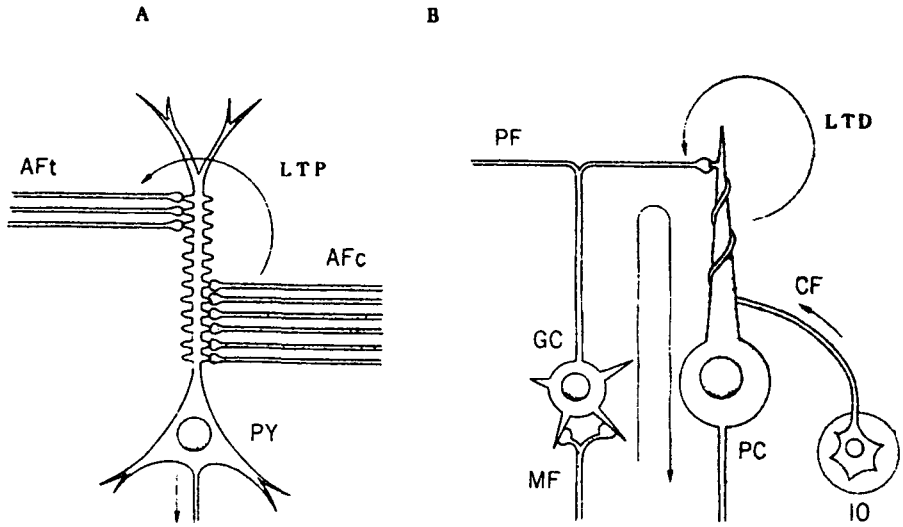

Fig. 1. Occurrence of LTP and LTD. B, LTP. PY, pyramidal cell, Afc, Aft, bundles of preysnaptic fibers for conditioning and testing, respectively. B, cerebellar LTD. PC, Purkinje cell. GC, granule cell. MF, mossy fiber. PF, parallel fiber. CF, climbing fiber. IO, inferior olive. Circles with arrow heads indicate occurrence of associative LTP in A and LTD in B

fibers alone causes neither of these effects.

To constitute a nerve network capable of learning, not only LTD but also potentiation would be necessary. In a theory of the cerebellum, it is assumed that the product of discharge frequencies in parallel fibers and climbing fibers determines the effect of their conjunctive activation (Fujita, 1982). If the product is larger than a certain value, LTD will occur, while if smaller, potentiation will emerge. An experimental counterpart of the postulated potentiation is that when parallel fibers alone are stimulated repetitively, this potentiates parallel fiber-Purkinje cell transmission (Sakurai, 1987). An analysis in cultured Purkinje cells revealed an increased quantal content, which indicates an augmented release of parallel fiber transmitters (Hirano, 1991). Phorbol esters facilitate the release of parallel fiber transmitter (Crepel and Jaillard, 1991a), and hence it is possible that PKC is involved in the anti-LTD potentiation. Whether or not potentiation also occurs on the postsynaptic side in the form of sensitization of AMPA receptors is still unclear.

Hippocampal and neocortical LTD: LTD also occurs in the hippocampal pyramidal cells when the two bundles are stimulated out of phase in the manner of anti-association, each testing stimulus (to b) falling between two successive stimuli (to c). LTD so induced lasted for some hours (Stanton and Sejnowsky, 1989). However, this type of LTD has not been confirmed by other laboratories (Paulsen et al, 1993). In the visual cortex, tetanic stimulation of an optic nerve induces LTP in those synapses supplied by the stimulated optic nerve to cortical cells. The same stimuli, however, induced LTD in those synapses derived from another optic nerve (Tsumoto and Suda, 1979). The occurrence of LTD is less frequent when the nontetanized optic nerve is deprived of spontaneous activity by intraocular injection of tetrodotoxin. Hence, spontaneous activity out of phase with tetanus stimuli seems to facilitate LTD. LTD has also been observed in the sensorimotor cortex (Bindman et al, 1988) and in the prefrontal

cortex (Hirsch and Crepel, 1990). In the neocortical pyramidal cells, whether LTP or LTD is induced depends on the membrane potential, LTD at a less depolarized state and LTP at a more depolarized level (Artola, Borcher and Singer, 1990). Since the intracellular Ca^{2+} concentration would vary according to membrane potential, it has been postulated that the driving force for LTD and LTP is a function of intracellular Ca^{2+} concentration (Tsumoto, 1993; Artola and Singer, 1993). Cellular and molecular processes underling these types of synaptic plasticity have extensively studied in recent years. Since a number of review articles are now available (Tsumoto, 1992; Ito, 1992; Bliss and Collingridge, 1993; Artola and Singer, 1993; Raymond, Blackstone and Huganir, 1993), the following sections will be devoted only to the cerebellar LTD which the author's own interest is centered around.

SIGNAL TRANSDUCTION FOR CEREBELLAR LTD

Events in parallel fiber synapses

Evidence accumulates to indicate that parallel fibers release L-glutamate as trans -mitters, which act upon a particular ionotropic subtype of glutamate receptors selective to AMPA. The transmission is effectively blocked by specific non-NMDA antagonists, CNQX (Hirano, 1990; Konnerth et al, 1990) and by a synthetic analog of Joro spider toxin, 1-acetylnaphthyl sperimin (Ajima et al, 1991). Purkinje cells are also equipped with a metabotropic subtype of glutamate receptors (Sugiyama et al, 1987; Masu et al, 1991). The recently cloned metabotropic glutamate receptor 1 $f\dot{c}$ subtype has been located in the postsynaptic membrane of dendritic spines of Purkinje cells which receive parallel fiber synapses (Gorcs et al, 1993).

AMPA receptors: LTD has been ascribed to reduction of glutamate sensitivity in parallel fiber synapses based for the following reasons. Prominent reduction of glutamate sensitivity in Purkinje cells indeed occurs in the situation mimicking LTD (Ito et al, 1982; Kano and Kato, 1987; Crepel and Krupa, 1988). A quantal analysis in tissue-cultured Purkinje cells revealed that the quantal size for parallel fiber transmitters representing postsynaptic receptor sensitivity was reduced during LTD, while the quantal content indicating the number of quantal units of the transmitter released from parallel fibers was unchanged (Hirano, 1991). The postulated reduction of glutamate sensitivity may occur for various causes. Conversion of activatable receptor molecules to a desensitized form would occur under prolonged action of agonists. Sequesteration or downregulation of glutamate receptors may also lead to reduction of activatable glutamate receptors in number. The fact that LTD is induced when glutamate is repeatedly released from parallel fibers in conjunction with climbing fiber signals favors the view that LTD is, at least initially, due to sustained desensitization of AMPA receptors. The postulated desensitization is such that it does not occur normally, but readily takes place under climbing fiber signals. LTD-like reduction of parallel fiber transmission was observed even when AMPA receptors were not activated (Daniel et al, 1992), but it is uncertain whether this form of reduction accounts for the real LTD. A more recent observation that under the influence of aniracetam, AMPA receptors change their reactivity during LTD, favors the desensitiza-tion hypothesis (Crepel et al, 1994).

Metabotropic glutamate receptors: By recording from Purkinje cells with a grease method, a slow depolarization followed by a hyperpolarization was observed after repetitive stimulation of parallel fibers (Batchelor and Garthwaite, 1993). These potentials were Ca^{2+} dependent and were replicated by perfusion of an exogenous agonist of metabotropic glu -tamate receptors, 1S, 3R-ACPD. Therefore,

parallel fiber signals seem to activate also metabotropic glutamate receptors.

Metabotropic receptors are linked through G -protein to both poduction of diacylglycerol (DAG) and inositol-phospholipid metabolism yielding IP3 . DAG in turn activates protein kinase C (PKC). Since block of metotropic glutamate receptors by MCPG, a specific antagonist, abolishes LTD (Hartell, 1994), activation of metabotropic glutamate receptors should constitute an important step of LTD induction.

Receptors for IP3 are abundant in Purkinje cells, especially in the endoplasmic reticulum which stores Ca^{2+} ions (Furuichi et al, 1989; Ross et al, 1989). Application of an agonist of metabotropic glutamate receptors, t-ACPD, facilitates phosphoinositide turnover in the cerebellum (Hwang et al, 1990). Stimulation of metabotropic glutamate receptors with glutamate or quisqualate leads to enhanced Ca^{2+} levels in the cerebellar cortex even in a Ca^{2+} -free medium (Llano et al, 1991). t-ACPD raises Ca^{2+} levels primarily in the soma but also, to a lesser degree, in a restricted part of the dendrites of Purkinje cells (Vranesic et al, 1991). However, a more recent study reveals that t-ACPD raises Ca^{2+} levels all over the dendritic trees of Purkinje cells (Hartel, N. personal communication).

AMPA responsiveness of Purkinje cells is persistently reduced when a rat cerebellar slice is exposed to quisqualate which excite both AMPA and metabotropic glutamate receptors. A similar reduction of AMPA responsiveness is induced by combined application of AMPA and t-ACPD (Ito and Karachot, 1990b). The quisqualate effects therefore could be due to activation of IP3 metabolism and consequent enhancement of intracellar Ca^{2+} ion levels.

Events induced by climbing fiber signals

A climbing fiber make contact with a Purkinje cell dendrite through numerous synaptic junctions. L-aspartate has been proposed as a likely transmitter for climbing fiber synapses, but more recent evidence does not favor this possibility. L-aspartate-like immuno-reactivity is sparse in climbing fiber terminals (Zhang et al, 1990). L-homocysteate has drawn attention because K^+-induced Ca^{2+}-dependent release from L-homocysteate from cerebellar slices is abolished by destruction of climbing fibers (Vollenweider et al, 1990). L-homo cysteate has therefore been proposed as a putative transmitter of climbing fibers; however, a recent immunohistochemical study has revealed localization of L-homocysteate in Bergmann glia fibers, but not in climbing fibers (Cuenod et al, 1990). A suggestion is then that L-homocysteate is released from glia cells under the influence of a messenger substance produced in climbing fiber-activated Purkinje cells, and that it intensifies action of genuine (yet unknown) climbing fiber transmitters on Purkinje cells.

Climbing fiber transmission is effectively blocked by CNQX (Knopfel et al, 1990a), but not by APV (Perkel et al, 1990). A synthetic analog of Joro spider toxin that blocks parallel fiber- Purkinje cell synapses does not affect climbing fiber synapses (Ajima et al, 1991). Hence, postsynaptic receptors in climbing fiber synapses have an intermediate pharmacological nature between NMDA and non-NMDA glutamate receptors.

Ca^{2+} entry: Climbing fiber-evoked EPSPs lead to generation of Ca^{2+}-dependent spikes and plateau potentials in Purkinje cell dendrites. Climbing fiber-induced enhancement of intracellular Ca^{2+} concentration has been demonstrated with microfluorometric measurements (Knopfel et al, 1990b; Ross et al, 1990; Konnerth et al, 1992). Ca^{2+} concen-tration is enhanced even in the periphery of Purkinje cell dendrites where climbing fiber synapses are absent, presumably due to conduction of Ca^{2+} spikes along dendrites.

Intradendritic injection of a Ca^{2+} chelator, EGTA or BAPTA, abolished LTD (Sakurai, 1990; Konnerth et al, 1992). Perfusion of a cerebellar slice with membrane-soluble Ca^{2+} chelator, BAPTA-AM, effectively abolished quisqualate-induced desensitization of AMPA

receptors (Ito and Karachot, 1990b). Ca^{2+} spikes induced directly by depolarizing membrane of a Purkinje cell lead to sustained reduction of glutamate or AMPA sensitivity when combined with application of glutamate or AMPA (Crepel and Krupa, 1988; Linden et al, 1991). These observations indicate a role of Ca^{2+} in induction of LTD.

Messengers linking parallel fiber and climbing fiber signals

Recent investigations suggest roles of a number of messenger substances in LTD. These messengers are activated by climbing fiber and parallel fiber signals and eventually lead to induction of LTD (Fig. 2).

Nitric Oxide: NO has recently drawn attention as a novel neuronal messenger (Bredt and Snyder, 1992). NO acts to stimulate guanylate cyclase to produce cGMP in cerebellar neurons (Garthwaite et al, 1988). Stimulation of a cerebellar slice causes a transient elevation of NO concentration (Shibuki and Okada, 1991). Since this NO response largely diminishes after 3-acetyl-pyridine destruction of climbing fibers, it is likely to be caused by climbing fiber impulses. A similar conclusion was reached by measurement of cGMP production (Southam and Garthwaite, 1991). Since NO synthase requires Ca^{2+}-activated calmodulin, it is natural to suppose that climbing fiber impulses activate NO production in Purkinje cells via elevation of intradendritic Ca^{2+} concentration. In accordance with this view, LTD as well as quisqualate-induced desensitization is abolished by hemoglobin which absorbs NO, and also by L-NMMA which inhibits production of NO (Ito and Karachot, 1990b; Shibuki and Okada, 1991). Further, sodium nitroprusside, which releases NO in solution, induces LTD when combined with parallel fiber activation (Shibuki and Okada, 1991). It also produces sustained reduction of AMPA sensitivity when combined with AMPA application (Ito and Karachot, 1990b).

However, NO synthase isolated from a cytosolic fraction of cerebellar tissues and cloned has been located immunohistochemically in a granule cell layer (Bredt and Snyder, 1992; Bredt et al, 1991:Ross et al, 1990). Basket cells and some mossy fibers have been labelled, but no evidence is available to support the presence of NO synthase in climbing fibers or Purkinje cells. This negative finding creates a difficulty in explaining the role of NO in LTD unless we

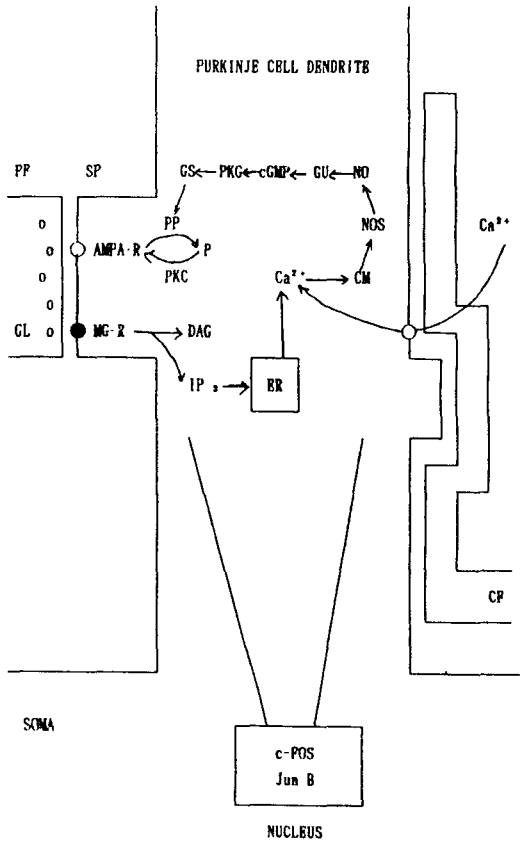

Fig. 2. Cellular and molecular events underlying LTD in Purkinje cells. PD, Purkinje cell dendrite. PF, parallel fibers. CF, climbing fibers. SP, spine. gl, L-glutamate. AMPA-R, AMPA receptor. MG-R, etabotropic glutamate receptors. CM, calmodulin. NOS, NO synthase. GU, guanylate cyclase. GS, G -substrate. PP, protein phosphatase.(modified from Ito and Karachot, 1990a, 1992).

assume a remote interaction between Purkinje cells and other types of cells via a messenger going out of the cell membrane (for example, arachidonic acid). Nevertheless, a possibility remains that a particulate fraction of cerebellar tissues contains another type of NO synthase. The cytochrome P450 reductase exists in Purkinje cells (Bredt et al, 1991), which produces CO instead of NO. Like NO, CO activates guanylate cyclase, but it is insensitive to L-NMMA, and therefore is unlikely to be involved in LTD.

Another complication is that there is no evidence that NO plays a role in the LTD induced in tissue-cultured Purkinje cells (Linden and Connor, 1992). Persistent reduction of AMPA sensitivity was induced by combined application of AMPA and membrane depolarization even under hemoglobin or L -NMMA. Thus, the current state of research pertaining to NO in the cerebellum is complicated, and further information is required before a conclusion can be reached as to the exact site of NO production and its role in LTD.

Guanylate cyclase and cGMP: Guanylate cyclase is located in Purkinje cells, especially in primary dendrites (Ariano et al, 1982). It may be expected that climbing fiber impulses act to enhance the cGMP level in Purkinje cells via elevation of Ca^{2+} and subsequently of NO. Bath application of membrane-soluble cGMP derivatives (Shibuki and Okada, 1991) or intracellular injection of cGMP or its derivative (Daniel et al, 1993; Hartel, 1994) indeed induces LTD when combined with parallel fiber stimulation. Bath application of cGMP derivatives causes sustained desensitization of AMPA receptors when combined with AMPA application (Ito and Karachot, 1990b).

Although earlier studies indicated the presence of cGMP in Purkinje cells, a recent immunohistochemical labeling revealed the abundance of cGMP in Bergmann glia fibers, Purkinje cell somata lacking cGMP (De Vente et al, 1990). Nevertheless, a low level of cGMP has been found also in Purkinje cells (Garthwaite, personal communication).

cGMP-dependent kinase and protein phosphatase: The abundance of cGMP -dependent kinase (PKG) in Purkinje cells has been revealed immunohistochemically (Lohman et al., 1981). A specific inhibitor of cytosolic PKG, KT5823, effectively blocks the quisqualate-induced desensitization of AMPA receptors (Ito and Karachot, 1990b) and also LTD induced by intradendritic injection of cGMP combined with parallel fiber stimulation (Hartel, 1994a).

Purkinje cells also contain a substrate specific to PKG, namely, G-substrate (Detre et al, 1984; Nairn et al, 1985). When phosphorylated by PKG, G-substrate acts to inhibit protein phosphatases 1 and 2A . The phosphorylated G-substrate shares some chemical properties with protein phosphatase 1 in other neural tissues. Involvement of G -substrate in LTD is suggested because inhibitors of protein phosphatases, okadaic acid and calyculin A, induce persistent reduction of AMPA responsiveness of Purkinje cells when combined with AMPA (Ito and Karachot, 1992). Since calyculin A is more potent than okadaic acid, protein phosphatase 1 but not 2A is likely to be involved in accordance with the chemical properties of G-substrate.

It seems that climbing fiber impulses eventually influence AMPA receptors through inhibition of protein phosphatase by shifting the balance between phosphorylation and dephosphorylation of AMPA receptors to phosphorylation (Fig. 2).

Protein Kinase C: Purkinje cells are rich in protein kinase C (PKC) (Kose et al, 1988). Phorbol esters which activate PKC induce desensitization of AMPA receptors and a PKC inhibitor stops LTD (Crepel and Jaillard, 1991a; Crepel and Krupa, 1988; Linden and Connor, 1991). PKC inhibitors also antagonize calyculin A in inducing sustained reduction of AMPA responsiveness of Purkinje cells (Ito and Karachot, 1992).

PKC may interact with guanylate cyclase by

phosphorylation (Crepel and Ardinat, 1991). However, since PKC inhibitors antagonize calyculin A in inducing desensitization of AMPA receptors (Ito and Karachot, 1992), it is probable that PKC acts to phosphorylate AMPA receptors, counterbalancing the dephosphorylating action of protein phosphatases (Fig. 2). In fact, phorbol esters act to reduce AMPA responsiveness of Purkinje cells when combined with AMPA. It is still unclear how PKC is activated in normal situations producing LTD, but a possibility is that activation of metabotropic glutamate receptors in parallel fiber synapses provides a drive to PKC.

Immediate early genes: Certain genes in nerve cell nuclei quickly react with chemical stimuli given to cell surface. It has been thought that a long term phase of synaptic plasticity is effected through turning on or off of certain genes involved in production of receptor and messenger molecules or those regulating synaptic structures. Very recently, combined application of AMPA and a membrane-soluble cGMP derivative has been found to induce expression of both c-Fos and Jun-B genes, which could conjointly form AP1 complex (Nakazawa et al, 1993). AP1 complex, if formed, would modify transcription of certain genes which could be involved in functional or morphological regulation of sy -naptic transmission at parallel fiber synapses (Fig. 2).

LTD VS. CEREBELLAR LEARNING

Hebb (1949) assummed a hypothetical synaptic plasticity, called Hebbian synapse, in order to explain capabilities of memory and learning in the central nervous system. Marr (1969) and Albus (1971) proposed neuronal network models of the cerebellum under an assumption that the cerebellar cortex contains synaptic plasticity as a memory element. The models consist of a self-organizing network like a simple perception (Albus, 1971; Gilbert, 1974) or an adaptive filter (Fujita, 1982). By incorporating such a network, an adaptive control system models have been proposed in order to explain cerebellar function devoted to motor learning (Ito, 1972, 1984; Kawato and Gomi, 1992). The above introduced findings of LTD substantiate the assumption of synaptic plasticity and thereby justify the neuronal network and control system models for the cerebellum. Thus, our knowledge at the synapse level can be linked with that at the system level by the aid of neuronal network and control system models. Furthermore, our knowledge at the synapse level provides new tools for testing validity of the hypothetical models by manipulating synaptic plasticity.

Error-driven adaptation in cerebellar circuitry

The cerebellum consists of numerous functional modules called corticonuclear microcomplexes in each of which a cortical microzone is associated with a small group of neurons in a cerebellar or vestibular nucleus and also with a small group of inferior olive neurons (Fig. 3A). Suppose that in performing a movement, a precerebellar nucleus sends command signals to a nuclear cell group, which in turn pass to a brainstem structure. While the command signals also pass to the microzone, climbing fiber afferents convey signals representing errors in the performance of the system in which the corticonuclear microcomplex is inserted. Climbing error signals would induce LTD in those mossy fiber-parallel fiber pathways to cerebellar Purkinje cells active at the moment of erroneous performance. These pathways would be depressed so that signal transfer via the microzone to the nuclear cell group would be modified, and as a consequence, the performance of the system would be improved toward minimization of errors.

Motor adaptation

As a typical example of corticonuclear

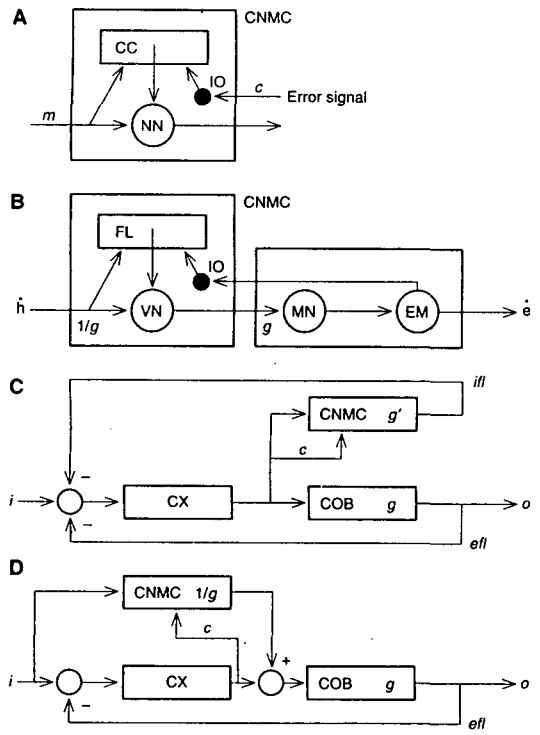

Fig. 3. *Models postulated for the control theories of the cerebellum. A, corticonuclear microcomplex (CNMC). m, mossy fiber pathway. c, climbing fiber pathway. CC, cerebellar cortex. NN, nuclear neuron. IO, inferior olive. B, adaptive control system for VOR. FL, flocculus. VN, vestibular relay nucleus MN motoneuron. EM, eye muscle. h, head velocity. e, eye velocity. C, "dynamics" control system model for voluntary movement. CX, motor cortex. COB, control object. i, input. o, output. efl, external feedback loop. ifl, internal feedback loop. D, "inverse dynamic" model control system for voluntary movement and also thought (1993b)*

microcomplexes, the cerebellar flocculus combined with a group of vestibular relay neurons is inserted to the vestibuloocular reflex (VOR) arc (Ito et al, 1971). The flocculus hypothesis of VOR control thus proposes that the flocculus as a modifiable sidepath enables the VOR to be conducted accurately in spite of the fact that VOR lacks feedback (Ito, 1972) (Fig. 3B). Supportive evidence for this model has been accumulated by lesioning the flocculus and also by recording from flocculus Purkinje cells (Ito, 1982, 1989). The above-described recent results on LTD provides a new tool for testing involvement of LTD in motor learning. Subdural injection of 0.1 ml of 5 fEV hemoglobin to the flocculus abolished adaptation of VOR without affecting dynamic charac teristics of the oculomotor system (Nagao and Ito, 1991). In passing, it is noted that injection of a fA-noradrenergic agnist, isopretenol, into rabbit's flocculus facilitates adaptation of VOR, while that of fA antagonist, sotalol, depressed the adaptation (van Neerven et al, 1990). Even though it is unclear how these drugs interfere with LTD, the results support the view that the flocculus plays an essential role in the VOR adaptation. Even though another synaptic plasticity is claimed to exist in vestibular relay cells (Lisberger and Sejnowsky, 1992), it should be a process subsidiary to Purkinje cell LTD (Ito, 1993a).

A similar scheme of adaptive feedforward control may apply to compound reactions such as posture, locomotion, and saccadic eye movement (see Ito, 1984) and classical conditioning of eye blink reflex (Thompson, 1987). A decerebrated cat walking on a treadmill can accomodate to a sudden change of belt velocity for one forelimb, and this accommodation is abolished by applying hemoglobin or L-NMMA to the forelimb area of the vermis (Yanagihara, 1993, 1994).

Learning in voluntary movement

Voluntary control of arm movement would be assisted likewise by the cerebellum (Gilbert and Thach, 1977). Since the paravermis of the cerebellum forms a loop pathway receiving command signals from the motor cortex and sending back its output signals to the motor cortex, the paravermis may form an internal feedback loop for operation of the motor cortex (Fig. 3C). While exercise is performed by the motor cortex by referring to visual and

other sensory feedback, the para-vermal circuitry could be adjusted so as to bear the dynamics of the skeletomuscular system of a limb to be controlled. The voluntary motor commands would act on a "dynamics" model formed in the paravermis, instead of acting on the actual skeletomuscular system, so that the external feedback loop is replaced by the internal loop through the cerebellar "dynamics" model (Ito, 1970, 1982). In this model, practice of a movement is a process for building and adjusting the dynamics" model in the cerebellum.

However, other areas of the cerebral and cerebellar cortices are connected in a parallel fashion, instead of a loop fashion. Therefore, another model assumes that the cerebellum forms a feedforward controller acting in parallel with a feedback controller of the cerebral cortex on the same control object (Kawato et al, 1987). The cerebellum would thus control a limb in a feedforward fashion, in place of the cerebral cortex operating in a feedback fasfion (Fig. 3D). When a feed-forward control system reproduces an arm trajectory equivalent to the instructed trajectory, the controller should bear a dynamics inversely equal to the dynamics of the skeletomuscular system of the limb (CNMC vs COB in Fig. 3D). Hence, after learning, the cerebellum should bear an inverse dynamics of the skeletomotor system of a limb.

"Dynamics" memory and learning

What is learnt in the above models of arm control, is the dynamics or inverse dynamics, instead of individual arm trajectory actually practiced. Kawato et al.'s (1987) simulation study demonstrated that after learning for a particular trajectory in the manner of Fig. 3C and D, a robot displays accurate smooth formation arm trajectory in any direction. Hence, a term "dynamics" learning is proposed for expressing this manner of learning. The cerebellar circuitry therefore retains "dynamics" memory but not memory of individual trajectories.The finger-nose test for cerebellar patients can be understood as due to loss of "dynamics" memory of the arm, without which precise arm trajectory formation in a feed -forward mode with eyes closed becomes impossible.

A study with positron emission tomography revealed an increase of regional blood circulation in cerebellar nuclei during brisk right-handed finger to thumb opposition with each digit in turn (Friston et al, 1992). The increase, apparently representing physiological activation, diminished when the trials were repeated. This observation could be interpreted as indicating that activation of cerebellar tissues is needed during formulation of a cerebellar model but not during maintenance of it.

The above scheme of the inverse dynamics model has been adopted to explain characteristic discharge patterns of Purkinje cells in monkey's ventral paraflocculus, which were found to match the expectation from the inverse dynamics of eyes (Komatsu et al, 1993).

Possible involvement of the cerebellum in the thought

Leiner et al. (1986) pointed out that the lateralmost part of human cerebellum has evolved in parallel with development of the cerebral association cortex which plays roles in mental function. In fact, lesions in the lateralmost part of the cerebellum do not yield motor symptoms. The cerebellum may endow mental dexterity just as motor dexterity (see also Leiner et al, 1989, 1991, 1993).

In developing a control system model of the cerebellum as mentioned above, the author pointed out the similarity of the thought to movement. In thought, ideas and concepts are manipulated just as limbs in a movement. Therefore, the adaptive control system model developed for explaining cerebellar motor learning may also apply to control of the thought (Ito 1990, 1993). From this viewpoint, control principles for movement and for thought are essentially of similar nature, once

movement and thought are represented in neuronal circuitry of the brain.

Since the hemipshere-dendate division of the cerebellum is connected to the cerebral association cortex, the inverse dynamic model (Fig. 3D) would apply to the thought control. What is an inverse dynamics model of a concept? Its concrete form will not be clear unless we know representation of a concept in the brain. Once we know how a concept is encoded within the neuronal network of the association cortex, one may figure out how it is encoded in the cerebellar neuronal circuitry in a reversed relationship.

Recent studies using positron emission tomography revealed that local blood circulation in the cerebellum is enhanced during a certain type of language processing, converting a noun to a verb with a related meaning (Petersen et al, 1990). Further, a patient with a hemispheric infarct exhibited larger chances of errors in noun-to-verb conversion, and the reaction time or the occurrence of errros was not reduced during repeated trials as contrasted to clear learning effect demonstrated in normal subjects (Fiez et al, 1992).

Involvement of the cerebellum in mental activity has thus become an important theme of clinical interest. A decrease of the size in the cerebellar hemispheres and vermal lobules VI through VII is associated with autism (Murakami et al, 1989). Complex behaviors requiring visual-spatial organization and planning and programming of daily activities are impaired in cerebellar patients (Boetz et al, 1989). The variability in performing rythmic tapping was increased in hemispheric lesions, apparently due to deficit in the internal timing system to determine when to make a response (Ivry et al, 1987). Further investigation is needed, but the presently proposed cerebellar thought control system could be a useful concept for understanding clinical features of cerebellar hemispheric lesions.

COMMENTS

An important theme of physiology is to reproduce complex system functions of the body based on our knowledge of cellular and molecular processes in tissues. In this article, the attempt to reproduce motor learning based on knowledge of LTD in the cerebellum is introduced. Adaptive control system models are proposed for reflexes, compound reactions, and voluntary movement control, and are expanded even to problems of mental control in human brain. Similar approach could be applied to other major nervous system functions such as affect, will, and consciousness. These functions could also be reproduced based on our knowledge of synaptic mechanisms if appropriate neuronal network and system models are introduced. To achieve this goal, it is essential to integrate heterogeneous ideas and concepts at synapse and system levels, which is an important task of of future physiology.

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