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LONG-TERM DEPRESSION

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INTRODUCTION

The term "long-term depression (LTD)" may apply to any form of long lasting depression in synaptic transmission. For example, lowering of transmission efficacy at the dentate area occurs following a low-frequency repetitive stimulation and is called long-term depression (Bramham & Srebro 1987). However, the scope of this article is limited to a special type of LTD that is present in the cerebellar cortex and is now regarded as a memory element for cerebellar motor learning.

Purkinje cells in the cerebellar cortex are supplied two distinct types of excitatory synapses, one from parallel fibers (axons of granule cells) and the other from climbing fibers (axons of inferior olive neurons). Repetition of nearly simultaneous arrival of signals form some parallel fibers and a climbing fiber to a Purkinje cell leads to a long-lasting depression of transmission from the parallel fibers to the Purkinje cell, i.e. LTD. Such synaptic plasticity was suggested to exist around 1970, when dissection of the neuronal network structure of the cerebellum, as summarized by Eccles, Ito & Szentágothai (1967), provoked theoretical considerations of the above-mentioned dual synaptic inputs to Purkinje cells. Whereas Brindley (1964), Marr (1969), and Grossberg (1969) suggested a potentiation of transmission to occur in the parallel fiber-Purkinje cell synapses conjunctively activated with a climbing fiber, Albus (1971) assumed a depression. Early studies aiming at experimental verification of these theoretical possibilities failed for some technical reasons, except for a few studies in which movement-related activity of Purkinje cells provided a support, though indirect, for Albus' view (Gilbert & Thach 1977, Ito 1977). A decade had passed before clear experimental evidence for LTD became available (Ito et al 1982).

LTD constitutes the four major types of synaptic plasticity together

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with long-term potentiation (LTP) (cf Landfield & Deadwyler 1988), sensitization (cf Byrne 1987), and sprouting of axon terminals (cf Tsukahara 1981). It is interesting to note that LTD is an addition to the ten types of synaptic plasticity that Brindley (1967) postulated to exist in nervous systems, including LTP and sensitization. Recent efforts have been devoted to extend the scope of LTD studies in two directions. Cellular and molecular mechanisms of LTD are investigated with both in vivo and in vitro cerebellar preparations, and the role of LTD as a memory element of the cerebellum is studied with neuronal circuitry dissection, lesion experiments, and recording from Purkinje cells in alert behaving animals. This article summarizes the outcomes of these two lines of recent investigations.

CHARACTERIZATION OF LONG-TERM DEPRESSION

Experimental Conditions for Induction and Detection

LTD can be induced by conjunctive stimulation of climbing fibers and parallel fibers, but not by stimulation of climbing fibers or parallel fibers alone. In studies of the cerebellar flocculus of nonanesthetized decerebrate rabbits, climbing fibers were stimulated at their site of origin in the inferior olive, whereas parallel fibers were stimulated indirectly by electrical stimulation of a vestibular nerve that projects mossy fiber afferents to the flocculus (Ito et al 1982). At the exposed cerebellar surface, parallel fibers were stimulated directly through a microelectrode inserted into the cortex (Ito & Kano 1982, Ekerot & Kano 1985). In in vitro slice preparations of guinea pig cerebellum, climbing fibers were stimulated with electrodes placed at the white matter and parallel fibers were stimulated with electrodes at the pial surface. Even though parallel fibers in slices were cut short at 300 μm length, pial stimulation effectively induced synaptic transmission from parallel fibers to Purkinje cells (Sakurai 1987).

The combined stimulation of climbing fibers and mossy or parallel fibers was repeated at 1–4 Hz, the normal range of discharge in climbing fibers, for 25 s to 10 min. Usually, climbing fiber stimulation was timed so as to precede mossy fiber or parallel fiber stimulation by 10 ms, but the timing of climbing fiber and parallel fiber stimulations has been found to be allowed a relatively wide latitude. Stimulation of parallel fibers during the period between 20 ms prior and 150 ms subsequent to the stimulation of climbing fibers is nearly equally potent in inducing LTD in terms of the probability of occurrence of LTD among trials (Ekerot & Kano 1985, 1988). This contrasts to the critical timing theoretically postulated (Marr 1969), and suggests that LTD depends on some variable factor such as enhanced correlation between the firing of parallel fibers and that of

climbing fibers, as assumed in Fujita's (1982a) theory. The idea of critical timing of collision with parallel fiber discharges seems also to be unrealistic in view of the characteristically slow, irregular pattern of climbing fiber discharge (see Ito 1984).

LTD is detected by recording responses of Purkinje cells to stimulation of the parallel fibers or mossy fibers involved in a conjunctive stimulation. In in vivo preparations, extracellular unit spikes are recorded from a Purkinje cell responding to parallel fiber or mossy fiber stimualtion. Only in vitro preparations allow stable intracellular recording from a soma or dendritic shaft of a Purkinje cell. Mass field potentials evoked in the molecular layer of the cerebellar cortex by stimulation of parallel fibers have a component representing postsynaptic excitation in cerebellar cortical neurons. This component, however, was reduced only by 20% at the most, and often the reduction was undetectable, even when prominent LTD occurred in unit spike recording from individual Purkinje cells (Ito & Kano 1982). This probably is because 20 times as many non-Purkinje cells (stellate cells and basket cells) as Purkinje cells are found in the molecular layer of the cerebellar cortex (see Ito 1984). Mass field potentials generated by these non-Purkinje cells would mask the LTD that should happen in the mass field potentials of Purkinje cell origin. Ineffectiveness of mass field potential recording makes studies of LTD relatively difficult, as contrasted to hippocampal LTP which is well represented by mass field potentials (Bliss & Lømo 1973).

Time Course and Magnitude

LTD consists of an initial phase lasting for about 10 min and a subsequent later phase. Determining the whole time course of LTD is still difficult because of technical difficulties inherent in continuous recording of extracellular unit spikes and intracellular postsynaptic potentials. With extracellular unit spike recording, 1 hr is usually the limit for stable, reliable recording, but in some cases, LTD was followed with reasonable stabiltity for 3 hr (M. Kano and M. Kato, personal communication). Intradendritic recording in slice preparations can hardly be continued stably for more than 1 hr, but it was noted that the depression frequently remained without appreciable recovery at 1 hr. An attempt has been made to follow the time course of LTD by means of a reflex testing (M. Ito and L. Karachot, unpublished). LTD occurring in a mossy fiber-parallel fiber-Purkinje cell pathway was tested by measuring a mossy fiber-evoked Purkinje cell inhibition on a vestibular nerve-evoked excitation of Deiters neurons with extracellular recording of mass field potentials in the nucleus of Deiters. LTD was followed in this way for up to 3 hr without appreciable recovery.

The magnitude of depression at the late phase of LTD is 40% in terms

of the firing index of Purkinje cell responses to 2 Hz stimulation of mossy fibers (Ito et al 1982). With direct stimulation of parallel fibers, the depression often amounted to 90% (Ekerot & Kano 1985). With intradendritic recording in slice preparations, the parallel fiber-evoked excitatory postsynaptic potentials (EPSPs) were reduced in their peak size by 10–50%, 30% on the average (Sakurai 1987).

Complications

It is remarkable that postsynaptic inhibition of Purkinje cell dendrites during conjunctive parallel fiber-climbing fiber stimulation prevents LTD from taking place, for the reason mentioned below (Ekerot & Kano 1985). If a strong stimulation of mossy fibers or parallel fibers is used for conjunctive stimulation with climbing fibers, LTD fails to occur because strong stimulation induces postsynaptic inhibition through the parallel fiber-stellate cell pathway. This may account, at least partly, for the earlier failure in detecting LTD. In an in vitro experiment, this complication was avoided by blocking stellate cell inhibition with picrotoxin (Sakurai 1987). These findings resemble those for LTP in the cerebral neocortex, where abundant postsynaptic inhibition appears to mask the occurrence of LTP (Artola & Singer 1987). No LTD was reported to occur in a cerebellum irrigated with artificial solution (Llinás et al 1981). The reason for this failure is still not clear, but stellate cell inhibition is a factor to be carefully examined as a possible cause.

Do climbing fiber signals act only for induction of LTD or do they play multiple roles? One role might be facilitation of parallel fiber-evoked responses (either excitation or inhibition) in Purkinje cells, which occurs when parallel fiber stimulation is combined with spontaneous complex spikes at 20–50 ms intervals (Ebner & Bloedel 1984). This facilitatory effect, however, is short-lasting, and should be a phenomenon independent of LTD. Another example of multiple climbing fiber roles is a membrane hyperpolarization that follows depolarizing responses of Purkinje cell dendrites to climbing fiber signals (Hounsgaard & Midtgaard 1985, Sakurai 1987). This hyperpolarization summates to a long-lasting membrane hyperpolarization when climbing fibers are stimulated repetitively. It probably underlies the prolonged silence occurring in unit spike activities of Purkinje cells after stimulation of climbing fibers (Rawson & Tilokskulchai 1981). This silence lasts only for a few minutes, however, and hence would not contribute to the major phase of LTD.

The prominent increase of simple spike discharge that follows destruction of the inferior olive (Colin et al 1980) is presumably due to removal of the membrane hyperpolarization built in Purkinje cell dendrites by spontaneous climbing fiber signals. The increase of simple spike discharge

diminished gradually in two weeks after destruction of the inferior olive (Benedetti et al 1984, Batini & Billard 1985). Climbing fiber destruction also induced reduction in the inhibitory action of Purkinje cells on Deiters neurons (Ito et al 1979). This reduction, outlasting the increased simple spike discharge (Lopiano & Savio 1986, Karachot et al 1987), would suggest another role of climbing fibers in remote trophic regulation of Purkinje cell axon terminals.

MOLECULAR MECHANISMS OF LONG-TERM DEPRESSION

Clear evidence is now available for involvement of Ca^{2+} ions and quisqualate-specific glutamate receptors in induction of LTD.

Ca^{2+} Ions

Climbing fiber impulses evoke a large dendritic spike potential followed by a plateau potential in Purkinje cell dendrites (Ekerot & Oscarsson 1981). These potentials represent inflow of Ca^{2+} ions into Purkinje cell dendrites (Stöckle & ten Bruggencate 1980, Llinás & Sugimori 1980). Postsynaptic inhibition produced in Purkinje cell dendrites through stellate cells effectively depresses this Ca^{2+} inflow (Campbell et al 1983). Involvement of Ca^{2+} ions in induction of LTD was initially suggested by the observation that stellate cell inhibition prevented LTD from occurring (Ekerot & Kano 1985). More direct evidence has recently been provided by injection of EGTA, a Ca^{2+} chelator, into Purkinje cell dendrites in slice preparations, which effectively abolished LTD (Sakurai 1988). This property resembles that of LTP, which is also blocked by injection of EGTA into dendrites of hippocampal pyramidal cells (Lynch et al 1983). A common role of Ca^{2+} ions may be suggested in both the LTD and LTP, even though the direction of the synaptic efficacy change is opposite in them.

Glutamate Receptors

Accumulating evidence indicates that the neurotransmitter released at parallel fiber-Purkinje cell synapses is L-glutamate (for references, see Ito 1984). A recent immunohistochemical labeling revealed enrichment of glutamate at parallel fiber synapses (Somogyi et al 1986), and a pharmacological study indicated a quisqualate-specific nature of postsynaptic receptors at parallel fiber-Purkinje cell synapses (Kano et al 1988).

Involvement of L-glutamate receptors in induction of LTD was first indicated with iontophoretic application of L-glutamate to a Purkinje cell through a microelectrode. Conjunctive application of L-glutamate with

electrical stimulation of climbing fibers at 4 Hz effectively induced long-lasting depression in glutamate sensitivity of that Purkinje cell tested with the same microelectrode (Ito et al 1982). The time course of this depression resembled that of LTD. This effect was not produced by application of L-glutamate or stimulation of climbing fibers alone. It is suggested that the LTD is essentially due to long-lasting lowering of glutamate sensitivity of Purkinje cells.

When L-glutamate was applied iontophoretically to a dendritic region of a Purkinje cell in conjunction with climbing fiber stimulation, synaptic transmission to the Purkinje cell from those parallel fibers passing through that dendritic region exhibited a long-lasting depression equivalent to LTD in both time course and magnitude (Kano & Kato 1987, 1988). This implies that the presence of L-glutamate in synaptic regions, either released from parallel fibers or applied iontophoretically, is necessary for induction of LTD. A similar effect was induced with iontophoretic application of quisqualate to a dendritic region, in place of L-glutamate, but with neither kainate nor aspartate (Kano & Kato 1987, 1988). This implies that quisqualate-specific glutamate receptors that mediate parallel fiber-Purkinje cell transmission are specifically involved in LTD.

Thus, LTD is presumed to be due to a reduction of sensitivity of quisqualate-specific glutamate receptors that occurs under the presence of L-glutamate in a synaptic region. Furthermore, when the excitatory action of iontophoretically applied quisqualate on Purkinje cells was blocked by simultaneous iontophoretic application of a glutamate antagonist, kynurenate, conjunctive stimulation of climbing fibers failed to induce a long-lasting depression of parallel fiber-Purkinje cell transmission (Kano & Kato 1988). This means that for eliciting LTD, L-glutamate or its agonist must react with quisqualate-specific receptors.

Taken together, these results indicate that LTD is due to desensitization of quisqualate-specific glutamate receptors at parallel fiber-Purkinje cell synapses. The glutamate receptor desensitization postulated in Purkinje cells contrasts to the sensitization of glutamate receptors suggested to underlie LTP in the hippocampus (Lynch & Baudry 1984). Testing of glutamate sensitivity of Purkinje cells may be recommended for detecting the postulated desensitization of glutamate receptors underlying LTD. However, selective testing of glutamate sensitivity only in those parallel fiber-Purkinje cell synapses involved in conjunctive stimulation with climbing fibers is technically unrealistic, because iontophoretically applied glutamate for this testing would diffuse to excite also those synapses not involved in the conjunctive stimulation and even extrasynaptic glutamate receptors. Therefore, no attempt has been made to test glutamate sensitivity of parallel fiber-Purkinje cell synapses undergoing LTD.

Molecular Process that Links Ca^{2+} Inflow to Desensitization of Glutamate Receptors

At present, no evidence suggests a particular molecular process that, following Ca^{2+} inflow, leads to desensitization of glutamate receptors in Purkinje cells. However, several possibilities may be raised on the basis of current knowledge of synaptic transmission.

One possibility is that the increased Ca^{2+} ions may directly facilitate desensitization of L-glutamate receptors. An analogy can be drawn from desensitization of acetylcholine receptors at neuromuscular junctions that is facilitated by Ca^{2+} ions (Miledi 1980). A biochemical binding study also demonstrates that elevated Ca^{2+} concentration facilitates desensitization of acetylcholine receptors (Changeux & Heidman 1987). The time course of the LTD can be simulated with kinetics data of Ca^{2+}-facilitated desensitization of acetylcholine receptor molecules. However, no data as yet suggest a facilitatory action of Ca^{2+} ions on glutamate receptor desensitization.

A second possibility is that Ca^{2+} ions facilitate glutamate receptor desensitization via a second messenger process. Since cGMP and cGMP-dependent protein kinase are specifically contained in Purkinje cells (Lohmann et al 1981), a role of cGMP in the LTD may be suggested. Ca^{2+} ions excite guanylate cyclase activity in cerebellar tissues (Ohga & Daly 1977), and this probably accounts for the enhanced cGMP level in the cerebellar cortex following activation of climbing fibers (Biggio & Guidotti 1976). It has also been reported that a high level of cGMP desensitizes glutamate receptors isolated from cerebellar tissues (Sharif & Roberts 1980). Therefore, the possibility has been suggested that climbing fiber signals cause Ca^{2+} inflow into Purkinje cell dendrites, consequently stimulate guanylate cyclase, and thereby enhance cGMP level in Purkinje cell dendrites; glutamate receptors at parallel fiber-Purkinje cell synapses will then be desensitized under conjoint influences of cGMP from the inside and L-glutamate from the outside (Ito 1987). Efforts to reproduce LTD with ionotophoretic application of cGMP analogues, however, has met a difficulty that cGMP analogues by themselves exert an excitatory action on Purkinje cells, probably due to their pharmacological effect (M. Ito and M. Kano, unpublished observation).

A third possibility is that Ca^{2+} ions in collaboration with L-glutamate induce a change in structural configuration of dendritic spines. Since parallel fiber-Purkinje cell synapses are located on the head of spines, the synaptic efficacy would decrease if constriction occurs in the neck of spines, and therefore one may imagine that a long-lasting constriction of spine necks underlies LTD. This contradicts the shortening of spines suggested

to underlie LTP in hippocampal pyramidal cells (Crick 1982, Koch & Poggio 1983, Kawato et al 1984). A recent study with rapid-freeze, deep-etch techniques revealed that actin filaments run along the axis of dendritic spines of Purkinje cells (Hirokawa 1988). This finding argues against the occurrence of constriction at spine necks, since these actin filaments would induce shortening, if any, but not constriction of spine necks.

So far, evidence is available only for postsynaptic events involved in LTD, and there is no evidence that LTD also involves a long-lasting presynaptic event such as decreased transmitter release. Presynaptic events have been suggested to contribute to LTP, based on an increased glutamate release from hippocampal tissues (Lynch et al 1985) and an increased quantal number in transmitter release (Yamamoto et al 1987). Because such measurements have not been performed in the cerebellum, argument cannot be made for or against this suggestion. The postsynaptic events described above, however, explain the genesis of LTD satisfactorily.

ROLES OF LONG-TERM DEPRESSION IN CEREBELLAR FUNCTION

A basic hypothesis so far put forward concerning the learning capabilities of the cerebellum is that a region or cerebellar cortex forms a modifiable side path into a reflex arc or into a command signal pathway for voluntary control through connections of mossy fibers and Purkinje cell axons, and that the signal transfer characteristics of this cerebellar path are modified by climbing fiber signals. Climbing fiber signals represent control errors in the system's performance, and act to depress wiring of the cerebellar network responsible for error generation, through induction of LTD (see Ito 1984).

This hypothesis of cerebellar learning can be critically examined by recording unit spikes from individual Purkinje cells in alert, behaving animals. Activation of Purkinje cells through climbing fibers is represented by generation of *complex* spikes, and activation through parallel fibers is reflected in generation of *simple* spikes, even though simple spike discharge is influenced also by the activities of cortical inhibitory neurons (for example, see Miyashita & Nagao 1984). A number of motor tasks have been introduced for relating Purkinje cell activities to cerebellar function in various animal species.

In an attempt to correlate Purkinje cell activity to behavior, it is crucial to record from Purkinje cells at the very site that is primarily involved in the behavior under examination. According to our current knowledge, the

cerebellum is composed of numerous narrow (only a few hundred microns wide) microzones, each receiving climbing fibers from a small separate group of inferior olive neurons (Oscarsson 1976). Each microzone projects Purkinje cell axons to a separate group of nuclear neurons and so is dedicated to a certain bodily function that in general is entirely unrelated to those of two neighboring microzones. Since mossy fiber afferents frequently branch in the cerebellum, they may supply collaterals to neighboring microzones and so induce common simple spike activities in them. Therefore, even if two Purkinje cells exhibit mutually related simple spike activities, this does not necessarily imply their belonging to the same microzone. Similarity in climbing fiber responses in two Purkinje cells should be a more reliable sign for their common microzonal origin, but caution is still needed because even climbing fibers issue collaterals innervating different mirozones (for references, see Ito 1984). The most reliable criterion to identify a microzone in a behaving animal is therefore provided by local cerebellar stimulation that induces a unique effect through Purkinje cell output from the microzone. Complex spike responses of Purkinje cells would next be considered, whereas simple spike responses are the least reliable index for the unity of a microzone. It must be emphasized that insufficient identification of microzones explains most discrepancies of the results reported from different research groups.

Provided that a proper microzone is identified in relation to a certain motor task, two lines of information would be crucial for testing the cerebellar learning hypothesis. First, complex spikes should represent control errors involved in performance of the motor task. Second, sustained correlated activities in complex and simple spikes should lead to a progressive modification in simple spike activity, provided that no disturbance occurs, such as blockade of climbing fiber-induced Ca^{2+} inflow into Purkinje cell dendrites by stellate cell inhibition (see above).

Vestibulo-ocular Reflex

The involvement of the cerebellar flocculus in adaptive control of the vestibulo-ocular reflex (VOR) has been studied extensively (for references, see Ito 1982, 1984). The cerebellar flocculus is connected to the VOR pathway in two ways, so the flocculus receives vestibular signals as a mossy fiber input and in turn projects Purkinje cell axons to relay cells of the VOR. Floccular Purkinje cells exhibit modulation of simple spike discharges in response to head rotation, and through this modulation, they modify activities of the VOR relay cells, and consequently the VOR. The flocculus also receives visual signals as a climbing fiber input monitoring the performance of the VOR.

Under sustained mismatching of visual and vestibular stimulation, the

VOR undergoes a progressive increase or decrease of its gain that leads to minimization of retinal errors under given stimulating conditions, as first demonstrated in human subjects wearing dove prism goggles (Gonshor & Melvill Jones 1976). The VOR adaptation reproduced in animals is abolished with lesioning of the flocculus and also with interruption of the visual climbing fiber pathway to the flocculus (for references, see Ito 1984). When a rabbit performs the VOR adaptation, floccular Purkinje cells exhibit complex spike activities representing retinal errors (Ghelarducci et al 1975, Miyashita & Nagao 1984, Nagao 1988a), and a progressive change takes place in their simple spike responsiveness to head rotation (Dufossé et al 1978). The direction of the change is consistent with the change of the VOR gain, and hence a causal relationship can be suggested (Ito 1977, 1982).

Taken together, these results strongly support the "Flocculus Hypothesis of the VOR Control" (Ito 1982) that the flocculus is the site of the VOR adaptation and that the LTD plays a key role there. Retinal error signals conveyed by climbing fibers to the flocculus would induce the LTD at those parallel fiber-Purkinje cell synapses transferring vestibular mossy fiber signals at that time, and so would modify transfer characteristics of the flocculus for vestibular mossy fiber signals. The VOR adaptation was reproduced successfully with a computer simulation based on this learning principle (Fujita 1982b).

The VOR adaptation is abolished after deprivation of monoamines from rat brain by means of 6-OHDA (Miyashita & Watanabe 1984). The role of these monoamines in the VOR adaptation is still unclear, but one possibility is that monoamines interfere with the occurrence of LTD. An analogy may be drawn from the fact that noradrenaline enhances the LTP in hippocampal slices (Johnston et al 1988).

An objection against the flocculus hypothesis has been raised based on the failure to detect a change of simple spike modulation correlated to VOR adaptation in Purkinje cells of monkey's flocculus (Miles & Lisberger 1981). This failure, however, is apparently due to ignorance of the microzone structure of the flocculus. When the microzone specifically involved in the horizontal VOR (H-zone) was identified with local stimulation that evoked horizontal eye movement, floccular Purkinje cells in monkeys indeed exhibited adaptive changes similar to those reported in rabbits (Watanabe 1984, 1985).

Another objection has been based on overestimation of the simple spike responsiveness of floccular Purkinje cells to eye velocity. If the eye velocity responsiveness predominates over vestibular responsiveness, the change observed in simple spike responses to head rotation after the VOR adaptation would merely be a reflection of increased or decreased eye velocity

due to adaptation, and would not indicate a change of the vestibular mossy fiber responsiveness of floccular Purkinje cells (Miles & Lisberger 1981). However, eye velocity responsiveness actually obtained in systematic surveys through the rabbit's floccular H-zone is generally low so that the reflection of changed eye velocity accounts only for a fraction of the simple spike responsiveness to head rotation actually obtained due to adaptation (Miyashita 1984, Nagao 1988b). Consequently, the change of simple spike responses to head rotation that occurs during VOR adaptation can be regarded as largely representing modification of vestibular mossy fiber responsiveness of floccular H-zone Purkinje cells, as postulated in the flocculus hypothesis.

Optokinetic Eye Movement Response

The optokinetic eye movement response (OKR) is eye movement following relatively slow movement of the visual environment. Its gain progressively increases under sustained optokinetic stimulation (Collewijn & Grootendorst 1979, Nagao 1988a). This adaptation was abolished by flocculectomy, and floccular H-zone Purkinje cells exhibited changes of simple spike responsiveness to optokinetic stimulation in parallel with the OKR adaptation. It has thus been proposed that the flocculus is the site of adaptation of not only the VOR but also OKR (Nagao 1988a). The observed change of simple spike discharge is correlated to complex spike discharge evoked by the optokinetic stimulation in a manner that suggests the LTD to be its cause. Since the same H-zone Purkinje cells and vestibular relay neurons are involved in both the VOR and OKR, the flocculus appears to adaptively control the sum of these two synergistic reflexes by referring to common retinal error signals. This manner of control has been postulated in terms of the flocculus hypothesis of the coordinated ocular reflex control (Ito 1982).

Classical Conditioning of the Eye Blink Reflex

Air-puff stimulation of the cornea evokes blinking with the eyelid and nictitating membrane, which can be classically conditioned with tone stimulation. Involvement of the cerebellum in this classic conditioning has recently been demonstrated (Thompson 1986). Auditory stimuli are conveyed to the cerebellum through mossy fiber afferents originating from the pontine nucleus, whereas corneal stimuli are forwarded through climbing fiber afferents. Stimulation of the cornea may imply an error which an eye blink had to prevent from occurring. This error representation by climbing fibers conforms to the general scheme of the cerebellular learning hypothesis.

A role of LTD in this classic conditioning has been suggested under

assumptions that tone-eye blink reaction is originally mediated by a cerebellar nucleus, and that it is normally suppressed by Purkinje cells. Repeated combined application of tone and corneal stimuli would induce LTD, which removes driving effects of tone stimuli on Purkinje cells, leading to release of the tone-eye blink reaction from Purkinje cell inhibition (Ito 1984).

Recording from Purkinje cells revealed simple spike activity generally consistent with the above view (Thompson 1986). In the conditioned animals, Thompson and his associates (Donegan et al 1985, Foy & Thompson 1986) found a class of Purkinje cells displaying a decrease in rate of simple spike discharge that preceded and modeled the learned behavioral eyelid response. In the untrained animals, they found Purkinje cells that showed increases in simple spike responses to tone stimuli. Further, the frequency of climbing fiber responses evoked by the corneal airpuff unconditioned stimuli decreased markedly as a result of training. These observations are strongly consistent with the hypothesized roles of LTD and climbing fiber signals in cerebellar learning. In one study, a variety of response patterns have been observed, probably because Purkinje cells were sampled without specifying the microzone responsible for the conditioning (Berthier & Moore 1986). Among Purkinje cells sampled along a track perpendicularly crossing microzones in a hemispheric portion of the lobulus simplex, one cell exhibited inhibitory responses to tone stimuli (record 3 of Berthier & Moore 1986), a finding that conforms to the above view. It is possible that this cell represents the proper microzone for the eye blink conditioning.

Combination of air puff-evoked simple spike responses and spontaneously discharging complex spikes has been reported to fail to produce an LTD-like effect, except for a short-term facilitation of air puff-evoked simple spike responses (Zuo & Bloedel 1987). This observation has been claimed to contradict the cerebellar learning hypothesis, but it is necessary to examine whether any factor prevents LTD from occurring, such as stellate cell inhibition evoked by mossy fiber signals (see above), before drawing this conclusion.

Locomotion

When walking is disturbed with an obstacle, an animal adaptively modifies patterns of limb movements to avoid the obstacle. In cats, Deiters neurons innervating the forelimb contralateral to the perturbed forelimb exhibited an increased discharge in response to the perturbation, apparently due to a decreased simple spike discharge in those vermal Purkinje cells innervating the Deiters neurons (Matsukawa & Udo 1985). Interlimb coordination during the cat's locomotion may be effected by linked activity of

vermal Purkinje cells and Deiters neurons. Perturbation frequently evoked complex spikes in these Purkinje cells, as has also been observed with another type of locomotor perturbation (Andersson & Armstrong 1987) and with a perturbed active forelimb movement (Gellman et al 1985). These complex spikes would represent control errors in locomotion, and thc pattern of the simple spike responsiveness to perturbation would be gradually modified during repeated trials by means of the LTD. Recording of these responses during the course of learning will provide a crucial test of this possibility.

Decerebrate locomoting ferrets acquired a conditioned movement of a perturbed forelimb to avoid contacting a bar (Bloedel et al 1987). This conditioning, however, was preserved even after extensive cerebellar lesions, and, therefore, does not represent cerebellar function. Instead, the cerebellum appeared to be critical for the organization of movement required to avoid the bar in successive trials while minimizing modification in the ongoing movement which is in fact a clear instance of learning. Purkinje cell responses related to acquisition of such organization have to be investigated in order to test the validity of the cerebellar learning hypothesis in this particular movement task.

Posture

Complex spikes in frog cerebellar Purkinje cells appear to be related to errors in postural compensation (Amat 1983). Rats can be operantly conditioned to walk on a rotating rod, and this learning is impaired by lesioning the red nucleus (Kennedy & Humphrey 1987). Since severance of the rubrospinal tract originating from the magnocellular red nucleus neurons was quickly compensated for, the impairment of learning should be acribed to destruction of parvocellular red nucleus neurons projecting to the inferior olive. A peculiar finding is that lesion of the red nucleus no longer impaired the learning after the rat had compensated for severance of the rubrospinal tract. The parvocellular red nucleus neurons appear to play a role in the process of compensating for severance of the rubrospinal tract but not in maintenance of the compensated behavior. An interesting task for the future will be to relate these observations with the unique structure of the rubro-olivo-dentate triangle (see Ito 1984).

Voluntary Movement

Neuronal organization dedicated to the control of smooth pursuit eye movement has been a subject of recent extensive investigations (see Eckmiller 1987). Activity of complex spikes in monkey cerebellum has been correlated to retinal slip signals in this voluntary movement control (Stone & Lisberger 1986), consistent with cerebellar learning hypothesis.

The monkey's step movements with an arm undergoes adaptative adjustment against changes in a handle load. Purkinje cells exhibited complex spike discharge during arm movement against an altered load, and at the same time spike discharges were reduced (Gilbert & Thach 1977). As soon as the arm movement was readjusted to the altered load, complex spike discharge returned to a control level, while simple spike discharges remained depressed. These observations have been interpreted in accordance with Albus' (1971) plasticity assumption and therefore are in agreement with the cerebellar learning hypothesis.

Purkinje cells in the monkey's cerebellum exhibit complex spike discharges at the onset of visually guided wrist tracking movement. In Mano et al's (1986) records, these complex spike discharges seem to represent control errors in pursuit, i.e. discrepancy between movements of the target visual cue and the cursor trace driven by wrist extension or flexion, in agreement with the cerebellar learning hypothesis. However, these authors considered the complex spikes only in relation with handle velocity, and further misinterpreted the occurrence of complex spike discharges seemingly irrelevant to the degree of training as contradictory to the cerebellar learning hypothesis. It is important to realize that errors in a visually guided tracking task cannot be nullified, no matter how much training is done, because the tracking movement is triggered by an error. Even in a learned state, control errors would remain, probably after their time duration could be minimized. It is crucial to evaluate precisely relationships between complex spike discharges and control errors in the visually guided wrist tracking to find a functional meaning of the complex spike discharges. Mano et al (1986) also claimed that their failure to detect a long-lasting depression of simple spike discharge following a spontaneous complex spike contradicts the cerebellar learning hypothesis. The hypothesis, however, predicts the occurrence of LTD only at those parallel fiber-Purkinje cell synapses repeatedly activated in conjunction with climbing fiber signals at a rate above a certain level. These prerequisite conditions for testing the cerebellar learning hypothesis do not seem to be fulfilled in their experiments.

SUMMARY

LTD has now been established as a synaptic plasticity specific to the cerebellum. Cellular and molecular mechanisms of LTD have been elucidated to some extent, but still a number of questions are left open. The most crucial question may concern its time course, as to how long the LTD lasts beyond the limit of the present maximum observation time of 3 hr, and whether and how it is eventually transformed to a permanent memory.

Molecular mechanisms underlying LTD should be investigated further in respect to Ca^{2+} binding and storage, protein kinase C, phosphorylation of glutamate receptors, GTP proteins, etc. The ineffectiveness of mass field potentials in representing LTD makes such studies relatively difficult, and a hope for future development may be placed in reproduction of LTD in tissue cultured Purkinje cells or even in isolated glutamate receptors in a simplified form.

The cerebellar neuronal network incorporating LTD as a memory element has been conceived as a simple perceptron-like (Albus 1971) or adaptive filter-like (Fujita 1982a) parallel processing computer. Such a neuronal computer incorporated in a reflex or a more complex movement system would endow the system with subtle capabilities of adaptation and learning. The scheme of the floccular control of the VOR closely resembles that of a self-tuning regulator, a type of adaptive control system. For cerebellar control of voluntary movements, however, another version of the adaptive control system, the model reference control system, seems to be more applicable (Ito 1986). This system continuously readjusts its dynamics by referring to errors derived through comparison of its performance with that of an internal model. It is important to note that a model for an unknown system can be built based on the same principle, by feeding errors derived from their comparison to adjust the model. It may thus be conceived that an internal model is built within the cerebellum in the manner of model reference adaptive control, and that an internal model so formed is utilized for adaptive control of movement. A recent simulation study successfully reproduced learning in formation of an arm trajectory based on these principles of model reference control (Kawato et al 1987).

On the experimental side, however, the complex neural organization for control of locomotion, posture, and voluntary movements still eludes full elucidation. Nevertheless, evidence is accumulating to support the cerebellar learning hypothesis. Some controversies have been raised against the hypothesis, but in view of the often insufficient data on which arguments are based, one may hope that these will be resolved in the future when more data are collected and examined for their consistency with the cerebellar learning hypothesis.

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