

MOLECULAR GENETIC ANALYSIS OF SYNAPTIC PLASTICITY, ACTIVITY-DEPENDENT NEURAL DEVELOPMENT, LEARNING, AND MEMORY IN THE MAMMALIAN BRAIN

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ABSTRACT

Recently, dozens of mutant mice generated with gene targeting or transgenic technologies have been shown to exhibit a distinct set of impairments in the brain and behavior. In this review, we discuss how studies of mutant mice have helped elucidate the mechanisms that underlie synaptic plasticity and the relationship of these synaptic mechanisms to the activity-dependent phase of neural development and learning and memory. We focus on the recent progress in the analysis of whisker-related pattern formation, elimination of climbing fibers, long-term potentiation, long-term depression, and various learning and memory tasks in mutant mice.

INTRODUCTION

One of the greatest challenges in neuroscience is understanding how the nervous system acquires, stores, and utilizes information derived from the sensory world. With the establishment of the “neuron doctrine” by Cajal (1894), which stated that the nervous system is made up of discrete units (i.e. neurons),

Table 1 Brief description of phenotypes in other mutant mice

Targeted gene product	Phenotype	References
Monoamine oxidase A (MAOA)	Disrupted barrel pattern in the somatosensory cortex; normal in the thalamus and trigeminal nuclei.	Cases et al 1996
Synapsin I and synapsin II	Impaired transmitter release during repetitive stimulation. Normal CA1 and mossy fiber LTP.	Rosahl et al 1995 Spillane et al 1995
Brain-derived neurotrophic factor (BDNF)	Reduced CA1 LTP.	Korte et al 1995
Prion protein	Impaired GABA _A -mediated inhibition; impaired CA1 LTP. Normal GABA _A -mediated inhibition; normal CA1 LTP. Normal spatial learning.	Collinge et al 1994 Lledo et al 1996
Tissue plasminogen activator (t-PA)	Deficient CA1 I-LTP.	Bueler et al 1992 Frey et al 1996
$\beta 2$ subunit of nicotinic acetylcholine receptor ($\beta 2$ -nAChR)	Abnormal avoidance learning; normal spatial learning.	Picciotto et al 1995
Neural-cell adhesion molecule (N-CAM)	Reduced olfactory bulb; impaired spatial learning.	Cremer et al 1994

neuroscientists including Cajal proposed that modifications might occur in the interaction between neurons. They suggested that certain neuronal modifications might underlie developmental processes as well as processes underlying learning and memory.

In 1949, Hebb proposed a well-defined rule of synaptic plasticity: Coincident activity in two connected neurons leads to strengthening of their connection. Hebb further postulated that associative learning could be based on this synaptic modification. Today we know that synaptic plasticity is expressed in many forms. Thus, Hebb's coincident rule can be applied only to some forms of synaptic plasticity. Yet the notion that neural activity leads to synaptic modifications remains central in our current understanding of activity-dependent development, learning, and memory in the brain.

Genetics has been a powerful tool to dissect complex biological processes. With the advent of embryonic stem-cell gene-targeting technology (Capecchi 1989), it became possible to produce a strain of mice with a *disruption* (also known as a gene knockout) or some other kind of mutation in a gene of interest. A mutant mouse strain created by the gene-targeting technology provides an unlimited supply of animals with the identical molecular lesion defined by the

mutation. Thus, the genetic approach provides an effective and highly specific means for investigating the function of a particular gene in developmental processes as well as in complex behaviors. The most significant advantage this approach offers is that it allows researchers to assess the relationships among phenomena observed at molecular, cellular, and systems levels.

During the past few years, a few dozen mutant mice have been generated using the gene-targeting or transgenic technologies, and some of them exhibited a distinct set of impairments in the brain and in their behavior. In this review, we discuss how studies of mutant mice have helped elucidate the mechanisms that underlie synaptic plasticity and the relationship of these synaptic mechanisms to activity-dependent neural development, learning, and memory. We discuss in detail only those gene knockout mice in which the relationship between synaptic plasticity and development or memory have been analyzed. Table 1 lists other mutant mice along with a brief description of their phenotypes. For coverage of the genetic manipulation technology per se, readers should refer to reviews by Capecchi (1989, 1994) and Zimmer (1992).

MECHANISMS OF DEVELOPMENTAL PLASTICITY

Sensory pathways of the brain generally develop from crudely wired networks to precisely organized systems through a refinement of synaptic connections. Studies of the visual system have demonstrated that neural activity plays a critical role in the refinement of wiring between developing neuronal groups. N-methyl-D-aspartate (NMDA) receptors, a subtype of postsynaptic glutamate receptors that are permeable to Ca^{2+} influx, are thought to transduce synaptic activity to Ca^{2+} -dependent cellular processes that result in the refinement (for reviews, see Constantine-Paton et al 1990, Shatz 1990).

NMDA Receptor in Whisker-Related Neuronal Patterns

In rodents, tactile hairs (e.g. whiskers and sinus hairs) on the snout are arranged in a discrete array and collectively form a unique sense organ that is important for the animal's exploratory behavior. The whisker-related neuronal pattern is first established in the brain stem and then in the dorsal thalamus as well as in the primary somatosensory cortex (Woolsey 1990). The role of neural activity and NMDA receptors in the development of the whisker-to-barrel system has been studied using various antagonists, such as TTX (a voltage-gated Na-channel blocker) and AP5 (an NMDA-receptor antagonist), applied either to the branches of the trigeminal nerve that innervates the whiskers (Henderson et al 1992) or directly to the barrel cortex in newborn rats (Chiaia et al 1992, Schlaggar et al 1993). Although lesions of the sensory periphery dramatically alter whisker-related patterns in the brain within a few days of birth (for a

review, see Woolsey 1990), these pharmacological experiments failed to prevent the emergence of the patterns.

A functional NMDA receptor is formed by the essential subunit NR1 (ξ) and at least one of four NR2 (ε) subunits (for review, see Nakanishi 1992). The NR1 subunit is expressed ubiquitously in the brain, whereas the four NR2 subunits are expressed in distinct regions. In mutant mice lacking the NR1 subunit, whisker-related barrelettes in the brain stem fail to form, although pathfinding, initial targeting, and crude topographic projection of trigeminal axons are unaffected (Li et al 1994). Similarly, the targeted inactivation of the NR2B (ε_2) subunit disrupts the formation of the barrelette pattern in the brain stem (Kutsuwada et al 1996). NR1 and NR2B mutant mice die prematurely before their whisker-related neuronal patterns in the thalamus and the somatosensory cortex can be examined. In contrast, mutant mice deficient in the NR2D subunit exhibit no abnormalities in any of the three types of whisker-related neuronal patterns (Ikeda et al 1995). These results demonstrate that synaptic activity mediated by NMDA receptors is essential for the formation of periphery-related neural patterns in the mammalian brain. In earlier pharmacological studies, inhibitors or antagonists may have been applied too late during development to disrupt activity-dependent refinement processes.

Upon Ca^{2+} entry through NMDA receptors, Ca^{2+} -dependent protein kinases such as Ca^{2+} /calmodulin-dependent kinase II (CaMKII) could be activated. In mice that lack the gene encoding αCaMKII , the development of anatomical barrel patterns seems normal (Glazewski et al 1996). The adult experience-dependent plasticity, as measured by the changes of neuronal firings in the barrel cortex in response to tactile inputs, is suppressed in the mutant mice.

PKC γ in Activity-Dependent Synapse Elimination in the Cerebellum

Activity-dependent refinement of neuronal pattern formation can be achieved either by creating or strengthening properly targeted synapses or by eliminating or weakening unwanted synapses. The latter forms of developmental synaptic plasticity are best seen in the cerebellum. In the cerebellar cortex, Purkinje cells (PCs) have two distinct types of excitatory synapses: one from parallel fibers (PFs), the other from climbing fibers (CFs). Synaptic innervation of PCs by CFs is modified dynamically during postnatal development. Around postnatal day five (P5) in rats, each PC is innervated by three to four CFs (Crepel 1982). Elimination of multiple CFs occurs during the second and third postnatal weeks until a one-to-one relation between CFs and PCs is attained at approximately P21. During this period of CF elimination, PF-PC synapses are continuously generated concurrent with the migration of granule cells from the external granular layer to the internal granular layer. Earlier studies using

X ray-induced mutants indicate that PF inputs to PCs are required for the elimination of surplus CFs (for a review, see Crepel 1982). One interesting hypothesis is that this synapse elimination results from competition among multiple CFs for a limiting factor (e.g. a neurotrophic factor) and that PF-PC activity fuels this competition (Crepel 1982).

The γ isoform of protein kinase C (PKC γ), a Ca²⁺- and phospholipid-dependent kinase, is highly expressed in PCs but not in other types of neurons in the cerebellum (Tanaka & Nishizuka 1994). The expression of PKC γ changes markedly during cerebellar development: It is very low at birth and reaches a peak around the third postnatal week (Herms et al 1993, Moriya & Tanaka 1994). This temporal pattern of PKC γ expression coincides with the developmental transition from multiple to single CF innervation onto each PC in wild-type mice (see Figures 1a and b). In mature PKC γ mutant mice, about 40% of PCs are still innervated by multiple CFs (see Figure 1c), whereas other aspects of the cerebellum appear normal (Kano et al 1995). Although this type of abnormal CF innervation of PCs has been observed in other gene knockout mice (e.g. GluR δ 2, mGluR1), in contrast to PKC γ mutant mice, these mutant mice harbor additional cerebellar impairments. For instance, synaptic contacts between PFs and dendritic spines of PCs occur half as often in GluR δ 2 mutant mice as they do in normal mice (Kashiwabuchi et al 1995). In addition, mutant mice lacking GluR δ 2 or a subunit of metabotropic glutamate receptor (mGluR1) are deficient in *long-term depression* (LTD), a form of cerebellar synaptic plasticity (Aiba et al 1994b, Kashiwabuchi et al 1995; M Kano, K Hashimoto, A Aiba & S Tonegawa, unpublished observations). In contrast, PKC γ mutant mice exhibit robust LTD. The specific impairment of CF elimination in the otherwise seemingly normal cerebellum of PKC γ mutant mice, combined with earlier studies (see Crepel 1982), suggests that PKC γ in PCs plays a role in the CF elimination by transducing the neural signals delivered at PF-PC and CF-PC synapses.

MECHANISMS OF SYNAPTIC PLASTICITY IN THE HIPPOCAMPUS

Long-term potentiation (LTP) is defined as a long-lasting increase of synaptic efficacy following a brief high-frequency stimulation. LTP was discovered by Bliss & Lomo (1973) in the dentate gyrus and since then has been studied widely as a promising cellular mechanism for learning and memory (Swanson et al 1982). In the hippocampus, synaptic potentiation can be classified according to its temporal duration (Bliss & Collingridge 1993, Malenka & Nicoll 1993). *Post-tetanic potentiation* (PTP) refers to synaptic enhancement that decays within minutes. *Short-term potentiation* (STP) presumably lasts

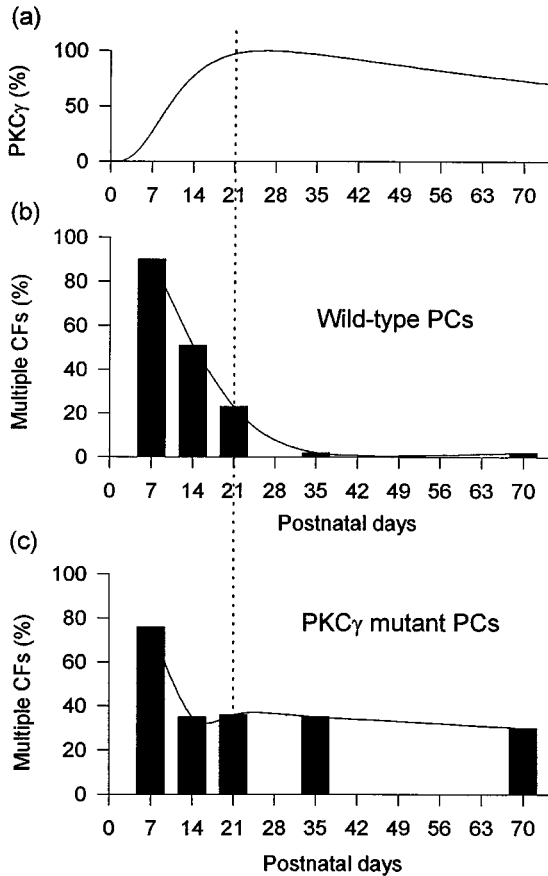


Figure 1 The role of PKC γ in the activity-dependent elimination of surplus CFs. (a) The stylized representation of the temporal pattern of PKC γ expression (see text). Developmental change in the percent of PCs innervated by two or more CFs in (b) wild-type or (c) PKC γ mutant mice (data taken from Kano et al 1995). Major elimination of CFs occurred around postnatal day 21 in wild-type mice when PKC γ expression peaked, whereas such elimination did not occur in the mutant mice. CFs, climbing fibers; PCs, Purkinje cells.

for 10–20 min. LTP can last at least 1 h. LTP has been observed in the three major excitatory synapses in the trisynaptic circuit of the hippocampus (see Figure 2). In this circuit, the perforant pathway runs from the pyramidal cells of the entorhinal area to the granule cells of the dentate gyrus (dentate LTP); the mossy fiber pathway runs from the granule cells of the DG to the CA3 pyramidal cells (mossy fiber LTP); and the Schaffer collateral pathway runs from the CA3 pyramidal cells to the CA1 pyramidal cells (CA1 LTP).

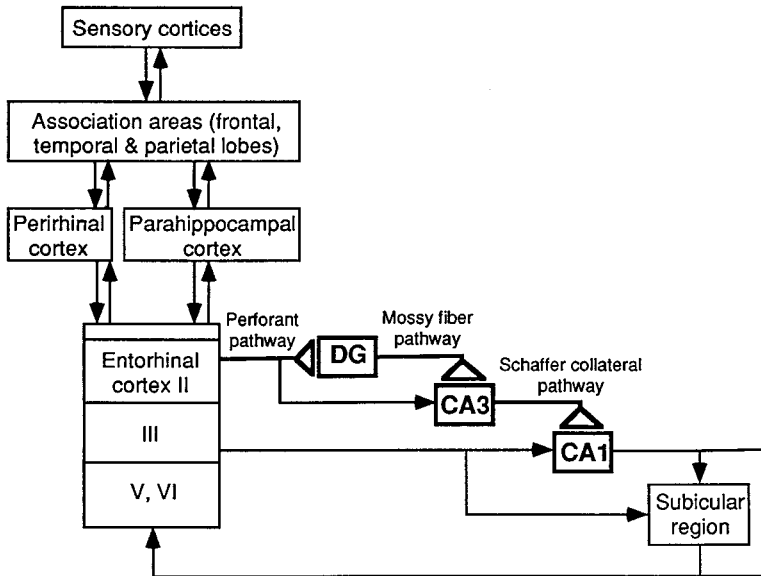


Figure 2 The trisynaptic circuit of the hippocampus as a part of the memory system residing in the medial temporal lobe (based on Amaral & Witter 1989, Witter 1993, Huang et al 1995). LTP has been observed in the perforant pathway (DG, the dentate gyrus), the mossy fiber pathway (CA3), and the Schaffer collateral pathway (CA1).

Types of hippocampal LTP can also be distinguished on the basis of their dependence on NMDA receptors (Bliss & Collingridge 1993, Nicoll & Malenka 1995). Both dentate LTP and CA1 LTP are initiated postsynaptically by the activation of NMDA receptors. In CA1 LTP, this activation leads to Ca^{2+} influx and subsequent activation of PKC, CaMKII, and tyrosine kinases (for review, see Bliss & Collingridge 1993, Malenka & Nicoll 1993). In addition, Bashir et al (1993) have suggested that mGluRs are required for the induction of CA1 LTP. However, the role of mGluRs remains uncertain because it is difficult to establish the efficacy and specificity of agonists or antagonists for this family of receptors (Chinestra et al 1993, Manzoni et al 1994). It is unclear whether dentate LTP shares these molecular cascades with CA1 LTP (see below). In contrast, it is quite certain that CA3 LTP is independent of NMDA receptors and is thought to be initiated presynaptically (Zalutsky & Nicoll 1990). The cAMP-dependent protein kinase A (PKA) has been shown to be critical for CA3 LTP (Huang et al 1994, Weisskopf et al 1994) but not for CA1 LTP (see also Huang & Kandel 1994).

The CA1 LTP and the mossy fiber LTP both are induced by a single train of tetanus. Another form of LTP, called late phase LTP (l-LTP), which is induced

Table 2 Summary of impairments in synaptic plasticity and hippocampus-dependent memory

Mutant mice	CA1 LTP	Mossy LTP	Dentate LTP	Spatial learning	Contextual conditioning	References
α CaMKII	Deficient	N/A	N/A	Deficient	Deficient	Silva et al 1992 Chen et al 1994
<i>fyn</i>	Impaired	N/A	N/A	Impaired	N/A	Grant et al 1992
PKC γ	Impaired	Normal	Normal	Impaired	Impaired	Abeliovich et al 1993a,b Hsia et al 1995 Chen et al, unpublished observations
mGluR1	Impaired	Normal	N/A	Impaired	Impaired	Aiba et al 1994a Hsia et al 1995
PKA (C β 1 or RI β)	Normal	Impaired	Normal	Impaired	N/A	Conquet et al 1994
	Normal	Deficient	N/A	Normal	Normal	Brandon et al 1995 Huang et al 1995
Thy-1	Normal	N/A	Impaired	Normal	N/A	Nosten-Bertrand et al 1996

by multiple trains of tetanus and which lasts for at least several hours, has been found in both the CA1 and CA3 regions (Frey et al 1993, Huang & Kandel 1994). In contrast to CA1 LTP, l-LTP is blocked by inhibitors of PKA and by inhibitors of protein synthesis (Frey et al 1993, Huang et al 1994, Nguyen et al 1994).

LTP at Schaffer Collateral-CA1 Synapses

PROTEIN KINASES The involvement of Ca²⁺-sensitive kinases in CA1 LTP has been confirmed by the observations that an LTP impairment occurs to various degrees in the hippocampal slices derived from several gene knockout mice (see Table 2). The impairment is severe and cannot be overcome by electrophysiological manipulations in α CaMKII mutant mice (Silva et al 1992a, Stevens et al 1994). The LTP impairment in mutant mice lacking a tyrosine kinase *fyn* occurs in response to a tetanus of low intensity but not to one of high intensity (Grant et al 1992). PKC γ mutant mice lack LTP induced by a conventional high-frequency tetanus but are capable of generating LTP-like potentiation when the tetanus is preceded by low-frequency (1 Hz) stimuli (Abeliovich et al 1993a). Transgenic mice expressing a constitutively active, autophosphorylated form of α CaMKII exhibit normal CA1 LTP upon a 100-Hz tetanus and show an enhanced capacity to generate LTD following lower frequency (1-10 Hz) stimulations (Mayford et al 1995). This observation indicates that α CaMKII regulates the frequency-response function for the production of LTP and LTD.

GLUTAMATE RECEPTORS NR1 is expressed ubiquitously in the brain, whereas among NR2 subunits only NR2A and NR2B are expressed in CA1 and CA3 pyramidal cells in the adult hippocampus (Monyer et al 1994). Targeted disruption of the NR2A ($\epsilon 1$) subunit gene results in a reduction of both the NMDA receptor current and the CA1 LTP (Sakimura et al 1995). Unfortunately, premature death of NR1 or NR2B mutant mice precludes analysis of synaptic plasticity in adult animals (Forrest et al 1994, Li et al 1994, Kutsuwada et al 1996).

Different phenotypes have been observed in the mGluR1 mutant mice generated by two research groups. Aiba et al (1994a) reported a reduced amplitude of CA1 LTP in its line of mGluR1 mutant mice. In contrast, Conquet et al (1994) reported normal CA1 LTP. Other contradictory findings have been reported on these two lines of mice (see below). The cause of these different phenotypes is unknown.

PRIMED LTP An interesting finding in gene knockout studies is the *priming effect* on CA1 LTP. In PKC γ mutant mice, LTP was absent after a conventional tetanus but could be induced when the tetanus was preceded by a 1-Hz stimulation lasting for 10 min (Abeliovich et al 1993a). Although the low-frequency stimulation induces LTD (Dudeck & Bear 1992), a prior induction of LTD is not required for the subsequent induction of CA1 LTP in PKC γ mutant mice. In contrast, priming had no effect in the induction of CA1 LTP in mGluR1 (Aiba et al 1994a) or α CaMKII mutant mice (Stevens et al 1994). Perhaps the low-frequency priming activates a second messenger system that can substitute for the deficient PKC γ .

NITRIC OXIDE AND CARBON MONOXIDE Researchers generally agree that the induction of CA1 LTP occurs postsynaptically (Bliss & Collingridge 1993), but whether the expression of LTP is presynaptic or postsynaptic or both remains controversial (e.g. Manabe & Nicoll 1994, Stevens & Wang 1994). If there is a presynaptic component in the expression of LTP, one has to assume that there are some retrograde messengers that travel from the postsynaptic site to the presynaptic terminal. Inhibitors for the production of two candidate retrograde molecules, nitric oxide (NO) and carbon monoxide (CO), are reported to block LTP in hippocampal slices (O'Dell et al 1991, Schuman & Madison 1991, Stevens & Wang 1993, Zhuo et al 1993). However, LTP is normal in mutant mice lacking either the neuronal isoform of nitric oxide synthase (nNOS) or the major cerebral isoform of CO-producing heme oxygenase (HO-2) (O'Dell et al 1994, Poss et al 1995). In O'Dell's and Poss's studies, inhibitors of NO or CO were still able to block LTP in the nNOS or HO-2 mutant mice, which suggests the involvement of other enzyme isoforms. A recent study showed that, as with nNOS mutant mice, CA1 LTP is normal in eNOS (the endothelial

isoform) mutant mice but impaired in the double mutant mice that lack both nNOS and eNOS (Son et al 1996). These results support the idea that NO is involved in CA1 LTP. Perhaps, nNOS and eNOS can compensate for each other in the single knockouts.

Late Phase LTP at Schaffer Collateral-CA1 Synapses

The cAMP-responsive element-binding protein (CREB) is an attractive candidate that mediates l-LTP, because this transcription factor activates the transcription of several genes with the cAMP-responsive element (CRE) sequence in response to increases in intracellular cAMP and Ca^{2+} . In mice deficient in the α and γ isoforms of CREB, the magnitude of CA1 LTP induced by one train of tetanus (100 Hz, 1s) was reduced during the initial 60 min after the tetanus, and it decayed to baseline 90 min later (Bourtchuladze et al 1994). Thus, there seems to be a greater impairment in l-LTP than in the early phase. But it is puzzling that LTP, which is not supposed to depend on protein synthesis, is affected by the CREB gene inactivation. It remains to be tested whether more specific impairments can be revealed if multiple trains of tetanus, a conventional protocol to induce l-LTP (Frey et al 1993, Huang et al 1994, Nguyen et al 1994), are used instead of a single train. Another puzzling result comes from mutant mice deficient for type I adenylyl cyclase (I-AC), which is responsible for generating cAMP. Wu et al (1995) found that the magnitude of CA1 LTP was reduced during the initial 30 min after three repeated trains of tetanus in I-AC mutant mice, whereas l-LTP (up to 4 h) was unaffected. Overall, mutant mice deficient in the cAMP pathway have not yet provided a clear picture of the specific role of cAMP and the cAMP-responsive transcription factor in l-LTP.

LTP at the Perforant-Granule Synapses and Mossy Fiber-CA3 Synapses

Dentate LTP is assumed to be generated by the same mechanisms as CA1 LTP (Bliss & Collingridge 1993). However, the notion that dentate LTP and CA1 LTP are generated by a common mechanism is challenged by two studies on mutant mice. We found that dentate LTP is unaltered in PKC γ mutant mice (C Chen, LC Butler, JO McNamaram & S Tonegawa, unpublished data), whereas CA1 LTP had been shown to be impaired (Abeliovich et al 1993a). Nosten-Bertrand et al (1996) showed that in vivo dentate LTP is impaired but CA1 LTP is normal in mutant mice deficient in the neuronal glycoprotein Thy-1. These results indicate that dentate LTP may be distinct from CA1 LTP in some biochemical cascades, although both depend on NMDA receptors.

Mutant mice lacking either the catalytic subunit isoform (C β 1) or the regulatory subunit isoform (RI β) of PKA are deficient in mossy fiber LTP but not in CA1 LTP (Brandon et al 1995, Huang et al 1995). These results confirm a

critical role of PKA in the induction of mossy fiber LTP, which was shown previously by pharmacological studies (Huang et al 1994, Weisskopf et al 1994). In contrast to PKA mutant mice, PKC γ mutant mice exhibit an apparently intact mossy fiber LTP (Hsia et al 1995). Conquet et al (1994) reported impaired mossy fiber LTP in their line of mGluR1 mutant mice, while Hsia et al (1995) showed that mossy fiber LTP is intact in Aiba et al's (1994a) mutant line. This is the same discrepancy that appeared in studies of CA1 LTP, and again, no obvious explanation can be given for these divergent results.

In sum, most results obtained with gene knockout mice complement those obtained by pharmacological studies; in some cases, gene knockout studies extend the pharmacological studies by specifying the role of a particular isoform. However, the genetic approach has yet to provide a complete picture of the molecular cascades essential for LTP. One future direction is to "rescue" LTP in mutant mice by pharmacological or genetic manipulations. Such rescue experiments can further define the extent and specificity of deficits caused by the single gene knockout and help to identify other molecules interacting with the missing gene product.

SYNAPTIC PLASTICITY AND HIPPOCAMPUS-DEPENDENT MEMORY

Hebb (1949) postulated that associative learning might be based on strengthening of synaptic connections among neurons that are coincidentally active. Hippocampal LTP appears to satisfy this coincidental activity rule (Bliss & Lomo 1973): Both presynaptic stimulation and postsynaptic depolarization are required for its induction; the potentiation is specific for the activated synapses; and it is long-lasting well beyond initial stimulation (see also Bliss & Collingridge 1993). These properties make LTP an attractive candidate mechanism for learning and memory.

The hippocampus is a major component of the medial temporal lobe, a brain system that plays an important role in declarative or relational memory (for review, see Eichenbaum et al 1992, Squire 1992). In this brain system, synaptic plasticity at the trisynaptic circuit of the hippocampus has been best studied in relation to learning and memory (see Figure 2). Previous lesion studies in rodents confirmed that two forms of declarative memory, spatial memory and contextual fear memory, depend on the intact hippocampus (Blanchard & Fial 1968, Morris et al 1982, Kim & Fanselow 1992, Phillips & LeDoux 1992, Chen et al 1996). Furthermore, both spatial memory and contextual fear memory have been shown to be disrupted by AP5, a specific antagonist for NMDA receptors, which could also block dentate LTP *in vivo* (Morris et al 1986, Kim et al 1991). These results support the notion that the form of LTP requiring the activation of

NMDA receptors is critical for these forms of hippocampus-dependent learning and memory (Swanson et al 1982, McNaughton et al 1986).

These combined pharmacological and behavioral studies have a number of caveats. For instance, since AP5 blocks NMDA receptor-dependent LTP and LTD, one cannot conclude which of these two forms of synaptic plasticity underlies the tested learning ability. Another uncertainty accompanying these studies is related to the site of synaptic plasticity. Is LTP (or LTD) at all three types of excitatory hippocampal synapses needed for spatial or contextual learning? Or, is LTP at one or two types of these synapses dispensable? Since each gene knockout mouse exhibits a distinct pattern of deficiency or impairment with respect to the form of synaptic plasticity and the type of hippocampal synapses (see above), the combined electrophysiological and behavioral studies of multiple gene knockout mice may be enlightening.

An Important Role of CA1 LTP in Spatial and Contextual Learning

The first (α CaMKII) and the second (*fyn*) gene knockout mice analyzed by a learning task (Morris water maze) provided data that are consistent with the notion that CA1 LTP underlies spatial learning (Grant et al 1992; Silva et al 1992a,b). Subsequently, PKC γ mutant mice, which are deficient in the conventionally induced CA1 LTP but are proficient in primed LTP, were shown to exhibit mild deficits in both spatial learning (Morris water maze) and contextual fear conditioning (Abeliovich et al 1993a,b). These results were interpreted to indicate that PKC γ deficiency results in a modification in the ability of CA1 synapses to have LTP induced and that this modification underlies the observed mild deficits in spatial and contextual learning.

More recently, Conquet et al's (1994) line of mGluR1 mutant mice was reported to have an impairment in spatial learning. As mentioned earlier, Conquet et al reported that CA1 LTP was normal in the hippocampal slices derived from their mGluR1 mutant mice. If these claims hold, a serious blow has been dealt to the notion that CA1 LTP underlies spatial learning. However, as was also mentioned earlier, the Aiba et al's (1994a,b) mGluR1 mutant mice have been reported to exhibit a reduced CA1 LTP and a moderate deficit in contextual fear conditioning. This latter work, in contrast to Conquet et al's, supports the hypothesis that CA1 LTP is the cellular substrate for contextual learning (Aiba et al 1994a,b). Both PKC γ (Abeliovich et al 1993a) and mGluR1 (Aiba et al 1994a) mutant mice exhibit apparently normal CA1 LTD. These data combined with the deficits in spatial and contextual conditioning observed in these mutant mice do not support the hypothesis that CA1 LTD plays a primary role in these forms of learning. This conclusion is further supported by the analysis of PKA mutant mice, which exhibited impaired CA1 LTD and normal spatial and

contextual learning (Brandon et al 1995, Huang et al 1995). Thus, except for Conquet et al's (1994) mGluR1 mutant study, researchers have correlated an impairment in CA1 LTP with an impairment in spatial learning and contextual fear conditioning (see Table 2).

Is CA1 I-LTP Important for Memory Consolidation?

The transfer of information from short-term memory to long-term storage in the nervous system is thought to require new protein synthesis, a conclusion based on experiments with fish, rodents, and birds, using a variety of tasks (for review, see Davis & Squire 1984). Previous work in the marine snail *Aplysia* suggests a link among the increase in intracellular cAMP levels, protein synthesis, and memory consolidation (Goelet et al 1986, Dash et al 1990, Alberini et al 1994). As mentioned earlier, in both the CA1 and CA3 regions of the hippocampus taken from normal rats, I-LTP induced by repeated high-frequency stimulations is blocked by antagonists or inhibitors of cAMP signaling and by inhibitors of protein synthesis (Frey et al 1993, Huang et al 1994, Nguyen et al 1994).

Studies of CREB mutant mice have suggested a role for the cAMP-responsive transcription factor in memory consolidation (Bourtchouladze et al 1994). CREB mutant mice display normal contextual fear memory at 30 min after training. However, they begin to lose the memory 1 h after training, and show even greater loss of memory 24 h later. Thus, it appears that the memory impairment, in CREB mutant mice, is selective for memories lasting longer than 30 min. Bourtchouladze et al (1994) suggest that the impairment in I-LTP (90–120 min after stimulation) in the CA1 area correlates with the long-term memory deficit. But this correlation is weakened by the observation that CREB mutant mice are also impaired in the consolidation of cued fear conditioning, a hippocampus-independent task.

The type I adenylyl cyclase (I-AC) mutant mice, which have reduced CA1 LTP, learn to find a hidden platform in the Morris water maze normally (Wu et al 1995), but they do not display a preference for the region where the platform had been after it is removed, suggesting a weak spatial memory. The memory impairment in I-AC mutant mice is correlated with an impairment in LTP that occurs within 30 min after the tetanus, whereas I-LTP seems normal in the mutant mice.

Dentate LTP and Mossy Fiber LTP May Be Dispensable for Spatial and Contextual Learning

Since dentate LTP and mossy fiber LTP are normal in PKC γ mutant mice (see above), and CA1 LTP and the contextual fear conditioning are impaired (Abeliovich et al 1993a, b), neither dentate LTP nor mossy fiber LTP seem to be able to support full-fledged contextual conditioning. Indeed, Huang et al

(1995) found that the defect of mossy fiber LTP in the mutant mice lacking the $C\beta 1$ or the $RI\beta$ isoform of PKA does not disrupt spatial and contextual learning, indicating that mossy fiber LTP is dispensable for these forms of learning. Dentate LTP also seems to be dispensable because the mutant mice deficient in *Thy-1* are impaired in dentate LTP but are unimpaired in spatial learning (Nosten-Bertrand et al 1996). These mutant mice exhibit normal CA1 LTP, which is again consistent with the notion that CA1 LTP is crucial for spatial learning.

In summary, most of the results from gene knockout mutant mice appear to support the view that LTP in the CA1 region is the primary synaptic mechanism for hippocampus-dependent spatial and contextual learning. In contrast to CA1 LTP, dentate LTP and mossy fiber LTP seem to be dispensable for these forms of learning as long as CA1 LTP is intact. However, these conclusions do not imply that these types of synaptic plasticity do not contribute to spatial and contextual learning in wild-type animals.

MECHANISMS OF SYNAPTIC PLASTICITY IN THE CEREBELLUM

In the cerebellum, LTD can be induced at PF-PC synapses following conjunctive stimulation of PFs and CFs (Ito et al 1982, Sakurai 1987, Ito 1989). The molecular mechanisms underlying cerebellar LTD are beginning to be elucidated (for review, see Linden 1994). In addition to an elevation of intracellular calcium (Sakurai 1990, Konnerth et al 1992), activation of both ionotropic AMPA receptors and mGluRs are thought to be necessary for LTD induction (Linden et al 1991, Daniel et al 1992, Hartell 1994). Antibodies against mGluR1, a subtype of mGluRs that is abundant in PCs (Shigemoto et al 1992, Fotuhi et al 1993), block LTD induction in a cell culture system (Shigemoto et al 1994). Activation of PKC by phorbol ester results in LTD (Crepel & Krupa 1988, Linden & Connor 1991), whereas inhibitors of PKC prevent the induction, but not the maintenance, of LTD (Linden & Connor 1991).

Cerebellar LTD is clearly deficient in the two independently generated lines of mGluR1 mutant mice (Aiba et al 1994b, Conquet et al 1994). In contrast, other electrophysiological properties, such as excitatory synaptic transmission and short-term synaptic plasticity at both PF-PC and CF-PC synapses, seem to be normal in these mutants. The anatomy of the cerebellum does not appear to be overly disturbed (based on examination with both light and electron microscopy). These data suggest that mGluR1 is essential for cerebellar LTD. Activation of mGluR1 at the PF-PC synapses results in the release of Ca^{2+} from intracellular stores and in the activation of PKC (Nishizuka 1988, Aramori & Nakanishi 1992). Although the Ca^{2+} release is too small to induce LTD, the

Ca²⁺ elevation that follows the activation of CF-PC synapses is much more pronounced (Konnerth et al 1992, Miyakawa et al 1992, Takei et al 1992). Based on previous pharmacological studies (Crepel & Krupa 1988, Linden & Connor 1991) and on the mGluR1 mutant (Aiba et al 1994b) studies, researchers have concluded that for the induction of LTD the activation of PKC rather than the Ca²⁺ release is the crucial event that follows the mGluR1 activation at PF-PC synapses.

Since PKC γ is the major isoform of PKC present in PCs, one might expect that LTD would be deficient or reduced in PKC γ mutant mice; however, this result was not found (Chen et al 1995). Nevertheless, PKC γ may play a role in the induction of LTD in wild-type mice. This possibility is suggested by the differential inhibitory effect of a PKC inhibitor, peptide PKC(19–36), on LTD induced in wild-type and PKC γ mutant mice. In agreement with earlier reports (Linden & Connor 1991, Hemart et al 1995), this inhibitor blocked the induction of LTD in wild-type slices. In contrast, in the mutant slices LTD was not abolished entirely by the inhibitor; only the magnitude was reduced (Chen et al 1995). This observation suggests that the mutant LTD involves other PKC isoforms that are less sensitive to the inhibitor than the one responsible for the wild-type LTD.

Another unexpected finding came from the analysis of mutant mice deficient in glial fibrillary acidic protein (GFAP). GFAP is an intermediate filament protein highly expressed in cerebellar Bergmann glia. It was reported that LTD is not induced in the cerebellar slices derived from GFAP mutant mice (Shibuki et al 1996). This finding suggests that LTD induction requires communication between PCs and Bergmann glia. In cultured cells, neurotransmitter-mediated communication between neurons and glia has been demonstrated (Nedergaard 1994, Parpura et al 1994), but the precise mechanism of glia-neuron communication in synaptic plasticity remains unknown. As mentioned earlier, cerebellar LTD is also impaired in GluR δ 2 mutant mice (Kashiwabuchi et al 1995).

SYNAPTIC PLASTICITY AND CEREBELLUM-DEPENDENT MEMORY

The classic neurological concept that the cerebellum is a site of motor control originates from observations that patients with cerebellar damage often exhibit motor-related clinical symptoms such as gait ataxia, dysmetria, hypotonia, and tremor (Dow & Moruzzi 1958). Thach et al (1992) suggested that the adaptive role of the cerebellar cortex is to combine simpler elements of movement into more complex coordinated acts. They also suggest that the cerebellum allows simple, stereotyped reflexes to adapt appropriately to different task conditions. These notions imply two distinct functions of the cerebellum: learning

associated with component movements (discrete motor learning) and smooth performance of compound movements (motor coordination).

Evidence suggests that stored in the cerebellum are the memory traces for discrete motor learning, such as adaptation of vestibulo-ocular reflex (VOR) (for a review, see Ito 1984) and classical conditioning of eyeblink response (McCormick & Thompson 1984, Yeo et al 1984, Lavond et al 1985, Thompson 1986, Skelton 1988, Daum et al 1993, Krupa et al 1993, Molchan et al 1994).

Like hippocampal LTP, cerebellar LTD also satisfies the properties postulated by Hebb (1949): LTD is induced when PF-PC synapses are activated within a certain short period during which postsynaptic depolarization occurs as a consequence of the activation of CF-PC synapses; the depression is specific to the activated PF-PC synapses; and it is long lasting (Ito 1989, Linden 1994). The associative nature of cerebellar LTD, by which temporally conjugated activation of two afferent pathways but not of each alone is required for its induction, makes this form of synaptic plasticity particularly suitable for associative learning and memory. Thus, LTD at PF-PC synapses has been suggested as a cellular substrate for discrete motor learning (Ito et al 1982, Ito 1989, Thompson et al 1993, Ito 1994, Chen & Thompson 1995). Furthermore, LTD has been proposed to underlie motor coordination (Ito 1984). However, little direct evidence has linked LTD to discrete motor learning. In addition, it is not clear whether motor coordination depends on an animal's ability to acquire discrete motor learning and in turn on the presence of cerebellar LTD. Recent progress made using gene knockout mice has shed some light on these issues.

Role of LTD in Associative Learning of Discrete Motor Responses

During eyeblink conditioning, an initially neutral tone (known as a conditioned stimulus or CS) is paired with an airpuff or a peri-orbital shock (known as an unconditioned stimulus or US). To achieve effective conditioning, the onset of the CS has to precede the US by about 250 ms. This temporal requirement for the CS and the US is a common feature in all forms of classical conditioning (Rescorla 1988). Before conditioning, the CS (tone) alone does not elicit an eyeblink, but the US (airpuff or shock) does (called the unconditioned response or UR). After more than 100 trials pairing the CS and US, the animal learns that the tone is a reliable predictor of the US, so that the tone alone evokes an eyeblink (called the conditioned response or CR). As training proceeds, there is a gradual increase in both the probability and the amplitude of tone-evoked eyeblinks (i.e. CRs).

Based on lesion, recording, and stimulation studies, Thompson (1986) proposed the putative neural circuit for eyeblink conditioning: The tone signal (CS) enters the cerebellum via mossy fibers, while the airpuff or shock signal

Table 3 Summary of impairments in motor behaviors, climbing fiber innervation, and synaptic plasticity in the cerebellum

Mutant mice	Motor coordination	Eyeblink conditioning	CF innervation	LTD	References
PKC γ	Impaired	Facilitated	Multiple	Normal	Chen et al 1995 Kano et al 1995
mGluR1	Impaired	Impaired	Multiple	Impaired	Aiba et al 1994a,b Conquet et al 1994 Kano et al, unpublished observations
GluR δ 2	Impaired	Not tested	Multiple	Impaired	Kashiwabuchi et al 1995
GFAP	Normal	Impaired	Normal	Impaired	Shibuki et al 1996

(US) enters via climbing fibers. Purkinje cells send outputs to a deep cerebellar nucleus, the interpositus nucleus, which in turn projects to the red nucleus in the brain stem. Two sensory signals converge on the PCs and deep nuclear neurons. According to this neural circuit, two forms of synaptic plasticity could be involved in associative learning of eyeblink response (see Figure 3). LTD at PF-PC synapses decreases the excitability of PCs, which send an inhibitory output, the sole output of the cerebellar cortex, to deep cerebellar nuclear neurons. Thus, LTD disinhibits the nuclear neurons. The second form of synaptic plasticity is long-term enhancement at excitatory synapses from mossy fibers to nuclear neurons, as one study has suggested (Racine et al 1986). The net effect of LTD at PF-PC synapses and the enhancement at mossy fiber-nuclear neuron synapses is to increase the excitability of nuclear neurons. Thus, the signal transfer function through the cerebellum in response to the CS (tone) is enhanced, leading to the emergence of the CR (Figure 3).

The role of cerebellar LTD in associative eyeblink conditioning has been examined in mGluR1 mutant mice generated by Aiba et al (1994b). Cerebellar LTD is clearly deficient in the mGluR1 mutant mice, and the amplitude of the conditioned response is reduced (Aiba et al 1994b). Thus, cerebellar LTD appears to be involved in eyeblink conditioning. This correlation is further supported by studies of other mutant mice (see Table 3). GFAP mutant mice have deficient LTD and impaired eyeblink conditioning (Shibuki et al 1995). Although eyeblink conditioning is markedly impaired, both mGluR1 and GFAP mutant mice still retain some degree of eyeblink conditioning (Aiba et al 1994b, Shibuki et al 1996). Thus, an apparently complete loss of LTD in the cerebellar cortex causes only a partial deficit in eyeblink conditioning, suggesting that the LTD plays only a partial role in this learning. This conclusion seems

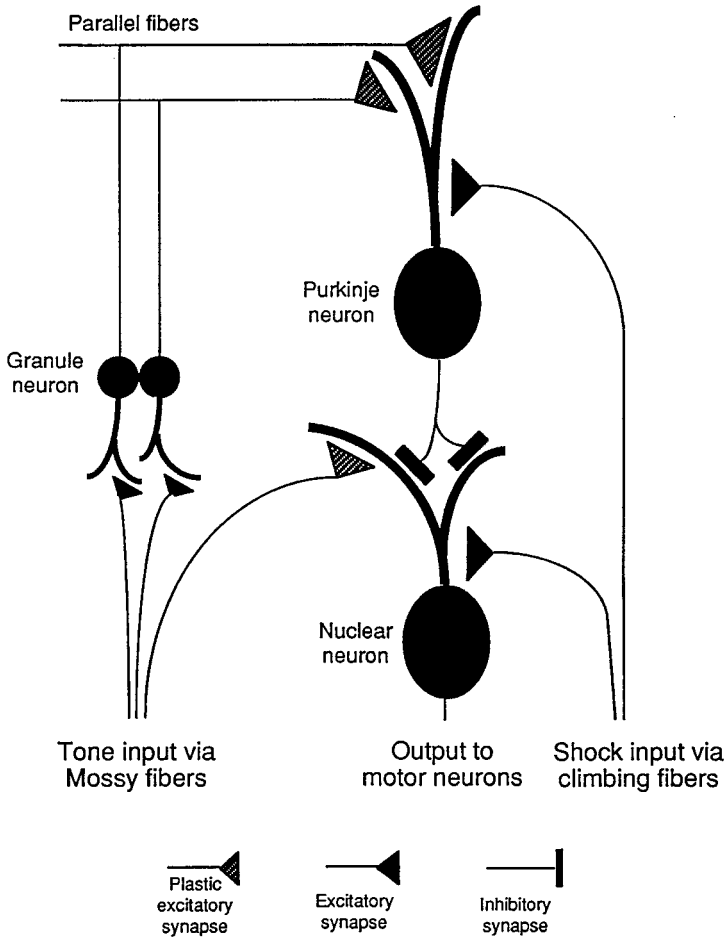


Figure 3 A model describing the role of synaptic plasticity in the cerebellum in eyeblink conditioning, based on the neural circuit proposed by Thompson (1986). Two forms of synaptic plasticity are postulated to support the conditioning: (a) LTD at PF-PC synapses decreases the excitability of PCs in response to the CS. Since PCs send an inhibitory output to deep cerebellar nuclear neurons, LTD reduces the inhibition in the latter. (b) LTP occurs at excitatory synapses from mossy fibers to nuclear neurons. The net effect of the LTD and the LTP is increased excitability of output nuclear neurons. As a result, the signal transfer function of the cerebellum is enhanced in response to the CS. CS, conditioned stimulus (i.e. tone); PCs, Purkinje cells.

to corroborate with the results of previous lesion studies: Lesions limited to the cerebellar cortex cause only partial impairment in eyeblink conditioning, whereas lesions in the interpositus nucleus, a deep cerebellar nucleus, result in a complete deficit (for review, see Thompson & Krupa 1994). It would be helpful if future studies evaluated the role of synaptic plasticity in the deep cerebellar nuclei.

Eyeblink conditioning is facilitated in the early stage of training in PKC γ mutant mice (Chen et al 1995). One possible explanation is that the multiple CF innervation that occurs in these mice (see above) results in a higher than normal number of impulses for PCs. During the acquisition of CRs, a higher number of complex spikes may be elicited in response to the US in PKC γ mutant PCs than in wild-type PCs. Consequently, LTD induction may be facilitated in the mutant mice, leading to a faster acquisition of CRs. The validity of this hypothesis could be tested by monitoring complex spikes in PCs that occur during eyeblink conditioning.

Role of LTD and Discrete Motor Learning in Movement Coordination

We now turn to the issue of whether LTD inducibility and discrete motor learning capability are required for motor coordination (see Table 3). mGluR1 mutant mice are deficient in LTD, are impaired in eyeblink conditioning, and exhibit motor discoordination (Aiba et al 1994b, Conquet et al 1994). PKC γ mutant mice retain LTD inducibility and are capable of eyeblink conditioning but are impaired in motor coordination (Chen et al 1995). Thus, PKC γ mutant mice seem to be able to acquire the memory for movement components but cannot properly combine them spatially and temporally to achieve complex coordinated acts. GFAP mutant mice exhibit a phenotype opposite to that of PKC γ : deficient LTD, impaired eyeblink conditioning, and normal motor coordination (Shibuki et al 1996).

The three mutant mice displaying motor discoordination all harbor multiple innervation of PCs by CFs in the mature cerebellum (see Table 3). On the basis of physiological deficits and behavioral consequences observed in these mutant mice, Chen et al (1995) proposed that mono-innervation of PCs by CFs is required for motor coordination (see Figure 4). The cerebellar cortex is composed of numerous distinct compartments known as *microzones*, which are thought to be the functional units that control component movement (Oscarsson 1979, Ito 1984). PCs in each microzone receive common excitatory inputs from CFs that emanate, in normal adult animals, from a distinct subgroup of olivary neurons in the brain stem. A compound movement requires a program that will coordinate many component movements in an orderly fashion (Thach et al 1992). It is generally believed that the basic program for a compound

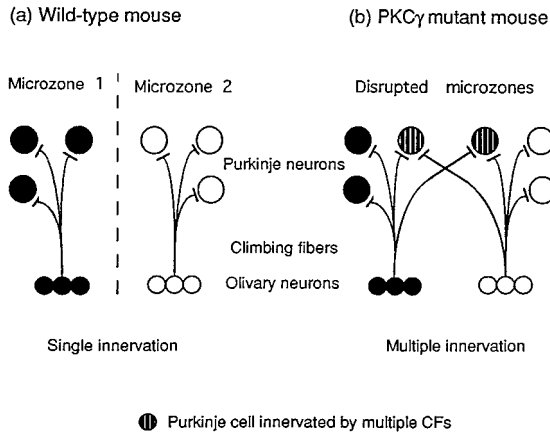


Figure 4 A schematic diagram depicting the role of CFs in motor coordination (modified from Chen et al 1995). Olivary neurons are organized into various subgroups (solid vs open circles). (a) In the wild-type cerebellum, each PC is innervated by only one CF and thus can be driven only by one subgroup of olivary neurons at a given time in a given movement. Each functional unit, which corresponds to a microzone, of PCs (solid vs open circles) presumably corresponds to each component movement in a coordinated act. (b) In $PKC\gamma$ mutant mice, certain PCs are innervated by multiple CFs. These PCs would be driven by mixed subgroups of olivary neurons (striped circles). Thus, multiple CF innervation could disrupt either the delivery of the refined motor program or the error-correction mechanism, eventually leading to impaired motor coordination in the mutant mice. CFs, climbing fibers; PCs, Purkinje cells.

movement, such as walking, resides in the cerebral cortex (Allen & Tsukahara 1974, Fetz 1993). Furthermore, such a basic cerebral program has to be modified or refined in the cerebellum if the animal is to perform the movement smoothly or skillfully (Ito 1984, Thach et al 1992).

Neuroscientists differ in their views on how the cerebellum and its associated structures accomplish program refinement. One view is that the refinement instruction for coordinated movements is generated in the inferior olive by dynamic synchronization of neuronal subgroups (Llinas & Yarom 1981, 1986; Llinas & Welsh 1993; Welsh et al 1995). According to this idea, CFs transmit to PCs signals for a refined program. In adult wild-type mice with mono-innervation of PCs by CFs, each PC presumably would follow the direction of one subgroup of synchronous olivary neurons at a given time in a given movement. By contrast, in either adult $PKC\gamma$, $mGluR1$, or $GluR\delta 2$ mutant mice or wild-type mice younger than three weeks, certain PCs with multiple CF innervation would receive inputs from olivary neurons of two or more different subgroups. This would make it difficult for such PCs to respond in synchrony with a particular subpopulation of olivary cells (see Figure 4). Thus,

the coherence of neural activity in each cerebellar functional unit would be disrupted and motor coordination impaired.

Another view is that CFs transmit to PCs so-called error signals that are detected by inferior olivary neurons as the difference between the intended and executed movements (Marr 1969, Albus 1971, Ito 1984, Gellman et al 1985, Thach et al 1992). The error signal transmitted by a CF to a PC will induce LTD in the PF-PC synapses (Ito 1989), which results in a suppression of the neural activity in the neural circuit responsible for the erroneous movement. The single innervation would assure exquisite specificity in the error-correction mechanism. In animals with multiple CF innervation, PCs of a microzone would receive not only the proper error signal but also other signals via the surplus CFs (see Figure 4). The strength of PF-PC synapses in certain microzones would be inappropriately modified by wrong error signals. As a consequence, the cerebellum would not be able to properly control compound movements. If LTD is a part of the error-correction mechanism, why do GFAP mutant mice, which lack LTD, show apparently normal motor coordination? One possibility is that the employed behavioral tests for motor coordination are not sensitive enough to detect subtle impairments. Another possibility is that other forms of synaptic plasticity are sufficient for the smooth operation of the tested behavioral tasks.

Recent data (see Table 3) are compatible with both views on the role of CFs in motor coordination: transmission of motor program-refining signals by dynamic synchronization of olivary neuronal subgroups (Llinas & Welsh 1993), or transmission of error-correction signals for synaptic plasticity (Ito 1984). The studies on gene knockout mice, especially those on PKC γ mutant mice, have provided an attractive hypothesis for the physiological role of the developmental elimination of the surplus CFs.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Studies on gene knockout mice have contributed significantly to our understanding of the mechanisms of synaptic plasticity and its role in the activity-dependent phase of neural development, learning, and memory. The specific progress made has demonstrated the following:

1. NMDA receptors are critically important for whisker-related pattern formation.
2. PKC γ is a key molecule for activity-dependent elimination of surplus CFs during cerebellar development.
3. NO is a strong candidate for a retrograde messenger for CA1 LTP.

4. CA1 LTP as opposed to mossy fiber or dentate LTP plays a particularly critical role in the hippocampus for spatial and contextual learning.
5. Cerebellar LTD contributes to discrete motor learning.
6. Mono-innervation of PCs by CFs is crucial for motor coordination.

Although gene knockout technology is useful in neuroscience, it has definite limitations. The major limitation is that there is no regional or temporal restriction in the deletion of the gene. Consequently, if the gene product is expressed and plays a role in neural development as well as in the functioning of the mature brain, it is often difficult to decide whether the observed abnormality was caused by the lack of one or the other of these dual roles. Likewise, when the gene product is widely expressed in the brain and is involved in multiple physiological processes it can be difficult to determine whether the lack of the gene product in the brain area of interest (e.g. hippocampus, cerebellum) is responsible for the observed higher-order abnormality. Another shortcoming of current gene knockout technology is not-so-infrequent cases of premature death of the mutant mice. This shortcoming often arises from a regionally unrestricted deletion of the gene.

These problems may be overcome by improvements of the technology that allow a gene deletion in a region-specific and/or temporally controlled fashion. The regional restriction of the gene knockout can be achieved by using region-specific promoters, such as the L7 promoter, which is reported to be active in cerebellar PCs and retinal rod bipolar neurons (Oberdick et al 1990). A region-specific promoter can drive the expression of the phage P1-derived Cre recombinase, which deletes the target gene that has been flanked by a pair of Cre-recognition sequences called *loxPs* (Sauer 1993). This method has been used successfully in the immune system to produce a cell-type-specific knockout of DNA polymerase β (Gu et al 1994).

Temporal control of gene expression has been achieved in many non-neural tissues in transgenic mice by a tetracycline-responsive promoter (Furth et al 1994) or an interferon-responsive promoter (Kuhn et al 1995). Tetracycline binds to a trans-activator (tTA) and prevents it from interacting with the *tet* operator sequence in the promoter region of the targeted gene, hence, turning off its expression (Furth et al 1994). Furthermore, a reverse trans-activator (rtTA) system has also been developed, in which tetracycline turns on the gene expression (Gossen et al 1995). Thus, a mouse can be produced in which the expression of the Cre recombinase can be controlled temporally by administration of tetracycline. Breeding of such mice with a *loxP*-gene *X-loxP* mouse can generate mice in which the deletion of gene X is controlled temporally by tetracycline administration. If one utilizes a tissue-specific promoter to drive

tTA or rtTA expression, both regional restriction and temporal control of the gene deletion can be achieved.

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