Functions and Roles of Glutamate Receptors in Synaptic Transmission and Plasticity

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Glutamate receptors mediate most excitatory synaptic transmission in the central nervous system and play an essential role in neural plasticity, neural development, and neurodegeneration (Nakanishi 1992, 1994; Hollmann and Heinemann 1994; Nakanishi and Masu 1994). There are two general groups of glutamate receptors (Nakanishi 1992, 1994; Hollmann and Heinemann 1994; Nakanishi and Masu 1994). Ionotropic glutamate receptors comprise ligand-gated cation channels and are subdivided into α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate type and *N*-methyl-D-aspartate (NMDA) type of receptors. Metabotropic glutamate receptors are G-protein-coupled receptors that modulate the production of intracellular second messengers. With the aid of a functional cloning strategy that combines electrophysiology and a *Xenopus* oocyte expression system, we cloned and characterized the NMDA receptor and mGluR (Masu et al. 1991; Moriyoshi et al. 1991). Both receptors exist as diverse members of receptor subunits and subtypes (Fig. 1) (Hollmann and Heinemann 1994; Nakanishi and Masu 1994). The NMDA receptor subunits are classified into two distinct groups. NMDAR1 is a key subunit that possesses all properties of the NMDA receptor-channel complex, whereas four NMDAR2 subunits (NMDAR2A–2D) serve as modulatory subunits that potentiate and differentiate NMDA receptor activity by heteromeric formation with NMDAR1. The mGluRs form a family consisting of at least eight different subtypes (mGluR1–mGluR8) (Fig. 1) (Nakanishi 1994; Pin and Duvoisin 1995). mGluR1 and mGluR5 are coupled to inositol triphosphate/Ca^{++} signal transduction and respond strongly to quisqualate. The other six subtypes are linked to the inhibitory cascade of cAMP formation. mGluR2 and mGluR3 react effectively with (2S, 1'R, 2'R, 3'R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) (Hayashi et al. 1993), whereas mGluR4, mGluR6, mGluR7, and mGluR8 respond potently to L-2-amino-4-phosphonobutyrate (L-AP4). The mGluR subtypes are thus subdivided into three groups according to their sequence similarities, signal transduction mechanisms, and agonist selectivities (Nakanishi 1994; Pin and Duvoisin 1995). It is thus important to explore roles of diverse members of glutamate receptors in brain function and dysfunction on the basis of the knowledge of the molecular diversity of glutamate receptors. This paper deals with some specialized functions of the diverse members of mGluRs in the central nervous system.

EXPERIMENTAL PROCEDURES

Targeted disruption of the mGluR2 and mGluR6 genes was carried out with the use of an embryonic cell line (Masu et al. 1995; Yokoi et al. 1996). ON and OFF responses were recorded from the superior colliculus with extracellular recording techniques (Masu et al. 1995). GABA-mediated inhibitory postsynaptic currents (IPSCs) at a mitral cell of the accessory olfactory bulb (AOB) were recorded by the slice patch-clamp recording methods after electrical stimulation of a granule cell (Hayashi et al. 1993). Basal synaptic transmission and paired-pulse facilitation at the mossy fiber-CA3 synapses were determined in hippocampal slices (Yokoi et al. 1996). Long-term potentiation (LTP) and long-term depression (LTD) at the mossy fiber-CA3 synapses were induced by tetanic stimulation (100 Hz, 1 sec) and low-frequency stimulation (1 Hz, 15 min), respectively (Yokoi et al. 1996). Immunostaining of mGluR2 and mGluR6 was carried out with use of the mGluR2/mGluR3 antibody and the mGluR6 antibody, respectively (Nomura et al. 1994; Yokoi et al. 1996).

RESULTS AND DISCUSSION

mGluR6 in Visual Information Processing

Visual signals are transmitted from photoreceptors to bipolar cells. A key process of visual information transmission is detecting visual contrasts (Dowling 1987; Shepherd and Koch 1990; Schiller 1992; DeVries and Baylor 1993). Photoreceptors hyperpolarize by light exposure and reduce glutamate release. In turn, ON and OFF bipolar cells depolarize and hyperpolarize, respectively. The opposite reaction occurs by dark stimulus. Thus, the visual signals are segregated into parallel ON-center and OFF-center pathways at the level of bipolar cells (Schiller 1992; Wässle et al. 1991; DeVries and Baylor 1993). Electrophysiological evidence has indicated that a putative L-AP4-sensitive mGluR subtype mediates postsynaptic responses of ON-bipolar cells by enhancing cGMP hydrolysis similar to that of signal transduction in photoreceptors (Nawy and Jahr 1990, 1991; Shiells and Falk 1990). We cloned mGluR6 from a retinal cDNA library. mGluR6

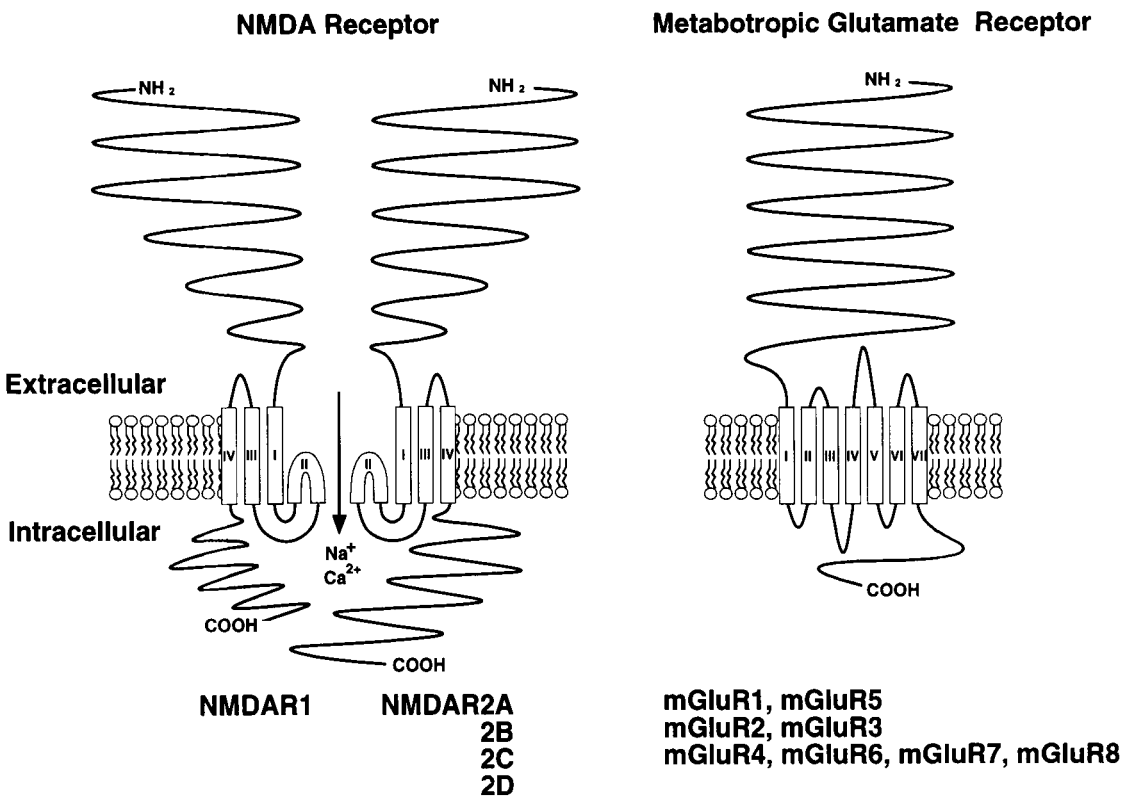

Figure 1. Structural models of NMDA receptors and mGluRs.

is exclusively expressed in the retinal bipolar cell layer and selectively responds to L-AP4 in CHO cells expressing the recombinant mGluR6 (Nakajima et al. 1993). mGluR6 immunoreactivity is restrictedly located at the outer plexiform layer where photoreceptors form synaptic contacts with bipolar cells (Fig. 2A) (Nomura et al. 1994). Furthermore, immunoelectron microscopy disclosed a specific and selective localization of mGluR6 at the postsynaptic site of rod (ON-type) bipolar cells (Nomura et al. 1994). In addition, mGluR6 is localized from the cell body to the postsynaptic site during retinal development, and this change in the mGluR6 subcellular localization agrees well with the synaptic formation between photoreceptors and bipolar cells (Nomura et al. 1994). These observations strongly suggest that mGluR6 functions as an mGluR subtype responsible for ON responses.

Taking advantage of the restricted expression of mGluR6 in retinal ON-bipolar cells, we generated knockout mice lacking mGluR6 expression (Masu et al. 1995). The homozygous mGluR6-deficient mice showed no apparent behavioral abnormality and no gross anatomical changes in the retinal cell organization or in the projection of optic nerve fibers to the brain. When electroretinograms were monitored in mGluR6-deficient mice, the b-wave that represents ON responses never appeared by exposure to any intensities of light (Masu et al. 1995). More direct evidence for the deficit of ON responses in the mGluR6 deficiency was indicated by recording field potentials from the superior colliculus, a major target of the optic nerve fibers. This recording indicated that the mGluR6 deficiency results in a complete loss of ON responses to light stimulus but unchanged OFF responses to dark stimulus (Fig. 2B) (Masu et al. 1995). Thus, mGluR6 is essential for synaptic transmission in the ON pathway.

Despite the lack of light-induced ON responses in mGluR6-deficient mice, they retained the ability to respond to visual inputs as assessed by shuttle box avoidance behavioral analysis in conjunction with light exposure (Masu et al. 1995). Furthermore, the mGluR6-deficient mice were unaltered in a light-dark cycle of daily locomotor activity as well as in light-stimulated induction of Fos immunoreactivity in the suprachiasmatic nuclei (H. Iwakabe et al., in prep.). These results demonstrate that the deficit of ON responses does not completely disrupt responsiveness to visual stimulation and lead to the conclusion that OFF responses also serve as an important signal for transmission of visual information.

We examined possible visual dysfunction that results from the deficit of ON responses (H. Iwakabe et al., in prep.). The pupillary response is controlled by different pretectal neurons that distinctly respond to light and dark stimuli (Clarke and Ikeda 1985). Thus, ON and OFF responses are thought to be critical for the pupillary reflex. Wild-type mice showed light-induced responses of pupillary contraction with an approximately straight-line relationship between the pupil diameter and the logarithmic units of light in-

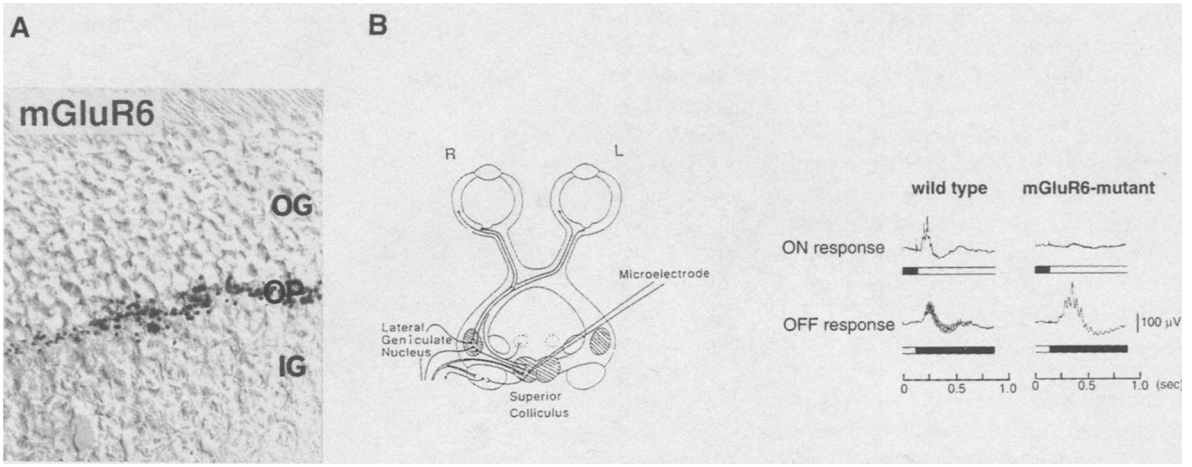

Figure 2. mGluR6 immunostaining in the retina and specific deficit of ON responses in mGluR6-deficient mice. (*A*) Intense punctate mGluR6 immunoreactivity is seen only at the outer plexiform layer (OP) of the wild-type retina (Nomura et al. 1994). (OG) Outer nuclear layer; (IG) inner nuclear layer. (*B*) In mGluR6-deficient mice, ON responses to light stimuli (*open bar*) were abolished without any changes in OFF responses to dark stimuli (*filled bar*) (*right*) when ON and OFF responses were recorded from the superior colliculus (*left*) (Masu et al. 1995).

tensity (H. Iwakabe et al., in prep.). In contrast, mutant mice exhibited little or no pupillary contraction at low luminances, and the pupillary contraction occurred only at high luminance (H. Iwakabe et al., in prep.). The intensity-response relationship in mutant mice was thus shifted toward high luminances by about two logarithmic units. Furthermore, the latency period preceding a pupillary contraction was markedly prolonged in mutant mice (H. Iwakabe et al., in prep.). Thus, the pupils of mutant mice respond to light stimulus, but the sensitivity and rapid responsiveness of light-induced pupillary contraction are markedly impaired in mGluR6-deficient mice. We next examined optokinetic nystagmus (OKN) to address the ability of mutant mice to discriminate visual contrasts (H. Iwakabe et al., in prep.). OKN represents a stereotyped pattern of eye movements that keep a moving subject on the retina. OKN thus provides a useful tool for evaluating the ability of detecting visual contrasts (Fig. 3). Wild-type mice showed robust OKN in response to the rotation of both high-contrast (30:1) and low-contrast (2:1) stripes but did not display OKN

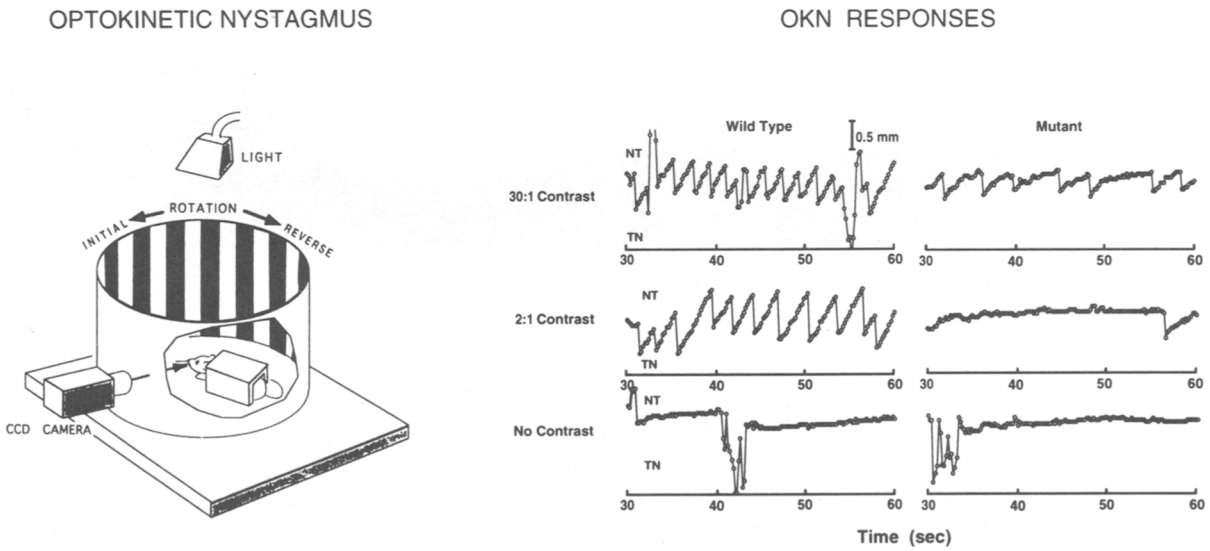

Figure 3. OKN in wild-type and mGluR6-deficient mice. The animal was restrained in the middle of an optokinetic drum with an alternating vertical stripe, and OKN was measured by rotating the optokinetic drum (*left*). mGluR6-deficient mice showed severe impairment in driving OKN in response to a low visual contrast (*right*) (H. Iwakabe et al., in prep.).

in the drum with no contrast. In mutant mice, the high-contrast stimulus did elicit OKN, but with a markedly reduced frequency. Furthermore, OKN was almost abolished in response to the low-contrast stimulus. Thus, the mGluR6 deficiency severely impairs the ability to drive OKN in response to visual contrasts, indicating that mGluR6 is essential for discrimination of visual contrasts (H. Iwakabe et al., in prep.).

On the basis of the characterization of mGluR6 together with the electrophysiological study of ON responses (Nawy and Jahr 1990, 1991; Shiells and Falk 1990), the synaptic mechanism in visual transmission from photoreceptors to ON-bipolar cells can be summarized as follows (Fig. 4) (Nakanishi 1994; Shiells 1994): When photoreceptors respond to light stimulus, cGMP is lowered through the stimulation of phosphodiesterase via transducin (Stryer 1991). This decrease in cGMP hyperpolarizes photoreceptors by closing the cGMP-gated ion channel and reduces glutamate release (Stryer 1991). In the absence of glutamate release, mGluR6 keeps inactive, and a high concentration of cGMP is maintained in ON-bipolar cells and depolarizes these cells by stimulating the cGMP-gated ion channel. Glutamate release from ON-bipolar cells, in turn, increases and excites the subsequent ON pathway.

The key role of mGluR6 in ON-bipolar cells is the conversion of membrane polarization of photoreceptors to an opposite polarization at the postsynaptic bipolar cells. In contrast, OFF-bipolar cells use postsynaptic AMPA receptors and preserve the sign of photoreceptors (de la Villa et al. 1995). Two distinct types of glutamate receptors are thus effectively utilized for evoking ON and OFF responses to the common glutamate transmitter (Fig. 4). Our investigation has provided the compelling evidence that a specific mGluR subtype plays an essential role in synaptic transmission. It is also noted that two G protein-coupled receptors, rhodopsin and mGluR6, are involved in an initial step of visual transmission and are capable of amplifying visual inputs via a second-messenger system (Fig. 4). The peripheral sensory organs receive graded external signals, and these graded signals must be converted to action potentials at an early step of information processing. Thus, the involvement of mGluR6 in visual transmission is important for discrimination and amplification of external signals prior to transmission of these signals to higher centers of the brain.

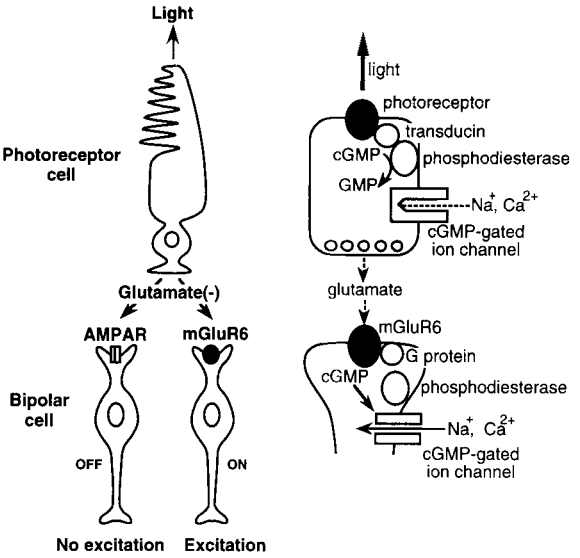

Figure 4. Model for the synaptic mechanism underlying the ON and OFF segregation and for the function of mGluR6 in the ON pathway. (AMPAR) AMPA receptor.

mGluR2 in Olfactory Memory Formation

Most mammalian species possess two olfactory systems, the main olfactory bulb and accessory olfactory bulb (AOB). In both systems, olfactory receptor neurons project axons to glomeruli where they make synaptic contacts with mitral cells (Shepherd and Koch 1990). Granule cells are inhibitory interneurons that form typical dendrodendritic synapses with mitral cells, and these synapses undergo reciprocal regulation (Brennan et al. 1990; Shepherd and Koch 1990); the mitral cell, when activated by olfactory receptor neurons, excites the granule cell via glutamate, and this depolarization in turn releases γ-aminobutyrate (GABA) from the granule cell and hyperpolarizes mitral cells (Fig. 5). The granule cell lacks an axon and forms divergent dendrodendritic synapses not only with an excited mitral cell, but also with a large number of unexcited neighboring mitral cells and is thought to cause both recurrent and lateral inhibition via inhibitory GABA transmission (Shepherd and Koch 1990).

In situ hybridization and immuno-electron-microscopic analysis indicated that mGluR2 is highly expressed and localized at the dendrites of granule cells in the AOB (Hayashi et al. 1993). In contrast, mGluR3 is not appreciably expressed in granule cells. Since DCG-IV was identified as a potent and selective agonist for mGluR2/mGluR3 (Hayashi et al. 1993), we investigated the role of mGluR2 in olfactory transmission by examining the effect of the DCG-IV-induced activation of mGluR2 on GABA transmission from the granule cell to the mitral cell (Fig. 5A) (Hayashi et al. 1993). Whole-cell recording of a mitral cell was performed by the slice patch-clamp recording method. When a granule cell was electrically stimulated, GABA-mediated IPSCs were evoked in a mitral cell. When mGluR2 was activated by the addition of DCG-IV in this system, the GABA-mediated IPSCs were markedly reduced in a reversible manner (Fig. 5A). This observation, as well as others, indicated that glutamate released from an excited mitral cell activates mGluR2 of the granule cell and relieves the mitral cell from the GABA-mediated inhibition (Hayashi et al.

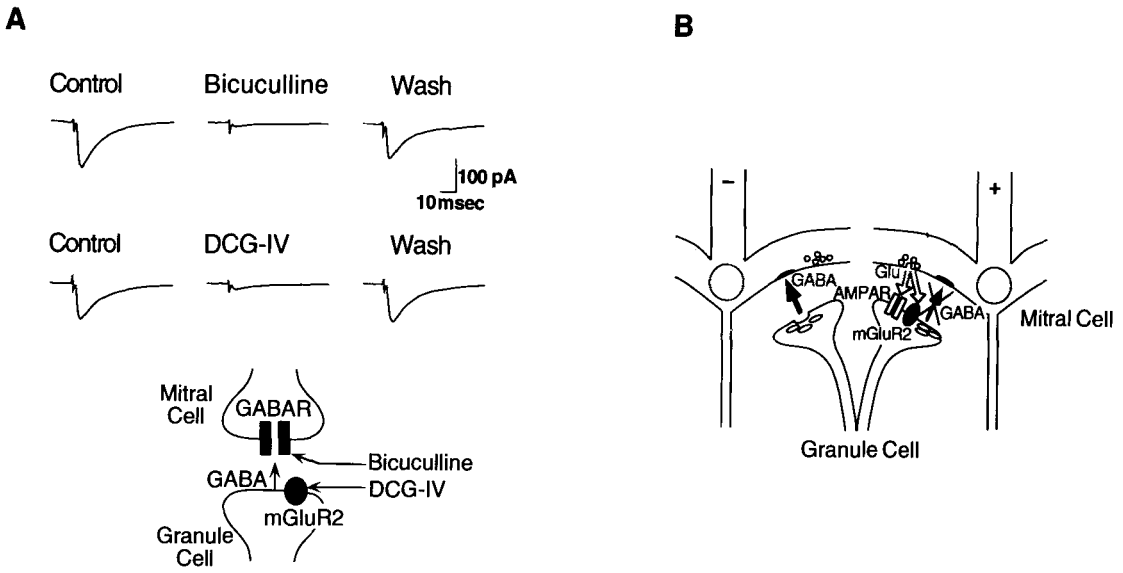

Figure 5. Suppression of GABA-mediated IPSCs by DCG-IV and a model for olfactory discrimination by mGluR2 activation. (*A*) GABA-mediated IPSCs that were blocked by the $GABA_A$ receptor antagonist bicuculline were markedly reduced by DCG-IV in a reversible manner (Hayashi et al. 1993). (GABAR) $GABA_A$ receptor. (*B*) Dendrodendritic synapses between a granule cell and mitral cells are thought to cause both recurrent inhibition and lateral inhibition. This model holds that the mGluR2 activation relieves recurrent inhibition by suppressing GABA release but keeps lateral inhibition. (AMPAR) AMPA receptor; (Glu) glutamate.

1993; Nakanishi 1995). Because this modulatory effect of mGluR2 is thought to be confined to the synapses of excited mitral cells, this modulation would relieve recurrent inhibition of excited mitral cells but keep lateral inhibition of unexcited neighboring mitral cells (Fig. 5B). The mechanism discussed here evidently enhances the signal-to-noise ratio between excited and unexcited mitral cells and would contribute to discrimination of olfactory transmission (Fig. 5). The synaptic circuitry of the second-order neurons is thus crucial for resolving and discriminating external sensory stimuli. Furthermore, AMPA receptors are used for exciting granule cells, whereas mGluR2 functions to relieve excited mitral cells from recurrent inhibition. Thus, the existence of two different types of glutamate receptors is again crucial for sensory information processing in the olfactory system.

The reciprocal dendrodendritic synapses in the AOB are known to have the capacity for the neural plasticity that is involved in the formation of olfactory memory (Brennan et al. 1990; Kaba and Nakanishi 1995). It is thus possible that the persistent activation of mGluR2 by glutamate results in prolonged excitation of olfactory transmission that may enhance synaptic efficacy responsible for memory formation. Female mice form an olfactory memory at mating, thus maintaining pregnancy during exposure to familiar pheromones of the stud male but evoking pregnancy block after exposure to unfamiliar male pheromones of a different strain (Brennan et al. 1990). This olfactory block of pregnancy, known as the Bruce effect, is caused by sustained norepinephrine in the AOB (Fig. 6A) (Brennan et al. 1990). Norepinephrine is enhanced in the AOB after mating and persistently excites mitral cells by reducing GABA transmission from granule cells, thus forming olfactory memory specific to pheromones exposed after mating. The activation of mGluR2 similarly reduces inhibitory GABA transmission and may create olfactory memory formation.

To investigate a possible role of mGluR2 in olfactory memory formation, we carried out the following animal behavioral analysis (Fig. 6B) (Kaba et al. 1994). mGluR2 agonists were infused into the AOB of females during exposure to male pheromones without mating. The females were then mated with a different strain and reexposed to test pheromones of the original strain. Under this protocol, the memory formation by drug infusions can be evaluated by measuring the protection of pregnancy block induced by the test pheromonal exposure. Infusion of DCG-IV, but not phosphate buffer alone, into the AOB during exposure to male pheromones reduced pregnancy block significantly. This formation of memory required the concomitant action of DCG-IV and of pheromonal exposure. More importantly, this memory was specific to the pheromones to which the females were exposed during infusion of DCG-IV. Furthermore, the α-adrenergic receptor antagonist phentolamine had no effect on the DCG-IV-mediated formation of memory, indicating the direct action of DCG-IV on mGluR2 rather than the enhancement of actions of norepinephrine. This study demonstrates that a specific mGluR subtype plays an important role in neural plasticity responsible for recognition memory formation (Kaba et al. 1994). The olfactory bulb is relatively simple in structural organization and synaptic opera-

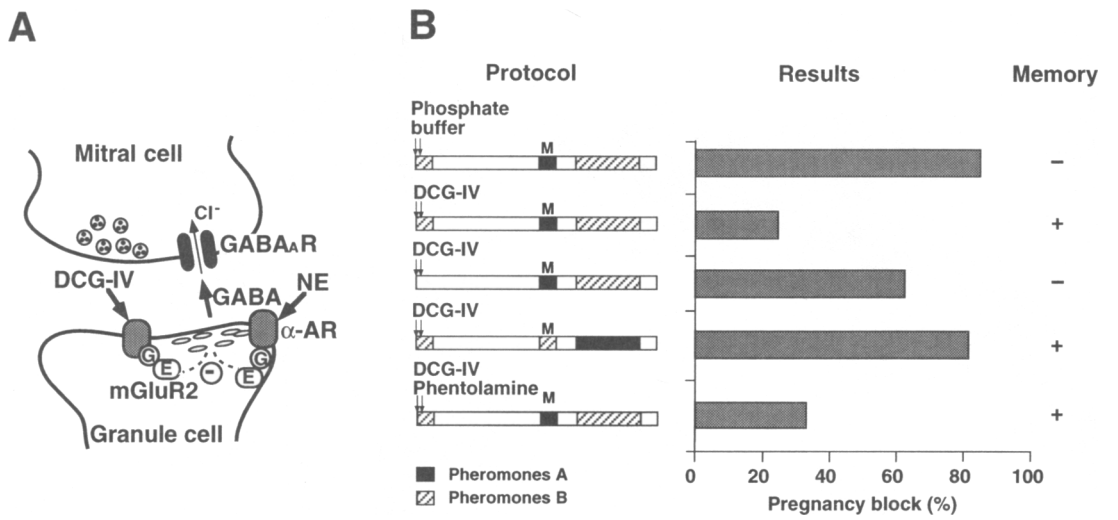

Figure 6. Microcircuitry in dendrodendritic synapses between a mitral cell and a granule cell in the AOB and olfactory memory formation by activation of mGluR2. (*A*) Inhibitory actions of both DCG-IV and norepinephrine (NE) on GABA release are indicated by a minus symbol; (GABA$_A$R) GABA$_A$ receptor; (α-AR) α-adrenergic receptor; (G) G protein; (E) intracellular effector. (*B*) In the protocol of behavioral analysis, shaded and filled boxes indicate exposures to pheromones of two different strains. Arrows show drug injections. (M) Mating. Data are taken from Kaba et al. (1994) and modified.

tion. This system should provide a useful behavioral paradigm for study of the mechanism underlying memory formation.

mGluR2 in Hippocampal Synaptic Plasticity

LTP and LTD at the CA1 region of the hippocampus are mediated by the activation of NMDA receptors at the postsynaptic cell (Bliss and Collingridge 1993; Nicoll and Malenka 1995). It is generally believed that these modifications in synaptic efficacy are of fundamental importance for memory acquisition and learning (Bliss and Collingridge 1993; Nicoll and Malenka 1995). In contrast, both LTP and LTD in hippocampal mossy fiber-CA3 synapses result entirely from changes in the presynaptic cell and are independent of NMDA receptor activation (Zalutsky and Nicoll 1990; Nicoll and Malenka 1995; Kobayashi et al. 1996). In situ hybridization study and immunostaining in combination with lesion analysis indicated that mGluR2 is predominantly expressed in granule cells of the hippocampal dentate gyrus and is distributed to mossy fibers (Shigemoto et al. 1995). Furthermore, immuno-electron microscopy revealed a unique localization of mGluR2 at the preterminal zone rather than the synaptic junction of mossy fibers (Fig. 7A) (Yokoi et al. 1996). To investigate the role of the presynaptic mGluR2 in synaptic transmission and plasticity in the mossy fiber-CA3 synapses, we generated mutant mice lacking mGluR2 (Yokoi et al. 1996).

The mGluR2-deficient mice grew normally and showed no behavioral abnormality, nor any gross anatomical changes in the brain. Neither field excitatory postsynaptic potentials (EPSPs) evoked by stimulation of mossy fibers nor paired-pulse facilitation at the mossy fiber-CA3 synapses was distinguishable between the wild-type and mutant mice (Yokoi et al. 1996). The mGluR2/mGluR3-selective agonist, DCG-IV, markedly and reversibly depressed EPSPs at the mossy fiber-CA3 synapses in wild-type mice, but this depression was greatly reduced in mutant mice (Yokoi et al. 1996). The presynaptic mGluR2 thus plays an important role in presynaptic inhibition. We investigated effects of mGluR2 deficiency on LTP and LTD at the mossy fiber-CA3 synapses. mGluR2-deficient mice showed normal LTP after tetanic stimulation (Yokoi et al. 1996). In contrast, LTD induced by low-frequency stimulation was significantly impaired in mutant mice (Fig. 7B) (Yokoi et al. 1996). In wild-type mice, EPSPs were facilitated during low-frequency stimulation and then decreased below control levels after low-frequency stimulation. This depression lasted for at least 45 minutes. In mutant mice, the facilitation of EPSPs and the subsequent short-term depression were unchanged. However, this depression was transient, and EPSPs returned gradually to control levels. Therefore, the presynaptic mGluR2 is not required for mossy fiber LTP but is essential for mossy fiber LTD.

We tested for the involvement of mossy fiber LTD in spatial learning by performing the Morris water-maze tasks. The wild-type and mutant mice showed no differences in their ability to perform either the visible- or hidden-platform tasks (Yokoi et al. 1996). In a transfer test, the wild-type and mutant mice exhibited no difference in either the time spent or the number of crossings in the trained quadrant. To evaluate spatial learning flexibility, we examined the ability of trained mice to adapt to a new platform location (Yokoi et al. 1996). The wild-type and mutant mice capably adapted to a new platform location with no significant dif-

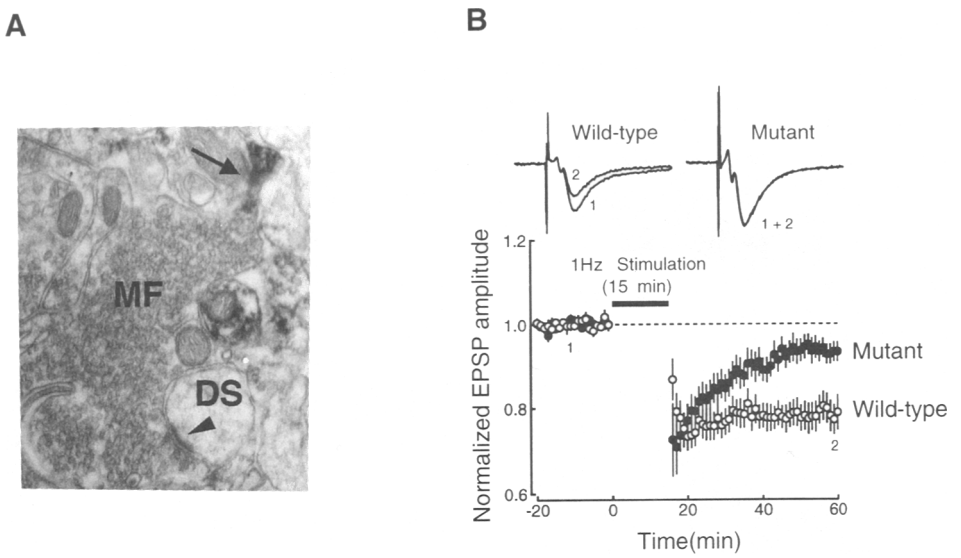

Figure 7. Immunoelectron microscopy of mGluR2 and LTD at mossy fiber-CA3 synapses. (*A*) mGluR2 immunostaining (*arrow*) is seen at the preterminal zone of a mossy fiber terminal (MF). (DS) A dendritic spine; (*arrowhead*) an active zone. (*B*) LTD recorded after low-frequency stimulation was impaired in mGluR2-deficient mice (Yokoi et al. 1996).

ference. These observations demonstrate that impairment of the CA3 LTD does not hinder spatial learning.

Recent evidence has shown that enhancement of calcium entry into the presynaptic terminal of the mossy fiber by tetanic stimulation activates Ca^{++}/calmodulin-sensitive adenylate cyclase 1, and this activation would in turn produce LTP through protein kinase A (Fig. 8) (Huang et al. 1994; Weisskopf et al. 1994; Nicoll and Malenka 1995). mGluR2 is coupled to inhibition of the cAMP cascade (Tanabe et al. 1992). Thus, taken together with the mechanism proposed for LTP induction (Huang et al. 1994; Nicoll and Malenka 1995), a model for induction of LTP and LTD at the mossy fiber-CA3 synapses is illustrated in Figure 8. At the mossy fiber-CA3 synapses, slowly accumulating glutamate during low-frequency stimulation activates the presynaptic mGluR2, and this activation of mGluR2 would inhibit the cAMP cascade and result in induction of LTD. It is thus conceivable that LTP and LTD can occur by up- and down-regulation of the cAMP cascade at the presynaptic site of the mossy fiber-CA3 synapses. Interestingly, the elimination of mossy fiber LTP by gene targeting of protein kinase A has been shown to have no effect on spatial learning (Huang et al. 1995). Thus, contrary to current theories about hippocampal function, neither LTP nor LTD at the mossy fiber-CA3 synapses appears to be required for spatial learning, although they may have a variety of other physiological roles. LTD, for example, may involve a dynamic modulation of information processing; an increase in the flexibility of circuit formation; protection of postsynaptic cells from overexcitation; or cancellation of acquired memory. Thus, the mGluR2-deficient mouse should serve as an interesting model to study the LTD-relevant brain function.

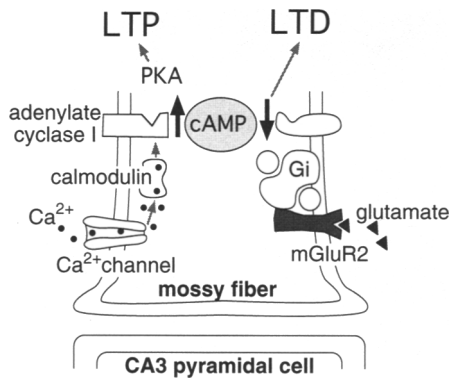

Figure 8. Model for induction of LTP and LTD at the mossy fiber-CA3 synapses.

CONCLUSION

Ionotropic glutamate receptors exert the rapid neuronal excitation characteristic of glutamate transmission. The mGluRs, on the other hand, mediate relatively slow responses by coupling to intracellular signal transduction, and less attention has been paid to the functions of mGluRs. However, glutamate is a common transmitter that acts on both the ionotropic receptors and mGluRs with similar efficacy. Furthermore, G-protein-coupled receptors have the potential to modulate a variety of intracellular signal transduction events. The mGluRs have thus been expected to share important roles in glutamate transmission with the ionotropic receptors. The different subtypes of the mGluR family play critical roles in synaptic transmission, modulation, and plasticity. The ionotropic recep-

tors also consist of diverse members of subunits that form different heteromeric receptors. Further studies of both individual subtypes of the mGluR family and different heteromeric assemblies of the ionotropic receptors will be important for an understanding of implications of glutamate transmission in brain function.

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