Analysis of Synaptic Plasticity and Memory in the Mammalian Brain by the Gene Knockout Technology

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Genetics has been a powerful tool to dissect complex biological processes. With the advent of the embryonic stem cell-gene targeting technology (Capecchi, 1989), also known as gene knockout, it is now possible to produce a strain of mouse with a disruption in a gene of interest. A few dozen strains of mutant mice using this technology, which exhibit a distinct set of impairments in the neural system and/or the behavior, have been generated during the last few years (see Mayford et al., 1995; Chen and Tonegawa, 1995). Here I will discuss how this technology has been used to help elucidate the mechanisms of long-term potentiation (LTP), long-term depression (LTD) and their relationship to learning and memory.

Molecular Mechanisms of LTP and Its Role in Hippocampus-dependent Memory

LTP, a long-lasting increase of synaptic efficacy as a result of a brief stimulation, is thought to be a cellular mechanism for learning and memory. Ca^{2+} influx through NMDA receptor channels and subsequent activation of protein kinases such as PKC and CaMKII are required for the induction of LTP(Bliss & Collingridge, 1993; Nicoll & Malenka, 1995). And mGluRs are also suggested to be required for LTP induction (Bashir et al., 1993), but this notion is hindered by difficultites in establishing the specificity of agonists or antagonists used (Chinestra et al., 1993; Manzoni et al., 1994).

The importance of glutamate receptors and kinases in LTP has been confirmed by observations in gene knockout mice, including those deficient in αCaMKII

(Silva et al., 1992a), tyrosine kinase fyn (Grant et al., 1992), PKCγ (Abeliovich et al., 1993a), mGluR1 (Aiba et al., 1994a), and NMDAR2B (Sakimura et al., 1995). The deficiency is most severe in αCaMKII mutant mouse in that only post-tetanic potentiation is spared (Silva et al., 1992a; also Stevens et al., 1994). The LTP impairment in fyn mutant mice is seen with the tetanus of low intensity but not with that of high intensity (Grant et al., 1992). PKCγ mutant mice, although lacking LTP induced by conventional high-frequency tetanus, are capable of generating LTP-like potentiation when the tetanus is preceded by low frequency stimuli (Abeliovich et al., 1993a). Targeted disruption of the NMDAR2A (ε1) subunit gene resulted in significant reduction of the NMDA receptor current and CA1 LTP (Sakimura et al., 1995). Two lines of mGluR1 mutant mice have been generated using different targeting vectors by two independent laboratories. Aiba et al. (1994a) reported a reduction of the amplitude of CA1 LTP in the mutant mice. However, Conquet et al. (1994) showed normal CA1 LTP, but impaired mossy fiber-CA3 LTP.

One interesting feature that emerged from knockout studies is a "priming effect" on CA1 LTP. As mentioned above, in PKCγ mutant mice, LTP was absent after conventional tetanus, but could be induced after priming the Shaeffer collaterals with 1 Hz stimulation lasting for 10 minutes (Abeliovich et al., 1993a), which is the stimulation protocol to induce LTD (Dudeck and Bear, 1992). Interestingly although the prior low frequency is a pre-requisite for LTD induction, a prior induction of LTD is not. In contrast, such a priming had no effect on LTP induction in mGluR1 mutant slices (Aiba et al., 1994a). This difference between PKCγ and mGluR1 mutant mice is interesting because PKC is one of down-stream components of mGluR-initiated second messenger cascade. The priming effect is also absent in the CA1 region of αCaMKII mutant mice (Stevens et al., 1994). The priming effect thus seems specific in rescuing LTP in PKCγ mutant mice. Therefore, the diversity of impairments in the mutant mice provides an opportunity to dissect functional roles of different receptor and second messenger systems in the induction of CA1 LTP. In some cases such as αCaMKII and PKCγ mutants, the gene knockout studies extended the pharmacological studies by specifying the role of a particular isoform.

The hippocampus is thought to play a key role in relational, contextual, spatial or declarative memory (for review see Squire, 1992; Eichenbaum et al., 1992). Spatial memory and contextual fear memory in rats can be disrupted by a specific antagonist for NMDA receptors that can also block hippocampal LTP in vivo (Morris et al., 1986; Kim et al., 1991). Studies of two mutant mice, αCaMKII (Silva et al., 1992a, 1992b) and fyn (Grant et al., 1992), provided support for LTP as a candidate mechanism underlying hippocampus-dependent spatial learning. Deficient in CA1 LTP, both αCaMKII and fyn mutant mice exhibit specific deficits in the acquisition and retention of spatial memory, with less severe deficits in non-spatial learning tasks. In fear conditioning, αCaMKII mutant mice show severely reduced fear responses (i.e. performance deficit) and appearant lack of both hippocampus-dependent and independent fear memories (Chen et al., 1994).

In contrast, PKCγ mutant mice, which lack conventional LTP but have primed LTP, exhibit a subtle deficit only in the retention test of spatial memory (Abeliovich et al., 1993b); the deficit in fear conditioning is restricted to the contextual memory tested 24 hours after conditioning, while performance in the contextual training

Table 1. Summary of Synaptic Plasticity and Hippocampus-dependent Memory

Mutant mice	αCaMKII	fyn	PKCγ	mGluR1
CA1 LTP	Deficient	Impaired	Impaired	Impaired
CA1 LTD	Deficient	Not tested	Normal	Normal
Spatial learning	Deficient	Impaired	Impaired	Not tested
Contextual fear conditioning	Deficient	Not tested	Impaired	Impaired
Reference	Silva et al., 1993a, b Stevens et al., 1994 Chen et al., 1994	Grant et al.,1992	Abeliovich et al., 1993a, b	Aiba et al., 1994a

phase or in the tone task appears normal. This suggests that the disruption of PKCγ leads to a specific deficit in the hippocampus-dependent memory. The line of mGluR1 mutant mice generated by Conquet et al. (1994) was reported to have an impairment in spatial learning. This result seems to agree with the effect of administrating mGluR antagonist, MCPG, which blocks spatial learning in wild-type animals (Riedel et al., 1994). The nature of behavioral deficits revealed with fear conditioning in the mGluR1 mutant mice generated by Aiba et al. (1994a) appears similar to PKCγ mutant mice.

In sum, the results from gene knockout mutant mice appear to support the notion that LTP in the CA1 region is a candidate synaptic mechanism important for the integrity of hippocampus-dependent memories (Table 1). Perhaps, other synaptic mechanisms such as hippocampal LTD and primed LTP may also play some roles.

Molecular mechanisms of LTD and Its Role in Cerebellar-dependent Discrete Motor Learning

In the cerebellum, LTD can be induced in parallel fiber (PF)-Purkinje cell (PC) synapses following conjunctive stimulation of PF and climbing fibers (CF) (Ito, 1989; Linden, 1994). An elevation of intracellular Ca^{2+} (Sakurai, 1990; Konnerth et al., 1992), and activation of both AMPA receptors and mGluRs are thought to be necessary for LTD induction (Linden et al., 1991; Daniel et al., 1992; Hartell, 1994). More recently, antibodies against mGluR1 was shown to block LTD induction in cell culture preparation (Shigemoto et al., 1994).

Cerebellar LTD is clearly deficient in both lines of mGluR1 mutant mice, suggesting that mGluR1 is essential for cerebellar LTD (Aiba et al., 1994b; Conquet et al., 1994). In contrast, other cerebellar electrophysiological properties, such as voltage-gated Ca^{2+} channels, excitatory transmission at CF- and PF-PC synapses, do not appear to be affected by the mutation. LTD appears to be normal in PKCγ mutant mice (Chen et al., 1995). But the application of a potent PKC inhibitor, peptide PKC(19-36) that abolished LTD in the wild-type mice (Linden and Conner, 1991; Hemart et al., 1995), decreased the magnitude but did not abolished LTD in the mutant mice. This suggests that the knockout of PKCγ modifies the efficacy of the inhibitor PKC(19-36) in blocking LTD, perhaps as a result of up-regulation of activity of other PKC isoforms that are less sensitive to the inhibitor. Cerebellar LTD is

Table 2. Summary of LTD and cerebellar function (from Chen et al., 1995)

Mutant mice	PKCγ	mGluR1	GluRδ2	GFAP
Motor coordination	Impaired	Impaired	Impaired	Normal
Eyeblink conditioning	Facilitated	Impaired	Not tested	Impaired
CF innervation	Multiple	Multiple	Multiple	Normal
LTD	Normal	Impaired	Impaired	Impaired
Reference	Chen et al., 1995 Kano et al., 1995	Aiba et al., 1994 Conquet et al., 1994 Unpublished results	Kashiwabuchi et al., 1995	Shibuki et al., 1995

deficient in mutant mice deficient for GluRδ2 (Kashiwabuchi et al., 1995), a subunit of a glutamate receptor with selective localization in Purkinje cells (Araki et al., 1993). LTD is also deficient in mutant mice deficient for GFAP, is an intermediate filament protein highly expressed in cerebellar Bergmann glia (Shibuki et al., 1995). This suggests that LTD induction requires certain communication between Purkinje cells and Bergmann glia (Nedergaard, 1994; Parpura et al., 1994).

It has been suggested that LTD may be the cellular mechanism underlying cerebellum-dependent motor learning, such as vestibular ocular reflex (VOR) and eyeblink conditioning (Ito, 1984, 1989; Thompson, 1986; Thompson et al., 1993). However, until recently little evidence had been available that directly linked LTD to motor learning. mGluR1 mutant mice are deficient in cerebellar LTD; although these mice are still able to learn eyeblink response, the magnitude of the conditioned response is reduced (Aiba et al., 1994). It thus appears that cerebellar LTD is not essential for associative learning of eyeblink response, but is clearly involved in this form of learning. The notion of the involvement of LTD in eyeblink conditioning is further supported by other mutant mice. Mutant mice lacking GFAP have deficient LTD and impaired eyeblink conditioning (Shibuki et al., 1995). In contrast, PKCγ mutant mice are not only unimpaired in eyeblink conditioning but show facilitated learning in the early stage of training; LTD is present in the mutant mice (Chen et al., 1995).

In sum, studies of the gene knockout mice suggest that cerebellar LTD contributes to motor learning of discrete reflexes (Table 2). In addition, mutant mice deficient for mGluR1, PKCγ and GluRδ2 show characteristic cerebellar symptoms such as ataxic gait and impaired movement coordination in association with multi CF innervation of PCs persisted into adulthood (Aiba et al., 1994b; Conquet et al., 1994). Therefore, mutant mice serve as a unique animal model for investigating synaptic dysfunction underlying those cerebellar symptoms.

References

Abeliovich, A., Chen, C., Goda, Y., Silva, A.J., Stevens, C.F., and Tonegawa, S. (1993a). Modified hippocampal long-term potentiation in PKCγ-mutant mice. *Cell 75,* 1253-1262.

Abeliovich, A., Paylor, R., Chen, C., Kim, J. J., Wehner, J. M., and Tonegawa, S. (1993b). PKCγ mutant mice exhibit mild deficits in spatial and contextual learning. *Cell 75,* 1263-1271.

Aiba, A., C.Chen, K.Herrup, C.Rosenmund, C.F.Stevens and S.Tonegawa (1994a). Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. *Cell 79,* 365-375.

Aiba, A., M.Kano, C. Chen, M.E.Stanton, G.D.Fox, K.Herrup, T.A.Zwingman and S. Tonegawa (1994b). Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. *Cell* 377-388.

Araki, K., Meguro, H., Kushiya, E., Takayama, C., Inoue, Y., and Mishina, M. (1993). Selective expression of the glutamate receptor channel δ2 subunit in cerebellar Purkinje cells. *Biochem. Biophys. Res. Commun. 197,* 1267-1276.

Bashir, Z.I., Bortolotto, Z.A., Davies, C.H., Berretta, N., Irving, A.J., Seal, A.J., Henley, J.M., Jane, D.E., Watkins, J.C., Collingridge, G.L. (1993)a. Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. *Nature 363,* 347-350.

Bliss, T. V. P., and Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature 361,* 31-39.

Capecchi, M.R. (1989). Altering the genome by homologous recombination. *Science 244,* 1288-1292.

Chen, C., D.G.Rainie, R.W.Greene and S.Tonegawa (1994). Abnormal fear response and aggressive behavior in mutant mice deficient for αCaM kinase II. *Science 266,* 291-294.

Chen, C. , Kano, M., Chen, L., Bao, S., Kim, J.J., Thompson, R.F., and Tonegawa, S. (1995). Distinction of movement coordination and discrete motor learning in mutant mice deficient for PKCγ. *Cell,* submitted.

Chen, C. and Tonegawa, S. (1995). Analysis of synaptic plasticity and memory in the mammalian brain by gene knockout approach. In (M. Baudry and J. L. Davis, ed.) Long-term Potentiation: *A Debate of Issues,* Part 3. MIT Press, Cambridge, MA.

Chinestra, P., Aniksztejn, L., Diabira, D., and Ben-Ari, Y. (1993). (RS)-a-methyl-4-carboxyphenyl-glycine neither prevents induction of LTP nor antagonizes metabotrophic glutamate receptors in CA1 hippocampal neurons. *J. Neurophys. 6,* 2684-2689.

Conquet, F., Bashir, Z.I., Davies, C.H., Daniel, H., Ferraguti, F., Bordi, F., Franz-Bacon, K., Reggiani, A., Matarese, V., Conde, F., Collingridge, G.L. and Crepel, F. (1994). Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. *Nature 372,* 237-243.

Crepel, F., and Krupa, M. (1988). Activation of proteik kinase C induces a long-term depression of glutamate sensitivity of cerebellar Purkinje cells. an in vitro study. *Brain Res. 458,* 397-401.

Daniel, H., Hemart, N., Jaillard, D., and Crepel, F. (1992). Coactivation of metabotropic glutamate receptors and voltage-gated calcium channels induces long-term depression in cerebellar Purkinje cells in vitro. *Exp. Brain Res. 90,* 327-331.

Dudek, S.M., and Bear, M.F. (1992). Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proc. Natl. Acad. Sci. USA 89,* 4363-4367.

Eichenbaum, H., Otto, T., and Cohen, N. J. (1992). The hippocampus: What does it do? *Behav. Neural Bio. 57,* 2-36.

Grant, S. G. N., O'Dell, T. J., Karl, K. A., Stein, P. L., Soriano, P., and Kandel, E. R. (1992). Impaired long-term poteintiation, spatial learning, and hippocampal development in fyn mutant mice. *Science 258,* 1903-1910.

Hartell, N.A. (1994). Induction of cerebellar long-term depression requires activation of glutamate metabotropic receptors. *Neuro Report 5,* 913-916.

Hemart, N., Daniel, H., Jaillard, D., and Crepel, F. (1995). Receptors and second messengers involved in long-term depression in rat cerebellar slices in vitro: a reappraisal. *Eur. J. Neurosci. 7,* 45-53.

Ito, M. (1984). *The Cerebellum and Neural Control.* Raven Press, New York.

Ito, M. (1989). Long-term depression. *Ann. Rev. Neurosci. 12,* 85 - 102.

Kashiwabuchi, N., Ikeda, K., Araki, K., Hirano, T., Shibuki, K., Takayama, C., Inoue, Y., Kutsuwada, T., Yagi, T., Kang, Y., Aizawa, S., and Mishina, M. (1995). Impairment of motor coordination, Purkinje cell synapse formation, and cerebellar long-term depression in GluRδ2 mutant mice. *Cell 81,* 245-252.

Kim, J. J., DeCola, J. P., Landeira-Fernandez, J., and Fanselow, M. S. (1991). N-Methyl-D-aspartate receptor antagonist APV blocks acquisition but not expression of fear conditioning. *Behav. Neurosci. 105,* 126-133.

Konnerth, A., Dreessen, J., and Augustin, G.J. (1992). Brief dendritic calcium signals initiate long-lasting synaptic depression in cerebellar Purkinje cells. *Proc. Natl. Acad. Sci. USA 89,* 7051-7055.

Linden, D.J. (1994). Long-term synaptic depression in the mammalian brain. *Neuron 12,* 457-472.

Linden, D.J., and Connor, J.A. (1991). Participation of postsynaptic PKC in cerebellar long-term depression in culture. *Science 254,* 1656-1659.

Linden, D.J., Dickinson, H., Smeyne, M., and Connor, J.A. (1991). A long-term depression of AMPA currents in cultured cerebellar Purkinje neurons. *Neuron 7,* 81-89.

Manzoni, O.J., Weisskopf, M.G., and Nicoll, R.A. (1994). MCPG antagonizes metabotropic glutamate receptors but not long-term potentiation in the hippocampus. *Eur. J. Neurosci. 6,* 1050-1054.

Martin, L.J., Blackstone, C.D., Huganir, R.L., Price, D.L. (1992) Cellular localization of a metabotropic glutamate receptor in rat brain. *Neuron 9,* 259-270.

Mayford, M., Abel, T. and Kandel, E.R. (1995). Transgenic approaches to cognition. *Curr. Opin. Neurobiol. 5,* 141-148.

Morris, R. G. M., Anderson, E., Lynch, G. S., and Baudry, M. (1986). Selective impairement of learning and blockage of long-term potentiation by the N-methyl-D-aspartate receptor antagonist AP5. *Nature 319,* 774-776.

Nedergaard, M. (1994). Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science 263,* 1768-1771.

Nicoll, R.A. and Malenka, R.C. (1995). Contrasting properties of two forms of long-term potentiation in the hippocampus. *Nature 377,* 115-118.

Riedel, G., Wetzel, W., and Reymann, K.G. (1994). (R,S)-a-methyl-4-carboxyphenylglycine (MCPG) blocks spatial learning in rats and long-term potentiation in the dentate gyrus in vivo. *Neurosci. Lett. 167,* 141-144.

Parpura, V., Basarsky, T.A., Liu, F., Jeftinija, K., Jeftinija, S., and Haydon, P.G. (1994). Glutamate-mediated astrocyte-neuron signaling. *Nature 369,* 744-747.

Shibuki, K., Gomi, H., Chen, L., Bao, S., Kim, J.J., Wakatsuki, H., Fujisaki, T., Fujimoto, K., Ikeda, T., Chen, C., Thompson, R.F., and Tonegawa, S. (1995). Deficient cerebellar long-term depression, impaired eyeblink conditioning and normal motor coordination in GFAP mutant mice. *Neuron,* submitted.

Sakimura, K., Kutsuwada, T., Ito, I., Manabe, T., Takayama, C., Kushiya, E., Yagi, T., Aizawa, S., Inoue, Y., Sugiyama, H. and Mishina, M. (1995). Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor ε1 subunit. *Nature 373,* 151-155.

Sakurai, M. (1990). Calcium is an intracellular mediator of the climbing fibre induction of cerebellar long-term depression. *Proc. Natl. Acad. Sci. USA 87,* 3383 - 3385.

Shigemoto, R., Abe, T., Nomura, S., Nakanishi, S., and Hirano, T. (1994). Antibodies inactivating mGluR1 metabotropic glutamate receptor block long-term depression in cultured Purkinje cells. *Neuron,* 12, 1245-1255.

Silva, A. J., Stevens, C. F., Tonegawa, S., and Wang, Y. (1992a). Deficient hippocampal long-term potentiation in a-calcium-calmodulin kinase II-mutant mice. Science 257, 201-206.

Silva, A. J., Paylor, R., Wehner, J. M., and Tonegawa, S. (1992b). Impaired spatial learning in a-calcium-calmodulin kinase II-mutant mice. *Science 257,* 206-211.

Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psych. Rev. 99,* 195-231.

Stevens, C.F., Tonegawa, S. and Wang, Y. (1994). The role of calcium-calmodulin kinase II in three forms of synaptic plasticity. *Curr. Biol. 4,* 687-693.

Thompson, R.F. (1986). The neurobiology of learning and memory. *Science 233,* 941-947.

Thompson, R.F., Thompson, J.K., Kim, J.J., Krupa, D.J., Norholm, A.F., and Chen, C. (1993). Synaptic plasticity and memory storage. In (M.Baudry, R.F.Thompson and J.L.Davis, ed.) *Synaptic plasticity: molecular, cellular, and functional aspects,* pp. 230-259. MIT Press, Cambridge, MA.