COINCIDENCE DETECTION in the nervous system

Molecular and cellular mechanisms

Consequence for learning and memory

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A genetic approach to synaptic plasticity and learning

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Transgenic mice overexpressing a specific gene and mice with a particular gene inactivated (knockouts) are now being used to dissect the molecular basis of synaptic plasticity. In particular, we are using them to examine molecules involved in learning and memory, and in activity-dependent modulation of synapses during development. Four examples are discussed here. The importance of phosphorylation and dephosphorylation in LTP is being investi-

gated in αCaMKII knockouts; LTD in the cerebellum and its role in motor learning are being examined in mGluR1 knockouts. In development, the contributions of the NMDA-R subunit 1 (NMDA-R1) and the anti-apoptosis gene *bcl-2* to the formation of a somatosensory map are being assessed with the help respectively of knockout and transgenic mice.

Synaptic plasticity in αCaMKII deficient mice

Compared with wild-type mice, induction of LTP and LTD was impaired in the hippocampus of mice lacking αCaMKII. In the CA1 region, tetanic stimulation did not result in an increase in synaptic strength, so LTP could not be established (Silva *et al.*, 1992). Low-frequency stimulation produced only a small reduction in synaptic strength, that is, weak LTD. In the wild-type mouse LTD can be reversed with tetanic stimulation but this did not occur in the knockout (Stevens *et al.*, 1994). We are now attempting to rescue the synaptic plasticity in these knockouts by introducing the wild-type αCaMKII gene into the hippocampal neurons using a vaccinia virus vector.

Pharmacological methods for rescuing LTP induction have also been pursued in hippocampal slices from the αCaMKII knockout. We have demonstrated that both short-term potentiation (STP) and LTP can be rescued in the CA1 region by pretreating the slices with ocadaic acid, a potent inhibitor of phosphatase (PP) 2A. This synaptic enhancement is inhibited by APV, i.e., it is dependent on NMDA-Rs. An LTP that is insensitive to APV inhibition, unlike the conventional CA1 LTP, was rescued by caliculin A, an inhibitor of PP-1 and PP-2A, and MK520, which inhibits PP-1 through the inhibition of PP-2B; STP was not restored by these treatments.

αCaMKII normally phosphorylates AMPA receptors and is possibly involved in activating the production of a retrograde messenger. Because LTP can be rescued in the absence of αCaMKII, we think it is likely that another endogenous calcium-activated kinase, for instance βCaMKII, attempts to phosphorylate these targets. However, in the absence of αCaMKII, the phosphatases dominate and LTP is not easily induced. The differential effects of the various phosphatase inhibitors indicate that LTP in CA1 appears in at least two forms, one fast rising and dependent on NMDA-Rs, the other slow rising and NMDA-R independent.

Synaptic plasticity in mGluR1 knockouts

mGluR1s are highly expressed in the hippocampus, particularly in CA2, CA3 and the dentate gyrus, but have not been found in the CA1 region. They are also highly expressed in cerebellar Purkinje neurons (*Figs 7, 12*). In hippocampal slices from our mGluR1 knockout mice, STP and LTD seemed to be normal but LTP was impaired (Aiba *et al.*, 1994a). However, Conquet *et al.* (1994) found a more complete deficiency in independently generated mGluR1 knockouts. In the cerebellum of our knockouts, LTD was clearly deficient, although synaptic transmission at the PF-Purkinje neuron and CF-Purkinje neuron synapses, voltage-gated calcium channels and short-term synaptic plasticity all seemed normal (Aiba *et al.*, 1994b).

Cerebellar LTD may participate in certain types of motor learning, such as classical conditioning of the eyeblink response, which seems to be stored in the cerebellar cortex and/or the deep cerebellar nuclei. We have subjected mGluR1 knockout mice to the classical eye-blink conditioning paradigm to test whether cerebellar LTD is involved in this motor learning. Eye-blink conditioning was adapted for use with mice by pairing a tone as conditioning stimulus with a small periocular electrical shock as the unconditioned stimulus. Control knockout animals received an unpaired stimulus regime. Conditioning was tested by measuring the electromyogram from the eyelid. In early stages of training, there was no difference between the knockouts and wild-type mice but after about 30 trials the response in the knockouts plateaued, whereas that in the wild-type animals continued to improve (Aiba *et al.*, 1994b).

Although LTD cannot be established in the mouse cerebellum when the mGluR1 is absent, only the later stages of learning seem to

be affected. Thus, the mGluR1 and LTD are not essential for the initial motor learning but in some way seem to modulate the learning process.

Genetic analysis of the development of a somatosensory map

In rodents, tactile sensation from the whiskers is transmitted by the trigeminal nerve to the trigeminal nucleus in the brain stem, from there to the thalamus and then to the somatosensory cortex. In each of these levels, the pattern of the whiskers on the face is replicated in a characteristic pattern involving both afferent axons and intrinsic neurons. The pattern, which can be detected by cytological methods, is known as the barrel field in the cortex, barreloids in the thalamus and barrelettes in the brain stem. Although the pattern formation is known to depend on whisker-mediated sensation, the cellular and molecular mechanisms that establish and maintain it are not understood (O'Leary *et al.*, 1994).

We have used NMDA-R1 knockouts to address the role of NMDA-R-dependent activity in the formation of these patterns. The barrelettes seen in the brain stem of the wild-type mouse can hardly be detected in the NMDA-R1 knockouts. This is somewhat surprising as it has previously been reported that the formation of barrelettes in normal postnatal mice was not prevented by injections of TTX, which blocks electrical activity, or of the NMDA-R antagonist APV (O'Leary *et al.*, 1994). We suggest that the molecular signals for the formation of barrelettes had already been delivered by the time the newborn animals received their injections.

Another mechanism that may be involved in the formation of barrelettes is the elimination of excess neurons by programmed cell death. Nerve growth factor (NGF) injected into E15-E18 rat embryos preserved excess neurons in the trigeminal ganglion and disrupted whisker-related pattern formation (Henderson *et al.*, 1994). This indicates an activity-dependent process in the formation of barrelettes : NGF is presumed to be unevenly distributed in the whisker pad and its effect depends on whether a particular sensory neuron innervates a whisker or terminates between whiskers. If the in-between ones do not get enough NGF and so undergo apoptotic cell death, a pruning process that removes superfluous sensory axons would occur, promoting the formation of the pattern.

To test this model, apoptotic cell death was examined histologically in NMDA-R1 knockout mice using the TUNEL assay. The newborn knockout mice showed a significant reduction in the number of apoptotic cells compared with equivalent wild-type mice. Another test of the programmed cell death model is to use transgenic mice that overexpress the *bcl-2* proto-oncogene, which blocks apoptotic cell death. In these animals, some parts of the nervous system are hypertrophied and transected axons survive an injury that normally leads to neuronal death. However, the barrels in the cortex and the barrelettes in the brain stem are normal.

Whisker-related pattern formation in the brain thus seems to be dependent on NMDA-Rs and probably requires their activation; the failure of barrelettes to form in the NMDA-R1 knockout mice is, however, probably not related to a blockage of naturally occurring cell death. The model we prefer consists of competition between the sensory neurons for a postulated limiting amount of a substance that enables the survival of synapses on their target neurons. Active synapses that are coincidentally stimulated with NGF may win the competition and be strengthened. Injecting NGF prevents barrelette formation by blocking cell death but if NGF is the substance that synapses compete for, injecting it into rat embryos could nullify the competition by providing excess of the survival substance.

Problems and prospects

The potential of genetically engineered mice in neuroscience has already been amply demonstrated. Knockout mice are powerful tools for determining whether a protein (enzyme, receptor, etc.) is essential for a particular neurobiological process or behaviour. However, the technology still has shortcomings. For instance, when the phenotype of a knockout mouse includes defects affecting

complex physiological functions or behaviour, it may be difficult to determine whether a mutation has its effect during development of the nervous system or in the functioning of the mature nervous system. Another shortcoming is that the knockout occurs in the entire nervous system, and throughout the body, hence it is sometimes difficult to attribute the defective phenotype to a lack of the normal gene function in a particular part of the nervous system or type of neuron. Substantial improvements will come if a given gene can be knocked out only in a certain restricted region of the central nervous system and/or during an ontogenically restricted period. The technology to achieve this is currently being developed in several laboratories including our own.