Neurobiology

Memory floxed

Richard G. M. Morris and Roger J. Morris

As the generations come and go, new memories are made while old ones fade away. The arrival of a new generation of mouse mutants, together with advances in their electrophysiological assessment, have now been heralded with the publication of four papers by Susumu Tonegawa, Eric Kandel and their colleagues, in *Cell*[1–4]. The importance of these new approaches goes well beyond the data obtained — they are a step towards bridging the intellectual divide between *in vivo* systems and cellular accounts of neuronal memory mechanisms.

Tonegawa and colleagues[1–3] have broken new ground by creating a mouse in which a targeted gene (a subunit of an excitatory glutamate receptor) has been inactivated, but only in specific neurons within a subregion of the hippocampus known as CA1. Many of these neurons are 'place' cells: they fire impulses only when the mouse is at a certain location. In an analysis of learning and place cells in the mutant mice, they find that they learn a spatial task poorly; that brain slices from them show a precise regional deficit in an important form of synaptic plasticity; and that single-neuron recordings in the whole animals reveal peculiarities in the firing pattern in the CA1 region. Kandel and colleagues[4] describe a mouse that has an engineered alteration in synaptic plasticity of a different kind, but which also shows changes in the patterns of cell firing.

The first generation of mouse mutants that were developed to explore memory and behaviour were created using homologous recombination to inactivate genes of interest[5]. Powerful though this technique may be, the regionally and temporally unrestricted nature of the resulting gene deletion can lead to developmental defects or premature death, and other pleiotropic effects can play havoc with attempts to interpret the effect of the deletion in the adult brain. Some of these problems are avoided by using the Cre/loxP system to restrict inactivation of the targeted gene to a specific subset of cells (see panel). Building on Mark Mayford's characterization of the α-calcium–calmodulin-dependent kinase II promoter (*αCaMKII*)[6], Tsien *et al.*[1,2] produced a mouse in which the gene for the R1 subunit of the *N*-methyl-D-aspartate (NMDA) receptor was excised only in CA1 pyramidal neurons.

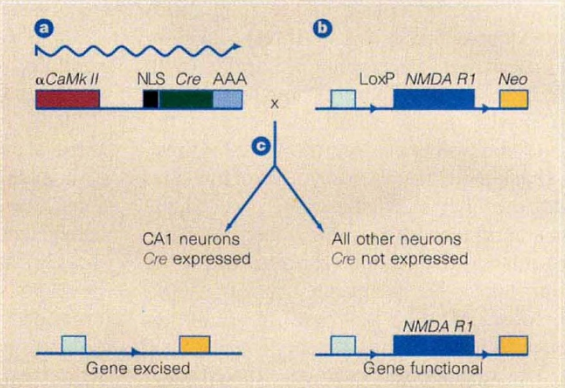
Having trapped their mouse, it was time to get it to tell its tale. The R1 subunit mediates a slow-rising and long-lasting excitatory postsynaptic current that is critical for certain forms of synaptic plasticity[7] and spatial learning[8] in the hippocampus. The mutants had no detectable NMDA-receptor-mediated currents in the CA1 region, yet signalling was normal in other parts of the hippocampus. Moreover, whereas long-term potentiation (LTP) and long-term depression were absent in the CA1 neurons, short-term plasticity was intact. The mice also showed reduced spatial learning in a water-maze (where the animal is required to use distal cues to swim to a platform that is hidden in a large pool).

These findings confirm, with greater anatomical precision, earlier studies using antagonists to the NMDA receptor, and they add one crucial piece of information. Gene deletion — even with the Cre/loxP system used here — is a chronic treatment, allowing the neural circuitry to develop compensatory mechanisms. Other hippocampal synapses support LTP, but (as these mice show) they cannot compensate for defective CA1 pyramidal neurons in supporting learning.

Striking new findings were obtained using single-cell recording techniques. The skull of a 30-gram mouse might seem to be too small to support a headcap containing a moveable electrode headstage and impedance-reducing circuits. But in a technical *tour de force*, McHugh *et al.*[3] miniaturized the device that was first developed by Bruce McNaughton and John O'Keefe, and they were rewarded for their efforts with two new results. First, although the CA1 pyramidal cells that fired in localized areas of space ('place fields') were clearly observed, the areas that they covered were larger in

Restricting gene inactivation to a subset of neurons

Cre is a bacteriophage recombinase that excises DNA between a pair of palindromic sequences called loxP sites[11]. If the loxP sites are introduced into a mouse so as to flank a targeted gene (referred to as the floxed gene, for 'flanked by loxP'), that gene will be excised in cells that express active Cre[12].



Transgenic technology – pro-nuclear injection of DNA into a fertilized oocyte – is used to express Cre. The *Cre* gene is placed under the control of regulatory elements that cause the Cre protein to be expressed only in specific subsets of cells. Tonegawa's group[1–3] used the promoter for the α-subunit of Ca^{2+}-calmodulin protein kinase II (*αCaMKII*) (**a**), a gene that is normally expressed postnatally in forebrain neurons[13]. The promoter (red) was added upstream of the *Cre* gene (dark green), and a nuclear localization signal (black) was added to the 5′ end of *Cre* to ensure the transport of Cre to the nucleus. A polyadenylation signal (light blue) was added to the 5′ end.

The expression pattern of the *αCaMKII* promoter is influenced by where the construct inserts into the host genome – a factor that varies in every transgenic line made. Fortunately, in one cell line, *Cre* was expressed in almost all CA1 pyramidal neurons, and only in these cells.

Tonegawa's group created a second line of transgenic mice by homologous recombination in embryonic stem cells. The flanking loxP sites were specifically introduced into introns so as not to inactivate the targeted (*NMDAR1*) gene (**b**): so the floxed gene was functionally normal unless the Cre recombinase was also expressed. The transgenic mice were then produced by injecting the floxed embryonic stem cells into a blastocyst.

The two elements were brought together by mating the mice, then backcrossing to obtain the F_2 recombinant generation, in which some mice were homozygous for floxed *NMDAR1*, and contained one copy of the *Cre* gene whose expression was restricted to CA1 neurons (**c**). In these mice, the *NMDAR1* gene was excised (in the third postnatal week of life) only from CA1 pyramidal neurons[2].

R.J.M. & R.G.M.M.