

# Genetic neuroscience of mammalian learning and memory

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Our primary research interest is to understand the molecular and cellular mechanisms on neuronal circuitry underlying the acquisition, consolidation and retrieval of hippocampus-dependent memory in rodents. We study these problems by producing genetically engineered (i.e. spatially targeted and/or temporally restricted) mice and analysing these mice by multifaceted methods including molecular and cellular biology, *in vitro* and *in vivo* physiology and behavioural studies. We attempt to identify deficits at each of the multiple levels of complexity in specific brain areas or cell types and deduce those deficits that underlie specific learning or memory. We will review our recent studies on the acquisition, consolidation and recall of memories that have been conducted with mouse strains in which genetic manipulations were targeted to specific types of cells in the hippocampus or forebrain of young adult mice.

**Keywords:** memory; hippocampus; *N*-methyl-D-aspartate receptors

## 1. THE GENERAL STRATEGY

An understanding of the brain mechanisms of cognition, such as learning and memory, requires the identification of the underlying events and processes occurring at multiple levels of complexity; from molecular, synaptic and cellular levels to neuronal ensemble and brain systems level. One would then have to deduce cause–consequence relationships among these multilevel events and processes. This is an enormous challenge because cognitive phenomena manifest themselves as behaviours of live animals, while many of the analytical methods for the study of underlying mechanisms rely on *in vitro* preparations. Among *in vivo* analytical methods, non-invasive imaging such as functional magnetic resonance imaging and positron emission tomography are powerful in correlating cognitive phenomena to the activities of neuronal ensembles or brain systems, but the current technology of this type cannot reach down to cellular or molecular levels (reviewed in Schacter & Wagner 1999). Single-unit and multi-unit recording techniques have been powerful in monitoring activities of individual neurons and neuronal ensembles as an animal undergoes a specific type of cognition (Wilson & McNaughton 1993; Wessberg *et al.* 2000). The recent advent of invasive imaging techniques is filling in the gaps between the levels of complexity addressed by the non-invasive imaging approach and the *in vivo* electrophysiology (reviewed in Helmchen & Denk 2002). However, all of these approaches are primarily designed to identify events or processes that *can* occur while the subjects or animals go through a specific cognitive behaviour but are, in principle, mute in identifying

lower-level events or processes that are *necessary* for the higher-level phenomenon. For this latter purpose, lesion and pharmacological intervention techniques have been widely used.

Lesion studies address whether a certain brain region or a global neuronal circuitry is necessary for a given cognitive function but are not designed to identify a specific type of molecule or cell necessary for a specific cognition. Traditionally, for an intervention at the molecular level, pharmacological administration has been used because of the availability of a range of receptor antagonists and enzyme inhibitors. Examples are AP5 for NRs and CNQX for AMPA receptors (Morris *et al.* 1986; Izquierdo & Medina 1997; Steele & Morris 1999). The advantages of a pharmacological intervention are: (i) not only can it block a physiological process at the molecular level, but (ii) it can also block a specific physiological process acutely and reversibly. However, it is difficult to target a drug to a specific brain area, for example, area CA1 of the hippocampus and it is even more difficult to do so reproducibly from one animal to another. This last point is particularly relevant in rodent memory research because most memory tasks require averaging of at least a dozen individual animals. In addition, many of the drugs used are not entirely specific to a single receptor or enzyme. As the question posed becomes increasingly sophisticated, the intrinsic limitation associated with a pharmacological blockade is emerging, namely the lack of cell type specificity—for instance, even if one manages to target the delivery of AP5 to area CA1, it will block NRs expressed on both excitatory pyramidal cells and inhibitory interneurons.

Given this background, it was desirable to develop an alternative intervention technique aimed at the molecular level. For relatively simple invertebrate systems such as fruit flies or worms, molecular genetics has been used for this purpose with substantial success (Benzer 1967; Brenner 1974). With the advent of the transgenic and

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One contribution of 30 to a Theme Issue 'Long-term potentiation: enhancing neuroscience for 30 years'.

<i>approach:</i>	molecular genetic	slice and culture electrophysiology cell biology biochemistry	multielectrode physiology of freely moving animals imaging	behavioural studies
<i>level of complexity:</i>	molecule	individual neurons and synapses	neuronal ensembles neuronal circuitries	cognition and behaviour

Figure 1. The general strategy based on cell type-restricted knockout mice. Approaches and techniques designed to identify a deficit or impairment at a particular level of complexity in the organization of the brain are listed. Analysis of targeted gene knockout mice can potentially allow the identification of causal relationships among events occurring at different levels of complexity.

'knockout' techniques, it became possible in the early 1990s to apply genetics or 'reverse genetics' to neuroscience of a mammalian (mouse) system for the study of cognitive mechanisms (Silva *et al.* 1992*a,b*). However, because of the inherent complexity of mammalian central nervous systems, it was necessary to add a spatial and/or temporal restriction to the genetic manipulations for them to be a truly effective approach. Thus, our experimental strategy was to create new mouse strains in which a gene of interest is deleted or overexpressed or the activity of its protein product is inhibited, in a limited brain area or, preferably, in a specific cell type of a restricted brain area of adult inbred mice (Tsien *et al.* 1996*a*; Nakazawa *et al.* 2002). We will then subject these genetically engineered mice, together with normal littermates, to a variety of analytical methods each designed to detect a defect or impairment at a particular level of complexity (figure 1). The advantages of this approach are that the blockade can be highly specific with respect to the gene and its protein product, the brain area and the type of cells. Furthermore, animal to animal reproducibility of the blockade is guaranteed. However, this type of genetic blockade is generally inferior to the pharmacological blockade with respect to temporal control. Nevertheless, there have been cases in the literature in which a reversible temporal control was combined with a certain degree of spatial restriction by genetic manipulations (Mayford *et al.* 1996; Mansuy *et al.* 1998).

## 2. MEMORY ACQUISITION

Using the Cre/loxP system (Sauer & Henderson 1988) we previously targeted a knockout of the obligatory NR subunit, NR1, to the CA1 pyramidal cells of young adult mice (Tsien *et al.* 1996*a,b*). These mice displayed impairments in the SC CA1 LTP and in spatial learning tested in the hidden platform version of the Morris watermaze. The mutant's inability to form normal memory representations as CA1 place cells (McHugh *et al.* 1996) suggested that this mutant mouse is defective in the acquisition rather than the retrieval of the memory. During recent years, we have demonstrated that the mutants are also impaired in non-spatial hippocampus-dependent learning; trace-fear conditioning (Huerta *et al.* 2000) and olfaction-based transverse patterning (Rondi-Reig *et al.* 2001). These findings provide the most cogent evidence for Hebb's hypothesis (Hebb 1949).

The standard Morris watermaze task tests animals' ability for 'reference memory', which is acquired incrementally over multiple trials and involves information constant across trials. Another type of memory supported by the hippocampus is 'episodic memory' (for humans) (Tulving 1972) or 'episodic-like memory' (for rodents) (Griffiths *et al.* 1999) which is acquired rapidly with one trial or one-time experience and involves trial- or event-specific information (Marr 1971; Tulving 1995). It is probable that different mechanisms underlie these two types of declarative memory (Squire 1994; Eichenbaum 1997; Vargha-Khadem *et al.* 1997). However, little is known about underlying differential mechanisms. We investigated two theories, both of which are based on mathematical modelling of memory systems. First, it has been suggested that the learning rate and the number of unambiguous patterns are greater in a network with bi-directional modifiability of synaptic strength than in a network with unidirectional modifiability (Willshaw & Dayan 1990). For instance, the information storage efficiency of a network with only LTP capability would be lower than that of a network with both LTP and LTD capability. We generated a mouse strain (CN-KO) in which the gene encoding the sole regulatory subunit of calcineurin in the brain (CNB1) is only deleted in the postnatal forebrain (Zeng *et al.* 2001). At the SC-CA1 synapses, LTD was significantly diminished while the LTP elicited by saturating stimulation (100 Hz, 1 s) was normal (a collaboration with Mark Bear). The mutant mice were normal in the acquisition and retrieval of spatial reference memory but were specifically impaired in two tasks for spatial episodic-like memory, namely a DMP version of the Morris watermaze (Steele & Morris 1999) and the working memory version of Olton's eight-arm radial maze (Olton & Papas 1979). These results support the notion that a network with bi-directional modifiability of synaptic strength plays a crucial role in the acquisition of episodic-like memory, while it is dispensable for reference memory.

In rodents, infusion of an NR antagonist into the hippocampus has been shown to result in a deficit in 'episodic-like' memory (Morris & Frey 1997; Steele & Morris 1999). However, to date, there has been no study, to our knowledge, that directly implicates a specific sub-field of the hippocampus or a specific protein therein in this mnemonic process. It has been suggested that recurrent networks with modifiable synaptic strength could support the rapid acquisition of memories of a one-time experience

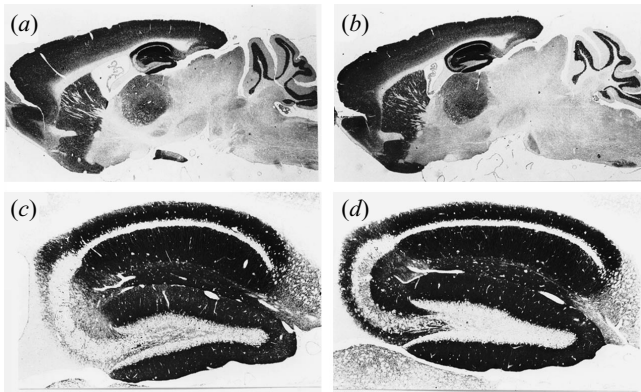


Figure 2. Immunoperoxidase staining of medial parasagittal sections of brains (*a,b*) or transverse sections (*c,d*) of the hippocampus derived from 18-week-old CA3-NR1 KO mice (*a,c*) and their control littermates (*b,d*) visualized with 3,3'-diaminobenzidine. The primary antibody used was specific for NR1. The immunoreactivity was specifically deficient in the apical and basal dendrite areas of mutant CA3.

(Marr 1971). The CA3 sub-field of the hippocampus is known to have a robust recurrent network with pyramidal cells receiving synaptic contacts from *ca.* 2% of other CA3 pyramidal cells (MacVicar & Dudek 1980; Miles & Traub 1986). Hebbian-type synaptic plasticity in the form of LTP has also been demonstrated at the synapses of recurrent collaterals in CA3 (Harris & Cotman 1986; Williams & Johnston 1988; Zalutsky & Nicoll 1990; Berger & Yeckel 1991). In conjunction with a set of inhibitory feedback inputs to CA3 pyramidal cells, such a CA3 recurrent network has been suggested to provide a mechanism for maintaining coherent information for short-term duration and to serve as a temporary storage site for short-term, episodic or working memories by reverberating activity in the recurrent collateral connections (Rawlins 1985; Wiebe *et al.* 1997; Kesner & Rolls 2001).

To test this proposal, we generated a knockout mouse (CA3-NR1 KO) in which the deletion of the NR1 gene is restricted to the CA3 pyramidal cells of an adult mouse (figure 2; Nakazawa *et al.* 2003). These mice were impaired in the spatial DMP task when the platform was placed in a novel location, but were normal when the platform location employed a few days earlier was reused. This behavioural deficit was highly specific in that the mutants were normal in the acquisition of spatial reference memory as tested by the standard hidden platform version of the Morris watermaze task (see below). In order to investigate the cellular mechanism underlying this specific behavioural impairment observed in the mutant mice, we monitored the activities of the pyramidal cells in CA1, the area downstream of CA3 and the site for the hippocampal output, before and after the animals entered a novel space from a familiar space. We found that the specificity of spatial tuning in the mutants was reduced during the first 15 min of exploration in the novel space compared with the same period in the familiar space. By contrast, no space shift-associated change of spatial tuning was observed when the mutant mice were returned 1 day later to the pair of spaces experienced on the previous day. The spatial tuning of CA1 place cells of control animals did not exhibit any space shift-associated changes. These results suggest that CA3 NRs, most probably those in the recur-

rent network, play a crucial role in rapid hippocampal encoding of a novel encounter and in one trial- or one experience-based rapid learning.

How does the lack of CA3 NRs lead to the decreased spatial specificity of CA1 pyramidal cells in the novel space? CA1 receives inputs from both the layer III stellate cells of the EC via the temporo-ammonic pathway, and from CA3 pyramidal cells via the SCs. During spatial exploration, cells in the superficial layer of the EC show spatially related responses with significantly lower specificity than that observed in CA3 (Barnes *et al.* 1990; Quirk *et al.* 1992; Frank *et al.* 2000), and it has been suggested that they provide a major source of input to CA1 (Vinogradova 1975; McNaughton *et al.* 1989; Brun *et al.* 2002), particularly during tasks that require encoding of novel information (Sybirska *et al.* 2000). The activity of the temporo-ammonic pathway has been shown to regulate the gating of CA1 spikes in EC-hippocampal slices (Remondes & Schuman 2002). Our result, demonstrating that CA1 place fields are less spatially tuned in the mutant animals when a new spatial representation is required, is consistent with the notion that a new spatial context is conveyed via the temporo-ammonic pathway. Moreover, our finding supports a long-standing hypothesis that the CA1 network acts as a comparator: detecting novelty or mismatches between actual sensory information from the EC and the expectation from memory in CA3 (Vinogradova 1970; Gray 1982; McNaughton *et al.* 1989; Moser & Paulsen 2001; Fyhn *et al.* 2002).

We propose that during exposure to a novel context, CA1 response is initially driven by the spatially broadly tuned, direct EC input. In control animals, NR function in CA3, perhaps via recurrent connections, allows the rapid formation of more spatially specific responses that can then drive correspondingly specific response in CA1 as the input through the SCs comes to dominate that from the EC. In CA3-NR KO mice, CA3 NR ablation leading to the lack of dominant CA3 input (Nakazawa *et al.* 2002, 2003) may result in the more gradual spatial refinement of CA1 place fields implemented by other hippocampal circuit plasticity. CA1 place field enlargement may be due to the prolonged influence of direct EC input to CA1 during this slow refinement process. Our results indicate that the reduced spatial tuning in mutants' CA1 lasts for at least 15 min (duration of place cell recording sessions). Thus, at some time between 15 min after the onset of exploration of a novel environment and the return to it 24 h later, normal place cell activity seems to be restored in the mutants. This reduced spatial tuning may, in turn, affect the accuracy of spatial learning during this period. The time-course of these physiological effects was roughly consistent with those of the behavioural deficits observed in the DMP tasks in which the mutants exhibited memory impairment for at least 20 min (four trials each taking *ca.* 2 min plus three inter-trial intervals of 5 min) in a test session with a novel platform location while they behaved normally for the platform locations experienced 4 days earlier.

### 3. MEMORY CONSOLIDATION

A critical feature of both memory consolidation and the formation of long-lasting synaptic plasticity is a requirement

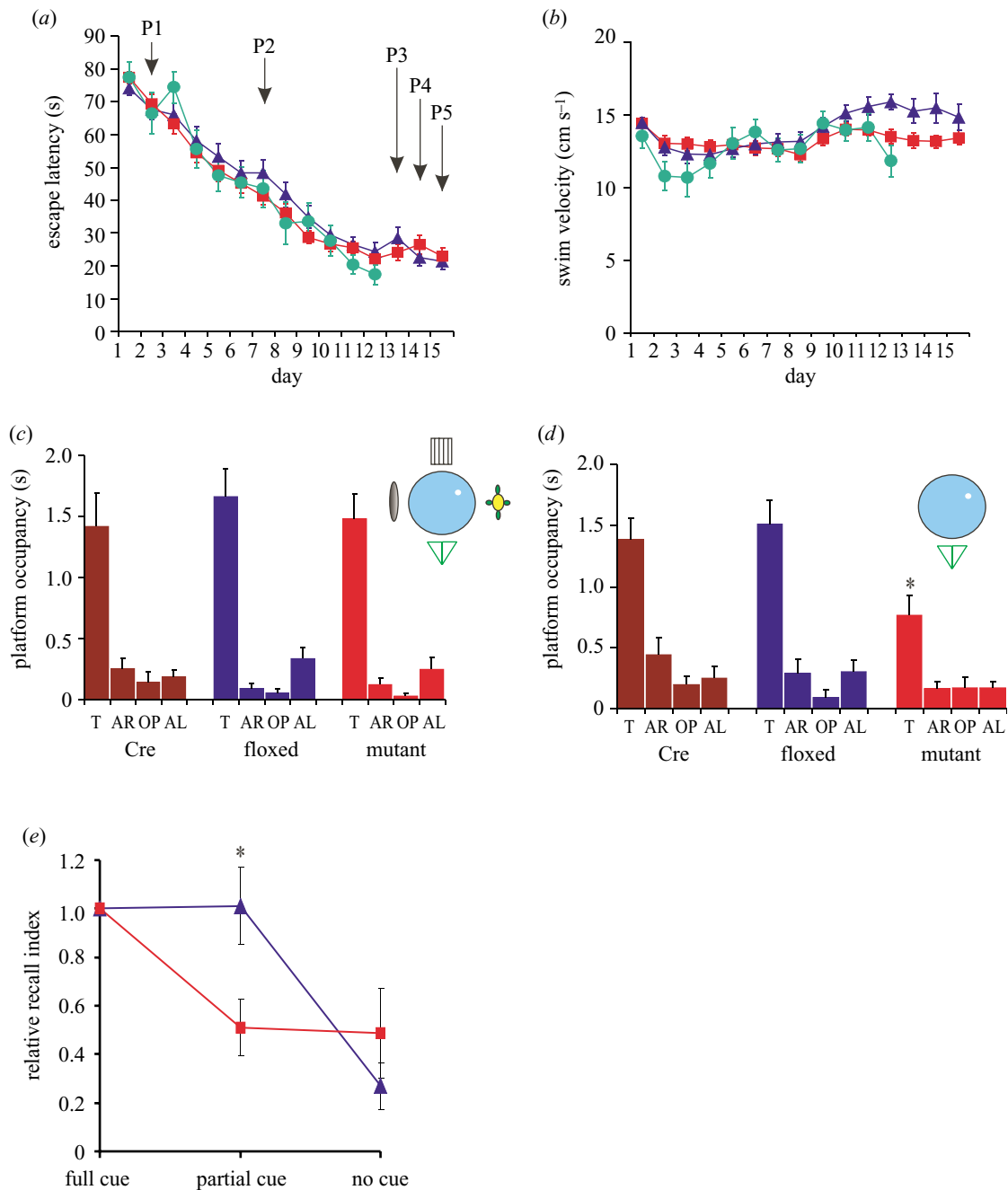


Figure 3. Performance of CA3-NR1 KO mice in the standard Morris watermaze task and recall capability under various cue conditions. (a,b) 18–24-week-old male CA3-NR1 KO mice (mutant, red,  $n = 44$ ), *fNR1* (blue,  $n = 37$ ), Cre ( $n = 14$ , not shown), and their wild-type littermates (green,  $n = 11$ ) were subjected to training trials under full-cue conditions. The four types of mouse did not differ significantly in (a) escape latency or (b) swimming velocity (genotype effect for each measure,  $F_{3,102} < 2.5$ ,  $p > 0.05$ ; genotype  $\times$  trial interaction for each measure,  $p > 0.05$ ). (c) Day 13 probe trial (P3) of randomly selected subsets of Cre ( $n = 14$ ), *fNR1* ( $n = 20$ ) and mutant ( $n = 23$ ) mice by absolute platform occupancy (time (s) the mice spent in the area which corresponded exactly to the area occupied by the platform during the training session) (Cre,  $F_{3,52} = 15.8$ ,  $p < 0.0001$ ; *fNR1*,  $F_{3,76} = 37.4$ ,  $p < 0.0001$ ; mutant,  $F_{3,88} = 35.5$ ,  $p < 0.0001$ ; Newman–Keuls *post hoc* comparison (the target platform position compared with all the other platform positions);  $p < 0.01$  for all genotypes). (d) The same sets of mice as in (c) were subjected to partial-cue probe trials on day 14 (P4) and absolute platform occupancy was assessed. Cre and *fNR1* mice exhibited similar recall under partial-cue conditions as under full-cue conditions (paired *t*-test,  $p > 0.9$  for each genotype), while recall by the mutant mice was impaired (paired *t*-test,  $*p < 0.01$ ). (e) Relative recall index (RRI, averaged ratio of the target platform occupancy of the partial-cue (P4) or no-cue (P5) probe trial to that of the full-cue (P3) probe trial for each animal) of *fNR1* mice ( $n = 18$ , blue) and mutant mice ( $n = 22$ , red). The RRI value difference between the *fNR1* and the mutant mice under the partial-cue conditions was significant ( $*p < 0.009$ , Mann–Whitney *U*-test), while that under no-cue conditions was not ( $p = 0.9$ , Mann–Whitney *U*-test). T, target quadrant; AR, adjacent right quadrant; OP, opposite quadrant; AL, adjacent left quadrant.

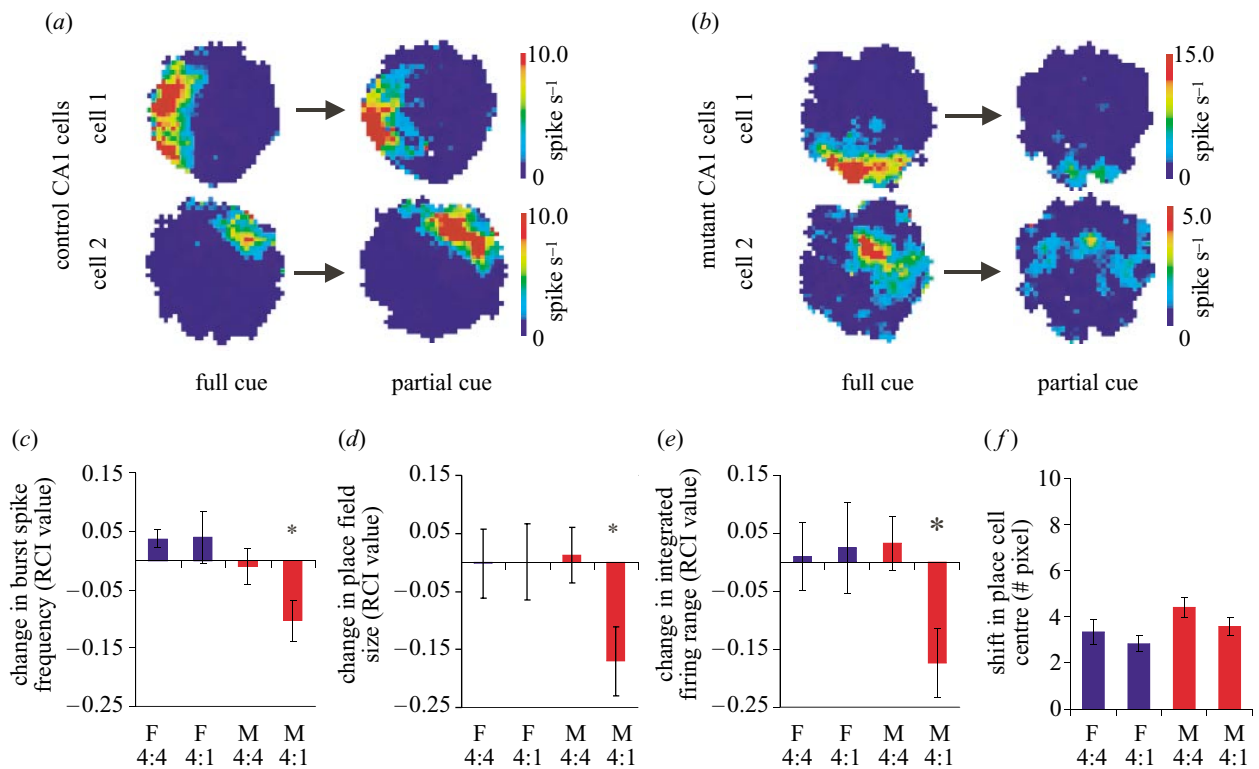


Figure 4. CA1 place cell activity in CA3-NR1 KO mice. (*a,b*) Examples of place fields that are representative of cells that showed no reduction (*fNR1*; (*a*)) and a reduction of field size (mutant; (*b*)) before and after partial cue removal. (*c–e*) Relative change in the place field properties for each cell recorded across two conditions quantified with a relative change index (RCI, defined as the difference between the cell's firing between two conditions divided by the sum of the cell's firing across the two conditions). Among the cells that were identified as the same cells throughout the two recording sessions, the average burst spike frequency ((*c*)  $F_{3,140} = 4.16$ ,  $p < 0.007$ ; Fisher's *post hoc* comparison (mutant 4 : 1 versus all the other three paradigms),  $*p < 0.05$ ), place field size over 1 Hz ((*d*)  $F_{3,140} = 2.68$ ,  $p < 0.049$ ; Fisher's *post hoc* comparison (mutant 4 : 1 versus all the other three paradigms),  $*p < 0.05$ ) and the integrated firing rate ((*e*)  $F_{3,140} = 3.20$ ,  $p < 0.025$ ; Fisher's *post hoc* comparison (mutant 4 : 1 versus all the other three paradigms),  $*p < 0.05$ ) were significantly reduced in the mutant animals (red bars) only after partial cue removal (4 : 1). By contrast, partial cue removal did not affect the CA1 place cell activity in the control mice (blue bars). (*f*) Location of the CA1 place field centre between the two recording sessions was not shifted regardless of genotype and cue manipulation ( $F_{3,140} = 2.15$ ,  $p = 0.097$ ). F, *fNR1* control mice; M, mutant mice.

for new mRNA and protein synthesis (Frey *et al.* 1988; Milner *et al.* 1998). Previous studies of memory consolidation have largely focused on the regulation of gene expression, establishing an important role for the transcription factor CREB in this process (Bourtchuladze *et al.* 1994; Bartsch *et al.* 1995). We have studied the roles of two kinases, CaMKIV and ERK, in memory consolidation and long-lasting synaptic plasticity using conditional gene engineering techniques.

Among several  $Ca^{2+}$ -dependent protein kinases that phosphorylate CREB at Ser-133 for its activation, CaMKIV is the only one detected predominantly in the nuclei of neurons (Jensen *et al.* 1991). However, global CaMKIV knockout mice exhibited developmental impairments and conflicting data regarding the role of this kinase in LTP and memory (Ho *et al.* 2000; Wu *et al.* 2000). We generated and analysed transgenic mice in which a dominant-negative form of CaMKIV (dnCaMKIV) inhibits  $Ca^{2+}$ -stimulated CaMKIV activity only in the postnatal forebrain (a collaboration with Tom Soderling) (Kang *et al.* 2001). In these transgenic mice, activity-induced CREB phosphorylation and *c-Fos* expression were significantly attenuated. Hippocampal L-LTP was also impaired whereas basic synaptic function and E-LTP

were unaffected. Further, these deficits correlated with impairments in long-term memory, specifically in its consolidation–retention phase but not in the acquisition phase. These results indicate that neural activity-dependent CaMKIV signalling in the neuronal nucleus plays an important role in the consolidation–retention of hippocampus-dependent long-term memory.

In another study in my laboratory (currently unpublished), Ray Kelleher and Arvind Govindarajan focused on the ERK signalling cascade which is known to play a central role in the response to mitogenic signals in many cell types by regulating the phosphorylation of key transcription factors. In neurons, the ERK pathway is activated in response to calcium influx and neurotrophin stimulation. Although previous studies relying on the use of pharmacological inhibitors have implicated ERK activation in LTP and memory (Impey *et al.* 1999; Orban *et al.* 1999), the underlying cellular and molecular mechanisms remain unclear. We generated transgenic mice in which ERK activation is inhibited by a dominant-negative ERK kinase (dnMEK1) transgene only in the postnatal forebrain. The protein synthesis-dependent portion of L-LTP was impaired at SC-CA1 synapses, whereas E-LTP, paired pulse facilitation, and basal synaptic transmission

were normal. Consistent with this selective impairment in hippocampal L-LTP, the mutant mice exhibited a selective impairment in long-term memory in contextual fear conditioning. We further investigated the role of the ERK signalling pathway in activity-dependent protein synthesis by applying transfection techniques to cultured hippocampal neurons. We propose that the ERK signalling pathway governs memory consolidation and long-lasting synaptic plasticity, at least in part, through a novel role in the regulation of protein synthesis in neurons.

#### 4. MEMORY RECALL

In the past, the neuroscience of associative memory has largely focused on the mechanisms underlying its acquisition and consolidation, while the mechanism of memory recall has been relatively ignored. In day-to-day life, recall of associative memory almost always occurs under the constraints of limited cues. For instance, recalling the rich content of interesting conversations with someone can be triggered merely by the subsequent sighting of that person. In the past, a study of the mechanism underlying this fundamental feature of memory recall, referred to as 'pattern completion,' has been limited to computational modelling. These theoretical studies proposed that a recurrent network with modifiable synaptic strength, such as that in hippocampal area CA3, could provide this pattern completion capability (Marr 1971; Gardner-Medwin 1976; Hopfield 1982; McNaughton & Morris 1987; Rolls 1989; Hasselmo *et al.* 1995). However, because of technical difficulties, at least 30 years have passed with virtually no experimental evidence for or against the hypothesis since David Marr's first publication on this subject (Marr 1971). We addressed this issue with CA3-NR1 KO mice (Nakazawa *et al.* 2002). A set of immunocytochemical and cytochemical experiments demonstrated the integrity of the cytoarchitecture of the mutant hippocampus (a collaboration with Masahiko Watanabe's laboratory). Whole-cell patch-clamp recordings performed on visually identified cells in acute hippocampal slices showed the normal intrinsic properties of CA3 pyramidal cells. The evoked *N*-methyl-D-aspartate currents were entirely missing at C/A-CA3 synapses while those at the MF-CA3 synapses as well as the medial perforant path-dentate gyrus synapses and SC-CA1 synapses were normal. LTP was deficient at C/A-CA3 synapses, whereas it was intact at MF-CA3 and SC-CA1 synapses (a collaboration with Dan Johnston's laboratory). The mutant mice were normal in the acquisition and retrieval of spatial memory tested in the hidden platform version of the Morris water-maze. However, when the memory of the location of the hidden platform was tested following removal of three of the four major extramaze cues (partial cue conditions), the mutants exhibited a clear deficit of memory retrieval compared with the control animals (figure 3). That this specific recall deficit was not due to faster loss of the memory was confirmed by demonstrating that the mutants reached the platform as fast as the control mice in a trial carried out 1 h after the partial cue probe test following the restoration of the platform and the complete set of cues (full-cue conditions).

To investigate the neural mechanisms that might underlie the specific recall deficit we examined the neurophysi-

ological consequences of CA3-NR1 deletion by analysing CA1 place cell activity. While the complex spike bursting was significantly reduced, the basic cellular properties of CA1 pyramidal cells, such as mean firing rate and spike width, were normal in the mutants. No significant differences were observed between the mutant and control mice in either place field size or average firing rate within a cell's place field (integrated firing rate). Furthermore, the ability of cells with overlapping place fields to fire in a coordinated manner did not differ between control and mutant mice (note that this is in contrast to CA1-NR1 KO mice). Thus, spatial information within CA1 is relatively preserved despite the loss of CA3 NRs providing a physiological correlate of the intact spatial performance of the CA3-NR1 KO mice in the Morris water-maze under full-cue conditions. We examined the effect of partial cue removal on CA1 output as follows. Mice were allowed to explore a familiar arena for 20–30 min under full-cue conditions, and then removed to their home cage. Following a 2 h delay, mice were returned to the open field with either the same four major extramaze cues present (full-cue conditions) or with three of the four cues removed (partial-cue conditions). In the control mice, there were no significant changes in place field properties associated with the change in the cue conditions; burst frequency, place field size or integrated firing rate was maintained upon partial cue removal. By contrast, mutant CA1 cells showed significant reduction in all of these properties (figure 4). Interestingly, however, the centre of individual place fields did not shift across conditions, suggesting that some reflection of past experience is maintained in the firing of mutant CA1 place cells even under conditions of partial cue removal. These physiological results are compatible with the behavioural results, suggesting that reductions in CA1 output as a consequence of reduced CA3 drive resulting from cue removal may make it more difficult for mutants to retrieve spatial memories. This impairment may underlie the inability of mutants to solve spatial memory tasks such as the water-maze when only partial distal cues are available.

A substantial portion of aged individuals exhibit deficits of memory recall (Gallagher & Rapp 1997). In early Alzheimer patients, retrieval is the first type of memory function to decline; such retrieval deficits may serve as an early predictor of Alzheimer's disease (Tuokko *et al.* 1991; Backman *et al.* 1999). Normal ageing produces a CA3-selective pattern of neurochemical alterations (Le Jeune *et al.* 1996; Kadar *et al.* 1998; Adams *et al.* 2001). Exposure to chronic stress, which can lead to memory deficits, also selectively causes atrophy in the apical dendrites of CA3 pyramidal cells (McEwen 1999). These results are consistent with our findings in mice that the CA3 region is critical for cognitive functions related to memory recall through pattern completion.

#### 5. CONCLUSIONS

The multifaceted analyses of the CA1-NR1 KO mice and CA3-NR1 KO mice dramatically illustrated the power of cell type-restricted, adult-onset gene manipulations in the study of molecular, cellular, neuronal ensemble and neural circuitry mechanisms underlying learning and memory. These studies provide evidence that the same neurotransmitter receptors, NRs, can play quite distinct

roles in the mnemonic process—memory acquisition versus memory recall—depending on where in the brain, and in which hippocampal circuitries, they are expressed. The desired level of temporal control of blockade has yet to be attained (i.e. reversibly regulated control of gene expression), but the exquisite cell-type specificity and developmentally late onset of the gene knockout attained in the mutant mice allowed us to dissect the mnemonic mechanisms at a level of resolution that could not be reached by other methods. In the future, it is expected that a number of genetically engineered mouse strains will be generated that harbour spatially restricted and/or temporally regulated expression of genes. Multifaceted analyses of these mice will help dissect mechanisms, not only for memory but also other cognitive functions.

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## GLOSSARY

- C/A: commissural/associational  
 CaMKIV: Ca<sup>2+</sup>/calmodulin-dependent protein kinase IV  
 CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione  
 CREB: cAMP response element binding protein  
 DMP: delayed-matching-to-place  
 EC: entorhinal cortex  
 E-LTP: early phase long-term potentiation  
 ERK: extracellular signal-regulated protein kinase  
 LTD: long-term depression  
 L-LTP: late phase long-term potentiation  
 LTP: long-term potentiation  
 MF: mossy fibre  
 NR: *N*-methyl-D-aspartate receptor  
 SC: Schaffer collateral