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NMDA RECEPTORS IN CA1 PYRAMIDAL CELLS ARE NEEDED FOR SPATIAL LEARNING, BUT NOT FOR NONSPATIAL LEARNING

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The major research interest in our laboratory is to uncover the molecular, cellular, and neuronal ensemble mechanisms that subserve learning, and the memory and associated cognitive functions of mammalian brains. Since these functions can be reliably measured only by monitoring the performance of live animals in behavioral tasks, it is important to develop an experimental approach that utilizes live animals but permits the identification of underlying molecules, cells, and neuronal subpopulations which play a crucial role in behavior. A potentially effective approach is to perturb the complex nervous system of a live animal with a treatment or agent whose direct effect is well known, and examine its far-reaching effects on behavior and cognition.

I. LESION, PHARMACEUTICAL, AND GENETIC APPROACHES

Table I lists several methods that have been used for this purpose. The lesion method is effective for assessing the global role of a particular brain region or a connection between brain regions, but it is not intended to identify underlying molecular mechanisms. Phar-

TABLE I
Comparison of Brain Manipulation Methods

Manipulation	Example	Strength	Weakness
Lesion/ablation	Hippocampua Medial septum	Glogbal function	Non molecular Insufficient reproducibility
Pharmacological blockade	AP5, many others	Temporal restriction Molecular	Often insufficient specificity Insufficient spatial restriction Insufficient reproducibility
Global gene knockout	αCaMKII fyn	Specificity Reproducibility	No temporal or spatial restriction Frequent lethality
Conditional (spatial and/or temporal) or inhibition knockout	NMDAR1	Specificity Spatial and temporal restriction	Labor intensive Costly

macological interventions can be powerful if the specificity of the drug is well known. The advantage of this approach is that the intervention can be executed, often reversibly, after the development of the nervous system is completed (temporal control). Disadvantages include a difficulty in restricting the area affected by the drug in a reproducible manner and the fact that the specificity of many compounds is insufficient or not fully understood.

In 1992, the advent of knockout mouse technology reached neuroscience (*5*, *11*, *12*). This method provides a highly specific means of deleting the function of a receptor, enzyme or other protein at the gene level. The animal to animal reproducibility of the intervention is excellent if the knockout mice are made in a pure genetic background. Nonetheless, the knockout technology in its original version has serious limitations. With this technique, the gene of interest is deleted in all cells and throughout the life of the animal: there is no spatial or temporal restriction. This often results in developmental and/or pleiotropic defects. Thus, in order to take advantage of the high specificity and reproducibility of the knockout technology, it is important to add spatial and/or temporal control.

Using the phage P1-derived Cre/loxP recombination system (*10*), we recently succeeded in developing a technique that permits a gene knockout restricted to a small region of the hippocampus, the CA1 area (*13*).

II. CA1-SPECIFIC NMDA RECEPTOR KNOCKOUT MOUSE IS IMPAIRED IN CA1 LTP, IN SPATIAL LEARNING AND IN CA-1 PLACE cELLS

We applied this technique to the NR1 gene, the gene encoding the essential subunit for all forms of N-methyl-D-aspartate (NMDA) receptors (*14*). The CA1-specific NR1 knockout mouse ($CA1^{KO}NR1$) grows normally, in stark contrast to the previously reported NR1 knockout mouse (*8*) with a global NR1 gene deletion. Both *in situ* hybridization and electrophysiological studies in brain slices supported the notion that the NR1 knockout is specific to the CA1 area. As expected, LTP (long term potentiation) was deficient at the Schaeffer collateral-CA1 synapses in the mutant mice, while the early component of the excitatory postsynaptic current (EPSC) mediated by AMPA receptors was intact (*14*). We examined whether the lack of NMDA receptor-mediated LTP in the CA1 region deprived the mutant mice of the ability to acquire spatial memory. Indeed, the mutant mice exhibited impaired spatial learning in the hidden-platform version of the Morris water maze but were normal in the nonspatial, cue-guided version of the same maze. These results strongly suggest that NMDA receptor-dependent synaptic plasticity in the CA1 area plays an essential role in spatial learning (*14*). In order to further investigate the mechanism subserving spatial learning, we examined the place-specific firing of CA1 pyramidal cells by applying the large-scale multiple electrode recording technique to freely behaving mutant mice. We discovered that although the CA1 pyramidal cells of these mice retain place-related activity, there was a significant decrease in the spatial specificity of individual place fields (*9*). We also found a striking deficit in the coordinated firing of pairs of neurons tuned to similar spatial locations. As far as we are aware, this is the first time that a relationship between synaptic plasticity and place cells was demonstrated. Our results indicate that NMDA receptor-mediated synaptic plasticity is necessary for the

proper representation of space in the CA1 region of the hippocam pus. For an ensemble code to provide accurate spatial information there must be robust covariance of the firing of cells that hav overlapping spatial fields (*9*). The lack of such covariance, which w observed in the mutant mice, would radically impair the ability of th animal to use a hippocampal ensemble code as a robust indicator c spatial location. Thus, the downstream brain regions will fail to lear anything about place from CA1 in the mutant mice. We propose tha this can explain why the mutant mice are deficient in spatial learnin

III. NR1 GENE DELETION OCCURS POSTDEVELOPMENTALLY AND ONLY I PYRAMIDAL CELLS

Since the publication of the results that are summarized above, w have further characterized the $CA1^{KO}NR1$ mice by various method The additional results are briefly described below.

First, by monitoring NMDA receptor-mediated EPSCs, v demonstrated that the NR1 deletion in the CA1 area does not occ until postnatal third week (P.T. Huerta and S. Tonegawa, unpu lished observation). This is presumably because the transcription promoter that controls the expression of the Cre transgene is acti only postnatally, and it takes some time after birth for the C recombinase to reach the threshold level required for efficient reco bination. Importantly, this delay in NR1 gene deletion provides additional advantage because it means that any adverse effect of t lack of NR1 gene on development would be minimal. Second, the is the effect of an animal's age on the CA1-specificity of knocko Both *in situ* hybridization and recording of NMDA recept mediated EPSC indicate that the CA1-specificity of the NR1 kno out is maintained until about five months of age, but thereafter t NR1 gene deletion spreads to some of the other forebrain areas, su as the dentate gyrus and some neocortex areas (P.T. Huerta, Kato, and S. Tonegawa, unpublished observation). Third, within age range described above, the NR1 knockout seems to be not o CA1-specific but also CA1 pyramidal cell-specific. Thus, we dem strated that NMDA receptor-mediated EPSCs are normal in inh tory interneurons in the stratum oriens of the CA1 area (P.T. Hue

and S. Tonegawa, unpublished observation). This observation was corroborated with results obtained by a confocal microscopic analysis of hippocampal slices derived from the Cre transgenic mouse line T50 (*13*) that had been crossed to a reporter mouse in which the expression of GFP (green fluorescence protein) depends on Cre/loxP recombination. Immunohistology of hippocampal slices with antibody for an inhibitory neuron marker (parvalbumin) indicated that GFP-derived fluorescence, a result of Cre/loxP recombination, occurs only in CA1 pyramidal cells (Z.J. Huang and S. Tonegawa, unpublished observation).

We subjected the $CA1^{KO}NR1$ mice to several other memory tasks that have been claimed to be hippocampus-dependent. We found that the mutant mice are clearly impaired in Kesner's cheese board maze, a dry land version of the water maze task (*7*). The mutant mice were also impaired in the spatial alternation task in Olton's T-maze (*6*). These results confirm that $CA1^{KO}NR1$ mice are deficient in spatial learning (P.T. Huerta and S. Tonegawa, unpublished observation). Although there is a general agreement as to the requirement of the hippocampus for spatial learning, that for nonspatial learning is uncertain. Nevertheless, Cohen and Eichenbaum provided cogent evidence for the concept that the function of the hippocampus may be in "relational learning" which encompasses spatial and some nonspatial learning (*2*). For instance, using ibotenic acid as the lesion agent, Bunsey and Eichenbaum demonstrated that the social transmission of food preference (STFP), a purely nonspatial task, required an intact hippocampus including CA1, CA3, dentate gyrus, and subiculum when a 24 hr delay was introduced between the socialization session and the test session (*1*). This study, however, did not address the question whether synaptic plasticity in any of these hippocampal areas is required for this task. It was therefore of considerable interest to examine whether $CA1^{KO}NR1$ mice can perform the STFP task. As shown in Fig. 1, mutant mice exhibited robust food preference either immediately or 24 hr after the socialization session (P.T. Huerta and S. Tonegawa, unpublished observation). In the earlier lesion study, Bunsey and Eichenbaum, in fact, concluded that CA1 and CA3 are not essential for STFP, although they regarded this conclusion as "tentative" because the lesion was

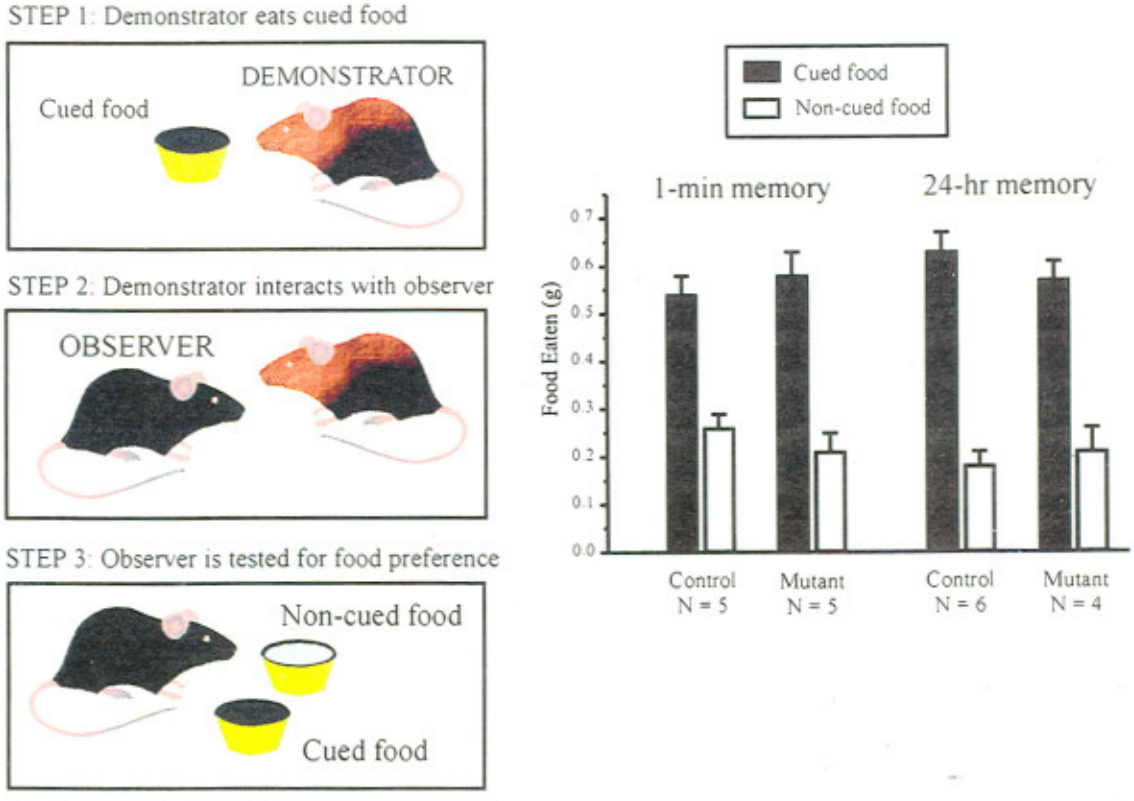

Fig. 1. CA1^{KO}NR1 mice show normal social transmission of food preference. The left panel gives a brief description of the experimental procedure. In step 1, a demonstrator mouse eats cued food (*i.e.*, chow mixed with cinnamon) for 2 hr. Subsequently, in step 2, the demonstrator interacts with observer mice for 5 min. It has been previously shown that during the interaction the observer associates carbon disulfide, a natural component of the animal's breath, with the odor of the food recently eaten by the demonstrator (Galef *et al.*, *Physiol. Behav.* **42**, 119–124, 1988). In step 3, following a delay of 1 min or 24 hr, the observer mouse is offered a choice of cued and non-cued food (*i.e.*, chow mixed with cocoa). The amount of cued and non-cued food eaten by the observer mice during 2 hr was measured and plotted in the right panel.

incomplete (63%) (*1*). Our results with CA1^{KO}NR1 mice confirm this earlier conclusion using a more specific and complete "molecular lesion".

In order to determine whether the dispensability of intact CA1 NMDA receptors applies to other nonspatial tasks, we subjected the CA1^{KO}NR1 mice to a novel object recognition task (*3*). In the literature, hippocampus dependency of this task is controversial in rodents. Wood and Phillips claimed that an integrity of CA1 is required based on an experiment carried out in rats that underwent transient forebrain ischemia combined with hemorrhagic hypotension (*15*). In

contrast, Aggleton and his colleagues reported that rats with fornix lesions performed normally in the object recognition task and rats with perirhinal lesions were impaired in the delayed (15 min) version of the task (*4*). As shown in Fig. 2, we found that CA1^{KO}NR1 mice performed normally in both short (1 min) and delayed (24 hr) versions of the novel objection recognition tasks (P.T. Huerta and S. Tonegawa, unpublished observation).

Taken together, our behavioral studies of CA1^{KO}NR1 mice

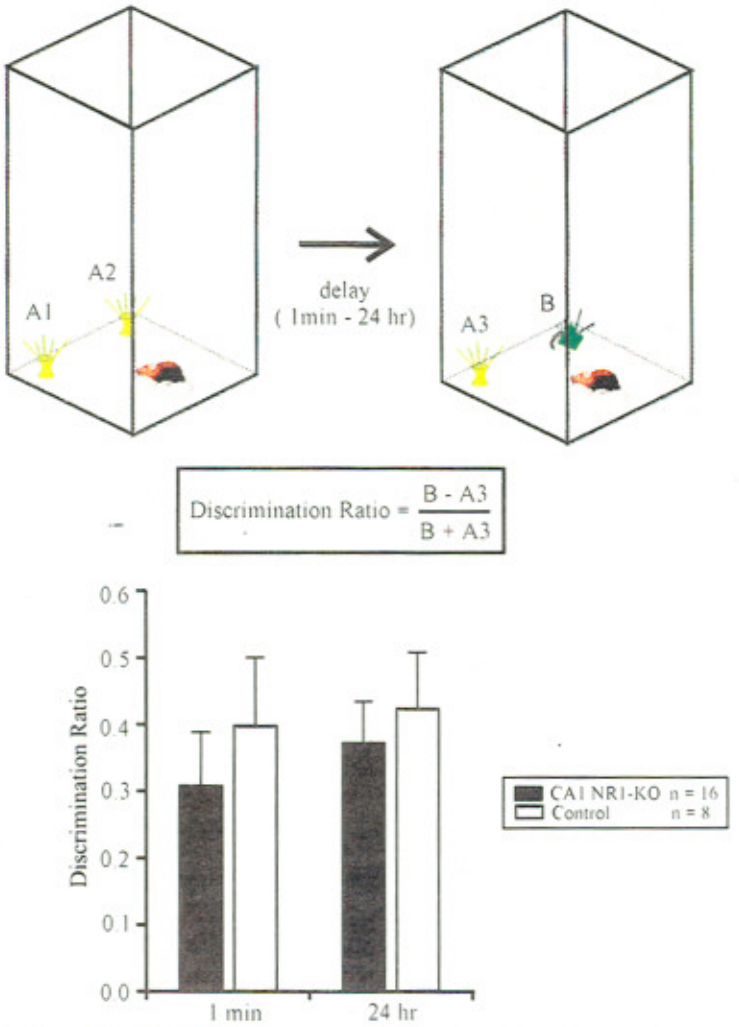

Fig. 2. CA1^{KO}NR1 mice are not impaired in the novel object recognition test. During the study phase, mice are exposed for 5 min to two identical objects (A1 and A2) located on the sides of the box. After a delay of either 1 min or 24 hr, mice are returned to the box and presented with an object similar to the ones in the study phase (A3) and a novel object (B). The time the mouse spends exploring A3 and B is measured, and used to produce a "discrimination ratio". Positive values indicate that the mouse preferred to explore the novel object.

combined with earlier lesion studies by others suggest a very interesting dichotomy for the role of the hippocampus in spatial and nonspatial memory. While an intact hippocampus may be needed not only in spatial tasks but also in at least some forms of nonspatial tasks, NMDA receptors within CA1 pyramid neurons may have differentiated roles in spatial *vs.* nonspatial tasks. For spatial tasks they are essential, while for nonspatial tasks they are not. While CA1 occupies a pivotal position in the major hippocampal pathway (trisynaptic loop) that receives inputs from the superficial layers of entorhinal cortex (ECs) and sends outputs back to the deeper layers of entorhinal cortex (ECd) via the subiculum (Sub), there are two shunt pathways consisting of ECs→Sub→ECd and ECs→CA1→Sub →ECd that do not involve the activation of CA1 NMDA receptors. It is therefore possible that the trisynaptic loop is essential for spatial tasks, while the subiculum shunt pathways are sufficient for the nonspatial tasks demanding "relational learning" such as STFP. Apart from the qualification mentioned above, Bunsey and Eichenbaum's lesion study is consistent with this hypothesis. The issue can be more rigorously tested when techniques become available for targeting of gene knockout to other subsystems of the hippocampus and associated cortices.

SUMMARY

We developed a conditional gene knockout technology with which a gene of interest can be deleted specifically in the CA1 pyramidal cells and applied this technique to the NMDA receptor gene NR1. The CA1-specific NR1 knockout (CA1-NR1 KO) mice are deficient in LTP and LTD at the Schaffer collateral CA1 synapses, in the coordinated firing of CA1 place cells and in the acquisition of spatial memory.

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