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>
<thead>
<tr>
<th>Manipulation</th>
<th>Example</th>
<th>Strength</th>
<th>Weakness</th>
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<tbody>
<tr>
<td>Lesion/ablation</td>
<td>Hippocampus</td>
<td>Global function</td>
<td>Non molecular</td>
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<td></td>
<td>Medial septum</td>
<td></td>
<td>Insufficient reproducibility</td>
</tr>
<tr>
<td>Pharmacological blockade</td>
<td>AP5, many others</td>
<td>Temporal restriction</td>
<td>Often insufficient specificity</td>
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<tr>
<td></td>
<td>Molecular</td>
<td></td>
<td>Insufficient spatial restriction</td>
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<td></td>
<td></td>
<td></td>
<td>Insufficient reproducibility</td>
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<tr>
<td>Global gene knockout</td>
<td>α-CaMKII fyn</td>
<td>Specificity</td>
<td>No temporal or spatial restriction</td>
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<td></td>
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<td>Reproducibility</td>
<td>Frequent lethality</td>
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<td>Conditional (spatial and/or temporal) or inhibition knockout</td>
<td>NMDAR1</td>
<td>Specificity</td>
<td>Labor intensive</td>
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<tr>
<td></td>
<td></td>
<td>Spatial and temporal restriction</td>
<td>Costly</td>
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Pharmacological 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\textsuperscript{K0}NR1) grows normally, in stark contrast to the previously reported NR1 knockout mouse (8) with a global NR1 gene deletion. Both \textit{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 hippocampus. For an ensemble code to provide accurate spatial information, there must be robust covariance of the firing of cells that have overlapping spatial fields (9). The lack of such covariance, which was observed in the mutant mice, would radically impair the ability of the animal to use a hippocampal ensemble code as a robust indicator of spatial location. Thus, the downstream brain regions will fail to learn anything about place from CA1 in the mutant mice. We propose that this can explain why the mutant mice are deficient in spatial learning.

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

Since the publication of the results that are summarized above, we have further characterized the CA1\textsuperscript{K0}NR1 mice by various methods. The additional results are briefly described below.

First, by monitoring NMDA receptor-mediated EPSCs, we demonstrated that the NR1 deletion in the CA1 area does not occur until postnatal third week (P.T. Huerta and S. Tonegawa, unpublished observation). This is presumably because the transcription promoter that controls the expression of the Cre transgene is active only postnatally, and it takes some time after birth for the Cre recombinase to reach the threshold level required for efficient recombination. Importantly, this delay in NR1 gene deletion provides an additional advantage because it means that any adverse effect of the lack of NR1 gene on development would be minimal. Second, the effect of an animal's age on the CA1-specificity of knockout was demonstrated both \textit{in situ} hybridization and recording of NMDA receptor-mediated EPSC indicate that the CA1-specificity of the NR1 knockout is maintained until about five months of age, but thereafter it spreads to some of the other forebrain areas, such as the dentate gyrus and some neocortex areas (P.T. Huerta, Kato, and S. Tonegawa, unpublished observation). Third, within the age range described above, the NR1 knockout seems to be not only CA1-specific but also CA1 pyramidal cell-specific. Thus, we demonstrated that NMDA receptor-mediated EPSCs are normal in inhibitory interneurons in the stratum oriens of the CA1 area (P.T. Hu...
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^K0NR1 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^K0NR1 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 (7). 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^K0NR1 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
STEP 1: Demonstrator eats cued food

**DEMONSTRATOR**

Cued food

STEP 2: Demonstrator interacts with observer

**OBSERVER**

Non-cued food

Cued food

STEP 3: Observer is tested for food preference

Fig. 1. CA1KO 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 CA1KO 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 CA1KO 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\textsuperscript{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\textsuperscript{KO}NR1 mice
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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