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27. Cryostat sections (7 μm) were prepared on gelatin-coated slides. The antisense RNA probes were prepared with T3 (D2R) and Sp6 (D1R) polymerases and α-^{35}S-labeled cytidine triphosphate (Amersham). The D1R and D2R probes were synthesized from 384–base pair–long (linearized by Bsu 36I) and 1680–base pair–long (linearized by Eco RI) mouse cDNA templates, respectively. Hybridization conditions and preparation of probes for retinoid receptors were as described [P. Dollé, E. Ruberte, P. Leroy, G. M. Morris-Kay, P. Chambon, *Development* **110**, 1133 (1990)]. All slides were exposed to Kodak NTB-2 autoradiography emulsion for 2 weeks.
28. For cocaine-induced behavior analysis, animals of each genotype were divided into two groups treated with an intraperitoneal injection of either cocaine (20 mg per kilogram of body weight) or an equivalent volume of saline. The open field test was carried out for 5 min, 25 min after the injection. Locomotion and stereotyped behaviors were scored as described in (8). Each animal was tested only once.
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Abnormal Hippocampal Spatial Representations in αCaMKII^{T286A} and CREB$^{\alpha\Delta-}$ Mice

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Hippocampal "place cells" fire selectively when an animal is in a specific location. The fine-tuning and stability of place cell firing was compared in two types of mutant mice with different long-term potentiation (LTP) and place learning impairments. Place cells from both mutants showed decreased spatial selectivity. Place cell stability was also deficient in both mutants and, consistent with the severities in their LTP and spatial learning deficits, was more affected in mice with a point mutation [threonine (T) at position 286 mutated to alanine (A)] in the α calmodulin kinase II (αCaMKII^{T286A}) than in mice deficient for the α and Δ isoforms of adenosine 3′5′-monophosphate–responsive element binding proteins (CREB$^{\alpha\Delta-}$). Thus, LTP appears to be important for the fine tuning and stabilization of place cells, and these place cell properties may be necessary for spatial learning.

The hippocampus and related brain structures play a critical role in spatial memory in rodents (*1*, *2*). Correspondingly, activity of the CA1 and CA3 pyramidal cells reflects an animal's position in space, reinforcing the hypothesis that the hippocampus mediates a maplike representation of the environment (*1*, *3*). In addition, hippocampal circuitry supports a variety of synaptic plasticity mechanisms including *N*-methyl-D-aspartate receptor (NMDAR)–dependent LTP (*4*). Current research with mutant mice (*5*–*7*) is aimed at drawing a closer connection between LTP, place cells, and spatial memory (*8*). Here we examined the spatial firing patterns of hippocampal cells in αCaMKII^{T286A} and CREB$^{\alpha\Delta-}$ mutant mice because these mutants differ substantially in the severity of their defects in synaptic plasticity and learning. The αCaMKII^{T286A} mice are severely impaired in spatial learning as well as in NMDAR-dependent LTP in the CA1 region of the hippocampus (*9*). In contrast, CREB$^{\alpha\Delta-}$ mice have reduced LTP and mild spatial learning deficits (*10*). Consequently, we expected to find more profound abnormalities in the spatial representations of αCaMKII^{T286A} mice than in those of CREB$^{\alpha\Delta-}$ mice.

Mice were repeatedly allowed to explore a four-arm radial maze that contained a large set of controlled stimuli, including local cues consisting of a distinctive surface on each maze arm, and distal cues composed of distinct three-dimensional objects on a curtain surrounding the maze (Fig. 1). Once hippocampal complex-spike cells were isolated (*11*), recordings were taken for a 5- to 10-min baseline trial in which all distal and local cues were in their usual configuration (session I). Immediately afterward we tested the reaction of cells to a rearrangement of the familiar cues by recording for an additional 5 to 10 min with the local and distal cues rotated 90° in opposite directions (session II). Finally, to assess the ability of place cells to recover their original representations, we recorded the cells again for 5 to 10 min with the

Table 1. Firing properties of place cells. Numbers in parentheses indicate standard errors.

Place cell parameter	Wild type	αCaMKII^{T286A}	CREB$^{\alpha\Delta-}$
No. of complex-spike cells	24	32	45
No. of place cells	12	14	24
Mean firing rate (Hz)*	0.678 (0.189)	1.239 (0.304)	0.844 (0.161)
No. of fields per cell	1.833 (0.297)	2.643 (0.401)	2.917 (0.345)
Place field size (pixels)	10.091 (1.147)	8.811 (0.910)	11.129 (1.185)
Infield firing rate (Hz)†	4.342 (0.467)	6.337 (0.776)‡§	3.607 (0.265)
Spatial selectivity‖	0.995 (0.081)	0.707 (0.035)‡	0.726 (0.037)‡

*The firing rate was calculated as the total number of spikes divided by total time (in seconds) spent in the maze. †Mean firing rates for pixels within the place fields. ‡Significantly different (Newman-Keuls tests, $P < 0.05$) from WT cells. §Significantly different (Newman-Keuls tests, $P < 0.05$) from CREB$^{\alpha\Delta-}$ cells. ‖Logarithm of the ratio of the mean firing rate within the field to the mean firing of all rates outside the field. A score of 1.0 indicates a 10-fold increase in firing inside the place field.

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local and distal cues in their original configuration (session III).

Here we describe the spatial firing patterns of complex-spike cells from three αCaMKII^{T286A} mice, six CREB$^{\alpha\Delta-}$ mice, and four wild-type (WT) littermates (*12*). In session I, about half of the neurons recorded from each group were identified as place cells, that is, cells with spatially selective activity characterized as place fields (Fig. 1) (*13*). As is typical of rat place cells (*3*), the overall firing rates of mouse place cells were ~1 Hz in all groups (Table 1). In addition, other standard firing parameters of these cells did not differ significantly among the groups, although in αCaMKII^{T286A} mice firing within the place field was elevated. However, spatial selectivity (the ratio of mean firing rate in, and mean firing rate out of the place fields) was abnormally low in both αCaMKII^{T286A} and CREB$^{\alpha\Delta-}$ mice [one-way analysis of variance, $F(2,126) = 7.835$, $P < 0.001$] (Table 1), as reflected by more scattered firing patterns (Fig. 1). According to previous observations, repeated exposure to a new environment results in a "focusing" of place fields, reflected by an increase in spatial selectivity (*14, 15*). This phenomenon is prevented by treatment with an NMDAR antagonist (*15*), which is also known to block some forms of LTP (*4*). One interpretation of the poor selectivity in the mutant place cells is that focusing did not occur because of their deficient capacity for LTP. These findings are consistent with previous reports of decreased spatial selectivity in two mutants with abnormal LTP (*6, 7*).

In session II, when the familiar cues were reconfigured, WT cells largely maintained the same spatial representations as in session I, whereas αCaMKII^{T286A} and CREB$^{\alpha\Delta-}$ cells showed new representations, indicating a marked instability of place representations in these mutant mice (Fig. 2). Two-thirds of WT place cells kept the identical spatial representations (*16*), or the representation changed only by rotation of all the place fields 90° in concert with the rotation of either the local or the distal cues (Same, Fig.

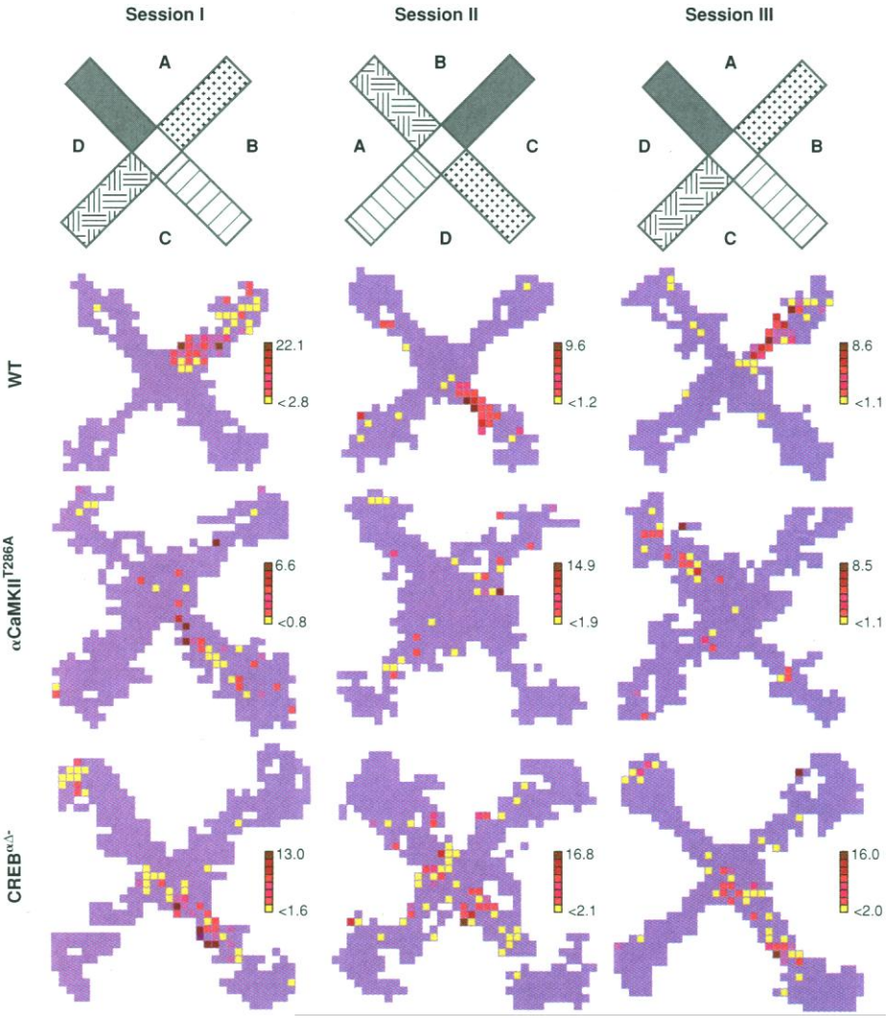

Fig. 1. Example of wild-type (WT), αCaMKII^{T286A}, and CREB$^{\alpha\Delta-}$ place cells. (**Top**) Diagram of the radial maze. Four distinct, brightly colored objects (distal cues A, B, C, and D) were placed on dark curtains surrounding the maze, and each of the 30-cm maze arms was covered with an insert that was distinct in texture, color, and odor (local cues indicated by shadings). The cue configuration was identical for sessions I and III. In session II, all four local cues were rotated 90° clockwise, and all four distal cues were rotated 90° counterclockwise. (**Bottom**) Spatial firing patterns of typical WT, αCaMKII^{T286A}, and CREB$^{\alpha\Delta-}$ cells. Blue pixels indicate locations visited at least three times without cell activity, and colored pixels indicate local activity rates (in hertz). The place field of the WT cell followed the rotated local cues in session II, and returned to the original location in session III. In the αCaMKII^{T286A} cell, spatial localized firing in session I was unstable, that is, it changed loci unpredictably across subsequent sessions. In the CREB$^{\alpha\Delta-}$ cell, multiple place fields developed in session I were degraded in session II, but were restored in session III.

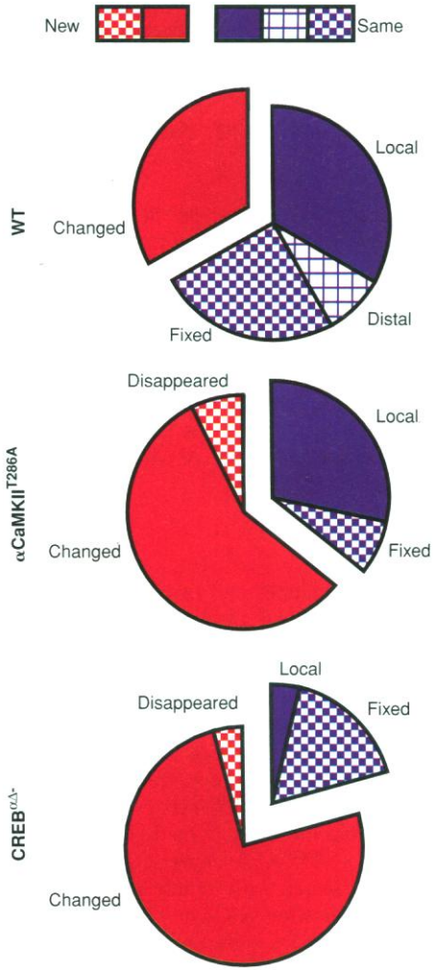

Fig. 2. Place cell responses to cue manipulations in session II. Cells maintaining place fields developed in session I (Same) include those that remained fixed in the same maze arm or rotated with the local or distal cues. Cells considered to have different representations (New) include those whose place fields changed location inconsistent with a simple rotation (*16*), and those in which the place field disappeared altogether.

2) (*17*). The remaining minority of cells developed different place fields, and no cells lost spatial firing altogether (New, Fig. 2). In contrast, only a minority of both αCaMKII^{T286A} and CREB$^{\alpha\Delta-}$ cells maintained their place fields, whereas most developed new place fields, and a few lost spatial selectivity altogether. The distributions of Same and New responses differed significantly between groups [χ^2 (2) = 7.317, $P < 0.05$], and both the αCaMKII^{T286A} and CREB$^{\alpha\Delta-}$ distributions differed significantly from that for WT cells ($P < 0.05$).

When the highly familiar environment was presented again in session III, two-thirds of the WT cells and half of the CREB$^{\alpha\Delta-}$ cells showed the same representations as in session I, whereas only a few αCaMKII^{T286A} cells did so. Compared with WT mice, the proportion of Same responses in αCaMKII^{T286A} mice was significantly decreased [χ^2 (1) = 5.418, $P < 0.02$], whereas that for CREB$^{\alpha\Delta-}$ was not [χ^2 (1) = 0.9, not significant]. Comparison of the patterns of Same and New responses across all three testing sessions (Fig. 3) showed that the distributions differed among the three groups [χ^2 (6) = 18.489, $P < 0.01$], and those for both the αCaMKII^{T286A} and CREB$^{\alpha\Delta-}$ cells significantly differed from that of WT cells ($P < 0.05$). In WT mice, the majority of cells maintained their initial spatial representations (with or without a rotation) in both sessions II and III (Fig. 3, Same-Same). In contrast, the largest proportions of cells in both mutant mice developed new spatial firing patterns during both sessions II and III (Fig. 3, New-New), indicating that they were less likely to be controlled by specific subsets of the stimuli present throughout testing. However, this finding is not entirely attributable to an overall instability of place fields in both types of mutants. A substantial proportion of CREB$^{\alpha\Delta-}$ cells had new place fields in session II but restored their initial fields in session III (Fig. 3, New-Same), suggesting that many of these cells did not "recognize" the familiar cue subsets when re-arranged. Nevertheless, they could recover their original representations when the familiar cue arrangement was restored. Unlike CREB$^{\alpha\Delta-}$ cells, a substantial proportion of αCaMKII^{T286A} cells that maintained their place representations in session II subsequently switched to a new spatial representation in session III (Fig. 3, Same-New), demonstrating a profound place cell instability in αCaMKII^{T286A} mice.

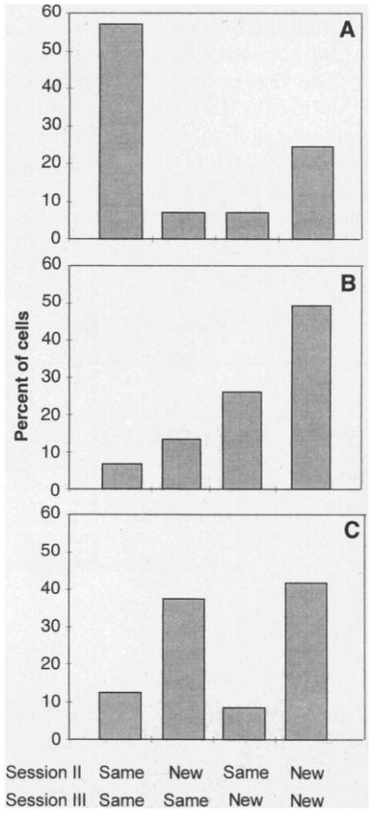

Fig. 3. Distributions of four response patterns of place cells in (**A**) WT, (**B**) αCaMKII^{T286A}, and (**C**) CREB$^{\alpha\Delta-}$ mice in sessions II and III. Same-Same are cells that maintained session I spatial representations across sessions II and III. New-Same are cells that developed new representations in session II but recovered their original representations in session III. Same-New are cells that retained the session I representation in session II but developed a new representation in session III. New-New are cells that developed new representations in sessions II and III.

In parallel with the LTP and spatial learning deficits of the mutant mice studied here, our results show that place cell stability is more severely affected in the αCaMKII^{T286A} mice than in the CREB$^{\alpha\Delta-}$mutants. Although the rotation of the cues disrupted place fields in the hippocampus of both mutants, only the cells in αCaMKII^{T286A} mice could not recover their representations when the cues were restored to their original configuration. These observations of place field instability, along with deficient LTP and impaired memory, are consistent with the proposal that NMDAR-dependent plasticity mediates the maintenance of representations for specific episodes as familiar items are reexperienced in repeated or novel configurations (*18*).

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11. Extracellular potentials were recorded with stereotrode- or tetrode-configured bundles of 25-μm Formvar-coated nichrome wires carried by a miniature microdrive (*19*). Under ketamine (50 mg per kilogram of body weight) and xylazine (2 mg/kg) anesthesia, the electrode array was implanted at 1.7 mm posterior to bregma, and 1.7 mm right of the midline, just above the dorsal hippocampus. The electrode was lowered gradually each day, after which recordings were evaluated for spike unit activity. Recordings were passed through a unity gain preamplifier-headstage, amplified (10,000 times) and bandpass-filtered (0.3 to 0.6 kHz low cut, and 5 to 6 kHz high cut), and digitized at 32 kHz (Data Translation DT2821) with Enhanced Discovery software (DataWave Technologies). Animal position was tracked with a video camera system (DataWave Technologies) that followed an incandescent headstage lamp at 60 Hz. Single cells were isolated by waveform parameters (Autocut, DataWave Technologies), and cell isolations were considered stable when clusters remained within fixed boundaries throughout testing.
12. Both αCaMKII^{T286A} and CREB$^{\alpha\Delta-}$ mutant mice were from the F_2 generation of a cross between strains 129 and C57BL/6. Data from WT littermates from both groups (n = 2 from each) were combined, because their genetic backgrounds were very similar and their results were statistically indistinguishable. Mice were housed individually and kept at 95% ad libitum body weight.
13. In characterizing place fields we used data only from when the mouse was moving at least 1 cm/s, and there were no significant group differences in running speeds (WT = 2.98 ± 0.33 cm/s; αCaMKII^{T286A} = 2.76 ± 0.17 cm/s; CREB$^{\alpha\Delta-}$ = 2.77 ± 0.22 cm/s). A place field was identified as a continuous area of at least 22.5 cm^2 (four pixels of 2.37 cm by 2.37 cm) where the average firing rate was at least two times the cell's overall firing rate. By these criteria a place cell could have multiple discontiguous place fields (see Fig. 1) that were considered separately in assessments of spatial selectivity.
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16. A place field was considered fixed or only rotated if all its pixels were within 15 cm (half of the arm's length) of the axial expanse of the original place field either in the same arm or the appropriate 90°-rotated arm, respectively. Fixed place fields were assumed to be under the control of unidentified stable environmental cues.
17. In rats distal cues control the largest proportion of place cells (*19*), but in WT mice local cues predominate. However, when local cues were removed and replaced with identical smooth black arms in a separate analysis, most place cells remained spatially selective. This result indicates that although place cells in mice attend preferentially to local cues, they also respond to distal cues.
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