We must therefore expect that a directed fishery on this small gadoid (landings in the Northeast Atlantic are about 3×10^5 t year^{-1}) will have a positive effect on the euphausiids, which in turn prey on copepods, a much more important food source for commercial fish species than euphausiids. Hence, fishing for Norway pout may have a cascading effect, leading to a build-up of nonutilized euphausiids. Triangles such as the one involving Norway pout, euphausiids, and copepods, and which may have a major effect on ecosystem stability, are increasingly being integrated in ecological theory (*21*), especially in fisheries biology (*22*).

Globally, trophic levels of fisheries landings appear to have declined in recent decades at a rate of about 0.1 per decade, without the landings themselves increasing substantially. It is likely that continuation of present trends will lead to widespread fisheries collapses and to more backward-bending curves such as in Fig. 5, whether or not they are due to a relaxation of top-down control (*23*). Therefore, we consider estimations of global potentials based on extrapolation of present trends or explicitly incorporating fishing-down-the-food-web strategies to be highly questionable. Also, we suggest that in the next decades fisheries management will have to emphasize the rebuilding of fish populations embedded within functional food webs, within large "no-take" marine protected areas (*24*).

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Impaired Locomotion and Dopamine Signaling in Retinoid Receptor Mutant Mice

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In the adult mouse, single and compound null mutations in the genes for retinoic acid receptor β and retinoid X receptors β and γ resulted in locomotor defects related to dysfunction of the mesolimbic dopamine signaling pathway. Expression of the D1 and D2 receptors for dopamine was reduced in the ventral striatum of mutant mice, and the response of double null mutant mice to cocaine, which affects dopamine signaling in the mesolimbic system, was blunted. Thus, retinoid receptors are involved in the regulation of brain functions, and retinoic acid signaling defects may contribute to pathologies such as Parkinson's disease and schizophrenia.

The retinoic acid (RA) signal is transduced by two nuclear receptor families, the retinoic acid receptors (RARα, RARβ, and RARγ) and the retinoid X receptors (RXRα, RXRβ, and RXRγ), which function as RAR-RXR heterodimers and play important roles during mouse embryonic development and postnatal life [(*1–4*) and references therein]. The high levels of expression of retinoid receptors in the brain and spinal cord (*5*), together with the RA responsiveness of various neurotransmitter pathways in vitro (*6*, *7*), suggest that retinoid signaling might be involved in the regulation of neural functions. The locomotor skills of knockout mice for the genes encoding RARβ, RARγ, RXRβ, and RXRγ, all of which are normally expressed in the striatum (*5*), were analyzed by open

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field and rotarod behavioral tests (*8*). The open field test revealed that RARβ-RXRβ, RARβ-RXRγ, and RXRβ-RXRγ double null mutant mice, but not the corresponding single mutants, exhibited statistically significant reductions in forward locomotion when compared with wild-type littermates (Fig. 1A). Furthermore, 40% of RARβ-RXRβ null mutants also showed backward locomotion. The frequency of rearings was significantly diminished in all double null mutants and in RXRβ and RXRγ single mutants (Fig. 1B). Using the rotarod test to measure motor coordination, we found that the fall latency was decreased in RARβ, RARβ-RXRβ, and RARβ-RXRγ null mutants (Fig. 1C), because most of these mice fell shortly after the beginning of the rotation. The performance of RXRβ-RXRγ null mutant mice, in spite of their normal RARβ gene expression, was also impaired. In contrast, RARα and RARγ null mice and RARα-RXRγ or RARγ-RXRγ double null mutant mice did not show any defects in these locomotor tests, even though both RARα and RARγ transcripts were expressed in the striatum (*9*) [Fig. 3V (Fig. 3 will be discussed more fully below)].

On the basis of these results, RARβ, RXRβ, and RXRγ appear to be involved specifically in the control of locomotor behaviors, although to different extents. The observation that the defects exhibited by RARβ-RXRβ, RARβ-RXRγ, and RXRβ-RXRγ double null mutants were similar suggests that heterodimers (*1*, *2*) of RARβ with either RXRβ or RXRγ are the functional receptor units involved in control of locomotion, and that RXRβ and RXRγ are functionally redundant. The above locomotor defects reveal a physiological function for RXRγ, because no obvious developmental or postnatal abnormalities could be previously ascribed to its knockout in single and compound mutants (*3*, *10*).

RARβ, RXRβ, and RXRγ are expressed in skeletal muscles, peripheral nervous system (PNS), and central nervous system (CNS) (*5*, *11*). Thus, defects in these structures could be at the origin of locomotor defects. The morphology and histology of skeletal muscles of all double null mutant mice appeared normal, and no abnormalities were detected in the development after birth of fast- and slow-twitch muscle fibers. The PNS of these mutant mice appeared anatomically normal during development [embryos 10.5 day after coitus stained with an antibody to neurofilament (*9*)] and after birth [morphological observations of sciatic and facial nerves in aged animals (*9*)]. Furthermore, muscle and PNS functions of RARβ, RXRβ, and RXRγ single and double null mutants were indistinguishable from those of wild-type littermates with

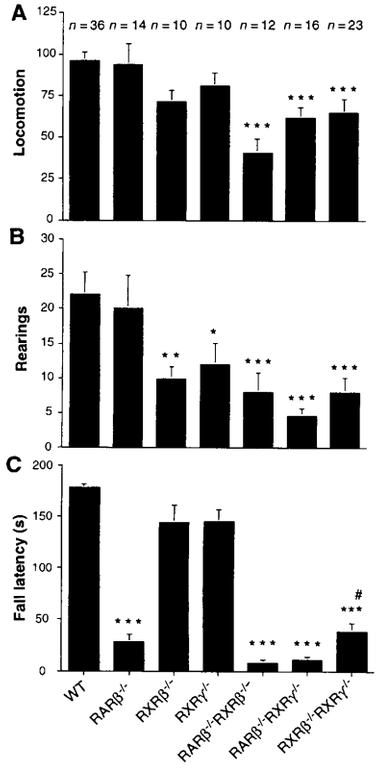

Fig. 1. Locomotor activity of RARβ$^{-/-}$, RXRβ$^{-/-}$, RXRγ$^{-/-}$, RARβ$^{-/-}$RXRβ$^{-/-}$, RARβ$^{-/-}$RXRγ$^{-/-}$, and RXRβ$^{-/-}$RXRγ$^{-/-}$ null mutant animals. In the open field test forward locomotion (**A**) (measured as the number of squares crossed) and the number of rearings (**B**) were scored during a 5-min test period. Rotarod performance (**C**) was determined as the time spent on the rotating rod. To avoid the possible effects of a mixed genetic background, we used large numbers (*n*) of animals in these tests. Data are expressed as means ± SEM, and groups were compared by one-way analysis of variance (ANOVA) with Welch correction [$F_{locomotion}(6,37) = 8.36$; $F_{rearings}(6,39) = 12.92$; $F_{latency}(6,59) = 12.62$]. Post hoc analysis was performed with the Bonferroni multiple *t* test with all possible 21 comparisons (BMDP) (*25*); ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ relative to wild-type (WT) littermates; #$P < 0.1$, relative to the RARβ$^{-/-}$RXRγ$^{-/-}$ group.

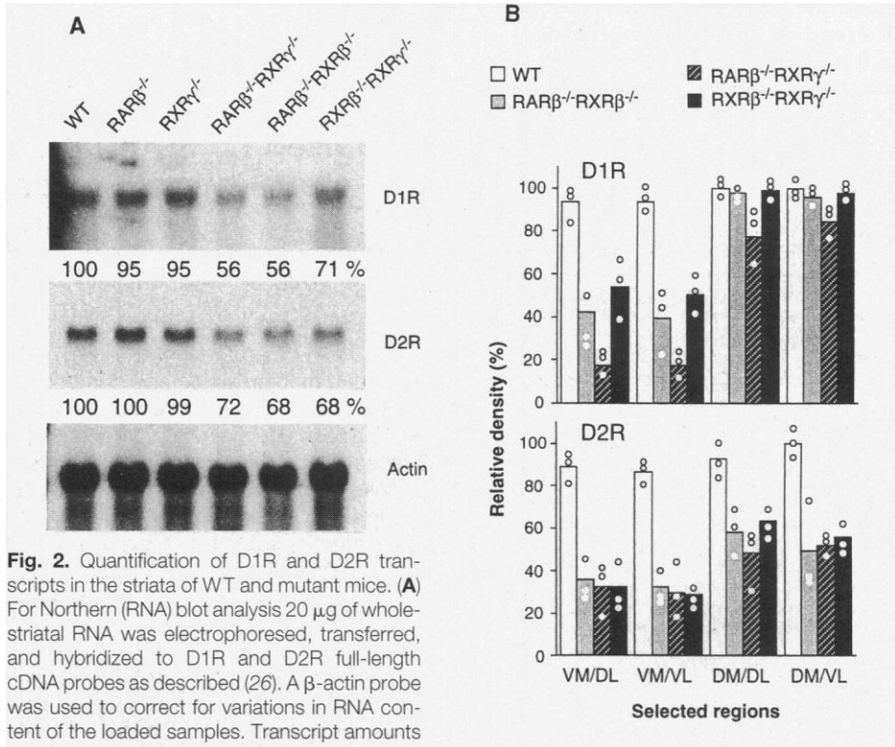

Fig. 2. Quantification of D1R and D2R transcripts in the striata of WT and mutant mice. (**A**) For Northern (RNA) blot analysis 20 μg of whole-striatal RNA was electrophoresed, transferred, and hybridized to D1R and D2R full-length cDNA probes as described (*26*). A β-actin probe was used to correct for variations in RNA content of the loaded samples. Transcript amounts were quantified with a phosphoimager (Fuji). After correction for variation in β-actin transcript amounts, D1R and D2R transcript amounts in RARβ$^{-/-}$, RXRγ$^{-/-}$, RARβ$^{-/-}$RXRβ$^{-/-}$, RARβ$^{-/-}$RXRγ$^{-/-}$, and RXRβ$^{-/-}$RXRγ$^{-/-}$ mutants were expressed relative to WT mice. The values given below the lanes represent the mean of at least three independent experiments, which did not differ by more than 10%. (**B**) Regional changes in D1R and D2R transcript expression (as revealed by in situ hybridization) were quantified densitometrically in selected striatal regions (see Fig. 3B). Data represent the ratios between the signal intensities measured in the different areas in individual WT and mutant animals, as indicated. In each case, the vertical bars correspond to mean values obtained from three animals, each animal being represented by a dot. VM/DL, VM/VL, DM/DL, and DM/VL are the ratios of signal intensities in the various areas as defined in Fig. 3B; for each animal, the area exhibiting the strongest signal intensity was taken as 100%.

respect to compound muscle action potentials, motor unit number, and absence of spontaneous activity in the gastrocnemius muscle (*12*). In addition, all of these mutant animals had normal balance reflexes, and no gross anatomical or histological abnormalities could be detected in their spinal cords. Thus, the muscle or PNS deficiencies are unlikely to account for the locomotor impairment, which may instead reflect a CNS dysfunction.

Dopamine signaling, which is predominantly mediated by D1 and D2 dopamine receptors (D1R and D2R) in the striatum, is involved in the control of motor planning (voluntary movements) (*13–15*). The strong striatal expression of RARβ and RXRγ (*5*) (see also Fig. 3, U and X), the similarity between the present locomotor defects and those of D2R knockout mice (*15*), and the responsiveness of D2R to RA in cultured cells (*6*, *16*) prompted us to analyze the expression of D1R and D2R (the most abundant dopamine receptors in the striatum) in our mutant mice. RARβ-RXRβ, RARβ-RXRγ, and RXRβ-RXRγ double null mutants, but not RARβ or RXRγ single mutants, reproducibly exhibited 40 and 30% reduction in whole-striatal D1R and D2R transcripts, respectively, when compared with wild-type controls (Fig. 2A). This reduction of D1R and D2R transcripts and receptor proteins was particularly marked in the medioventral regions of the striatum, including the shell and the core of the nucleus accumbens, and the mediodorsal part of the caudate putamen [Fig. 3, E to P (*9*)]. In contrast, expression of these receptors persisted, with no significant variations in ventrolateral and dorsolateral striatal regions (compare Fig. 3, panel E to panels F through H, panel I to panels J through L, and panel M to panels N through P). The reduction of D1R and D2R transcripts in the ventromedial compared with ventrolateral striatal region in each animal ranged from 50 to 80%, depending on the type of double null mutants tested (Fig. 2B).

To investigate whether the reduction of dopaminergic receptor expression could re-

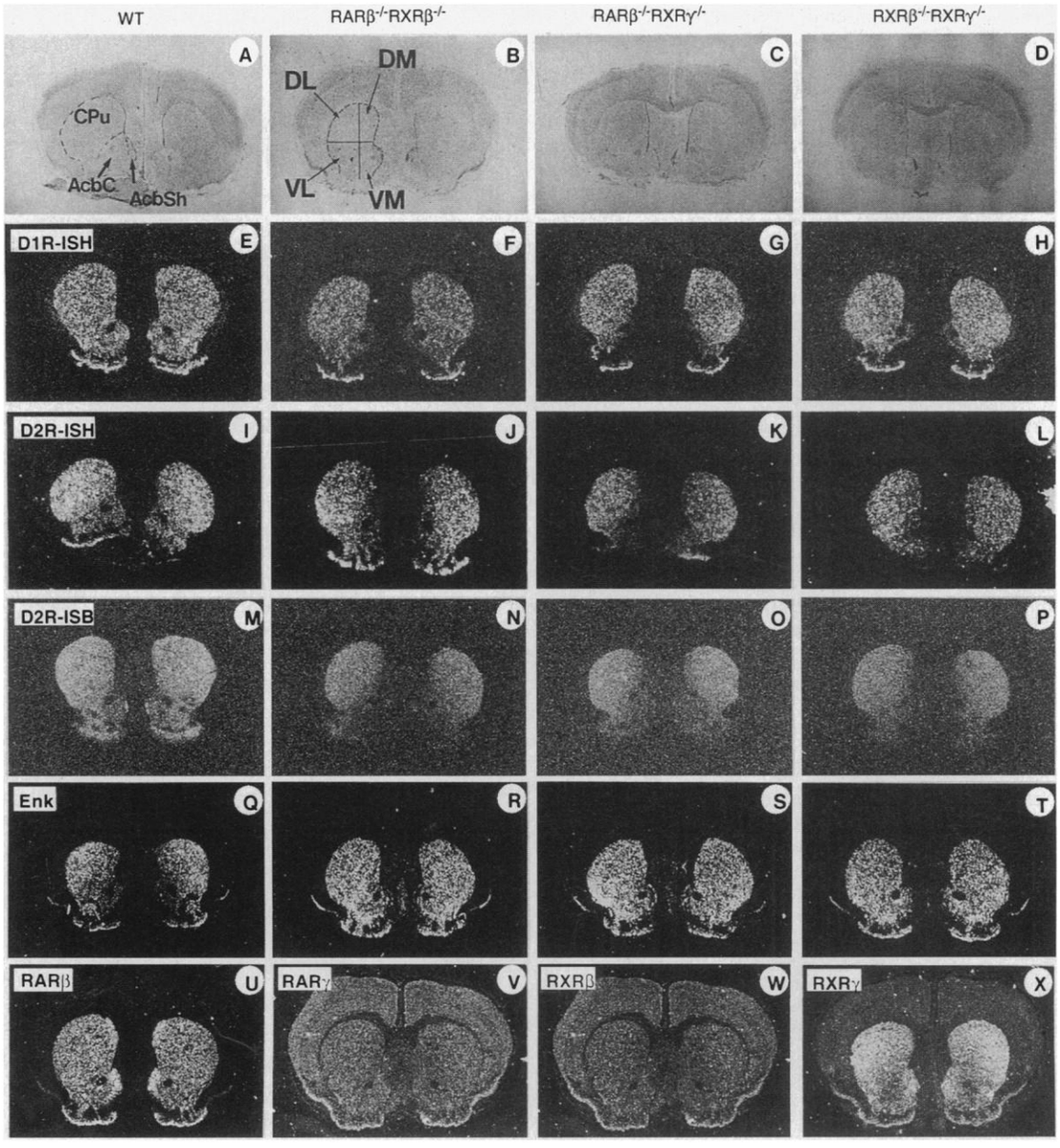

Fig. 3. Analysis of D1R, D2R, and enkephalin (Enk) expression in brains of WT and mutant mice. The views of selected section planes are presented for histological identification (**A** to **D**). In situ hybridization (ISH) with antisense RNA probes corresponding to D1R (**E** to **H**), D2R (**I** to **L**), and Enk (**Q** to **T**) were carried out as described (*27*) with WT and RARβ$^{-/-}$RXRβ$^{-/-}$, RARβ$^{-/-}$RXRγ$^{-/-}$, and RXRβ$^{-/-}$RXRγ$^{-/-}$ mutant sections. In situ binding (ISB) of the ^{125}I-labeled D2R-specific antagonist sulpride (**M** to **P**) was performed as described (*15*). Each experiment was performed with at least three animals with similar results. The expression pattern of RARβ, RARγ, RXRβ, and RXRγ transcripts in the striatum of WT mice is shown as indicated (**U** to **X**). Sense probes did not give any signal in any of these experiments (*9*). Note that although the RXRβ transcript signal in (W) was very weak, the RXRβ protein was readily detected immunohistochemically in the whole striatum (*5*). CPu, caudate putamen; AcbC, core of the nucleus accumbens; AcbSh, shell of the nucleus accumbens; DL, dorsolateral striatum; DM, dorsomedial striatum; VL, ventrolateral striatum; and VD, ventrodorsal striatum.

flect the absence of the cells expressing them, we tested the expression of enkephalin, a known marker of D2R-containing neurons. The expected increase (*15*) in enkephalin expression was observed in the nucleus accumbens region where D2R expression was reduced (Fig. 3, Q to T), indicating that the neurons that normally express D2R were present. Furthermore, the histology of the mutant striata appeared normal (Fig. 3, A to D), and no increase in apoptosis was detected (*9*). Thus, the reduced amounts of D1R and D2R transcripts appear to result from an altered control of their expression, and the lack of retinoid receptors does not seem to affect the development of striatal neurons. The characterization of a putative RA response element in the D2R promoter (*16*) suggests that its expression could be altered at the transcriptional level. Moreover, the simultaneous reduction of both D1R and D2R transcripts in the same brain area indicates that the expression of these two genes could be, at least partially, similarly controlled.

The ventral striatum belongs to the mesolimbic dopaminergic system, whose neurons project from the ventral tegmental area to the nucleus accumbens and the olfactory tubercule. Dysfunction of dopamine signaling in the ventral striatum, induced by lesions or infusions of D2R antagonists, reduces motor activity in rats and delays the initiation of the execution of some stereotyped behaviors (*17*, *18*). Thus, the reduction of D1R and D2R expression in this area could generate the behavioral abnormalities observed in the retinoid receptor mutants. On the other hand, it is unlikely that these abnormalities are due to reduced dopamine concentrations for the following reasons: (i) with the exception of RXRβ transcripts, the present retinoid receptors are not expressed in the mesencephalic regions (substantia nigra and ventral tegmental area) where dopaminergic neurons arise (*5*); (ii) the expression of tyrosine hydroxylase (the limiting enzyme in catecholamine synthesis) is apparently not altered in these areas; and (iii) the expression of D2R, which in these regions controls dopamine release, is also not affected (*9*).

Cocaine-induced hyperlocomotor activity was used to assess the dopaminergic pathway integrity in the mesolimbic system (*14*, *19*, *20*). Cocaine interferes with the dopamine signaling through its binding to the dopamine transporter (*19*). In the presence of cocaine the uptake of released dopamine is blocked. This leads to increased dopamine concentration in synapses, which results in hyperlocomotion (*19*). No statistically significant cocaine-mediated increase of locomotion or rearings was observed in the RARβ-RXRβ, RARβ-RXRγ, and RXRβ-RXRγ null mutants, when compared with the corresponding saline-treated animals (Fig. 4). Thus, as D1R-null mice (*14*), these mutants do not exhibit the cocaine locomotor-activating effects. In view of the existing synergism between D1R and D2R in these effects (*14*), we propose that the concomitant decrease of these receptors in the ventromedial area of the striatum leads to a phenotype that resembles that of D1R-null mice.

Taken together, the decrease of D1R and D2R expression in the ventral striatum of RARβ-RXRβ, RARβ-RXRγ, and RXRβ-RXRγ null mutant mice and the impaired response of these mice to cocaine indicate that retinoids are involved in controlling the function of the dopaminergic mesolimbic pathway. The reduction of D1R and D2R expression occurred preferentially in the ventral striatum, indicating that additional factors are involved in the control of D1R and D2R expression in the dorsal striatum, because RARβ, RXRβ, and RXRγ are expressed in entire caudate putamen and nucleus accumbens structures (*5*) (see also legend to Fig. 3, U to X). Some locomotor defects in RARβ-RXRβ null mutants (backward locomotion) were more severe than expected from the level of reduction in D1R and D2R transcripts, when compared with RARβ-RXRγ null mice. Thus, besides D1R and D2R, additional dopamine receptors [for example D3R (*13*)] and neurotransmitter pathways might also be affected in the retinoid receptor mutants.

Our findings, together with the localization of a RA-synthesizing enzyme in mesostriatal dopaminergic neurons (*21*), suggest that altered vitamin A signaling could be implicated in the etiology of pathologies (for example Parkinson's disease and schizophrenia) that have been linked to dysfunction of dopaminergic systems. Moreover, the orphan nuclear receptor Nurr1, a putative heterodimerization partner of RXRs, appears to be required for the formation of dopamine-producing neurons (*22*). With the broad distribution of retinoid receptors in the CNS (*5*), additional brain functions might be modulated by retinoid signaling. Because many RXR-RAR double null mutant mice exhibit highly pleiotropic defects and die in utero or at birth (*2*, *3*), spatiotemporally controlled somatic mutations in the CNS (*23*) are required to further investigate the functions of retinoid signaling in the brain.

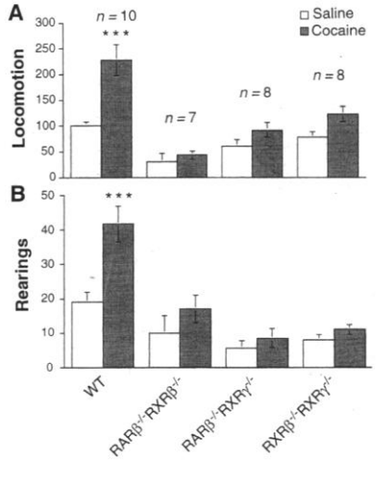

Fig. 4. Effects of cocaine on motor behavior of RARβ$^{-/-}$RXRβ$^{-/-}$, RARβ$^{-/-}$RXRγ$^{-/-}$, and RXRβ$^{-/-}$RXRγ$^{-/-}$ mice. The locomotion (**A**) (measured as in Fig. 1) and rearing behaviors (**B**) of WT and double null mutant males treated with saline or cocaine were examined in the open field test (*28*) (*n* corresponds to the number of animals in each group). Data were analyzed by two-way ANOVA with the Brown-Forsythe correction, because the variances were not equal [$F_{locomotion}(3,26) = 6.47$, $P < 0.005$; $F_{rearings}(3,29) = 7.85$, $P < 0.001$]. The effects of the cocaine treatments were then compared in post hoc analyses with the Bonferroni multiple *t* test with α adjusted for 16 comparisons [that is, four comparisons of saline- and cocaine-treated groups within each genotype, six comparisons between each saline-treated group, and six comparisons between each cocaine-treated group (BMDP) (*25*)]; ***$P < 0.001$, relative to saline-treated animals of the same genotype.

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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

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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.