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7. The enhancer trap line E2-3-27 was isolated by V. Hartenstein and Y. N. Jan [*Roux's Arch. Dev. Biol.* **201**, 194 (1992)].
8. aCC, RP2, and the most medial U innervate dorsal muscles 1, 2, and 10, respectively. RP5 innervates ventral muscle 12 [H. Sink and P. M. Whitington, *J. Neurobiol.* **22**, 298 (1991); M. Landgraf, T. Bossing, G. M. Technau, M. Bate, *J. Neurosci.* **17**, 9642 (1997)].
9. Isolation of *caps* genomic and cDNA clones was according to standard methods.
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13. The COOH-terminal peptide sequence AAGGYPYIAGNSRMIPVTEL (see caption to Fig. 2 for amino acid abbreviations) of CAPS was used to immunize rabbits. The specificity of the antiserum was confirmed by its failure to stain *caps* mutant embryos and larvae.
14. We cannot detect CAPS protein expression on the surface of muscles before the formation of synapses. Like Fasciclin II, CAPS may shift from diffuse surface distribution to concentration at neuromuscular junctions [K. Zito, R. D. Fetter, C. S. Goodman, E. Y. Isacoff, *Neuron* **19**, 1007 (1997)].
15. E. Shishido *et al.*, data not shown.
16. The *caps* gene was mapped to 70A of polytene chromosome, a region uncovered by a deficiency, *Df(3L)Ly*. *caps* mutant alleles were generated by imprecise excision of the P-element in E2-3-27. Two of them, $caps^{65.2}$ and $caps^{124.2}$, which showed the lowest viability and contained the largest deletions, were used in this study and gave similar results.
17. Only a few (7%) $caps^{65.2}$/*Df(3L)Ly* individuals survive to adulthood.
18. Monoclonal antibody 1D4 (antibody to Fasciclin II) (*4*), 2D5 (antibody to Fasciclin III) [N. H. Patel, P. M. Snow, C. S. Goodman, *Cell* **48**, 975 (1987)], mAb 22C10 [S. C. Fujita, S. L. Zipursky, S. Benzer, A. Ferrus, S. L. Shotwell, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7929 (1982)], and the *ftz-tau-lacZ* transgene [C. A. Callahan and J. B. Thomas, *ibid.* **91**, 5972 (1994)] were used to evaluate axon architectures in the CNS and in the periphery. Muscle morphology was examined by Nomarsky optics.
19. No obvious abnormalities were seen in the nerve terminals of other muscles.
20. Neuromuscular terminals were visualized with mAb 1D4 (see *18*).
21. In $caps^{65.2}$/*Df(3L)Ly* third-instar larvae, 26.1% (n = 283) of the muscle 12 terminals contained abnormal arborization on muscle 13. In contrast, only 1.9% (n = 53) showed the abnormalities in control (+/*Df(3L)Ly*) individuals.
22. Muscle 12 is innervated by three types of boutons: large (type Ib), small (type Is), and intermediate (type II) [X. Jia, M. Gorczyca, V. Budnik, *J. Neurobiol.* **24**, 1025 (1993)]. All three types of boutons are found in the ectopic endings on muscle 13 in $caps^{65.2}$/*Df(3L)Ly*, at frequencies of 71, 15, and 15%, respectively (n = 34).
23. Ectopic expression of *caps* was induced by GAL4-UAS system [A. H. Brand and N. Perrimon, *Development* **118**, 401 (1993)]. Two independent transformant lines, *UAS-caps-Ia* (on the first chromosome) and *UAS-caps-Ib* (on the third chromosome), were used and gave similar results. *G14-GAL4* drives expression in all body-wall muscles from midstage 12 (D. Lin and C. S. Goodman, unpublished results; kindly provided by C. S. Goodman).
24. Ectopic and increased expression of *caps* in all muscles by *G14-GAL4* did not affect muscle development and adhesion (*34*).
25. In 72% of the hemisegments of *G14-GAL4*/+; *UAS-caps-Ib*/+ third-instar larvae (n = 149), the muscle 12 terminal contained one or more collaterals that extended to muscle 13. In 25% of the hemisegments, the nerve branch extended further, to either muscle 5, 6, 8, or 30. However, these aberrations were observed in only 2% of the hemisegments of control larvae (*UAS-caps-Ib*/+, n = 53). No obvious neuromuscular defects were seen in other regions of the body wall.
26. Ectopic endings made by type Ib motorneurons in $caps^{65.2}$/*Df(3L)Ly* larvae contained 3.9 ± 1.3 (n = 12) boutons, whereas those in *G14-GAL4*/+; *UAS-caps-Ib*/+ contained 15.7 ± 1.7 (n = 14) boutons.
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28. In *G14-GAL4*/+; *UAS-caps-Ib*/+ larvae, 31% (n = 91) of muscle 13 contained ectopic type III endings. Type III boutons were observed only in muscles 12 and 13, excluding the possibility that *caps* induces type III morphology. Ectopic synapses with type Ib and type II are found in ~40% and 20% of muscle 13, respectively. More than one type of bouton is found in some cases.
29. Ectopic endings on muscle 13 were observed in 5 of 11 segments in *G14-GAL4*/+; *UAS-caps-Ib*/+ first-instar larvae.
30. Mhc^{82}-*GAL4* drives expression from first-instar larval stage [G. W. Davis, C. M. Schuster, C. S. Goodman, *Neuron* **19**, 561 (1997); G. W. Davis, C. S. Goodman, *Nature* **392**, 82 (1998)].
31. This was further supported by the observation that overexpression of *caps* in muscle 13 but not in 12 by *H94-GAL4* (*30*) can induce the formation of the ectopic synapses by muscle 12 motorneurons (E. Shishido *et al.*, data not shown).
32. *ftz-tau-lacZ* transgene (*18*) was used to specifically visualize muscle 12 motorneurons in the embryos.
33. This contrasts with the phenotype caused by ectopic expression of *fasciclin II* on muscle 13, where many muscle 12 motor axons establish their synapses on muscle 13 before reaching muscle 12 (*30*). Even when *caps* overexpression has been induced in muscle 13 but not in 12 by *H94-GAL4* (*31*), most muscle 12 motorneurons initially contact muscle 12.
34. A. Nose, T. Umeda, M. Takeichi, *Development* **124**, 1433 (1997); S. Raghavan and R. A. H. White, *Neuron* **18**, 873 (1997).
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36. This is consistent with the lack of increased muscle-muscle adhesion when *caps* is overexpressed (*24*). Expression of *caps* in S2 cells was conducted as described (*6*).
37. E. Shishido *et al.*, data not shown.
38. D. J. Matthes, H. Sink, A. L. Kelodkin, C. S. Goodman, *Cell* **81**, 631 (1995); K. J. Mitchell *et al.*, *Neuron* **17**, 203 (1996); D. Rose *et al.*, *Development* **124**, 1561 (1997).
39. A rabbit antiserum (GC1) that specifically stains type III endings was fortuitously obtained in our laboratory.

40. We thank V. Hartenstein and Y. N. Jan for making their collection of enhancer trap lines available. We also thank D. Lin and C. S. Goodman for GAL4 lines, Y. Shimoda and M. Kokubo for technical assistance, A. Chiba and M. Yoshihara for technical advice, and A. Chiba and S. Ritzenthaler for comments on the manuscript. Supported by research grants to A.N. and M.T. from the Ministry for Education, Science and Culture of Japan.

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Abolition of Long-Term Stability of New Hippocampal Place Cell Maps by NMDA Receptor Blockade

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Hippocampal pyramidal cells are called place cells because each cell tends to fire only when the animal is in a particular part of the environment—the cell's firing field. Acute pharmacological blockade of *N*-methyl-D-aspartate (NMDA) glutamate receptors was used to investigate how NMDA-based synaptic plasticity participates in the formation and maintenance of the firing fields. The results suggest that the formation and short-term stability of firing fields in a new environment involve plasticity that is independent of NMDA receptor activation. By contrast, the long-term stabilization of newly established firing fields required normal NMDA receptor function and, therefore, may be related to other NMDA-dependent processes such as long-term potentiation and spatial learning.

The ability of rodents to learn and remember features of a new environment is thought to require the formation in the animal's brain of a cognitive map—a neural representation of space. In 1971, O'Keefe and Dostrovsky (*1*) proposed that a rat's position in space is encoded by the coordinated activity of individual hippocampal pyramidal cells [place cells, recently reviewed by (*2*)]. Such encoding is possible because each place cell tends to fire only when the rat (or mouse) is in a cell-specific part of the current environment, the cell's "firing field." The conjoint activity of place cells is therefore thought to be the basis of a map of the environment that the animal uses for solving spatial problems. In this sense, the cognitive map serves as a cellular substrate for spatial memory (*3*). Place cells have two other properties that make them attractive as elements of a spatial memory system. The first is environmental stabili-

ty—a given cell has the same firing field in each of many exposures to the same environment, for as long as the cell is identifiable (up to 6 months) (4). The other is environmental specificity—the firing field of a place cell in one environment does not predict its field in a second, distinct environment (5, 6). Thus, when an animal is put into a new environment, each pyramidal cell changes its positional firing pattern in an entirely unpredictable fashion: The field of any cell can change in firing rate, shape, or position (or a combination of all three) or turn off or on, irrespective of what other cells do. This process is called "remapping" and reflects the formation of a new hippocampal map for the novel environment. Once formed, this new map is also stable and does not interfere with existing maps of familiar environments (7, 8).

That rodents can rapidly form stable representations of new environments raises the following question: What are the cellular mechanisms whereby firing fields are first formed and once formed are then maintained? One candidate mechanism is long-term potentiation (LTP) or, more precisely, the plastic processes that underlie LTP. LTP is a long-lasting, activity-dependent enhancement of synaptic strength that has been extensively studied in the hippocampus (9). One type of LTP that appears to be important for spatial memory is the NMDA receptor–dependent form that occurs at the Schaffer collateral pathway that connects pyramidal cells of the CA3 region to those of the CA1 region. Typically, pharmacological or genetic disruption of this type of LTP results in impaired performance in tasks that require spatial memory (10). Thus, NMDA receptors may play an essential role in the formation and maintenance of spatial maps. To test this idea, we used the competitive NMDA receptor antagonist CPP [(±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid; RB, Natick, Massachusetts] and addressed three questions: (i) Does acute blockade of NMDA receptors throughout the brain produce a degradation of the positional firing patterns of CA1 place cells in a familiar environment? (ii) Does acute blockade of NMDA receptors prevent remapping when the rat is put into a new environment? (iii) Finally, does this blockade affect the short- or long-term stability of newly formed place fields?

Our experimental strategy, summarized in Fig. 1, was based on the stability of place cells in a familiar environment and the development of new fields by the same cells in a novel environment (6, 7). We used a 76-cm-diameter gray cylinder with a white cue card as the familiar environment and a geometrically identical white cylinder with a black cue card as the novel environment. Animals were injected with either CPP or saline before their first exposure to the novel environment. Examples of positional firing patterns in the two environments of four pyramidal cells simultaneously recorded from a saline-injected rat (Fig. 2, A and B) and four pyramidal cells simultaneously recorded from a CPP-injected rat (Fig. 2, C and D) are shown in Fig. 2 (11). Each row shows firing rate maps for a single cell during 10 recording sessions over 2 days. The maps are grouped first according to the recording apparatus (gray cylinder on the left; white cylinder on the right) and then by the time order of the session.

These maps illustrate four basic findings. First, blocking NMDA receptors did not interfere with a previously formed map. In the familiar gray cylinder, each cell had the same firing pattern after the injection as it did before (sessions D1G0 and D1G1), showing that place cells are as stable in a familiar environment after injections of CPP as they are after injection of saline. Second, blocking NMDA receptors did not prevent remapping in a novel environment. During the first exposure of the rats to the novel white cylinder (D1W1), the firing patterns of the place cells did not obviously resemble those in the gray cylinder (D1G1). Third, despite the blockade of NMDA receptors, the remapping seen in the novel white cylinder during the first session on day 1 persisted for at least 1.5 hours until the second session in the white cylinder

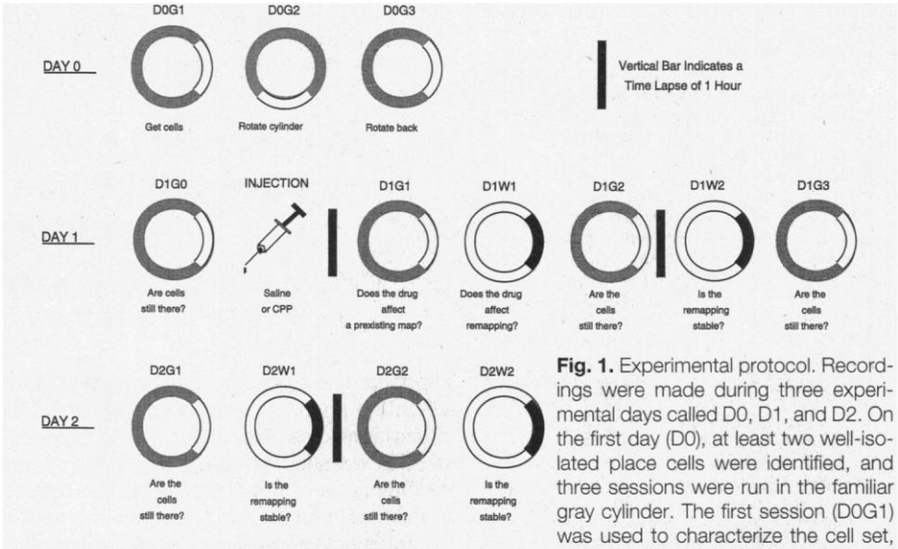

Fig. 1. Experimental protocol. Recordings were made during three experimental days called D0, D1, and D2. On the first day (D0), at least two well-isolated place cells were identified, and three sessions were run in the familiar gray cylinder. The first session (D0G1) was used to characterize the cell set, the second (D0G2) to test the effects of rotating the cue card, and the third (D0G3) to test whether the card rotation effects were reversible. For all rats, we found that rotating the white cue card 90° caused equal rotations of each firing field and that the field rotations were reversible, establishing that visual stimuli controlled firing fields. At the start of the next day (D1), a gray cylinder session (D1G0) was run to see if the cell recordings were stable. If so, the rat was injected with either physiological saline or CPP (10 mg/kg) and returned to its home cage for 1 hour. Three sessions were then run in the familiar gray cylinder, the novel white cylinder, and the familiar cylinder (D1G1, D1W1, and D1G2, respectively). Session D1G1 allowed us to ask if the drug affected established place cells, session D1W1 allowed us to see if CPP interfered with remapping, and session D1G2 allowed us to tell if exposure to the new environment disrupted the established firing fields. These sessions took a total of about 1 hour, after which the rat was returned to its home cage for 1 hour. Two more sessions were then run in the white cylinder and the gray cylinder. Session D1W2 allowed us to ask if the remapping in the novel environment was stable, and session D1G3 provided both a check of cell stability and a baseline for day 2 recordings. On day 2, after the drug had ceased to act (see Fig. 4), recording sessions were divided into two pairs separated by 1 hour in the home cage. Session D2G1 allowed us to check for cell stability in the familiar environment, and session D2W1 allowed us to see if the remapping on the first day was stable. Session D2G2 provided yet another check of cell stability, and session D2W2 allowed us to see if the firing fields in the white cylinder were the same as in session D2W1.

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(D1W2). Fourth, the most profound effect of blocking NMDA receptors was to abolish the long-term stability of the map in the novel environment. In the saline-injected rat, the remapping established on day 1 (D1W1 or D1W2) remained stable on day 2 (D2W1 and D2W2), but in the CPP-injected rat the new map formed on day 1 was replaced on day 2 with another new map. Thus, CPP prevented the first remapping from being stabilized.

To quantify these results, we compared positional firing patterns in pairs of sessions to look for stability or remapping before, during, and after NMDA channel blockade. We calculated a "similarity" score for a session pair by computing the correlation between the firing rates on a pixel-by-pixel basis (*12*). The mean similarity for the cells of each rat was computed and then averaged across rats to get group means (Fig. 3). The high similarity for preinjection and postinjection sessions in the gray cylinder (D1G0/D1G1) indicates that established firing fields are not significantly affected by CPP. This persistence is in agreement with studies showing that NMDA blockade does not interfere with established LTP in slices or with previously formed spatial memories in intact animals (*9*, *10*). Moreover, CPP did not degrade the firing properties of individual place cells. We measured several properties of firing fields and found no significant effect of drug on field size, peak firing rate, coherence, information content, or signal-to-noise ratio. CPP also did not have any significant effect on average running speed in the familiar environment (*13*).

When the same cells were recorded during the animal's first introduction to the novel white cylinder, a remapping was seen for all saline- (6 of 6) and most CPP- (5 of 6) injected rats (comparison D1G1/D1W1, Fig. 3). For both saline- and CPP-injected

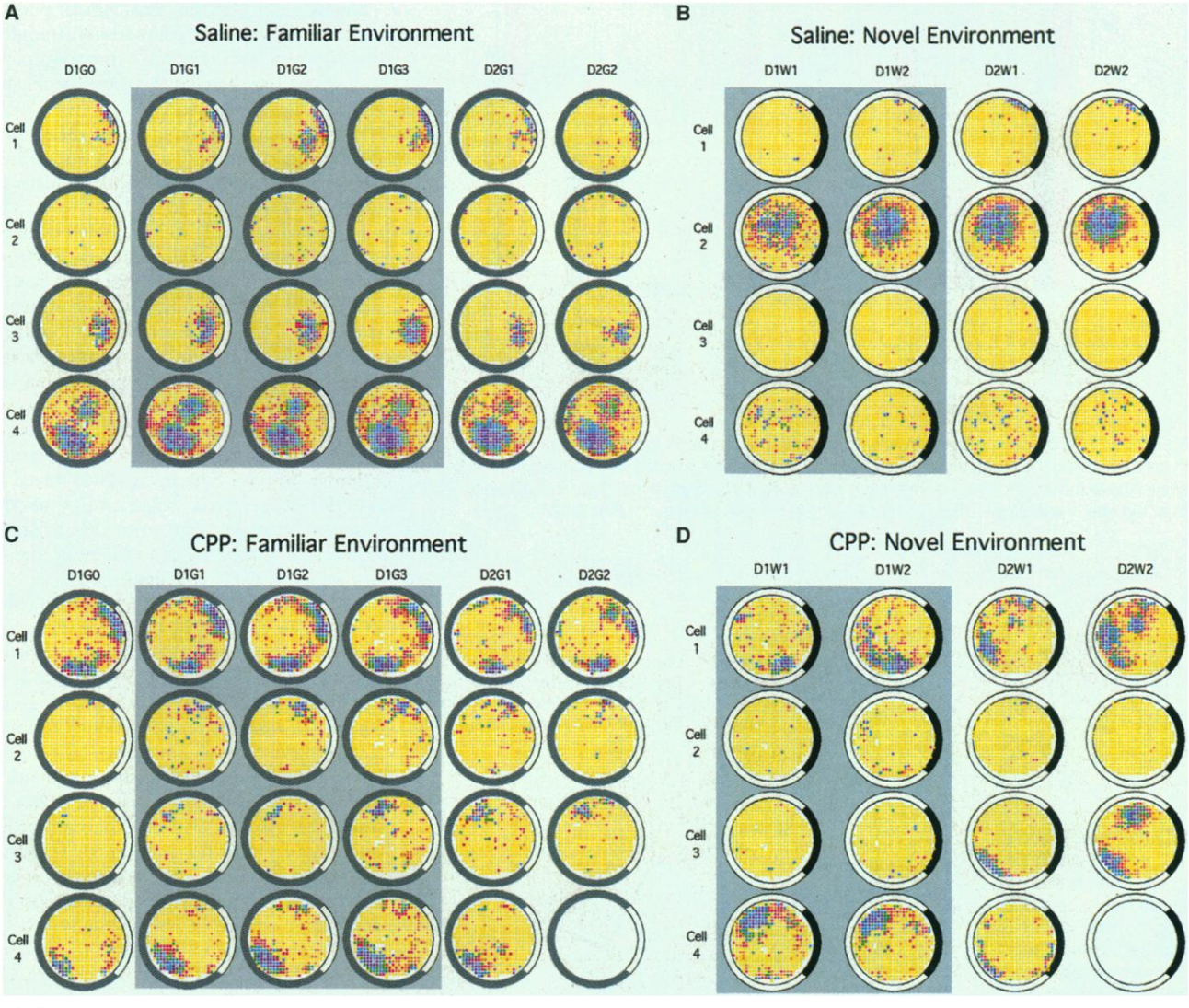

Fig. 2. Examples of the firing fields of four cells from a saline-injected rat and four cells from a CPP-injected rat for all sessions on days 1 and 2 of the protocol. Each square pixel in a rate map represents a 3.5 cm by 3.5 cm area in the apparatus. Yellow encodes regions that the rat visited in which the cell never fired. Orange, red, green, blue, and purple pixels encode progressively higher firing rates and are autoscaled in each session. A gray pixel signifies the field center, and white pixels were not visited. See (*6*, *7*) for details. (**A** and **B**) Saline-injected rat in familiar (A) and novel (B) environments. Firing rate maps for each pyramidal cell are shown as a row. The rate maps are sorted first according to the recording chamber as indicated by the outlines around each map and then according to time order. The gray backgrounds highlight all day 1 postinjection sessions. (**C** and **D**) CPP-injected rat in familiar (C) and novel (D) environments . Firing rate maps for four pyramidal cells recorded for the 10-session protocol spanning 2 days. Cell 4 was lost before the last two sessions (D2G2 and D2W2).

rats, the first session in the gray cylinder (D1G1) was significantly more similar to later gray cylinder sessions than to the first session in the white cylinder (D1W1) (*14*). Furthermore, in all but the one CPP animal that did not remap, the firing fields of simultaneously recorded cells in the novel environment changed independently of each other. Comparing the first two white cylinder sessions (D1W1/D1W2) indicates that the remapping was stable for at least 1.5 hours for both saline- and CPP-injected rats. However, the initial remapping in rats injected with CPP tended to be less complete than in saline rats. By inspecting rate maps, we saw that the firing pattern in the novel environment partially resembled the pattern in the familiar environment for one or more cells in each rat. This residual discharge was absent in the second white cylinder session (D1G1/D1W2) except in the one rat injected with CPP that did not remap.

Although CPP had only relatively minor effects on remapping during day 1, it abolished the long-term stability of the newly formed map: A second remapping occurred for each CPP rat on day 2, as if they had not previously seen the white cylinder (D1W2/D2W1, mean similarity = 0.03 ± 0.02). The second remapping also did not resemble the original gray cylinder map (D2G1/D2W1, mean similarity = 0.01 ± 0.02). By contrast, the day 1 remapping was stable on day 2 in all saline-injected rats [D1W2/D2W1, mean similarity = 0.47 ± 0.05, $t(10) = 7.08$, $P < 0.001$ compared with CPP]. For CPP-injected rats, the new firing patterns in the white cylinder on day 2 (after the drug's effects had worn off) (*15*) were stable for at least 1.5 hours (D2W1/D2W2). The firing fields in the gray cylinder persisted from the first to the last session (familiar environment, D1G1/D2G2) in both groups, indicating that the recordings were stable for the duration of the experiment.

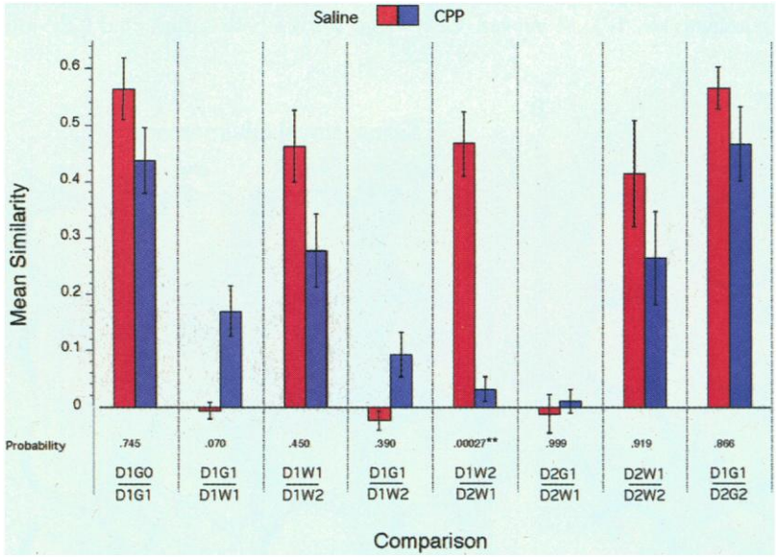

Fig. 3. Comparisons of firing pattern similarity in selected pairs of sessions. Each comparison is shown as two bars indicating the mean similarity score (±SEM) for saline-injected rats (red) and CPP-injected rats (blue). Above the label for each comparison is the probability that the mean similarity for saline- and CPP-injected rats is equal by *t* tests, corrected for the number of tests. **, $P < 0.001$.

One possible explanation of the second remapping is that CPP acts as a discriminative stimulus for state-dependent learning (*16*). In this state-dependent view, the combination of CPP and the novel cylinder on day 1 is effectively a different environment than the novel cylinder on day 2, when CPP has ceased to act. According to this explanation, however, the combination of CPP and the familiar cylinder should cause a remapping from the predrug map in the familiar cylinder, which did not occur. In addition, we made a second injection of CPP in one rat on day 2 after the two white cylinder sessions. The firing fields stayed in the day 2 patterns, suggesting that the remapping on day 2 was not due to state-dependent learning but rather was due to instability of the day 1 map.

To determine how effectively the 10-mg/kg dose of CPP blocks NMDA receptors, we examined primed-burst potentiation (*17*) in awake, freely moving rats (Fig. 4) (*18*). Primed-burst potentiation is an activity-dependent enhancement of synaptic strength that is similar to LTP in its dependence on NMDA receptor activation but is of shorter duration, so that it can be tested repeatedly without saturation. A primed burst with no drug on day 0 caused robust potentiation of the population spike. By contrast, there was no potentiation on day 1 when primed bursts were delivered 1.5 and 3 hours after CPP injection, showing effective blockade of NMDA receptors. On day 2, a primed burst again caused potentiation, showing the drug had ceased to act. There was a significant effect of drug condition [$F(3,12) = 7.40$, $P < 0.01$] and a significant condition × time interaction [$F(18,72) = 2.60$, $P < 0.01$] in a two-way analysis of variance comparing the four conditions (before CPP injection and 90

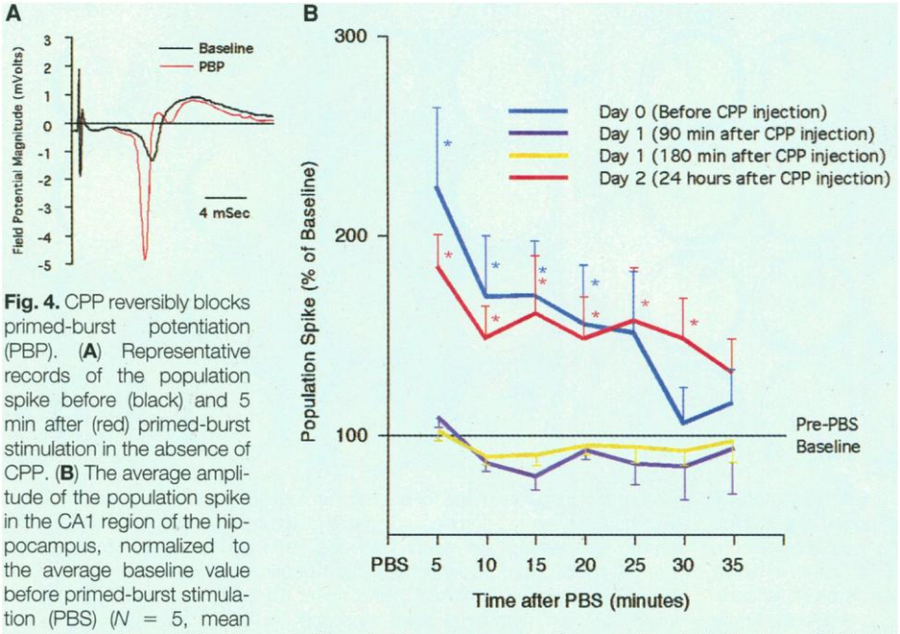

Fig. 4. CPP reversibly blocks primed-burst potentiation (PBP). (**A**) Representative records of the population spike before (black) and 5 min after (red) primed-burst stimulation in the absence of CPP. (**B**) The average amplitude of the population spike in the CA1 region of the hippocampus, normalized to the average baseline value before primed-burst stimulation (PBS) ($N = 5$, mean baseline = 1.33 ± 0.12 mV, not significantly different among the four conditions). Vertical bars, SEM; *, $P < 0.05$ compared with baseline.

min, 180 min, and 24 hours after injection). Subsequent analysis showed that the 90- and 180-min conditions were significantly different from baseline and 24 hours. Thus, the dose of CPP used for place cell recordings effectively blocked NMDA receptors at the time of day 1 recordings and ceased to act during day 2 recordings.

In a previous study investigating the role of NMDA receptors in spatial mapping, McHugh *et al.* (*19*) recorded from mice with a selective knockout of the NMDA receptor in pyramidal cells of the CA1 region. They found that place cells in the CA1 region of the knockout mice had firing fields that were somewhat abnormal but were stable for at least 1 hour. McHugh *et al.* (*19*) attributed the inability of NMDA receptor subunit 1 knockout mice to solve spatial problems to a defect in a higher order property of place cells: that cells with overlapping fields in the knockout mice do not tend to fire at the same time and therefore do not properly signal the animal's position.

Because these results suggested that fairly normal CA1 place cell activity is still possible when NMDA receptors are deleted from the CA1 region, we asked whether a more widespread blockade of NMDA receptors might cause a greater disruption of CA1 place cells. For example, does the formation of place cells in the CA1 region require normal NMDA-mediated LTP in other parts of the hippocampus or the neocortex? We therefore used global pharmacological blockade of NMDA receptors in all brain areas. A pharmacological blockade also offered the advantage of temporal control, allowing us to investigate the effects of NMDA receptor blockade on both the maintenance of a previously formed place cell map and the establishment of a new map in a novel environment.

Our results with acute, global interference with NMDA receptors confirm the main conclusion of McHugh *et al.* (*19*) that NMDA receptors must be available for place cells to be normal and extend that conclusion by showing more precisely the role played by NMDA receptors in the formation and long-term maintenance of a place cell map. However, our results also differ in one respect from those of McHugh *et al.* (*19*). Whereas they found that chronic knockout of NMDA receptors in CA1 resulted in CA1 place cells with somewhat enlarged and diffuse firing fields, we found that CPP had no effect on field size or quality. One possible explanation for this difference is that McHugh *et al.* (*19*) were able to eliminate NMDA receptor subunit 1 protein completely by genetic means, but the dose of CPP that we used may not have been sufficient to block NMDA receptors completely. However, the dose we used is twice as great as needed to impair spatial memory (*20*) and is sufficient to reversibly prevent primed-burst potentiation (Fig. 4) and hippocampal LTP (*21*). A second possibility is that in the study of McHugh *et al.* (*19*) the gene encoding the NMDA receptor subunit 1 protein was knocked out during a period in the development of the mapping system when NMDA receptor expression is still required for the formation of normal synaptic organization (*22*).

Consistent with previous studies on LTP and learning, we found that the hippocampal representation of an already familiar environment was unaffected by global NMDA receptor blockade [(*9*, *10*); see also (*23*)]. A surprising result of our study was that NMDA-dependent processes are also not required for creating new firing fields in a novel environment or for the short-term maintenance of the new fields, although they are required for long-term maintenance of the new fields. The maintained quality of firing fields in the familiar environment and the development of crisp new fields in a new environment suggest that the place cell system receives adequate sensory information despite blockade of NMDA receptors and therefore that the deficits caused by system-wide NMDA receptors blockade are not due to interference with sensory systems. Reliable location-specific firing in a new environment requires that cell activity becomes linked to a sensory configuration that exists only within the cell's firing field. How can this linking occur if NMDA receptors are blocked? Perhaps place cells are tuned to certain stimuli by genetic or developmental events before the rat enters the new environment. In this case, the resemblance of firing fields in the two environments might be expected to reflect the resemblance of the environments to each other. Remapping is, however, often complete even when a new environment closely resembles the old environment (*6*, *7*). Moreover, it is hard to understand why preexisting tuning would allow the same pyramidal cells to have fields that are different during days 1 and 2 in the same environment, as happened with the CPP animals.

Our data, therefore, suggest the interesting possibility that, in addition to the NMDA-dependent plasticity essential for long-term stability of a new map, there exists a second, more labile and NMDA-independent form of plasticity that is sufficient to allow firing fields to form and to be maintained for 1.5 hours. The possibility that there are two forms of plasticity for different phases of spatial memory processes is consistent with topological mapping theories, which require firing fields to be established first by some unspecified mechanism, after which NMDA-dependent plasticity encodes the distance between the fields, creating a map that can be used to solve navigational problems (*24*). This more labile form of plasticity could be sufficient to subserve working memory in the radial arm maze, which persists for several hours during NMDA receptor blockade (*25*), and might also contribute to the ability of rats to learn other spatial tasks during blockade of NMDA receptors (*26*).

The most profound effect of NMDA receptor blockade was to disrupt the long-term (16 to 24 hour) stability of a newly formed firing field map: The first set of fields disappeared and was replaced by a second, newer set of fields the next day. McHugh *et al.* (*19*) did not investigate 24-hour stability. However, studies of several other types of mutant mice with deficits in both LTP and spatial learning have found that they also have as a common feature place fields with short- or long-term instability, although the fields in these mutant mice have other abnormal properties as well (*27*). Our results indicate that acute pharmacological blockade of NMDA receptor–dependent processes produces a selective deficit in long-term stabilization of new firing fields, with little effect on other firing field properties. Because some forms of both spatial learning and LTP are also NMDA-dependent (*9*, *10*), our results suggest that these three phenomena may be related: The same plasticity mechanisms that underlie the long-term maintenance of LTP may be required for long-term stabilization of new place field maps (either in hippocampus or in other brain areas), which in turn may be necessary for spatial memory.

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11. The methods used in this study are fundamentally similar to those previously summarized in detail (*6*, *7*). Briefly, food-deprived adult male Long-Evans rats (Harlan) were trained to chase randomly dropped food pellets (Bio-Serv) in a 51-cm-high gray cylinder (76-cm diameter) with a white card mounted on one wall to provide an asymmetrical cue. After being thoroughly familiarized with the task and environment, animals were implanted stereotactically (anterior, −2 mm; lateral, 1.8 mm; dorsal, 2 mm) with a drivable 10-wire (25-μm nichrome) microelectrode array about 1 mm dorsal to the CA1 hippocampal pyramidal cell layer. At least 5 days after surgery, animals were screened for units while chasing pellets in the gray cylinder. Units were amplified 10,000 times and bandpass filtered at 300 to 10,000 Hz. The amplifier output was digitized at 40 kHz. When at least two place cells could be simultaneously recorded in the gray cylinder, cue control was tested by rotating the cylinder 90°. In all cases, all fields rotated 90° (*28*). The next day, after one 8-min test session (D1G0) in the gray cylinder (the familiar environment), animals were injected intraperitoneally (ip) with either saline or CPP (10 mg/kg) and returned to their cages for an hour. All other recording sessions were 16 min. The intervals between sessions are shown in the legend for Fig. 1. Animals were always returned to their home cage between recording sessions. Units were analyzed offline with the Discovery CP Analysis and Autocut software packages (Datawave, Longmont, CO). Although it is possible that in some cases a "unit" was really two neurons, this would have worked against our main findings by increasing the variability of the data.
12. The rate maps produced by a given cell in two sessions were treated as two lists of numbers for the calculation of a correlation coefficient. The values of correlation coefficients for all cells from an animal were averaged to provide a mean "similarity" score. Cells that had an overall firing rate of less than 0.1 spikes per second in both sessions were excluded from quantitative analysis.
13. Peak firing rate is the rate in the three by three block of pixels with the highest average rate. Coherence is the *z* transform of the correlation coefficient between the rate of a pixel and the average rate of its eight nearest neighbors. Signal-to-noise ratio is the number of in-field spikes divided by the total number of spikes in a session. Information content = $\Sigma P_i(R_i/R)\log_2(R_i/R)$, where i is the bin number, P_i is the probability for occupancy of bin i, R_i is the mean firing rate for bin i, and R is the overall mean firing rate (from E. Markus *et al.* [*Hippocampus* **4**, 410 (1994)]). Running speed was calculated every 0.25 s and averaged across the session.
14. For saline-injected rats, the similarity of D1G1 to D1W1 was −0.006 ± 0.014, and the similarity of D1G1 to D2G2 was 0.565 ± 0.037 [t (10) = 6.55, $P = 6.5 \times 10^{-5}$ comparing the two similarity scores]. For CPP-injected rats, the similarity of D1G1 to D1W1 was 0.170 ± 0.044, and the similarity of D1G1 to D2G2 was 0.466 ± 0.065 [t (10) = 3.32, $P = 7.8 \times 10^{-3}$].
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18. Male Long-Evans rats (Charles River Laboratories) were anesthetized and implanted with two twisted pairs of Teflon-coated stainless steel wires (127-μm diameter) for stimulation and recording. The recording electrode was positioned in the pyramidal layer of the CA1 region of the hippocampus, and the stimulating electrode was positioned in the contralateral ventral hippocampal commissure. Electrode positions were confirmed by postmortem histology. After at least 5 days of recovery, evoked potentials were recorded in awake, freely behaving animals. The electroencephalogram was also recorded to insure that there were no after discharges and that the animals were not asleep. Population spike amplitude was measured between the negative peak and the maximum positive peak of the evoked potential. The stimulation intensity was adjusted to produce a population spike that was 30% of maximum at the beginning of the experiment, and the evoked potential was tested once every 12 s for at least 40 min. After a 5-min baseline period, primed-burst potentiation was induced during behavioral immobility, with a single stimulus pulse followed 170 ms later by a four-pulse, 200-Hz burst at an intensity that produced a maximal population spike. This procedure was repeated four times: during a baseline session, the next day both 90 and 180 min after injection of CPP (10 mg/kg ip), and again 24 hours after the CPP injection.
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Edge Effects and the Extinction of Populations Inside Protected Areas

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Theory predicts that small populations may be driven to extinction by random fluctuations in demography and loss of genetic diversity through drift. However, population size is a poor predictor of extinction in large carnivores inhabiting protected areas. Conflict with people on reserve borders is the major cause of mortality in such populations, so that border areas represent population sinks. The species most likely to disappear from small reserves are those that range widely—and are therefore most exposed to threats on reserve borders—irrespective of population size. Conservation efforts that combat only stochastic processes are therefore unlikely to avert extinction.

The contention that small populations are vulnerable to extinction through stochastic processes has a sound theoretical basis in both demography and population genetics (*1*). Management of small populations has therefore dominated both the theory and practice of conservation biology for nearly 20 years (*2*). However, most empirical evidence supporting this contention is indirect, because direct measures of size are rarely available for populations that have subsequently become extinct (*3*).

If small populations are vulnerable, large carnivores should be especially extinction-prone because their trophic position constrains them to living at low population densities. However, carnivore populations are also exposed to strong external pressures because their requirements conflict with those of local people. Where large carnivores survive outside protected areas, intentional or accidental killing by humans frequently limits their numbers (*4*). Even within protected areas, conflict with humans is usually the single most important cause of adult mortality (*5*). Most of this mortality occurs when carnivores range beyond reserve borders (*5*); such deaths account for proportions of mortality comparable with those known to cause decline in harvested populations of the same species (*4*, *5*). Border areas of reserves may therefore become population sinks. Such sinks will have the greatest impact on overall population dynamics in small reserves with high perimeter:area ratios and in species that range widely and therefore come into frequent contact with reserve borders. In large carnivores, then, both stochastic processes and strong edge effects could contribute to the extinction of isolated populations.

We investigated the relative importance of these two factors by compiling data on population extinctions for 10 species of large carnivores (Table 1). For each species, we chose a geographic region within the species' historic range in which

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