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22. Mutations were introduced into the EAF plasmid by homologous recombination after first introducing the gene-specific changes into the respective fragments subcloned into pBlueScript (Stratagene). We used a limited polymerase chain reaction (PCR)–based mutational strategy to reduce the chance of introducing secondary mutations; minimal regions of the PCR-amplified products containing the desired changes were ligated into existing subclones. The limited PCR regions could easily be sequenced and shown to be free of extraneous mutations. To generate the desired alteration on the EAF plasmid in B171-8, we performed suicide vector–directed homologous double recombination as described in (*7*, *11*). We generated B171-8ΔAcm by replacing the pilinencoding *bfpA* gene with a chloramphenicol acetyltransferase gene; transcription of the remaining *bfp* operon genes was unaffected (*11*, *16*). B171-8T::Gm has sustained an insertional disruption of *bfpT* (*13*). Wild-type phenotypes were restored by providing the mutants with a normal copy of the respective gene in trans on a low-copy-number plasmid.
23. Healthy adult volunteers (18 to 48 years old) gave written, informed consent for participation in this study. All volunteers provided a medical history and underwent physical examination and laboratory testing. The study was performed in the General Clinical Research Center (GCRC) at Stanford Hospital (Stanford University, Palo Alto, CA). The challenge organisms were resuscitated from frozen stocks of organisms that were confirmed to be O111 antiserum reactive, EAF plasmid positive, and *eaeA* hybridization positive. Each mutant carried its characteristic mutation as assessed by DNA sequence, mRNA, and protein immunoblot analysis. The cultures were grown overnight in Luria broth, washed three times in phosphate-buffered saline (0.85%), visually inspected for the absence of bacterial clumps, and adjusted to the appropriate OD_{600} for each inoculation. Volunteers took nothing by mouth for 2 hours before challenge. They ingested 150 ml of a 1.3% sodium bicarbonate solution 1 minute before ingesting 30 ml of the dose of challenge bacteria in phosphate-buffered saline plus 1.3% sodium bicarbonate. All stool samples were collected for 48 hours. The nursing staff were unaware of the nature of the challenge bacteria and scored the stool specimens as formed/semiformed or liquid. The end point of this study was development of diarrhea as indicated by the volume and number of liquid stools produced in the first 48 hours. Forty-eight hours after ingesting the bacteria the volunteers were treated with ciprofloxacin and were released from the GCRC after their stools were determined to be negative for *E. coli*.
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28. In-frame deletion mutations of *bfpD* (B171-8ΔD) and *bfpF* (B171-8ΔF) were constructed from convenient restriction sites within those genes. Site-directed mutations in the nucleotide triphosphate binding regions of BfpD (B171-8D^{ma}) and BfpF (B171-8F^{ma}) were generated by a modification of the Stratagene QuikChange mutagenesis strategy. A pair of primers carrying the intended nucleotide changes but otherwise complementary to the sequences encoding the Walker box A region of either protein were used for amplification. To minimize unintentional changes in the nucleotide sequence, we used the limited PCR-based strategy (*22*) to replace the homologous wild-type fragment that had been cloned into a modified version of suicide plasmid pGP704 (*31*). The appropriate loci of the EAF plasmid were replaced by the intended mutations by using the suicide vector–driven homologous recombination strategy employed above. Complementation of the D and F mutations was accomplished by providing a copy of the wild-type gene on a low-copy-number plasmid, which restored the normal LA and autoaggregation phenotypes.
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Drosophila Synapse Formation: Regulation by Transmembrane Protein with Leu-Rich Repeats, CAPRICIOUS

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Upon reaching the target region, neuronal growth cones transiently search through potential targets and form synaptic connections with only a subset of these. The *capricious* (*caps*) gene may regulate these processes in *Drosophila*. *caps* encodes a transmembrane protein with leucine-rich repeats (LRRs). During the formation of neuromuscular synapses, *caps* is expressed in a small number of synaptic partners, including muscle 12 and the motorneurons that innervate it. Loss-of-function and ectopic expression of *caps* alter the target specificity of muscle 12 motorneurons, indicating a role for *caps* in selective synapse formation.

The final step in formation of neural connectivity involves the recognition of target cells. Although earlier events of growth cone guidance greatly restrict the target region, neurons still have to choose a specific synaptic partner from among several potential targets (*1*). We studied synapse formation in the neuromuscular system of *Drosophila melanogaster*. In each abdominal hemisegment of *Drosophila* larvae, ~40 motorneurons innervate 30 muscle fibers in a specific manner (*2*). Once a motor axon enters its target region during late embryogenesis, its growth cone searches over the surface of many muscles but withdraws from most of these contacts, forming stable synapses only with its own target or

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targets (3–5). Here, we describe the *caps* gene that regulates the formation of some of the selective synaptic connections in this system.

We screened for enhancer trap lines that express a reporter gene in specific muscle fibers during the establishment of motorneuron innervation (6). *caps* was identified by analysis of one such line, E2-3-27 (7). In E2-3-27 embryos, the reporter (*caps*-LacZ) is expressed in four dorsal (1, 2, 9, and 10) and six ventral (12, 14 to 17, and 28) muscles (Fig. 1A). In muscle 12, *caps*-LacZ and *caps* RNA (see below) are expressed in a single nucleus of the syncytial muscle, near the contact site of the motorneuronal growth cone (Fig. 1, B, C, and F). *caps*-LacZ is also expressed in central nervous system (CNS) motorneurons aCC, RP2, RP5, and the most medial U, all of which innervate *caps*-LacZ–positive muscles (Fig. 1, D and E) (8). *caps*-LacZ is not expressed in motorneurons that have been identified as innervating *caps*-negative muscles (for example, RP1, RP3, and RP4). Thus, the expression of *caps*-LacZ is correlated with neuromuscular specificity (Fig. 1H).

We cloned the genomic DNA flanking the P-element insertion site (Fig. 2A) and identified a gene (*caps*) whose expression pattern was identical to that of the reporter gene (Fig. 1, F and G) (9). *caps* encodes a transmembrane protein with 14 Leu-rich repeats (LRRs) in its extracellular domain (Fig. 2, B and C). LRR, a ~24 amino acid motif found in various proteins from sources as diverse as yeast to human, may mediate protein–protein interactions (10). Among the proteins with LRRs, CAPS protein was most closely related to the product of the *tartan* gene from *Drosophila* (11), with amino acid similarity extending beyond the LRR region into the cytoplasmic region. Proteins with LRRs expressed on the cell surface may function in cell adhesion or recognition (6, 12). CAPS protein is expressed on the surface of developing motor axons (Fig. 1I) (13). In first-instar larvae, CAPS protein was detected in the mature synaptic sites of all *caps*-positive muscles (Fig. 1, J to L) (14, 15).

To determine the function of *caps* in vivo, we first generated *caps* loss-of-function mutant alleles, which lack the first exon (Fig. 2A) and do not express CAPS protein detectable by our antibody (16). Most of the *caps* mutants die late in embryogenesis or soon after hatching, although a few survive to adulthood (17). Although no gross developmental defects were found in the CNS or musculature of *caps* mutant embryos and larvae (18), the target specificity of muscle 12 motorneurons was altered (19). In wild-type larvae, muscle 12 is innervated by the terminal branch of ISNb, including the RP5 axon, which projects to the boundary between muscles 12 and 13 and forms synaptic endings exclusively on muscle 12 (Fig. 3A) (20). In contrast, in *caps* mutant larvae, the terminal branch is often accompanied by additional varicosities on muscle 13, a neighboring *caps*-negative muscle (Fig. 3B) (21, 22). Thus, *caps* restricts arborization of the nerve terminal to muscle 12.

Ectopic overexpression of *caps* in all embryonic muscles by *G14-GAL4* driver caused formation of more ectopic synapses (23, 24). In ~70% of the hemisegments, the ISNb terminal formed one or more additional collaterals that formed more robust synaptic endings on muscle 13 (Fig. 3C) (25, 26). The ectopic nerve endings contained type III boutons, which are typical of muscle 12 but not muscle 13 neuromuscular synapses (Fig. 3, D and E) (22, 27, 28). Since the ectopic synapses were present in the first-instar larvae, *caps* may function while the connections are being formed (29). This possibility is further supported by the absence of such ectopic endings when *caps* expression was induced after completion of synaptogenesis by Mhc^{82}-*GAL4* (30).

We propose that *caps* mediates selective synapse formation. The loss-of-function phenotype may result from improper recognition of the target muscle, whereas the

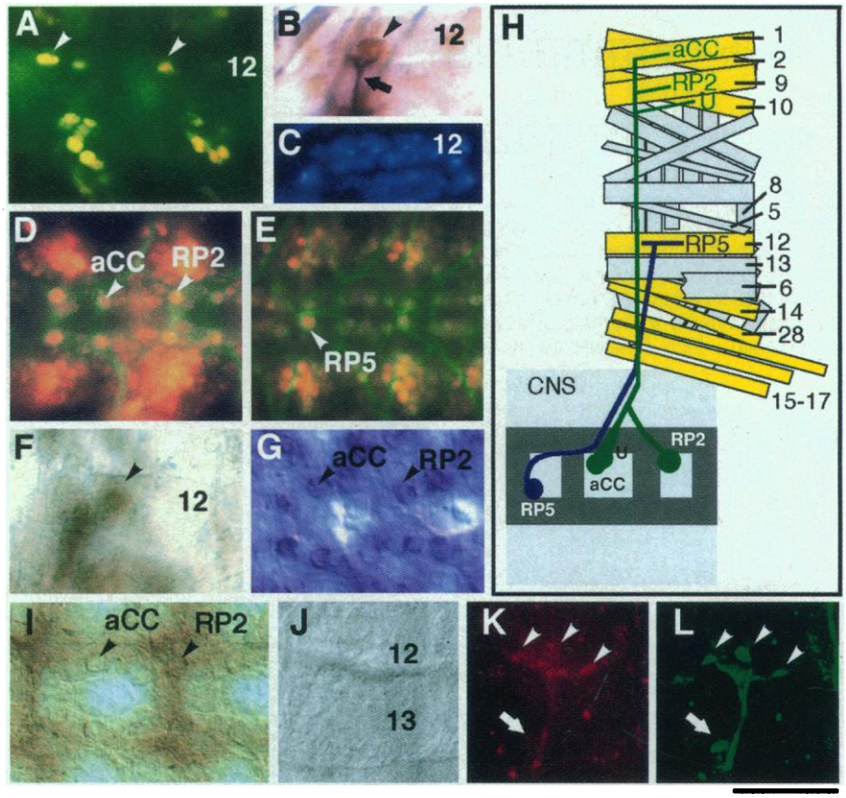

Fig. 1. Expression of *caps* in motorneurons and muscles. (**A** to **E**) *caps*-LacZ expression. Stage 15 E2-3-27 embryos stained with an antibody to LacZ. (A) Expression in a single nucleus in muscle 12 (arrowheads) and in nuclei of other ventral muscles 14 to 17 and 28 (Cy3; red). Muscle morphology visualized with fluorescein isothiocyanate (FITC)-phalloidin (green). (B) Double staining with antibodies to LacZ (arrowhead; brown; horseradish peroxidase reaction) and to Fasciclin II (arrow; purple; alkaline phosphatase reaction), which visualizes the motor axons. (C) 4′,6′-diamidino-2-phenylindole staining to visualize all nuclei (blue). (D and E) Expression in a subset of motorneurons in the CNS (arrowheads; Cy3; red). Subset of CNS axons visualized with monoclonal antibody (mAb) 22C10 (FITC; green) (18). (**F** and **G**) *caps* RNA expression in a single nucleus in muscle 12 [(F), arrowhead] and in motorneurons aCC and RP2 [(G), arrowheads]. (**H**) Hemisegment structure. *caps*-positive muscles (yellow), *caps*-positive motorneurons in the intersegmental nerve (ISN; green) and in intersegmental nerve b (ISNb; blue) are shown. Still to be determined is whether motorneurons that innervate other *caps*-positive muscles (9, 14 to 17, 28) also express *caps*. (**I** to **L**) CAPS protein localization. (I) CNS of a stage 15 embryo. (J to L) A first-instar larva. The same preparation is visualized with Nomarsky optics (J), or with antibodies to CAPS [(K) Cy3; red] or to Fasciclin II [(L) FITC; green]. Fasciclin II is expressed on all neuromuscular synapses (4). CAPS is detected at the synapses on muscle 12 (arrowheads) but not on muscle 13 (arrows). Bar: 30 μm in (A), (D), and (E); 20 μm in (B), (C), (F), (G), and (I) to (L).

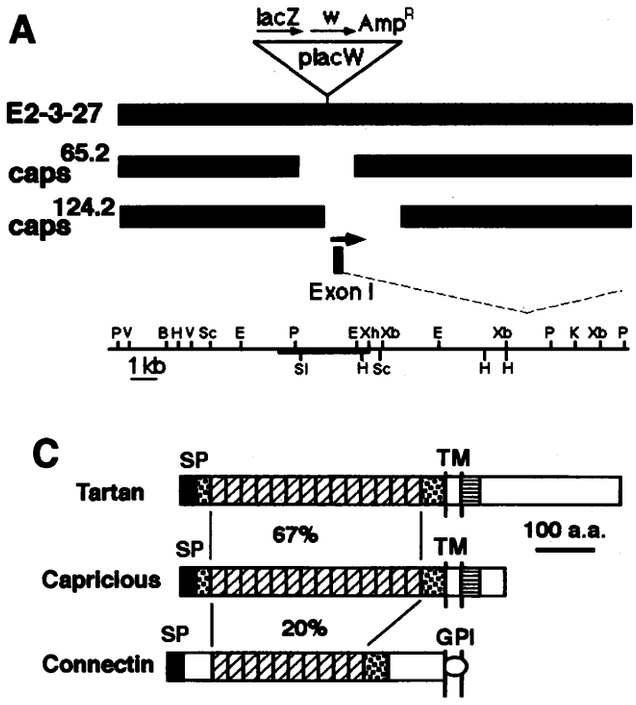

Fig. 2. (**A**) Configuration of the *caps* gene in E2-3-27 enhancer trap line and in two *caps* alleles. The horizontal bar at the bottom of (A) indicates restriction mapping of the region. E, Eco RI; H, Hind III; K, Kpn I; P, Pst I; Sc, Sac I; Sl, Sal I; V, Eco RV; Xb, Xba I; and Xh, Xho I. (**B**) The deduced amino acid sequence of CAPS protein. The signal peptide is underlined, and the transmembrane domain is double underlined. Arrowheads indicate conserved Cys residues in the NH_2-terminal and COOH-terminal flanking regions of LRRs. Single-letter codes for amino acids are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (**C**) Diagram showing the domain structure of Tartan (*11*), CAPS, and Connectin (*6*). Hatched rectangles indicate LRR, and dotted boxes denote NH_2-terminal and COOH-terminal flanking regions. Conserved regions in the cytoplasmic domain of Tartan and CAPS are shaded with horizontal lines. SP, signal peptide; TM, transmembrane domain; GPI, GPI-anchor. The bar at the bottom of (C) shows the length of 100 amino acids.

B

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1   MSLAPHLGQAFSLCLCLCLCLVLATLPVALGLANCPNGCECDDDTLMVNC

51  GEGTLDVLPIALNPAIQRLVIKNNKLKTIDSSMQFYAQLTFLDLSFNDML
        LRR1              LRR2

101 TIPERSFAYHAKLQELHLDHNKIGQVSNKTFLGLSTISVLNLRGNLIAEL
        LRR3              LRR4

151 EYRTFSPMVKLAELNLGHNRISHIDPHALDGLDNLRVLYLDDNTLTTVPG
        LRR5              LRR6

201 ELTFQALHSLAELYLGTNSFMTIPGGAFQDLKGLTRLDLRGAGLHNISGD
        LRR7              LRR8

251 ALKGLVSLRFVDLSDNRLPAIPTAAFQRLGRLEQLNIGQNDFEVISSGAF
        LRR9              LRR10

301 SGLRELRHLELTGAQRLRRVESGAFSGNTNLEHLNLSSNKQLNELSSIAL
    LRR11             LRR12

351 GGLPHLSTVVLKANQLSSLDEGLVPWADLQTLDLSENPFECDCRLLWLRH
    LRR13             LRR14

401 LLVSRNASGQYAPVICAYPTALRDLPLAHLAEPLLGCAHGAASKQAIIGI

451 LVVACAGLITTLALVLYTCRHRIREMLKGHSALGRKEREYQKTFSDEEYM

501 SRPPPGGGGVHPAAGGYPYIAGNSRMIPVTEL
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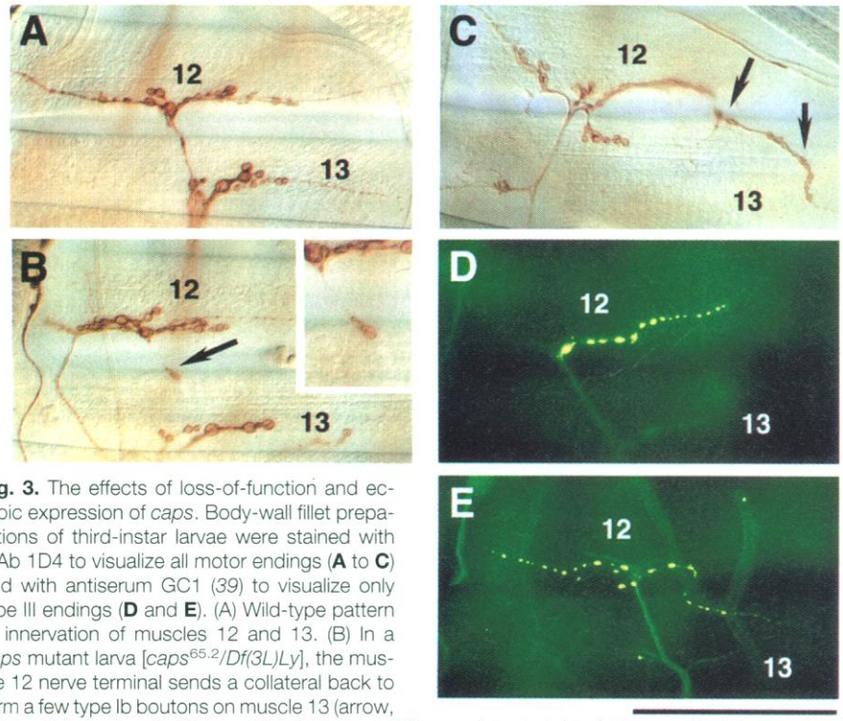

Fig. 3. The effects of loss-of-function and ectopic expression of *caps*. Body-wall fillet preparations of third-instar larvae were stained with mAb 1D4 to visualize all motor endings (**A** to **C**) and with antiserum GC1 (*39*) to visualize only type III endings (**D** and **E**). (A) Wild-type pattern of innervation of muscles 12 and 13. (B) In a *caps* mutant larva [$caps^{65.2}$/*Df(3L)Ly*], the muscle 12 nerve terminal sends a collateral back to form a few type Ib boutons on muscle 13 (arrow, enlarged in the inset). (C) In a *G14-GAL4/+*; *UAS-caps-Ib/+* individual, the muscle 12 nerve terminal projects toward and forms several type Ib boutons on muscle 13 (arrows). (D) Wild type showing exclusively type III motorneuron innervation of muscle 12. (E) In a *G14-GAL4/+*; *UAS-caps-Ib/+* larva, the type III motorneuron turns back and innervates muscle 13. Bar: 200 μm.

extra synapses on muscle 13 could reflect retention of inappropriate synaptic contacts. In contrast, the gain-of-function phenotype could indicate that the nerve terminal is attracted to muscle 13 and other muscles by ectopic *caps* (*31*). In both cases, however, muscle 12 motorneurons reach their target region normally (*32*) and extend along muscle 12 before making ectopic synapses on muscle 13 (*33*). Thus, *caps* may stabilize specific motorneuronal contacts during a late phase of target selection.

The expression of *caps* on both sides of the synaptic partners suggests that *caps* functions homophilically, as has been proposed for the candidate target recognition molecules, Connectin and Fasciclin III (*6*, *34*, *35*). However, expression of *caps* in S2 cells did not promote cell aggregation (*36*, *37*). Thus, *caps* may mediate synaptic target recognition through cell-cell signaling rather than adhesion.

Other molecules implicated in *Drosophila* neuromuscular target recognition (*35*, *38*) include another LRR protein, Connectin, which is expressed both pre- and postsynaptically on a different subset of motorneurons and muscles (*6*, *34*). Thus, neuromuscular connections may be specified in part by a combination of this group of genes in *Drosophila*.

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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)].
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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.
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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*).
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39. A rabbit antiserum (GC1) that specifically stains type III endings was fortuitously obtained in our laboratory.

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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-