the functionally determined topological map (*19*). We also conclude that the functional operation of the cerebral cortex depends on the pattern of thalamocortical connectivity. Segregation of TCAs in sensory cortex promotes independence of processing inputs that characterize neighboring, but nonadjacent, groups of sensory receptors such as whisker follicles. Overlap of TCAs in barrelless mice generates receptive fields of cortical neurons that are more appropriate to a continuous and less discriminate representation of the tactile periphery. This overlap excludes the possibility that single-whisker information can be processed separately within a discrete group of cortical neurons before further intracortical relay. Most theories on sensory discrimination and map modification by sensory experience depend on precise spatiotemporal ordering of sensory inputs (*20*). The defective temporal differentiation and impaired spatial separation of sensory inputs uncovered here within the somatosensory cortex of barrelless mice provide a model for testing such hypotheses.

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16. Mystacial whiskers are distributed in five horizontal rows (named A through E). For the DG uptake experiments, the caudalmost whiskers of rows A, C, and E were clipped on the left side. Animals were injected intraperitoneally with 2-[1-^{14}C]deoxy-D-glucose (16.5 μCi per 100 g of body mass) and placed in an object-filled cage for 45 min, after which they were anesthetized and their brains processed for autoradiography [E. Welker, S. B. Rao, J. Dörfl, P. Melzer, H. Van der Loos, *J. Neurosci.* **12**, 153 (1992)].
17. Recordings were made from mice under urethane anesthesia (2 mg per gram of body mass, administered by intraperitoneal injections of a 10% solution in distilled water). Units were situated 350 to 480 μm below the pial surface. The center whisker was defined as that giving the largest response magnitude. Data for the surround whisker refer to the one with the shortest latency. Response magnitudes were measured by the mean number of spikes generated per 50 deflections [E. Welker, M. Armstrong-James, H. Van der Loos, R. Kraftsik, *Eur. J. Neurosci.* **5**, 691 (1993)].
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22. Our dear collaborator Hendrik Van der Loos died in October 1993. We thank N. Trapp and G. Skabardonis for technical assistance and S. Catsicas, P. G. H. Clarke, A. Fine, J.-P. Hornung, G. M. Innocenti, and J. H. Kaas for comments on the manuscript. Supported by the Swiss National Science Foundation (grant 31-39184), the Wellcome Trust (M.A.-J.), and the Medical Research Council of Canada (grant MT-12941).

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A Neural Tetraspanin, Encoded by *late bloomer*, That Facilitates Synapse Formation

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Upon contacting its postsynaptic target, a neuronal growth cone transforms into a presynaptic terminal. A membrane component on the growth cone that facilitates synapse formation was identified by means of a complementary DNA–based screen followed by genetic analysis. The *late bloomer* (*lbl*) gene in *Drosophila* encodes a member of the tetraspanin family of cell surface proteins. LBL protein is transiently expressed on motor axons, growth cones, and terminal arbors. In *lbl* mutant embryos, the growth cone of the RP3 motoneuron contacts its target muscles, but synapse formation is delayed and neighboring motoneurons display an increase in ectopic sprouting.

As neuronal growth cones encounter a variety of guidance cues, they steer toward attractive signals and away from repulsive ones (*1*). Ultimately, they reach their targets, shut down their motility machinery, retract their filopodia, and transform into presynaptic terminals. In *Drosophila*, genetic analysis can be used to dissect the molecular machinery of synapse formation at the embryonic neuromuscular junction (*2*). In each abdominal hemisegment, some 40 motoneurons make specific synaptic connections with 30 identified muscle fibers. For example, the RP3 motoneuron forms synapses on muscles 7 and 6 (*3*).

We designed a complementary DNA (cDNA)–based reverse genetic approach to identify molecules involved in growth cone guidance, target recognition, and synapse formation (*4*). In an effort to bias our search toward molecules involved in cell-cell interactions, we purified mRNA from rough endoplasmic reticulum–bound polysomes and used it to prepare a cDNA library that was enriched and then normalized for clones encoding membrane and secreted proteins. We initially screened 850 clones by large-scale whole mount in situ hybridization to identify clones that hybridize to subsets of neurons or their targets during development. Here, we describe the *lbl* gene that functions to facilitate the establishment of neuromuscular synapses.

An *lbl* cDNA, *p3B6*, was identified as a clone that hybridizes to a specific subset of neurons in the embryonic central nervous system (CNS). All motoneurons that can be uniquely identified express *lbl*, including aCC, RP1 through RP5, the three U's, and the VUMs; for example, aCC (a motoneuron) expresses *lbl*, whereas its sibling, pCC (an interneuron), does not (Fig. 1A). A lateral cluster of neurons that is coincident with the major cluster of other motoneurons also expresses *lbl* (Fig. 1B), which further suggests that *lbl* is expressed specifically by motoneurons (*5*). Some peripheral sensory neurons also express *lbl*, but transiently and in much smaller amounts (*6*).

The nearly exclusive restriction of *lbl* expression by motoneurons was confirmed

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with the use of an antiserum raised against an LBL fusion protein (*7*). All of the CNS axons that express LBL protein exit the CNS in one of the two major peripheral motor nerves (5) (Fig. 1C). In the body wall, all motor nerve branches stained with the antiserum (Fig. 1D), which indicates that most, if not all, motor axons express LBL. The protein is distributed along the entire length of the motor axons, including their growth cones and presynaptic terminals (Fig. 1D). Motor axons stained in the absence of detergent, indicating that LBL is a cell surface protein. The expression of LBL on motor axons is transient; in third-instar larvae, no expression was detected.

An *lbl* cDNA clone was sequenced (*8*), revealing an open reading frame of 208 amino acids (Fig. 2A). A database search (*9*) for proteins that share sequence similarity with the deduced LBL sequence revealed eight proteins with highly significant similarity scores (Fig. 2A); all of these proteins are members of the tetraspanin family (*10*), which currently includes ~15 vertebrate proteins and one invertebrate protein. Like LBL, all tetraspanins are relatively small proteins (<350 amino acids) with four putative transmembrane domains (Fig. 2B) (*11*).

To determine the function of *lbl*, we identified two P-elements [*P(w+)S64* and *P(w+)Y13*] that are inserted in the 5′ untranslated region of the *lbl* gene and that disrupt its expression (*12*). The *P(w+)Y13* insertion eliminates all expression of *lbl* transcript in embryos; lbl^{Y13} homozygous mutant flies are viable and fertile. To identify potential defects in motor axons and synapses, we stained lbl^{Y13} mutant embryos with the monoclonal antibody (mAb) 1D4 (*13*). In *lbl* mutant embryos, motor axons follow their appropriate pathways and reach their correct muscle target region by stage early 16 (*14*), as in control embryos. However, although they reach their targets, *lbl* growth cones do not transform into presynaptic terminals in the normal fashion. For example, in control embryos, the RP3 motor axon forms synapses in the cleft between muscles 7 and 6 by stage late 16 (Fig. 3A). In contrast, in *lbl* mutant embryos, the RP3 terminal arbor is often absent at stages late 16 (Fig. 3B) and early 17 (Fig. 3C) (*15*). A second phenotype observed is the presence

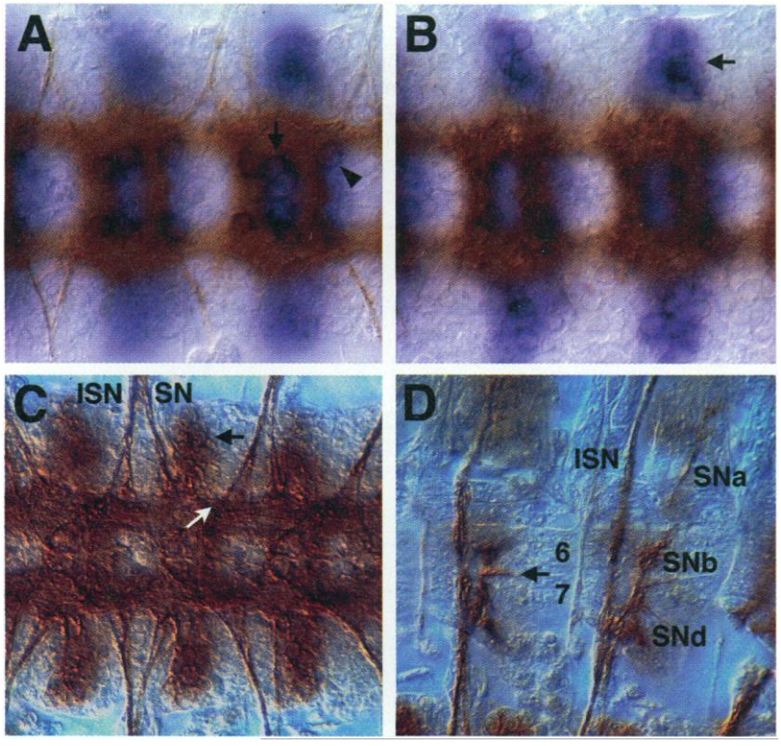

Fig. 1. In the developing CNS, *lbl* mRNA and LBL protein are expressed by motoneurons. (**A** and **B**) Ventral nerve cord of a stage 14 embryo hybridized with the *lbl p3B6* cDNA probe (blue); axons (brown) were immunostained with BP102 mAb. In (A), the dorsal focal plane is shown; *lbl* expression is detected in the RP motoneurons (arrow points to RP1, RP4, and RP3) and in aCC (arrowhead), but not in aCC's sibling, the interneuron pCC. In (B), a more ventral focal plane, *lbl* is expressed in discrete lateral clusters of neurons (arrow) that are coincident with the locations of major clusters of motoneurons. (**C** and **D**) Stage 16 embryo stained with an LBL-specific antiserum. In (C), LBL protein is expressed on motoneuron axons exiting the CNS in the intersegmental (ISN) and segmental (SN) nerves. RP axons (white arrow) exit in ISN; the black arrow points to a lateral cluster of motoneuron cell bodies. In (D), a ventral region of the body wall, the focus is on ventral longitudinal muscles 7 and 6. The LBL protein is expressed on all motor nerves (ISN, SNa, SNb, and SNd). The arrow marks the RP3 terminal arbor in the cleft between muscles 7 and 6.

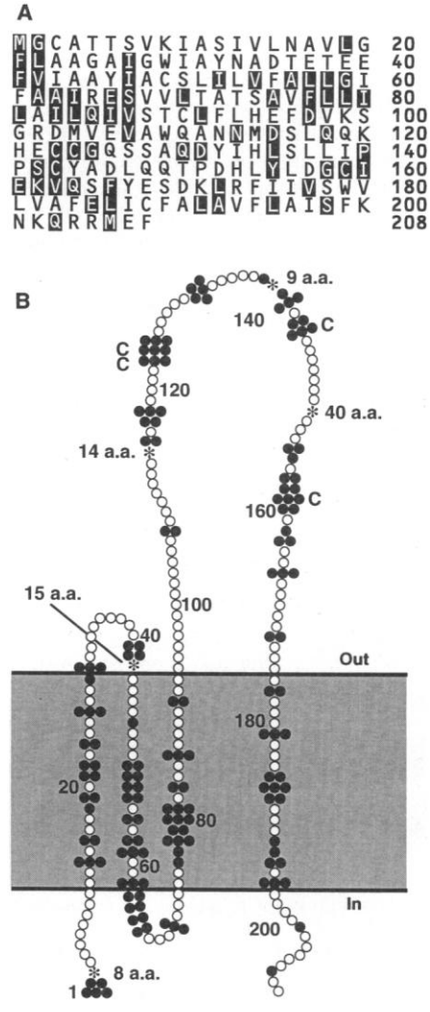

Fig. 2. The *lbl* gene encodes a member of the tetraspanin family of transmembrane proteins. (**A**) The deduced LBL amino acid sequence (GenBank accession number U49081). Amino acids in reverse type are identical with at least three of eight other tetraspanins including CD37, CD53, CD63, CD82, A15, CO-029, IM23, and PETA-3 (*26*). Abbreviations for the amino acid residues are as follows: 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. (**B**) Predicted membrane orientation of LBL on the basis of homology with other tetraspanins and staining of embryos with an LBL antiserum raised against the large extracellular "loop" (*7*). The shaded area represents the cell membrane. Amino acid residues conserved between LBL and four or five (●), six or seven (●●), or all eight (●●●) family members listed in (A) are indicated. Conserved cysteines are marked C as reference points. Asterisks denote locations where one or more of the other eight tetraspanins varies significantly in length; numbers of amino acids (a.a.) show the largest insertions.

of ectopic axonal arborizations onto muscles 7 and 6 from the transverse nerve (TN) (Fig. 3, B and C) (*15*). Because LBL is not expressed on TN axons, the ectopic processes that extend from the TN onto muscles 7 and 6 appear to be a secondary consequence of the lack of RP3 innervation (*16*). In both first- and third-instar larvae, muscles 7 and 6 in all *lbl* mutant segments have apparently normal RP3 synaptic arbors (Fig. 3D) (*17*). Moreover, all ectopic TN arborizations have been retracted. Thus, the ability of RP3 to establish a synapse on muscles 7 and 6 is impaired in *lbl* mutant embryos, but by the end of embryogenesis, synapses do form.

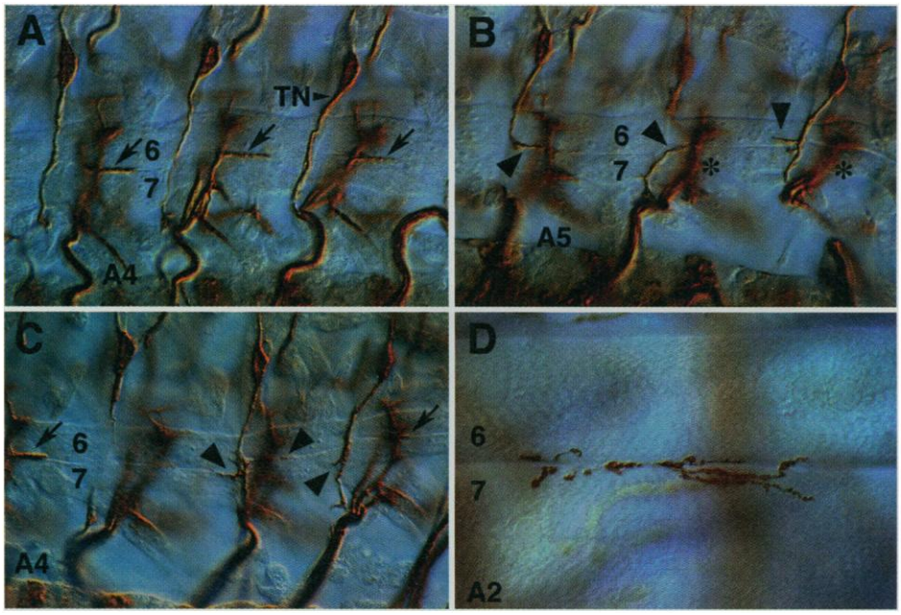

Fig. 3. The RP3 synaptic terminal arbor on muscles 7 and 6 is absent or abnormal in *lbl* mutant embryos. Embryos (**A** to **C**) and larvae (**D**) were stained with 1D4 mAb (*4*) to visualize motor axons and TN axons. A2, A4, and A5 refer to abdominal hemisegments 2, 4, and 5. In (A), three hemisegments of a wild-type (w^{1118}) control embryo at stage late 16 are shown. The RP3 terminal arbor is established in the cleft between muscles 7 and 6 (arrows). TN axons grow along a glial-like cell process extending from the nerve cord and typically do not branch onto muscles 7 or 6. In (B), an lbl^{Y13} mutant embryo at stage late 16 is shown; the RP3 terminal arbor is absent from the cleft between muscles 7 and 6 in all three segments, even though the SNb motor axons including RP3 are just adjacent to these muscles (asterisks). Instead, TN axons (arrowheads) ectopically arborize onto muscles 7 and 6 and sometimes enter the cleft between muscles 7 and 6. In (C), an lbl^{Y13} mutant embryo at stage early 17 is shown; the four hemisegments are (from left to right) A4 to A7. The RP3 terminal arbor is absent from A5 and A6, and TN axons (arrowheads) have instead ectopically extended into the cleft between muscles 7 and 6 in these segments. A4 has a phenotypically "wild-type" RP3 terminal arbor (arrow); A7 has an RP3 terminal arbor in the cleft that is smaller than in the wild type (arrow). In (D), muscles 7 and 6 from segment A2 of a lbl^{Y13} mutant third-instar larva are shown. A phenotypically wild-type RP3 synapse is seen in all segments at this stage.

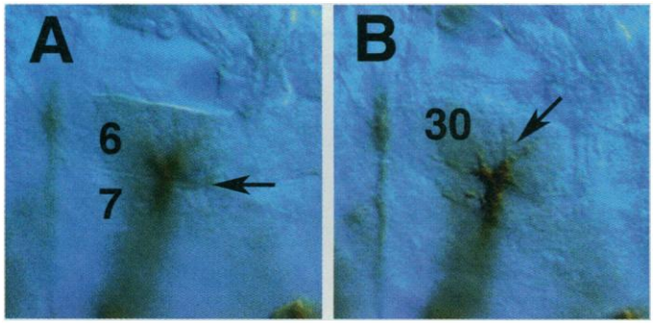

Fig. 4. The RP3 growth cone reaches its target region in *lbl* mutant embryos. Photomicrographs of a stage late 16 filleted embryo are shown in which the cell body of the RP3 motoneuron was penetrated with a microelectrode and filled with Lucifer Yellow (LY), then processed by horseradish peroxidase immunocytochemistry with an antibody to LY. (**A**) and (**B**) are two different focal planes of the same preparation. The focal plane in (A) is on the cleft between muscles 7 and 6 (arrow), where the RP3 growth cone would have transformed into a synaptic terminal arbor in wild-type embryos. The focal plane in (B) is external to muscles 7 and 6 and just internal to muscles 14 and 30. This is the region where the RP3 growth cone (arrow) is often located in *lbl* mutant embryos at this stage.

To further confirm that the primary defect in *lbl* mutant embryos is in synapse formation and not pathfinding, we used intracellular injection of Lucifer Yellow into the RP3 cell body to examine the location of the RP3 growth cone (*18*). In all stage mid- to late 16 *lbl* mutant RP3 dye fills (n = 13), the RP3 growth cone followed its normal pathway and was adjacent to muscles 7 and 6 (Fig. 4). RP3 had not formed its terminal arbor on muscles 7 and 6 in any of these stage 16 dye fills, but rather remained a growth cone contacting these and neighboring muscles. Thus, the primary defect associated with loss-of-function mutations in the *lbl* gene appears to be the impaired ability of an identified motoneuron growth cone (RP3) to respond to its muscle targets (muscles 7 and 6) and to transform into a presynaptic terminal arbor. LBL does not appear to function in pathfinding or in the recognition of the correct target region. Rather, LBL is expressed nearly exclusively on motoneurons and appears to function as a key component that facilitates the formation of neuromuscular connections.

Clues to LBL function during synapse formation are provided by other tetraspanins. Several tetraspanins are components of receptor complexes in the immune system (*19*), and some deliver costimulatory signals for cell activation (*20*) or promote an increase in intracellular Ca^{2+} (*21*, *22*). Tetraspanins can increase adhesion and decrease motility (*23*). Two tetraspanins function as suppressors of metastasis (*24*). One tetraspanin (CD9) is dynamically expressed on both neurons and glia in the developing and mature mammalian nervous system (*22*, *25*).

By analogy with the functions of other tetraspanins, we suggest that LBL may function as part of a receptor complex on motoneuron growth cones during synapse formation. LBL function would increase signal transduction (possibly by increasing intracellular Ca^{2+}) through this putative presynaptic target recognition complex and would promote both an increase in adhesion and a decrease in motility, thus facilitating the growth cone's transformation into a presynaptic terminal. Presumably, other components of the hypothetical presynaptic recognition complex can still function in the absence of LBL, albeit in a less robust fashion, because the synapse ultimately does form in *lbl* mutant embryos. The function of LBL in promoting motoneuron synapse formation, and its nearly exclusive expression by motoneurons, lead us to predict that other tetraspanins may function during the forma-

tion of synapses elsewhere in the nervous system of *Drosophila* and probably in mammals as well.

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4. A detailed description of the methods is available from the authors or at http://fruitfly.berkeley.edu (C. C. Kopczynski, T. Serano, G. R. Rubin, C. S. Goodman, in preparation).
5. LBL appears to be expressed by all motoneurons whose axons exit in the two major motor nerves (the intersegmental and segmental nerves) and innervate somatic body wall muscles; LBL is probably not expressed by motor axons that exit other nerves and innervate visceral and respiratory muscles.
6. The small amount of *lbl* expression in peripheral sensory neurons begins at stage 14 and disappears by stage early 17.
7. A 189–base pair (bp) Pst fragment from the *p3B6* cDNA was cloned into the Pst site of pQE31 (Qiagen) to fuse a six-histidine affinity tag onto the NH_2-terminus of LBL amino acids 85 to 148. This region corresponds to the last 8 amino acids of TM3 and the first 55 amino acids of the large extracellular loop. The fusion protein was expressed with QIAexpress (Qiagen) and injected into mice, and antiserum was tested histologically on embryos. The specificity of the antiserum for LBL was confirmed by its failure to stain lbl^{Y13} embryos.
8. A 1.2-kb insert in cDNA clone *p3B6.6* was subcloned as six smaller fragments into Bluescript SK+ (Stratagene). Double-stranded template DNA was sequenced on both strands by means of the Autoread sequencing kit (Pharmacia) and ALF automated sequencer (Pharmacia).
9. DNA and protein databases were searched with the program BLAST [S. F. Altschul *et al.*, *J. Mol. Biol.* **215**, 403 (1990)].
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11. CD37 and CD53, two tetraspanins most closely related to LBL, share 29% amino acid identity with one another but only 19% and 16% amino acid identity, respectively, with LBL. Sequence similarity among tetraspanins is highest in and between TM2 and TM3; in this region, CD37 and CD53 share 39% amino acid identity, whereas LBL shares 27% amino acid identity with CD37 and 25% identity with CD53.
12. The *lbl* gene was mapped to polytene band 42F1 by in situ hybridization. Two P-element stocks containing insertions in the 42F1 region, S64 and Y13, were obtained from the Berkeley *Drosophila* Genome Center. The genomic DNA flanking each of these P-elements was isolated by the inverse polymerase chain reaction [B. Dalby, A. J. Pereira, L. S. B. Goldstein, *Genetics* **139**, 757 (1995)] and sequenced. Both P-elements were inserted in a 5′ untranslated exon of the *lbl* gene. In line *P(w+)S64* the P-element was inserted 225 bp upstream, whereas *P(w+)Y13* was inserted 151 bp upstream of the start of translation. Each stock was backcrossed for 10 generations against the parental w^{1118} background to remove any modifiers of the mutant phenotype.
13. The 1D4 mAb recognizes Fasciclin II, a marker for motor axons, growth cones, and terminal arbors [D. Van Vactor *et al.*, *Cell* **73**, 1137 (1993)].
14. Staging of embryos was done with attention to head involution, CNS condensation, gut morphology, and 1D4 mAb staining (*13*) [J. A. Campos-Ortega and V. Hartenstein, *The Embryonic Development of* Drosophila melanogaster (Springer-Verlag, Berlin, 1985)].
15. There appears to be a reciprocal relation between RP3 innervation and ectopic sprouting onto muscles 7 and 6 in *lbl* mutant embryos. At stage late 16, the RP3 terminal arbor was absent in 50% of hemisegments (n = 132); 41% (n = 109) displayed ectopic TN arborizations. At stage early 17, the RP3 terminal arbor was missing in 22% of hemisegments (n = 137) and ectopic TN arborizations were seen in 49% (n = 136). In contrast, in control (w^{1118}) embryos, the RP3 terminal arbor was absent in only 12% of stage late 16 hemisegments (n = 128) and in only 2% of stage early 17 hemisegments (n = 118). Ectopic TN arborizations were observed in 11% of stage late 16 control embryos (n = 114) and in 3% of stage early 17 control embryos (n = 127).
16. This tendency of the TN to sprout onto muscles 6 and 7 in the absence of a functional RP3 synapse has been observed previously [H. Keshishian *et al.*, *J. Neurobiol.* **24**, 757 (1993); J. Jarecki and H. Keshishian, *J. Neurosci.* **15**, 8177 (1995).
17. First-instar larvae were selected 0 to 5 hours after hatching and dissected. Synaptic boutons were visualized with a polyclonal antibody to synaptotagmin [J. T. Littleton *et al.*, *Development* **118**, 1077 (1993)]. Boutons were counted in segments A2 to A5. The mean numbers of boutons (±SD) were 14.5 ± 3.5 for the control (Canton S) (n = 37) and 14.3 ± 3.4 for the *lbl* mutant (n = 67).
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Replay of Neuronal Firing Sequences in Rat Hippocampus During Sleep Following Spatial Experience

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The correlated activity of rat hippocampal pyramidal cells during sleep reflects the activity of those cells during earlier spatial exploration. Now the patterns of activity during sleep have also been found to reflect the order in which the cells fired during spatial exploration. This relation was reliably stronger for sleep after the behavioral session than before it; thus, the activity during sleep reflects changes produced by experience. This memory for temporal order of neuronal firing could be produced by an interaction between the temporal integration properties of long-term potentiation and the phase shifting of spike activity with respect to the hippocampal theta rhythm.

Several lines of circumstantial evidence point to a role for the hippocampus in memory, including numerous reports of amnesia or learning deficits after hippocampal damage (*1*) and of the presence in several parts of the hippocampus of a robust and long-lasting form of Hebbian synaptic modification known as long-term potentiation (*2*). Much of the data can be accounted for by a theory postulating that the hippocampus is the heart of a system capable of storing memory traces on the basis of a single, brief experience, after which these traces are "consolidated" gradually into more permanent storage in the neocortex (*3*–*5*). A memory system of this sort would, however, be seriously lacking if it were incapable of encoding information about the order in which events occurred.

Recent studies indicate that hippocampal unit activity during sleep reflects the activity patterns that occurred during the experience that preceded the sleep interval. Pyramidal cells in the CA3 and CA1 regions of the rat hippocampus have long been known to fire in a spatially specific manner in a variety of behavioral paradigms, with each cell firing when the rat is in a particular area of the environment (*6*):

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