types) resistant to dihydropyridines, ω-CgTx, and ω-Aga-IVA. The P-type channels and the resistant channels must reside together in glutamatergic terminals because strong depolarizations are able to overcome block by ω-Aga-IVA to maximally stimulate secretion. Thus L-, N-, and P-type channels may account for only part of the Ca^{2+} current in mammalian brain.

In conclusion, we have shown that ω-Aga-IIIA and ω-Aga-IVA block glutamate release from rat brain synaptosomes, a process resistant to ω-CgTx. This pharmacological evidence suggests that in rat brain, a P-type Ca^{2+} channel has a prominent role in neurotransmission at glutamatergic synapses and that the role for N-type channels in this process remains obscure. These results show that ω-agatoxins are important tools for identifying and characterizing presynaptic Ca^{2+} channels involved in neurotransmitter release and thus will prove useful in efforts to understand the ways in which neurosecretion and synaptic transmission are regulated.

REFERENCES AND NOTES

1. G. J. Augustine, M. P. Charlton, S. J. Smith, *Annu. Rev. Neurosci.* **10**, 633 (1987).
2. M. C. Nowycky, A. P. Fox, R. W. Tsien, *Nature* **316**, 440 (1985).
3. L. J. Regan, D. W. Sah, B. P. Bean, *Neuron* **6**, 269 (1991).
4. L. J. Regan, *J. Neurosci.* **11**, 2259 (1991).
5. D. J. Mogul and A. P. Fox, *J. Physiol. (London)* **433**, 259 (1991).
6. J. R. Lemos and M. C. Nowycky, *Neuron* **2**, 1419 (1989).
7. E. F. Stanley and G. Goping, *J. Neurosci.* **11**, 985 (1991).
8. C. A. Lindgren and J. W. Moore, *J. Physiol. (London)* **414**, 201 (1989).
9. L. M. Kerr and D. Yoshikami, *Nature* **308**, 282 (1984).
10. J. Rivier *et al.*, *J. Biol. Chem.* **262**, 1194 (1987).
11. V. J. Venema, K. M. Swiderek, T. D. Lee, G. M. Hathaway, M. E. Adams, *ibid.* **267**, 2610 (1992).
12. I. J. Reynolds *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8804 (1986).
13. J. B. Suszkiw, M. M. Murawsky, M. Shi, *J. Neurochem.* **52**, 1260 (1989).
14. A. L. Horne and J. A. Kemp, *Br. J. Pharmacol.* **103**, 1733 (1991).
15. P. M. Lundy, R. Frew, T. W. Fuller, M. G. Hamilton, *Eur. J. Pharmacol.* **206**, 61 (1991).
16. T. J. Turner and S. M. Goldin, *Biochemistry* **25**, 586 (1989).
17. F. Hofmann and E. Habermann, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **341**, 200 (1990).
18. I. M. Mintz *et al.*, *Nature* **355**, 827 (1992).
19. R. Llinás, M. Sugimori, J.-W. Lin, B. Cherksey, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1689 (1989).
20. O. D. Uchitel *et al.*, *ibid.* **89**, 3330 (1992).
21. B. M. Olivera *et al.*, *Ann. N.Y. Acad. Sci.* **635**, 114 (1991).
22. I. M. Mintz, V. J. Venema, M. E. Adams, B. P. Bean, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6628 (1991).
23. I. M. Mintz, M. E. Adams, B. P. Bean, *Soc. Neurosci. Abstr.* **18**, 9 (1992).
24. T. J. Turner, L. B. Pearce, S. M. Goldin, *Anal. Biochem.* **178**, 8 (1989).
25. T. J. Turner, unpublished results.
26. The effects of ω-Aga-IIIA and ω-Aga-IVA on cumulative, Ca^{2+}-dependent glutamate release were analyzed with the use of both double-reciprocal and Hill plots of the average values for release at each concentration of toxin tested. The IC_{50} value reported for ω-Aga-IIIA was obtained from double-reciprocal plots. We determined the potency of ω-Aga-IVA both by double-reciprocal and Hill analyses, using the I_{max} (maximal inhibition) value derived from the double-reciprocal plot for the Hill analysis. The value reported is that for the double-reciprocal analysis; the Hill analysis indicated an apparent K_i (inhibition constant) of 28.5 nM.
27. We have used the Na^+ channel activator batrachotoxin in Na^+-free solutions to increase the Na^+ permeability of synaptosomes, which are then depolarized by superfusion with a stimulating buffer containing 30 to 145 mM NaCl. The duration of the depolarization is limited by inclusion of the Na^+ channel blocker tetrodotoxin. The binding of tetrodotoxin to the Na^+ channel is a simple second-order reaction, so that the duration of the depolarization can be estimated from the product of the tetrodotoxin concentration and the forward rate constant for binding, estimated to be 10 to 30 $\mu M^{-1}\ s^{-1}$. At a concentration of 30 μM tetrodotoxin, we calculate that the depolarization will last ~10 ms [T. J. Tuner and K. Dunlap, *Soc. Neurosci. Abstr.* **17**, 577 (1991); *J. Cell. Biochem.* **16**, 225 (1992)].
28. R. McKinnon and C. Miller, *J. Gen. Physiol.* **91**, 335 (1988).
29. Reversal of the Na^+- and K^+-dependent glutamate transporter in the synaptic plasma membrane mediated the Ca^{2+}-independent glutamate release, as confirmed by the ability of transport inhibitors such as dihydrokainic acid and *threo*-β-hydroxyaspartic acid to completely block this component (T. J. Turner, unpublished results).
30. We performed release experiments in groups of 16 with at least one run for each experimental condition, using synaptosomes prepared immediately before each experiment. To minimize any time-dependent variability in the release rates, we randomized the order of runs for each experimental condition from day to day. Error bars for release rates, which were omitted for clarity, were generally less than 10% of the average values and did not exceed 20%.

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Effects of Kinesin Mutations on Neuronal Functions

M. Gho,* K. McDonald, B. Ganetzky, W. M. Saxton

Kinesin is believed to generate force for the movement of organelles in anterograde axonal transport. The identification of genes that encode kinesin-like proteins suggests that other motors may provide anterograde force instead of or in addition to kinesin. To gain insight into the specific functions of kinesin, the effects of mutations in the kinesin heavy chain gene (*khc*) on the physiology and ultrastructure of *Drosophila* larval neurons were studied. Mutations in *khc* impair both action potential propagation in axons and neurotransmitter release at nerve terminals but have no apparent effect on the concentration of synaptic vesicles in nerve terminal cytoplasm. Thus kinesin is required in vivo for normal neuronal function and may be active in the transport of ion channels and components of the synaptic release machinery to their appropriate cellular locations. Kinesin appears not to be required for the anterograde transport of synaptic vesicles or their components.

Kinesin is a ubiquitous mechanochemical adenosine triphosphatase (ATPase) that can move toward the plus ends of microtubules in vitro (*1*, *2*). The native molecule is a heterotetramer consisting of two identical heavy chains and two light chains (*2*). The kinesin heavy chain, and more specifically the NH_2-terminal head of the heavy chain, appears to contain all of the mechanochemical elements necessary for generating microtubule-based movements (*3*). The light chains and the COOH-terminal tail of the heavy chain are thought to be involved in binding kinesin to vesicles or other cargo destined for microtubule-based transport (*2*, *4*).

On the basis of the polarity of kinesin's movement and its presence in neural cells, it was originally suggested that kinesin might act as a motor for anterograde axonal transport (*1*). It since has been shown that vesicle movement in axoplasm (both anterograde and retrograde) can be inhibited in vitro by an antibody that binds to the kinesin heavy chain (*5*). It also has been shown that kinesin heavy chain (*khc*) mutations in *Drosophila* cause behavioral defects that suggest a requirement for kinesin in normal neuromuscular functions (*6*). Although these data provide a persuasive argument that kinesin acts as a motor for axonal transport, the argument remains indirect.

Kinesin is a member of a superfamily of kinesin-like proteins that share similar mechanochemical domains (*7*), and multiple members of the kinesin superfamily are co-expressed in neural and other tissues (*8*). Thus, if kinesin is a motor for anterograde axonal transport, it is likely to function in the context of a complex set of anterograde motors. Consequently, understanding anterograde axonal transport and other micro-

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tubule-based transport processes will require critical analyses of the specific functions of different motor proteins.

We have taken advantage of *khc* mutations to study the effects of impaired kinesin function on specific physiological functions of motor neurons in *Drosophila* larvae. Action potential propagation by segmental nerves and synaptic transmission at neuromuscular junctions were tested in both the second (A2) and sixth (A6) abdominal segments of control and *khc* mutant larvae (*9*). Two hypomorphic *khc* alleles (khc^5 and khc^6) were used to construct both control and mutant genotypes (*10*) because these alleles are severe enough to cause a strong behavioral phenotype in larvae (6), yet mild enough to permit larval growth to an appropriate size for manipulations. Similar results were obtained with both alleles, and the data were pooled.

Figure 1 shows recordings of compound action potentials and corresponding excitatory junctional potentials (EJPs) evoked by stimulation of segmental nerves in control and *khc* mutant larvae (9). Recordings from segment A6 indicate that *khc* mutations caused a fourfold reduction in the mean amplitudes of both compound action potentials and EJPs (Table 1) (*11*). Similar differences were observed for segment A2, but the short segmental nerve that innervates A2 made the dual recordings and subsequent quantification of compound action potentials difficult. Our best recordings (greatest amplitudes) from A2 are shown in Fig. 1.

The voltage required to evoke responses in *khc* mutant larvae was twice that for control larvae (legend to Fig. 1). Even with the increased voltage, only about one out of four nerve stimuli evoked a response. Control larvae never failed to respond under the same conditions. Because it usually was possible to elicit compound action potentials and corresponding EJPs, functional motor neurons were present in *khc* mutants. However, the frequent failures and the reduced amplitudes of compound action potentials in mutant nerves indicate that axonal function was impaired. A compound action potential is the sum of many simple action potentials in the individual sensory and motor axons within the segmental nerve. Thus, reduced compound action potential amplitude could reflect a reduction in the proportion of axons that fire in response to a given stimulation or a reduction in the action potential amplitudes produced by individual axons. In either case, *khc* mutations cause impaired action potential propagation. Electron micrographs of cross sections of larval nerves (not shown) indicate that *khc* mutations do not alter the number of axons in a nerve bundle.

The observation that EJP amplitudes in *khc* mutants are reduced relative to controls suggest that wild-type kinesin is required for proper synaptic transmission. To analyze whether the reduction in synaptic transmission resulted from defective release of neurotransmitter or in the postsynaptic response to neurotransmitter, we studied spontaneous miniature excitatory junctional currents (mEJCs) in control and *khc* mutant muscles (Fig. 2). Such currents are thought to be evoked in a muscle cell by the random spontaneous release of single quanta of a neurotransmitter from the presynaptic terminal, probably due to the exocytosis of individual synaptic vesicles (*12*). The mEJC amplitude serves as a measure of postsynaptic response to quantal stimulation by neurotransmitter. Under voltage-clamp conditions (*13*), the mean amplitude of mEJCs was not appreciably different in control and *khc* mutant muscles (Fig. 2B). Furthermore, comparable results were obtained at different muscle membrane potentials (V_H = −60, −80, −100, and −120 mV) indicating by extrapolation that the reversal potential of the postsynaptic currents was not modified by the *khc* mutations. These results show that postsynaptic response to neurotransmitter was normal in

Table 1. Mean amplitudes of compound action potentials (CAP) and excitatory junctional potentials (EJP) in segment A6.

Larvae	CAP (μV)	EJP (mV)
Control (n = 9)	366 ± 130	23 ± 9
khc mutant (n = 10)	86 ± 46	5 ± 3

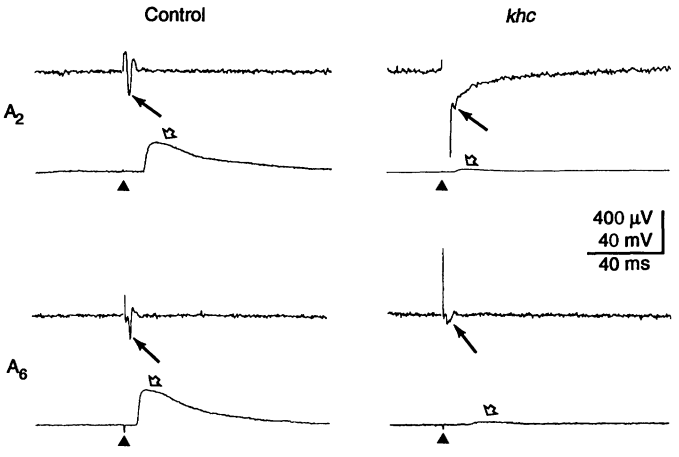

Fig. 1. The effects of *khc* mutations on action potentials and synaptic transmission. Segmental nerves for A2 and A6 were stimulated (triangles) and recordings were made of compound action potential amplitude (filled arrows) and the corresponding EJP amplitudes (open arrows) from muscle 6 in control (left) and *khc* mutant (right) larvae. The upper panels show responses obtained from segment A2 and the lower panels those obtained from segment A6. The large stimulus artifact that precedes the action potential in mutants was a result of the strong stimulation required to evoke a measurable response. Stimulation with reversed polarity did not evoke any responses. Care was taken to evoke all-or-none EJP responses associated with a measurable delay of at least 2 ms, indicating that the response was elicited by an axonal action potential rather than by direct depolarization of the terminal. Stimulation parameters were 5 V and 0.1 ms for controls and 10 V and 0.1 ms for mutants.

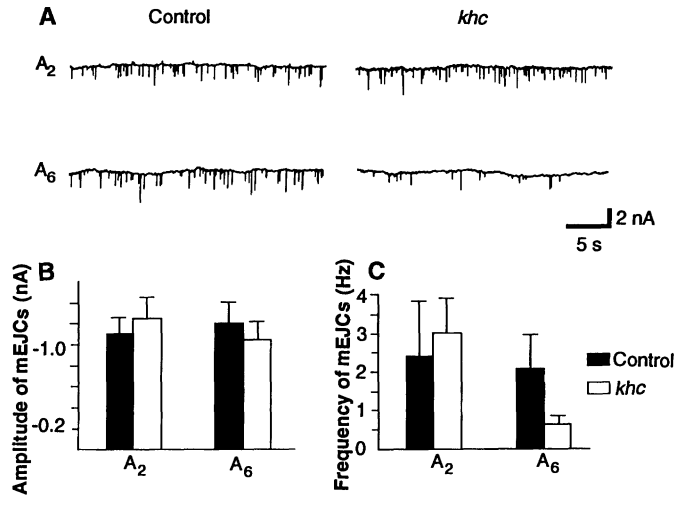

Fig. 2. Spontaneous miniature excitatory junctional currents recorded from control and *khc* mutant muscles. (**A**) Representative traces of mEJCs obtained from muscle 6 in the second (A2) and sixth (A6) abdominal segments. Histograms show mean and standard deviation values for the amplitude (**B**) and frequency (**C**) of mEJCs in control and *khc* mutant muscles. In (C) the values for segment A6 are significantly different as determined by the Student's test ($P < 0.01$). The number of animals tested was five controls and five mutants for segment A2 and six controls and six mutants for segment A6. Data were collected for at least 30 s from each muscle. To obtain good resolution in mEJC recordings, the holding potential was −100 mV. The external calcium concentration was 0.15 mM.

khc mutants. Therefore, the effect of *khc* mutations on synaptic transmission must be due to impaired neuronal function.

Although the amplitude of mEJCs was not affected by *khc* mutations, in segment A6 the frequency of mEJCs was depressed fourfold relative to that of controls in both hypertonic (Fig. 2C) (9) and normal saline solutions (not shown). These results suggest that *khc* mutations affect the function of the axon terminal itself, at least in the more posterior segment. An occurrence versus amplitude histogram was prepared for mEJCs recorded from segment A6 of mutant and wild-type larvae to address the possibility that the reduction in mEJC frequency resulted from the absence of a distinct subpopulation of synaptic vesicles. The amplitude histograms from *khc* mutants and controls were not distinguishable (not shown). Thus it is unlikely that kinesin is responsible for the preferential transport of a particular class of synaptic vesicles to motor terminals.

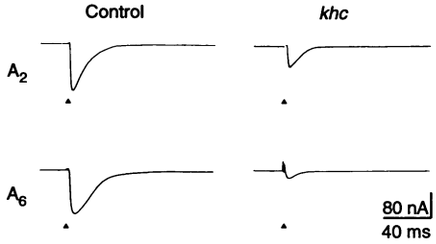

Fig. 3. The effect of *khc* mutations on excitatory junctional currents evoked by direct electrotonic stimulation of the presynaptic terminal. Terminals were stimulated (triangles) and recordings of EJCs were taken from muscle 6 of segments A2 and A6 of control and *khc* mutant larvae. The bathing solution contained 1 μM tetrodotoxin. Stimulation parameters were 5 V and 1 ms.

Table 2. The number of synaptic vesicles (SVs) per cubic micrometer of cytoplasm estimated from electron micrographs of sections of boutons on muscles 6 and 7.

Animal	Segment	Sections	SVs/μm³
		Mutant	
1	A2	13	1440
	A6	19	1750
2	A6	12	2280
3	A6	23	2390
		Control	
1	A2	32	2100
	A6	12	1320
2	A6	9	2260
3	A6	26	1790

The analysis of EJP amplitudes after nerve stimulation (Fig. 1) revealed that *khc* mutations reduce the amount of evoked neurotransmitter release from motor neurons. This result could be either a direct consequence of impaired presynaptic terminal function or an indirect consequence of impaired action potential propagation in motor axons. To evaluate the function of presynaptic terminals in the absence of axonal conduction, excitatory junctional currents (EJCs) evoked by direct electrotonic stimulation of the presynaptic terminal were measured (Fig. 3) (*14*). The mean amplitude of EJCs evoked by direct stimulation in segment A2 of controls was 159 ± 35 nA (n = 3 larvae). Under similar conditions the mean amplitude in *khc* mutants was 61 ± 20 nA (n = 4 larvae). The reduction in amplitude in *khc* mutants was more extreme in segment A6: 118 ± 27 nA for controls (n = 7 larvae) and 25 ± 16 nA for *khc* mutants (n = 6 larvae). These observations show that *khc* mutations inhibit the function of presynaptic terminals in both anterior and posterior segments.

The preceding observations provide direct evidence that kinesin is required for proper neuronal function. In addition, comparison of results from segments A2 and A6 suggest that the effect of impaired kinesin function on motor terminals is more severe in posterior segments. Motor terminals in posterior segments are served by relatively long axons, and thus there is a correlation between phenotype severity and axon length. This correlation supports the hypothesis that kinesin functions in vivo as a motor for axonal transport.

The axonal components transported by kinesin have not yet been identified. Hall and Hedgecock (*15*) have reported a severe reduction in synaptic vesicle concentration in nerve terminals of nematodes carrying mutations in *unc*-104, a *Caenorhabditis elegans* gene that encodes a kinesin-like protein (*16*). This suggests that UNC-104 protein is responsible for the anterograde transport of synaptic vesicle components (*15*). A similar role for kinesin might explain the impaired transmitter release reported here for *khc* mutants.

To determine if the defects in transmitter release in *khc* mutants were due to reduced transport of synaptic vesicle components, we examined nerve terminals by electron microscopy (*17*). In *khc* mutant nerve terminals corresponding to those assayed by electrophysiology, synaptic vesicles appeared to be abundant (Fig. 4). Morphometric analysis confirmed that the concentrations of synaptic vesicles in the terminals of *khc* and control larvae were not different (Table 2). These results, bolstered by our observations that the frequency of spontaneous transmitter release in *khc* mutants was normal in segment A2 (Fig. 2) while the evoked transmitter release was substantially reduced (Fig. 3), suggests defects in the mechanism of transmitter release. Kinesin must be responsible for the transport of material other than synaptic vesicle components. Presumably the components of synaptic vesicles in *Drosophila* are transported by a kinesin-like protein that is similar to UNC-104.

Both of the defects of *khc* mutant neurons—faulty action potential propagation and impaired neurotransmitter release—could be explained by reduced anterograde transport of ion channels. Voltage-gated sodium and potassium channels are likely to be carried in the membranes of vesicles that are transported along microtubules to points of insertion into the axonal plasma membrane (*18*). Kinesin could be responsible for transporting such channel-bearing vesicles. Impaired kinesin function would

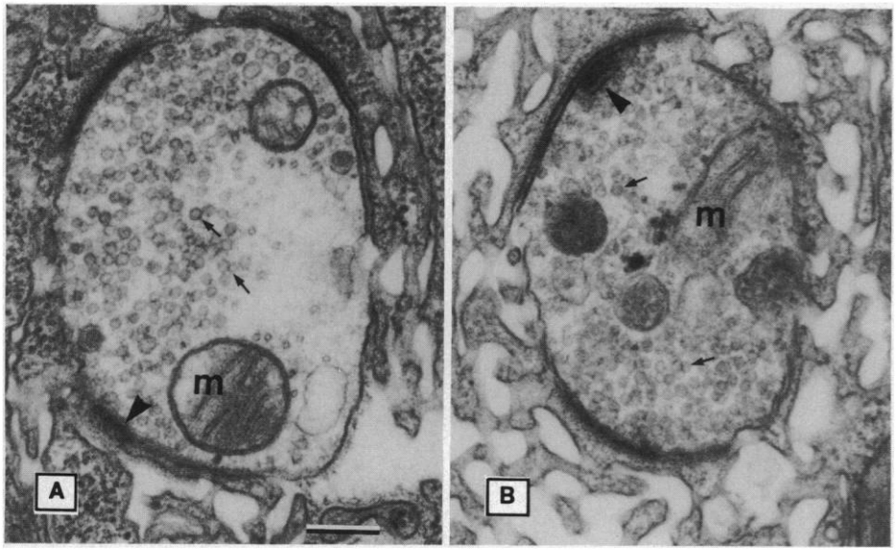

Fig. 4. A comparison of the ultrastructure of nerve terminals from wild-type and *khc* mutant larvae. Synaptic vesicles (arrows), mitochondria (m), and dense bodies (arrowheads) are noted in sections of boutons from junction of muscles 6 and 7 in segment A6 of wild-type (**A**) and *khc* mutant (**B**) larvae. Scale bar, 0.2 μm.

then lead to reduced sodium and potassium channel density in the axonal membrane and consequently to a reduced capacity to propagate action potentials. Similarly, impaired kinesin function could lead to reduced calcium channel density in terminal membranes, causing impaired neurotransmitter release. However, reduced anterograde transport of other terminal components could also cause impaired transmitter release. These include presynaptic membrane components responsible for forming release sites and cytoplasmic proteins that are active in preparing synaptic vesicles for productive association with release sites.

REFERENCES AND NOTES

1. R. D. Vale, T. S. Reese, M. P. Sheetz, *Cell* **42**, 39 (1985); R. D. Vale *et al.*, *ibid.* **43**, 623 (1985).
2. R. B. Vallee and H. S. Shpetner, *Annu. Rev. Biochem.* **59**, 909 (1990); S. T. Brady, *Neuron* **7**, 521 (1991).
3. J. T. Yang, W. M. Saxton, R. J. Stewart, E. C. Raff, L. S. B. Goldstein, *Science* **249**, 42 (1990).
4. P. J. Hollenbeck and J. A. Swanson, *Nature* **346**, 864 (1990); F. K. Gyoeva and V. I. Gelfand, *ibid.* **353**, 445 (1991).
5. S. T. Brady, K. K. Pfister, G. S. Bloom, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1061 (1990).
6. W. M. Saxton, J. Hicks, L. S. B. Goldstein, E. C. Raff, *Cell* **64**, 1093 (1991).
7. R. D. Vale and L. S. B. Goldstein, *ibid.* **60**, 883 (1990); L. S. B. Goldstein, *Trends Cell Biol.* **1**, 93 (1991).
8. R. J. Stewart, P. A. Pesavento, D. N. Woerpel, L. S. B. Goldstein, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8470 (1991).
9. Conventional intracellular voltage and two-microelectrode voltage-clamp recordings were made from body wall muscle 6 [for nomenclature see J. Johansen, M. E. Halpern, K. M. Johansen, H. Keshishian, *J. Neurosci.* **9**, 710 (1989)] of third instar larvae that were dissected as described by L. Y. Jan and Y. N. Jan [*J. Physiol. (London)* **262**, 189 (1976)] and maintained in hypertonic saline to prevent muscle contraction (129 mM NaCl, 2 mM KCl, 4 mM $MgCl_2$, 5.4 mM $CaCl_2$, 5 mM Hepes, pH 7.1, and 353 nM sucrose). Normal saline (36 mM sucrose) was also used in some experiments. Microelectrodes were filed with 3 M KCl. During conventional voltage recordings, membrane potential was held at −60 mV by using a bridge circuit unless otherwise indicated. The bridge was continually checked by hyperpolarizing current pulses. Segmental nerves were stimulated via a suction electrode at 0.3 Hz. Compound action potentials were recorded extracellularly by a second suction electrode placed midway between the central nervous system and the appropriate segment.
10. Hemizygous mutant genotypes ($khc^m/Df(2R)Jp6$) were constructed by using either khc^5 and khc^6 and a deletion that removes the *khc* gene (*Df2R)Jp6*). Control larvae were of two types: (i) those with the second chromosome genotype $khc^m/Df(2R)Jp6$ but carrying a transposon that includes the wild-type *khc* gene (*P-khc*$^+$) on the third chromosome or (ii) those with the second chromosome genotype $khc^m/+$. Results from these two different types of control animals were indistinguishable and were pooled.
11. The amplitude of a compound action potential was measured by drawing a line between the two positive peaks that flanked the negative one and measuring the vertical distance between that line and the negative peak.
12. J. E. Heuser *et al.*, *J. Cell Biol.* **81**, 275 (1979).
13. The measurement of synaptic currents with the voltage-clamp technique avoided the nonlinear summation that occurs when synaptic potential is used as a measurement of synaptic activity [G. J. Augustine, M. P. Charlton, S. J. Smith, *J. Physiol. (London)* **367**, 163 (1985)]. Also, the voltage clamp permitted comparison of data from different cells independent of input membrane resistance.
14. Direct (electrotonic) stimulation of the presynaptic terminal was accomplished by adding tetrodotoxin (1 μM) to the bathing solution to block axonal action potential propagation. The segmental nerve was then cut, drawn into the suction electrode and stimulated as close to the terminal as possible [C.-F. Wu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4047 (1978)]. A series of stimuli of increasing voltage was used to determine the plateau level for transmitter release, and suprathreshold stimulation was then used for each test.
15. D. H. Hall and E. M. Hedgecock, *Cell* **65**, 837 (1991).
16. A. J. Otsuka *et al.*, *Neuron* **6**, 113 (1991).
17. Larvae were dissected in hypertonic saline as described in (*9*), fixed in 2% glutaraldehyde, 100 mM Na cacodylate (pH 7.2) for 1 hour at 25°C, and post-fixed, stained, and embedded as described [K. McDonald, *J. Ultrastruct. Res.* **86**, 107 (1984)]. Transverse serial 100-nm sections were cut from appropriate regions, post-stained with uranium and lead, and photographed at ×21,000 on a Phillips CM10 electron microscope operating at 80 or 100 kV (K. McDonald, *ibid.*). Micrographs of sections were analyzed by eye for the number of small (30 to 100 nm in diameter) electron lucent vesicles present in bouton profiles. The area of each bouton profile was then determined with computer digitization of the circumference, and volume was calculated by multiplication by the section thickness. Microscopy and analysis were performed by different people. Samples and micrographs were coded to avoid bias until analysis was complete.
18. T. Brismar and W. F. Gilly, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1459 (1987); W. F. Wonderlin and R. J. French, *ibid.* **88**, 4391 (1991).

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