including the borders of the brain image. All of our maps were screened for such artifacts, and the images were rejected if artifacts were present. However, this was uncommon because of our head restraining system and the fact that head movements are naturally uncommon during finger movements.
9. Four paired *t* tests were done to assess the statistical significance of the difference between activation during contralateral and ipsilateral movements in (i) the right hemisphere of right-handed subjects, (ii) the right hemisphere of left-handed subjects, (iii) the left hemisphere of right-handed subjects, and (iv) the left hemisphere of left-handed subjects.
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The *Caenorhabditis elegans unc-17* Gene: A Putative Vesicular Acetylcholine Transporter

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Mutations in the *unc-17* gene of the nematode *Caenorhabditis elegans* produce deficits in neuromuscular function. This gene was cloned and complementary DNAs were sequenced. On the basis of sequence similarity to mammalian vesicular transporters of biogenic amines and of localization to synaptic vesicles of cholinergic neurons in *C. elegans*, *unc-17* likely encodes the vesicular transporter of acetylcholine. Mutations that eliminated all *unc-17* gene function were lethal, suggesting that the acetylcholine transporter is essential. Molecular analysis of *unc-17* mutations will allow the correlation of specific parts of the gene (and the protein) with observed functional defects. The mutants will also be useful for the isolation of extragenic suppressors, which could identify genes encoding proteins that interact with UNC-17.

Mutations in the *unc-17* gene of *C. elegans* were first described by Brenner (*1*). These mutations result in impaired neuromuscular function that leads to jerky, coiling locomotion (*1*, *2*) as well as abnormal pharyngeal pumping and defecation (*3*). The role of acetylcholine as an excitatory neurotransmitter at nematode neuromuscular junctions (*4*) suggests that *unc-17* might be involved in cholinergic processes. Brenner (*1*) also observed that *unc-17* mutants were resistant to cholinesterase inhibitors. The direct effect of cholinesterase inhibition is a rise in the synaptic concentrations of acetylcholine. Therefore, genetic resistance may result from decreased synthesis or release of the transmitter or from decreased response to the transmitter. Additional evidence for cholinergic involvement came from the accumulation of high concentrations of acetylcholine in *unc-17* mutants (*5*) and the genetic interactions between *unc-17* and the closely linked *cha-1* gene, which encodes choline acetyltransferase (ChAT, the acetylcholine synthetic enzyme) (*2*, *6*).

We cloned the *unc-17* genomic region by walking from the *cha-1* gene (*7*). Putative *unc-17* complementary DNAs (cDNAs) were isolated from two different libraries, one prepared by Barstead and Waterston (*8*) and one purchased from Stratagene. These libraries were initially probed with genomic clones from the region and then with an *unc-17* cDNA, RM#51P (*9*). Five independent cDNAs from the region were isolated and RM#51P was completely sequenced (*10*). The inserts from the remaining cDNA clones were characterized by restriction enzyme analysis with high-resolution gels and by sequencing of the 5′ and 3′ ends. The *unc-17* cDNA with the longest 5′ sequence, RM#125P, was 48 base pairs (bp) longer than RM#51P and appeared to be the result of trans-splicing, with six nucleotides derived from the 22-nucleotide spliced leader SL1 (*11*). This cDNA was thus only 16 bases short of full length. The total length of RM#125P was 1.9 kb. Consistent with this length, a single, nonabundant mRNA of approximately 2.0 kb has been detected in Northern blots of wild-type RNA {either total or polyadenylate [poly(A)]–selected RNA} probed with *unc-17* cDNAs (*12*). We believe that these cDNAs correspond to the *unc-17* gene for several reasons. First, the injection of cosmid F57G7 (containing the complete coding sequence of the cDNA) completely rescues the mutant phenotypes of *unc-17(e245)* animals (*13*). In addition, three independent *unc-17* mutations have been associated with alterations in the cDNA sequence (*14*). Finally, two *unc-17* mutations led to decreased immunohistochemical staining, with the use of an antibody raised against a cDNA-encoded peptide (*14*).

The translation of the open reading frame from the first methionine codon (81 bp from the trans-splice acceptor site) gives a predicted protein (UNC-17) with 532 amino acids, a mass of 58.5 kD, and an isoelectric point of 5.29 (Fig. 1). Two rat proteins have sequence similarity to UNC-17: (i) the synaptic vesicle monoamine transporter (MAT or SVAT) and (ii) the chromaffin granule amine transporter (CGAT). These proteins transport biogenic amines (catecholamines, serotonin, and perhaps histamine) into synaptic vesicles or chromaffin granules (*15*, *16*) and are 62% identical (*16*). The *C. elegans* UNC-17 protein is 37% identical to CGAT and 39% identical to SVAT (Fig. 1) and appears to belong to the same gene family of proteins with 12 transmembrane domains (*17*). These characteristics suggest that it might also be a vesicular neurotransmitter transporter.

To determine the cellular and subcellular localization of UNC-17, antibodies against UNC-17–specific peptides were raised (*18*). Anti–UNC-17 staining was observed in most regions of the nervous system, including the nerve ring, the ventral and dorsal nerve cords, and the pharyngeal nervous system (Fig. 2, A and B). Many, but not all, of the identifiable neurons contained the UNC-17 antigen. Within individual cells, anti–UNC-17 binding was punctate and appeared to be concentrated near synaptic regions (Fig. 2, A and B). Double labeling with antibodies to the *C. elegans* synaptic vesicle protein synaptotagmin (*19*) showed colocalization of the two antigens within a subset of neurons (Fig. 2, B and C). This colocalization suggests that in the cells that contained UNC-17, this protein was associated with synaptic vesicles.

To confirm that UNC-17 was associated with synaptic vesicles, we took advantage of the properties of mutations in the *unc-104* gene, which encodes a kinesin-related protein required for the axonal transport of synaptic vesicles (*20*). Viable *unc-104* mutants accumulate large numbers of synaptic vesicles in their cell bodies and have few vesicles in their processes. In *unc-104* ani-

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mals, anti–UNC-17 staining was restricted to neuronal cell bodies (Fig. 2D), the pattern expected for a synaptic vesicle component. As in wild-type animals, the anti–UNC-17 staining in *unc-104* mutants corresponded to a subset of anti-synaptotagmin staining.

The restriction of UNC-17 to cell bodies in *unc-104* mutants permitted identification of many of the cells in which it was expressed. The neurons in the pharynx containing UNC-17 were shown to correspond exactly to those that were labeled with antibodies to C. *elegans* ChAT (*21*) (Fig. 3), which suggests that UNC-17 was present only in cholinergic neurons. Comparable results were observed in other parts of the nervous system. Taken together, they support the hypothesis that UNC-17 is associated with synaptic vesicles in most, if not all, cholinergic neurons.

More than 20 alleles of *unc-17* that are viable as homozygotes have been identified: their phenotypes vary from mild (almost normal behavior and development) to severe (almost paralyzed, poor growth). Two of these mutants, *md1447* and *p279* (*14*), show a severe and uniform decrease in UNC-17 staining throughout the nervous system. Both mutants showed normal anti-ChAT and anti-synaptotagmin staining.

We have also been able to isolate, in a noncomplementation screen (*22*), two alleles of *unc-17* (*md64* and *md75*) that are

```
UNC-17   MGFNVPVINRDSEILKADAKKWLEQQDNQKKCVLVIVSIALLLDNMLYMVIVPIIPKYLRDIH...............NYQVTFEGYHN
SVAT     ........mal-DLVl...lR--rdsrhsR-lI-f--fL--------lt-V-----s--ys-Kheknsteiq.....ttrpeLvvstses
CGAT     ..........mlqVVlgapqRl-kegrqsR-l---V-fV--------lt-V---V-tF-yatefkdsnsslhrgpsvssQqaL-spaFst
                                                      TM1

ETSQLANGTYLVRE...............................VGGRINFLDEELELGWLFASKALLQIFVNPFSGYIIDRVGYEIPMILGLCTMFFS
if-yyn-s-v-Itgnatgtlpggqshkatstqhtvanttvpsdcpsed-dll.n-nVqV-l------tV-Llt---i-lLtn-I--p---fA-f-i--i-
if-ffd-t-tt-e-hvpfrvt...wtngtipppvteassvpknncLq-iefleE-nVrI-i-------M-Ll----v-pLtn-I--h---fV-fmi--l-
                                                               TM2                          TM3

TAIFALGKSYGVLLFARSLQGFGSAFADTSGLAMIADRFTEENERSAALGIALAFISFGCLVAPPFGSVLYSLAGKPVPFLILSFVCLADAIAVFMVINP
-VM--fss--af--i------i--scssva-Mg-L-svY-DDe--gkpM----ggLam-v--g--------efV--tA---V-aaLv-L-gAIqlf-LQ-
-LM--fsqT-al-fv--T---i--sfssva--q-L-svY-Dny--gr-M----qqLal-l--qa-----M-efV--ss-----a-La-L-qALqlcILw-
                          TM4                        TM5                          TM6

HRRGTDSHGEKVQGTPMWRLFMDPFIACCSGALIMANVSLAFLEPTITTWMSEMMPDTPGWLVGVIWLPPFFPHVLGVYVTVKMLRAFPHHTWAIAMVGL
s-vqpE-q....k---Ltt-lk--Y-Liaa-sIcf--MgI-m---aLpi--m-t-c.Srk-qL--Af--asisyLI-tnIfgiLAHkmgR..-LcaLL-M
sKvspE-a....m--sLlt-lk--Y-Lvaa-sIcl--MqV-i----Lpi--mqt-c.S-e-qL-LAf--asvayLI-tnLfqvLAHkmgR..-LcsL--M
                                TM7                                TM8

AMEGIACFAIPYTTSVMGLVIPLSFVCFGIALIDTSLLPMLGHLVDTRHVSVYGSVYAIADISYSLAYAFGPIIAGWIVTNWGFTALNIIIFATNVTYAP
VIv--silc--FaknIy--Ia-nfg-g-a-fMV-S-MM-IM-y---l--------------VaFcmg--i--sag-a-Akai--pw-mt--gIidIaF--
VAv--sllcV-lahnIf--Ig-naqLq-a-qMV-S--M-IM-y---l--t-----------VaFcvqF-i--stg-v--qvi--pw-mV--qti-Ii---
 TM9                           TM10                     TM11                      TM12

VLFLLRKVHSYDTLGAKGDTAEMTQLNSSAPAGGYNGKPEATTAESYQGWEDQQSYQNQAQIPNHAVSFQDSRPQAEFPAGYDPLNPQW
Lc-f--sp......p--eEkMaIlmdhncpikrkmytqnnVqSypigdde-sesd
Lccf-qnp......p--eEkraIlsqecptetqm-tfqkptkafplgensD-pssge
```

Fig. 1. Deduced amino acid sequence of UNC-17 and alignment with SVAT and CGAT sequences. The standard single-letter abbreviations for amino acids are used (*23*); the cDNA sequence has been assigned GenBank accession number L19621. The synthetic peptide used as immunogen is italicized. Dots indicate gaps that have been introduced to optimize alignment; dashes indicate identity with the UNC-17 sequence. Uppercase letters denote similarity to the UNC-17 sequence (similarity groups: AILMV, C, DE, FWY, G, HKR, NQ, P, ST). Underlined sequences indicate the putative transmembrane domains (TM1 to TM12) as presented for the CGAT and SVAT sequences (*16*). Amino acid totals: UNC-17, 532; SVAT, 515; CGAT, 521.

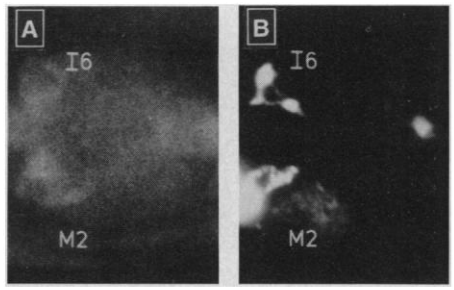

Fig. 3. Immunostaining with anti-ChAT and anti–UNC-17 antibodies in *C. elegans* larvae. (**A**) In the pharynx of a wild-type larva, two identified cells, the motorneuron M2 and the interneuron I6 (*24*), stain diffusely and uniformly with anti-ChAT antibody (*21*). However, the other 10 pharyngeal neuron cell bodies in this region (visualized by Nomarski optics) are not stained. (**B**) In the pharynx of an *unc-104*(*e1265*) mutant larva, anti–UNC-17 staining is concentrated in a patchy manner in the cell bodies of particular neurons. The image shows the same region of the pharynx shown in (A). Only the M2 and I6 neurons are positive for UNC-17 staining.

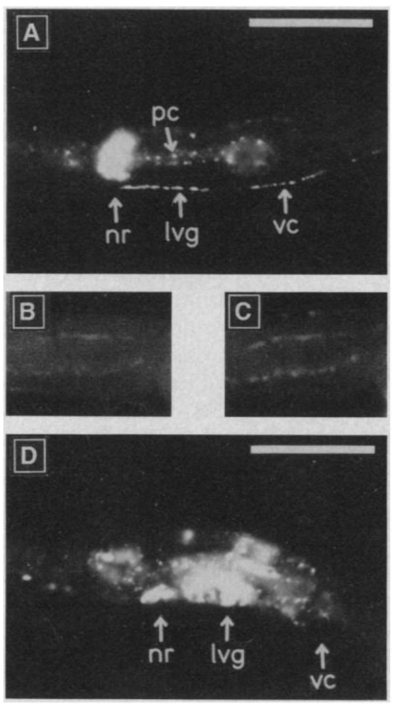

Fig. 2. Immunostaining of *C. elegans* larvae with antibodies to an UNC-17 peptide and a synaptic vesicle protein. (**A**) Anterior nervous system in a wild-type L2 larva stained with anti–UNC-17. Punctate staining is especially dense in the circumferential nerve ring (nr), the region with the most extensive neuropile. Staining is less dense in the lateral and ventral ganglia (lvg), which contain many neuronal cell bodies but relatively few synapses. Punctate staining is also seen along the ventral nerve cord (vc) and a pharyngeal nerve cord (pc). (**B**) Anti–UNC-17 staining in two of the pharyngeal nerve cords appears as lines of punctate staining in this higher magnification view of the isthmus of the wild-type L2 larva. (**C**) Staining with an antibody to *C. elegans* synaptotagmin (*19*), a synaptic vesicle protein, in the same animal shown in (B). Three nerve cords are seen as lines of punctate staining. The pattern of staining in two of these cords corresponds to the pattern seen with anti–UNC-17, but the staining in one cord is unique to anti-synaptotagmin. (**D**) The L2 larva with the *unc-104*(*e1265*) mutation shows much less UNC-17 staining than does the wild type in the neuropile-rich nerve ring and exhibits increased staining in particular cell bodies in the lateral and ventral ganglia. Scale bars, 20 μm.

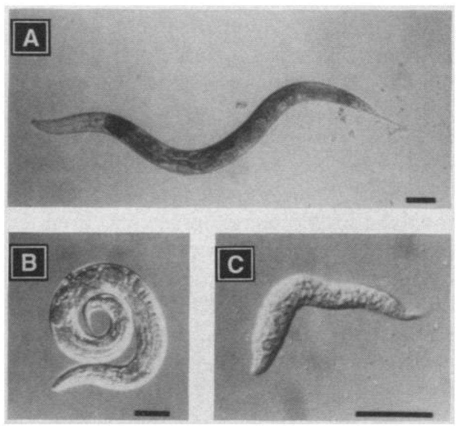

Fig. 4. Differential interference contrast (Nomarski) images of *C. elegans* wild-type and *unc-17* mutants. (**A**) Body posture of the adult wild-type hermaphrodite during forward locomotion. (**B**) Adult hermaphrodite homozygous for the *unc-17*(*e245*) mutation. These animals grow and reproduce but are severely uncoordinated and often assume a coiled posture. (**C**) Terminal phenotype of animals homozygous for the recessive lethal *unc-17*(*md64*) mutation. These larvae are extremely uncoordinated, do not grow, and die several days after hatching. Scale bars, 150 μm.

lethal as homozygotes (*14*). Animals homozygous for either allele undergo apparently normal embryogenesis and hatch. The newly hatched animals are small and coiled and resemble an extreme version of young larvae homozygous for severe viable alleles of *unc-17* (Fig. 4). These animals do not grow or feed, can barely move, and usually die within a few days. This phenotype is the same that was observed with lethal alleles of *cha-1* that eliminate acetylcholine synthesis (*6*). These characteristics suggest that cholinergic function is completely lost in the lethal *unc-17* mutants. The lethal *unc-17* alleles are fully recessive to wild type, and animals heteroallelic for either lethal allele and a viable *unc-17* allele display a stronger phenotype than do the corresponding viable mutant homozygotes. Therefore, we conclude that the lethal phenotype represents the null (or near-null) *unc-17* phenotype and that *unc-17* encodes a function essential for survival.

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13. The transformation protocol was according to C. C. Mello, J. M. Kramer, D. Stinchcomb, and V. Ambros [*EMBO J.* **10**, 3959 (1991)].
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21. Antibodies against *C. elegans* ChAT were prepared in the same manner as were the UNC-17 antibodies (*18*), with the use of the peptide TPVNSNPGYIFPKVKFETK (coupled to rabbit serum albumin) as immunogen. The *C. elegans* ChAT protein is a soluble enzyme [J. B. Rand and R. L. Russell, *J. Neurochem.* **44**, 189 (1985)]; ChAT immunostaining is diffuse throughout cholinergic neurons and is not altered in *unc-104* mutants.
22. Male wild-type L4 animals were mutagenized with ethyl methane sulfonate (*1*) and, after reaching maturity, were mated with *unc-33*(*e204*) *unc-17*(*p1156*) *dpy-13*(*e184*) hermaphrodites. The anomalous allele *p1156* fails to complement both *cha-1* and *unc-17* mutations (*2, 6*). After 1 day, the mated hermaphrodites were transferred to medium containing the cholinesterase inhibitor aldicarb (0.5 mM). These hermaphrodites eventually died after laying ~100 eggs each. The self-progeny, which are homozygous for *dpy-13*, grew poorly on aldicarb. Most cross progeny were heterozygous for *p1156* and died. However, new *unc-17* or *cha-1* alleles induced by the mutagenic treatment led to heteroallelic Unc or Cha cross progeny that were heterozygous for the recessive *dpy-13* marker and that grew well in the presence of aldicarb. The new mutations were characterized by standard methods (*1, 2*) and included viable and lethal alleles of *unc-17* and *cha-1*. Mutations that were lethal as homozygotes were maintained as balanced heterozygotes with the balancer chromosome *nT1* [E. L. Ferguson and H. R. Horvitz, *Genetics* **110**, 17 (1985)].
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