

(SH2) domain (22). Unlike receptor-SH2 interactions, EGF receptor-adaptin association does not occur at 4°C. Moreover, the extent of receptor-adaptin association is greater than that between receptors and proteins that contain SH2 domains (22). Alternatively, the interaction between the EGF receptor and adaptins may be more stable than the association of the growth factor receptors with SH2 domain-containing proteins.

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Functional Magnetic Resonance Imaging of Motor Cortex: Hemispheric Asymmetry and Handedness

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A hemispheric asymmetry in the functional activation of the human motor cortex during contralateral (C) and ipsilateral (I) finger movements, especially in right-handed subjects, was documented with nuclear magnetic resonance imaging at high field strength (4 tesla). Whereas the right motor cortex was activated mostly during contralateral finger movements in both right-handed (C/I mean area of activation = 36.8) and left-handed (C/I = 29.9) subjects, the left motor cortex was activated substantially during ipsilateral movements in left-handed subjects (C/I = 5.4) and even more so in right-handed subjects (C/I = 1.3).

Two basic aspects of human motor function are hemispheric dominance and handedness. The dominant roles of the left hemisphere for speech and the right hemisphere for visuospatial tasks and for the spatial distribution of attention are well documented (1). With regard to motor function, the deficits resulting from a left hemispheric lesion are more pronounced than those resulting from a right hemispheric lesion (2), although some specific deficits depend on the task (3). Damage to the cortex around the central sulcus, or lesion of its output at the internal capsule, leads to paralysis or paresis of the contralateral hand, but the motor function of the ipsilateral hand is differentially affected by lesions in the left or the right hemisphere: Left hemispheric lesions result in motor dysfunction of the ipsilateral (left) hand, whereas lesions on the right side leave the motor function of the ipsilateral (right) hand relatively unaffected (2). However, little attention has been paid to the handedness of subjects whose motor functions have been studied after cortical brain damage (2, 3). We assessed the possible asymmetry of activation in the right and left motor cortex and tried to determine the relations, if any, between this activation and handedness.

Fifteen healthy human subjects (4) participated in the study; ten were right-handed (five women and five men) and five were left-handed (one woman and four men) (5). Subjects lay supine in a 4-T nuclear magnetic resonance (NMR) instrument (6). They were instructed to make repetitive opposition movements of the thumb on each of the remaining four fingers and to exert moderate pressure at each contact while NMR images of each hemisphere were acquired before, during, and after the task (Fig. 1) (7). For each hemisphere, the task was performed successively by each hand in random order. The surface area of activation within the precentral gyrus of each hemisphere during the movement of each hand was calculated (8), and differences in this area between the activation obtained during movements of the contralateral and ipsilateral fingers were determined (9). The ratio of the mean area of contralateral/ipsilateral (C/I) activation was also calculated. This study extends previous work on functional activation of the motor cortex that was done with high-field NMR (10).

There was a hemispheric asymmetry in the functional activation of the motor cortex during contralateral and ipsilateral movements, especially in right-handed subjects (Figs. 2 and 3) (11). Thus, whereas the right motor cortex was activated mostly during contralateral finger movements in both right-handed (C/I = 36.8) and left-handed (C/I = 29.9) subjects, the left motor cortex was activated substantially during ipsilateral movements in left-handed subjects (C/I = 5.4) and even more so in right-handed subjects (C/I = 1.3).

The involvement of the left motor cortex in ipsilateral hand movements could explain the well-documented significant deficits produced by left, but not right, hemispheric lesions on the motor performance of the ipsilateral hand (2, 3). In general, some activation of the human mo-

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Fig. 1. Experimental design for task performance and data acquisition.

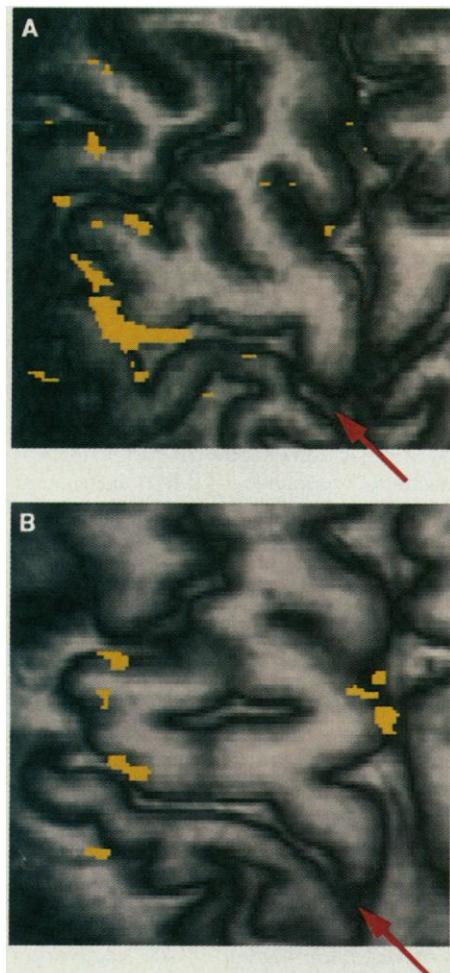


Fig. 2. Functional activation of the left motor cortex during right (contralateral) finger movements in (A) a left-handed and (B) a right-handed subject; the area activated (yellow) is greater in the left-handed subject. The red arrow indicates the central sulcus; medial is to the right and anterior is at the top.

tor cortex during ipsilateral movements could be expected on the basis of anatomic evidence that about 10 to 15% of fibers in the lateral cortical spinal tracts of humans are uncrossed (12). Functional activation studies of the sensorimotor cortex with positron emission tomography (PET) during ipsilateral movements in healthy subjects have detected either changes during movements of the arm but not hand (13) or no changes at all (14). However, a PET study on patients with hemiplegia (15) documented a functional activation during ipsilateral movements performed with the affected hand, but no ipsilateral activation

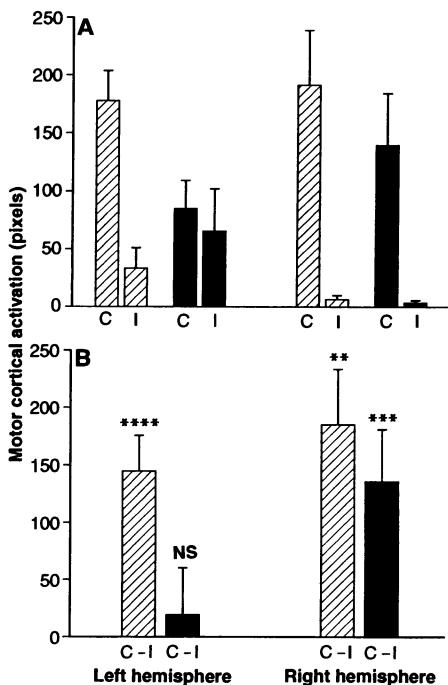


Fig. 3. (A) Motor cortical activation during contralateral (C) and ipsilateral (I) finger movements. Bars are mean \pm SEM. For left-handed (hatched) and right-handed (solid) subjects, $n = 5$ and 10 , respectively. (B) Quantitative differences and their statistical testing between contralateral and ipsilateral (C - I) activation. Significance levels of paired t tests: **, $P < 0.02$; ***, $P < 0.015$; ****, $P < 0.009$; NS, not significant.

was found during movements made by the normal hand. The present finding that the hemispheric asymmetry observed seems to be more pronounced in right-handed subjects underscores the complexity of the interplay of these factors even at the level of the precentral gyrus.

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4. Informed consent was obtained from the subjects according to a protocol approved by the University of Minnesota Institutional Review Committee. The mean age (\pm SD) of the subjects was 31.6 ± 6.5 years.
5. All subjects completed the Edinburgh Inventory for handedness [R. C. Oldfield, *Neuropsychologia* **9**, 97 (1971)].
6. All experiments were performed on a 4-T whole

body system [SIS Co. (Sunnyvale, CA)/Siemens (Erlangen, Germany)] with actively shielded gradient coils. The home-built MR radio-frequency (rf) antenna was an anatomically fitted, parallel two-loop surface coil (14 cm by 18 cm). The rf power deposition was kept below the Food and Drug Administration specific absorption rate guidelines. The head of each subject was restrained.

7. To locate the precentral gyrus, we acquired multislice axial images weighted with longitudinal relaxation time (T_1) with a refocused, fast, low-angle shot (FLASH) pulse sequence [A. Haase, J. Frahm, W. Hanicke, K.-D. Merboldt, *J. Magn. Reson.* **67**, 257 (1986)] based on a magnetization-prepared, rapid gradient-recalled echo sequence [echo time (TE) = 7 ms, repetition time (TR) = 13 ms, and 0.5-cm slice thickness] with 128 phase-encoding steps segmented in four blocks of 32 interleaved steps. From multislice anatomic axial images, an oblique plane between axial and sagittal planes was separately defined for each hemisphere along the central sulcus for images weighted with the apparent transverse relaxation time (T_2^*); in the oblique plane, the precentral gyrus was located anterior to the central sulcus. The oblique plane was verified by multislice oblique anatomic images. The frequency of the movements was 1.5 Hz. This and the time to start and stop the movements were indicated by a light-emitting diode placed in the magnet in front of the subject. All subjects performed successively with each hand while data were collected from each hemisphere; the serial order of the hemispheres and the hands within a hemisphere were randomized. Data pertaining to the functional activation of the motor cortex were collected before, during, and after the task as single slices. Consecutive single-slice, gradient-echo, oblique images were acquired with a single scan using a T_2^* -weighted, refocused FLASH pulse sequence; typically 30 images were collected consecutively (5.2 s each): 10 before, 10 during, and 10 after task performance. Typical acquisition parameters for T_2^* -weighted imaging: TE = 35 ms, TR = 49 ms, interscan delay = 2 s, flip angle = 16° , slice thickness = 1.2 cm, and total acquisition time = 5.2 s. The 128 complex pairs in the readout domain and 64 phase-encoding steps were recorded in a 16 cm by 13 cm field of view (FOV), that is, the in-plane pixel resolution was 1.3 mm by 2.0 mm. The phase encoding steps were centrally reordered to minimize inflow effects. The acquired data were zero-filled to 256×256 and then Fourier-transformed. All subsequent analysis, including the functional maps, had 256×256 pixel points in a 16 cm by 13 cm FOV.

8. For every pixel, two means (\pm SD) were computed: one from the images acquired during the performance of the task ("task" mean, $n = 10$ images) and another from pooled images acquired before and after task performance (7) ("baseline" mean); for this latter mean, 16 images were used (eight immediately preceding task performance and the last eight of the post-task period). The pre- and post-task images were pooled to account for a possible drift in the baseline unrelated to the task; we did not use the first two images of the post-task period to avoid possible contamination of the baseline with task effects extending beyond the end of the task period. A Student's t test was then performed ($P < 0.0001$) to assess whether there was a statistically significant change of the signal during the task as compared with the baseline. A binary, functional activation map was then constructed consisting of only those pixels that showed such a statistically significant change. We overlaid this map on a T_1 -weighted anatomic image to locate the activation site. The area of activation used for comparisons between conditions was the number of the activated pixels located within the precentral gyrus. The activated area was well localized, as described previously (10). Possible artifacts caused by movement of the head induce spatially interleaved positive and negative alterations in image intensity and usually cover large areas,

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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11. These effects cannot be accounted for by the sex of the subjects: There were no statistically significant differences in these effects between men

- and women within the right- or left-handed subjects (*t* test, $P > 0.2$ for all comparisons). Asymmetrical hemispheric activation during contralateral movement has also been documented using Xe¹³³ inhalation [J. H. Hasley, U. W. Blauenstein, E. M. Wilson, E. H. Wills, *Neurology* **29**, 21 (1979)].
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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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