Central Synaptic Inputs to Identified Leech Neurons Determined by Peripheral Targets

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Developing Retzius (Rz) neurons in different segments of the central nervous system of the medicinal leech have different peripheral targets: Rz cells in standard segments innervate the body wall, whereas Rz cells in the reproductive segments innervate reproductive tissue. Early removal of reproductive tissue primordia causes reproductive Rz cells to develop morphologically like their standard segmental homologs, suggesting that Rz cells depend on peripheral targets for signals that determine their central and peripheral morphology. Furthermore, after removal of reproductive tissue, reproductive Rz cells also receive synaptic inputs normally appropriate for standard Rz cells. These results suggest that the functional identity of these neurons is specified by the target they contact during embryogenesis.

THE DEVELOPMENT OF APPROPRIATE function by a neuron may depend on many external influences, among which is the neuron's target. Selection of the correct target can ensure a neuron's survival (*1*), and may also allow it to acquire characteristics that are essential for its appropriate function (*2, 3*). We showed earlier that morphological features of certain identified neurons in the leech are determined in part by contact with a specific target during embryogenesis (*3*). We now report that these neurons require the same specific target to receive appropriate synaptic inputs.

A pair of serotonergic Retzius (Rz) cells is found in each segment of the leech central nervous system (Fig. 1A). Rz cells in most segments innervate the body wall of their own and adjacent segments (*4–6*). These neurons modulate the activity of other neurons, glands, and muscles by releasing serotonin (*7, 8*); they receive synaptic inputs from identified cutaneous mechanosensory neurons and from neurons that generate the swimming motor program (*8–10*). In contrast, Rz cells in segments 5 and 6 [Rz(5,6)] innervate the reproductive tissue, which is found only in those segments (*5, 9, 11*); these neurons do not receive the same synaptic inputs as their standard segmental homologs (*9, 10*). Although there are a number of morphological and physiological differences among mature Rz cells, all Rz cells early in their development have similar central and peripheral projections (*9, 11*). Rz(5,6) become different from their segmental homologs only after contact with their target, the reproductive tissue (*5, 9, 11*). Removal of reproductive tissue early in embryogenesis causes Rz(5,6) to develop a central arbor like that of standard Rz cells and to innervate the body wall (*3, 6*). We show here that, after such ablations, the synaptic inputs to Rz(5,6) also develop in a manner appropriate for standard Rz cells. These results suggest that the functional identity of these neurons is specified by contact with their peripheral target during embryogenesis.

Neuronal cell bodies cluster to form ganglia in the anterior segments of the leech embryo during the seventh day of development (*12*), and loose clumps of primordial reproductive tissue are also first apparent at this time (*5*). Rz cells initiate neurite outgrowth during the eighth day of embryogenesis. We removed reproductive tissue primordia from leech embryos early in the tenth day of development, the time when Rz cell processes first exit the ganglion (*3, 5*). Sham operations were performed in other embryos by removing similar amounts of tissue near the reproductive tissue. After the experimental and control leeches developed to a juvenile stage (*13*), they were dissected and prepared for electrophysiological recording (Fig. 1B).

In juvenile leeches, standard Rz cells responded to a number of stimuli in the same way as they do in the nervous system of adult leeches. Cutaneous stimulation (prodding or pinching the tail) caused excitation of these neurons (56 of 56 cells in 25 indviduals) (Fig. 2A). Often, especially in response to strong mechanical stimuli, swimming was elicited; in such cases, the Rz cells remained excited throughout the episode of swimming (37 of 37 cells in 15 individuals) (Fig. 2B). Standard Rz cells also received excitatory synaptic input from the P cells, mechanosensory neurons responsive to pressure on the skin (16 of 17 cells in 11 individuals) (Fig. 2C). Each Rz cell received similar input from each of the four P cells in its own ganglion. As in adults (*10*), this input facilitated strongly; a single action potential in the P cell usually did not cause an excitatory postsynaptic potential (EPSP). Therefore, we always tested for this connection with depolarizations producing pairs of action potentials in the P cell, and we looked for an EPSP in the Rz cell after the second P cell action potential.

In normal untreated animals, Rz(5,6) responded very differently to these mechanical stimuli. Prodding or pinching the tail did not excite Rz(5,6) (0 of 12 cells excited in nine individuals) (Fig. 2D); repeated stimulation sometimes resulted in inhibition. Eliciting swimming also had an inhibitory or no effect on these neurons (0 of 6 cells excited in five individuals) (Fig. 2E), and stimulating P cells was likewise ineffective in exciting

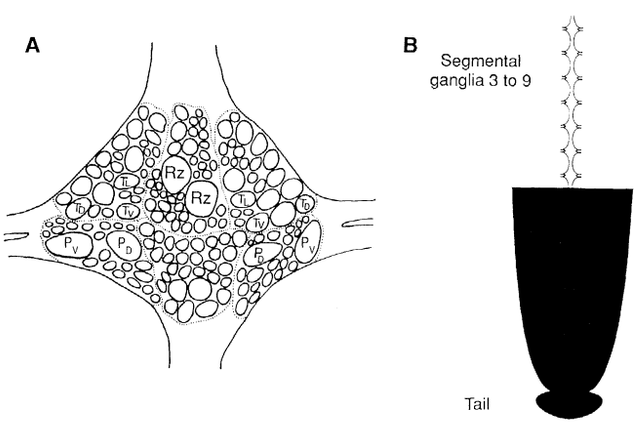

Fig. 1. (**A**) Schematic drawing of the ventral surface of a standard leech ganglion. Some of the neurons of interest are indicated by abbreviations: Rz, Retzius cells; P_V and P_D, ventral and dorsal pressure mechanosensory neurons, respectively; and T_L, T_V, and T_D, lateral, ventral, and dorsal touch mechanosensory neurons, respectively. (**B**) Schematic drawing of a partially dissected leech preparation. Ganglia 3 to 9 have been exposed; the anterior brain and ganglia 1 and 2 have been removed, and connections to the tail brain have been severed to make swimming easier to elicit. The body of the leech was pinned down in the denervated segment 10; posterior to segment 10, the leech was free to move in the recording chamber. The tail could be stimulated by pinching it with forceps, and it was observed for swimming and other behaviors. Rz, P, and T cells were identified on the basis of their size, position, and distinctive action potential wave forms (*18, 19*). Standard techniques for recording and stimulating were used (*19*), except as indicated. Ganglia were viewed under Nomarski optics on the stationary stage of a Zeiss standard microscope; recordings were made with 0.1*M* potassium acetate–filled microelectrodes with resistances of approximately 100 megohms.

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Rz(5,6) (0 of 7 cells excited in six individuals) (Fig. 2F). As with standard Rz cells, these observations on Rz(5,6) in juveniles were identical to those in adult leeches (*10*).

In contrast, when recordings were made in animals from which the reproductive tissue had been removed, Rz(5,6) received synaptic inputs that were qualitatively appropriate for standard Rz cells. Rz(5,6) were now excited by prodding or pinching the tail (12 of 17 cells in eight individuals), although not always sufficiently to elicit action potentials (Fig. 3A). Furthermore, Rz(5,6) in these experimental animals were sometimes excited during episodes of swimming (5 of 11 cells in six individuals) (Fig. 3B) and also received EPSPs from P cells (11 of 13 cells in eight individuals) (Fig. 3C). Rz(5,6) in control animals receiving sham operations were no different from those in untreated controls. They were not excited by tail stimulation (0 of 10 cells in four individuals), by episodes of swimming (0 of 5 cells in three individuals), or by P cell stimulation (0 of 8 cells in four individuals) (Fig. 3, D to F).

We tested for the presence of nonspecific, aberrant connections to Rz cells in some of the experimental animals by stimulating other identified cutaneous mechanosensory neurons, the touch (T) cells. Neither standard Rz cells nor Rz(5,6) normally received synaptic input from the T cells. None of the Rz(5,6) tested in experimental animals received input from T cell stimulation (0 of 5 cells in three individuals), although all of these Rz cells received EPSPs in response to P cell stimulation. Thus, ablating the reproductive tissue did not cause connections to the Rz cells to be made indiscriminately.

The excitatory connections made to Rz(5,6) after ablation were less reliable and seemed to be weaker than those normally made to standard Rz cells. Most cells received excitatory input from some combination of tail pinch, swimming, or P cell firing, but no hierarchy of connectivity was apparent. These results suggest that the excitatory inputs to Rz cells constitute separate pathways that can be influenced independently of one another. It is possible that the manipulations cause changes by modifying neurons in the pathway to the Rz cells. Ganglia 5 and 6 contain many additional neurons that are not found in standard ganglia (*14*). These neurons, which arise postembryonically, do not appear after early ablation of male reproducitve tissue (*15*). The absence of these extra neurons cannot, however, explain our results because the normal segment-specific pattern of synaptic connectivity of Rz(5,6) and standard Rz cells is apparent as early as 15 days of embryogenesis (*16*); long before the extra neurons appear. In experimental animals, Rz(5,6) cells probably play an active role in developing their new pattern of connectivity, since they grow additional neuropilar processes after ablation of reproductive tissue (*3*). These new processes may now contact presynaptic neurons normally appropriate for standard Rz cells, or postsynaptic sites may be modified, for example, by the addition, substitution, or loss of particular neurotransmitter receptors.

The mechanism for the target-dependent specification of reproductive Rz cells is still unknown. It is clear, however, that both the structure and function of a Rz cell are affected by the target it contacts during embryogenesis. Such interactions may be important in the appropriate development of neurons in many, if not all, nervous systems (*17*).

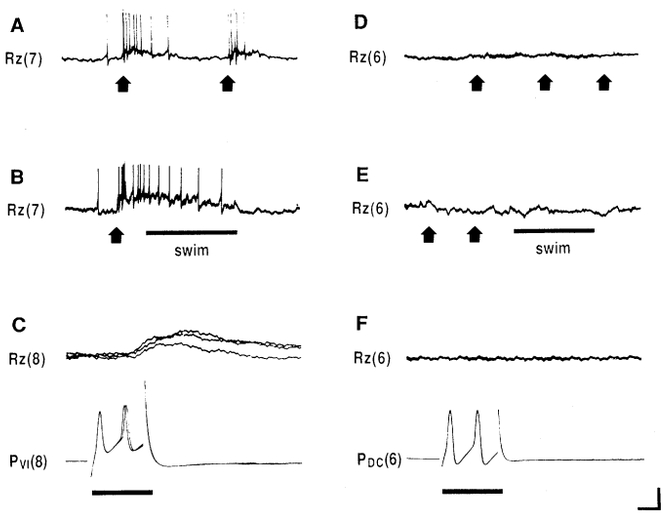

Fig. 2. Standard Rz cells (A to C) received excitatory inputs lacking in Rz(5,6) cells (D to F). Common time and voltage calibration bars are shown at the lower right. (**A**) Rz(7) excited by tail pinches, indicated by arrows (10 mV, 1 s). (**B**) Rz(7) depolarized during a swimming episode. A tail pinch occurred at the arrow; the duration of swimming is indicated by the bar (10 m V, 1 s). (**C**) Stimulation of a ventral ipsilateral P cell in ganglion 8 excites Rz(8). Three sweeps are superimposed. The P cell was depolarized to give two action potentials; the bar indicates the duration of the depolarizing pulse. Large transients at the beginning and end of depolarization are capacitative artifacts (Rz, 2 mV; P, 20 mV; 20 ms). (**D**) Rz(6) is unaffected by tail pinches, indicated by arrows (5 mV, 1 s). (**E**) Rz(6) is unaffected or slightly inhibited just before and during swimming (2 mV, 2 s). (**F**) Rz(6) does not receive an EPSP from P(6) (Rz, 2 mV; P, 20 mV; 20 ms). Subscripts of P cells identify type (ventral or dorsal) and location (ipsilateral or contralateral) of the P cell.

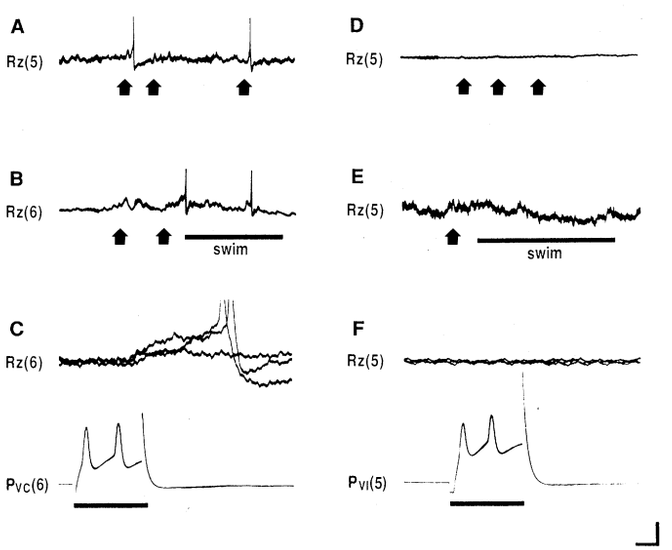

Fig. 3. Responses of Rz(5,6) in animals with reproductive tissue ablated (A to C) and animals receiving sham operations (D to F). Common time and voltage calibration bars are shown at the lower right. (**A**) Rz(5) receives excitatory input after tail pinches, indicated by arrows (5 mV, 1 s). (**B**) Rz(6) is depolarized before and during a swimming episode. Tail pinches occurred at the arrows; the duration of swimming is indicated by the bar (10 mV, 1 s). (**C**) Rz(6) excited by P(6) stimulation. Three sweeps are superimposed; in each of two sweeps, the Rz cell fired an action potential (the tops are clipped in the recording). The P cell was depolarized to give two action potentials; the bar indicates the duration of the depolarizing pulse. Large transients at the beginning and end of depolarization are capacitative artifacts (Rz, 2 mV; P, 20 mV; 20 ms). (**D**) In a sham-operated control, Rz(5) is unaffected by tail pinches, indicated by arrows (5 mV, 1 s). (**E**) Rz(5) is slightly inhibited during swimming (2 mV, 2 s). (**F**) Rz(5) does not receive an EPSP from P(5) (Rz, 2 mV; P, 20 mV; 20 ms). Subscripts of P cells identify type (ventral or dorsal) and location (ipsilateral or contralateral) of the P cell.

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elk, Tissue-Specific *ets*-Related Genes on Chromosomes X and 14 near Translocation Breakpoints

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The *myb-ets*–containing acute leukemia virus, E26, transforms myeloblasts and erythroblasts in culture and causes a mixed erythroid and myeloid leukemia in chicks. Genes (*ets*-1, *ets*-2, and *erg*) with variable relatedness to the v-*ets* oncogene of the E26 virus have been identified, cloned, and characterized in several species. Two new members (*elk*-1 and *elk*-2) of the *ets* oncogene superfamily have now been identified. Nucleotide sequence analysis of the *elk*-1 cDNA clone revealed that this gene encodes a 428-residue protein whose predicted amino acid sequence showed 82% similarity to the 3′ region of v-*ets*. The *elk* or related sequences appear to be transcriptionally active in testis and lung. The *elk* cDNA probe detects two loci in the human genome, *elk*-1 and *elk*-2, which map to chromosome regions Xp11.2 and 14q32.3, respectively. These loci are near the translocation breakpoint seen in the t(X;18) (p11.2;q11.2), which is characteristic of synovial sarcoma, and the chromosome 14q32 breakpoints seen in ataxia telangiectasia and other T cell malignancies. This suggests the possibility that rearrangements of *elk* loci may be involved in pathogenesis of certain tumors.

E26 IS A REPLICATION-DEFECTIVE retrovirus whose genome harbors two oncogenes, v-*myb* and v-*ets* (*1, 2*). E26 causes erythroblastosis and myeloblastosis in chickens and transforms myeloblasts, erythroblasts, and quail embryo fibroblasts in culture (*3–5*). Beug *et al.* (*6*) have shown that the v-*myb* domain of E26 causes myeloblast transformation and that v-*ets* is responsible for erythroblast and fibroblast transformation. The v-*myb* and v-*ets* oncogenes are expressed as a 135-kD polyprotein (p135 *gag-myb-ets*) that is localized in the nucleus of transformed cells (*7–9*) and exhibits DNA binding activity (*10*). Sequences homologous to v-*ets* are present in the genome of organisms widely separated phylogenetically, namely, chicken [Ck-c *ets*-1 and -2 (*11–13*)], mouse [M-*ets*-2 (*14*)], human [Hu-c *ets*-1 and -2, as well as *erg* (*14–18*)], sea urchin [Su(LV)-*ets*-2 (*19*)] and *Drosophila* [D-*ets*-2 (*20*)]. In humans, *ets*-1 and *ets*-2 and *erg* have been mapped near chromosome regions (*1, 18, 21*) that are involved in translocations characteristic of certain leukemias and lymphomas (*22–25*). In this study, we report the molecular cloning, chromosomal localization, and expression of two *ets*-like genes *elk*-1 and *elk*-2.

A COLO 320 cDNA library in the λ gt10 vector (*17, 18*) was screened with Hu-*ets*-2 cDNA (*26*). A clone designated λ 11 was found that contained *elk*-1 sequences (Fig. 1A). *elk*-1 cDNA, which is derived from the *elk*-1 locus, detects a closely related unlinked gene, *elk*-2. The *elk*-1 cDNA clone is ~2.9 kb in length. A computer analysis of the nucleotide sequence revealed that the 5′ region of *elk*-1 (nucleotides 306 to 603) showed ~67% homology with only the 3′ region (nucleotides 2044 to 2334) of the v-*ets* oncogene. Thus, the *elk*-1 cDNA identified a single domain of similarity with the v-*ets* oncogene: this is unlike Hu-*ets*-2 and *erg*, which have two v-*ets*–related domains. The deduced amino acid sequence of *elk*-1 cDNA is ~82% homologous in its 5′ region with the 3′ end of v-*ets* (Fig. 1B). The longest open reading frame in *elk*-1, starting with a methionine codon at position 316 in the nucleotide sequence (Fig. 1A), could encode a 428–amino acid polypeptide with an estimated relative molecular mass of ~45 kD. Since a stop codon is present in the same frame farther upstream (Fig. 1A), the ATG at position 316 may be used as the initiation codon. The initiation codon at residue 316 is preceded by another initiation codon (nucleotide 115) in the same reading frame upstream of the termination codon (nucleotide 277) that precedes the long open reading frame. Thus, a potential polypeptide composed of 54 amino acids could also be synthesized from *elk*-1 mRNA in the bicistronic manner proposed for some eukaryotic mRNAs. A similar phenomenon was observed in *erg*-1 and *erg*-2 mRNAs (*17, 18*). There are four in-frame ATGs located in the 5′ portion of the sequence, one of which essentially conforms to the Kozak consensus sequence (*27*).

The deduced amino acid sequence of the *elk*-1 polypeptide shows that it has a primary length of 428 amino acids and is rich in proline (62 residues), serine (44 residues), leucine (42 residues), glycine (40 residues), and alanine (37 residues). It bears one potential glycosylation site at amino acid position 51. A plot of hydropathicity values, as determined by the method of Kyte and

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