the observation by HEBBLE investigators of a high-velocity, southward-flowing current deeper than 4000 m with an increased velocity below 4500 m. The highest inferred velocity occurs at approximately 4900 m (Fig. 2), where the highest current velocity in the HEBBLE area was 72 cm/sec (*9*, *10*) and the greatest light-scattering in the nepheloid layer is found (*6*). The width of the high-velocity core of the WBUC is approximately 300 km (Fig. 1). The eastern margin of the WBUC core occurs on the abyssal plain at water depths of 5100 to 5400 m and is characterized by a transition in silt mean values similar to that observed on the western margin. The existence of two such well-defined margins may be due to the long residence time of the WBUC core in this channel or to the meandering of the WBUC high-velocity core within this zone during the time represented by the geologic samples. Since our velocity parameters indicate that the margins are quite abrupt, we suggest that the high-velocity WBUC has flowed in a narrow (300 km) channeled area between 4440 and 5200 m for the time represented in our samples (~ 100 to 200 years).

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16. Most samples were taken within 4 cm of the core top of large-diameter gravity cores taken aboard the R.V. *Eastward*. The remaining samples were taken within 4 cm of the core top of trigger-weight and gravity cores from existing collections. No attempt was made to verify that core tops represent recent sediments since we are interested in detecting conditions that persist on the present sea floor. Naturally, sediment beneath the high-velocity WBUC will be a lag deposit containing older, reworked material, but the presence of this material indicates the present hydrodynamic environment. Cores shown on Fig. 1 but not on Fig. 2 were analyzed for particle size, but the data are not shown since those cores are near large slump deposits (*15*).
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18. The noncarbonate fraction in the silt size range from 4 to 8 ϕ or 62 to 4 μm is isolated, and the mean size is determined by analysis of the particle size distribution with an Elzone (Particle Data, Inc.) electronic sensing instrument [see (*13*) and (*17*) for analytical methods]. The particle size analysis is restricted to the noncarbonate fraction in order to eliminate the effects of carbonate dissolution on the size distribution, so that only effects produced by the bottom current will prevail. The silt size range only is analyzed since those sizes are most sensitive to winnowing by bottom currents with a velocity common in the modern ocean.
19. One determines the magnetic grain long-axis alignment by measuring the anisotropy of magnetic susceptibility (AMS) on a torsion fiber magnetometer. A standardized AMS parameter, F_s, is sensitive to the efficiency of long-axis alignment [see (*13*) and (*17*) for analytical methods]. More efficient alignment is interpreted as evidence of increased bottom-current velocity.
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10 September 1981; revised 26 October 1981

Survival and Differentiation of Identified Embryonic Neurons in the Absence of Their Target Muscles

Abstract. *Although prevented from contacting their target muscles, identified limb-innervating neurons in grasshopper embryos survive and differentiate to maturity.*

The survival and differentiation of motoneurons and autonomic neurons in vertebrates are influenced decisively by the cells their axons contact during development; deprived of their target muscles, these neurons usually die (*1*). Central neurons in insects are arranged in ganglia, each of which corresponds to and innervates a particular body segment. Thoracic segments have limbs and their ganglia contain limb-innervating neurons; these cells are absent from ganglia in the abdominal segments, which lack limbs. During embryogenesis, motoneurons in each segment send axons to the periphery to innervate developing muscles, and shortly thereafter many neurons die (*2*, *3*). The mechanisms that regulate the survival and differentiation of these insect neurons are unknown. Here we report experiments in which limb buds were removed from insect embryos early in development. Our results show that two identified insect neurons need not contact their target muscle in order to survive and differentiate.

We removed limb buds from early embryos of the grasshoppers *Locusta migratoria* and *Schistocerca americana*. For our experiments in *Locusta* we chose the largest limb motoneuron, the fast extensor tibia (Feti) (*4*), which innervates the extensor tibia muscle of the metathoracic leg. Since Feti sends an axon to only one leg, we removed the metathoracic leg on one side and used the homologous cell on the unoperated side as a control. For our experiments in *Schistocerca* we chose the largest modulatory neuron, the dorsal unpaired median extensor tibia (Dumeti) (*5*), which also innervates the extensor tibia muscle. Since the axon of Dumeti bifurcates and extends into both metathoracic legs, we removed these legs and used the homologous cell from unoperated animals as a control. Both Feti and Dumeti can be recognized in normal embryos by the large size and position of the cell bodies and by their axonal and dendritic morphology (Figs. 1 and 2).

In experiments with *Locusta*, the limb buds were removed from embryos within the egg (*6*). The damage caused by the operation varied. In some cases proximal fragments of the leg remained, and these animals were later rejected. In others the leg was completely removed (serial sections of animals 2 to 3 days after the operation confirmed that no limb muscles were present on the operated side). Sixty-two successful operations were performed; in 25 the limb bud was removed at or before the appearance of

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the Feti neuron (25 percent of embryo development) (*7, 8*) and in 37 the bud was removed between this stage and the time axons first enter the limb (*7*) (25 to 30 percent of development).

In experiments with *Schistocerca*, limb buds were removed from embryos that were taken out of their egg cases and allowed to continue development in hanging drop cultures (*9*) through a modification of Mueller's method (*10*), such that the embryos develop in vitro from 40 percent of development to hatching (*11*). Limb buds were removed at 40 percent of development or at 50 percent. At 40 percent, the growth cones of Dumeti are well within the central nervous system, while at 50 percent they are just leaving the central nervous system but still are far from the limb bud and target muscle (*12*). At least six successfully operated and cultured embryos were assayed at each stage along with four cultured controls.

In normal locusts the Feti neuron can be identified by its large cell body (*13*) after 55 percent of embryonic development. The cell body is visualized in the living ganglion by Nomarski optics (Fig. 1) or by staining with toluidine blue (*14*).

In every operated animal examined between 55 percent of development and adulthood, the Feti neuron was present and was of normal size, both on the operated (N = 62) and the control (N = 44) sides of the segment (Fig. 1, a and c). The axonal and dendritic anatomy of the Feti neuron was determined by intracellular injection of the fluorescent dye Lucifer yellow (*12*) or by cobalt backfilling of peripheral nerves (*15*). In every experimental and control Feti observed (between 65 percent of development and second instar), the axon made its characteristic turn within the ganglion (N = 32 and 6 for the operated and control sides, respectively) and the cell produced a normal dendritic tree (N = 14 and 6) (Fig. 1, b and d). Action potentials could be evoked from cells on both operated (N = 7) and control (N = 5) sides (insets in Fig. 1, b and d). However, the differentiation of Feti on the operated side was not completely normal. In 3 of 32 neurons assayed, the dendritic tree included an additional anterior branch that appeared to terminate in the metathoracic ganglion. Furthermore, the pattern of peripheral nerves on the operated side was disturbed, and, in some cases, the axon of Feti branched and followed more than one peripheral pathway. Although sometimes Feti branches over inappropriate muscles, we do not know if it succeeds in innervating them.

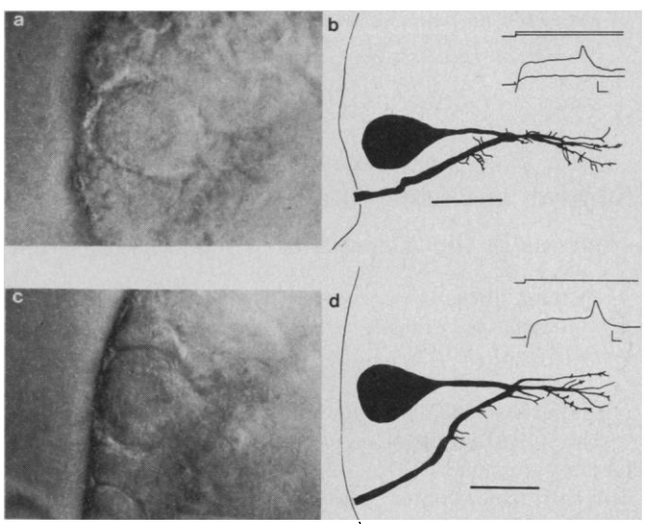

Fig. 1. Differentiation of the Feti neuron in the metathoracic ganglion of *Locusta migratoria* embryos following removal of the metathoracic limb bud at 25 to 30 percent of embryonic development. (a and c) Photomicrographs of the cell body on control (a) and operated (c) sides of the ganglion at 83 percent of development (×700). (b and d) Camera lucida drawings of Feti on control (b) and operated (d) sides of the ganglion. Cobalt was used to stain the cell traced in (b); Lucifer yellow was used in (d). Scale bar, 50 μm. Insets show intracellular recordings of action potentials from control and experimental cells at 90 percent of development. Horizontal bar, 5 msec; vertical bar, 1 nA (upper trace), 5 mV (lower trace).

The differentiation of Dumeti can be followed from the time of its formation from the median neuroblast to its maturation (*12*). In normal embryos its axons reach their targets by 60 percent of embryonic development; between 55 and 70 percent the cell follows a characteristic sequence of morphological and physiological differentiation (*12, 16*). In our embryo cultures Dumeti neurons generally held to this timetable of differentiation in both operated and unoperated insects (Fig. 2); however, the developmental stages in cultured embryos proceeded about 30 percent slower than in embyros left in ovo. The operated Dumeti neuron becomes electrically uncoupled and excitable between 55 and 60 percent of development with the normal distribution of Na^+ and Ca^{2+} currents in the soma and axons, its cell body greatly enlarges after 60 percent, and its dendrites branch in their characteristic manner. However, the differentiation of the Dumeti neuron in operated insects was not completely normal. The cell often retained more than one axon branch and followed more than one peripheral pathway. Furthermore, although its cell body enlarged greatly, it often was somewhat smaller than normal (and thus was only as large as what normally are the next largest progeny of the median neuroblast).

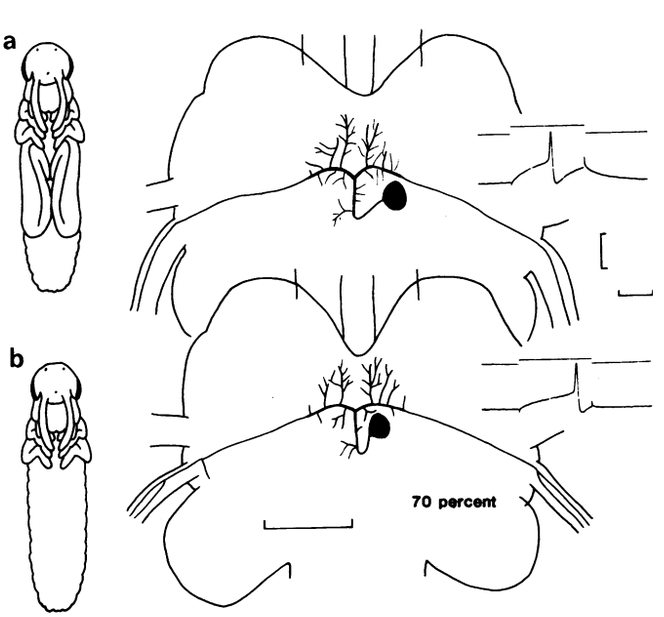

Fig. 2. Differentiation of the Dumeti neuron in the metathoracic ganglion of *Schistocerca americana* embryos grown outside of the egg case in hanging drop cultures from 43 to 70 percent of development. (a) Control embryo. (b) Embryo in which both metathoracic legs were removed at 43 percent. Lucifer yellow was used to stain both neurons (camera lucida drawings). Scale bar, 100 μm. Insets on right show intracellular recordings of action potentials. Horizontal bar, 20 msec; vertical bar, 1 nA (upper trace), 40 mV (lower trace).

In insects many different sensory neurons can differentiate in the absence of their central interneurons (*17*). Similarly,

we have found that when Feti and Dumeti neurons are prevented from reaching their target muscles, they still survive and differentiate. We conclude that specific instructions from the target muscle play little if any part in the development of these neurons, although we cannot exclude the possibility that contact with some muscle, albeit the wrong one, might be a general condition for their survival.

Neurons in the central nervous system of insects are generated by segmental sets of embryonic precursor cells (*18*). In each embryonic segment the set of precursors is similar, yet the final pattern and number of neurons are strikingly different. Segment-specific differences in cell survival have been described (*19*). For example, limb-innervating neurons homologous by cell lineage to Feti and Dumeti are produced in the abdominal segments, but they do not survive (*3*). Our experiments suggest that the differential survival of limb-innervating neurons is independent of the presence (in the thorax) or absence (in the abdomen) of limb muscles. The segment-specific pattern of muscles depends on the pattern of underlying ectoderm (*20*), but this determinative sequence appears not to extend to the nervous system through the muscles. Segment-specific neuron differentiation might be an intrinsic, inherited commitment of neurons, or it might be extrinsically controlled by their segmental environment. Our experiments do not distinguish between these alternatives. However, they rule out one of the possible extrinsic determinants, the target muscles.

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28 July 1981; revised 14 October 1981

Natural Antibodies to Human Retrovirus HTLV in a Cluster of Japanese Patients with Adult T Cell Leukemia

Abstract. *Human T cell lymphoma leukemia virus (HTLV) is a human retrovirus (RNA tumor virus) that was originally isolated from a few patients with leukemias or lymphomas involving mature T lymphocytes. Here we report that the serum of Japanese patients with adult T cell leukemia, but not the serum of tested normal donors, contains high titers of antibodies to HTLV. These observations, together with data from Japan showing that adult T cell leukemia is endemic in southwest Japan, suggest that HTLV is involved in a subtype of human T cell malignancy, including Japanese adult T cell leukemia.*

A human T cell lymphoma leukemia virus (HTLV) was previously isolated from cells of patients with cutaneous T cell lymphoma and leukemia (CTCL) (*1, 2*) and shown to be unrelated to other retroviruses by nucleic acid hybridization studies (*3*) and by immunologic studies of its major core protein, p24 (*4*), and reverse transcriptase (*5*). HTLV is an acquired, not endogenous, retrovirus as shown by the presence of proviral sequences in DNA from the neoplastic T cells of the patient from whom HTLV was first isolated and the absence of such sequences both in B cells of the same patient (*6*) and in other sources of DNA from normal people (*3*). The previous reports of natural antibodies to HTLV proteins in the serum of some patients with CTCL (*7, 8*) and the absence of similar antibodies in the serum of numerous randomly selected normal donors are consistent with the interpretation that HTLV is acquired by infection and suggest a possible etiologic role of HTLV with human T cell malignancies.

In studying the association of HTLV with human T cell neoplasias we have recently focused on a documented clustering of Japanese patients with adult T cell leukemia (ATL). This leukemia, a subtype of T cell malignancy, occurs with high frequency and in striking clusters on the islands of Kyushu and Shikoku in southwestern Japan (*9, 10*). Among its unique clinical features are adult onset, frequent skin involvement, and common organ involvement. The leukemic cells have convoluted nuclei and ultrastructurally are similar to a small cell variant form of Sézary syndrome, another malignancy of mature T cells (*11*). Adult T cell leukemia differs from classical Sézary T cell leukemia in that people with ATL show no typical Sézary cells, no leukemic cells in the epidermis despite intradermal or subcutaneous infiltration, an infiltration of leukemic cells into the bone marrow, and a shorter survival time (*9*). Nevertheless, because similarities exist between ATL and Sézary syndrome, the leukemic form of CTCL, and because the geographic clustering of Japanese ATL patients also suggests a possible viral etiology, we examined the serum of Japanese patients with lymphoid malignancies for the presence of natural antibodies to HTLV.

Serum samples were surveyed for HTLV-specific antibodies by means of both an indirect membrane immune fluorescence assay and a solid-phase radioimmunoassay (RIA). The latter is one of the methods we previously used to de-

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