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11. Although the timing of the insulin cycle as a signal to the insulin-sensitive peripheral tissues is compatible with insulin-mediated acceleration and deceleration of glucose transport contributing to the plasma glucose cycle, the longer time constants associated with transcapillary and interstitial diffusion of insulin in the periphery would serve to damp an insulin-mediated peripheral cycle. It is also likely that different degrees of damping will be observed among various insulin-sensitive pathways depending on their individual time constants. For example, insulin stimulation of protein synthesis would probably display very low amplitude oscillation compared to insulin-mediated glucose transport in muscle and compared to inhibition of lipolysis in adipose tissue. In support of the conclusion that total glucose utilization is relatively constant, the downstroke phase of the plasma glucose cycle closely fits a single exponential function with a fractional rate ($k = -0.017$ per minute) that approximates 75 percent of the rate of total body glucose utilization determined with [3-$^3H_1$]glucose ($k = -0.023$ per minute).
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Synapses Between Neurons Regenerate Accurately After Destruction of Ensheathing Glial Cells in the Leech

Abstract. *Individual glial cells that ensheathe axons in the central nervous system of the leech were destroyed by intracellular injection of protease. The axons were then severed, and regeneration by particular neurons was studied physiologically and morphologically. Although certain axons sprouted more in the absence of the glial cell, functional synapses were accurately regenerated with normal frequency.*

It has been proposed that the glial cells that surround neurons in vertebrates and invertebrates play a variety of roles in nerve regeneration. For example, glia may guide regenerating axons in the central nervous system (CNS) of lower vertebrates (*1*) and in the peripheral nervous system of all vertebrates (*2*). On the other hand, glia may interfere with axon growth in the mammalian CNS (*3*), where regeneration occurs only to a limited extent. Invertebrate systems are particularly useful in the study of nerve regeneration, since the regeneration is accurate and can be examined at the level of individual cells (*4*). However, the involvement of glia in regeneration in invertebrate systems has not been established.

The CNS of the leech contains large glial cells and hence offers an opportunity to test directly the role of glia in nerve regeneration. After nerve injury in the leech CNS, the glial cell sheath survives; the glia are thus candidates for guiding regenerating axons (*5*). In the present study, we destroyed individual glial cells in the CNS of the leech *Hirudo medicinalis* by intracellular injection of a protease and observed the regeneration of axons and synapses in the absence of the ensheathing glial cell. While the growth pattern of regenerating axons was atypical in some cases, the axons still reestablished correct synaptic connections.

The leech CNS consists of a chain of ganglia which contain nerve cell bodies and synaptic neuropil and which are linked by three axon bundles called connectives. Each lateral connective, containing several thousand axons, is ensheathed between any two ganglia by a single glial cell several millimeters long. The medial connective, containing about 100 axons, is usually ensheathed along part of its length by one of the two connective glial cells and along the remainder of its length by the other glial cell.

One or both of the connective glial cells were killed by pressure injection into the cell of protease (Subtilopeptidase A, type VIII, Sigma; 6 mg/ml in 0.2*M* KCl containing 0.2 percent fast green FCF) through beveled recording electrodes (*6*). By observing the coloration of the glial cell, we could control the injection and destroy the glial cell without measurably affecting the axons it surrounded (Fig. 1, A and B). Destruction of the glial cell was verified by light microscopy of 2-μm-thick sections and by electron microscopy of ultrathin sections (*5, 7*). Axon health was monitored by physiological tests of impulse conduction and by electron microscopy. Glial cell killing resulted in an increase in the number of microglia (Fig. 1B), macrophage-like cells which exist in the normal leech nerve cord (*8*) and in greater numbers in the injured nerve cord (*5*). As judged from their appearance under the electron microscope, these cells participated in the removal of glial debris and in time ensheathed portions of some axons (Fig. 1C). In general, protease-injected glia were gone after 1 week, at which time the axons of the connectives were severed near one ganglion by crushing the correctives with fine forceps (*5, 7*). At various times during the ensuing 24 weeks the preparations were dissected from the animal and examined for regeneration (*5, 7*).

Regeneration by interneurons and mechanosensory neurons was studied. The S cell, an interneuron that occurs singly in each ganglion, projects an anterior and a posterior axon in the medial connective. Each S axon forms an electrical synapse near its tip midway along the connective with the axon of the S cell in the adjacent ganglion (*7, 9*). The two pressure (P) and two nociceptive (N) mechanosensory neurons on each side of every ganglion project axons in the ipsilateral connectives and form chemical synapses with motor neurons in neighboring ganglia (*4*). With glia present, both the S axon and the sensory cell axons can regenerate after a lesion of the connectives and reestablish correct synaptic connections (*7, 10*).

To test physiologically for regeneration of the S axon in the absence of glia, we recorded simultaneously with intracellular electrodes from the axotomized S cell and its target S cell in the adjacent ganglion. Synapse regeneration, evidenced by electrical coupling between the two S cells (Fig. 2A), occurred in all 12 preparations tested 4 weeks or more after injury. This is comparable to the frequency of S cell regeneration found previously in the presence of the glial sheath (*7*).

The physiological test for regeneration of P and N cells involved stimulating the cell body in the ganglion anterior to the injected glial cell and recording from the ipsilateral L motor neuron in the adjacent posterior ganglion. The L cell innervates longitudinal muscle and is one of the normal synaptic targets of P and N cells. Successful regeneration, evidenced by a unitary synaptic potential in

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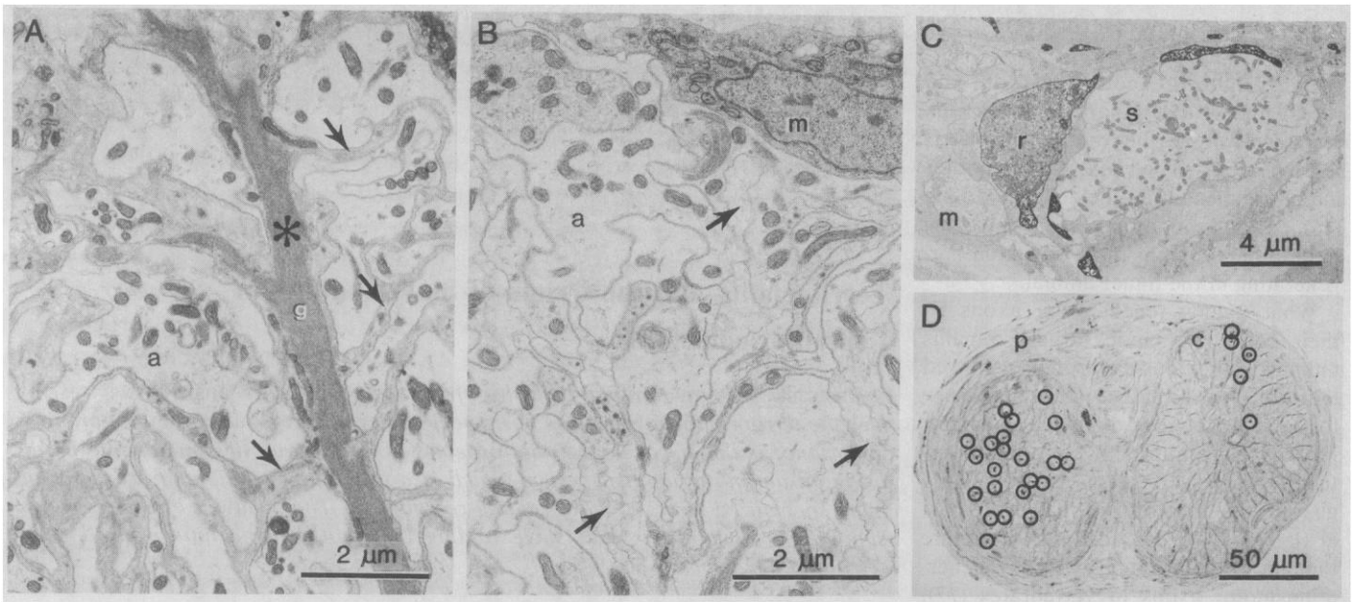

Fig. 1. (A and B) Electron micrographs of a cross section through the lateral connectives of a leech nerve cord. (A) Uninjected (control) lateral connective with glial cell (*g*) intact. The glial cell contains characteristic electron-dense bundles of intermediate filaments (asterisk) and ensheathes axons (*a*) with a branching network of processes (arrows). (B) Lateral connective 1 week after the glial cell was injected with protease. Some glial debris is still present (arrows). A microglial cell (*m*) is also visible. The axons (*a*) appear morphologically normal. (C) Electron micrograph of a cross section through the medial connective 5 weeks after the ensheathing glial cell was killed and 4 weeks after the axons were severed. The regenerating S axon (*r*) has been injected with horseradish peroxidase, and a number of sprouts can be seen. Physiological tests showed that the S axon had established synaptic contact with its normal target, the S cell of the adjacent ganglion. The severed distal stump of the S axon (*s*) remains intact. A microglial cell is also present. (D) Light micrograph of a cross section through the nerve cord 7 weeks after one connective glial cell (at the left) was killed and 8 weeks after the axons were severed. The horseradish peroxidase–stained regenerating processes of one P cell on the protease-injected side (*p*) and another on the control side (*c*) are circled. Sprouts are more numerous and more widely distributed on the protease-injected side.

the L cell evoked at fixed latency by an action potential in the P or N cell, occurred for 15 of 60 axons with glial sheaths and for 10 of 64 axons without glial sheaths (Fig. 2B). The difference between these two groups is not significant.

Next, the preparations were examined morphologically. Horseradish peroxidase was pressure-injected into the bodies of the regenerating cells and, in some cases, into the bodies of the target cells. The enzyme was allowed to diffuse for 12 to 60 hours before fixation and staining with diaminobenzidine (*7, 11*). The growth pattern of the S axon remained essentially unchanged in the absence of the glial cell. The regenerating S axon grew in a normal fashion (Fig. 1C), with sprouts concentrated in the dorsal quadrant of the medial connective, and stopped within 1 month at the normal site of synapse with the adjacent target S axon. In contrast, regenerating sensory axons whose glial sheath had been destroyed sprouted more and the sprouts were more widely dispersed throughout the connective than those of regenerating sensory axons whose glial sheath remained intact (Fig. 1D). Although sensory axons normally sprout more extensively in response to injury than S axons, the cause of increased sprouting by severed sensory axons in the absence of the glial cell is unknown. Removal of the glial cell may remove a physical constraint to the growth of sensory axons. Another possibility is that degeneration of the killed glial cell induces sprouting. Whatever the cause, the increased sprouting of sensory axons did not impair their regenerative ability.

The isolated portion of a severed S axon, referred to as the distal stump, survives normally for months after injury (*7, 9*). In the absence of a glial sheath, distal stumps of S cells also survived for long times (Fig. 1C). Thus, while metabolic support of distal axon stumps by glia might occur in other invertebrates (*12*), in the leech the large glial cells are not required for S axon stump survival. Sensory axon distal stumps can survive for months in the peripheral nerves (*13*) and for at least 1 month in the connectives (*14*), but it is not known whether

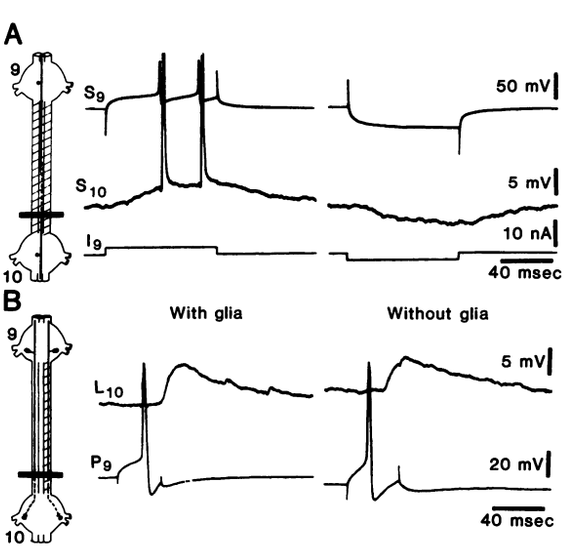

Fig. 2. Records showing the regeneration of correct synaptic connections by axons whose glial sheath was destroyed. At the left are schematic representations of the preparations. Glial destruction is indicated by hatching and regenerated axons by dotted lines. (A) Successful S cell regeneration, evidenced by electrical coupling between the severed, regenerating S cell in ganglion 10 (S_{10}) and its synaptic target, S_9. The lesion was made 31 days earlier and was preceded by the killing of both connective glial cells. (B) Records showing that two P neurons, one on the control side and the other on the protease-treated side, have successfully regenerated synaptic connections with L neurons 8 weeks after injury. The excitatory synaptic potentials recorded from the L neurons following action potentials in the regenerated P neurons appear normal.

sensory cell stumps can survive without an ensheathing glial cell.

Our results are consistent with the recent finding that synaptic connections are selectively formed between isolated leech neurons placed next to one another in culture medium in the absence of glia (*15*). Such conditions are, however, quite different from those for axons regenerating in the intact animal.

What guides regenerating axons to their correct synaptic targets remains unknown. Previous experiments showed that the synaptic target is not necessary for correct growth of the S cell axon (*16*). Moreover, as in the CNS of other invertebrates and vertebrates, neurons and glia in the leech CNS have no basal lamina, an extracellular structure thought to direct the growth of regenerating motor axons in vertebrates (*17*). One possible guide for axon growth in the leech is the distal axon stump. The regenerating S axon can grow along its severed stump and can form electrical synapses with it (*7, 9*). The increased sprouting of some regenerating axons after the loss of glia suggests that glia guide or restrict axon growth. However, as our experiments show, the ensheathing glial cell is not required for accurate regeneration of axons and synapses in the leech CNS.

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Territoriality and the Origin of Slave Raiding in Leptothoracine Ants

Abstract. *The slave-raiding behavior of* Harpagoxenus canadensis *closely resembles the territorial behavior of its host species,* Leptothorax muscorum. *Of primary importance is the discovery that both species of ants recruit nest mates into battle using an alarm-recruitment system which is a probable evolutionary precursor of more specialized forms of slave-raiding recruitment. The behavior of these species supports the hypothesis that slave raiding in leptothoracine ants evolved from territorial behavior.*

Slave-making ants are social parasites which supplement the labor force in their colonies by raiding other ant nests. Typically, the slave makers capture brood which is reared in their colonies to produce slave workers. Slavery is best known and most dramatic in its obligatory form, but less specialized forms of facultative intra- and interspecific slavery also occur (*1–3*). The evolutionary origin of ant slavery has been debated since the time of Darwin. However, slavery has evolved independently in several ant genera belonging to at least two subfamilies (*3*). Hence, a variety of evolutionary explanations may be necessary.

Among leptothoracine ants, two hypotheses have been advanced to explain the evolution of slave raiding. The territorial hypothesis maintains that slave raiding evolved from territorial fighting and opportunistic brood predation among colonies of closely related species (*2*). The alternative transport hypothesis contends that slave raiding evolved from brood transport among nests within colonies of a nonparasitic polydomous (multiple nests) and polygynous (multiple queens) species (*4*). Observations of the slave-raiding behavior of *Harpagoxenus canadensis* and of the territorial behavior of its host species, *Leptothorax muscorum*, as described in this report, support the territorial hypothesis.

The four species of the ant genus *Harpagoxenus* are morphologically well adapted for fighting. They are larger than their *Leptothorax* host species, with disproportionately larger heads and mandibles. In addition, inscribed on the dorsal surface of their heads is a pair of longitudinal grooves (scrobes) into which the antennal scapes can be folded for protection during fights. Both *H. americanus* and *H. sublaevis* have been shown to be obligatory slave makers (*5*), but *H. zaisanicus* and *H. canadensis* have been considered slave makers only by analogy and because they occur in mixed colonies with *Leptothorax* workers (*6, 7*). We collected colonies of *H. canadensis* and *L. muscorum* in northwestern Quebec. Interactions between *H. canadensis* and *L. muscorum* colonies ($N = 10$) and between pairs of *L. muscorum* colonies ($N = 6$) were observed in laboratory arenas (*8*).

During *H. canadensis* slave raids, members of opposing colonies were mutually antagonistic, with both slave makers and slaves being involved in the fighting. Even when outnumbered 2 to 1, slave-maker colonies consistently overwhelmed target colonies, raided their nests, and appropriated their broods. Workers and reproductives eclosed from captured brood. The workers remained as functional colony members (slaves), but the reproductives soon left in apparent attempts to conduct mating flights.

Interactions between *L. muscorum* colonies also always involved fighting. In four of six replicate experiments, the larger colony overran the smaller colony's nest and transported the captured brood back to its own nest, where it was mutilated and eaten. Colonies in the other two experiments coexisted for the duration of the experiment (3 days), apparently because the smaller colony erected a barricade of debris in its nest entrance. These observations indicate that *L. muscorum* colonies are territorial and capable of conducting territorial raids and engaging in opportunistic brood predation. The territorial hypothesis postulates that these behaviors were characteristic of the ancestors of lepto-

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