NADPH (− NADPH) the amount of aminoparathion, determined by LSC of TLC isolates of "aminoparathion areas," increased up to 30 minutes, and remained the same thereafter. In experiments with NADPH-fortified incubation mixtures (Table 1 and +NADPH in Fig. 2) addition of the cofactor resulted in a marked increase in aminoparathion formation, which reached its peak after 60 minutes of incubation. At that time, three times more aminoparathion had been produced with NADPH as compared to those assays, to which no coenzyme had been added. After the first 60 minutes of incubation, the amount of aminoparathion recovered from the hexane fraction dropped sharply, while the radioactivity in the total water-acetone fraction increased constantly (Fig. 2). Although the radioactivity in this fraction was not only due to the degradation of aminoparathion, there was indication that the aminoparathion had been metabolized into water-soluble products. The water fraction—obtained from the 120- and 180-minute assays—were combined and reextracted with chloroform. This removed over 50 percent of the radioactivity from the water. The extracted radiocarbon content in the chloroform was then resolved by TLC and autoradiography. LSC of isolated areas indicated that only 7 percent of the totally recovered radioactivity was located in the area that corresponded to aminoparathion, but 23 and 22 percent were located in those areas that corresponded to *p*-nitrophenol and *p*-aminophenol, respectively. Of the totally recovered radioactivity, 40 percent was located in the parathion area, and 8 percent in the paraoxon area.

In separate tests NADPH-fortified incubation mixtures were extracted after different incubation times. Analyses by GLC of the hexane fraction showed the presence of 1.35 μg of aminoparathion per incubation mixture after 15 minutes of incubation, 5.75 μg after 30 minutes, 7.1 μg after 60 minutes, 5.6 μg after 120 minutes, and 0.9 μg after 180 minutes.

Since oxygen is required for the functioning of oxidative enzymes, the activity of the nitroreductase should not be dependent on the presence of oxygen. To prove this point, triplicate assays were conducted with and without atmospheric oxygen (or a very low partial pressure of oxygen) in the 10-ml Erlenmeyer flasks. This was accomplished by bubbling nitrogen for 2 minutes through the assay mixture (10,000*g* supernatant as the enzyme source) and immediate sealing of the flasks with four layers of Saran wrap. Controls were conducted with incubation mixtures that had not been purged with nitrogen and were kept in open Erlenmeyer flasks. After incubation and extraction, the formation of aminoparathion was confirmed by the described test procedures. Results obtained by GLC of the hexane phase showed that oxygen was not required for the nitroreductase activity; the amount of aminoparathion produced in the absence of atmospheric oxygen was 11.17 ± 0.52 μg per incubation mixture and 9.80 ± 0.96 μg in its presence. Differences observed under the two experimental conditions are not significant.

To investigate the effects of some enzyme inhibitors, duplicate tests were conducted in which sesamex or SKF 525-A—a known inhibitor of microsomal enzymes—were added to the regular incubation mixture at $5 \times 10^{-4}M$ as described (*6*). Results showed that, in the presence of these chemicals, the amount of aminoparathion produced per incubation mixture was only 45 ± 1 percent (with sesamex) and 63 ± 0 percent (with SKF 525-A) of the amount that was produced in control tests.

No protein measurements of the various subcellular fractions were performed to determine specific enzyme activity. However, based on the amounts of aminoparathion produced, differences in enzyme activity were evident in assays conducted at different dates with one fly strain, and also in assays conducted simultaneously with all three strains. Both the CSMA and P_2/sel strain were a good source for the nitroreductase, whereas the F58-W strain was a poor source.

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Neural Regeneration: Delayed Formation of Central Contacts by Insect Sensory Cells

Abstract. *Correlated anatomical and electrophysiological results demonstrate that sensory neurons, which differentiate de novo within the epidermis of regenerate abdominal cerci of crickets, enter the terminal ganglion and form functional central connections even when regeneration of the cerci is delayed through the greater part of postembryonic development. Stimulation of regenerate cerci evokes activity in giant interneurons which is normal by several physiological criteria.*

Paired cerci, which form sensory appendages on the 11th abdominal segment of orthopteroid insects, are exceptionally well developed in crickets (Fig. 1A). They are clothed with numerous sensory hairs (Fig. 1C) and other mechanoreceptors. Cell bodies of sensory neurons situated in the cercal epidermis send dendrites to the sensilla, and axons which travel via the purely sensory cercal nerve to the terminal abdominal ganglion of the central nervous system (Fig. 1D) (*1*). There they contact collaterals of giant interneurons which send axons anteriorly through the paired connectives of the ventral nerve cord (*2*) (Fig. 1G).

As with insect appendages in general, new abdominal cerci regenerate after amputation in immature stages (instars), growing vigorously and developing all components of normal cerci within several instars. They are repeatedly formed when regenerates are amputated following each molt. Regeneration of functional cerci can thus be prevented during any desired sequence of instars, after which regeneration may be allowed to proceed.

Their cell bodies having been re-

moved with an amputated cercus, axons in the cercal nerve degenerate within several days (*3*). When cercal regeneration is repeatedly interrupted, the proximal stump of the cercal nerve withers until no trace of it is evident on the surface of the terminal ganglion (Fig. 1F). Thus the regeneration of functional connections by sensory fibers from a delayed regenerate requires that the axons reach the terminal ganglion, penetrate its sheath, and then recognize appropriate interneurons upon which to terminate. The prolonged absence of cercal afferents has no significant effect on the growth of the giant interneurons as judged by the cytological appearance of the cell bodies or the diameter of their major processes, the giant axons (Fig. 1G).

The regenerative capacity of the cerci suggested their suitability for the study of central connections formed by sensory fibers which arise de novo, rather than regenerating from preexisting sensory cells as is the rule in comparable experiments on vertebrates. This report demonstrates that axons originating in regenerate cerci do form functional contacts with giant interneurons even after prolonged absence of cercal input to the central nervous system.

Abdominal cerci were amputated from newly hatched first instar larvae of the house cricket *Acheta domesticus*. Regenerates were similarly removed following each subsequent molt until the seventh, or antepenultimate, instar, a period of 7 to 8 weeks at 30°C. Cerci were then allowed to regenerate during the seventh, eighth, and ninth instars, so that the adult animals bore cerci up to one-third the normal length. Sensilla of the regenerates appeared to be qualitatively similar to the normal. Cerci, nerves, terminal ganglia, and connectives containing the giant axons of regenerate animals were examined by light- and electron-microscopic techniques and compared with tissue from normal animals.

The technique for electrophysiological recordings was designed to be as repeatable as possible, so that differences among individual animals could be recognized. Each of the two connectives immediately anterior to the terminal ganglion was lifted onto a pair of silver electrodes and surrounded with petroleum jelly, which provided both insulation and protection against desiccation. The responses recorded in

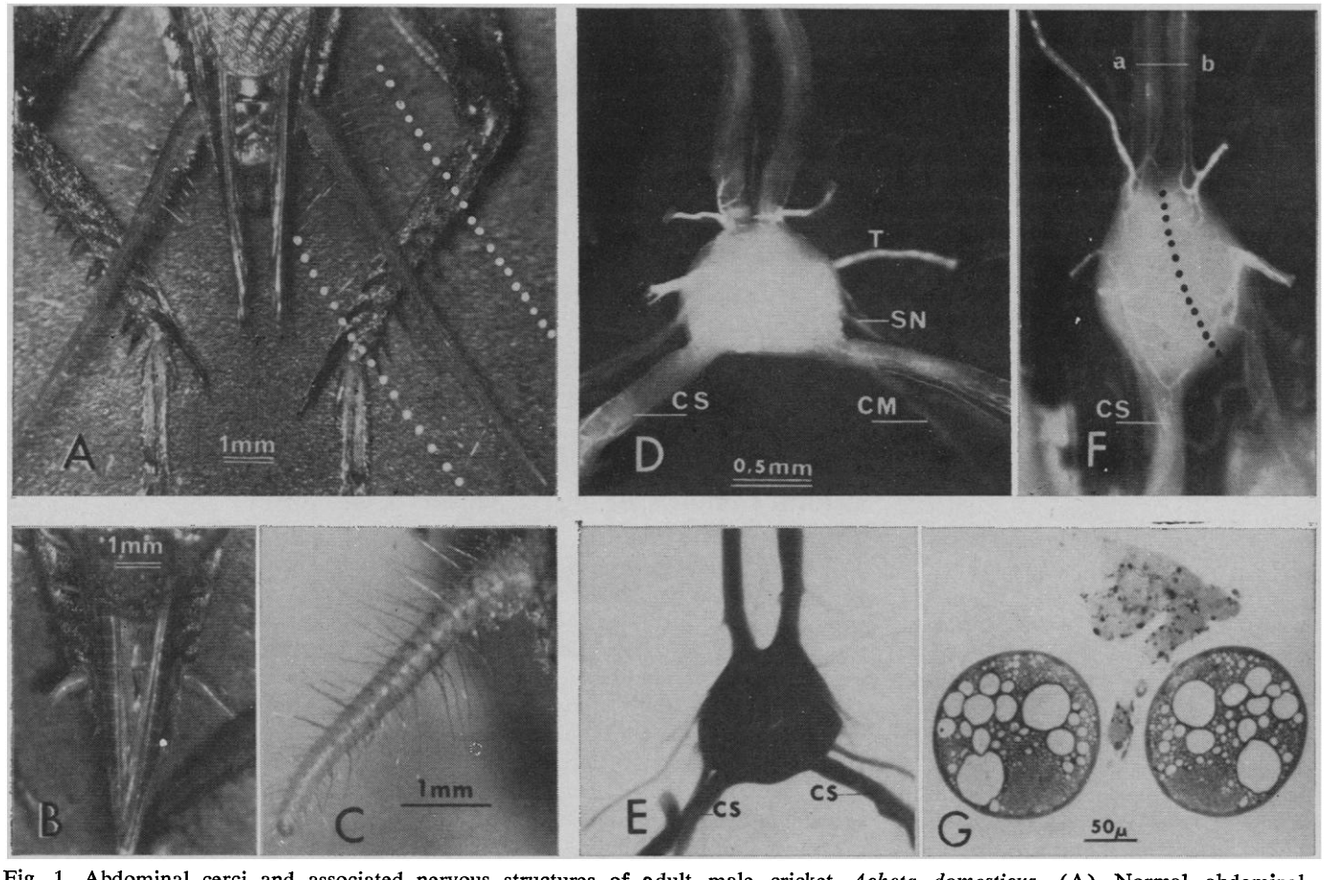

Fig. 1. Abdominal cerci and associated nervous structures of adult male cricket *Acheta domesticus*. (A) Normal abdominal cerci. Dotted lines delimit tips of longest sensory hairs, some of which are visible near the base. (B) Regenerate cerci. The left cercus is a typical single instar regenerate; that on the right is a second instar regenerate on which some sensory hairs are present but not visible in the photograph. Scale as in A. (C) Detail of third instar regenerate showing numerous long mobile sensory hairs (trichoid sensilla). (D) Normal terminal ganglion and associated nerves. Cercal sensory nerves (*CS*) enter the ganglion at its posterior angles, lower left and right. A cercal motor nerve (*CM*) innervating extrinsic cercal musculature is visible at right. The nerves (*SN*) are segmental and genital mixed nerves. Paired ventral nerve cord connectives leave the ganglion above. Tracheal trunks (*T*) enter the ganglion anteriorly and laterally. Fresh preparation. (E) Terminal ganglion with paired third instar regenerate cercal nerves from cerci comparable to that shown in Fig. 1C. Labeling as in Fig. 1D. Material fixed in osmium. (F) Terminal ganglion of cricket raised without right cercus. Note absence of right cercal sensory nerve and significant atrophy with consequent distortion of the ganglion denoted by line marking the anatomical midline. Labeling as in Fig. 1D. Scales of Fig. 1, D–F, are identical. (G) Cross section of ventral nerve cord shown in Fig. 1F. Note near symmetry of giant interneuron numbers and area despite asymmetry of ganglion due to absence of cercal afferents on right side.

this way were stable for many hours; some animals have yielded records for several days. The records were assumed to originate from the population of giant fibers (Fig. 1G), a group of axons which appear strikingly uniform in size and position when examined histologically.

Recordings from normal animals show units with ongoing spike activity, which can be either accelerated or inhibited by appropriate sensory input. Other units are normally silent, and their spikes are added as new components to the multiunit recording in response to defined stimuli. The properties of units with purely evoked responses are the best understood and were tested most rigorously in regenerate animals (*4*).

Spikes of large amplitude (3 to 5 mv in the best preparations) are elicited by stimuli such as air puffs and tone pulses. These cells receive strictly ipsilateral excitatory and bilateral inhibitory inputs. In these experiments with regenerate animals, the presence of the crossed inhibitory component was inferred from the enhancement of the response after removal of the contralateral cercus. The ipsilateral inhibitory component was not examined.

In most preparations there is a well-defined population of ongoing spikes with an amplitude of about 1.5 mv and a frequency of 25 spikes per second, and a heterogeneous population having amplitudes of 1 mv and less. For the present we make only the following points. The activity of these neurons is ascending and reflects cercal input. If both of the cerci are removed, the frequency of 1.5-mv spikes in both connectives falls to a very low (though usually not zero) level. In contrast to the evoked response described above, both the excitatory and the inhibitory inputs are bilateral.

Figure 2 shows recordings taken from a cricket which had been deprived of all cercal input for the first six instars and had been permitted to develop cerci for only the last three instars. Despite this severe interference with normal development, the physiological responses were virtually indistinguishable from those of normal animals. The large units were normally silent, but were readily excited by air puffs and tones. Their response was sustained to strong stimuli, but phasic when the stimulus was weak. The enhanced response occurring in the left connective after removal of the right cercus was an indication of crossed inhibition. Ongoing activity was present, and while both cerci were present there was a fairly well-defined set of spikes with an amplitude one-third as great as that of the evoked response. This was reduced more or less symmetrically by removal of one cercus, indicating bilateral excitatory input.

Up to this time we have tested 13 experimental animals for the large-spiked evoked response. It has been present in all cases in which regenerate cerci were present (12 out of 13); it was lacking in the one case in which cercal regeneration did not occur. Clearly, axons from sensory hairs found their way to the interneurons which form our assay for the quality of synaptic regeneration. The excitatory input was exclusively ipsilateral in the nine cases in which regeneration was bilateral. In three of these cases crossed inhibition was tested systematically with tone pulse stimulation, and was present as in normal animals.

Recordings were made from two animals which were raised with a normal cercus on one side and no trace of a regenerate on the other (Fig. 1F). In both cases a very weak evoked response was present on the side lacking a cercus; this response disappeared when the normal cercus was removed during the experiment. Apparently some axons from the single cercus entered the ganglion and crossed the midline to form functional excitatory connections with giant neurons on the opposite side. These would not have been formed during normal symmetric development or regeneration (*5*).

All experimental animals with regenerated cerci showed the usual varieties of ongoing activity and the expected changes when cerci were removed during the course of recording. Ongoing activity in the one animal tested which lacked cerci throughout development was indistinguishable from activity in a normal animal both of whose cerci had been removed either during recording or several days earlier.

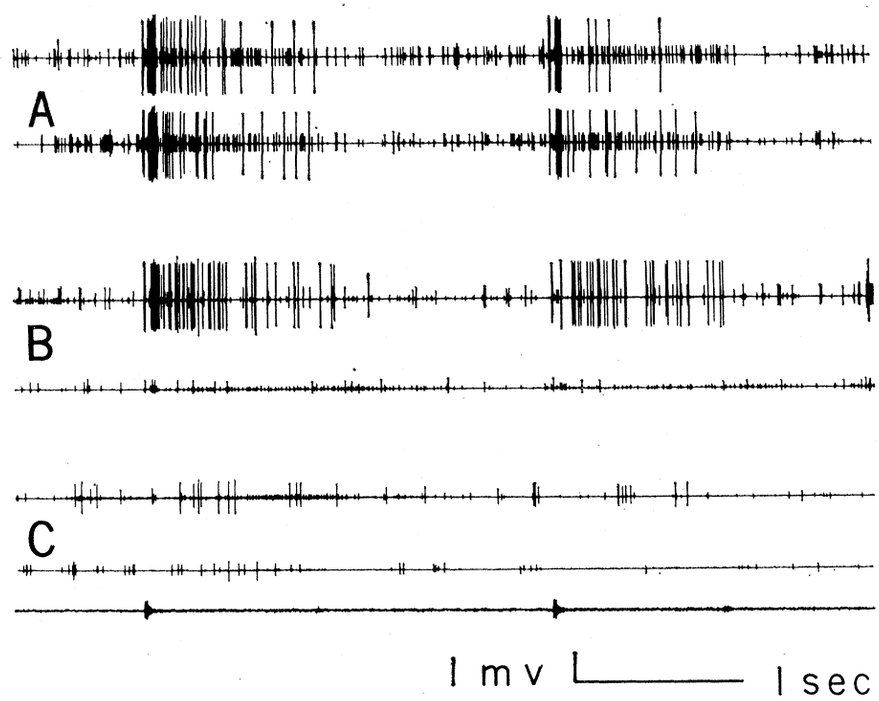

Fig. 2. Evoked and ongoing activity recorded in the ventral nerve cord of an adult male cricket. Cerci were removed at hatching, and subsequent regenerates were also removed until the seventh instar. Paired regenerates developed through the final three instars. In all cases, the upper trace was recorded from the left connective and the lower from the right; the bottommost trace is a stimulus monitor responding primarily to the sound of the solenoid valve controlling air flow. (A) Both cerci present. Responses to a strong, 1-second air puff evoked in both connectives. Ongoing activity conspicuous and approximately symmetric. (B) Right cercus removed. Response in right connective abolished; response in left connective enhanced, indicating release from inhibition. Ongoing activity much reduced in both connectives. (C) Left cercus removed. No trace of a response on either side. Ongoing activity further reduced.

We recognize two limitations on the interpretation of these results. First cercal nerve connections with the giant interneurons are established during embryonic development. The axons so formed, and removed at hatching in our experiments, number no more than 250 (*1*), but nevertheless do represent a short-lived projection of the sensory surface of the cerci upon the giant interneurons. Second, we cannot exclude the possibility that beyond the fifth instar small numbers of regenerate axons reach the ganglion from premolt regenerates within a given instar. But since the sensilla are ensheathed by cuticle, their neurons would not carry sensory information, and in any case their early amputation ensures that they would have degenerated long before subsequent afferent fibers could regenerate.

It should be emphasized that our results demonstrate only the correct connections made by the regenerate. We cannot yet exclude the possibility that the responses we have studied are mediated by only a small fraction of the contacts established by regenerating axons, and that wrong or random contacts have been made but not recognized. We expect more detailed physiological mapping and correlated degeneration studies to provide a more complete picture of the regeneration process.

Most of the growth of arthropod sensory systems occurs during the postembryonic period between hatching and the final molt to the adult stage. Our results indicate that interneurons can be deprived of their dominant sensory input for most of this period without degenerating or losing their capacity to reestablish correct connections with the sensory periphery. This stands in striking contrast to the dependence of mammalian sensory systems upon normal sensory inflow, especially during their early development (*6*).

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2. Anatomical and physiological aspects of the homologous system in the cockroach *Periplaneta americana* are described by K. D. Roeder [*J. Exp. Zool.* **108**, 243 (1948)], R. Farley and N. S. Milburn [*J. Insect Physiol.* **15**, 457 (1969)], and D. Dagan and I. Parnas [*J. Exp. Biol.* **52**, 313 (1970)]. J. S. Edwards and T. Sahota [*J. Exp. Zool.* **166**, 387 (1968)] demonstrated giant fiber activity evoked by regenerate cerci but did not compare the quality of the regenerate activity with the normal.
3. Orthograde degeneration in cercal sensory fibers is detectable within several hours of removal of a cercus. This contrasts with the prolonged survival of arthropod motor fibers [for example, R. Hoy, A. D. Bittner, D. Kennedy, *Science* **156**, 251 (1967)] and interneurons [P. S. Boulton and C. H. F. Rowell, *Z. Zellforsch. Mikroskop. Anat.* **101**, 119 (1969)].
4. A detailed analysis of the response properties of these neurons and other members of the giant fiber population is in preparation.
5. New cercal axons could, in principle, synapse on many interneurons in the terminal ganglion, including the contralateral giants. We saw no contralateral evoked responses in the nine cases of bilateral regeneration, but did see them in both cases of unilateral development. We take this to indicate a degree of specificity and bilateral interaction in the regeneration process. On the basis of evidence not detailed here, we believe that crossed inhibition is mediated by at least one interneuron interposed between the cercal axons and the giant fibers, so that its presence in three out of three symmetrical regenerates is a further indication of successful synapse formation.
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Culex (Melanoconion) aikenii: Natural Vector in Panama of Endemic Venezuelan Encephalitis

Abstract. *Experiments performed in an endemic area of Venezuelan equine encephalitis in the Panama Canal Zone demonstrated transmission of Venezuelan equine encephalitis virus from naturally infected* Culex aikenii *mosquitoes to laboratory hamsters. Results of experiments indicate that* Culex aikenii *is an efficient natural vector and the principal species of mosquito transmitting Venezuelan equine encephalitis in this endemic zone.*

Venezuelan equine encephalitis (VEE) is a disease of man and equines, widespread throughout Middle America, from Florida south to Ecuador and the Guianas. Two forms of the disease are known, an endemic one present in wet, lowland, coastal areas and the epidemic form which reportedly occurs in explosive outbreaks in drier areas of Central and northern South America and involves thousands of humans and equines. *Culex* mosquitoes of the subgenus *Melanoconion* have come under suspicion as vectors of VEE virus in several endemic areas of the Caribbean region (*1*). Evidence has been based mainly on the frequency of isolation of virus from laboratory mice inoculated directly with macerated wild-caught mosquitoes. However, proof of either natural or experimental transmission through the bites of these mosquitoes has been lacking. Therefore, no conclusive evidence has been produced on the vectorship of these or any other species of mosquitoes in the transmission of endemic VEE.

The discovery, through exposure of sentinel hamsters, of a focus of intense VEE activity in the Panama Canal Zone in August 1970 led to development of a project aimed at determining whether *Culex* (*Melanoconion*) mosquitoes were active in the transmission of VEE virus in that area. Work was carried out along the Chilibre River, which empties into the Chagres River about 500 m upstream from the area of Juan Mina, between Madden Dam and the Canal Zone town of Gamboa. All-night collections with human bait and sporadic hand-collections from sentinel hamsters showed that the most common mosquito biting man and hamsters during the study period was *Culex* (*Melanoconion*) *aikenii* (Aiken and Rowland), 1906 (*2*). This preliminary report presents results of experiments on VEE transmission with wild-caught *C. aikenii* and laboratory hamsters.

Female mosquitoes used in these experiments were captured singly while attempting to bite human subjects ex-

Table 1. Results of exposures of hamsters to the bites of wild-caught *C. aikenii*. Six hamsters were infected (+) with VEE; five were not (−).

Hamster No.	Mosquitoes (No.)		Infection
	Allowed to feed	Engorged	
LH-6555	1161	273	−
LH-6556	180	56	+
LH-6564	210	62	+
LH-6622	411	236	+
LH-6623	284	209	+
LH-6625	889	160	−
LH-6626	66	24	+
LH-6631	106	15	+
LH-6767	70	43	−
LH-6768	579	85	−
LH-6769	271	40	−