Development of Locomotor Patterns in the Absence of Peripheral Sense Organs and Muscles

(lobster/swimmeret motoneurons/invertebrate)

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ABSTRACT The role of peripheral sense organs and muscles in specifying the circuitry of the central nervous system during ontogeny was tested in larval lobsters. Presumptive locomotor appendages, the abdominal swimmerets, were extirpated before their differentiation. Electrophysiological recordings made 2-4 trophysiological developmental stage. of corresponding motor reflexes and normal patterns of rhythmic locomotor output appeared in the swimmeret motoneurons at the usual developmental stage. Therefore, target muscles and sense organs are unnecessary to the differentiation of normal motor output patterns in this simple invertebrate locomotor system.

The role of the periphery in determining the circuitry of the central nervous system has been studied and debated for nearly a century (1–5). According to one view, peripheral sense organs and muscles provide the central nervous system with essential information for specifying and modifying synaptic connections. Sensory feedback from movements in a developing motor system, for example, could help organize the corresponding central connections. Similarly, a muscle could, in principle, "instruct" its motoneurons to form appropriate central connections by means of biochemical "messages" conveyed centrally from the muscle—the well-known hypothesis of myotopic specification. Such hypotheses are significant not only for ontogeny and regeneration, but also for neuronal plasticity in general; if the periphery can indeed influence central circuitry, the underlying mechanisms could have implications for theories of learning and memory.

Evidence on the role of the periphery in establishing central circuitry consists largely of behavioral observations (3). Such evidence suggests that in some motor systems, at least, sensory input or feedback may not be necessary to the formation of normal movements during development (6-11) or after limb transplantation (12). Decisive tests using electrophysiological methods, however, are lacking. Myotopic specification seemed an attractive explanation for transplantation data (12-14), but more recent studies support alternative mechanisms (4, 15-17), and in any case a possible ontogenetic role for myotopic specification has not been studied previously.

The present study was undertaken to test the role of peripheral structures in specifying central nervous connections during the ontogeny of a simple locomotor system, the abdominal swimmerets of the lobster. This motor system was chosen because its neuronal organization is well understood in adults (18-29) and because it develops largely after larvae hatch from the egg (5, 30, 31). Swimmeret sense organs and muscles are undifferentiated at hatching, but the appendages are fully developed and capable of rhythmic locomotor movements 3 weeks later, when the larvae molt to the fourth larval stage (5). Thus it was possible to interfere with the normal developmental sequence by extirpation of presumptive swimmeret tissue, and to examine the effects on the subsequent differentiation of the locomotor output patterns by recording from the corresponding motoneurons.

MATERIALS AND METHODS

First-stage lobster larvae (Homoarus americanus) were collected within an hour of hatching at the Massachusetts State Lobster Hatchery, Martha's Vineyard, Mass. For extirpation of swimmeret limb buds, specimens were restrained upside down, transilluminated, and viewed with a binocular microscope. A micromanipulated heat filament, consisting of a thin (80-μm) nichrome wire attached to line voltage through a 12-V step-down transformer and a switch-controlled potentiometer, was briefly applied to the ventral limb bud and to the lateral abdomen immediately dorsal to the limb bud. This operation resulted in complete destruction of presumptive swimmeret tissue (Fig. 1). By methods detailed elsewhere (32), operated specimens were reared to or beyond the fourth larval stage, by which time the swimmerets are normally fully operational. Electrophysiological recordings were then made from the swimmeret nerves (the segmental first nerve roots of the abdominal ganglia) in normal and operated segments, using extracellular glass-capillary suction electrodes. Specimens used for histological studies were fixed in alcoholic Bouin's solution, embedded in paraffin, sectioned at 10 μm, and stained with Mallory's Triple. The extirpation operation was performed on 1200 larvae, of which 60 survived for use in these experiments.

RESULTS

Histological observations

Fig. 1A illustrates a late-season (July) lobster larva immediately after hatching. The swimmerets are represented by ventral limb buds and by presumptive muscle tissue in the lateral abdomen. Light and electron microscope studies have shown that swimmeret sense organs and muscle tissue are not yet differentiated at this stage of development (5). The segmental first nerve root, which innervates the ipsilateral swimmeret of the corresponding abdominal segment, is present when the larvae hatch, but differentiated neuromuscular
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Junctions are absent from presumptive swimmeret muscle tissue (5). Mature neuromuscular junctions are readily identified 2-3 days later; however, in the swimmeret muscle of late first-stage larvae (5).

Fig. 1B illustrates a normal fourth-stage larva, about 20 days after hatching. The swimmerets are now fully developed and used as the sole means of forward locomotion by the pelagic larvae. Fig. 1C shows a fourth-stage specimen whose swimmeret limb buds were unilaterally extirpated early in the first larval stage. The swimmeret on the operated side is completely absent. Fig. 1D illustrates that the extirpation operation did not cause the corresponding swimmeret nerve to degenerate; instead, the nerve invariably grew and terminated blindly in undifferentiated "scar" tissue in the immediate region of the operation. No evidence of regenerated swimmeret sense organs or muscles was detected in most operated animals that were examined histologically, including the specimens used in the electrophysiological experiments described below.

Electrophysiological observations

The above data demonstrate that the extirpation operation effectively prevented contact between the swimmeret nerve and its usual target sense organs and muscles. The role of these peripheral structures in differentiation of the central motor program could therefore be tested by comparison of extracellular recordings made from first roots in normal (control) and extirpated (experimental) abdominal segments in fourth-stage and older larvae. Fig. 2A shows a typical recording from a normal first nerve root during "voluntary" swimmeret beating, induced by directing a jet of water onto the anterior end of the animal. The motor output pattern shows features described previously in adults (20, 21, 26). Recordings from experimental motoneurons, i.e., motoneurons deprived of

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**Fig. 1.** Transverse sections of larval lobsters. (A) Early first stage, showing a swimmeret limb bud (lb) and lateral tissue (lt), destined to form the swimmeret appendage and muscles of the adult. (B) Normal fourth-stage larva, showing fully developed swimmerets. (C) Fourth-stage larva, showing the effect of unilateral extirpation of presumptive swimmeret tissue in the early first stage. (D) As in C but a different specimen, showing blind termination of the swimmeret nerve (first abdominal nerve root; arrow) within "scar" tissue in the immediate area of the extirpation. Scale, 250 μm in A and D, 500 μm in B and C.
Fig. 2. Extracellular recordings from the swimmeret nerves of sixth-stage larvae during "voluntary" swimmeret beating. (A) Normal (unoperated) specimen. (B) and (C) Presumptive swimmeret destroyed in the early first stage. P and R, power stroke and return stroke discharge, respectively, identified by visibly correlating the bursts with the movements of remaining normal appendages. The two traces in C are continuous. Time mark: 50 msec in A and B, 100 msec in C.

contact with peripheral sense organs and muscles by the extirpation operation, showed the same features, including: (1) rhythmic bursts of action potentials in the motoneurons; (2) concurrent bursts of impulses in synergistic motoneurons; and (3) alternating bursts in antagonistic motoneurons (Fig. 2, B and C). Moreover, experimental motoneurons made normal reflex connections in the central nervous system with afferent pathways from other swimmerets (Fig. 3, A and B) and with descending influences (Fig. 3C). It may be concluded that the development of normal patterns of locomotor output in the swimmeret motoneurons does not require the presence of differentiated swimmeret sense organs and target muscle.

Survival of larvae after extirpation of all eight presumptive swimmerets could not be obtained, and each experimental animal, therefore, had at least one remaining normal swimmeret. Contact between experimental first nerve roots and the remaining normal appendage(s) by way of a peripheral route is excluded by the histological data presented above. Central routing of afferent influences from the normal swimmeret(s) to experimental motoneurons is unlikely, for such influences are known to project posteriorly only one body segment (33). In many of the present experiments only a single anterior-most appendage remained on the second abdominal segment, and yet the posterior-most (experimental) motoneurons, located in the fifth abdominal segment, showed normal motor output patterns, including the absence of intersegmental reflexes initiated from the anterior-most appendage(s). Central routing of experimental motoneurons to

Fig. 3. Reflex and descending connections of motoneurons deprived of contact with the target appendage. (A) Superimposed traces showing response of an experimental swimmeret nerve to stimulation of the contralateral (normal) swimmeret nerve. (B) Reflex response of an experimental swimmeret nerve to pinching the rami of the contralateral (normal) appendage. (C) Response of swimmeret nerves on normal (N) and operated (O) sides of the same abdominal segment to mechanical stimulation of the head region. Arrow in C designates the approximate beginning of the stimulus in both B and C. Time mark: 2 msec in A, 20 msec in B, and 50 msec in C.
normal swimmerets, perhaps by means of central branches of the experimental motoneurons, is also unlikely, since 1:1 synchronization of impulses in experimental and normal swimmeret nerves was invariably absent (e.g., Fig. 4). These data indicate that experimental motoneurons were effectively isolated from the remaining normal appendage(s), as required by the experimental protocol. Indirect, long-distance influences from the normal appendage(s) to the experimental motoneurons cannot be excluded by the present experiments, but any such influences imply a much different mechanism from sensory and myotypic specification. Jacobson has, in fact, concluded that in vertebrates, at least, long-distance influences from the periphery to the central nervous system are unlikely (1).

DISCUSSION

The evidence presented here and elsewhere (5) demonstrates that the peripheral and central components of the lobster swimmeret system develop concurrently. Destruction of peripheral components of the system before their differentiation, however, did not alter the normal ontogenetic sequence or timetable of central components. Normal locomotor output patterns appeared at the usual time even though the corresponding motoneurons were deprived of sensory feedback from swimmeret sense organs, and even though the motoneurons were prevented from contacting differentiated swimmeret muscle. It follows that in this simple invertebrate locomotor system, at least, the correct differentiation of central nervous circuitry does not require direct information from peripheral target structures. Independence between the center and the periphery apparently characterizes not only the generation of adult motor output patterns (34), but also the development of specific central connections of motoneurons during ontogeny.

The generality of these findings cannot, of course, be judged until comparable experiments are performed on vertebrate species. The present study nevertheless supports the view that the ontogenetic information for specifying central circuitry of motor systems is contained largely, and perhaps entirely, within the central nervous system. In this case, further analysis of the developmental mechanisms underlying synaptic specificity in motor systems is likely to prove technically difficult at best.

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