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Postembryonic Development of Adult Motor Patterns in Crickets: A Neural Analysis

Abstract. *Adult crickets have stereotyped patterns of motor output which are generated by the central nervous system, and which serve as a standard against which emerging nymphal patterns can be measured. The neural circuits generating these patterns are not functional at hatching. The pattern elements appear in an ordered sequence over the course of the last four molts. The circuits are completely functional before the final molt. Circuits which might be prematurely activated are suppressed in the nymph by descending inhibition from the brain.*

Invertebrate behavior often reflects stereotyped patterns of motor neuron firing, called motor programs. The programs are generated by small groups of central neurons which can operate without phasic sensory feedback. Patterns of this type underlie locust flight, cricket stridulation, swimmeret beating in crayfish, and swimming and siphon withdrawal in mollusks (*1*). These patterns are so highly stereotyped in an individual, or between conspecifics, that they provide an excellent standard against which the activity of immature animals can be measured. In the case of stridulation, and presumably in all such programs, the patterns are not learned; the neural circuits generating them must be constructed during the course of development on the basis of genetically stored information. We have examined the appearance of two such motor programs during postembryonic growth, and have found that the circuits are not functional at the time of hatching from the egg, that elements of the pattern emerge in an ordered sequence over the course of the latter half of the nymphal life-span, that the circuits are complete before the final molt, and that completed circuits which might be accidentally activated are suppressed by descending inhibition.

The behavior patterns investigated were song production (stridulation) and flight in the field cricket *Teleogryllus commodus*. Both are exclusively adult behaviors, dependent upon the presence of the wings, which do not appear until the final molt. Furthermore, nymphs do not attempt to move their small wing pads (*2*), which are clearly visible, in the pattern appropriate for either behavior. This is particularly interesting in the case of aggressive stridulation because the nymphs do display other behaviors associated with aggressive interactions. Stereotyped motor programs underlie both behaviors; this was demonstrated first for locust flight, and later confirmed for cricket stridulation and flight (*3*).

We characterized nymphal behavior by recording action potentials with fine wires (20 μm) placed in identified muscles. Since the muscles are neurogenic (each nerve impulse produces a single muscle action potential), and since they are innervated by only one to three neurons, this procedure provides a precise monitor of identified motor neuron activity. The motor pattern of flight was elicited by activating the tarsal reflex (loss of contact with the substrate), and suspending the animal in a wind stream. Song patterns were released by making heat lesions in inhibitory areas (mushroom bodies) of the brain (*4*).

The crickets develop gradually into the adult form after nine, ten, or eleven molts (twelve stages including adult). Most individuals reach adulthood after ten molts. The number of molts an animal has completed can be determined by painting it, and repainting after each molt or, in a carefully controlled colony, by measuring skeletal size (we have done both). The structure of the wing pads, in late instar nymphs, indicates how many molts the animal will undergo before becoming an adult. In the case of song production, we recorded only from last instars; in the case of flight, we went as far back in development as any features of the pattern appeared (about ten animals were examined at each stage).

The acoustical repertoire of *T. commodus* is large, and the songs, although stereotyped, are complex (*5*). These insects use a variety of types of sound pulses, intervals between pulses, and numbers of pulses in a chirp. Last instar nymphs are able to generate nearly perfect motor patterns for the aggressive song, the courtship song, and the most highly structured part of one of the calling songs (Fig. 1). The last consists of five to seven loud pulses, with long intervals between the pulses, switching abruptly into a series of six to ten soft pulses with short intervals between pulses. The motor pattern produced by the nymph can be identified

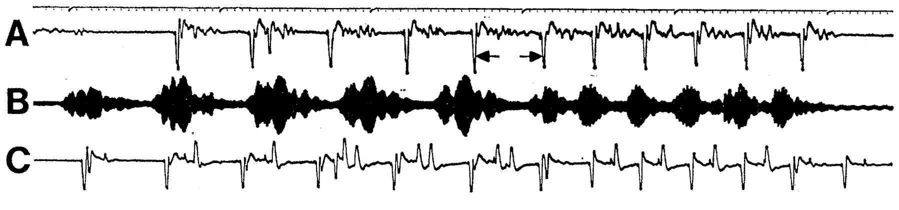
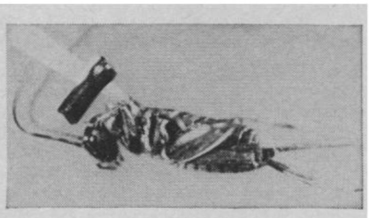
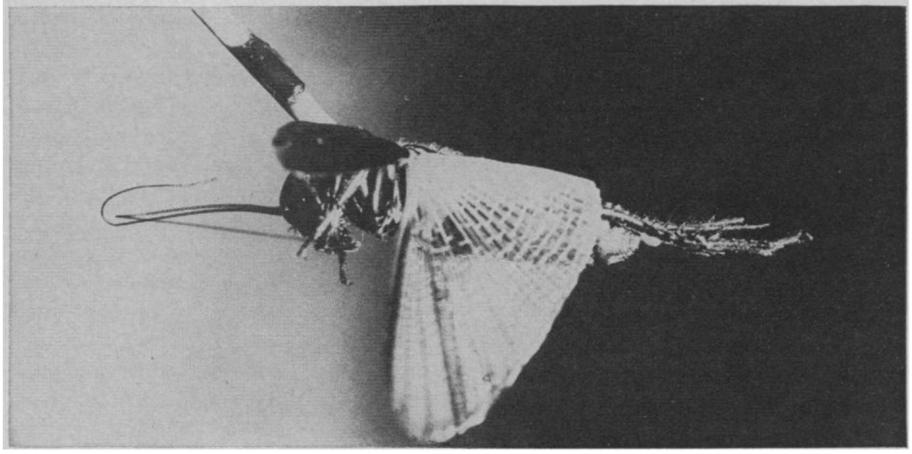


Fig. 1. Comparison of motor output of a nymph with the actual song of an adult. (A and C) Muscle action potentials recorded from a last instar nymph. (B) Sound pulses recorded from an adult (see text). (A) Second basalar muscle (wing opener); note how closely the number and spacing of nymphal muscle potentials correspond to the adult pattern. (C) Push-pull recording from subalar muscle (downward spikes, wing opener), and remotor muscle (upward spikes, wing closer); this demonstrates reciprocal firing of antagonists (closing movement produces sound pulse). Arrows indicate the switch (long intervals to left; short intervals to right), in both adult and nymphal patterns, from long to short intervals between pulses. Time calibration: 5 msec per small division.

Fig. 2. The flight posture of adult and nymphal (seventh instar) crickets in a wind tunnel. Important features are (i) antennae pointed ahead, (ii) fore- and midlegs drawn close to body, (iii) abdomen stiffened, and (iv) hind legs straight and parallel to body axis. The seventh instar nymphs were the youngest which displayed flight posture and motor patterning. Size calibration: black bar equals 5 mm (animals shown to same scale).





as the correct one for adult calling because it has (i) the correct number of bursts (corresponding to sound pulses) in both the loud and soft parts of the chirp, (ii) the correct interval between bursts, (iii) alternate firing of motor neurons for wing opening and wing closing, and (iv) the presence and correct timing of the switch from long to short intervals between pulses (Fig. 1). Therefore the last instar nymph has an essentially complete neural circuit for generating the adult pattern. Since the pattern cannot be elicited unless brain lesions are made, inhibition from higher centers must suppress the neural circuit during nymphal life. The neurons may ultimately be under the control of the hormonal system which mediates the change to the adult form.

The motor system underlying flight is not confined to the wing muscles but encompasses a large constellation of motor neurons distributed throughout the body. Its activation places the animal in an aerodynamically optimum posture characterized by (i) pointing the antennae straight into the wind stream, (ii) drawing the fore- and midlegs up close to the body, (iii) stiffening the abdomen (used for steering), and (iv) pointing the hind legs straight back (Fig. 2). Nymphs as young as seventh instar also assume this posture (Fig. 2). Although hard to quantify, there is a marked gradation from adults to early nymphs in the difficulty of getting the animals to assume the flight posture and to generate flight motor patterns. This certainly reflects some incompleteness of the neural system, either in the sensory input or in the motor output circuit for initiation of flight.

We examined the development of the neuronal firing pattern underlying flight, by recording in each animal from four sets of muscles, including the hind wing (metathoracic) depressors, the hind wing elevators, the fore wing (mesothoracic) depressors, and the fore wing elevators. In the adult flight pattern, the antagonists (depressors and elevators) in each body segment alternate rhythmically in burst production; the hind wings lead the fore wings, so the metathoracic units fire before their mesothoracic counterparts (see Figs. 2 and 3B).

The first features of the flight pattern to appear during development are in nymphs which must still go through four molts before reaching adulthood (Fig. 3G). Almost none of these animals show any flight activity, but some produce a few spikes at the flight frequency in the hind wing depressor neurons. All other flight units are silent, presumably indicating that the appropriate synapses have not been completed. In the next instar slightly longer bursts occur from hind wing depressors, and doublets (two spikes per burst) begin to appear (Fig. 3F). Multiple firing of units, particularly in doublets, is usually characteristic of adult flight. Two examples of development in the next instar (second from last) are shown (Fig. 3, C and D). For the first time, long trains of spikes equivalent to dozens of wing strokes are generated. The full complement of motor neurons of the muscle being monitored (subalar) is recruited (Fig. 3C). The full range of multiple firing seen in the adult, up to five spikes per burst, occurs (Fig. 3D). Finally, the system is just on the verge of activating the hind wing elevators and the fore wing units; in some animals, brief bursts of these neurons appear (Fig. 3C), although in others they are not recruited despite intense driving of the hind wing depressors (as judged by the number of spikes per burst; Fig. 3D). Some of the difference between individuals may represent the establishment of functional synaptic contacts during the course of this nymphal stage. Newly activated units may fire slightly out of correct phase (compare Fig. 3B with Fig. 3C), indicating that the timing mechanism of the neural circuit is not fully functional. In the final instar (Fig. 3B) all of the elements of the adult pattern are active and all fire in the correct phase. As in the case of stridulation, the circuit generating the pattern is essentially complete at this stage.

Although the correct pattern can be generated by nymphs, they never maintain the burst frequency (corresponding to rate of wing strokes) found in the adults. The high frequency of bursts can be attributed to the sensory input from four neurons, the stretch receptors, one of which is coupled to each wing (*6*). If these are destroyed, the frequency falls back to the nymphal level. Since no wing flapping occurs in the nymph, the input from these receptors would not be available to the circuit generating the pattern, and would account for the low output frequency. This view would also explain why (i) there is a major increase in frequency between the last instar and the adult, but no increase between successive nymphal stages, and (ii) there is no increase in frequency between stridulation patterns of last instar nymphs and adults, where the stretch receptors have little effect (*7*), although the same motor neurons are involved.

Figure 3E is representative of nymphs that undergo nine rather than the usual ten molts. This animal is an eighth instar nymph (as in Fig. 3F) but only two molts from adulthood (as in Fig.

3, D and C). It can generate sustained bursts from the hind wing depressors, but can produce neither activity in other muscles nor the full range of multiple firing of the adult. Since the pattern is intermediate between that of its chronological peers and of its peers in proximity to adulthood, the wiring up of the flight system must be accelerated if the animal is to delete a molt.

The sequence of pattern changes during development follows the same general course in all individuals; for example, the hind wing depressors are always the first neurons active in the flight rhythm. This indicates that the order of establishment of functional synaptic contacts in the growing neuropile is determinate rather than random. At all stages there is a correspondence between the development of the structural machinery of flight, the wing pads, and the emergence of functional neuronal connectivity generating the flight pattern.

Since the neural connectivity underlying both flight and stridulatory behavior is completed during nymphal life, it is interesting to note that premature activation of the circuits is avoided by two different mechanisms. A nymph may often be placed in a situation which would elicit aggressive stridulation, and so it is appropriate that this circuit is suppressed by descending inhibition. Such inhibition is unnecessary in the flight system where the animal is extremely unlikely to find itself in the correct stimulus situation: suspension in a wind stream.

A great deal of work has been done on the anatomy of developing insect nervous systems, and this information has recently been reviewed (*8*). In some neural centers, particularly the brain, addition of new neurons continues until late larval life (*8*, *9*). However, in cricket segmental ganglia, all of the neuron cell bodies appear to be present at hatching. Nevertheless, the ganglia undergo a large increase in size during postembryonic development, which reflects the addition of glial cells, an increase in soma size, and the growth of the neuropile (where synaptic contacts occur) (*10*). The degree to which central neuronal connections are functional is not known, except from observations that walking, eating, grooming, and other behaviors occur; larval grasshoppers, for example, can produce stridulatory movements of the legs (*11*). In the periphery, some cricket flight muscles and neuromuscular synapses are operational as early as the seventh instar (*12*).

Our study shows that although neuronal cell bodies may be present at hatching, neural circuits underlying exclusively adult motor programs are not functional in early instars. Elements of the pattern appear in a specific sequence during several nymphal stages. This indicates that operational synapses between the programming neurons are established in a determined order (a less likely alternative is that inhibitory synapses are sequentially inactivated). The process is completed by the last instar. Programs for adult behavior which might be activated in nymphal life are held in check by neurons in the brain. The investigation of the postembryonic emergence of motor programs provides a physiological substrate that bridges descriptions of the ontogeny of insect behavior and of the anatomy of the developing nervous system.

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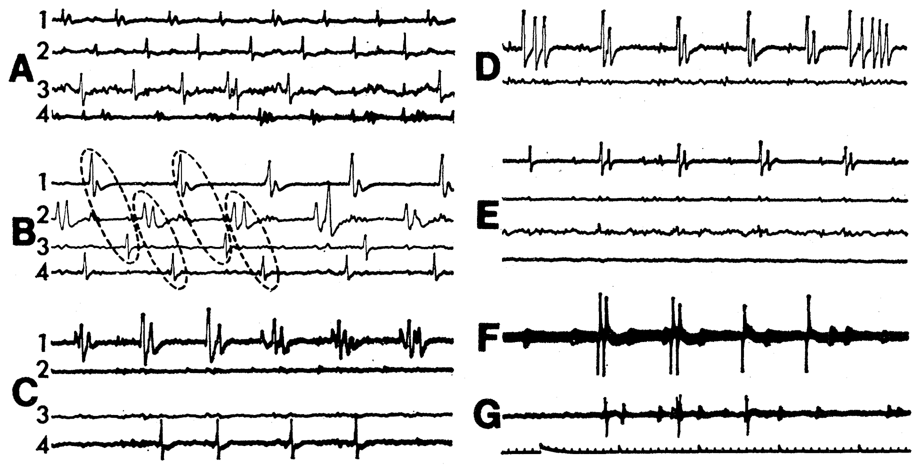

Fig. 3. The progressive development of adult flight pattern in nymphal crickets. The recordings are muscle action potentials that reflect the discharge of single motor neurons. In each record (A–G), the top trace is the hind wing subalar muscle (wing depressor); the second trace (if any) is the hind wing remotor muscle (wing elevator); the third trace (if any) is the fore wing subalar muscle (wing depressor); the fourth trace (if any) is the fore wing remotor muscle (wing elevator). Recordings were made from all four muscles in each animal, but some traces were deleted if the muscles were inactive in flight (one could confirm accurate electrode placement because the muscles are bifunctional and used in walking). In the adult pattern, the wing depressors and elevators alternate in each segment, and the hind wings lead the fore wings; broken lines (B) indicate this phase lag. (A) adult; (B) last instar; (C) second to last instar; (D) second to last instar; (F) third to last instar; (G) fourth to last instar; (E) this nymph, while also a second to last instar, will become an adult one molt earlier than the other animals of the figure, that is, after the ninth molt rather than the tenth (see text). The figure illustrates the gradual emergence of the adult motor pattern during nymphal development; key features are (i) appearance of short bursts in the hind wing depressors, (ii) generation of sustained bursts, (iii) recruitment of antagonists and of the forewing motor units, (iv) development of the adult burst frequency (further discussion in text). In the second to last instar, it appears that some small units of the hind wing elevators are active, for example D, second trace. Time calibration: 5 msec per small division.

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