ethanolamine receptors are totally insensitive to octopamine.

The observation that three types of conductance increase responses to phenylethanolamine have been found is consistent with the suggestion by Swann and Carpenter (*9*) that a single class of neurotransmitter binding site in this preparation may be coupled to at least three different ionophores. The three ionophores mediate Na^+, Cl^-, and K^+ conductance changes, respectively. This model provides an explanation for the occurrence of at least three different ionic responses to several transmitters (*7–9*) and for the similarities in several properties of the responses to different neurotransmitters, but resulting from the same ionic conductance.

These experiments provide strong evidence that phenylethanolamine may have a direct role in synaptic transmission in *Aplysia*. The presence of specific receptors for a substance present in nervous tissue does not necessarily prove a neurotransmitter role. However, the facts that the receptors are localized to the natural synaptic region, are found on only a small minority of neurons, and are usually highly specific for phenylethanolamine suggest that these responses do not result from some nonspecific membrane interaction.

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10. It is somewhat surprising that the Cl^- response was only abolished and not reversed in Cl^--free seawater. This was not a function of duration of exposure to the Cl^--free solution and also was not due to any effect on membrane resistance. Whereas we have always been able to reverse responses to acetylcholine and γ-aminobutyric acid, where receptors are located on the cell body, the apparent Cl^- responses to several other putative neurotransmitters with receptors located only in the neuropil could not be reversed (D. O. Carpenter, J. W. Swann, P. J. Yarowsky, *J. Neurobiol.*, in press).

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Ecdysis: Neural Orchestration of a Complex Behavioral Performance

Abstract. Cricket ecdysis (molting) requires continuously changing output in hundreds of motoneurons over a period of several hours, and exhibits considerable plasticity. Despite this complexity, analysis of identified motor units reveals a highly organized three-layered infrastructure, and indicates that the "small systems" paradigm currently applied to simple invertebrate motor programs can be extended to much more sophisticated behavioral performances.

The goal of neurobiology is to explain behavior in terms of the operation of the nervous system. Success in fairly complete analysis has so far been confined primarily to invertebrate behaviors. Here, the "small systems" approach, investigation of identified nerve cells, has been very fruitful at the level of synaptic function, diversity, and plasticity and, more recently, at the level of motor program generation, sensory modulation of motor output, executive control of motor output, and decision-making (*1*, *2*). However, the behaviors examined have been conspicuously restricted both spatially, to a portion of the body, and temporally, to acts that are episodic (crayfish tail flips; *Aplysia* gill withdrawal), rhythmic (leech swimming; locust flight), or tonic (postural) and have a duration or repetition time on the order of milliseconds (*1*, *2*). Behavior treated by ethologists, on the other hand, tends to involve extensive sequences of motor patterns (waterfowl or fish courtship; ant or bee foraging; caddis-fly nest building) and to occur on a time scale of minutes, hours, or days. A question of considerable import has been whether or not such "real" behaviors involve a quantum jump in sophistication that renders them inaccessible to the concepts and techniques of small systems neurobiology.

In this report we analyze a complex invertebrate behavior, cricket ecdysis (molting), in terms of the output of identified motoneurons. The behavior lasts over 4 hours and demands the carefully controlled coordination of practically every muscle in the animal in a sophisticated series of operations; it is normally stereotyped but can evince substantial flexibility under unusual conditions. Analysis reveals that the complete performance is brought about by the hierarchically controlled integration of numerous subelements, each of which appears comparable to the simpler motor programs previously analyzed. The main features are (i) sequential activation of about 48 relatively discrete motor programs, (ii) temporal and spatial coordination of concurrently active motor programs by a bout rhythm generator, and (iii) modulation of the bout rhythm and clustering of the programs into four major phases. This multilayered arrangement is effected by the interaction of central neural elements, sensory feedback, and very likely endocrine triggering. Therefore, to the degree that this behavior is typical, complex performances are brought about by the incorporation of many simpler motor programs, of the sort already studied, with the addition of overall coordinating and controlling elements.

Crickets, *Teleogryllus oceanicus*, were raised in the laboratory and isolated before the final molt. Ecdyses were videotaped (Sony V-32; AVC 3210 videocamera; 1:1.8 zoom lens with three-stage close-up lens) and analyzed on a frame-by-frame basis (20 msec per frame). Participation of single, identified motor units was determined by intramuscular recording from 25-μm insulated silver electrodes and stored on tape (TEAC TCA 40). In some cases, the behavior and an oscilloscope display of motor unit discharge were simultaneously videotaped. Recordings from 22 muscles were made during 77 ecdyses, 11 complete ecdyses were videotaped, and additional manipulations or observations were performed on another 66 animals, for a total of 154 ecdyses analyzed.

The ecdysial process is a mechanically difficult task effected by the following four operations. (i) A preparatory phase serves to loosen and split the old exuvia and to anchor it to the substrate. It involves restless locomotor and grooming activity; rhythmic leg movements, which fix the tarsal claws into the substrate; abdominal contractions and air-swallowing to exert pressure on the ecdysial line where splitting will occur; and assumption of a characteristic posture that facilitates emergence. (ii) The ecdysial phase extracts the animal from the old cuticle. Peristaltic abdominal waves propel the body forward and increase hemolymph pressure to widen the ecdysial split as appendages are extricated by a complex sequence of muscle contractions pulling them up and forward. (iii) The expansional phase inflates the new cuticle, protects appendages during hardening, and folds the wings. The cricket enters a period of general immobility, except for a small set of motor patterns necessary for proper expansion and curing of the new cuticle. (iv) The exuvial phase, which reclaims nutrients, commences with a release from immobility that can be quite abrupt. If not already on the exuvia, the cricket walks directly to it and begins consumption, which lasts about 40 minutes. Additional curing-related behaviors, such as alternate bending of the ovipositor, occur during this final period. A detailed description of each motor act, the contribution of each of the 22 muscles studied, and quantitative analysis of the sequence of activities and their temporal features can be found in Carlson (*3*).

We consider a motor program to be a series of contractions with a particular coordination of a defined set of muscles. Every phase comprises a set of motor programs (preparatory, 6; ecdysial, 30; expansional, 8; and exuvial, 4), each of which subserves a particular mechanical task. A typical program, as characterized by the activity of two identified motor units, is illustrated in Fig. 1. The program, lasting about 5 to 6 minutes, produces alternating contractions of bilaterally homologous muscles that gradually draw the forelegs out of their old exoskeletal sheaths. Several lines of evidence indicate that programs such as this are relatively discrete entities rather than artificial divisions of a continuous act. (i) Each program begins with a low level of activity, rises to a peak, and tapers off toward the end (Figs. 1 and 2). Activity level is determined by the number of motor units active, the frequency of impulses within a burst, the frequency of bursts, and the number of bursts in a bout (Fig. 2), all of which covary. (ii) Different programs, even those that overlap, peak independently (Fig. 2). (iii) Several programs can be reactivated at different times in ecdysis within a completely altered sensory-motor context. (iv) In some cases, serially homologous programs are temporally displaced; for example, middle and foreleg extraction programs occur together, but the hindleg program is delayed several minutes. (v) Depending on sensory circumstances, there are backup programs (for example, if the antennae are not successfully extracted by the beginning of the expansional phase, an infrequently used program is activated in which the forelegs are placed on the antennal flagellae and the head is rotated upward to withdraw them); optional programs, which are normally used but are not absolutely

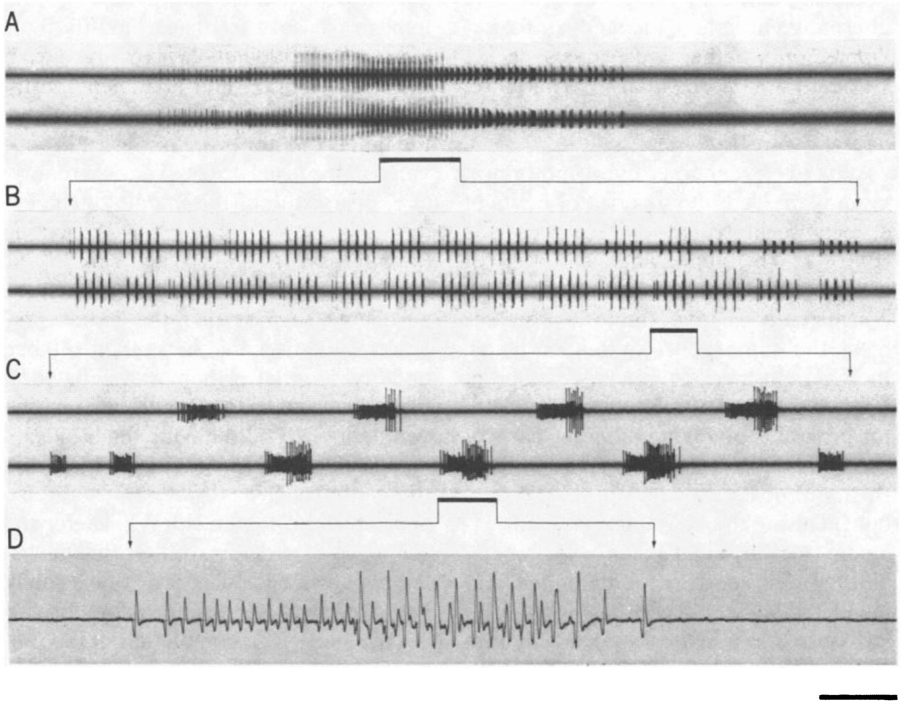

Fig. 1. Foreleg extraction motor program: recordings of action potentials in the two motor units of prothoracic muscle 71d (*3*) resulting in leg extraction. In each record, the upper trace is from the left muscle and the lower from the right. (A) The complete output of the muscle during the ecdysial phase. (B) Expansion of (A) showing that the program is divided into rhythmically occurring periods of activity (bouts). All concurrently active programs have the same bout rhythm. (C) Expansion of (B) showing that a single bout consists of rhythmical bursts of action potentials resulting in alternating contractions of the homologous muscles. (D) Expansion of (C) showing that a burst consists of multiple action potentials in two motor units. Recruitment conforms to the size principle in that the smaller unit is activated first and terminated last at the burst, bout, and motor program levels. This indicates independently variable sources of driving for the motoneurons for each of these levels. Calibrations: (A) 100 seconds, (B) 5 seconds, (C) 0.5 second, and (D) 50 msec.

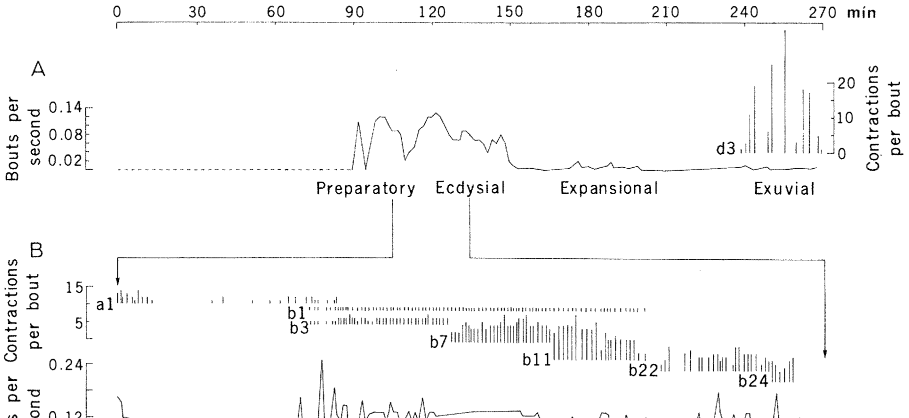

Fig. 2. Motor program, bout, and phase organization of ecdysis. (A) The bout frequency during a complete ecdysis is shown as a function of time (upper scale; all motor programs active at any particular time have the same bout frequency). Four major phases of activity (preparatory, ecdysial, expansional, and exuvial) are separated by low-activity transitions (the dashed line corresponds to the early preparatory phase, when bouts are irregular; the subpeak within this phase is not a consistent feature). (B) Expansion of (A) showing bout frequency during the most active part of ecdysis (lower scale). Above the bout frequency graphs are sequences of vertical lines showing, on the same time scale, when eight different sample motor programs are active [(A) d3; (B) a1, b1, b3, b7, b11, b22, and b24; programs are described in Carlson (*3*)]. Each line represents a single bout; the height of the line signifies the number of separate muscle contractions in the bout (Fig. 1) according to the scales for contractions per bout. This measure of bout intensity covaries with other indices (see text) and indicates that each program has its own activity peak.

necessary for a successful ecdysis; and alternative programs (depending, for example, on whether the ecdysis occurs vertically or horizontally). Therefore, each program appears to have its own neural controls.

With two exceptions, every motor program is divided into a series of bouts of activity separated by quiescent periods (Fig. 1). The appearance and rate of these bouts define the bout rhythm. All concurrently active motor programs have the same bout rhythm, although their activity periods may have different relative timing. The relative timing of motor programs involving different parts of the body is longitudinally ordered with the more posterior occurring earlier in a bout. During the ecdysial phase, for example, a bout might begin with a metachronal contraction of the abdominal segments, which would continue forward as leg contractions in the thorax and mouthpart contractions in the head. The bout rhythm therefore provides a timing cue for the coordination of whatever motor programs are active. Although the bout rhythm can only be measured in terms of particular motor programs, it occurs throughout the entire ecdysis and thus transcends the activity of any single motor program. Consequently, it must be generated by a separate layer of neural elements above that of local control of motor programs.

In every ecdysis, the bout rhythm itself undergoes a systematic modulation resulting in four frequency peaks (Fig. 2) separated by intervals of low bout rates. These peaks are coincident with the major phases described above. Although the set of motor programs that is active changes continuously during ecdysis, the onset of each new phase is also characterized by especially rapid turnover in the program complement. Therefore, the division into four phases is a biologically meaningful aspect of the organization of ecdysis; it must reflect a further layer of control, either neural or hormonal, which is superimposed on the bout rhythm and motor program generators.

We have some information on the roles in ecdysis of (i) motor programs generated within the central nervous system, (ii) sensory feedback, and (iii) hormones. If the old cuticle is removed from the head, thorax, and abdomen shortly before the onset of ecdysial behavior, the first six motor programs are still activated in the usual order. If cuticle removal is delayed until the middle of the ecdysial phase, programs for extricating the hind legs, abdomen, and cerci occur in normal sequence. A variety of such experiments suggest that the motor programs of ecdysis will be similar to those underlying other orthopteran behaviors, including flight, walking, singing, and ventilation (*1*, *4*), in having the basic elements of coordination generated within the central nervous system. There also appears to be some central basis for the sequence of programs rather than a system in which each new program is solely activated by sensory information signaling the successful completion of the previous program.

Experiments in retarding or accelerating removal of the old exuvia do, however, reveal a powerful sensory influence on certain motor programs. If, for example, a thread is tied around the neck, thereby preventing leg extraction, the program is continued for as long as 30 minutes instead of the usual 5 to 6. Retarding extraction by waxing the old exoskeleton to the new also greatly prolongs the activity of the large motor units. Certain programs are abruptly terminated within a single bout of successful appendage extrication, indicating a decisive sensory cue, but other programs may continue for several bouts after extrication. Programs can be reactivated if the sensory situation changes; for example, if the tarsal claws are dislodged during the preparatory phase, anchoring can be repeated. Sensory information is also a critical factor at the choice points of backup, optional, and alternative programs. Similar effects of sensory feedback have been described in orthopteran embryonic ecdysis (*5*). Therefore, a continuous exchange of information between the central nervous system and peripheral sensors underlies execution of the behavior.

It is unlikely that neural elements alone control this behavior. Ecdysis has many features in common with moth eclosion, which has been shown to be hormonally triggered (*6*). In preliminary experiments with brain extracts from pharate crickets, some ecdysial motor programs have been elicited in injectees. Therefore, the possible importance of endocrine systems in controlling the behavior cannot be disregarded.

What does analysis of ecdysis suggest about the organization of sequential, long-term behavior in insects? The behavior is not a single, prolonged, and mystifyingly complex motor program. Instead, it is composed of well-defined subelements, one subserving mechanical tasks (motor programs) and two providing hierarchical control (bout rhythm and phases). The subelements can be analyzed in terms of the sensory and central neural features seen underlying simple behaviors. Therefore, it appears feasible to extend the analysis of behavior at the level of identified neurons to very sophisticated performances.

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