

nonfollicular ovarian tissues or secretions thereof are not essential for LH to have an initial effect on the follicle. The similarity between the effect of LH in vitro and in vivo is illustrated by the normal histologic appearance of corpora lutea developing under the kidney capsule.

The site and physiologic mechanism of action of LH on the Graafian follicle are obscure. The hormone may act on the peripheral (thecal) tissues of the follicle or it may enter the follicle to stimulate the granulosa cells directly. An action of LH on the follicle wall might account, at least in part, for the propensity of LH-stimulated follicles to vascularize when autotransplanted. The importance of capillary penetration in the early stages of luteinization is unknown, but it is apparent from results of this study that absence of a blood supply does not preclude the initial action of LH on the follicle.

The rabbit is an especially useful

species in which to investigate luteinization, since the follicles remain in readiness to ovulate and luteinize. However, the procedure described here can be adapted to investigations of luteinization and corpus luteum formation in other species, including those that ovulate spontaneously.

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large cells which are easy to impale with microelectrodes and from which it is possible to continuously record synaptic potentials for hours (5, 6) and even days (7). The molluscan preparation in which synaptic physiology has been studied in particular detail is the abdominal ganglion of *Aplysia*. This ganglion contains about 1800 cells of which perhaps 1000 have been categorized as members of different functional clusters and 30 have been identified as unique individuals that can be recognized reliably from preparation to preparation (6). A number of the central connections between different identified cells have been mapped and several interneurons common to the identified cells have been either identified or inferred (8).

Despite a substantial background of neurophysiological information on *Aplysia* (for reviews, see 6, 9), relatively few studies have examined the behavior of this animal (10, 11) and little is known about the control of behavior by the nervous system. Our report here is part of a larger study designed to investigate the role of the abdominal ganglion in the behavior of *Aplysia* (4, 6, 11) and to delineate neural systems that control behavioral reflexes which might prove modifiable.

A striking and reliable response in the intact *Aplysia* is a defensive withdrawal reflex of the external organs of the mantle cavity. When the siphon or the mantle shelf is touched, the siphon, the mantle shelf, and the gill contract and withdraw into the mantle cavity (Fig. 1, A1 and A2). This response has a short latency but occasionally a second, long latency response occurs. The excitatory receptive field of this reflex is centered on the siphon and on the margin of the mantle shelf (Fig. 1, A3). A similar but not quite identical behavioral response also occurs spontaneously, sometimes recurring at intervals of 40 to 300 seconds. Both the evoked and the spontaneous withdrawal responses persist after the connectives leading to the rest of the central nervous system are cut, and both are abolished by removal of the abdominal ganglion, an indication that these responses are controlled by cells in this ganglion.

To study the cellular basis of the evoked and spontaneous withdrawal responses we used a semi-intact preparation (Fig. 1B). The animal was prepared by slitting it open and removing the digestive system. The parapodia were cut back and pinned, and the

Neuronal Controls of a Behavioral Response Mediated by the Abdominal Ganglion of *Aplysia*

Abstract. *Tactile stimulation of the siphon and mantle shelf in Aplysia causes a characteristic withdrawal response of the external organs of the mantle cavity. A similar response also occurs spontaneously. Both responses are mediated by the abdominal ganglion and therefore provide an opportunity for correlating cellular functioning and behavior in a relatively simple and well-studied neuronal system. The withdrawal responses are controlled by five identified motor cells which receive two types of synaptic inputs. One set of excitatory connections, activated by tactile stimulation of the siphon and mantle shelf, mediates the defensive withdrawal reflex. A second set of connections is activated by a spontaneous burst of activity in a group of closely coupled interneurons which are excitatory to some of the motor cells and inhibitory to the others. This second set of connections mediates the spontaneous withdrawal response. These two inputs can therefore switch the same population of motor cells from a simple reflex to a more complex, internally organized response.*

The cellular neurophysiological analysis of behavior and learning requires that neurons mediating a particular behavioral sequence be identified and their interconnections be specified. It is difficult to meet this requirement in the vertebrate central nervous system, but it is becoming increasingly more feasible in certain invertebrates. The nervous systems of higher invertebrates have the advantage of containing relatively few cells, many of which are directly accessible for investigation. For example, in arthropods, one can record from individual identified sensory, interneuronal, and motor elements and describe their functional properties (1). One of the striking findings to emerge

from this work is that single central neurons (command elements) can initiate complex behavioral sequences (2). This fortunate circumstance, which also occurs in mollusks (3, 4); reduces the task of a behavioral analysis. However, the advantages offered by arthropods are somewhat counterbalanced by the difficulty in recording synaptic potentials from their central neurons. As a result, it has generally not been possible to analyze the mechanisms involved in the synaptic transformations of neural information within the arthropod central nervous system. This limitation can be overcome in certain mollusks, particularly in marine gastropods, whose nervous system contains many

head was removed, all of the major ganglia except for the abdominal ganglion being eliminated. Qualitatively similar results were obtained in preparations in which the head and the remaining nervous system were not removed. The abdominal ganglion with its peripheral nerves intact was pinned to a small stage that could be transilluminated so as to facilitate the location and identification of cells on either the dorsal or ventral surface (Fig. 1B). Up to three cells at a time were impaled by means of glass microelectrodes, utilizing conventional intracellular recording and stimulating techniques described previously (6). The movements of the different external organs of the mantle cavity were recorded by means of a photocell.

We first attempted to define the motoneurons for this reflex by firing different identified cells intracellularly and observing the movements of the organs of the mantle cavity. By this means we found that some identified cells produced contractions of the ex-

ternal organs of the mantle cavity (the gill, the siphon, and the mantle shelf), whereas other cells influenced internal organs beneath the mantle cavity (for example, the heart and the sexual apparatus). We now describe five cells which contract the siphon, gill, and mantle shelf (Fig. 1C), the organs involved in the withdrawal response (12).

When one of these motor cells was fired rapidly it usually produced a smooth movement of the innervated organ. Individual spikes usually did not produce discrete synchronized twitches (Fig. 2, A1). However, there are a number of reasons for believing that the movements result from direct innervation of the organs, and are not mediated by interneurons. First, the responses do not readily fatigue. Second, the responses are not blocked when either the ganglion or the whole preparation is perfused with seawater containing a high concentration of Ca^{++} which blocks the firing of interneurons by raising their threshold. Third, all the motor cells send axons into one or

more nerves that innervate the organs whose responses they modulate. Finally, two cells (LD-G and L7) occasionally produce individual twitches that are exactly synchronous with single spikes (Fig. 2, A2).

Having identified at least some of the motoneurons innervating the siphon, the gill, and the mantle shelf, we next mapped the tactile receptive field of these motor cells by stimulating the external body surface of the animal with light brush strokes. All five motor cells were found to receive large excitatory postsynaptic potentials (EPSP's) producing a brisk repetitive spike discharge following tactile stimulation of the external organs of the mantle cavity (Fig. 2B). The center of the excitatory receptive field was located at the siphon and at the edge of the mantle shelf and dropped off progressively with distance; there was no inhibitory surround. The receptive field of these five motor cells was identical to that of the defensive withdrawal reflex (Fig. 1, A3) indicating that these cells constitute part of the motor component of this reflex.

The effectiveness of the different motoneurons in producing contraction varied. For example, of the four motoneurons which innervate the gill one (LD-G) was particularly effective; removing it from the reflex pathway by direct hyperpolarization could produce a relatively large decrease in the total reflex contraction (Fig. 2B). The pattern of motor responses of the different motoneurons also varied (Fig. 3A). Three of the five motor cells produced movements largely limited to the gill. One cell produced movement only of the siphon, and one cell produced movements of the gills, the siphon, and the mantle shelf. Thus, the motor component of this reflex consists of individual elements with both a restricted and overlapping distribution. Such motor organization has also been described in other invertebrates (1-3). The sensory component of the defensive reflex is represented in the diagram of Fig. 3A by the line coming from the siphon. The break in the line indicates that we do not know the extent to which this excitatory afferent input is monosynaptic or is mediated by interneurons.

The five motor elements differ not only in their field of innervation but also in their electrophysiological and pharmacological properties (6). A question that arises is why are different cell types involved in a simple

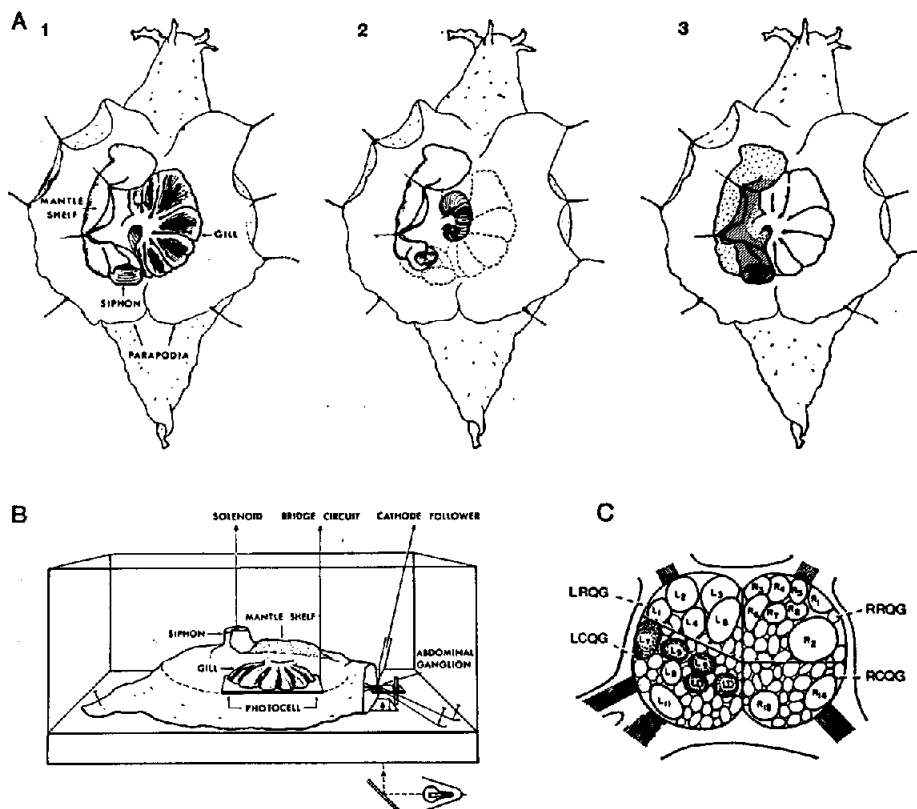


Fig. 1. (A) Dorsal view of an intact animal. The parapodia and mantle shelf have been retracted to reveal the gill. (A1) Position of organs in unstimulated condition. (A2) Position of organs during withdrawal reflex following tactile stimulation within receptive field. (A3) Tactile receptive field for withdrawal reflex. The area which produces strong effects is heavily stippled. The surrounding area (dotted) produces weaker effects. It was also possible to get weak effects by very strong stimulation outside of the main receptive field. (B) Experimental arrangement. A pull on the solenoid or a brush stroke was used as a tactile stimulus. (C) Dorsal surface of the abdominal ganglion. The identified cells (shaded) which are motoneurons to the organs of the mantle cavity are located in the left caudal quarter ganglion (LCQG).

withdrawal response which could, in principle, be mediated by a single cell with wide innervation. An important clue was found in the analysis of the central connections received by the various motor cells. These neurons receive a number of different spontaneous postsynaptic potentials (PSP's) attributable to different interneurons. A particularly striking synaptic event is a burst of PSP's which occurs simultaneously in all five cells. This PSP burst is attributable to several, closely coupled interneurons, one of which is Interneuron II (6, 13). In some cells (L7, L9-1, L9-2), the burst consists of inhibitory postsynaptic potentials (IPSP's), while in others (LD-G, LD-S) it consists of EPSP's (Fig. 2C). This burst of interneurons reflects an internally organized pattern of neural activity since it occurs in the totally isolated ganglion (6, 13). In both the isolated ganglion and the semi-intact preparation the burst occurs spontaneously at a rate comparable to that of the spontaneous withdrawal response seen in intact animals. In the semi-intact preparation the burst was invariably associated with a contraction of the external organs of the mantle cavity (Fig. 2C). In addition to occurring spontaneously, the interneuron burst and its associated withdrawal response sometimes could be triggered by a tactile stimulus. Under some circumstances, a single sensory stimulus gave rise to two consecutive responses: (i) a short-latency withdrawal response produced by a direct reflex discharge of the motoneurons, and (ii) a long-latency withdrawal response associated with the interneuron burst. The threshold for the long-latency response was on occasion less than for the direct reflex, so that only the long-latency response resulted from a tactile stimulus.

These data suggest the existence of a second motor system, independent of the defensive reflex and are consistent with the behavioral findings that the withdrawal response can occur spontaneously. Presumably, the short-latency evoked reflex and the spontaneous withdrawal have different functions for the animal. The evoked reflex withdrawal is clearly defensive. The spontaneous withdrawal may be involved in respiratory regulation or perhaps, in association with parapodial movements, it is concerned with expelling secretory substances or debris from the mantle cavity.

Figure 3B illustrates the cellular components of this second motor system.

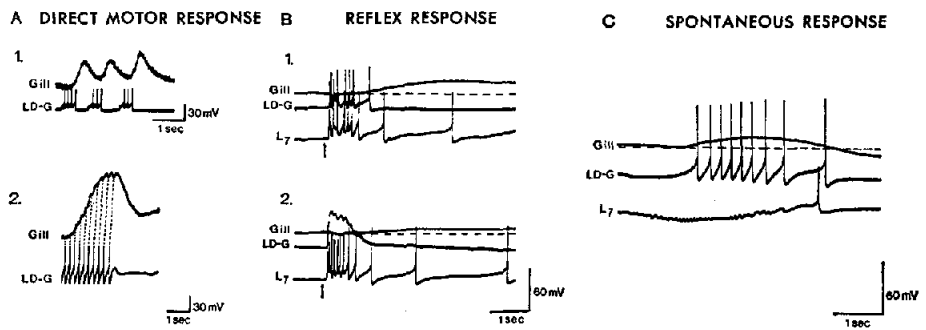


Fig. 2. Gill responses. Upper traces in parts A, B, and C represent the output of a photo-cell placed under the gill. Lower traces are intracellular recordings from motoneurons causing gill contraction. (A1) Smooth contraction produced by small number of spikes in cell LD-G. (A2) Individual twitches produced by LD-G after it was first rapidly fired to potentiate its effects. (B) Reflex response of gill and intracellular record from two motor neurons of the gill. In parts B1 and B2, an identical tactile stimulus was applied to the siphon (arrow). (B2) Reduction in motor response after removal of cell LD-G from the reflex path by directly hyperpolarizing the cell. The large EPSP's that ordinarily underlie the spiking in cell LD-G are unmasked by this procedure. (C) Spontaneous gill contractions and simultaneous excitation of LD-G and inhibition of L7. The onset of gill contraction precedes the discharge of cell LD-G, an indication that one or more still unidentified cells or a direct connection from one of the interneurons are responsible for the early phase of this response.

tem. This response involves the same population of motor cells as the evoked defensive reflex but instead of all of the cells receiving an excitatory input, some cells (indicated by the dark outline), are inhibited and others are excited by the interneurons. The interneurons can fire spontaneously but also can be triggered into activity by means of either a direct or indirect excitatory input from the siphon and the mantle

shelf, as indicated by the broken line.

These experiments suggest that under varying conditions, a single effector response can be controlled in each of the three modes by which behavior can be generated (i) as a simple reflex directly driven by sensory input, (ii) as a spontaneous central command or an internally organized sequence of neuronal activity, and (iii) as a central command which is triggered or re-

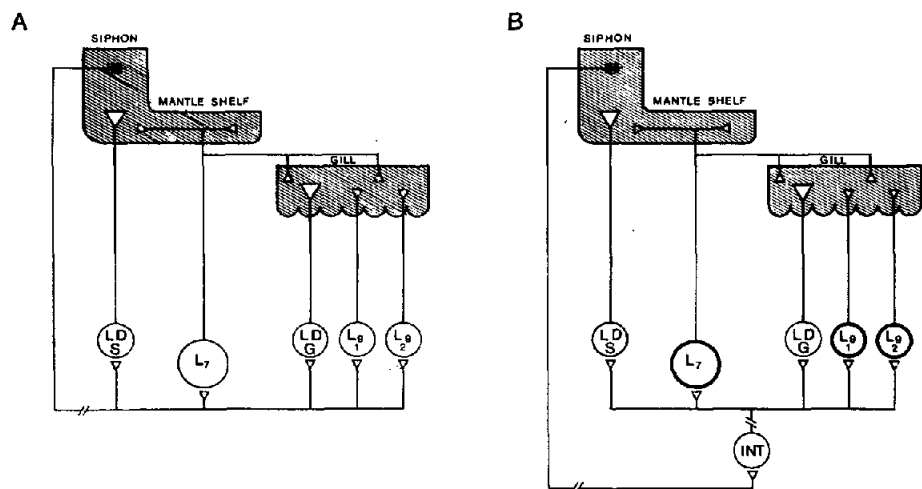


Fig. 3. Diagram of neural system controlling the defensive withdrawal reflex of organs of the mantle cavity (part A) and of the triggered and spontaneous withdrawal responses (part B). The large triangles represent the output of motor cells that produce a strong contraction whereas the small triangles represent the output of cells that produce weaker motor effects. In part B cells that are inhibited during the interneuron burst are outlined with a thick line, while those excited during the interneuron burst are outlined with a thin line. "Int" represents interneuron II and other closely coupled interneurons. In addition to innervating the motoneurons the interneurons may make direct connections with the peripheral organs. The sensory input is represented by the broken line coming from the siphon, although the actual receptive field is larger, as indicated in Fig. 1 A3.

leased by a stimulus that does not directly drive the reflex. These three modes of producing a behavioral response involve different combinations of the same population of motor elements. In the simple withdrawal reflex all of the known motor cells are activated, whereas in the spontaneous and triggered withdrawal one subset of these cells is activated and the other is inhibited. Thus, as a result of multiple inputs, the different members of the same population of motor cells can be switched between either a simple reflex or a more complex response that shares certain features with innate or instinctive behaviors (14).

In view of its advantages for cellular neurophysiological studies this preparation may prove useful for analyzing the neuronal mechanisms of learning. Initial experiments indicate that the behavioral reflex responses can be modified to show simple learning, such as quasi conditioning (sensitization), habituation, and dishabituation (15). Because the withdrawal response can occur either reflexly or spontaneously, it may also prove possible to study more complex behavioral modifications using either classical or operant conditioning paradigms.

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12. The dorsal surface of left caudal quarter ganglion in which these motoneurons are

located consists of cells L7 and L11 and three clusters of cells (LB, LC, and LD) each of which consists of cells having a number of common properties. The most prominent members of the LB and LC clusters were previously designated L8 and L9, respectively, but it was otherwise impossible to distinguish between the cells within the clusters (6). On the basis of motor function these groups can now be further subdivided. Most cells in group LB have no grossly observable motor effects on the external organs of the mantle cavity. A few LB cells, including L8, produce small and inconstant movements but these effects are so small that these cells have not been included as motoneurons in our analysis. In addition to L9 (now called L9-1) the LC group has a second prominent cell which we call L9-2 which also produces motor movement. Two cells of the LD cluster, LD-G and LD-S, produce motor effects but other LD cells appear not to do so. The five

motor cells described probably constitute a major component of the motor outflow of the withdrawal responses but some motor cells have almost certainly been missed. Although the four gill motoneurons produce different types of gill contractions, these differences will not be described in the present report.

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Learning Sets in an Invertebrate

Abstract. Eight isopods, *Porcellio scaber*, were trained with water reinforcement to turn in a T-maze to criterion of correct responses. They were then tested through nine reversals of the turning response. An analysis of variance indicated that mean errors to criterion declined significantly over reversals ($F = 4.78$, $d.f. = 9/63$, $P < .001$).

In the study of learning capacities of various species, the phenomenon of learning sets (1) has seemed to separate vertebrates from the invertebrates. Attempts to demonstrate learning sets in invertebrates, other than the highly specialized octopus (2), have been unsuccessful. Thompson (3) found that although the isopod *Armadillidium vulgare* could learn to reverse a position response in a T-maze, succeeding reversals produced no significant decrement in mean trials or errors to criterion. Harless (4) obtained similar results with *Porcellio scaber*. Where Thompson used the application of an electric shock contingent on a wrong turn in the T-maze, Harless made the onset of a bright light contingent on a wrong turn in a Y-maze. Both investigators report that while certain individual data seemed to show a reduction in errors over reversals, the group data failed to show any significant statistical evidence of a learning set phenomenon.

Pietsch (5) studied this phenomenon

in *P. scaber* but used a different reinforcement technique. Her animals began running a T-maze illuminated with a bright light previously demonstrated as aversive. When an animal turned in the correct direction the light was removed. The animals were able to learn 8 reversals, and mean errors appeared to decline over reversals; however, statistical analysis failed to demonstrate the significance of the trend.

These studies involved aversive stimulation. Thompson and Harless used avoidance conditioning, and Pietsch used escape conditioning. However, it is appropriate to investigate other reinforcement techniques before concluding that these animals are unable to demonstrate learning sets. The fact that lower vertebrates such as newts and terrapins can demonstrate learning sets (6), as well as the efficiency with which some invertebrates spatially orient themselves while foraging for food, calls for further work (7). We have investigated learning sets in *P.*

Table 1. Number of errors to criterion for each subject.

Subject	Reversal									
	0	1	2	3	4	5	6	7	8	9
1	20	29	27	11	16	23	9	14	6	1
2	29	48	7	15	12	7	11	7	8	5
3	38	23	29	33	27	29	15	6	15	23
4	1	7	21	15	10	1	19	6	11	1
5	38	23	9	11	7	5	1	1	0	16
6	17	23	20	9	16	14	14	17	15	14
7	21	15	15	9	16	20	1	3	7	7
8	56	21	21	11	30	12	19	14	7	2
\bar{X}	27.5	23.6	18.6	14.3	16.8	13.9	11.1	8.5	8.6	8.6