Habituation and Dishabituation: Interactions between Peripheral and Central Nervous Systems in Aplysia

Abstract. *The withdrawal response of the isolated siphon of* Aplysia *habituates to a light stimulus and dishabituates to a tactile stimulus, and vice versa, with or without connections to the central nervous system. The peripheral nervous system can dishabituate or enhance the response mediated by the central nervous system and vice versa. Normally the adaptive siphon withdrawal response of the intact animal must be mediated by the integrated activity of the peripheral and central nervous systems.*

Efforts to elucidate the neuronal mechanisms of complex behavior have met with only limited success. This has resulted in a search for systems with a reduced number of neurons (*1*) that mediate elementary adaptive behavioral responses, such as habituation. Habituation, a decreased response to repeated stimulation; dishabituation, the immediate restoration of a habituated response after the presentation of a novel stimulus; and facilitation, the enhancement of a response with repeated stimulation, have been studied in the spinal cord of cats (*2*), in lampreys (*3*), and in invertebrates. Studies on crustaceans and mollusks have shown that these phenomena can occur at the neuromuscular junction (*4, 5*), in peripheral neural circuits of *Aplysia* (*6, 7*), and at central neuron synapses (*5, 8–10*). Kupfermann *et al.* (*11*) have confirmed Peretz's earlier observation (*7*) on habituation of the response of the gill of *Aplysia* and have suggested that there are parallel independent neuronal pathways for habituation of the gill; that is, a central circuit through the abdominal ganglion and a peripheral circuit. A related reflex, siphon withdrawal in the intact animal, habituates to a tactile stimulus and shows retention of habituation between trials (*12*).

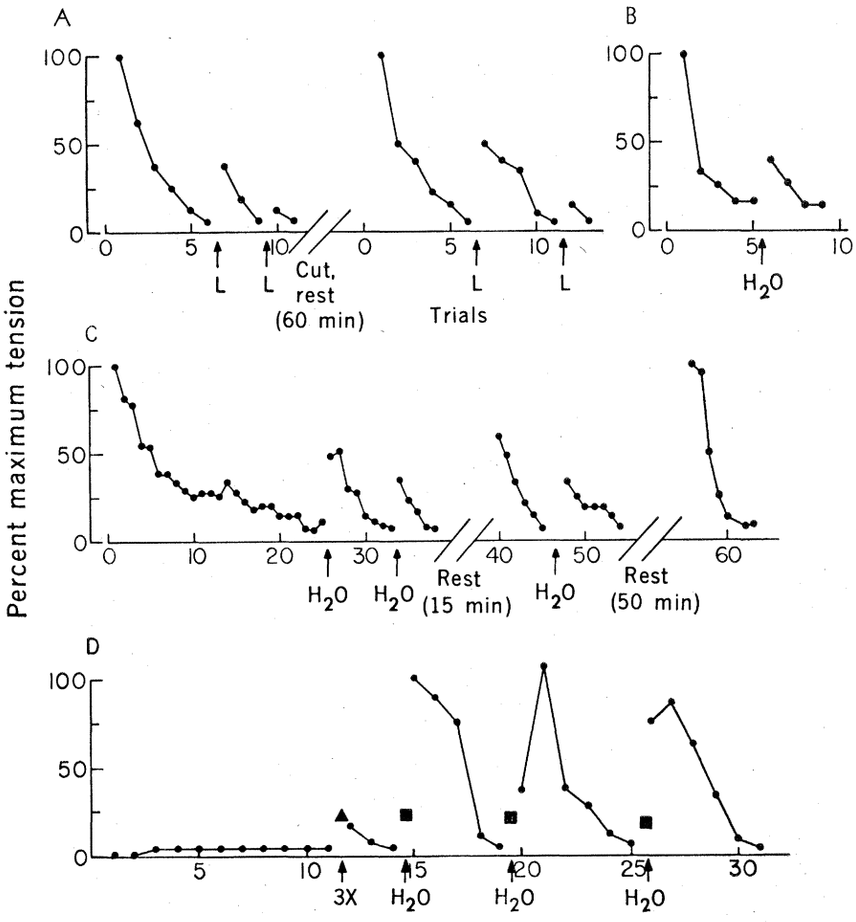

Fig. 1. Habituation and dishabituation in three types of preparations. The relative tension developed by siphon withdrawal in response to repeated stimulation (trials) is shown. (A) The response of the siphon to the tactile stimulus applied to a semi-isolated preparation habituated and was dishabituated by the light stimulus (L), habituated again, and was dishabituated by the light stimulus again. The siphon nerve was then severed (cut) and after a 60-minute rest the isolated siphon preparation was stimulated with water drops. The response habituated and was dishabituated by the light stimulus as it did with the abdominal ganglion intact. (B) The response of an intact animal habituated to the light stimulus and was dishabituated by the tactile simulus (H_2O). (C) The response of an isolated siphon habituated to the light stimulus and was dishabituated by the tactile stimulus (H_2O) and recovered with rest. (D) The response to electrical stimulation of the siphon nerve of the isolated siphon was enhanced by repeated electrical stimulation (triangle at 3×). This repeated stimulation also enhanced the successive responses to single stimuli. The tactile stimulus (H_2O) produced a response (solid square) and enhanced the next responses to a single shock. This response decayed and was again enhanced by the tactile stimulus. The interstimulus interval is 30 seconds in (A), (C), and (D); 90 seconds in (B).

We now show that the siphon withdrawal response of the isolated siphon without any attached central neurons will habituate to tactile or light stimuli applied to the siphon or to electrical stimulation of the distal siphon nerve. The habituated responses can be dishabituated by each of the other stimuli. The adaptive withdrawal response of the isolated siphon is essentially the same as the response of the siphon of the intact animal or a preparation of the siphon and abdominal ganglion. The peripheral system, which can separately mediate the response, and the central system interact and are parts of one integrated system.

We used three types of preparations of *Aplysia californica* as follows: (i) an intact, restrained animal in which the same area of the siphon, mantle, and gill could be repeatedly stimulated; (ii) a semi-isolated siphon preparation that consisted of the siphon, mantle, and gill with the abdominal ganglion connected by the siphon nerve; and (iii) an isolated siphon preparation that consisted of the siphon, mantle, and distal siphon nerve without any connections to the abdominal ganglion or other parts of the central nervous system. The distal siphon nerve of the isolated preparation was left long enough so that afferent activity in this nerve from the siphon could be recorded with a suction electrode, and also so that the siphon nerve could be electrically stimulated with 1-msec pulses from a Grass stimulator to evoke siphon withdrawal. In semi-isolated

preparations intracellular recordings were made from central neurons by means of conventional techniques. In all three preparations a thread was attached from the siphon to a Grass tension transducer and withdrawal tension was measured concurrently with electrical activity on a polygraph or on an oscilloscope. The tactile stimulus was a drop of seawater applied to the siphon of the isolated preparations and a brief jet of seawater (*13*) to the siphon of the intact animal. The light stimulus was white incandescent light of 1000 lumen/m^2 delivered to the siphon. The siphon was submerged under several inches of water and tested with light to verify that the siphon was responding to the light and not heat. All isolated preparations were run at 22°C and the intact animals were run at 15°C in artificial seawater (Instant Ocean).

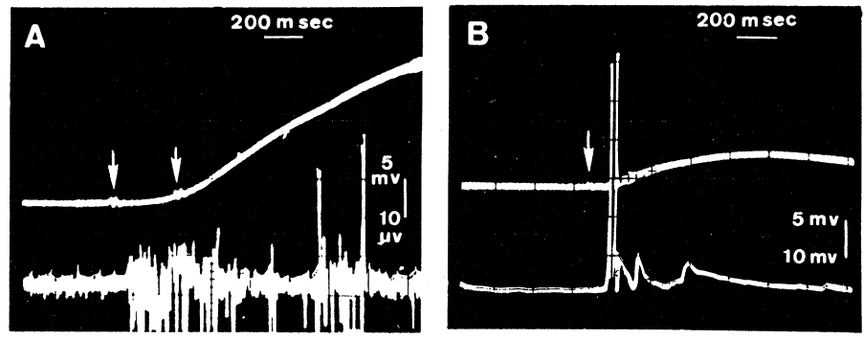

Fig. 2. Siphon tension and afferent neuronal activity evoked by tactile siphon stimulation. (A) The siphon tension (upper trace) and extracellularly recorded afferent activity from the siphon nerve (lower trace) evoked by water drops (arrows) in an isolated siphon are shown. The afferent activity occurred after a latency less than 150 msec and continued well after the contraction started. (B) The siphon tension (upper trace) and intracellular activity of a central neuron (lower trace) are shown in response to a drop of water (arrow) on the siphon of a semi-intact preparation. The latency for contraction and the evoked activity are both approximately 100 msec.

In the intact animal the siphon withdrawal response habituated to repeated light stimulation and was dishabituated by presentation of the tactile stimulus (Fig. 1B). The withdrawal response in the intact animal also habituated to tactile stimulation and was dishabituated by the light stimulus. The response of the semi-isolated preparation habituated to either repeated tactile or light stimulation and could be dishabituated by each of the other stimuli as in the intact animal. The response of a semi-isolated preparation decreased with repeated tactile stimulation (Fig. 1A). The decremented response was dishabituated by the presentation of the light stimulus. The siphon nerve of this preparation was severed and the abdominal ganglion was removed. After a 60-minute rest, the now-isolated siphon habituated to repeated tactile stimulation and was dishabituated by light. The isolated siphon response also habituated to repeated light stimulation and was dishabituated by tactile stimulation or recovered with rest (Fig. 1C). These semi-isolated and isolated preparations exhibited the parametric characteristics of habituation (*14*). Our results show that the peripheral nervous system alone can mediate habituation and dishabituation of the siphon withdrawal response.

In order to study the contribution of the abdominal ganglion (central neurons) to habituation and dishabituation in the semi-isolated preparation, we employed a sucrose block to the siphon nerve to stop communication between the central and peripheral systems. The siphon was stimulated repeatedly with water drops, and the withdrawal response habituated. Then the sucrose block (*15*) was applied to the siphon nerve for 20 minutes while stimulation of the siphon continued. During the block the central neurons should recover with rest and the peripheral circuit should remain habituated. When the sucrose block was removed the siphon withdrawal response was enhanced. This indicated that central neurons are involved in siphon withdrawal. These central neurons do habituate and recover with rest and influence the peripheral circuits. Similar results have been used to demonstrate central habituation (*5, 16*). However, in this case we know that the isolated periphery will habituate as well. Therefore, there are two circuits that can mediate habituation and dishabituation.

The influence of the peripheral system on the central system was demonstrated in the isolated siphon preparation. Electrical stimulation of the distal siphon nerve evoked a withdrawal response and thus electrical stimulation could be used to mimic the contribution of central neurons to the siphon withdrawal. Stimulation at high voltages produces a maximum response that does not readily habituate, but stimulation at moderate or low voltages (near threshold) produces a response that does habituate (*17*). The habituated responses can be dishabituated by either light or tactile stimulation of the siphon, which shows that the peripheral system influences the peripheral terminals of the central system. Conversely, the habituated response to tactile or light stimulation of the periphery could be dishabituated by electrical stimulation of the siphon nerve (*17*). Responses of the siphon to stimulation of the siphon nerve near threshold voltages (Fig. 1D) were enhanced by higher frequency electrical stimulation (3X) or by tactile stimulation of the siphon (H_2O). This enhancement of a response by the interjection of a new stimulus could be produced by a mechanism such as heterosynaptic facilitation that has been demonstrated in *Aplysia* (*10, 18*). This enhanced response decayed with the same time course as habituation (Fig. 1D). Often the enhancement of the response was not fully developed until the second test stimulus was given. This kind of response has been reported before by Bruner and Kehoe (*5*) in the dishabituation of synaptic potentials in *Aplysia* neurons. Thus, the peripheral system can enhance the response elicited by the central neurons. These findings on the influences that each system exerts on the other suggests that the peripheral and central systems are part of one integrated system.

Extracellular recordings from the siphon nerve of an isolated siphon preparation indicated that afferent activity was evoked by light or tactile (Fig. 2A) stimulation of the siphon. Simultaneous recording of the siphon tension showed that the latency of contraction to tactile stimulation was approximately 150 msec. The evoked afferent activity occurred after a similar latency (Fig. 2A) and normally would have activated central neurons. Some of the later afferent activity evoked could be due to sensing of the peripheral contraction as well as a response to the tactile stimulation of the siphon.

Intracellular recordings from neurons in the abdominal ganglion of semi-isolated preparations during siphon

stimulation indicated that there are several types of excitatory and inhibitory synaptic responses. Some of these synaptic responses decremented to repeated stimulation of the siphon but were not definitely related to habituation of the siphon withdrawal. Figure 2B shows a central neuron that responded to tactile stimulation of the siphon. Action potentials in these cells were not preceded by synaptic potentials, which suggests that the spike was initiated in a peripheral process of the cell and conducted to the soma. This also suggests that the neuron is a central sensory cell with processes in the periphery. However, this cell could also have been activated by a synapse with a peripheral sensory neuron. The arrangement of central sensory cells has been noted before in *Aplysia, Spisula,* and in the leech (*9, 19*). The latency of siphon contraction in Fig. 2B was approximately 100 msec, and the neuronal action potentials were concurrent with the onset of contraction. Since contraction was initiated at the same time that information reached the central neurons, it seems likely that the contribution of the central neurons to the siphon response in the intact animal must occur no sooner than the contraction initiated by the peripheral system.

At this time we have evidence that there are neurons in the isolated siphon preparation, as there are in other peripheral structures of *Aplysia* (*7, 20*). The number of neurons in the peripheral and central systems that mediate the siphon responses is unknown as is the nature of the receptors for light and tactile stimuli. Our results show that the nervous system which supports the adaptive behavior of siphon withdrawal is composed of a central and a peripheral system. These two systems influence each other and are, therefore, parts of an integrated system. Kupfermann *et al.* (*11*) proposed that the peripheral and central systems which control the gill withdrawal response of *Aplysia* are independent systems, yet state in the same paper that the systems may interact. Our results show that the peripheral system alone is capable of mediating a considerable amount of adaptive response and that the interaction between the central and peripheral systems may be quite complex.

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13. The jet of seawater (2-second duration) was produced by a commercially available Water Pik. This has been used previously (*16*).
14. R. Thompson and W. Spencer, *Psychol. Rev.* **173**, 16 (1966). The data presented here show five of the nine parametric characteristics of habituation. These are: (i) response decrement, usually as an exponential decay; (ii) spontaneous recovery with rest; (iii) dishabituation; (iv) habituation of dishabituation; (v) habituation over series.
15. The sucrose block was arranged by positioning a glass pipette over the nerve to direct a stream of isotonic sucrose over the nerve. The block was complete in 4 minutes as verified by monitoring the electrical activity. Shutting off the sucrose flow unblocked the nerve in 30 seconds. A piece of tubing under the nerve siphoned off the used sucrose.
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Rat Origin of CHB Cells

Growing interest in neurobiology has created a great demand for established cell strains of neurological origin. Several established cell strains of both neuronal (*1*) and glial (*2*) origin have been provided. One glial cell strain, CHB, which was first reported by Lightbody *et al.* (*3*), has been derived from a human astrocytoma. We wish to warn the scientific community that existing cultures of CHB cells and lines derived from them, although possessing some differentiated characteristics of glial cells, are of rat origin and not human origin.

Cytological examination of the chromosome numbers and karyotypes of CHB cultures in several laboratories indicated that these cells are of rat origin. Furthermore, T. B. Shows (Roswell Park Memorial Institute) compared the starch gel electrophoretic patterns of three soluble enzymes (glucose-phosphate isomerase, aspartate aminotransferase, and malate dehydrogenase) present in extracts of CHB cells, WI-38 human cells, and rat kidney. The isozyme profiles confirmed that CHB cells were of rat origin. Cultures of CHB cells from several laboratories have been examined in hopes of discovering the human glial cell strain; however, no human strain has been found. The relation of CHB cells to other known rat glial cell strains has not been established, but cytological and biochemical differences indicate that CHB is not identical to the widely studied C-6 strain of rat glial cells (*2*).

Publications in which some or all of the results reported were obtained with CHB cells or derived cells are cited (*4*).

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