

# Interaction of Chemosensory, Visual, and Statocyst Pathways in *Hermisenda crassicornis*

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**ABSTRACT** Neurons in the cerebropleural ganglia (CPG), photoreceptors in the eye, optic ganglion cells, and statocyst hair cells of the nudibranch mollusk *Hermisenda crassicornis* responded in specific ways, as recorded intracellularly, to stimulation of the chemosensory pathway originating at the tentacular chemoreceptors as well as to stimulation of the visual pathway originating at the photoreceptors. Synaptic inhibition of photoreceptors occurs via the chemosensory pathway. The possible significance of such intersensory interaction is discussed with reference to preliminary investigation of the animal's gustatory behavior and possible neural mechanisms of behavioral choice.

## INTRODUCTION

Stereotypic behavior of gastropod mollusks has recently aroused considerable interest, particularly because of the possibility of identifying neural mechanisms underlying such behavior. Examples of stereotypic gastropod responses include gill withdrawal of *Aplysia* (Kupfermann and Kandel, 1969; Peretz, 1970); tentacle contraction of *Aplysia* (Bruner and Kehoe, 1970); escape response of *Tritonia* (Dorsett et al., 1969); feeding response of *Aplysia* (Frings and Frings, 1965; Preston and Lee, 1973; Jahan-Parwar, 1972; Kupfermann, 1974); phototaxis and the clinging response of *Hermisenda* (Alkon, 1973b, 1974); and inking in *Aplysia* (Carew and Kandel, 1977).

Another question of recent interest concerns choice behavior in response to simultaneous presentation of two or more stimuli, each of which alone elicits distinct stereotypic behaviors. Davis and Mpitsos (1971) investigated choice behavior of *Pleurobranchaea* using food, sexual, and/or gravitational stimuli. It also has recently been shown that there is clear synaptic interaction between the visual and statocyst pathways of *Hermisenda* (Alkon, 1973a, 1975a, b). This interaction may account for choice behaviors involving stereotypic, phototactic, and geotactic responses.

The nudibranch mollusk *Hermisenda* possesses, according to past work (Agersborg, 1922, 1925), little perception of distant food stimuli, but clear perception of food stimuli on contact. Food substances applied to the *Hermisenda* tentacle or the oral region elicit a stereotypic feeding sequence. This

consists of arousal, orientation, mouth opening, and consummatory action similar to that described for *Aplysia*. In the present study, we investigate the interaction of chemosensation with sensory perception mediated by the visual and vestibular pathways. We recorded intracellular neuronal responses to stimulation of the *Hermisenda* tentacles with palpable food substances, extracts thereof, or amino acids. Neurons investigated include those with specific loci on the cerebropleural ganglia (CPG), photoreceptors within the eyes, optic ganglion cells, and statocyst hair cells. Electrical as well as natural stimulation of the tentacular pathway produced synaptic inhibition of photoreceptors, optic ganglion cells, and hair cells. We will consider the possible physiologic significance of this intersensory synaptic interaction in relation to mechanisms of behavioral choice for this animal.

#### MATERIALS AND METHODS

##### *Electrophysiology*

*Hermisenda* were provided by Mr. Michael Morris of Sea Life Supply (Sand City, Calif.). In these experiments the circumesophageal nervous system was isolated with intact tentacles but no rhinophores. Two peripheral nerves (N.2 and N.3, see Russell, 1929) innervating the tentacle as well as the mouth remained intact. The isolated nervous system was placed in a perfusion chamber and was pinned with stainless steel needles. The tentacle was then separated from the circumesophageal nervous system by a thin plastic barrier. The tentacular nerves passed through a small groove on the lower border of the barrier. To prevent solutions on each side of the barrier from mixing, Vaseline was placed around the groove and the barrier boundaries. Thus, the chamber was divided in two; one compartment for bathing the tentacle contained ~1 ml and the other 2 ml of seawater (Fig. 1). The tentacle was constantly perfused with seawater (3–7 ml/min), which was obtained by cotton filtering seawater at Woods Hole, Mass. The same results were obtained by perfusion with artificial seawater ( $\text{Na}^+$ , 482.5;  $\text{Mg}^{2+}$ , 55;  $\text{Ca}^{2+}$ , 10;  $\text{K}^+$ , 10;  $\text{Cl}^-$ , 620;  $\text{HCO}_3^-$ , 2.5 mM/liter.)

For natural stimulation of chemoreceptors in the tentacle, solutions of amino acids, squid extract, and egg yolk ( $\geq 10^{-4}$  g/liter) were added to circulating seawater in a small reservoir connected to the bathing chamber by a polyethylene tube or directly into the bathing chamber at a rate of 0.5–4 ml/min, 0.05–1.0 ml at a time. Applications were spaced by at least 5 min to avoid receptor adaptation. Stock solutions of  $10^{-2}$  M glycine and DL-methionine were made and diluted with natural seawater.

For electrical stimulation of the tentacular nerves, the tentacle was cut, and the rostral cut ends of N.3 and/or N.2 nerves were dissected out 2–5 mm in length. The nerves were stimulated by applying brief electrical pulses (up to 50  $\mu\text{A}$ ) through a suction electrode.

Conditions of illumination and intracellular recording have been described in detail elsewhere (Alkon, 1973a, 1975c). Latency of electrophysiologic events was measured by photographs taken on a Kymograph camera (Grass Instrument Co., Quincy, Mass.). Other records presented here were made on a Brush recorder (Gould, Inc., Instrument Systems Div., Cleveland, Ohio).

##### *Behavior*

To investigate contact chemoreception, 15 animals were starved for 7 days and maintained at 12–14°C with a 12-h dark, 12-h light ( $6.3 \times 10^3$  ergs-cm $^{-2}$ -s $^{-1}$ ) cycle. Stimuli were filter paper (no. 1 Whatman) squares (0.5 cm $^2$ ) soaked either in seawater or in

squid mantle extract. Squid extract was prepared by blending 30 g of squid mantle muscle with 100 ml of seawater for 3 min, centrifuging at 5,000 rpm, and then ultracentrifuging for 1 h at 40,000 rpm. The supernate was then recovered for use as a chemical stimulus.

We applied filter paper squares to parts of the animal with a pair of forceps. We monitored the resulting behavior for 2 min. A positive feeding response, defined as a bite on the paper surface, was recorded as a plus. A withdrawal, defined as a turning away from the stimulus, was recorded as a minus. Any other behavior was recorded as zero.

To investigate distance chemoreception and the animals' behavior in a choice situation, each animal was placed in a  $22.5 \times 7.5$  cm Rubbermaid opaque container (Rubbermaid, Inc., Wooster, Ohio). Within each container was 350 cm<sup>3</sup> of seawater to a depth of 2 cm. For visual stimulation a spot of light ( $6.3 \times 10^8$  ergs-cm<sup>-2</sup>-s<sup>-1</sup>) was projected on the con-

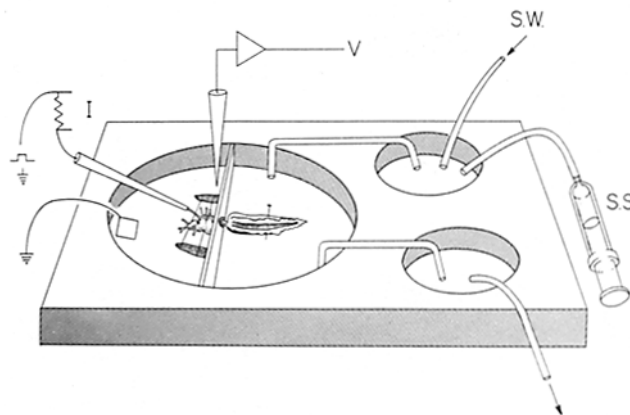


FIGURE 1. Chamber for tentacle perfusion and intracellular recording from *Hermisenda* nervous system. Bathing chamber is divided in two, one compartment contains ~2 ml and the other 1 ml of seawater. Each of two small reservoirs can accommodate 1 ml of solution and are connected by polyethylene tubes to bathing chamber. The tentacle was constantly perfused with seawater (S.W.) at the rate of 3–7 ml/min. Food solution (S.S.) was injected with syringe into the reservoir or in the bathing chamber. *I* and *V*; measurement of current and voltage, respectively.

tainer's bottom at one end. As a food stimulus, a piece (1 cm<sup>2</sup>) of squid mantle was placed on the container's bottom at one end. For the choice experiments, we placed animals ( $n = 10$ ) in the center of containers with light and food stimuli at opposite ends. For other experiments, animals were exposed to light alone ( $n = 10$ ) or to food alone ( $n = 20$ ). At the beginning of all experiments animals were placed facing one of the side walls so as to start in a neutral direction. Position of each animal was recorded after 10 and 20 min. If during testing an animal reached a piece of squid and began feeding, the food was removed. Thus all animals were essentially unfed during the testing period.

## RESULTS

### *Responses of Neurons to Natural Stimulation of Tentacle Chemoreceptors*

In the isolated circumesophageal nervous system of *Hermisenda*, large neurons ( $\geq 70$   $\mu$ m in diam) are located in the upper (cerebral) and lower (pleural) portion of the CPG (Detwiler and Alkon, 1973). In contrast, there are many

small ( $\leq 30 \mu\text{m}$  in diam) and middle-sized neurons in the central region of the ganglia. Small neurons on the ventral side of the CPG responded to natural stimuli (squid extract, egg yolk) with numerous excitatory synaptic potentials (EPSP's; see Materials and Methods for details of stimulus conditions). These EPSP's summated with higher concentrations of stimulus solutions to cause steady membrane depolarization and increased impulse activity (Fig. 2). Glycine solutions ( $\geq 10^{-8}$  M) and solutions of DL-methionine ( $\geq 10^{-10}$  M) also caused EPSP's and depolarization of ventral CPG neurons (Fig. 3). A few of the neurons studied in this ventral area and on the dorsal side (5/100) responded with hyperpolarization to light (Fig. 4).

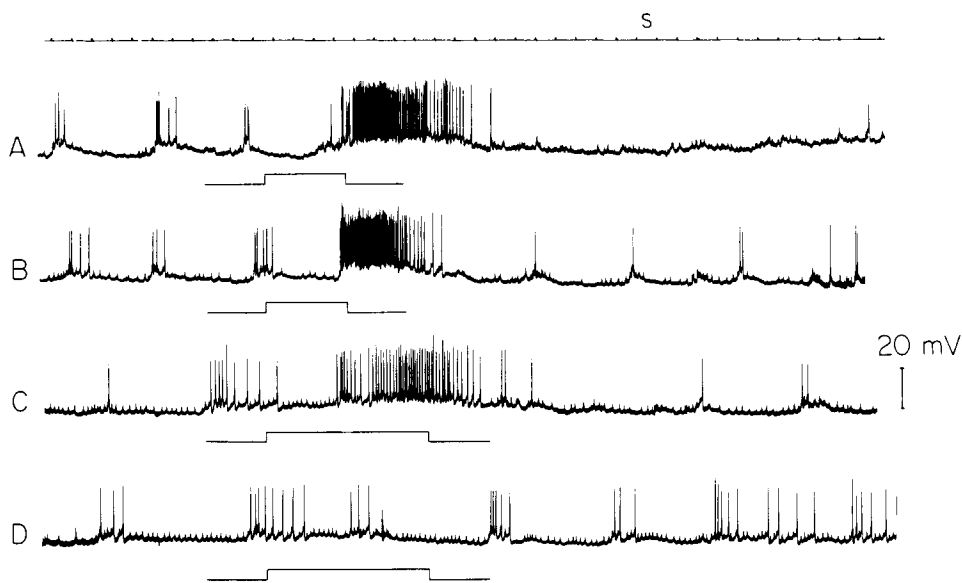
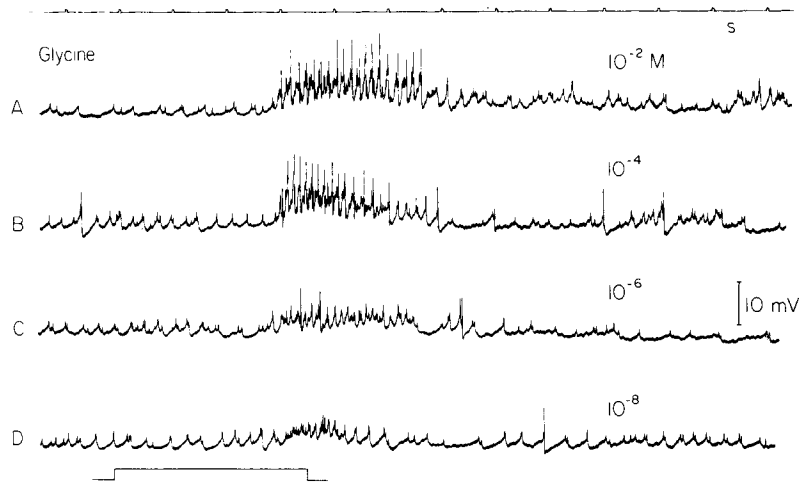


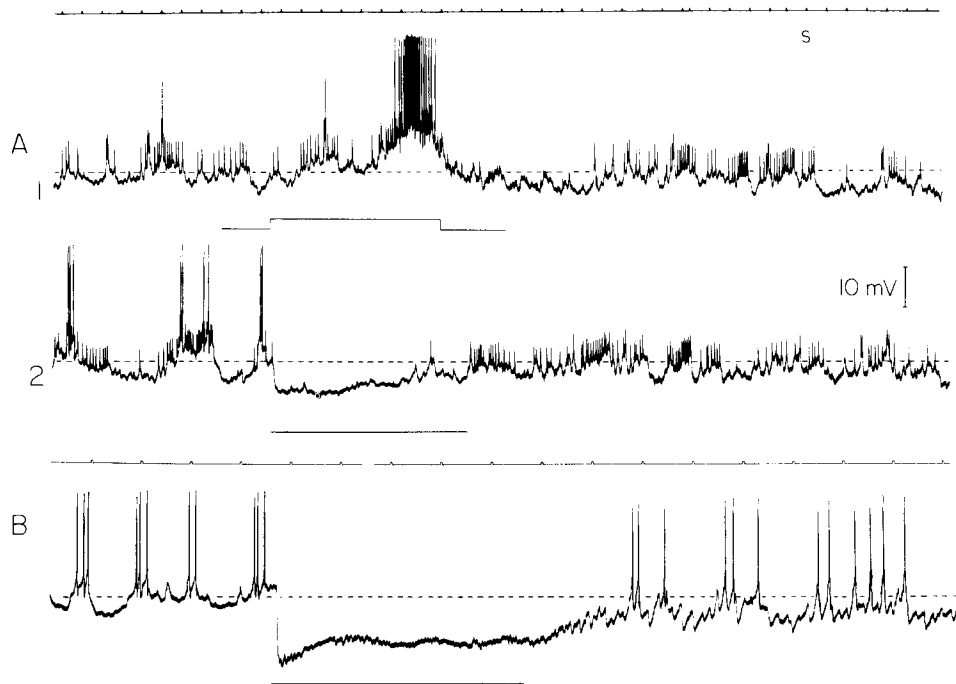
FIGURE 2. Excitation of a chemosensory interneuron in the CPG in response to egg yolk solution. (A) 0.1 ml of egg yolk solution, undiluted, was injected into the reservoir during the time indicated by the monitor signal beneath the recording trace. (B) 0.1 ml,  $10^{-3}$  dilution; (C) 0.2 ml,  $10^{-5}$  dilution; (D) 0.2 ml of seawater as control.

Type B photoreceptors in the eyes of *Hermisenda* hyperpolarized in response to natural tentacle stimulation. Spontaneous as well as light-induced impulse activity of type B photoreceptors could be reduced or eliminated by such stimulation (Fig. 5). Type A photoreceptors, unlike type B cells, were unresponsive to natural stimulation of the tentacle (Table I). The two type A photoreceptors in each eye have been previously distinguished from the three type B cells by features of morphology, impulse generation, light sensitivity, and synaptic interaction (Alkon, 1975a).

Touch receptors in the tentacle (Agersborg, 1925) might also respond to perfusion with food and chemical substances. To eliminate this possibility, we touched the tentacle at several points along its length with a silver wire attached to a piezoelectric device (Alkon and Bak, 1973). Such light pressure produced no observable responses in the photoreceptors.



**FIGURE 3.** Excitation of chemosensory interneuron within the CPG in response to glycine. (A) 0.1 ml of  $10^{-2}$  M glycine was injected into the reservoir during the time indicated by the monitor signal beneath the recording trace. (B)  $10^{-4}$  M. (C)  $10^{-6}$  M. (D)  $10^{-8}$  M.



**FIGURE 4.** Responses of CPG neurons to egg yolk solution and to light stimuli. (A) 1, Depolarization and superposed impulse activity in response to egg yolk solution, 0.2 ml,  $10^{-6}$  dilution, applied as indicated in the lower trace. 2, Hyperpolarization of the same cell in response to light flash (intensity:  $\sim 10^3$  ergs-cm $^{-2}$ -s $^{-1}$ ), indicated by monitor traces beneath intracellular record. (B) Another CPG neuron which responded to light but not to food stimuli. Light (intensity:  $\sim 10^3$  ergs-cm $^{-2}$ -s $^{-1}$ ), indicated by monitor trace beneath intracellular record. Dashed line shows approximate level of resting membrane potential.

Natural stimulation of the tentacle also eliminated spontaneous inhibitory synaptic potentials (IPSP's) recorded from hair cells in the caudal portion of each of the two statocysts (Fig. 6). These IPSP's were previously shown to be eliminated by a light step as well as by type B impulse trains (Alkon, 1975c). They probably arise from ipsilateral optic ganglion cell firing since spontaneous impulse activity of optic ganglion cells is also eliminated by impulse trains in ipsilateral type B photoreceptors (Alkon, 1973b). The results suggested, then, that optic ganglion cells also receive inhibition from the chemosensory pathway.

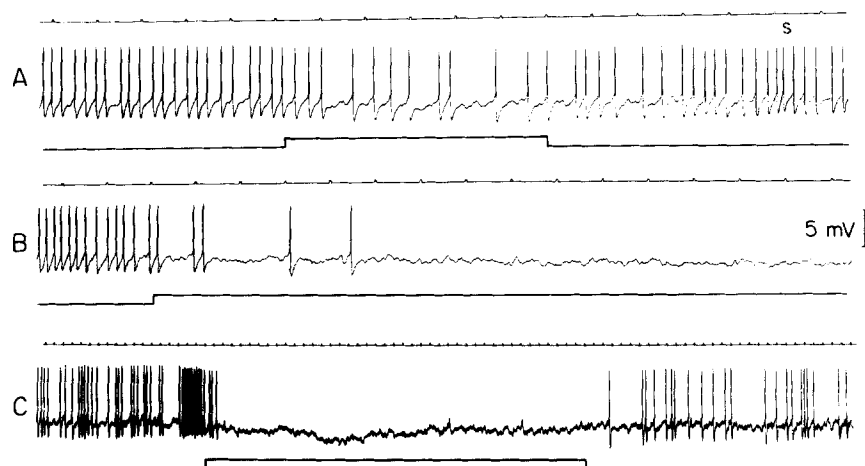


FIGURE 5. Inhibition of impulse activity in a type B photoreceptor cell in response to natural stimulation of the tentacle during dim illumination. Squid extracts were applied as indicated in lower traces, 0.2 ml (A), 0.6 ml (B), and 0.8 ml (C). In (B) and (C), hyperpolarizing current ( $-1.12$  nA) was applied to reduce impulse activity during constant illumination (intensity:  $\sim 10^2$  ergs-cm $^{-2}$ -s $^{-1}$ ).

TABLE I  
EFFECTS OF FOOD AND ELECTRICAL STIMULATION OF CHEMOSENSORY PATHWAY ON PHOTORECEPTOR CELLS IN THE EYE, STATOCYST HAIR CELLS, AND CPG NEURONS

Neural cells	Responses to food stimulation of tentacle	No.*	Responses to electrical stimulation of tentacle nerve	No.*
Type B photoreceptor	Inhibition of impulse activity	7/21	IPSP's	35/35
Type A photoreceptor	Inhibition of impulse activity	0/7	IPSP's	6/9
Statocyst hair	Inhibition, disinhibition, or facilitation	1/6	IPSP's, disinhibition, or facilitation	5/10
Neurons in the CPG	Excitation with or without delayed inhibition	11/26	EPSP's or IPSP's	44/55

\* No. of cells responding/no. of cells tested.

*Responses of Neurons to Electrical Stimulation of the Tentacle Nerves*

Small neurons and middle-sized neurons on the ventral CPG depolarized and increased impulse activity in response to electrical stimulation of the ipsilateral tentacular nerves (Fig. 7 A and Table I). These responses occurred with a latency of  $\sim 10$  ms (Fig. 8 B). The responses had a clear threshold and followed without failure to repetitive stimulation (20 Hz). These neurons were located in the same group of neurons which responded to food stimulation with increased impulse activity (Fig. 2).

Other small neurons, located mainly on the dorsal side of the CPG, hyperpolarized in response to electrical stimulation of the ipsilateral tentacular nerves (Fig. 7 B). These responses occurred with a longer latency of  $\sim 20$  ms (Fig. 8 C).

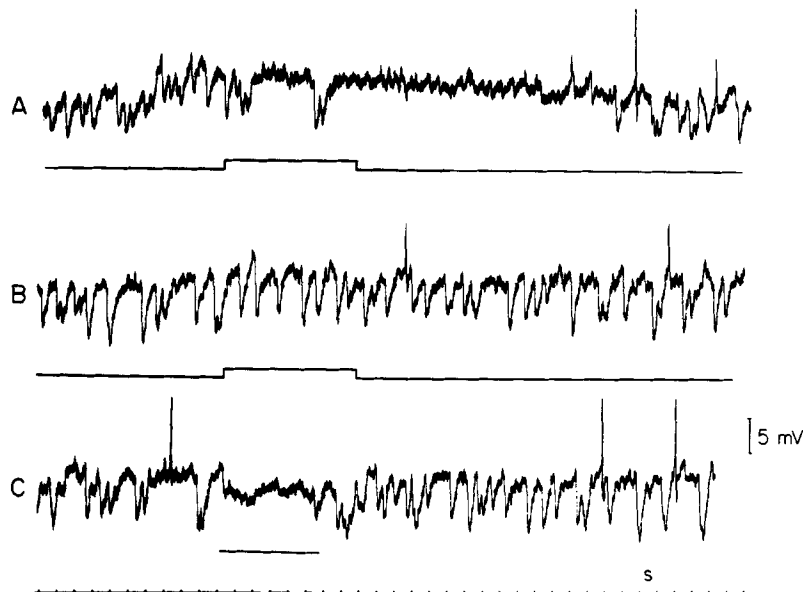


FIGURE 6. Disinhibition of hair cells in response to food or light stimuli. (A) Spontaneous IPSP's were suppressed by perfusion of the tentacle with squid extract (0.2 ml, as indicated in the lower trace). (B) Control of 0.2 ml seawater. (C) IPSP's were also suppressed in response to light (bar), intensity:  $10^8$  ergs-cm $^{-2}$ -s $^{-1}$ .

Perfusion of the nervous system with 20 mM CoCl $_2$  reversibly eliminated both the depolarizing and hyperpolarizing neuronal responses to electrical stimulation of the tentacles. This concentration of CoCl $_2$  was found in the past to reliably eliminate synaptic interactions in *Hermisenda* without significantly altering electrical characteristics of the neuronal membranes (Detwiler and Alkon, 1973; cf. Weakly, 1973).

Still other neurons (between 30 and 70  $\mu$ m in diam) in the CPG responded with antidromic firing to tentacular nerve stimulation (Fig. 8 A). This antidromic firing occurred with a latency of 2–5 ms and followed without failure repetitive stimuli up to 100 Hz. The axons of these neurons, then, probably course within

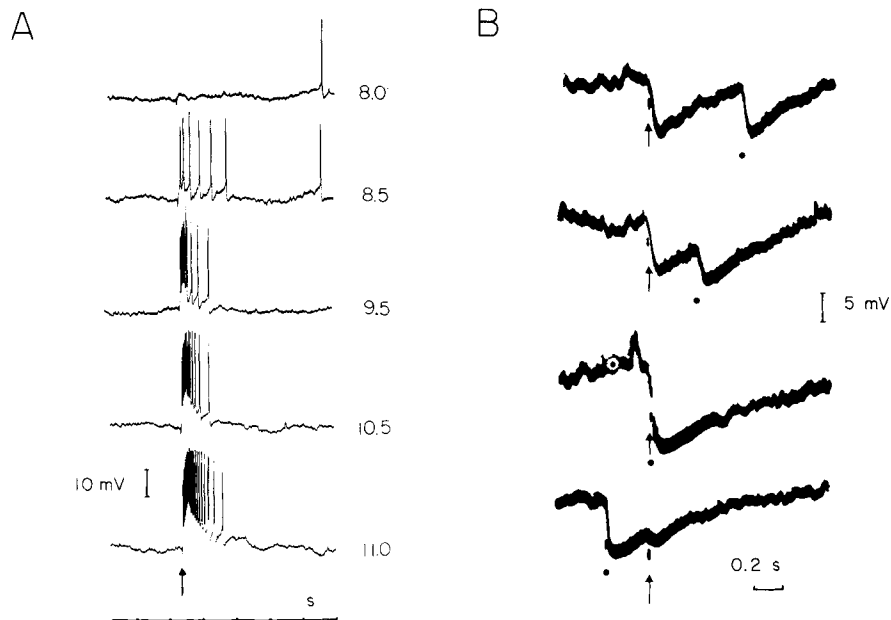


FIGURE 7. Excitation and inhibition of CPG neurons in response to tentacular nerve stimulation. (A) Excitation. Intensity of stimulus current ( $\mu\text{A}$ ) is indicated on the right side of each trace. (B) IPSP's in response to N. 2 (filled circle) and N. 3 (arrow) stimulation. Electrical stimulus (duration, 5 ms) is indicated by arrow. Each of the responses illustrated was recorded from different neurons.

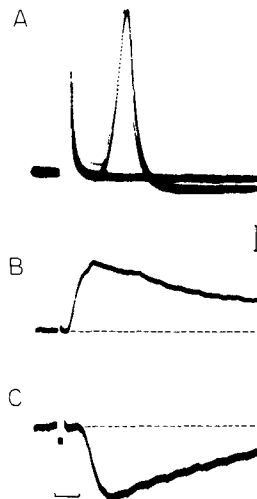


FIGURE 8. Response latency of CPG neurons with tentacular nerve stimulation. (A) Antidromic firing. (B) EPSP. (C) IPSP. N.2(A) and N.3 (B and C) were stimulated by 1-ms pulse (A and B), 5-ms pulse (C). Calibration, 10 mV for (A) and (B), 5 mV for (C); 2 ms for (A), 20 ms for (B) and (C). Each of the responses illustrated was recorded from different neurons.



the tentacular nerve. These cells could receive input from the tentacular chemoreceptors and, in turn, produce via synaptic interaction, the responses recorded in other CPG neurons described above. However, since these neurons had weak synaptic responses to electrical as well as natural stimuli, they are more likely within the efferent rather than the afferent part of the tentacular chemosensory pathway.

Type B photoreceptors hyperpolarized (5–15 mV) in response to electrical stimulation of the ipsilateral tentacular nerves (Fig. 9A). The latency of this hyperpolarization was  $\sim 18$  ms. Membrane conductance, measured by applying hyperpolarizing current pulses, increased during the hyperpolarization (Fig. 9B). For these measurements, we inserted two electrodes into the same type B

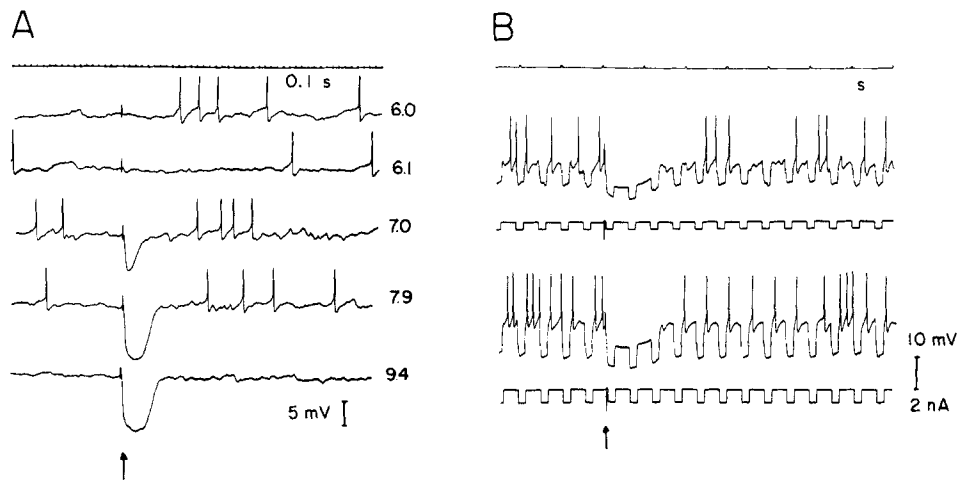


FIGURE 9. Inhibition of type B photoreceptors in response to electrical stimulation of tentacular nerve. (A) IPSP's. Intensity ( $\mu\text{A}$ ) of stimulus current (duration, 5 ms) is indicated on the right side of each trace. (B) Membrane conductance change during IPSP's. Two microelectrodes were inserted into the same type B photoreceptor; one for current injection, the other for measuring membrane potential. Traces beneath recordings indicate hyperpolarizing current pulses, 0.4 nA and 0.8 nA.

photoreceptor simultaneously. One electrode provided a means of current injection, the other of recording membrane potential. The hyperpolarizing response of the type B photoreceptor reversed when the cell's membrane potential was shifted to more negative levels with steady current injection. The response also reversibly disappeared during perfusion with 20 mM  $\text{CoCl}_2$ . The hyperpolarization fatigued substantially with repetitive stimulation of the tentacular nerve (Fig. 10B). Taken together, these observations demonstrated the synaptic basis of the hyperpolarizing response of type B photoreceptors. This synaptic inhibition was usually of sufficient magnitude to significantly reduce photoreceptor impulse activity elicited by a light stimulus (Fig. 11).

Electrical stimulation of ipsilateral tentacular nerves also reduced the fre-

quency of spontaneous EPSP's (Fig. 10 A) in type B photoreceptors (Alkon, 1976a). Thus, type B photoreceptors received direct synaptic inhibition from the chemosensory pathway and indirect inhibition through the reduced frequency of spontaneous EPSP's. All of these effects on type B cells occurred in response to stimulation of either N.2 or N.3, but not N.4 or N.1 (the rhinophore nerve).

Unlike type B cells, all of which received IPSP's (5–15 mV), only some of the type A photoreceptors (60%) showed small hyperpolarization (2–5 mV) in response to tentacular nerve stimulation (Table I). Latency, membrane conductance change, and reversal potential of this hyperpolarization were approximately the same as measured in type B cells (Fig. 12).

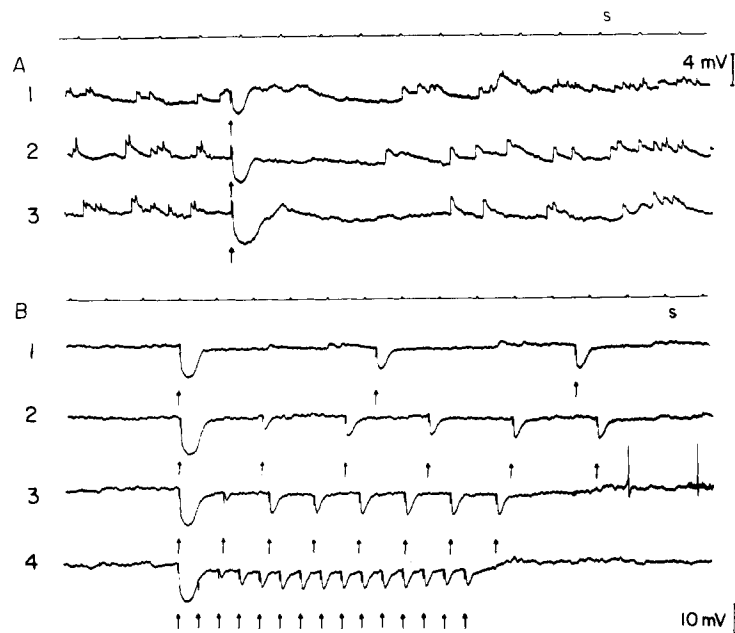


FIGURE 10. Responses of type B photoreceptors to tentacular nerve stimulation. (A) Spontaneous EPSP's were suppressed by electrical stimulation (5 ms) of tentacular nerves. 1, 11.5  $\mu$ A. 2, 13  $\mu$ A. 3, 18  $\mu$ A. (B) Decrease in size of IPSP's by repetitive stimulation (18  $\mu$ A) of tentacular nerves.

In the optic ganglion cells, electrical stimulation of the ipsilateral tentacular nerves produced marked hyperpolarization and cessation of impulse activity. This hyperpolarization occurred with a latency of  $\sim$ 18 ms and fatigued easily with repetitive stimulation (0.1–1.0 Hz). These results are consistent with the explanation that disinhibition of hair cells by natural stimuli (Fig. 6) arises as a consequence of inhibition of impulse activity of the optic ganglion and type B cells.

Hair cells, in response to electrical stimulation of the ipsilateral tentacular nerves showed three types of responses: inhibition, disinhibition, and inhibition followed by facilitation (Fig. 13). The facilitation could be attributed, at least in

part, to inhibition of photoreceptor cells and optic ganglion cells. Thus, for some hair cells, both light and food stimuli evoked similar responses.

#### *Chemosensory Behavior*

Of 15 animals tested, none responded positively (by biting at a test paper square) to tentacle or mouth contact with a filter paper soaked in seawater (Table II). All animals responded positively to tentacle or mouth contact with a

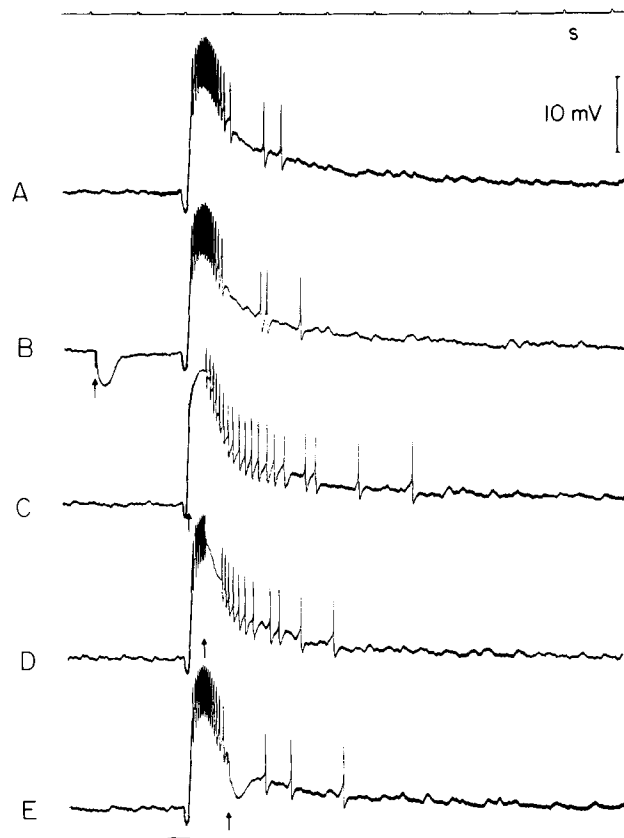


FIGURE 11. Modification of type B photoreceptor responses to light flash by tentacular nerve stimulation. (A) Response to light flash, intensity:  $10^3 \text{ ergs-cm}^{-2}\text{-s}^{-1}$ , indicated by bar in the bottom trace. (B) Electrical stimulation, 5 ms,  $15 \mu\text{A}$ , of the tentacular nerve (as indicated by arrow) preceded light flash. (C-E) N. 3 nerve was stimulated during response to light flash. Note that tentacle stimulation can reduce impulse activity of a type B cell in response to light.

filter paper soaked in squid extract. A positive response rarely resulted from rhinophore or tail contact with a paper soaked in squid extract (Table II). Positive responses with tail contact were mediated by the tentacle. Thus, tail contact resulted in the animal turning and exploring the filter paper square with the tentacles. Similarly the occasional withdrawal response encountered with a control stimulus applied to the tail followed tentacle exploration.

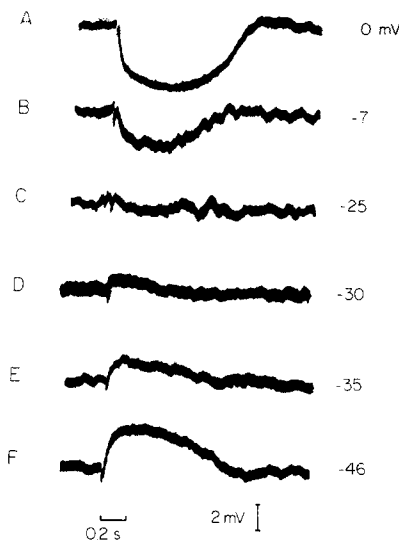


FIGURE 12. Reversal of IPSP's in a type A photoreceptor. IPSP's were evoked by stimulation, 5 ms,  $15 \mu\text{A}$ , of N. 3 nerve. Membrane potential was hyperpolarized from resting level (as indicated on the right side of each trace) by injecting steady negative current through the recording electrode.

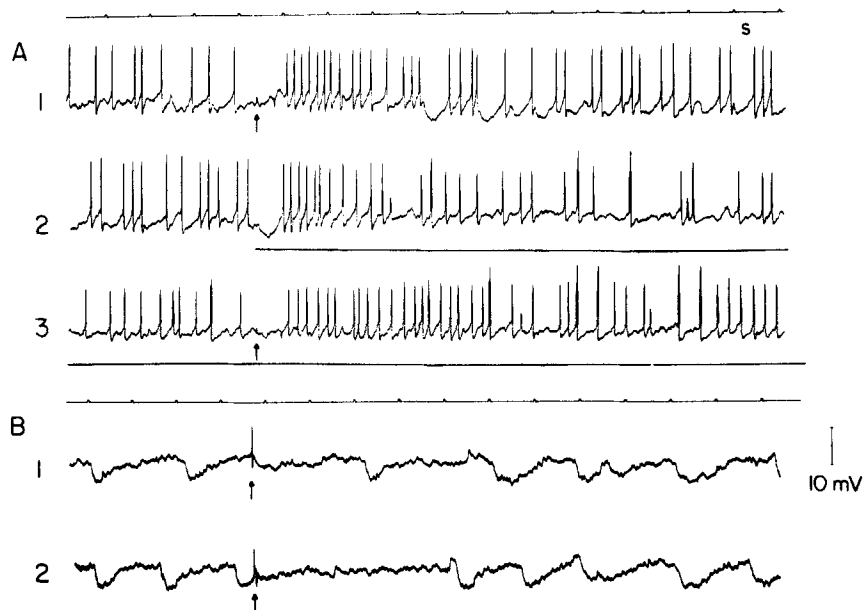


FIGURE 13. Responses of hair cells to electrical stimulation of tentacular nerve and to light flash. (A) Inhibition followed by facilitation of impulse activity was evoked by stimulation, 5 ms,  $15 \mu\text{A}$ , of tentacular nerves (stimulus indicated by arrow, 1) and by light flash, intensity:  $10^9 \text{ ergs}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$  (bar, 2), 3. During response to light, tentacular nerve was stimulated. Durations of current pulses were 400 ms (1) and 500 ms (3). (B) Spontaneous IPSP's in another hair cell were suppressed by electrical stimulation of the tentacular nerve (onset indicated by arrows). 1, 5 ms. 2, 50 ms pulse durations.

Additional animals (see Materials and Methods) were subjected to one of three treatments. First, a choice situation was established, with food at one end and a light spot at the other end of rectangular containers. In this situation, animals at first moved predominantly toward the light which was at maximum intensity 4.5 cm distant from the initial test position. Throughout the 7 days of testing, with progressive starvation (Fig. 14 A) more animals moved toward the food and away from the light ( $P < 0.05$ , as tested by a  $t$  test linear regression analysis; see Winer, 1962). However, the same animals did not make the same choice from day to day. Other animals were presented with a light spot alone. Over 7 days of testing with light, the response to light did not change significantly with time (Fig. 14 B) as tested by a linear regression analysis. For the third treatment, food alone was presented. As the animals were progressively starved over the 7 days of testing, the number of responses to the food (Fig. 14 C) increased significantly ( $P < 0.05$ , as tested by a linear regression analysis).

TABLE II  
CONTACT CHEMORECEPTION

Stimuli	Response
Seawater on filter paper	
Mouth	0
Tentacles	0
Rhinophores	0
Dorsal part of tail	2(-)*
Squid extract on filter paper	
Mouth	15(+)
Tentacles	15(+)
Rhinophores	2(+)
Dorsal part of tail	3(+)

$n = 15$ ; Values in table indicate number of animals responding to contact stimulation.

(+) = Bite taken from filter paper.

(-) = Withdrawal and no bite.

\* = Subsequent touch with tentacle and no bite.

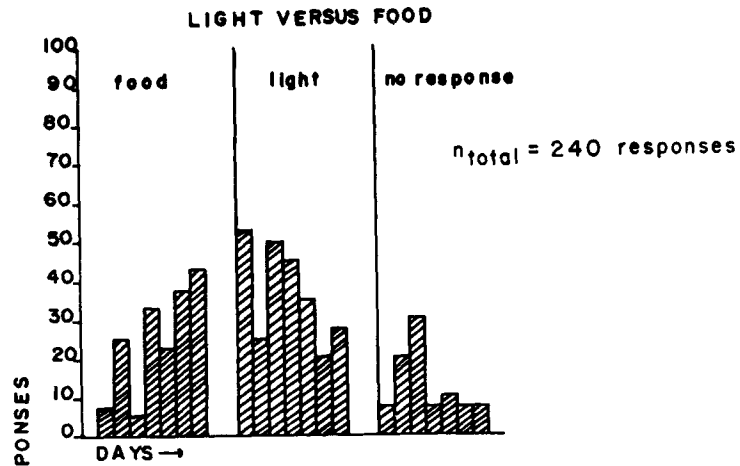
When the 7 days of data are summed for each treatment, the majority of responses (50–60%) are seen to be directed toward light or food when either is presented alone. When presented together, about 36% of the responses are directed toward the light and 25% toward the food. The number of responses to light alone was significantly different from that to light when presented in a choice situation with food (chi-square test,  $P < 0.01$ ,  $df = 1$ ).

#### DISCUSSION

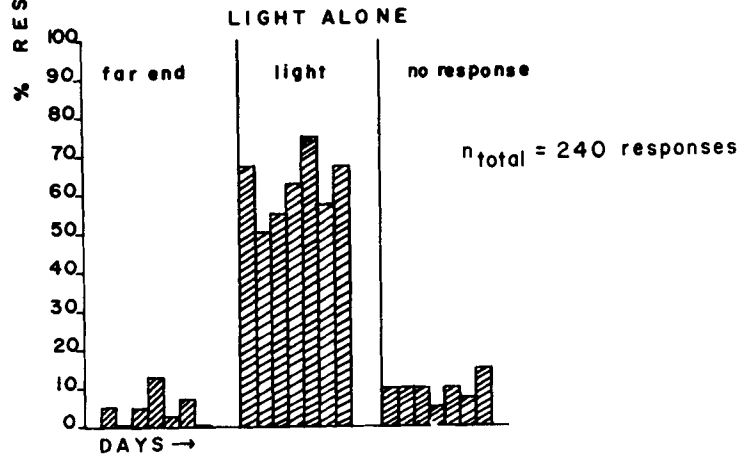
Our behavioral observations confirmed Agersborg's findings (1922, 1925) that the oral tentacles and mouth region of *Hermisenda* are highly sensitive to palatable foods, while rhinophores possess little or no taste function. Agersborg further states, however, that even hungry animals did not respond to distant food stimuli (~1 cm). In our laboratory, animals, starved for several days, quickly find food >4.5 cm distant.

We also showed in the present study that neurons in the CPG respond with depolarization or hyperpolarization to natural and electrical stimulation of the

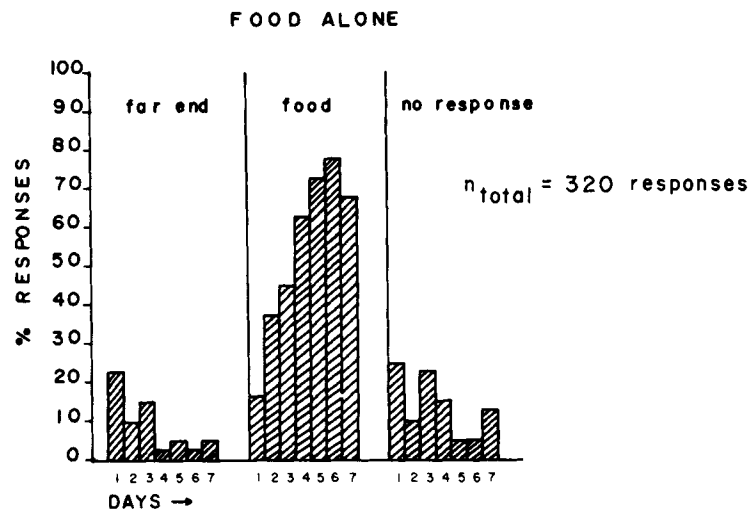
A



B



C



chemosensory pathway. It seems reasonable that chemosensory tentacular fibers have direct excitatory synaptic actions on CPG neurons since increasing concentrations of food stimuli caused graded increases in depolarization of CPG neurons. Furthermore, electrical stimulation of tentacular nerves produced, in these neurons, depolarization of short and fixed latency ( $\sim 10$  ms). Hyperpolarization of CPG neurons, however, occurred after electrical stimulation of the tentacle with a greater delay ( $\sim 20$  ms). Hyperpolarization of CPG neurons, then, as well as photoreceptors and optic ganglion cells, more likely represents synaptic inhibition from other CPG neurons which receive direct excitation from chemosensory tentacular fibers (Fig. 15). As shown in Table I, neuronal responses to electrical stimulation of the tentacle nerve were larger and more

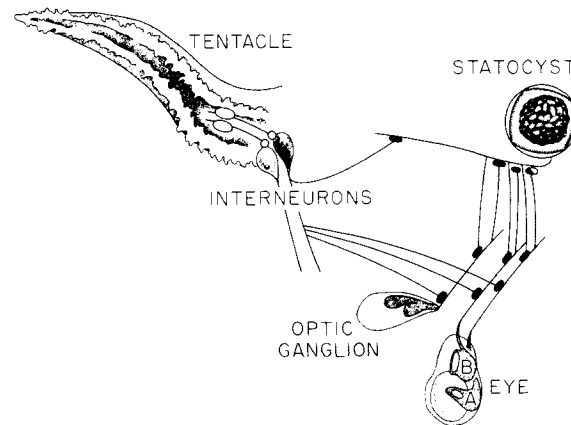


FIGURE 15. Diagram of interaction of chemosensory, visual, and statocyst sensory systems. Closed circles indicate inhibitory presynaptic endings. Open circles indicate excitatory presynaptic endings. Half-closed circle indicates a presynaptic ending with a synaptic inhibitory effect combined with a nonsynaptic excitatory effect.

frequently observed than neuronal responses to natural tentacular stimulation. This is not unreasonable, since more nerve fibers are fired synchronously and maximally (including those fibers from the mouth parts) by electrical stimulation than by natural stimulation of the tentacle. Thus, type B photoreceptors were always hyperpolarized in response to electrical stimuli, but only 30% responded to natural stimulation of the tentacle. The frequency of finding chemosensory interneurons using natural stimuli was  $\sim 40\%$  as compared to 80% with electrical stimulation.

When the three sensory systems (visual, vestibular, and chemosensory) are compared, differences are apparent. First, the tentacles and mouth parts are large, probably containing hundreds of receptors, whereas the eyes and

FIGURE 14 (*opposite*). (A) Percent responses directed toward food or light spot,  $n = 10$  animals, 7 days of testing. (B) Percent responses directed toward or away from light spot alone,  $n = 10$  animals, 7 days of testing. (C) Percent responses directed toward food alone,  $n = 20$ , 7 days of testing.

statocysts are small. Each eye contains five photoreceptors and each statocyst contains 12 hair cells. The tentacles are external structures which can be oriented while the eyes and statocysts are internal structures which are fixed adjacent to the circumesophageal nervous system. Our results also indicate that the effect of afferent tentacular fibers on CPG neurons is excitatory while these CPG neurons receive inhibitory input from afferent visual fibers. Finally, the number of CPG neurons which responded to tentacular stimulation was large while few CPG neurons responded to visual stimuli.

These differences (structural, receptor number, synaptic effects, and number of higher order neurons) suggest that the chemosensory pathway is more developed than either the visual or vestibular systems. Thus, a greater portion of the nervous system and its receptor organs appears to be responsible for chemosensation than for vision or gravitational sensation. This might also be reflected in a dominance, under specific environmental conditions, of chemosensory behavior over visual and vestibular responses. The findings of this study demonstrated that a conflict of choice occurs when visual and chemosensory stimuli are presented simultaneously. With starvation, the animal's response to food progressively predominates over the response to light (Fig. 14). This dominance may result from increased inhibition of photoreceptors and optic ganglion cells by the chemosensory pathway. Possible neural mechanisms responsible for this dominance, of course, range from changes in chemoreceptor adaptation to facilitation of synapses within the chemosensory pathway.

The intersensory interaction between sensory modalities identified in this study might also provide a neural basis for a behavioral hierarchy such as has been described for related species (cf. Davis, 1971). This hierarchy would also be expected to depend on environmental conditions such as food scarcity, oceanic turbulence, etc. Such intersensory interaction might also allow for specific neural and behavioral modification after repetitive sensory pairing, as has already been demonstrated for paired visual and vestibular stimuli (Alkon, 1976a, 1976b). Vertebrate hair cells also receive efferent input from the central nervous system (Wersäll and Flock, 1965). The chemosensory inhibition of photoreceptor in *Hermisenda*, however, might simply reflect the need for multiple neuron functions in a primitive nervous system which has an insufficient neuron number for the more specialized function possible in vertebrates (cf. Alkon, 1976c).

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