Function of an Eyestalk Ganglion, the *Medulla Terminalis*, in Olfactory Integration in the Lobster, *Panulirus argus**

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Summary. Ipsilateral antennular dysfunction resulting from total unilateral eyestalk ablation in spiny lobsters does not occur when visual input is restricted by an opaque cap over one eyestalk, or when optic ganglia alone (eg. *lamina ganglionaris, medulla externa, medulla interna*) are removed. Antennular dysfunction appears only when connections between the most proximal of the four eyestalk ganglia, the *medulla terminalis*, and the remainder of the cerebral ganglia (brain) are interrupted. We conclude that neural processing of olfactory input from the antennule involves structures in the *medulla terminalis*.

Introduction

Four highly differentiated ganglia occur in the eyestalks of most decapod Crustacea. The most distal presumably receive and process visual input, and are accordingly termed the optic ganglia. The fourth and most proximal ganglion mass, the *medulla terminalis*, is more heterogeneous, receiving extensive input from both higher order visual interneurons and from other regions of the brain, including the olfactory and accessory lobes of the deutocerebrum (MAYNARD, 1965). Although regarded as important, the exact role of eyestalk ganglia in normal non-visual brain function has remained largely a matter of conjecture with little experimental evidence.

In the Caribbean spiny lobster, *Panulirus argus* LATREILLE, MAYNARD and DINGLE (1963) showed that the initiation of feeding normally caused by olfactory antennular stimulation is abolished ipsilaterally upon removal of one eyestalk with its contained ganglia. Later experiments of EISENSTEIN and MILL (1965) suggest that learning in crayfish with food as a positive reward is disturbed by a similar operation. Neither study, however, attempted further localization of the critical region in the eyestalk ganglia.

The experiments reported in this paper show that antennular ol-
factory dysfunction in *Panulirus argus* does not occur following removal
of the optic ganglia alone, but does appear after subsequent ablation
of the *medulla terminalis* (including the hemiellipsoid body). The hypo-
thesis that the *medulla terminalis* is critical for normal olfactory in-
formation processing in the brain is thus supported.

Methods

Animals. Sixteen adult *Panulirus argus* Latreille of both sexes were chosen
from a stock collected by local fishermen and maintained in live cars or large
holding tanks with running sea water at the Bermuda Biological Station. During
the course of observations, animals were isolated and maintained in individual
aquaria with glass fronts and backs; in a few instances larger aquaria containing
two experimental lobsters were used. Over the course of observations, which in
some cases lasted up to 7 weeks, lobsters either fasted or were maintained on
minimal diets of fish.

Immediately after transfer from the holding tanks to individual aquaria, many
of the lobsters showed reduced feeding activity, or reduced responses to chemical
stimuli in the presence of a observer. During this period unresponsive animals
displayed avoidance or defensive activity upon the appearance of the investigator
in front of the observation aquarium. According to the individual lobster, periods
of from a few hours to a few days were required for habituation to the experimental
environment. Experimental manipulation was not begun until active feeding re-
sponses occurred regularly.

Test Procedure. In the usual test procedure a stimulus swab soaked in fish
juice was rapidly touched to the terminal, aesthetask-bearing portion of the outer
flagellum of one antennule (Laverack, 1964). The response was noted and the
swab then touched to the other antennule, sometimes after being resoaked in fish
juice. In many instances, particularly if antennular stimulation was ineffective, the
swab was touched to a parieopod dactyl also. A series of stimuli with a control sea
water swab preceded or followed the fish juice series. With a few exceptions,
lobsters were tested with fish juice no oftener than once or twice a day; on some
occasions several days elapsed between tests.

Responses to experimental stimuli were considered positive when antennular
stimulation caused immediate movement toward the stimulus locus and reaching,
grasping movements of the first two pairs of parieopods (Maynard and Dingle,
1963). Usually such responses were very obvious. In negative responses no move-
ment toward the stimulus occurred, only retreat or avoidance; or parieopod dactyl
grasping began several seconds after contact between the stimulus swab and
flagellum, suggesting convection of the stimulus to chemoreceptors on the dactyls.
This latter response did not involve the directional grasping characteristic of imme-
diate responses to antennular stimulation. Flagellar cleaning responses or directional
antennular movements were sometimes noted when stimuli did not evoke feeding
responses (Maynard and Dingle, 1963), but these abortive responses were counted
as negative in this study.

Stimulus. The olfactory stimulus, "fish juice", was made by macerating small
anchovies in sea water (Maynard and Dingle, 1963). In our experience, stimulus
localization was most easily and consistently obtained by soaking a small cloth
swab attached to a glass rod in fish juice, and then rapidly applying it to the
desired site of stimulation. If only responses occurring within the first few seconds

of stimulation are counted, artifacts caused by stimulus diffusion or convection to other receptor sites (eg. parieopod dactyls) are minimized. The few occasions in which the locus of the effective stimulus was ambiguous were discarded from further consideration. Undoubtedly the applied stimulus often combined touch and olfaction, rather than olfaction alone, but for the present study this is irrelevant.

An identical swab soaked with sea water was used regularly with the test stimuli to control for mechanical or visual stimulation alone. The control swab never evoked any portion of the feeding response.

Recording. Observations were usually dictated into a portable tape recorder at the time of the experiment and later transcribed. Two experimental lobsters were
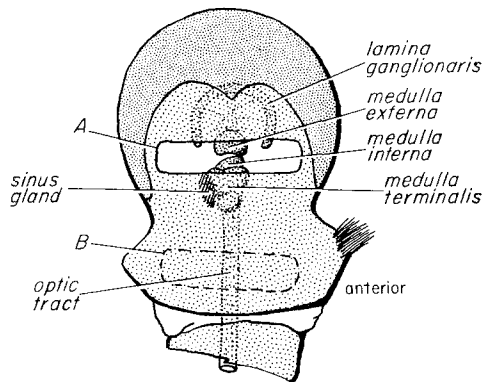

Fig. 1. Dorsal view of left eyestalk of *Panulirus argus*, anterior to right. *A*. Size and location of opening made in cuticle for optic ganglion ablation. *B* Approximate location of opening made in cuticle for optic tract transection

photographed with a Bolex 16 H movie camera at 32 frames per second for detailed analysis of response sequences.

Operative Procedures. Operative procedures never lasted more than a few minutes, and during them the lobster was restrained, unanaesthetized in air.

1. Blindfolding. Single eyestalks were capped with the tied mouth-ends of colored rubber balloons, or short lengths of black, opaque rubber tubing. Weak diffuse illumination was visible through the balloon rubber cap, the tubing caps were completely light-tight.

2. Eyestalk ablation. Eyestalks were cut off between the first and second segments, and the proximal stump briefly clamped to facilitate clotting. Haemolymph loss was minimal.

3. Optic Ganglion ablation. A horizontal slit was made in the dorsal cuticle of the eyestalk just above the junction of *medulla terminalis* and *medulla interna* (Fig. 1). Scissors inserted into the slit then separated all distal from the proximal ganglion or ganglia. The slit was filled with a compression pad of tissue paper to reduce bleeding. Within two or three days, all tissue distal to the cut degenerated.

4. Optic Tract transection. The same procedure was followed, except the slit in the distal eyestalk segment was proximal to the *medulla terminalis*.

5. Control operations. Cuticle and hypodermis were opened as above, and some bleeding permitted. Ganglia and nerves were not cut. The wound was filled with a compression pad.

Lobsters survived all operations. Where possible the less extensive operations, eg. blindfolding, optic ganglion ablation, control operations, were followed after an observational period ranging from 7 to 48 days by total unilateral eyestalk removal. Effects of eyestalk ablation were immediate, and as shown in a previous paper, permanent (Maynard and Dingle, 1963).

Histological Control. Eyestalks of operated animals were opened and examined for gross morphological structure after an observation series was completed. They were then fixed in Bouins and later embedded and sectioned for histological examination.

Season and Molt Stage. Experiments were made during June, July, and August, 1967. All lobsters that were used were in an intermolt stage and showed no evidence of premolt behavior (see Maynard and Dingle, 1963).

Results

In normal animals, antennular stimulation with fish juice usually elicited a directional feeding response unless the lobster was alarmed, nearing or recovering from molt, or satiated with food. If the stimulus shifted from one antennule to the other, the response continued or recurred, but differed in that the directed component shifted as one or the other antennule was stimulated. Generally, if one antennule elicited a response, the other did also. Occasional variations in the strength of the response elicited from the two sides occurred, but were difficult to evaluate because of the inherently poor control for stimulus intensity, interstimulus interval, and arousal state. At the present level of analysis, *response* or *no response* is sufficient distinction.

The effect of eyestalk ablation reported by Maynard and Dingle (1963) is fully confirmed. Removal of an eyestalk always resulted in subsequent failure to initiate feeding upon adequate stimulation of the ipsilateral antennule. The effectiveness of the contralateral antennule was apparently unaltered, or possibly augmented.

Table 1 summarizes the results from the two experimental and two control groups. The experiments were designed to answer the two questions: 1. Does loss of normal patterned visual input in one eye result in ipsilateral antennular dysfunction? 2. Are the outer optic ganglia necessary for ipsilateral antennular function in the feeding response?

During a test, first one and then the other antennule was stimulated within a short time. The test was considered positive when stimulation of either or both antennules produced a feeding response. Lobsters responded positively only about 64% of the time over all. There was considerable variation among individuals, and over time within a single individual. Frequently lobsters which did not respond to antennule stimulation were also unresponsive to the normally more effective stimulation of parieopod dactyls or mouthparts, thus indicating general reduction in feeding activity at the time of testing. The first column of

Table 1. *Responses to antennular stimulation*[a]

Lobster	Normal Control		One eye covered		Operative control		Optic gang. removed		Optic tr. severed		Eyestalk removed	
	a	b	a	b	a	b	a	b	a	b	a	b
1	—	—	0/2	0								
2	—	—	0/4	0								
3	1/1	0	3/4	0								
4	1/1	0	8/13	1								
5	1/1	0	8/8	1							3/3	3
6	1/1	0			11/15	1					3/5	3
7	2/5	0			2/7	1					1/1	1
8	5/7	0			4/8	0					1/1	1
9	5/7	0					3/4	0			1/1	1
10	2/8	0					6/6	0				
11	3/7	0					5/6	0			2/2	2
12	1/1	0					1/6	1				
13	1/1	0					7/7	3			3/5	3
14	1/1	0							3/5	3		
15	3/3	0							2/2	2		
16	2/2	0							0/4	0		
Totals	29/46	0	19/31	2	17/30	2	22/29	4	5/11	5	14/18	14
% respon.	63%		61%		57%		76%		45%		78%	
Relative asymmetry		0		0.11		0.12		0.18		1.0		1.0

[a] First one antennule and then the other was stimulated in each test.

Column a. Number of tests with a positive response/total number of tests (see text). — *Column b.* Number of positive tests in which only one antennule evoked the feeding response = *number of asymmetrical tests.* — *Relative asymmetry.* Number of asymmetrical tests/number of positive tests.

each test series in Table 1 gives the number of positive responses divided by the total number of tests for each individual and for each experimental series. Under the conditions of this experiment there is no significant difference in responsiveness among the various test groups.

Asymmetrical responses, in which stimulation of one antennule produced feeding whereas stimulation of the other did not, are indicated in the second column of figures for each test series. With the exception of the single asymmetrical response of control animal No 6 (see Table 1), the ineffective antennule in all asymmetrical responses was ipsilateral to the treated eyestalk. Asymmetrical responses were never observed in completely normal animals, whereas they were characteristic of all positive tests with animals having the eyestalk removed completely,

or the optic tract severed. The relative number of asymmetrical re-
sponses compared with the number of positive tests provides an appro-
priate measure of antennular dysfunction.

If individual tests are assumed independent for purposes of statistical
analysis, and if the maximum probability of an asymmetric response in
any test series is estimated by the percent of asymmetric posi-
tive tests observed, then the significance of variations in antennular
dysfunction among the six groups can be calculated (Table 2). The most

Table 2. *Probability matrix giving the likelihood that the relative asymmetry of experimental series differ according to chance*

		Normal control	One eye covered	Oper- ative control	Optic gang. removed	Optic tr. severed	Eyestalk removed
		$n_i=0$	$n_i=2$	$n_i=2$	$n_i=4$	$n_i=5$	$n_i=14$
Normal Control	$p_i=0.033$[a] $n=29$	—	NS	NS	<0.005	<0.005	<0.005
One eye covered	$p_i=0.105$ $n=19$	NS	—	NS	NS	<0.005	<0.005
Operative control	$p_i=0.12$ $n=17$	NS	NS	—	NS	<0.005	<0.005
Optic gang. removed	$p_i=0.18$ $n=22$	~0.02	NS	NS	—	<0.005	<0.005
Optic tr. severed	$p_i=0.835$[a] $n=5$	<0.005	<0.005	<0.005	<0.005	—	NS
Eyestalk removed	$p_i=0.933$[a] $n=14$	<0.005	<0.005	<0.005	<0.005	NS	—

[a] $p_i=$representing the probability of an asymmetrical response, is considered to be no greater than 0.033 ($^1/_{30}$) for the normal control series, and no less than 0.835 ($^5/_6$) or 0.933 ($^{14}/_{15}$) for the two series in which the optic tract is severed or the eyestalk removed. These represent the most conservative estimates consistent with experimental data. $n=$represents the total number of positive tests in each series. $n_i=$represents the observed number of asymmetrical responses in each series.

important conclusion is that animals with eyes covered or with optic
ganglia removed do not differ significantly from operative controls in
antennular dysfunction, whereas removal of the remaining eyestalk
ganglion, the *medulla terminalis*, produces immediate and complete
dysfunction.

There is a tendency for slight dysfunction following any eyestalk
manipulation, but this is generally at the limits of significance. One
individual, No 13, among the group of five lacking optic ganglia tended
to give more asymmetrical responses and is responsible for the apparently

significant differences between the normal series and the series with optic ganglia removed. In this individual, moreover, positive responses to ipsilateral stimulation were somewhat weaker and often had a longer
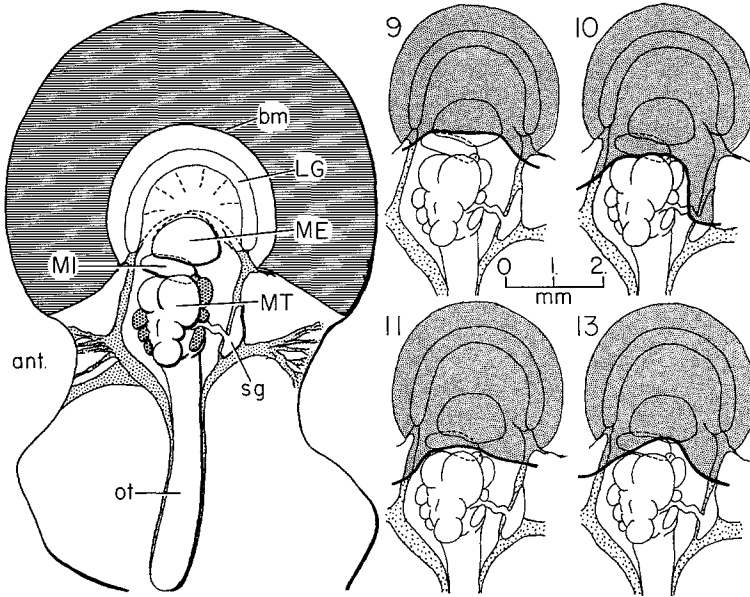

Fig. 2. Eyestalk ganglia remaining after removal of "optic" ganglia. *To left:* Diagram of intact eyestalk and ganglia reconstructed from serial sections; viewed from above, anterior to left. The ganglia are surrounded by a thick sheath of connective tissue (lightly stippled) which is attached laterally to the sides of the eyestalk. Clumps of neuron cell bodies associated with the *medulla terminalis (MT)* and visible from a dorsal view are heavily stippled. Neuron cell bodies associated with the three optic ganglia, *lamina ganglionaris (LG)*, *medulla externa (ME)*, and *medulla interna (MI)* are not shown. The sinus gland (*sg*) is connected to the *medulla terminalis* by the sinus gland nerve. The optic tract (*ot*) and the basement membrane of the retinal layer (*bm*) are obvious. Various lobes of the medulla terminalis are illustrated, but not labeled. *To right:* Diagrams of ganglia remaining in operated eyestalks of Lobsters no 9, 10, 11, and 13 at the time of testing. The heavy line indicates the approximate location of the cut. All ganglia and portions of ganglia distal to the line (stippled) were missing. Ganglia proximal to the line (unstippled) remained (see text)

latency than responses initiated by stimulation of the antennule on the unoperated side.

Histological Observations. Eyestalks of four of the five lobsters in the series lacking optic ganglia were removed 5—10 days after the initial operation. The portions of the eyestalk ganglia remaining after operation — determined from reconstructions of serial sections — are diagrammed in Fig. 2. In all four, the *medulla terminalis* including the hemiellipsoid

body was intact, and in all four the *lamina ganglionaris* and most of the *medulla externa* was missing. The packing of the *medulla terminalis*, *medulla interna*, and *medulla externa* was such, however, that a clean cut between ganglia was nearly impossible. Accordingly, a portion of the *medulla interna* was present in all cases, ranging from less than 5% of the total ganglion mass and including only portions of the most proximal layers in Lobster No 10 to the entire ganglion in Lobster No 9. In Lobsters No 11 and 13 about half the *medulla interna* remained. Proximal portions of the *medulla externa* were present in Lobsters No 9 and 13.

Variations in the amount of optic ganglia remaining were not correlated with behavioral responses to antennular stimulation. Surprisingly, no obvious structural basis for the asymmetrical response of Lobster No 13 was found. Possibly some less obvious pathology was involved.

In all four lobsters, a cuticular scab had formed over the surface of the cut, and a new epithelium was in the process of formation. Some debris and pigment granules were evident immediately beneath the scab, but no extensive region of moribund tissue. Blood cells accumulated around regions of the remaining ganglia within the outer neural sheath, suggesting an inflammatory reaction, but neither neuropil nor remaining nerve cells showed obvious evidence of degeneration, even those bordering the cut where fibers were undoubtedly severed appeared normal. Although there was no histological evidence that the nerve tissue of the ganglia remaining was pathologic, it is necessary to add that special techniques were not used, and that signs of degeneration in crustacean tissue are imperfectly known.

Discussion

The experiments reported here and in an earlier paper (MAYNARD and DINGLE, 1963) support the view that the *medulla terminalis* should not be counted among the true optic ganglia, but should be considered an important segment of the brain associated with higher order integration (BULLOCK and HORRIDGE, 1965; HANSTRÖM, 1925, 1948; WIERSMA, BUSH, and WATERMAN, 1964; WIERSMA and YAMAGUCHI, 1966). They also provide preliminary information about the nature of one of the integrations involved. It is clear that satisfactory utilization of olfactory input from the antennules in initiating feeding requires the structural integrity of the *medulla terminalis*. And it is very likely that such olfactory computation may procede in the complete absence of visual or optic ganglion input of any sort: the loss of *lamina ganglionaris*, *medulla externa*, and as much as 95% of the *medulla interna* causes no appreciable alteration of feeding activity induced by chemical stimulation of the ipsilateral antennule. We suggest that the role of the *medulla terminalis* is to participate in the initiation and maintenance of

specifically induced, directed exploratory behavior. Although the evidence presented here deals only with food-getting, one may reasonably wonder whether other similar activities associated with sex (RYAN, 1966), social interaction, or migratory movements may not also require the *medulla terminalis*.

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