

Resumido por el autor, Edward C. Day.

La fisiología del sistema nervioso de los tunicados.

I. La relación del ganglio nervioso con las reacciones sensoriales.

Los bordes de los sifones de *Ascidia mentula* son mas sensibles a la acción de los estímulos táctiles, vibratorios y químicos que los de otras especies. La reacción sensorial es local cuando el estímulo táctil es muy débil, pero bajo la acción de uno mas intenso ambos sifones responden de un modo coordinado transcurriendo un periodo latente, que dura próximamente medio segundo, durante el paso del impulso nervioso desde un sifón al otro. La dirección de este impulso es reversible. *Ascidia mentula* es también sensible a los efectos del HCl, NaCl, NaOH y la quinina, pero no a los de la solución de azúcar de caña al 50 por ciento; es también muy sensible a los efectos de las vibraciones de la habitación en que esté colocada, pero por completo indiferente a la luz solar. Los sifones amputados retienen la sensibilidad durante cinco a seis dias. Los individuos desprovistos de sifones los regeneran aun cuando se haya separado el ganglio con uno de ellos. La extirpación del ganglio interrumpe la coordinación, disminuyendo el tono muscular y la sensibilidad. El tejido ganglionar se regenera, estableciéndose la coordinación al cabo de cuatro a seis semanas. *Ascidia atra* es semejante a *Ascidia mentula* en estructura y reacciones sensitivas. En oposición, a los tunicados mencionados, *Ciona intestinalis* es decididamente sensible a la acción de un rayo solar y el área sensible está localizada alrededor del ganglio. Los sifones de *Ciona* separados del cuerpo exhiben contracciones automáticas rítmicas.

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THE PHYSIOLOGY OF THE NERVOUS SYSTEM OF THE TUNICATE

I. THE RELATION OF THE NERVE GANGLION TO SENSORY RESPONSES

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INTRODUCTION

When one beholds a solitary ascidian in normal surroundings, one comes to the conclusion that such an organism needs no complicated nervous system to cope with the exigencies of its environment. Unless the observer disturbs the ascidian by stamping on the sand, he may watch it for a considerable length of time without detecting any movements which would indicate it to be an animate object. From time to time, however, it is

seized with convulsions. The attacks, though violent, are brief, and soon the animal is as erect and motionless as before.

These sudden contractions at irregular intervals are the only vigorous muscular movements which the tunicate makes. They are performed in order to clear the pharyngeal sac of foreign particles, or to expel faeces, sperm, and mature ova from the atrial chamber. When the animal is strongly stimulated around either siphon, this characteristic vomiting reaction occurs. Stamping on the sand or clapping two stones under water will call forth a similar response. The lobes of either siphon are capable, however, of individual response and react to feeble stimulation with a local curling in of the lobe involved; a stronger stimulus causes puckering of the other lobes as well, while a still more vigorous application of the stimulus elicits an almost simultaneous closure of both siphons, the one stimulated being usually the first to respond.

This response of the siphons being an easily observed reaction, it served as a good indicator of the normal functioning of the nervous system. The circlet of tentacles just inside the aperture of the oral siphon which forms in some species a hairy strainer to prevent too large bodies from being swept into the pharynx with the ingoing current, could likewise be used as a reaction indicator, though with less success.

The lip-lobe reaction was by far the most delicate of the two, and through it the general irritability of the animal and the conductivity of the ganglion could be studied under varied experimental conditions. Through this reaction the relation of the central nervous system to the sense organs and musculature could be fairly well ascertained, but as an index of the relation of the ganglion to the activity of the visceral organs this reaction was useless.

While a few preliminary studies were made upon *Ascidia atra* Lesueur, a species found on the coral reefs of Bermuda, most of the investigations were carried out on *Ascidia mentula*, *Ciona intestinalis*, and *Ascidia mammillata*, but chiefly on *Ascidia mentula*, at the Naples Zoological Station.

My interest was first aroused in the subject of tunicate reactions during a zoological expedition from Harvard University to the Bermudas in 1910, and it led to later investigations carried out in 1913 at Naples. For the opportunity of enjoying the facilities of the Naples Station I am indebted, on the one hand, to Harvard University for a traveling fellowship, and on the other, to the Smithsonian Institution for the use of its research table there. I wish here to express my thanks to the various members of the Naples staff for their services and many kindnesses during my sojourn.

In how far *Ascidia atra* differs from *Ascidia mentula* in the finer details I cannot say, but in the gross anatomy they are very similar, although in color the former is bluish black and the latter a milky white. Both are from 4 to 6 inches in length, have a smooth outer tunic unornamented with protuberances, hairs, or other local modifications. The margins of incurrent and excurrent siphons of both species are lobed. The only record of the number of lobes which I have for *Ascidia atra* is a general statement that the number on the incurrent siphon ranges from 7 to 11, while that for the excurrent is 5 to 6. Hecht ('18) says 8 on the oral and 6 on the atrial. For *Ascidia mentula* the average number for the former is 9 and for the latter 6. The interlobular margins of both siphons of *Ascidia mentula* are edged with red pigment, while the lobes themselves are, as a rule, uncolored. Just proximal to these red edges are situated isolated pigment spots; a single one usually between each lobe on the oral siphon, and two or more in a cluster between each lobe on the aboral siphon. Occasionally an animal was brought to the laboratory which was totally brick red. *Ascidia atra*, on the other hand, is a velvety bluish black all over, due to the presence of pigment in the outer tunic. I never ran across one, however, where pigment was restricted to only the margins of the siphons. *Ascidia mammillata*, like *Ascidia mentula*, is whitish except for the pigment spots, in this case black, around the incurrent and excurrent apertures. *Ciona intestinalis* has red pigment spots on the margins of the siphons. Mention is made of these details of pigmentation because they

will later be referred to in connection with the sensitivity of the animals to light.

The procedure of experimentation was first to study the activities of the normal, undisturbed animal under as natural conditions as possible; second, to record its reactions to stimulations of various kinds, and, third, to discover the effect of various operative procedures such as incisions, amputations of siphons, and extirpation of the ganglion, upon the sensory reactions and the beat of the heart. Studies made upon the heart-beat will be given in a subsequent paper.

TACTILE REACTIONS OF NORMAL *ASCIDIA MENTULA*

A. Observations on unstimulated individuals

By the term unstimulated is meant cases in which I introduced no stimulating agent myself, although the animals were never entirely free from disturbing influences.

The animals remain motionless for long intervals with both siphons wide open when they are in their natural environment of shore-water. I have observed the same thing in the case of animals attached to the walls of large cement tanks in the Naples Aquarium, where natural conditions have been duplicated as nearly as possible. In the laboratory, although the tanks were large and provided with running sea-water, the animals in them were not entirely insulated from disturbing vibrations in the room.

A protocol was kept of continuous observations on two specimens of *Ascidia mentula* for a period of about two hours and the time recorded for every closure of both incurrent and excurrent siphons. The animals were kept singly in large glass jars furnished with running sea-water. Both animals were very responsive to vibratory disturbances and closed their siphons whenever a door shut or somebody walked across the floor. Hecht ('18) finds *Ascidia atra* also extremely sensitive to vibrations in the room. The response consisted simply of a puckering of the marginal lobes of the siphons; seldom did the whole body contract. When the room was quiet for a length of time, for one

of the animals no reactions were observed, while for the other frequent contractions were noticed, especially of the atrial siphon. This siphon proved to contain one or two parasitic crustacea, 3 to 4 mm. long, partially embedded in the siphon near its tip, and whenever they kicked up a rumpus the siphon closed, and the closing of this siphon often induced closure of the other. The parasitized atrial siphon being in a quasi continuous state of irritation responded more readily to the vibrations in the room than did the unexcited siphon. Sometimes small crabs were found living symbiotically in the pharyngeal sacs of the tunicates.

B. Responses to tactile stimulation

To penciling with a bristle the siphons give local responses, thus only the lip-lobe on the stimulated side of the siphon puckers in, provided that the stimulus is feeble; to a stronger stimulus, however, all lobes of the stimulated siphon respond, and if the penciling be of sufficient strength, the closure of the incurrent siphon is followed by closure of the excurrent siphon as well.

The base and body of the animal are insensitive to penciling, while the necks of the siphons are slightly sensitive, though less so than the lip-lobes. The margins of the two siphons are therefore the most sensitive areas of the entire body. As to relative responsiveness, little difference could be discovered between oral and aboral siphons; in one or two cases the aboral or excurrent siphon was the more sensitive of the two.

EFFECT OF OPERATIONS UPON THE TACTILE RESPONSE

A. Slitting the siphons

When the oral siphon was slit lengthwise for half an inch, cleaving it in two parts, and the lobes of one side were stimulated, there was a response first in that half, then after a second's latent period a response in the other half. A reversal of the

sequence occurred by stimulating the other half first. Thus the wave of stimulation could travel in either direction around the cut. No effort was made to determine whether this stimulation wave was of the nature of a nerve impulse, or whether it was simply a wave of muscular contraction which depends for its completion upon the continuity of the muscle tissues; nor further, whether the length of the latent period was dependent upon the intensity of the stimulus to any degree, or in how far it was affected by the depth of the incision.

B. Amputation of siphons

When an *Ascidia mentula* was narcotized with cocain, the oral siphon amputated below the circling of oral tentacles, and both the animal and the amputated piece were put into fresh sea-water and allowed to recover, the aboral siphon showed a return of sensitivity in a little over an hour while the amputated siphon did not regain sensitivity until two or three days after the operation. When the operation was performed without first narcotizing, recovery of the amputated piece took place in twelve to fourteen hours, whether it was the oral or aboral siphon.

Similar operations on the siphons of *Ciona intestinalis* revealed a greater recuperative power than that of *Ascidia mentula*, amputated siphons recovering sensitivity twenty-five minutes after the operation.

The siphons possess, therefore, an irritability which is independent of any connection with the nerve ganglion. The threshold of stimulation lies higher for amputated siphons than for the intact siphons, it requiring a stronger stimulation to elicit a response. This diminution in sensitivity was probably occasioned in part by the insufficient blood supply due to the operation, with a consequent lack of oxygen and an accumulation of catabolic products, and in part by the interruption of the nerve reflex. The presence or absence of the rings of oral tentacles made no obvious difference in the general responsiveness of the amputated oral siphon.

Not only did these amputated siphons exhibit independent irritability, but in the case of *Ciona*, an automatic rhythm was also manifested by the severed oral siphons. A siphon which had been cut off distal to the cirlet of oral tentacles was observed two days after the operation to execute a series of rhythmical contractions which consisted of the periodic curling of the lip-lobes either inward or outward and the occasional constriction of the neck region. There was no special sequence in which the several lobes took part in the contraction, for a contraction would start on one side and be followed by a puckering, sometimes of the opposite side, sometimes of an adjacent part. The proximal portion of the amputated piece, i.e., the region of the cut margin, played no rôle in these contractions, the rhythmical movements being restricted to the oral margin and to the directly underlying portion of the neck. In fact, when the proximal part was stimulated, the response occurred not in the proximal end, but in the distal after a short latent period, the impulse having traveled from the less irritable region of stimulation to the more irritable region of the sensitive lobes before producing a response. No attempt was made to analyze the underlying phenomena of this response or to seek an explanation of the rhythmicity. No such rhythm was observed for amputated siphons of *Ascidia mentula*. As it has frequently been demonstrated that muscle tissue may be thrown into rhythmical contractions by the presence of various salts in the water, this rhythmical contraction in the *Ciona* siphon might have been due to the automatic response of either the muscle or the nerve tissue to the stimulating effect of the sea-water on the cut surfaces. Whether sensory cells are present in the siphon regions or not I have made no histological examination to ascertain, but nerve fibers can be seen extending out into the lip-lobes.

Tunicates whose siphons had been amputated regenerated new ones in the course of about three weeks, but amputated pieces lived only five or six days and died without any sign of beginning to regenerate a new body.

The three following protocols will give the history of such an operation with its subsequent effects both on the amputated siphons and on the desiphonated body:

Protocol 1. *Ascidia mentula* nos. 10, 11, and 12. April 17, 1913. Narcotized all three animals with cocaine in sea-water and cut off the tips of the siphons at XX' on the oral or incurrent siphon (distal to oral tentacles) and at YY' on the aboral or excurrent siphon (fig. 1). Returned animals to running sea-water.

April 18. Amputated pieces A and C are sensitive to tactile stimulation (B pieces not tested).

April 19. A and C respond to tactile stimulation, but not to tapping on the jar containing them. One of the bodies B is sensitive to the tapping and responds by ejecting water from the siphons. Pharyngeal sacs have fallen away from the cut surfaces of the siphons.

April 23. A and C pieces are dead. (The supply of running water had got accidentally shut off.) The body pieces, B, are alive and appear to be regenerating new tips.

May 9. New siphons have been formed on all three animals. These lie within the old cut tips and appear to have been developed from the pharyngeal sac. Distinct lip-lobes and interlobular pigment spots are visible on most of them: 7 to 8 lobes on 10C, 8 on 11A, 6 on 11C, 5 on 12A; on 10A and 12C the siphons are too puckered up to make out the number.

Protocol 2. *Ascidia m.* no. 5. April 12, 1913. Cocainized the animal (400 cc. sea-water + 1 cc. 5 per cent cocain), and amputated oral siphon below the circlet of oral tentacles at XX' (fig. 2). Procedure was as follows:

5:10 P.M. Animal placed in cocain solution.

6:10 P.M. Benumbed; operated on, rinsed in fresh sea-water and returned to running sea-water.

6:45 P.M. Excurrent siphon sensitive to tactile stimulation; amputated incurrent siphon A, unresponsive.

April 15. A is responsive around marginal lobes; tentacles not. Lobes pucker in response to tactile stimulation and to rap on substratum. B is very sensitive both on cut surface and on excurrent siphon.

April 16. A and B respond to tap on jar.

April 17. B responds to tap on jar; A, too, if tap is strong.

April 19. B responds to tap on jar; A no longer.

May 9. B has regenerated a new incurrent siphon with six small lip-lobes and a new ring of pharyngeal tentacles. New siphon puckers shut upon tapping the jar.

Protocol 3. *Ascidia mentula* no. 17. May 10. Incurrent siphon amputated to include the nerve ganglion. (Since the object of this experiment was to study the effects of the operation on the heart beat, no attention was paid to the amputated piece.)

June 3. A new incurrent siphon and ganglion have regenerated; six lobes to the siphon (fig. 3).

That the nerve ganglion is quite dispensable to the response of the siphons to stimulation is evident from the foregoing results. Amputating the siphons, however, involved cutting off the blood-supply from the severed part, and the effect of breaking all connections with the ganglion was so complicated with interrupting the circulation that no safe conclusions could be drawn with regard to the nervous control exerted by the ganglion. By removing the ganglion, therefore, without at the same time injuring the circulation, its relation to the siphonal responses could be separately determined.

C. Extirpation of the ganglion

Four *Ascidiae mentulae* were operated upon by excising the ganglia, and they lived long enough for the ganglia to regenerate—a period of about one month.

Immediately following the operation on two of the animals, the oral siphons began to open, but their irritability to stimulation was greatly reduced. Since the operation was performed without the use of a narcotic, this diminution of sensitiveness was probably due to shock. With the lapse of a few days irritability gradually increased, but it never attained the level of the normal animal until towards the end of the month when the nervous tissue had regenerated. Thus, although the ascidians responded to penciling and to tapping on the jar in which they lay, a greater strength of stimulus was required; the animals were no longer disturbed by those extraneous vibrations from the closing of doors and the treading of feet which had produced responses prior to the operation.

Another animal which had had the ganglion destroyed by painting it with nitric acid, also opened its oral siphon immediately (1 minute) after the operation, but kept its aboral siphon closed for some time afterwards. Three days later, both siphons were open and responded to tactile stimulation, but the responses were of an inferior order.

Two marked changes in the reactions of the animals were produced by these operations on the ganglia: 1) a decrease in

general sensitiveness and, 2) a complete break in the coördination of the siphons. While each siphon was capable of responding when stimulated directly, it did not join in the response when the other siphon was stimulated, as was the case before the operation. This state of incoördination lasted until the ganglionic tissue regenerated, about four to five weeks later.

A brief history of a single case will illustrate the course of events following an operation on the ganglion.

Protocol 4. *Ascidia mentula* no. 2. April 12. Animal removed from water and ganglion extirpated. Oral siphon opened immediately after operation and before animal was returned to the water; aboral siphon remained closed.

April 15. Siphons both responsive to tactile stimulation with a bristle and to tapping on table or jar with a scalpel. No coördination between the two. The tentacles are also sensitive to penciling, and when they are touched the oral siphon closes.

April 26. Siphons both responsive to stimulation. No coördination.

May 5. Faint signs of recoördination between the siphons.

May 9. Coördination of siphons definitely reestablished. Out of ten trials in which the oral siphon was stimulated with a camel's-hair brush, the oral siphon responded ten times and the aboral seven, the sequence of contraction being from oral to aboral with a latent period of about one-half second between the two responses. When the aboral was stimulated, out of ten trials the oral siphon responded five times to its ten, the sequence being aboral to oral. The regenerated ganglion can be recognized as a small whitish body lying in the region of the original one.

The other three animals which had been operated upon at the same time as the one whose history has been given above, had also regenerated their ganglia, and, upon being tested, showed that the coördination of the siphons had likewise been reestablished. The fifth ascidian, whose ganglion had been destroyed with nitric acid, died a week after the operation. Up to that time, however, the siphons retained their independent sensitivity, but no coördination existed between them.

Two weeks earlier, but three weeks after the removal of the ganglia, none of the four ascidians exhibited any signs of coördination, although the siphons responded independently to direct stimulation. At the end of five weeks, however, the stimulation of one siphon brought about not only the closure of

that siphon, but also the closure of the other siphon as well. The unstimulated siphon did not always respond, owing apparently to the exhausted condition of the ganglion which seemed to fatigue quickly with too frequent an application of the stimulus. As no method was employed either for measuring the strength of the stimulus or for graphically recording the response, no exact comparison could be made between the original normal responses and those subsequent to regeneration of the ganglion. The sensitivity of the animal as a whole, however, did not seem so great after the restoration of ganglionic tissues as before the ganglion had been extirpated, for although the siphons responded readily to feeble penciling with a camel's-hair brush, they did not respond to vibratory disturbances, such as the shutting of doors and the treading of feet. This inability to respond to vibrations may find its explanation in the ease with which the ganglion is fatigued, because, even though the ganglion were composed of nervous tissue of a higher degree of sensitivity than before, the depressing effect of frequent vibratory stimulations would prevent the state of irritability from attaining to a maximum. The latent period between the responses of the in- and excurrent siphons was not measured, but it was about one-quarter to one-half second.

Just what the histological condition of the ganglion is at the time of restoration of the power of coördination it would be of interest to know. The neurogenesis, however, of the regenerating ganglion and its relation to restoration of physiological function is a problem which still awaits investigation.

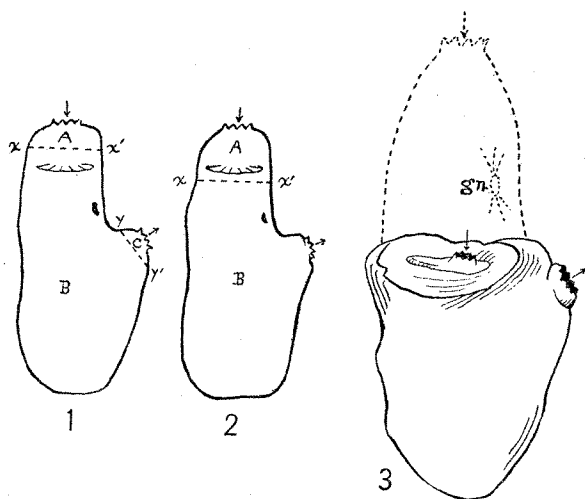
REACTION OF ASCIDIA MENTULA TO CHEMICAL SUBSTANCES

While the following are but a few incidental tests of a tentative nature, they throw a little light on the chemical sense of the animal. In the work of Hecht ('18) will be found an intensive treatment of the reactions of *Ascidia atra* to chemicals.

Needle-like crystals of quinine ($\frac{1}{2}$ mm. in length) were dropped on to the oral tentacles of three animals. After lying on them for a moment or two, they were ejected. A thread, knotted on

the end, weighted with a bit of tinfoil and soaked in a chemical solution of NaCl 2n, NaOH n/10, or quinine 10 per cent, and lowered on to the tentacles likewise evoked an ejection reaction.

I operated on six *Ascidiae mentulae* as follows: three incurrent siphons were severed proximal to the oral tentacles (fig. 2) and three distal to them (fig. 1); the excurrent siphons of the latter



Figs. 1 and 2 Two *Ascidiae mentulae* operated on as shown by dotted lines: in Fig. 1 the incurrent siphon (A) was amputated distal to the circlet of oral tentacles, in Fig. 2 proximal to the tentacles; excurrent siphon (B) also cut off of the first animal. Nerve ganglion is shown as a black spot near the crotch of the siphons.

Fig. 3 A regenerated *Ascidia mentula*. Twenty-four days after the incurrent siphon had been cut off carrying the nerve ganglion (gn) with it, a new siphon and ganglion had been regenerated and a coordinate response of both siphons was obtained upon stimulating either siphon independently.

three were also amputated a half inch from the tip. The amputated pieces opened immediately after the operation, but were unresponsive to chemical stimulation.

The following day when crystals of quinine were dropped on to the tentacles of the amputated siphons, it caused them to rise up. Crystals dropped on the lip-lobes of both incurrent and excurrent amputated siphons caused local contractions of the

lobes. In the case of the incurrent siphon, the chemical stimulation of one lobe often induced closure of the whole siphon. On one of the excurrent siphons there was also a complete closure effected. The contraction in this case was progressive in both directions from the point of stimulation around the rim of the siphon. The lip-lobes were more sensitive than the tentacles to the quinine. The inner lining of the pharynx also proved sensitive to the quinine and when stimulated brought about a contraction of the pharyngeal papillae and of ten a closure of one or both siphons.

The lip-lobes, tentacles, and pharyngeal lining were also sensitive to solutions of HCl, NaCl 2n, NaOH n/10, while to 50 per cent cane-sugar the same regions were unresponsive. The tentacles move about when stimulated, but do not contract, for they are stiff and apparently non-muscular organs. Often they gave an upward flip when stimulated. As this might have been due to the sudden ejection of water from the incurrent siphon, a siphon was amputated to include the tentacles, and the tentacles were again stimulated: they responded by rising up in a concerted reaction, but not so abruptly as before. When the ganglion was extirpated, this response of the tentacles could not be elicited. More data would probably have shown the contrary to be true for this latter case, since the erection of the tentacles occurs for an amputated siphon which is minus the ganglion and it ought also to occur for the intact siphon which is minus the ganglion.

REACTIONS OF ASCIDIA MENTULA TO VIBRATIONS

A normally sensitive individual closes its siphon to the slightest vibrations produced by disturbances in the room or by tapping on the jar in which it lies. If the incurrent siphon be amputated to include the circle of tentacles, it is still capable of responding; but if it is excised distal to the tentacles, it no longer responds. The amputated excurrent siphon like the incurrent without its tentacles also proves to be inert to vibrations. If the ganglion be extirpated from an animal with both siphons intact, the

vibratory stimulus, if sufficiently vigorous, will still induce closure of both siphons after the effects of shock have passed.

Since both siphons respond without the ganglion and since the excurrent siphon which lacks the circlet of tentacles is about as sensitive as the incurrent siphon which has them, it would seem that neither circlet of tentacles nor ganglion is the receptive organ for the vibrations, but that the lip-lobes themselves are capable of responding to the vibrations immediately. Hecht ('18) finds the lip-lobes to be the vibration receptors in *Ascidia atra*.

SUMMARY OF REACTIONS OF ASCIDIA MENTULA

A. Normal animal to tactile stimulation

1. The margins of the siphons are the most sensitive part of the animal, and close when stimulated.

2. To feeble stimulation the response is local and restricted to a single lobe of the siphon. To a stronger stimulus the whole siphon closes. Further increase of strength of the stimulus produces a closure of the other siphon as well.

3. There is a short latent period of about one-half second between the responses of the two siphons.

4. The sequence of closure is reversible; the impulse travels in either direction from one siphon through the nerve ganglion to the other, depending upon which siphon is stimulated.

5. Sometimes one siphon is more sensitive than the other and takes the initiative in the response when the two are stimulated simultaneously, as by vibrations in the room.

B. Operated animals to tactile stimulation

a. With siphons amputated. 1. In a siphon partially slit longitudinally, each half responds locally to a feeble stimulus, while stronger stimulation sends the impulse around the cut and produces a response of the two halves in sequence.

2. Amputated siphons retain their sensitivity for five or six days and then die.

3. The desiphonated bodies of the operated animals recover sensitivity after the operation, live and regenerate new siphons.

4. The ganglion is not necessary to the process of regeneration of amputated siphons, as regeneration occurs even if the ganglion be amputated with the siphon. A new ganglionic mass appears in addition to the new siphon.

b. With ganglion removed. 5. Extirpation of the ganglion has two main effects: *a)* an interruption of coördination between the siphons; *b)* a reduction of tone and general irritability of the animal.

6. The ganglion regenerates in from four to six weeks.

7. Coördination is reestablished with regeneration of the ganglion, and irritability is restored to almost its original degree.

8. The new ganglion is very quickly fatigued.

C. Normal animal to chemical stimulation

Lip-lobes, oral tentacles, and pharyngeal lining are sensitive to solutions of HCl, NaCl 2n, NaOH n/10, but are insensitive to 50 per cent cane-sugar solution. Quinine crystals applied to the lip-lobes cause siphons to close; applied to the oral tentacles, they cause these to flip up.

D. Operated animal to chemical stimulation

Quinine crystals applied to the lip-lobes or oral tentacles of amputated siphons produce the same but less vigorous response as for the normal animal.

E. Normal animal to vibratory stimulation

Both siphons are sensitive to disturbing vibrations in the room.

F. Operated animals to vibrations

Amputated incurrent siphons respond only provided they are cut off to include the circlet of tentacles; amputated excurrent siphons are insensitive. Deganglionate animals respond with closure of both siphons, but a more vigorous stimulation than normal is needed to elicit the response.

SENSORY RESPONSES OF ASCIDIA ATRA LESUEUR

Since my experiments on *Ascidia atra* were of a preliminary character, there are only a few which may be mentioned here. For a good account of the physiology of this species reference should be made to the research of Hecht ('18). The response to various forms of stimulation are briefly as follows:

Tactile stimulation. When the outer surface of the test was stimulated with a bristle, the base and column of the animal were found to be insensitive, the necks of the siphons moderately sensitive, and the margins of the aperatures most sensitive of all. According to the strength of stimulation, the siphons respond independently or coördinately.

Vibratory stimulation. Tapping on the jar or, when the animals are in their normal habitat, stamping on the sand or clapping two stones together under water causes the siphons to close.

Chemical responses. Acetic acid 1, 0.1, and 0.01 per cent strength when pipetted on the incurrent siphon caused it to close, but had no effect on the excurrent. A weaker solution of the acid, 0.001 per cent, and distilled water were both without effect.

An animal put in one-half per cent solution of ether in sea-water became totally narcotized in eight minutes. It bent over double on itself during the process, closed its siphons, and became quite insensitive to tactile stimulation. When returned to running sea-water again, it revived in twenty to twenty-five minutes; the animal straightened somewhat, both siphons opened and responded to stimulation with a bristle.

Either siphon could be locally anesthetized by pipetting a 1 per cent solution of chloroform in sea-water on to it. With both siphons rendered insensitive in this way, the animal still exhibited the ciliary current entering the oral and issuing from the aboral siphon, and it also gave vomiting reactions from time to time, forcibly ejecting water from both siphons and thereby indicating that the body musculature was still active.

Operations. A few amputations and incisions were tried on *Ascidia atra*, but the animals did not live more than a day or two after the operations, due apparently to adverse laboratory conditions: owing to the presence of iron rust in the laboratory water, it was impossible to keep the tunicates alive for more than four or five days. No experiments were tried on amputated siphons. An animal, however, from which the ganglion had been excised, recovered and displayed irritability of both siphons to tactile stimulation. As the animal died shortly after, no regeneration had time to occur.

Light reactions. Both oral and aboral siphons of *Ascidia atra* were tested for sensitivity to sunlight, but no evidence of a positive nature was obtained. When the animals lay in jars of running sea-water and kept in semidarkness, they closed the siphons periodically at approximately one-minute intervals, and when sunlight was focused on the siphon it could not be found to have any effect on these contraction-intervals.

EXPERIMENTS ON *CIONA* INTESTINALIS

For sake of comparison, a few operative experiments were performed on *Ciona* in which the siphons were amputated at various levels and records of reactions made for both amputated pieces and the bodies. The results are given in protocol 5 below, and by diagrams in fig. 5.

1. Effect of operative experiments on Ciona intestinalis, Animals 7, 8 and 9.

April 17, 1913. Three specimens of *Ciona intestinalis* were operated upon, after first narcotizing with cocain, by amputating the siphons as indicated by the dotted lines in figure 4. It will be noted that in the operation on animal 9 the nerve ganglion (black spot at the crotch) was included in the amputated piece.

From the protocol it will be noted that:

a. Ten minutes after the operation all the amputated pieces except 7 B were unresponsive to stimulation; the amputated excurrent siphon 7 B showed automatic rhythmical contractions.

PROTOCOL 5

Siphons of three specimens of Ciona were amputated as indicated in figure 4 by dotted lines

DATE	HOUR	CIONA NUMBER 7	CIONA NUMBER 8	CIONA NUMBER 9
April 17, 1913	12.20 P.M.	Siphons amputated	Siphons amputated	Siphons amputated
April 17, 1913	12.30 P.M.	Piece A, no reaction	Piece A, no reaction	Piece A, no reaction
		Piece B, irregular automatic contractions	Piece B, no reaction	Piece A, no reaction
April 17, 1913	4.45 P.M.	A, reacts to tactile stimulus	Piece A, reacts to tactile stimulus; also automatic contractions	A, reacts to tactile stimulus; also automatic contractions
				B, reacts to tactile stimulus
		N.B.—7A and 8A have long latent period. 8A reacts locally to stimulation of lip-lobe; often required repeated stimulations to elicit a response.		
April 18, 1913	12.15 P.M.	A, no reaction	A, automatic contractions	A, automatic contractions
		B, feeble response to tactile stimulus.	B, reacts to tactile stimulus	B, reacts to tactile stimulus
April 19, 1913	4.45 P.M.	A, reacts feebly to strong stimulus.	A, reacts to tactile stimulus; also automatic contractions	A, reacts to tactile stimulus
				B, also reacts
		B, reacts to strong stimulus. Cut surfaces of body also reacts	B, reacts to stimulus	

PROTOCOL 5—Continued

DATE	HOUR	CIONA NUMBER 7	CIONA NUMBER 8	CIONA NUMBER 9
April 23, 1913	3.30 P.M.	A, feeble; contracts only locally on one side B, feeble contractions	A, exhibits rhythmical contractions of the oral margin	A, shrunken and inert; outside peeling off, but there are new lip-lobes apparently regenerating in the center of the mass as indicated by 8 new red pigment spots B, reacts to stimulation

b. Four hours later all of the amputated pieces were responsive, and the amputated incurrent siphons 8A and 9A also exhibited rhythmical automatic contractions.

c. The latent period of response was lengthened as a result of the operation.

d. 9A, the piece with the ganglion included, gave visible evidence of regenerating a new siphon in the center of the degenerating amputated one.

e. B showed little progress in the regeneration of the lost parts for the same length of time.

2. Experiments with amputated siphon 8A

a. When the amputated piece was stimulated at its proximal or cut end, the impulse traveled distally and elicited a response first at the margin. This response was of two orders depending on the strength of stimulus: first, a local puckering in of one or two of the lip-lobes, and, second, a constriction of the neck region just proximal to the margin, followed by a pursing of the whole distal end. These responses are shown by diagrams 25 and 24, respectively, in figure 5. The asterisk indicates the point stimulated.

b. Rhythmical contractions occurred every minute or two involving local portions of the oral margin. These periodic contractions are shown by the series of diagrams in figure 5. The letters D and P indicate the distal and proximal ends, respectively. The arrows indicate the points at which contraction occurred.

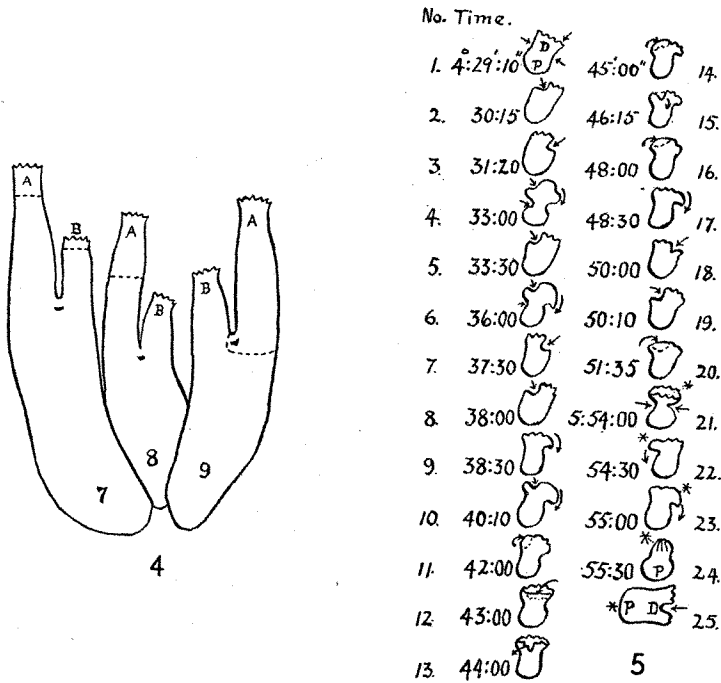


Fig. 4 Three *Ciona intestinalis* operated on as indicated by the dotted lines. The nerve ganglion is represented by the black spot at the crotch of the siphons. In animal No. 9 the ganglion was amputated along with the incurrent siphon.

Fig. 5 A series of diagrams showing automatic rhythmical contractions in an amputated incurrent siphon of *Ciona intestinalis* (8A of Fig. 4). These occurred every minute, more or less, as will be seen from time recorded in minutes and seconds to the left of each figure. The distal end of the siphon is uppermost in all figures except No. 25. D distal; P proximal. Arrows indicate the points and direction of contraction (puckering in, curling out or pursing). The asterisk means a stimulus applied at that point.

There was a certain circularity in the movement of the local contractions around the oral rim, especially seen in diagrams 15 to 20, where the invaginated part occupies successively progressive positions around the margin. These single local contractions seemed to summate in effect and to produce, after the completion of two or three, a more general reaction of the whole margin as in nos. 4, 6, 10, 21, 24.

REACTIONS OF TUNICATES TO LIGHT

From a consideration of the distribution of pigment one might well be skeptical of the photoreceptive nature of these pigmented regions. As a rule, in *Ascidia mentula* the pigment is restricted to a narrow interlobular band at the rim and to one or two subjacent spots, but often it is more or less extensively distributed over the siphons, sometimes coloring even the whole body surface. Another genus, *Cynthia papillosa*, is characteristically red all over, and *Ascidia atra* is entirely black; in *Ciona intestinalis*, again, the spots are red, while in *Ascidia mammillata* they are black, and in both they are localized on the margins of the siphons. From this diverse distribution and color of the pigment, therefore, one would hardly expect it to be associated with any photic sensitivity of the animal. *Ascidia mentula*, *Ciona* and *Ascidia atra* were tested at Naples while *Ascidia atra* had previously been tested at Bermuda.

The surface of the animal's body was explored with a pencil of sunlight. For the results on *Ascidia atra*, see page 323. The three following tables give the results for *Ascidia mentula* and *Ciona*; in tables 1 and 2 the diameter of the beam employed was 15 mm. while in table 3 it was cut down to 5 mm. The method of illuminating the animal was to reflect the beam of sunlight with a plane mirror through a cardboard diaphragm of the specified aperture upon the animal as it lay in a depth of 4 to 5 cm. of sea-water. Four regions of the body were tested in turn in this manner—incurrent siphon, ganglionic region, excurrent siphon, and the main part of the body. Contraction of the siphons was taken as indicator for the response. If no response

TABLE 1

Reaction of Ascidia mentula to a beam of sunlight 15 mm.² Animals 1 and 3 were red pigmented; 2, 4, and 5 white. The ganglion of each animal was made more accessible to light by cutting away part of the tunic. The tests were made on the second day following the operation

ANIMAL	TRIAL	INCURR. SIPHON	GANGLIONIC REGION	EXCURR. SIPHON	BODY
1	1	20''-0	20''-0	20''-0	20-0
	2	20''-0	20''-0	20''-0	
	3	20''-0	20''-0	20''-0	
	4	20''-0	20''-0	12''-+	
	5	20''-0	20''-0	10''-+	
2	1	20''-0	20''-0	20''-0	20''-0
	2	20''-0	20''-0	2''-+	
	3	6''-+	6''-+	20''-0	
	4	20''-0	20''-0	20''-0	
	5	20''-0	20''-0	20''-0	
3	1	20''-0	20''-0	20''-0	20''-0
	2	20''-0	20''-0	20''-0	
	3	20''-0	20''-0	20''-0	
	4	10''-+	20''-0	20''-0	
	5	20''-0	20''-0	6''-+	
4	1	20''-0	20''-0	20''-0	20''-0
	2	20''-0	20''-0	20''-0	
	3	20''-0	20''-0	20''-0	
	4	15''-+	20''-0	20''-0	
	5	20''-0	20''-0	20''-0	
5	1	20''-0	20''-0	20''-0	0
	2	20''-0	20''-0	20''-0	
	3	10''-+	20''-0	20''-0	
	4	20''-0	2''-0	20''-0	
	5	20''-0	2''-0	20''-0	
Total + reactions for 25 trials		4	1	5	0

TABLE 2

Reactions of *Ciona intestinalis* to a beam of sunlight 15 mm.² Animal not operated on as in case of *Ascidia m.*

ANIMAL	TRIAL	INCURR. SIPHON	GANGLIONIC REGION	EXCURR. SIPHON	BODY
1	1	10''-0	3''-+	30''-0	15''-0
	2	30''-0	5''-+		15''-0
	3	30''-0	3''-+		15''-0
	4	30''-0	3''-+		15''-0
	5	30''-0	5''-+		15''-0
2	1	10''-0	4''-+	30''-0	15''-0
	2	30''-0	6''-+		3''-+
	3	30''-0	5''-+		15''-0
	4	30''-0	5''-+		15''-0
	5	30''-0	9''-+		15''-0
3	1	6''-+	6''-+		15''-0
	2	30''-0	5''-+		15''-0
	3	30''-0	8''-+		15''-0
	4	30''-0	6''-+		15''-0
	5	12''-+	-		15''-0
4	1	10''-0	4''-+	8''-+	15''-0
	2	30''-0	3''-+		15''-0
	3	30''-0	4''-+		15''-0
	4	6''-+	5''-+		15''-0
	5	30''-0	5''-+		15''-0
5	1	10''-0	3''-+	30''-0	15''-0
	2	30''-0	4''-+		15''-0
	3	30''-0	3''-+		15''-0
	4	30''-0	4''-+		15''-0
	5	21''-+	2''-+		15''-0
6	1	10''-0	4½''-+	5''-+	15''-0
	2	30''-0	3½''-+		15''-0
	3	15''-+	3½''-+		15''-0
	4	30''-0	-		15''-0
	5	30''-0	-		15''-0
7	1	10''-0	5½''-+	8''-+	15''-0
	2	30''-0	4''-+		15''-0
	3	30''-0	6''-+		15''-0
	4	30''-0	5''-+		15''-0
	5	30''-0	9''-+		15''-0
Total + reactions		5 in 35 trials	32 in 32 trials	3 in 6 trials	1 in 35

TABLE 3

In this table the results are given for a more critical test on Ciona which was made with a beam of sunlight 5 mm.² in area

ANIMAL	TRIALS	INCURR. SIPHON	GANGLIONIC REGION	EXCURR. SIPHON
1	1	10''-0	8''-+	10''-0
	2	10''-0	7''-+	10''-0
	3	10''-0	9''-+	10''-0
	4	10''-0	7''-+	10''-0
	5	10''-0	10''-0	10''-0
2	1	10''-0	7''-+	10''-0
	2	10''-0	4''-+	10''-0
	3	10''-0	6''-+	10''-0
	4	10''-0	5''-+	10''-0
	5	10''-0	5''-+	10''-0
3	1	10''-0	8''-+	10''-0
	2	10''-0	5''-+	10''-0
	3	10''-0	5''-+	10''-0
	4	10''-0		10''-0
	5	10''-0	4''-+	10''-0
4	1	10''-0	10''-0	10''-0
	2	10''-0	10''-0	10''-0
	3	10''-0	6''-+	10''-0
	4	10''-0	8''-+	10''-0
	5	10''-0	10''-0	10''-0
5	1	10''-0	8''-+	10''-0
	2	10''-0	10''-0	10''-0
	3	10''-0	5''-+	10''-0
	4	10''-0	8''-+	10''-0
	5	10''-0	5''-+	10''-0
6	1	10''-0	6''-+	10''-0
	2	10''-0	10''-0	10''-0
	3	10''-0	6''-+	10''-0
	4	10''-0	8''-+	10''-0
	5	10''-0	5''-+	10''-0
7	1	10''-0	4''-+	10''-0
	2	10''-0	10''-0	10''-0
	3	4''-+		10''-0
	4	4''-0	5''-+	10''-0
	5	4''-0	8''-+	10''-0
Total + reactions.....		1 in 35 trials	26 in 33 trials	0 in 35 trials

Summary of tables 2 and 3

A. TOTAL REACTIONS	INCURR. SIPHON	GANGLIONIC REGION	EXCURR. SIPHON	BODY
For 15-mm. ² beam.....	5 out of 35 trials.	30 out of 32 trials.	3 out of 6 trials.	1 out of 35 trials.
For 5-mm. ² beam.....	1 out of 35 trials.	26 out of 33 trials.	0 out of 35 trials.	
Summary for both.....	6 out of 70 trials.	58 out of 65 trials.	3 out of 41 trials.	1 out of 35 trials.
B. Average time of response:				
For 15-mm. ² beam.....		4.2 sec.		
For 5-mm. ² beam.....		8.9 sec.		

was obtained before the lapse of twenty seconds, the reaction was counted as negative and recorded as 20''-0, as in trial 1, table 1, but if a contraction occurred, as in trial 4, at the end of twelve seconds, it was recorded 12''-+, indicating a positive response. In table 3 ten seconds was the time limit allowed for the response.

It will be seen from table 1 that the body of the animal gave no response out of the five times tested, the ganglionic region but 1 out of 25, the incurrent siphon 4 out of 25 and the excurrent 5 out of 25. Since all the animals were exceedingly sensitive to stimulation by tapping on the jar, the small number of responses here obtained indicate little if any sensitivity to light.

Table 2 indicates, in contrast to the results obtained for *Ascidia m.*, that the ganglionic area of *Ciona* is decidedly sensitive to light, yielding 32 responses out of 32 stimulations, while the siphons and body are relatively insensitive. The body responded but once in 35 trials, the incurrent siphon 5 in 35, and the excurrent 3 times in 6 trials. Since the excurrent siphon is much shorter than the incurrent, the 15-mm.² beam could not be focused upon it without its overlapping the ganglionic region somewhat, it was therefore not tested as frequently as the other parts, but left for a more critical test with a smaller beam.

Table 3 gives the results of this test. Stimulation was made with a beam 5 mm. in cross-section. The pencil of light was small enough to afford a more restricted localization of the stimulus both on the siphons and over the ganglionic area. Under these conditions the ganglion again exhibited its decided sensitivity to light in comparison with the insensitiveness of the siphons, yielding 26 responses out of 33 trials to only one response for the incurrent and none for the excurrent out of 35 trials apiece.

From the summarized table it will be noted that when the ganglionic region was stimulated the animal gave 58 responses (closure of siphons) out of 65 trials, while to stimulations of the other regions, the incurrent siphon yielded 6 out of 70, the excurrent 3 out of 41, and the body 1 response out of 35 trails. It was the larger of the two beams of light which was effective in eliciting responses when siphons or body were stimulated. It will also be observed that the latent period of response was longer for the smaller beam (8.9 seconds) than for the larger one (4.2 seconds); i.e., the larger the area stimulated, the shorter is the reaction time.

When the body region was illuminated with a beam about 4 cm. in diameter, it squirmed and contracted in that region, while both siphons remained open. Only two of the five *Cionae* responded in this manner, however. The reaction time in the one case was 18 seconds and in the other 26 seconds. Although the animals lay submerged in a depth of $4\frac{1}{2}$ cm. of sea-water, this comparatively slow reaction time may have been due to the thermal effect rather than to the actinic effect. A thermometer placed at that depth and illuminated for twenty seconds showed a rise of temperature of $0.2^{\circ}\text{C}.$, but no experiment was performed to decide the point in question.

SUMMARY OF THE RESULTS OF PREVIOUS INVESTIGATORS

Since Hecht ('18) has carefully reviewed the literature of the subject, I give below, from my own reading, only a brief summary of the results of both neurohistological and physiological studies made by earlier investigators.

A. Neurohistological observations (Van Beneden and Julin, '84)

1. The ganglion consists of a central fibrillar substance surrounded by a peripheral layer of cells.

2. The largest cells lie to the outside.

3. There are localized groups of cells chiefly at the anterior end and on the ventral side of the ganglion which are suggestive of motor centers.

4. Large nerve trunks run forward to the oral and backward to the aboral siphon.

5. These break up into branches to form a nerve net about the muscle fibers.

6. There are said to be club, brush, and plate-like motor nerve-endings present.

7. No specialized sensory nerve-endings have been described.

8. A cord of large ganglion cells extends down into the viscera.

9. The ganglion develops from the cerebral vesicle of the larva.

10. The visceral cord develops from the epithelial wall of the central canal of the larval nervous system.

11. The regenerating ganglion develops out of a derivative of the same embryonic tissue from which the original ganglion developed. (Schultze, '00).

B. Physiological observations (for Ciona intestinalis)

12. The siphons are the most sensitive parts.

13. Rapid stimulation (prodding) is more effective than slow continued pressure for the same intensity of stimulus (Poli-manti, '10).

14. Duration of contraction increases with increase in the strength of stimulation (Kinoshita, '10).

15. The aboral siphon is the most sensitive, judged by the shortness of the latent period; but judged by the duration of contraction, the oral is the most sensitive (Polimanti, '10).

16. Successive stimulations cause both a decrease in vigor and duration of contractions (Kinoshita, '10).

17. Contraction of the siphon begins at the tip and travels downward, while relaxation begins at the base and travels upward (Polimanti, '10).

18. There are four types of reflexes: 1) the individual, involving only the local region stimulated; 2) the protective, in which the impulse travels swiftly from siphon to siphon via the ganglion; 3) the general, which spreads more slowly from siphon to siphon via the base of the animal; 4) the ejection reflex (Jordan, '08).

19. Narcotizing reagents are: cocain, magnesium sulphate, chloral hydrate, aceto-chloroform, quinine sulphate, nicotine, hydrochloride (Kinoshita, '10), and morphine in weak doses (Polimanti, '10).

20. Excitative reagents are: Strychnine, and morphine in strong doses (Polimanti, '10).

21. Curare is a partial narcotic according to Kinoshita ('10), but an excitant according to Polimanti ('10).

22. As to the effect of temperature: 30°C. causes frequent opening and closing of siphons; 32° begins to have a benumbing effect, and 35° causes animal to shrink and become unresponsive (Polimanti, '10).

23. For the effect of light only negative results were obtained by Kinoshita ('10).

24. Extirpation of the ganglion produces four chief effects: interruption in the coördination of the siphons, decrease of sensitivity, increase of latent period and a lengthening of the duration of contraction (Kinoshita, '10; Polimanti, '10).

25. The ganglion regulates reflexes in a feeble way either by inhibiting or facilitating them (Jordan, '08; Polimanti, '10).

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