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Aspects Of The Oculomotor System Of Callinectes Sapidus

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By

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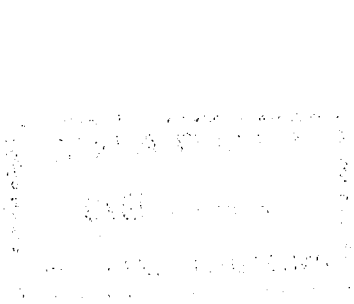

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This dissertation, written and submitted by

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ABSTRACT

An isolated perfused preparation was developed for the study of several aspects of the oculomotor system of the blue crab, Callinectes sapidus. The system for eyestalk rotation was investigated on an extracellular level. Two antagonistic pairs of muscles under visual and statocyst control were found to be responsible for stabilization and rotation of the eyestalk. The primary sensory input to the muscles appears to be from the statocysts, with both static position sense and dynamic acceleration components influencing the motor response. Two sensory feedback systems from mechanoreceptive hairs were found which influence the response of the eyestalks to statocyst input. The function of one system appears to be to allow the animal to differentiate between statocyst stimulation caused by whole body movement and that caused by movement of the basal segment of the antennule in which the statocyst is lodged. The second negative feedback system appears to have a multiple function. It is believed to function to null out the tonic excitatory position sense input from the statocysts when it is necessary for the eye to make a movement which is contrary to the position sense input as, for example, when the animal is following a visual target whose direction is opposite to that of the statocyst drive. It also produces reciprocal inhibition of the antagonist muscle. In addition, this system may be responsible for the incomplete compensation seen in compensatory eye movements made in response to pitch of the body.

A preliminary survey of the oculomotor neurons and interneurons in the cerebral ganglion established the potential of this ganglion for intracellular recording from components of the oculomotor system. Recordings were made from both motorneurons and interneurons. The recording from interneurons of the

oculomotor system was particularly good. Eye movements could be elicted in response to visual and tactile stimuli while recording from the ganglion. The preparation appears to be an excellent system in which to undertake an extensive analysis of intracellular events in the neuronal network underlying stereotyped eye movements and could lead to an understanding of the neuronal basis for such movements.

In the course of the above work on the oculomotor system, some observations were made on the cor frontale which controls the blood pressure to the cerebral system. The cor frontale had been thought to function as a heart regulating blood flow to the cerebral ganglion. It appears from this work that the cor frontale may not function as a heart but rather as a resistive mechanism for regulation of the blood pressure, more like the vertebrate arteriole. Furthermore, the function of this organ may not be to protect the flow to the cerebral ganglion but rather to insure the constancy of the pressure in the peripheral sensory, integrative and oculomotor apparatus of the eyecup.

GENERAL INTRODUCTION:

The oculomotor control system mediates the most highly coordinated, finely controlled movements of the body. The characteristics of this system have been the subject of intensive research using many different animals and approaches in different laboratories. In a recent comprehensive review of the subject, the eye movements are divided into four major subdivisions: the saccadic, the smooth pursuit, the vergence and the vestibular systems (Robinson, 1968). Most animals with finely controlled motile eyes possess at least three of these systems, (with vergence being found only in animals with binocular vision). The study of the neural mechanisms underlying these movements is being approached by several paths. From a recent symposium conducted by the Smith-Kettlewell Institute and the resultant book, The Control of Eye Movements (Bach-y-Rita, Collins and Hyde, 1971) a limitation of the present approaches to the study of the neural mechanisms controlling eye movement is seen. From the approach using psychophysical data and electrophysiological recording from motor and sensory elements of the system in the periphery, inferences can be drawn as to the central neural mechanisms responsible for the observed input-output relations. However, this data does not yield information on the specific cellular mechanisms responsible for the generation of eye movements. There is also a growing school of researchers approaching the central genesis of eye movements by electrophysiological recording from the central neurons of the oculomotor system of vertebrates, primarily in fish cats and monkeys. This approach is limited by the technical difficulties of recording from the neurons in the oculomotor system in higher animals, particularly when the recording is on the intracellular level. Not only are the neurons located in the less accessible sub-cortical areas of the brain but the

location of the interneuronal levels of the control system is spread throughout the subcortical area and largely unknown.

In order to have access to all the neuronal elements of the oculomotor system, the author chose to work with an animal with a very simple nervous system which possessed the range of eye movements found in higher forms. This approach is a common one which has been used successfully in many areas of neurophysiology. In vision, the most notable example of this is the work on lateral inhibition (Hartline, 1956) which was first discovered in the very primitive horseshoe crab, Limulus, and has proved to be a universal principle of sensory systems in higher animals. Since the decapod crab had been shown to exhibit a wide range of eye movements and extensive work had been done on the peripheral characteristics of the oculomotor control system, this animal was chosen for study. The following thesis is an investigation of several aspects of eye movements in a decapod preparation which has been developed for the study of the oculomotor control system on a neuronal level.

The presence of complex eye movements in decapod crustacea has been known for some time and the characterization of these movements investigated (Bethe, 1897, 1903 ; Von Buddenbrock, 1930, Von Buddenbrock and Friedrich, 1933; Dijkgraaf, 1955, 1956 a, b, c; Kunze, 1961, 1963). Most recently, the laboratory of G. A. Horridge has carried out an extensive study of the anatomy and movement capabilities of the eye and the peripheral electrophysiological correlates of movement of the eye in the decapod crab, Carcinus maenus. (Barnes and Horridge, 1969; Burrows and Horridge, 1968, a, b, d; Horridge, 1966, 1968; Horridge and Burrows, 1968, c, e, f; Horridge and Sandeman, 1964). The following will sum up some of this work, particularly as related to the study of the central mechanisms controlling eye movement.

ANATOMY OF THE PERIPHERAL OCULOMOTOR SYSTEM

The oculomotor system of Carcinus maenus, a common European and American shore crab used by the laboratories of G. A. Horridge for study of the oculomotor system, follows a pattern common to decapods. The morphological terminology used for Carcinus was taken from that for Callinectes sapidus, the American blue crab, (Cockran, 1935) in which the most extensive dissection of the system had been done. The oculomotor apparatus consists of an eyecup connected to an eyestalk by a flexible arthrodial joint. The eyestalks of each side are united in the midline by a middle cylinder. The eyecup contains the ommitidia and the first four levels of neural integration of visual information. Nine muscles support the eyecup in a flexible joint with the eyestalk. In addition two pairs of muscles control rotation and stabilization of the eyestalk. The control of eye movement is a function of the activity of fast and slow fiber systems of these eleven pairs of muscles. The motor supply to the nine eyecup muscles is carried in the optic and oculomotor nerves. The former contains only two motor axons and a large number of sensory, primarily visual, fibers. The latter contains approximately thirty motor axons and a small unknown number of mechanoreceptive fibers. The two motor neurons in the optic nerve mediate the withdrawal reflex (Sandeman, 1967). Those of the oculomotor nerve are primarily optokinetic and geotatic in function. The origons of the two nerves, the optic and oculomotor, in the central ganglia are separate. (Hanstrom, 1928).

MOVEMENT CAPABILITIES OF THE EYE

Movement of the eyecup upon the eyestalk consists of a total excursion of 30 degrees in the horizontal plane, 70 degrees in the vertical plane and 50 degrees rotation about the longitudinal axis. The joint is formed by a

flexible arthrodial membrane which joins the eyecup to the eyestalk and the relative position of the eyecup on this stalk is controlled by the balance of activity in the nine muscles. This balance is the result of a central program since interference with the action of one muscle does not change the impulse pattern to any of the other muscles. There is no evidence of a proprioceptive interaction between the muscles.

The movements of the decapod eye consist of three reflex movements in response to specific stimuli and four small amplitude movements which require no sensory input. The reflex movements are:

1. an optokinetic nystagmus in the horizontal plane
2. statocyst controlled movements to maintain an absolute position of the eye in space
3. withdrawal movements of the eyecup into the socket in response to mechanical stimulation

The small amplitude movements which are superimposed on the above reflex movements are:

1. a tremor of 2 - 5 cps and 0.05 - 0.2° amplitude
2. a flick or saccade with 0.05 - 0.2° amplitude and an initial fast phase and slow return phase
3. scanning movements of 0.5 - 1° amplitude
4. a slow drift in the absence of light or visual contrasts

MUSCLE ELECTROPHYSIOLOGY AND EYE MOVEMENT

Two general classes of activity, tonic and phasic, can be recorded from the eye muscles and correlate with the presence of fast and slow muscle fiber structure and innervation. Within these general classifications, fibers of intermediate type are found. The situation is similar to that in vertebrate eye muscle fibers (Hess and Pilar, 1963, Bach-y-Rita and Ito, 1966) although

the absolute values differ.

Structurally, the fast and slow muscle fiber systems in the crab differ in sarcomere length, fibrillar organization, endplate organization and arrangemen of sarcoplasmic reticulum. Physiologically, the fibers differ in contraction speed, and in degree of excitability of the membrane, ranging from a totally electrically inexcitable membrane to one with the production of spikes upon depolarization. The intracellular recording from phasic muscle fibers shows resting potentials from 65-80 mv., junction potentials of 20-30 mv., short rise time and decay times and show facilitation. Tonic activity is recorded as a steady discharge which increases during certain movements. Tonic muscle fibers have resting potentials of 50-60 mv., junction potentials of 5-15 mv., slower rise and decay times and no facilitation. One muscle may show both types of activity and intermediate values between the two general types, recorded from different fibers in the same muscle. An interesting aspect of the fast and slow fiber system is that the fast fiber system is active only during movement of the eye and only in definite phases of that movement. This seen most clearly in optokinetic nystagmus where the fast fiber system does not come in until near the end of the slow phase, is then inhibited centrally, and the fast fiber system of the opposing direction then comes in for the fast phase of optokinetic nystagmus in the opposite direction.

OPTOKINETIC NYSTAGMUS IN DECAPODS

Optokinetic nystagmus in decapods consists of the typical slow forward phase and a rapid return phase. In the slow phase, the eyecups follow the rotation of the environment (most effectively, the rotation of a striped drum). The eyes are then rapidly flicked back in the opposite direction during the fast

phase. The extent of the movement in Carcinus ranges from 2 to 20 degrees and varies with the drum speed. The effective drum speeds for evoking movement are from 0.0048 degrees to 5 degrees per second. The velocity gain is around unity at high drum speeds, (1 - 5° /sec) increases to 15 for lower speeds(.01°/sec) and decreases again at the lowest speeds. (Horridge and Sandeman, 1964).

THE SLOW PHASE OF OPTOKINETIC NYSTAGMUS

The slow phase of optokinetic nystagmus is controlled by the interaction of the central mechanisms with the parameters of the effective visual stimulus. (The effective visual stimulus is the slip speed which is the difference between the velocity of the moving drum and the velocity of the resultant eye movement). The efferent impulse pattern to the muscles is characteristic for the slip speed and consists of a tonic firing rate which increases in frequency with the duration of the slow phase and a phasic firing which comes in at high frequency only near the end of the slow phase. By clamping one eye and blinding the other, the blind eye is driven by the immobile seeing eye to make the optokinetic movements appropriate for the visual stimulus with no interference due to the lack of movement in the clamped seeing eye.

The appropriate impulse pattern in response to the slip speed is generated by the central ganglia irrespective of whether or not the eye is allowed to move. Peripheral movement thus has no control over the efferent response pattern to the visual stimulus.

If spontaneous withdrawal of the eye interrupts the slow phase, upon extension the eye returns to the position at which it was interrupted and firing resumes at the frequency characteristic of that point.

THE FAST PHASE OF OPTOKINETIC NYSTAGMUS

A set of experiments were devised to demonstrate that the onset of the fast phase of optokinetic nystagmus is part of a centrally determined program without the influence of proprioception. It can be shown by the following experiment that the onset of the fast phase depends on a central program and is not dependent upon the position reached by the eye in the slow phase or dependent upon muscle tension generated by the position of the eye. The oculomotor nerve is cut on one side and the optic nerve is cut on the opposite side. In this way, the seeing eye cannot move and the moving eye cannot see. The efferent impulse pattern to the muscles of the moving eye and the cut oculomotor nerve in response to a moving striped drum is normal and equal in both eyes. The same appropriate impulse pattern is put out by the brain regardless of whether the driven eye is intact, removed or clamped. The impulse pattern determining the onset of the fast phase is determined only by the effective visual stimulus to the seeing eye.

Further evidence that the pattern of nystagmus is centrally determined is seen in the following experiment, in which both eyecups of the crab are cemented into their sockets and one eye is blinded. A striped drum is moved a few degrees and stopped. If the seeing eye were not clamped, its movement would evoke a relative movement in the opposite direction which would cut off the nystagmus. When the eyes are clamped, the patterned output for nystagmus continues for up to one minute after the drum is stopped. The onset of the fast phase occurs with the same frequency as seen in the nystagmus evoked by the presence of the moving drum. It appears to be the "run down" of a pattern generator which is normally cut off by the relative movement in the opposite direction of a moving eye across a stationary drum.

The central nervous system is capable of some degree of plasticity by which the position in the slow phase at which the fast phase occurs can be altered. The discharge to one muscle, number 21, is primarily responsible for the slow phase toward the midline. The fast phase has been shown to occur when the discharge to this muscle reaches a certain frequency. By applying an electrical shock to the eye just before the discharge to muscle 21 reaches the frequency for the onset of the fast phase, the subsequent fast phases will begin earlier at a lower discharge frequency than before. The animal has modified the central program to make the fast phase occur earlier at the lower discharge frequency and thus avoids the shocks. It will continue to show this earlier onset of the fast phase for up to an hour after the shock procedure is eliminated.

TONIC AND PHASIC SYSTEMS IN PARALLEL

The eye muscles of most animals are composed of a spectrum of muscle types ranging from extremely fast to very slow (Hess and Pilar, 1963; Bach-y-Rita and Ito, 1966; Burrows and Horridge, 1968, a). The movement detection system is likewise composed of elements of differing time constants, ranging from those which register very fast movements to those registering extremely slow movement (Wiersma, Bush and Waterman, 1964). The motor system and the movement detection system must interact to produce several types of eye movements. The interaction of these two systems could take place in three ways. One could have slow muscle fibers directly driven by the sensory elements which register slow movement and the fast muscle fibers directly driven by the sensory elements monitoring fast movement. This is the "in parallel" theory. Alternatively, there could exist a common interneuronal pool in which the input from movement detection units of all time constants is fed. The out-

put of this pool could then be used in either of two ways. When the control system dictated the need for fast or slow movement, the interneuronal pool would send out activation only to the necessary fast or slow muscle fibers. Alternatively, the interneuronal output could be issued en masse to all motoneurons and the thresholds of the individual motoneurons would be set to determine which elements fired. The latter idea is very popularly known as the "size principle" of motoneurons (Henneman, 1965, Davis, 1971). This question can only be settled by stimulating and recording from specific units on the sensory and motor side of the system. The oculomotor system of the decapod is suited for analysis of this question because the sensory units of differing time constants can be isolated in the optic nerve (Wiersma, Bush and Waterman, 1965) and, as will be shown in the following work, the tonic and phasic oculomotor neurons can be separated for recording centrally.

There is some evidence favoring an "in parallel" system in Carcinus. Using optokinetic nystagmus, a means of adapting out the fast components of the visual response by initially habituating the eye to the rapid oscillations of the drum. In optokinetic nystagmus, there exists a period at the very beginning of the response when the eye is stationary while the drum is moving. The response of the eye is a spurt of movement until the eye catches up with the drum. This response may be more than 100% of the stimulus amplitude. By recording from the motor axons, it can be shown that this fast spurt of movement is carried out by the fast muscle fiber system. This initial spurt can be removed from the response, however, by first habituating the eye to a rapid oscillation of the drum. The response to movement of the drum then begins with the steady state response of the tonic muscle system, without the usual initial phasic response, and reaches ultimately the same eye drum relation of the normal response. (Horridge and Burrows, 1968, e).

This would seem to indicate that there are two separate systems, as in the "in parallel" theory, with one driven by slow and one by fast movement detection systems. There is one problem with this conclusion, however. The phasic system is active during large displacements of the eye even when the velocity of movement is slow.

The preceding material is an introduction to the work which has been done using the oculomotor system of decapods. The sophistication of the oculomotor system in this animal which has been demonstrated by psychophysical methods and analysis in the periphery encourages a central analysis of events in the cerebral ganglion which are responsible for observations in the periphery. The following thesis has been aimed at the development of this preparation for central analysis. An isolated perfused preparation was developed to work with the cerebral ganglion. This is necessary since the cerebral ganglion, unlike the peripheral parts of the nervous system, must be constantly perfused to maintain function of the neural tissue. The work with this preparation then falls into three divisions. The first section deals with the system which controls eyestalk rotation. The response to this system to visual and geotatic control was explored and the presence of two negative feedback systems involved in regulation of eyestalk rotation discovered. The second section is aimed at the development of intracellular analysis of the central neurons involved in oculomotor control in the cerebral ganglion. The final section is an example of scientific fallout. A pair of muscles which had been classified as oculomotor (Cockran, 1935) were observed to be part of a cerebral blood pressure regulating system. Because the regulation of the cerebral blood pressure is of vital importance to the functioning of the optic and oculomotor system, the work on the pressure regulating system is included here.

II. THE NEURAL AND MUSCULAR SYSTEM FOR EYESTALK ROTATION

INTRODUCTION:

The complete structure for movement of the eye in the blue crab is a complex arrangement consisting of an eyecup, an eyestalk and a middle cylinder. The eyecup, containing the ommitidia and the first four levels of sensory integration, projects from the eyestalk on a flexible joint. The eyestalks are connected to each other in the anterior central portion of the animal by a middle cylinder. The major part of eye movement control is carried out by nine pairs of muscles of the eyecup - eyestalk joint. There are, in addition, two pairs of muscles which control the movement of the eyestalk and, by stabilization or counter rotation of the eyestalk, keep the eyecup in a position in which it is able to maintain the largest array of ommitidia horizontal in space. These muscles, the musculus oculi basalis anterior, or muscle 15 and the musculus oculi basalis posterior, or muscle 17, are the subject of the following work. Muscle 15 arises from the posterior lateral surface of the middle cylinder and extends back over the cerebral ganglion to insert on an apodeme which attaches to an epistome under the cerebral ganglion. Muscle 17 attaches to the ventral carapace and to the ventral part of the middle cylinder. It is muscle 15 which is the primary subject of the following work although some observations on muscle 17 as the antagonist to muscle 15 are included.

There is no published report on the muscles of the eyestalk of Callinectes, with the exception of their anatomical description (Cockran, 1935). In another decapod, the Hawaiian swimming crab, Podophthalmus vigil, an extensive study of the electrophysiological and ultrastructural characteristics of the levator of the eyestalk, the analogy of Muscle 15 in Callinectes, was done in an in vitro preparation. (Hoyle and McNeill, 1968 a).

The levator in Podophthalmus was shown to contain two general categories of muscle fibers, slow, pink fibers and fast, white fibers which were found within separate bundles in the muscle. Four axons, two large and two smaller ones, supply the muscle. Although extensive analysis of the anatomical and electrophysiological characteristics of the muscle was done, no exact correlations of muscle fiber type with muscle function could be made because the work was done in an in vitro preparation. Speculations of functional correlations are made solely on the basis of visual following movements. In the following work on Callinectes, the muscle is left intact in the animal and its function investigated with the use of natural stimuli to use the sensory channels of the animal rather than electrical stimulation. In addition to the visually oriented functions of the eyestalk considered in Podophthalmus, it will be shown that in Callinectes the muscle is also used in statocyst mediated compensatory movements of the eyes.

The sensory physiology of the statocyst system has been investigated in some detail (Cohen, 1955, 1960; Dijkgraaf, 1955, 1956, a, b ; Cohen and Dijkgraaf, 1960). The statocyst in crustacea has been shown to function as the geotatic or gravitational receptor in much the same manner as the vestibular system in higher forms. In the statocyst, the static position sense is mediated by the statocyst hairs in contact with a calcarous statolith. Angular acceleration about the three major body axis is mediated by a second type of thread hair. The influence of these two receptor types will be analyzed in relation to compensatory movements of the eyestalk of Callinectes. In addition, two systems of mechanoreceptive sensory hairs on the eyestalk and antennule basal segment have been described which appear to mediate three and possibly four, negative feedback systems whose function is to null statocyst input.

MATERIALS AND METHODS

The animal preparation consisted of an isolated perfused portion of the anterior carapace containing the eyes, the cerebral ganglion, the statocysts and variable portions of the muscular and neural tissue of the esophageal region (see fig. 2). The dorsal artery was cannulated and the preparation perfused with a solution of ionic content similar to the blood of Callinectes sapidus (Perkins and Wright, 1969). The solution was buffered with Tes (Sigma Co.) and adjusted to a pH. of 7.5. Approximately 100 mg% glucose was added and oxygen bubbled through the solution before use. The perfusion pressure was kept at an experimentally determined optimal value by suspending the perfusion bottle three feet above the preparation. All experiments were done at room temperature. Under these conditions, the preparation could be maintained in good condition for 5 to 6 hours. During this time, reflex movements of the eyes could be repeatedly elicited, using visual, tactile or statocyst stimulation.

For intracellular recording, KCl filled microelectrodes were used with tip resistances ranging from 5 to 60 Mohms, depending upon the recording aims. A unity gain capacity compensating electrometer (Bak, 1958) with a variable gain Dana preamplifier was used for intracellular work. For extracellular recording, suction electrodes were used. The extracellular amplification was initially done with a Tektronic 122 amplifier and in later stages of the work, a 4 channel differential a.c. amplifier with a fixed gain of 1500 was used. Permanent records were initially made from a Tektronix storage oscilloscope with a Polaroid oscilloscope camera. In later stages a Grass C-4 camera was used for continuous records.

A movement detection device and tilt table were constructed for use in experiments on pitch compensation. The movement of the crab's eyestalk was measured by use of a capacitative movement transducer which did not impede eye movement (Sandeman, 1968). The transducer is capable of measurement of angular displacement of 0.01 degree over a 25 degree range and is linear for displacements up to 15mm. Calibration was done several times in each experiment by moving the wand a known distance between the sensors manually and the data was calculated trigonometrically to obtain angular excursion.

A tilt table was used from which the degree of tilt could be read out on the oscilloscope. A voltage proportional to the degree of tilt was obtained by the use of a potentiometer and 1.5 volt battery linked mechanically with the axis of the table. Pitch and roll was obtained in sine wave, step and ramp form by manual rotation of the axis of the table. The crab was placed on the table in the pitch or roll axis and held in place with modeling clay at the tips of the carapace.

A modification of the whole nerve technique (Iles and Mulloney, 1971) was developed to fill the axons to muscle 15. In the original method, a pool of procion yellow dye is formed with vaseline on a glass slide. The cut end of the nerve to be filled is placed in the pool of dye. The cathode is placed in the pool of dye and the anode placed in the solution containing the animal tissue. When current in the y amp range is passed across the electrodes, the dye can be observed to pass up the axons toward the ganglion to fill the cell bodies. This method was modified to fill the shorter axons of muscle 15 where it was necessary to have the dye closer to the ganglion. A small glass bowl was blown in the end of a heated piece of glass capillary tubing, - 1 mm. in diameter, and the edges of the bowl covered with silicone gel, which will form a water-tight

Fig. 1 *Callinectes sapidus*, the Blue Crab

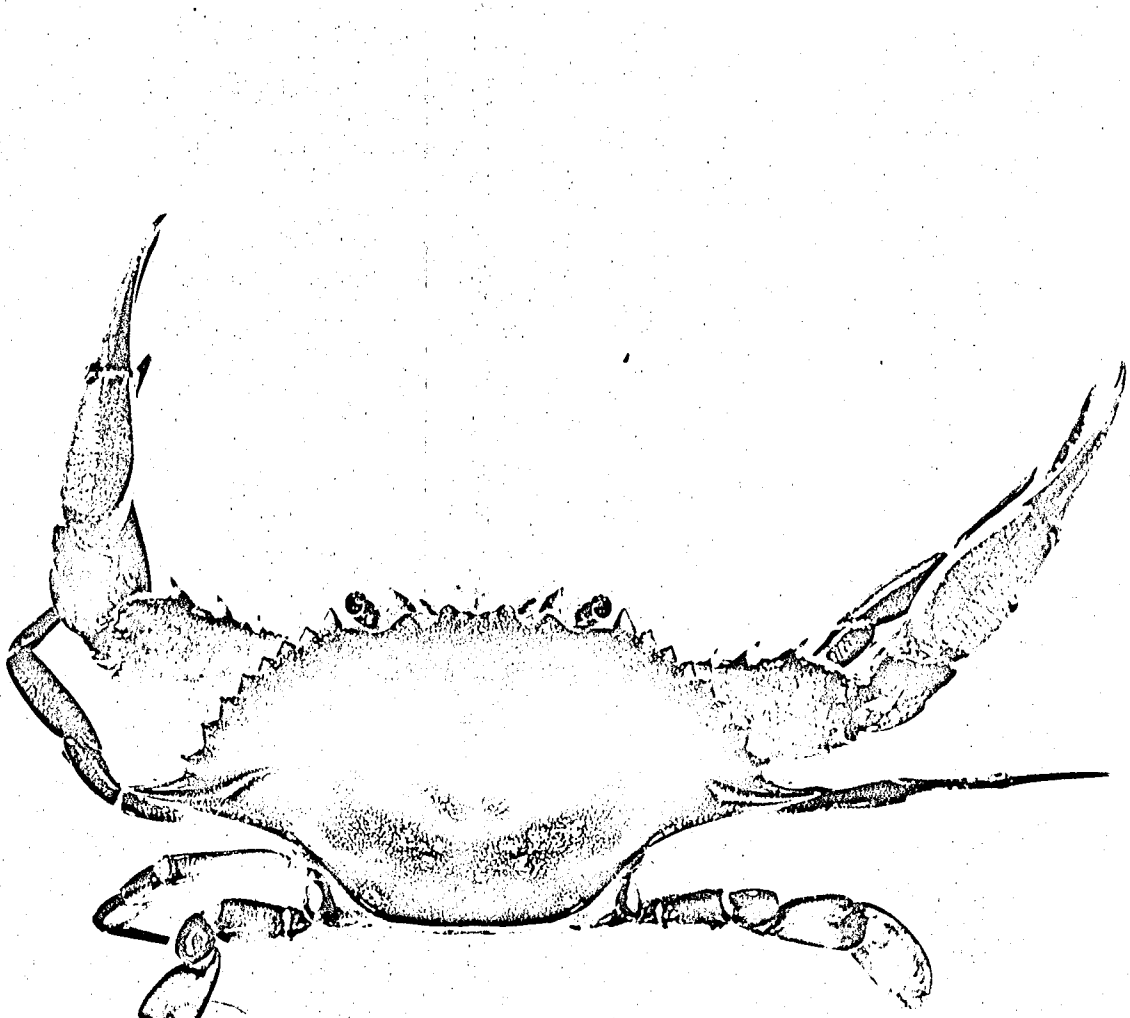
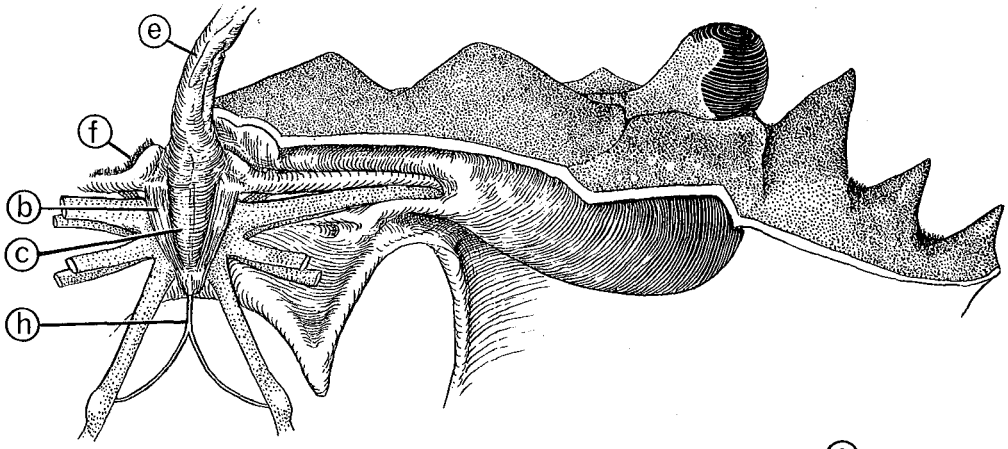


Fig. 2 Cerebral System of Callinectes sapidus illustrating relation of cerebral ganglion (j) to muscles of the eyestalk (b and d), cor frontale (c, g, h, i) and sensory hairs of eyestalk (f). Common apodeme (a) is the point of attachment for muscles 15 and 16.



a.— common apodeme

b.— muscle 15 and nerve

c.— cor frontale muscles 16

d.— nerve to muscle 17

e.— dorsal artery

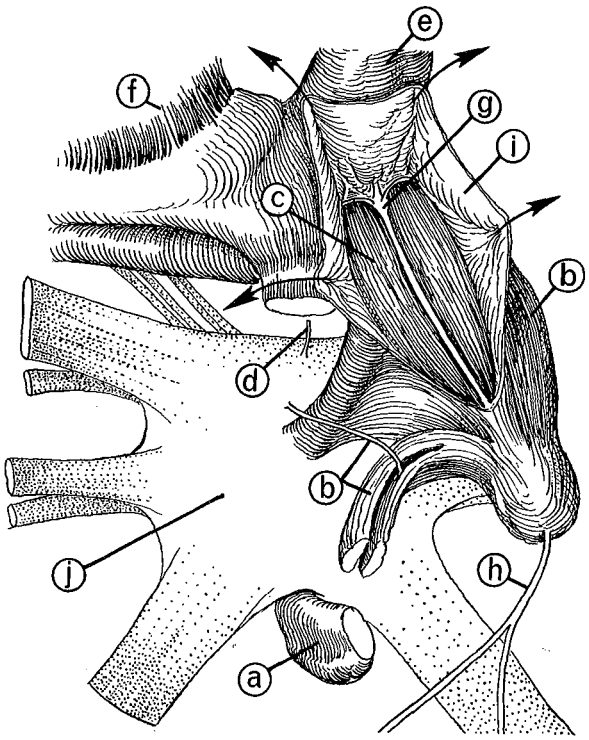
f.— sensory hairs on eye-stalk

g.— ventricular ganglion

h.— ventricular nerve

i.— sinus wall of cor frontale

j.— cerebral ganglion



did not permit this to be tested (see discussion). The fourth axon, of intermediate size, was considered by Hoyle and McNeill to be a peripheral inhibitor on the basis of scanty anatomical evidence. This axon was never seen to fire nor was any evidence of peripheral inhibition seen. It is possible that the two intermediate axons were actually branches from the same motor neuron in the central ganglion. The nerve to muscle 15 is very short, between 3 to 5 mm. in length and the splitting usually associated with the periphery could have taken place in the ganglion. The spikes from the two axons would then appear as one in the peripheral recording since the distance was not great enough for small differences in conduction velocity to be seen as separate spikes. An attempt was made to test this by filling the axons with procion yellow by the whole nerve technique. Although the axons filled by this method, it was not possible to get the somata to fill. The axons could be seen extending into the cerebral ganglion to an area containing large somata but no definite assignment of axons to somata could be made.

TWO DIFFERENT EXCITATORY JUNCTIONAL POTENTIALS FROM THE SAME AXION

In the course of frequent extracellular recording from the intermediate size axon and the muscle fibers corresponding to this axon, it was noticed that the amplitude and time course of the excitatory junction potential differed with the location of the suction electrode on the muscle. In an intracellular investigation, this was shown to be the case. The ejps from different muscle fibers, supplied by the same motor axon, differed greatly in amplitude, time course, facilitation and summation. This shown in figs. 4 and 5 where the intracellular electrode is sampling the muscle fibers while the extracellular suction electrode continuously monitors the firing of the axon.

Fig. 4 Differences in excitatory junctional potentials of two muscle fibers supplied by the same axon. Upper traces, intracellularly recorded junctional potential; lower traces, extracellular record of axon discharge. Same time and voltage scales in both traces. Calibration marker is for intracellular records, 100 msec. horizontal, 10 mv vertical marker.

RESPONSE OF MUSCLE 15 TO VISUAL STIMULI

Several types of stimuli were used to assess the response of muscle 15 to visual stimuli (fig. 7). The records are taken from different preparations so the baseline frequency differs. What is illustrated is the difference in firing frequency with the onset of the stimulus. In a is seen the unpatterned discharge recorded extracellularly from the nerve, typical of the preparation in the resting state. The movement of a small black rectangular object across the visual field in any direction and at any speed, with the exception of extremely slow or fast movement, will evoke the patterned discharge seen in b. In c and d is illustrated the response of the nerve and muscle to the turning on and off of a light above the preparation. In e, an unexpected response of the muscle is seen. When the carapace of the animal is touched, the eye normally retracts into the socket. During this retraction, muscle 15 fires vigorously. In f, retraction is further investigated. The eye has been caused to retract prior to the beginning of this record. A small rectangular black object was then passed in front of the retracted eye. The patterning and the increased firing rate is still seen when the eye is retracted.

THE ROLE OF MUSCLE 15 IN COMPENSATION FOR PITCH AND ROLL

a. RESPONSE RECORDED FROM THE MUSCLE IN THE PERFUSED PREPARATION:

Contraction of muscle 15 results in the rotation of the eystalk in a counter-clockwise motion causing a movement of the eyes in an outward and upward direction. This is the response which would be expected as a compensatory movemement to pitch and, perhaps, roll, of the animal. The following set of experiments were designed to ascertain the function of the muscle in pitch and

Fig. 7 Response of muscle 15 in isolated perfused preparation to visual and tactile stimuli. (a) control, extracellular recording from muscle, note unpatterned firing rate typical of unstimulated animal. (b) patterning of activity in response to movement of dark rectangular object, extracellular recording from left nerve, upper trace and right muscle, lower trace. (c) response to onset of light, upper trace, light sensor, up signals light on; middle trace, extracellular from muscle; lower trace, intracellular record from the same muscle. (d) response to light off. Traces same as in c, down on light sensor signals light off. (e) response to tactile stimulus to carapace resulting in retraction of the eye upper trace, extracellular from muscle; lower trace, intracellular from the same muscle. (f) response to movement of object of a above, while eye is retracted; upper trace, intracellular record from muscle, lower trace, record of movement stimulus, movement sensor lags behind response because the eye perceives the stimulus before the stimulus object pases before the sensor.

time scale as shown by marker in lower trace of b is 400 msec per interval.

Fig. 8 Ganglionic inputs controlling response of muscle 15 to pitch. Extracellular recording from the nerve and/or muscle of the right and left sides. Experiment done in the isolated perfused preparation. Time scale, lower trace marker intervals equals 400 msec. between markers.

a. control record, animal horizontal with ganglionic inputs intact.

b. control pitch 30° downward in animal with ganglionic inputs intact.

c. optic nerves cut, animal horizontal

d. optic nerves cut, 30° pitch downward

e. statocysts nerves cut, animal horizontal

f. statocysts nerves cut, 30° pitch downward

Note response to pitch in b of intact animal is still seen in d after section of optic nerves but all response is destroyed after section of statocysts. Change in time course of response in middle trace of d is due to slippage of electrode away from muscle and closer to nerve. Note difference in time course of muscle ejps of right and left sides, see fig.4for further illustration. Loose coupling between the right and left sides is also apparent.

b. RESPONSE TO PITCH RECORDED FROM MUSCLE 15 IN THE INTACT PREPARATION:

The response of muscle 15 to 45 degree pitch and roll as ascertained in the intact animal using a tilt table and small tungsten electrodes placed in the muscle. The results are illustrated in fig. 9. The muscle was active in response to pitch downward, inhibited in pitch upward and unresponsive to roll to either side. These results confirm those obtained in the experiments of the preceding section using the isolated, perfused preparation.

ROLE OF MUSCLE 17 AS ANTAGONIST TO MUSCLE 15 IN PITCH

It has been shown that muscle 15 is responsible for compensation for pitch forward and inhibited in pitch backward. Since the animal exhibits compensation for pitch backward, the muscle responsible for this was sought. On the ventral side of the eyestalk is a muscle pair, muscle 17, (terminology, Cockran, 1935) which is in a position to act as antagonist to muscle 15 in rotation of the eyestalk. That this is the case is shown in fig. 10 where the increase in firing rate to pitch backward parallels that of muscle 15 to pitch forward. The inhibition or cessation of firing seen in muscle 15 when the animal is pitched in the null direction (backwards for muscle 15) is not as strongly seen in muscle 17. This is also illustrated in fig. 11, when the animal is pitched forward and backward while recording simultaneously from both muscles.

The nerve containing the axons to muscle 17 exits from the ganglion anterior to the nerve to muscle 15. A cross section of this nerve taken at 3 points is seen in fig. 12. It is difficult to determine the number of motoneurons supplying muscle 17 by looking at the cross sections of the axons. There is considerable splitting of the axons during the entire course of the nerve. In

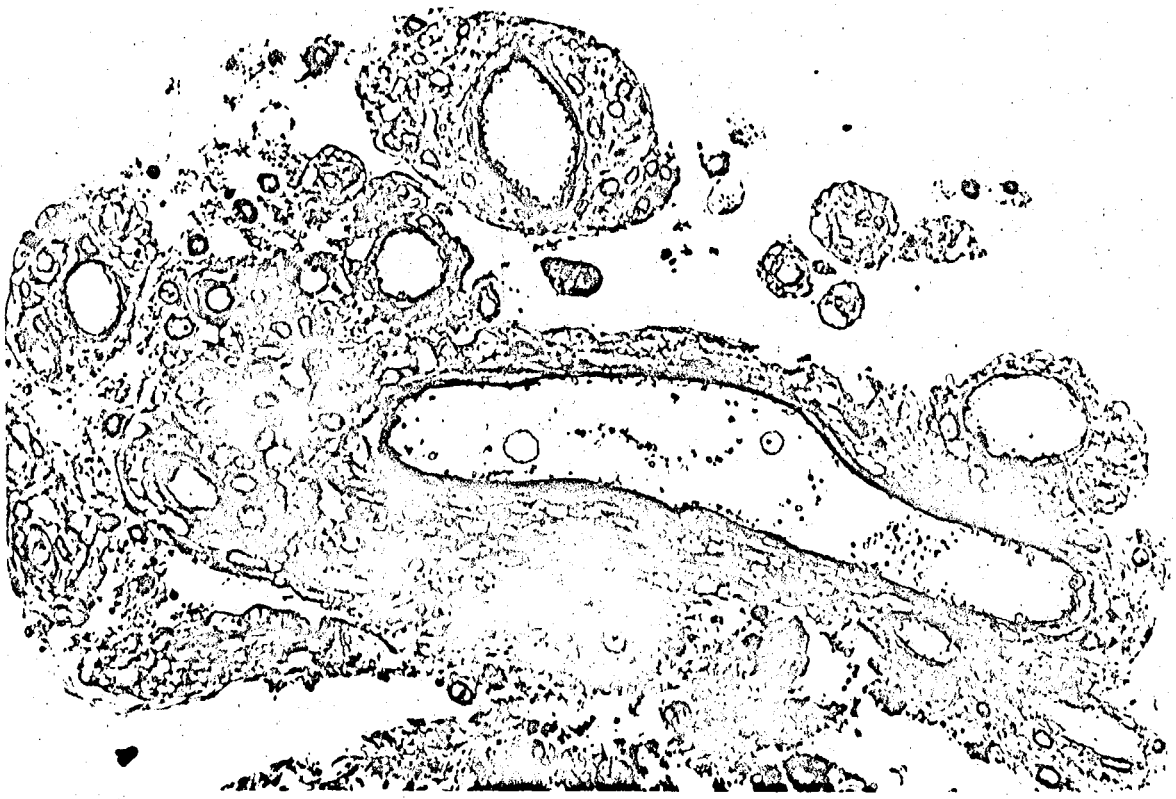
Fig. 9 Response of muscle 15 to pitch and roll. Extracellular recording from right muscle in intact animal. (a) animal horizontal. (b) 45° pitch downward. (c) 45° pitch upward. (d) 45° roll to left. (e) 45° roll to right. Time scale 100 msec. per division.

Fig. 10 Function of muscle 17 in pitch. Firing of muscle 17 recorded extracellularly from the muscle at varying degrees of pitch. (a) 15 degrees pitch upward. (b) level or resting position. (c) 10 degrees pitch forward. (d) 15 degrees pitch forward.

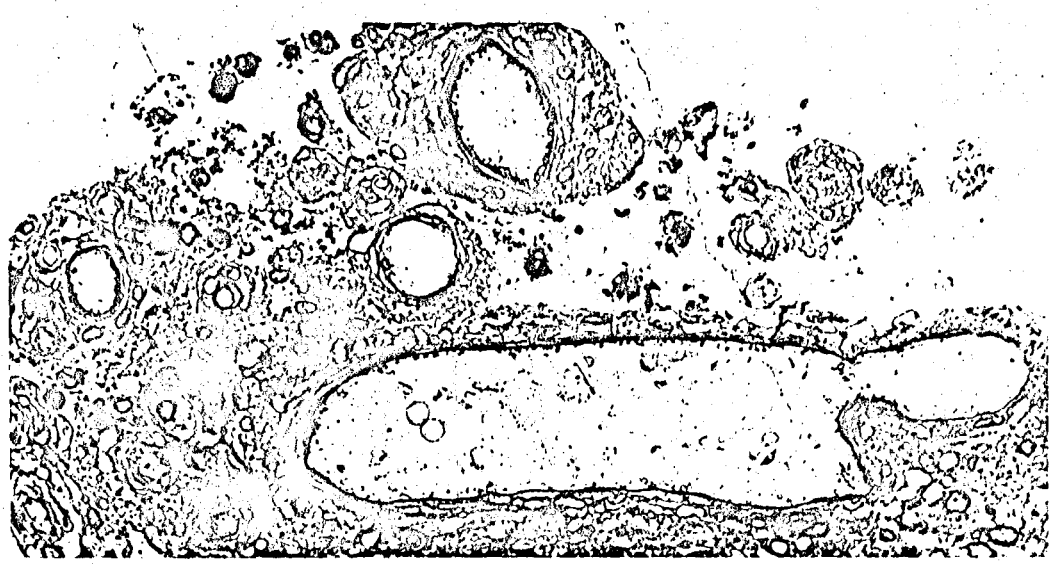
time scale: 0.5 sec.

Fig. 11 Simultaneous firing frequency of Muscle 17 and 15 to different degrees of pitch.

Pitch	Muscle 17 Firing Frequency	Muscle 15 Firing Frequency
level	7	1
5° forward	5	3
10° forward	4	7
15° forward	4	13
20° forward	3	11
level	11	1
5° backward	10	0
10° backward	12	0
15° backward	15	0
20° backward	21	0
4° forward	5	4



A



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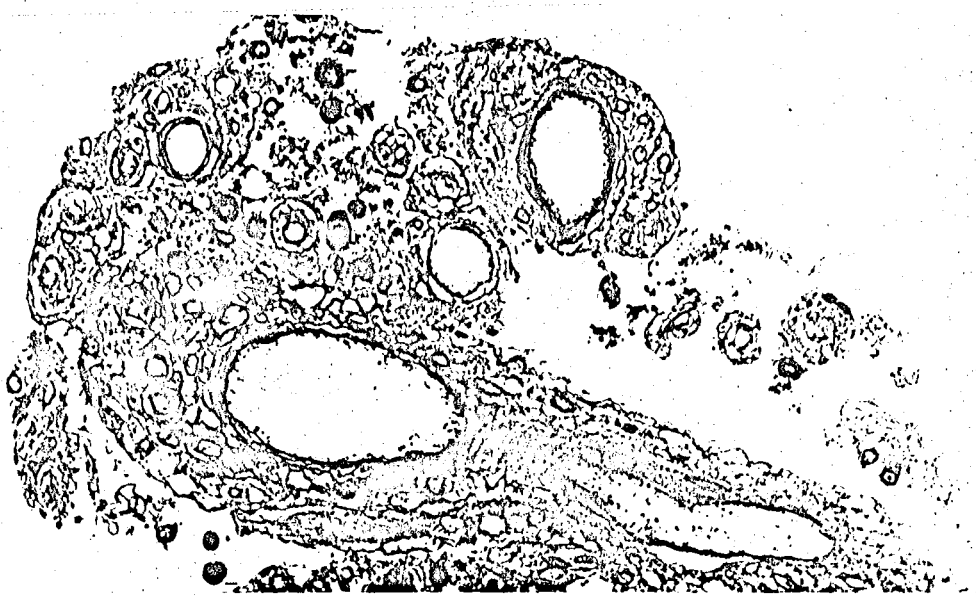


C

extracellular recording from the muscle one large and one small amplitude response with different frequencies of firing can be seen (fig. 13) indicating at least two motoneurons controlling the muscle response. The small amplitude e j p shows a regular tonic firing rate and superimposed on this at irregular intervals is a response of approximately 20 times greater amplitude and time course.

MEASUREMENT OF THE COMPENSATION OF THE EYESTALK FOR PITCH OF THE ANIMAL

Using the intact crab and a capacitative movement detection device (see Methods) the compensation of the eyestalk in response to pitch of the animal was measured. Pitch was restricted to values between 5 and 30°. Sine waves, ramp and step movements in the pitch plane were generated manually using the pitch table. The percentage compensation of movement of the eyestalk for movement of the body was typically under 10%. This is illustrated in fig. 14 in which the sine wave movement of the eyestalk, upper trace, varies between 0.9 degrees and 1.0 degrees in response to pitch of 10 degrees. The response to a step pitch shows an initial fast phase and a slower movement to a maintained value (fig. 15). The majority of the compensatory movement of the eyestalk to pitch showed between 8 and 10 percent compensation. There were a few exceptions to this however. In two preparations the responses showed 75 and 80% compensation. The response was consistent throughout the experiment showing the high compensation for all degrees of pitch. In addition, one preparation showed a high degree of tremor and instability. The eyestalk rotation is usually a smooth well-controlled movement. In this preparation, however, (see fig. 16) the response was larger than 10%, varied with each pitch of the animal and showed the illustrated tremor for the duration of the experiment (3-4 hours). These two exceptions to the

Fig. 13 The two types of excitatory junction potentials recorded extracellularly from muscle 17. The small response, seen as a notch, particularly prominent on the third large spike, is present as a tonic discharge in this muscle and probably corresponds to the small axons seen in fig. 12. The large signal is a phasic discharge which must correspond to the larger axon of the nerve to muscle 17.

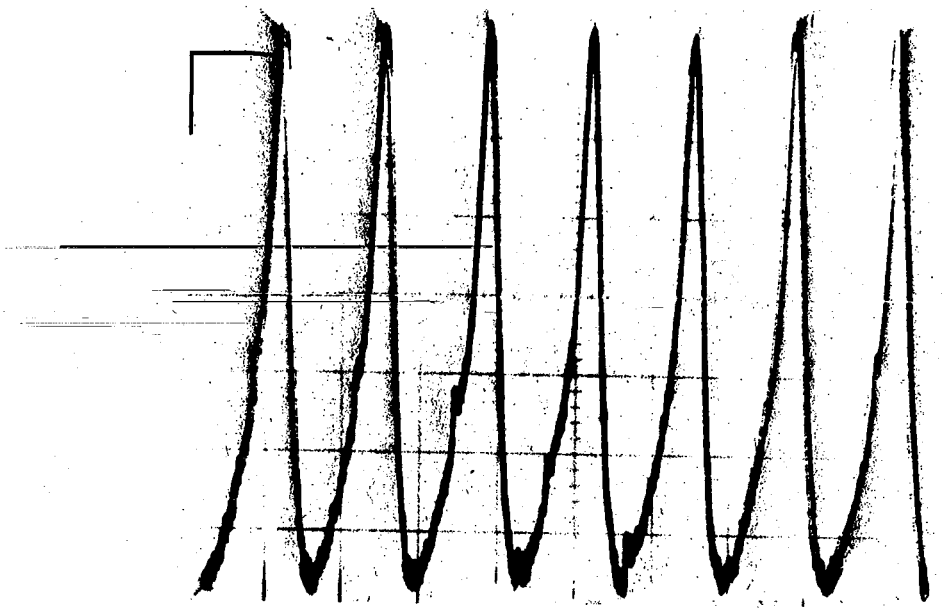


Fig. 14 Compensatory response of the eyestalk to quasi sine wave movements in forward pitch of the animal. Upper trace of each pair is the compensatory movement of the eystalk in response to forward pitch of the whole animal seen in the lower trace. Upper response in A is the normal response of approximately 10% compensation. B illustrates an infrequent recording of much greater compensation, in this case, approximately 70%. Scale: A, 1 degree, upper trace, for 10 degrees pitch in lower trace; lower set, 7 degrees upper trace and 10 degrees pitch in lower record, time 1 sec.

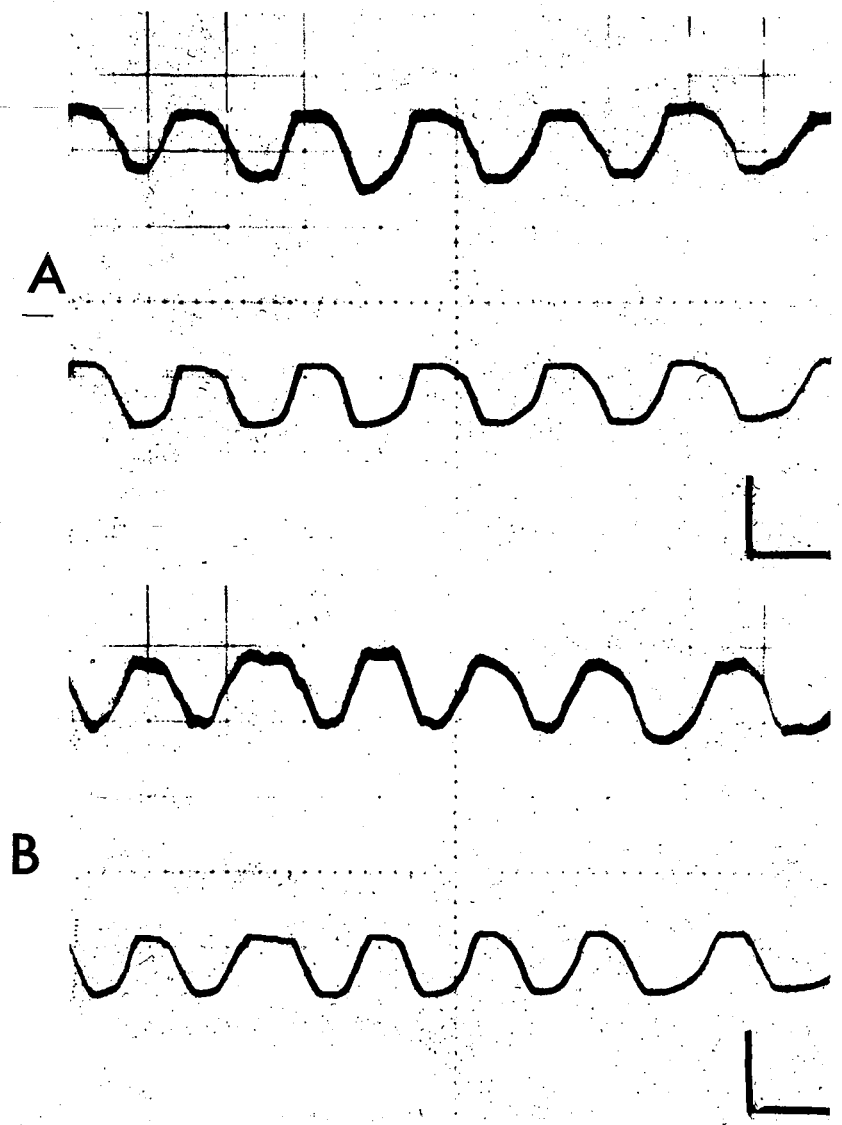

A
B

Fig. 15 Compensatory Response of the eyestalk to step movement in forward pitch of the animal. Response of eyestalk, upper trace; step movement of animal, lower trace. Scale: upper trace, 0.5 degrees; lower trace, 5 degrees; time, 1 second.

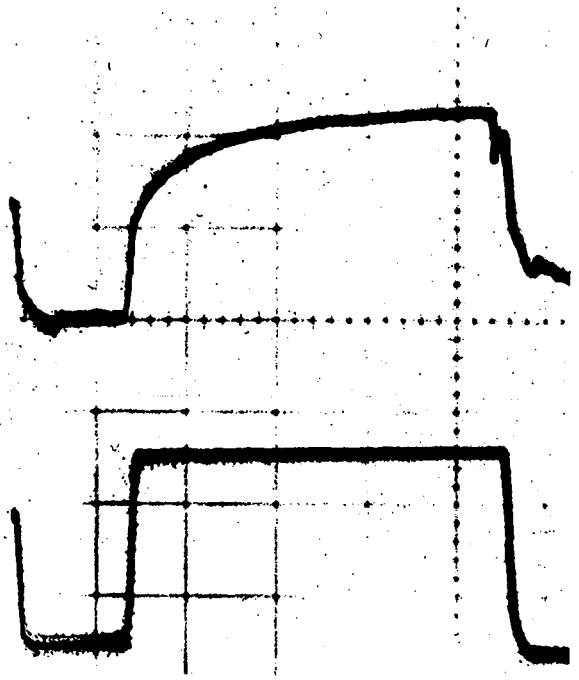
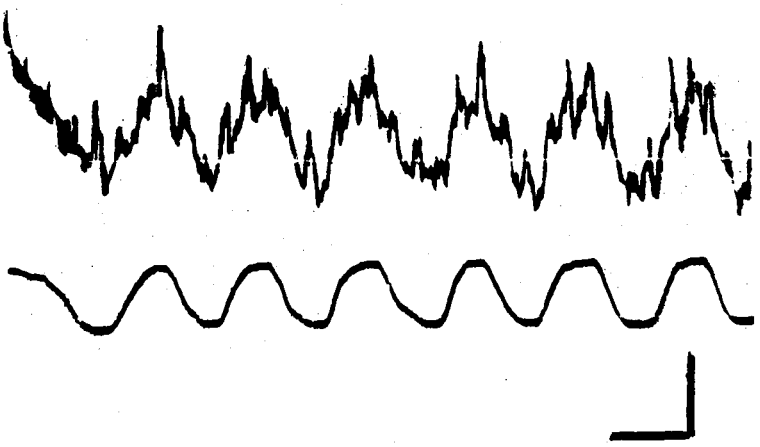


Fig. 16 Tremor and high compensation of eyestalk (upper trace) seen in response to pitch (lower trace) in one preparation. Scale: upper trace, 1.5 degrees; lower trace, 10^{o} degrees, time, 1 sec.

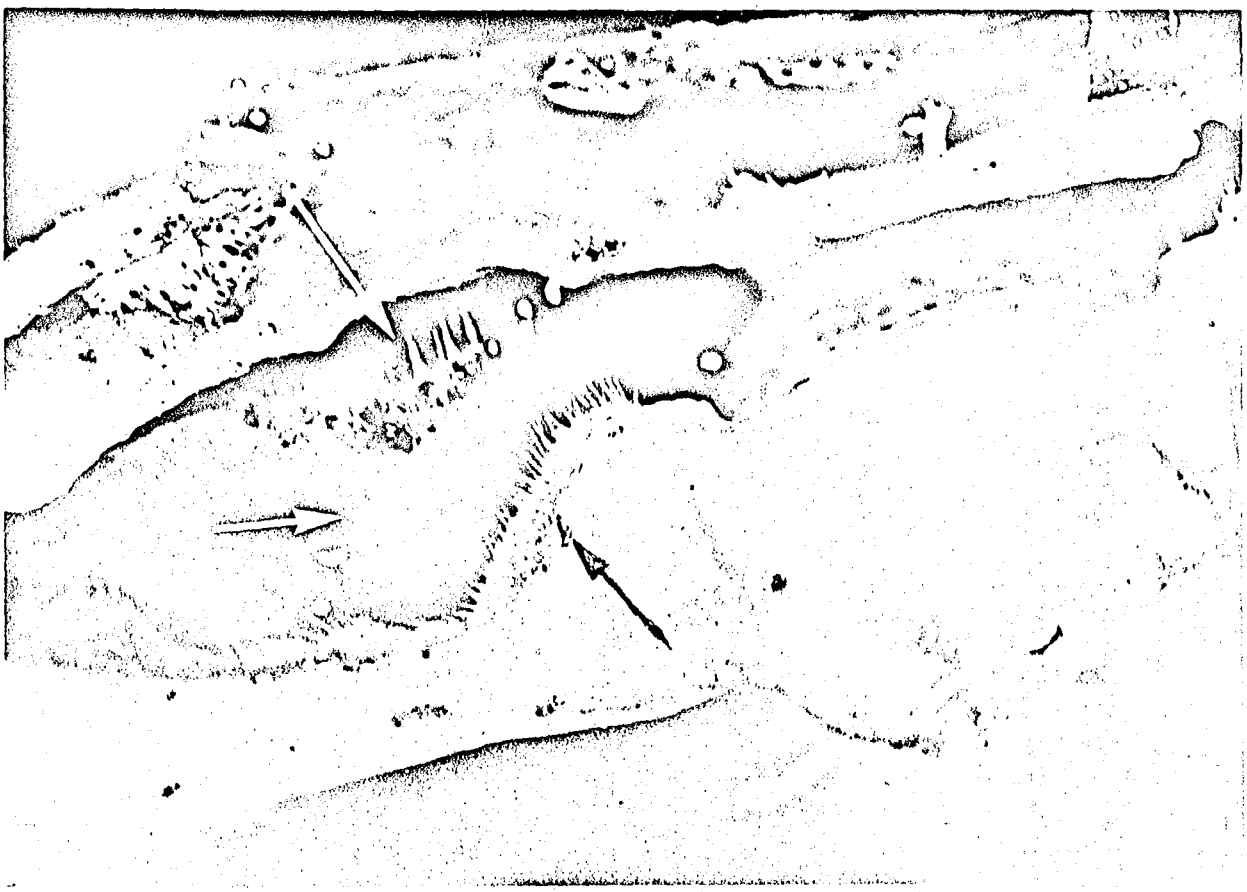


usual compensatory response are believed to be due to interference with a feedback mechanism (see discussion).

MECHANORECEPTIVE SENSORY HAIRS REGULATING MUSCLE 15 AND 17:

a. NEGATIVE FEEDBACK FROM ANTENNULAR SENSORY HAIR SYSTEM: Callinectes has a pair of small antennules which are constantly in motion. The statocysts are lodged in the basal segment of the antennules and move in common with antennule movement. On the dorsal surface of the basal segment are many sensory hairs (figs. 17 and 18). These hairs are long and thread-like and cover most of the surface of the basal segment, being especially numerous around the edges of the basal segment. The hairs extend upward to the under surface of the carapace over the antennule basal segment. The under surface of the carapace above the basal segment also has a number of these sensory hairs. When the animal moves the antennule, the sensory hairs are stimulated by the shearing movement of the hairs against the opposite surface. When the sensory hairs are displaced with a glass needle, an inhibition of activity is seen in both muscle 15 and 17 (fig. 19). The inhibition does not appear to show adaptation, being reproducible for repeated trials over a period of time. The same inhibition can be produced by stimulation of the hairs of the contralateral basal segment. If the sensory hairs are displaced by natural movements of the antennule which the animal is constantly making, this inhibition is not seen. Repeated stimulation of the sensory hairs evoked a movement response of the antennules, which did not cause inhibition of the muscles. The results of attempts to record the same inhibition seen in the eyestalk muscles from the nerves to the leg muscles were equivocal. Inhibition of certain fibers was sometimes seen but this result was not an invariable finding.

Fig. 17 Sensory hairs on eyestalk, (arrow, foreground) and antennular basal segment, (arrow, background). Eyestalk has been pulled away from the posterior aspect of the antennule against which it normally rotates. Note projection of antennular hairs toward overhanging carapace and basal segment of the antennule against which the eyestalk hairs rotate (horizontal arrow).



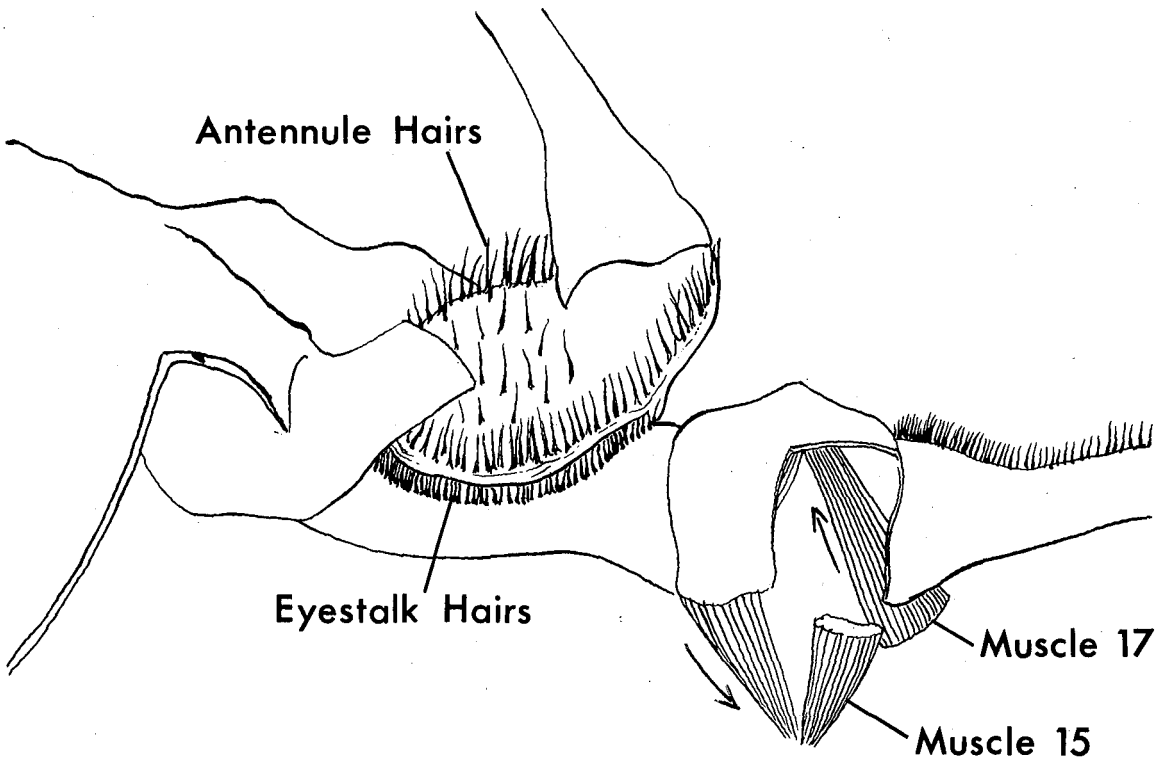
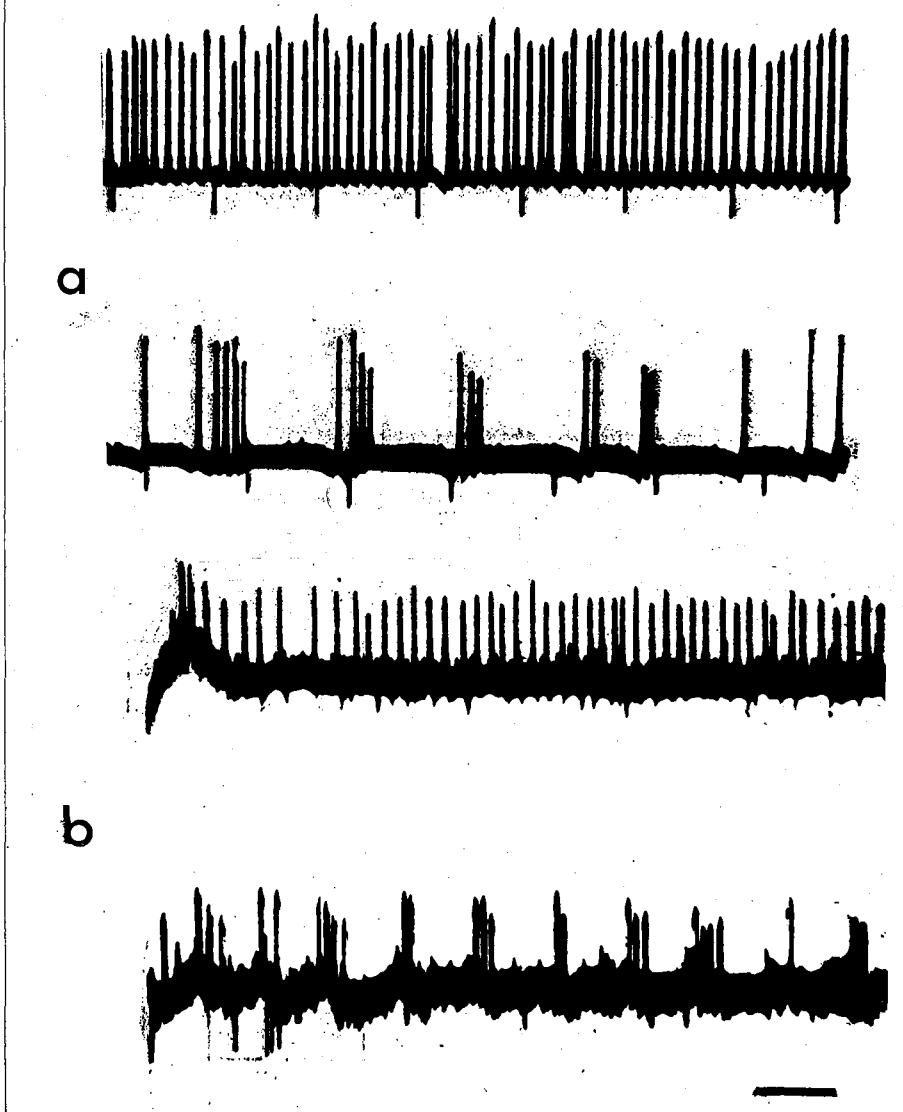


Fig. 18 . Antennule and Eyestalk of Callinectes sapidus illustrating sensory hair systems. Rotation of the eyestalk deflects eyestalk hairs against posterior portion of the antennule basal segment. Movement of the antennule deflects antennule hairs against the overlaying carapace.

Fig. 19 Inhibition of firing of muscle 15 and 17 following stimulation of antennular basal segment hairs. Upper traces in a and b, control level of firing: lower traces show inhibition following deflection of sensory hairs. a, muscle 15 ; b, muscle 17 . scale 0.5 sec.


a
b

To ascertain the behavioral function of the antennular hairs, the animal was tested behaviorally for several functions before and after removal of the hairs by cautery. The following functions were checked:

1. Compensatory eye movements
2. Movement of swimming appendages
3. Righting movements when the animal is inverted

The functions were tested with movement of the entire body and with movement of the antennules alone, since it was suspected that the hairs function to allow the animal to differentiate between the two means of stimulation of the statocysts.

After removal of the antennular hairs, compensatory eye movements to pitch were normal until the animal was completely turned over (180°). The normal animal in this position maintains the eyes approximately level with the carapace. The operated animal extends the eyes out and downward in exactly the same position (reversed by 180°) they would be extended if the animal were right side up. It appears that the animal is unable to tell whether it is upside down or right side up.

If the antennules were moved with forceps with sensory hairs intact, no eye movement was observed. After removal of the hairs, small movements of the eyes were seen in response to movement of the antennules. The movements were jerky and irregular and appeared to be opposite in direction to movement of the antennules.

Movement of the antennules with hairs intact has no influence on leg movement. After removal of the hairs, movement of the antennule resulted in a circular swimming motion by the periopod, the last leg of the crab, which is

adapted as a swimming paddle and also used in righting movements. When the operated animals were placed back in their retaining tank, swimming movements appeared normal. However, if the animal was turned over by 180° onto its back, it either did not attempt to right itself or attempted to right by pitch in the opposite direction than that seen in the normal animal. The normal animal rights itself by pitch in the anterior posterior direction, raising the frontal end up over the caudal end. The inverted operated animals which attempted to right did so by attempting pitch in the opposite direction using the rear periopod to raise the caudal end up to pitch over the frontal end.

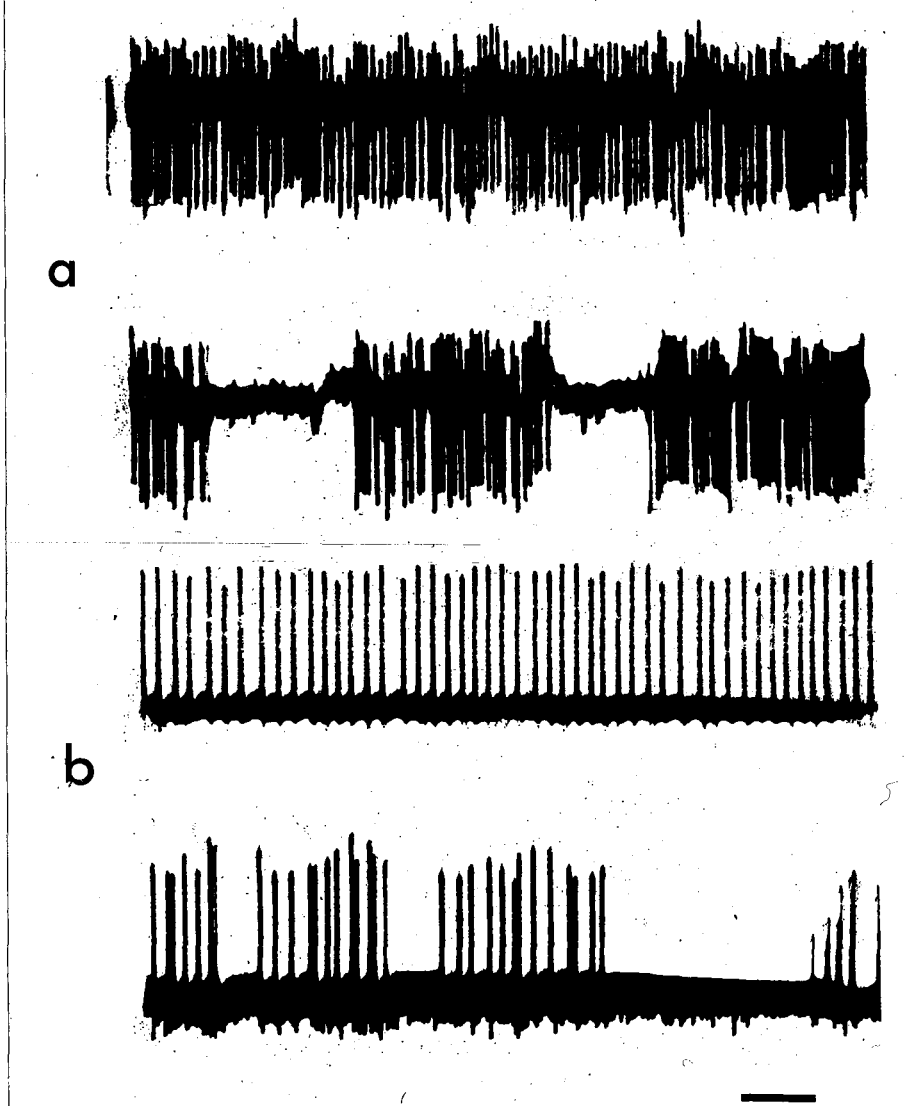
b. NEGATIVE FEEDBACK FROM EYESTALK SENSORY HAIRS:

On the anterior surface of the eyestalk which abuts the posterior surface of the basal segment of the antennule, there is a dense round patch of short bristle like sensory hairs (figs. 17 and 18). These hairs continue in a thin line down the eyestalk for about 1 cm. toward the eyecup, becoming more thread like. The hairs are so placed that a shearing force will be exerted on them as they pass the posterior surface of the basal segment of the antennule during rotation of the eyestalk. The influence of these hairs on the muscles which rotate the eyestalk was ascertained by recording from muscle 15 and 17 while displacing the hairs in several directions. A shearing force applied across the hairs, perpendicular to the direction of displacement by rotation of the eyestalk, was without effect. The tonic firing rate of the muscle, which has been shown above to be due to position sense input from the statocyst, continues without change. A displacement of the hairs in both direction of the dorso-ventral axis of the animal, the axis of displacement in rotation of the eyestalk, resulted in inhibition on the tonic discharge of muscle 15 (fig. 20). (The same effect seemed to be true for muscle 17 although, because of the inaccessibility of this muscle, the results were not as easily obtained.) The latency and duration of the inhibition is short. It was necessary to sweep past a row of hairs for the inhibition to be produced. No effect was seen to the stimulation of less than five hairs. The effect was, as for the antennule basal segment hairs, contralateral. Stimulation of the hairs of the opposite eyestalk produced inhibition of the same characteristics as stimulation of the ipsilateral hairs. With repeated stimulation of the hairs, the response was not as reproducible as for the antennular hairs. This was not adaptation or fatigue since the response decrement could still be seen an hour later when the response to antennular hairs was still

strong. The decrement may be due to damage to the sensory hairs. The hairs of the eyestalk are short, stiff and bristle like and strong displacement could result in damage to the base. Those of the antennule are thin threads which, being more pliable, are less liable to damage.

Using the whole animal on the tilt table, the effect of removal of the sensory hairs on the compensatory movements of the eyestalk was tested. A small hole was made in the carapace and the hairs on the eyestalk burned off with a small cautery. Movement of the eyestalk in response to pitch was tested as in previous experiments. The response to 10, 20 and 30 degree pitch of sine wave and step form was tested before and after removal of sensory hairs. There does not appear to be a significant difference in response after removal of the sensory hairs. The response is only slightly sluggish and more irregular, but the percent compensation does not appear to differ.

Fig. 20 Bidirectional sensitivity in the horizontal axis of eyestalk sensory hairs resulting in inhibition of firing of muscle 15. Upper traces, control firing level: lower traces, a. deflection of sensory hairs by passage of glass needle in an upward direction. b. deflection of sensory hairs by passage of glass needle down across the hairs. time scale: 0.5 sec.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DISCUSSION

MUSCLE FUNCTION AND FIBER COMPOSITION:

The movement of the eyestalk is under the control of two antagonistic pairs of muscles, muscle 15 and 17. Acting cooperatively, the 2 muscle pairs stabilize the eyestalk in a fixed position in space; acting antagonistically, muscle 15 rotates the eyestalk counter-clockwise around the eyestalk axis while muscle 17 controls rotation clockwise around the same axis. The majority of the data above was collected from work with muscle 15 but muscle 17 appears to operate in an analogous manner.

Muscle 15 is to be a complex muscle mediating movements originating from several sensory systems. The sensory drive appears to be differentially distributed to the motor axons to modulate the muscle output. The best example of this is seen in the characteristics of the intermediate axon which is part of a sensory to motor transduction of position sense input from the statocyst to the oculomotor system for unidirectional pitch responses. This one axon is responsible for maintaining the eyestalk in a constant position in space, probably by the influence of type I non-adapting position sense receptors in the statocyst. In addition, the same axon is under visual control although this does not appear to be a strong synaptic input as evidenced by the inability of the visual input to fire the muscle when the statocyst input is removed. In Callinectes, the non-adapting position sense receptors in the statocyst appear to keep the muscle at a constant low level of tension and the visual and acceleration input is superimposed on this.

Although four axons supply muscle 15, only two of them, the intermediate and the slow one, fired with consistancy. It is not likely that the silence of

the additional fibers was due to the dissected perfused preparation since, when the recording was done in a few preparations of the whole animal, these fibers were not seen. A more likely explanation is that the silent fibers fire only for specific situations which could not be duplicated under these experimental conditions. In the case of the fastest fiber, this is most easily seen. Callinectes is an extremely fast swimming crab. The movements on the tilt table used to induce responses in the intermediate axon may not have been of sufficient acceleration to excite the fast axon. If electrical stimulation of the statocyst nerve had been used, it may have evoked the firing of the larger axon. Among the optomotor fibers found in Carcinus (Wiersma and Fiore, 1971) there was a class of large fibers which could only be made to fire by fast rotation about the pitch axis, with an optimal speed of 90 degrees per second. Callinectes is a much faster swimming crab than Carcinus and it is to be expected that the acceleration factors in the oculomotor control would be corresponding greater. It is doubtful that the acceleration or velocity of movement used in these experiments would excite a fiber which was used in the maximum swimming activity of the animal.

The lack of firing of one of the two intermediate axons is not as easily explained. This fiber was labeled as a peripheral inhibitor in Podophthalmus on scanty evidence (Hoyle and McNeill, 1968). There was no evidence of peripheral inhibition seen in Callinectes. A more likely explanation is that what is seen anatomically as two axons peripherally is, in fact, branches of the same motoneuron which have split immediately after leaving the ganglion. This splitting is a common occurrence in axons as they near the muscle. Since the nerve is very short and the axons are of the same size, if they belong to the same motoneuron they may be recorded as one spike when the cell fired since the distance

traveled is not sufficient for differences in conduction velocity to register as two spikes. The splitting of the large axon to muscle 17 (fig. 12) is a good illustration of the danger of using peripheral axon counts in short nerves as indicative of the number of motoneurons centrally.

EXCITATORY JUNCTIONAL POTENTIAL DIFFERENCES:

The presence of ejps of two distinctly different amplitudes, time course and faciliation properties in response to the firing of one motoneuron has been described previously in other systems (Bittner, 1968, Atwood, Hoyle and Smyth, 1965, Wiersma, 1951). The ejp differences are attributed to differentiation in the nerve terminals rather than differences in the characteristic of the muscle membrane, e.g., the input resistance, properities of transmitter quanta, etc. (Bittner, 1968). Bittner was able to show, by recording the tension produced by a single muscle fiber, that equal depolarization of the two types of muscle fibers produced equal amounts of tension. The size of the ejp was shown to depend on the frequency of firing. As the firing frequency increased, the smaller ejp shows a greater facilitation and hence a greater tension is produced by the muscle. By this frequency control, one motoneuron is able to grade tension over a larger range than with a single type of ejp. In Callinectes, there are two distinctly different classes of ejps in separate muscle fibers in the firing of a single motoneuron (see figs. 4 & 5). It is possible to see, even at the limited frequency range in these experiments, that there is more facilitation in the smaller ejp than in the larger. One would expect, under conditions evoking high frequency discharge, that the smaller ejp would show a far greater increase in amplitude by facilitation than would be seen in the large ejp. By this means, it is possible by a peripheral mechanism at the level of the muscle to achieve, with frequency modulation, a greater tension range with use of a single motoneuron.

VISUAL CONTROL OF EYESTALK ROTATION:

The action of the eyestalk muscles is a forward and outward (or downward and outward in the case of muscle 17) extension and rotation of the eyecups. Optic input to the eyestalk muscles could serve two visual functions; to rotate the eyestalks in following movements in the pitch axis and to provide a stable base for the complex movements of the eyecup muscles in any axis. The response of muscle 15 to the variety of visual inputs (fig. 7) may indicate that the eyestalk is functioning as a stabilizer for the action of the nine eyecup muscles. The fact that visual input alone, when the statocyst input is cut, is not sufficient to cause firing of the muscle is an indication of the subsidiary function of visual control over eyestalk movements. Tonic input from the statocysts appears to be necessary to keep the motoneuron at a firing threshold and the visual input then results in an increase in the basal firing rate.

STATOCYST CONTROL OF EYESTALK MUSCLES:

The statocyst input to muscle 15 appears to have two components, a static position sense drive and a dynamic acceleration response. The static function is evidenced by a steady low firing frequency which is seen when the animal is in a resting position after all the inputs to the ganglion have been cut with the exception of those from the statocyst. If the animal is pitched forward and left in the new position, the steady firing continues but at a higher frequency, proportional to the degree of pitch (figs. 9 & 10). The dynamic response to acceleration is seen in an initial increase in firing rate which soon adapts to a maintained level. This division of position and acceleration responses is seen more clearly in the compensatory response of the eyestalk

to a step movement on the tilt table (fig. 15). The initial response is a rapid compensatory movement of the eyestalk bringing it close to the final position. A slower movement then brings the eyestalk to a final position which is maintained. The division of static and dynamic components of a compensatory response in the movement of the whole eye has been shown to be the result of activation of two types of receptors in the statocysts (Cohen and Dijkgraaf, 1960). Elimination of the thread hairs of the statocyst eliminates the response of the eye to acceleration, resulting in a compensatory movement which is composed of a slow approach to the final position. The slow compensatory response is controlled by the shorter statocyst hairs in contact with the statolith which mediate position sense.

There does not appear to be an appreciable contribution of the eyestalk in compensation for roll. This is consistent with the fusion of the middle cylinder with the eyestalks resulting in a rigid structure which does not allow movement of one eyestalk alone. Since the eyestalks appear to act as a unit, roll compensation must be carried out solely at the eyecup-eyestalk joint.

NEGATIVE FEEDBACK SYSTEMS INFLUENCING EYESTALK ROTATION:

a. Antennular basal segment system: All animals with geotatic receptor located in a moveable part of the body must be able to differentiate between stimulation of the receptor caused by movement of the whole body and that resulting from movement of the appendage in which the geotatic receptor is located. In man, this is a question of differentiating between stimulation of the vestibular system produced by movement of the whole body and that resulting from movement of the head alone. In crustaceans, the geotatic receptor is located in the basal segment of the antennule, which moves frequently. The central nervous system must have some knowledge of this movement of the antennule. This could be achieved by either efference copy or reafference. In efference copy, the central

nervous system would receive information about movement of the antennule by a signal which duplicated the motor impulses to the antennular muscles. In reafference, the feedback would consist of information from sensory receptors which signalled movement after it had been initiated.

An example of reafference which differentiates between body movement and movement of the appendage containing the geotatic receptor is the elastic strand found in the lobster, Panulirus argus (Schone and Schone, 1969, Schone, 1971). An elastic strand runs between the antennule and the muscle which raises the antennule so that when the antennule is lifted, the receptor is stretched. The function of the elastic strand was confirmed by recording eye movements to body and antennule movements before and after cutting the strand. The differentiation between the two movements is lost after cutting the elastic strand.

Reafference also appears to be used by Callinectes for the same differentiation. The sensory hairs which are found on the basal segment of the antennule in Callinectes feed back to the eyestalk system to null statocyst input to the eyestalk muscles. If the antennule alone moves stimulating the basal segment hairs and the statocyst, no change in the firing rate of the eyestalk muscles should be seen since the positive sign of the statocyst input and the negative sign of the antennule hairs will cancel. If the antennule hairs alone are stimulated, the negative sign of the input should result in an inhibition of the firing of the muscles. The latter is seen when recording from muscle 15 and 17 of the eyestalk. Inhibition of the tonic position sense statocyst input to the muscles is an invariable result of stimulation of the antennular hairs without concomittent movement of the statocysts.

The behavioral results of removing the antennular hairs agrees with the electrophysiological recording. After the antennular hairs are removed, the animal makes eye and periopod responses to antennular movement which could be the normal response to stimulation of the statocysts by body movement. The antennular sensory hair system thus appears to function to differentiate between stimulation of the statocyst by movement of the antennule and stimulation of the statocyst by body movement.

The response of the eyes when the animal is inverted does not appear to be related to the above differentiation mechanism. Rather, it may be the result of interference with a sensory mechanism which allows the animal to distinguish an upright from an inverted position. The statocyst is unable to signal inversion of the body because of the geometry of the receptors. However, the hairs on the under surface of the carapace above the antennular basal segment may be able to signal inversion of the body since they will be maximally stimulated by the weight of the antennular basal segment upon inversion. When the intact animal is inverted, the eyes are extended out and up, in line with the horizontal axis of the carapace. After the antennular and carapace hairs are removed and the animal is inverted, the animal extends the eyes outward and downward. This is exactly the same position to which the intact animal would extend the eyes if it were upright by 180 degrees. It appears from this that the animal is unable to tell that it is inverted by 180 degrees; it cannot tell up from down in its own body position. For this hypothesis to be so, it is necessary that the statocyst give the same response when the animal is upright or inverted. This is not impossible since the statolith position sense hairs are cemented into the statolith (Cohen, 1965) and the same hairs which are stimulated by a shearing movement in the upright position will be stimulated by the weight of the

statolith when the animal is inverted.

b. EYESTALK SENSORY HAIR SYSTEM:

The eyestalk in Callinectes rotates around the dorso-ventral axis by the action of two antagonistic pairs of muscles. In the course of this rotation, a group of sensory hairs on the eyestalk are stimulated and feed back onto the muscles as inhibition. The eyestalk muscles are under the influence of a tonic excitatory position sense input from the statocysts and the inhibition from the sensory hairs is modultaing this tonic excitatory drive. The two most obvious functions of the inhibition would be the production of compensatory eye movements or the reciprocal inhibition of antagonistic pairs of muscles. To produce compensatory eye movements, the inhibition must act upon the agonist muscle; to produce reciprocal inhibition, the inhibition must act upon the antagonist muscle.

From the experimental results it appears that the deflection of the hairs by movement of the eyestalk in either direction produces inhibition of both antagonist and agonist muscle. This appears to be paradoxical; the eyestalk should then be unable to move since its movement is inhibiting further movement. However, it is a question of the balance of excitatory and inhibitory inputs to each muscle to obtain the desired functional use of the eyestalk. It is then possible to produce both compensatory eye movements concomitantly with inhibition of the antagonist. This is achieved by the addition of an increased excitatory drive from the statocysts to one muscle. The sequency of events may be as follows. The animal is pitched forward, the statocysts send an excitatory drive to the agonist muscle to rotate the eyestalk in compensation for pitch. The eyestalk begins to rotate. This rotation deflects the sensory hairs on the eyestalk. The output of

the sensory hairs feeds back onto the agonist muscle and reduces its output thus producing the 10% compensation of the eyestalk instead of the 100% compensation seen in the legs in response to the same statocyst stimulation. At the same time, the output of the sensory hairs is being fed to the antagonist muscle as inhibition. This inhibition of the antagonist in rotation of the eyestalk may appear strange since it facilitates the action of the agonist at the same time that the action of the agonist is being limited by the same sensory receptor. However, the inhibition of the antagonist may function simply to make the movement of the agonist more efficient and smooth. As seen in fig. 9, 10 and 11, there is still some tonic excitatory drive to the antagonist muscle when the animal is in a stationary position of pitch. The reciprocal inhibition would function to decrease this excitation to the antagonist when the eyestalk began to move, making more efficient use of the agonist. If both agonist and antagonist receive equal amounts of inhibition from deflection of the sensory hairs, the increased statocyst drive to the agonist will produce the 10% compensatory movement.

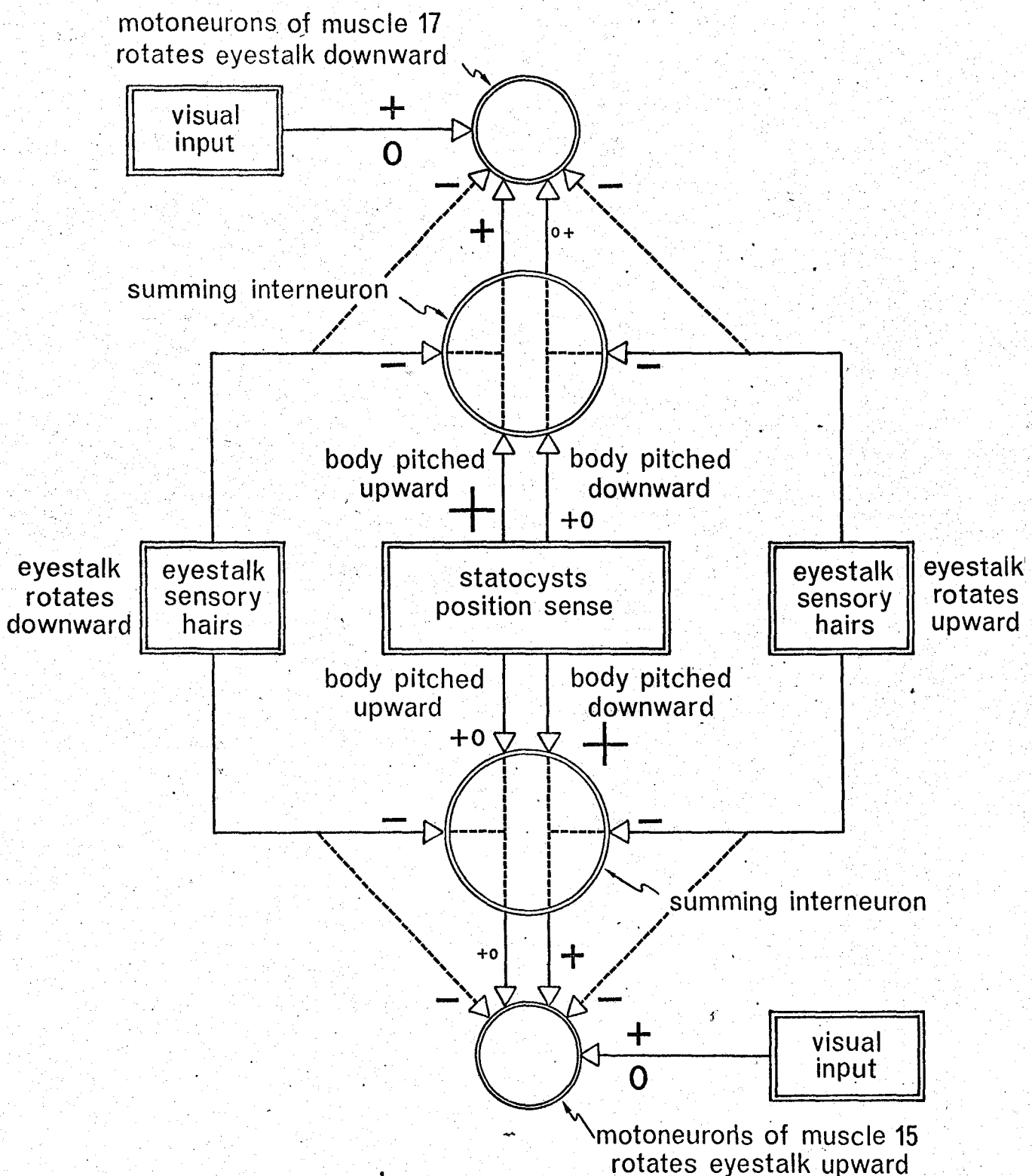
The production of compensatory eye movements by this mechanism requires only that 10% of the statocyst drive in the "on" direction escape inhibition. The inhibitory circuit limited to the eye answers the long standing question of how the same sensory receptor, the statocysts, could drive one reaction, the righting responses of the animal, to complete compensation and another reaction, eye movements, to such a small compensation. The second question asked of compensatory eye movements concerns their function. Why does the animal show incomplete compensation for pitch? This can be answered logically by considering the visual and statocyst information coming to the central nervous system about the animal's position in space. If the eyes were to compensate completely to the same degree as the body, the eyes, which can respond more quickly than the body,

would constantly be signalling information to the central nervous system that the body was in equilibrium while the statocysts would be signalling a disequilibrium. Having the eyes lag behind the body avoids conflict of visual and statocyst information as to body position.

There is an additional situation where the inhibition produced by the eyestalk sensory hairs may be used to null statocyst input. Such a situation occurs when the animal is pitched in one direction and may need to make a visually directed movement in the opposite direction. This would be the case when the animal is swimming in one direction of pitch and sees prey moving in the opposite direction of pitch. If the animal is able to make following movements with the eyes before it has changed the direction of pitch, the following movement of the eyes is opposite to the direction of pitch. Also, even in the resting position of the animal, in which the anterior end of the carapace is pitched 15 degrees above the horizontal, the visual input must overrule the tonic statocyst input in order for the animal to make a visual inspection of anything at a lower level than the eyes. Any visual guidance of the claws to prey on the sea floor must require some downward rotation of the eyestalk and this rotation is counter to the statocyst input. It is possible that the animal does not make use of the eyes for such movement but relies on the chemoreceptors on the claws and mouth parts. However, a model (fig. 20) can be devised whereby the animal is able to make visually directed movements which can both oppose the statocyst movement and escape the eyestalk inhibitory system. This can be done by inserting an interneuron between the statocyst and the motoneuron. The eyestalk inhibitory hairs feed into this interneuron. This interneuron accomplishes one purpose; it prevents the inhibitory input from the eyestalk hairs from feeding onto the

Fig. 20[1]

Model of the eyestalk feedback control system. The model can operate with or without the summing interneuron, depending upon the inclusion of the visual input. In the production of compensatory eye movements and reciprocal inhibition, the summing interneuron is not necessary and inhibition would act either directly upon the motoneuron or upon an interneuron. The interneuron is added to allow visually directed movements to escape the eyestalk sensory hair inhibition and to oppose statocyst directed movements. Plus and minus signs indicate excitation and inhibition, respectively. Scale of sign indicates the magnitude of the outputs. For further explanation see text.


motoneurons of muscle 17
rotates eyestalk downward
visual input
+
0
−
−
+
0+
summing interneuron
−
−
body pitched upward
body pitched downward
+
+0
eyestalk rotates downward
eyestalk sensory hairs
statocysts position sense
eyestalk sensory hairs
eyestalk rotates upward
body pitched upward
body pitched downward
+0
+
−
−
summing interneuron
+0
+
−
−
+
0
visual input
motoneurons of muscle 15
rotates eyestalk upward

motoneuron directly. Since the interneuron can signal only degrees of excitation, the excitatory visual input can drive the motoneuron and the inhibition from eyestalk rotation does not cancel the visual input but is felt only as a lessened excitation from statocyst drive. Since when the visual input is attempting to act antagonistically to the statocyst drive, the visually driven motoneuron is receiving less positive drive from the statocyst and will see only a decreased positivity from the interneuron, not inhibition. The opposing motoneuron which is the agonist for statocyst input will be under a decreased drive from the interneuron, due to inhibition from the eyestalk hairs and will also lack the excitatory drive from the visual input. In this way, the visual input may predominate and the eyes will be able to execute visually directed movements free from the inhibitory system and in opposition to statocyst drive.

The above oculomotor model can be tested in future experimental work by intracellular recording from the motoneurons of the eyestalk rotation system. The entire motor system appears to contain at the most seven motor neurons and perhaps as few as four. The resolution of the relationship between the motoneurons, the statocysts and the inhibitory sensory hairs may prove to have wider applications for oculomotor control systems in general. It is possible that the inhibitory control system in Callinectes may have a correlation with the inhibitory stretch reflex which feeds back onto the stretched muscle in cats (Back-y-Rita, in press). The extraocular muscles serve much as the same function for all animals and it would not be surprising to find similar control mechanisms throughout the animal world.

SUMMARY

The control system for stabilization and rotation of the eyestalk in Callinectes has been shown to involve two antagonistic pairs of muscles under visual and statocyst control. The primary sensory input to the muscles appears to be from the statocysts with both static position sense and dynamic acceleration components. The static position sense input has been shown to be proportional to the degree of pitch of the animal. Two motor axons are responsible for the tonic statocyst and visual control of the muscle. The larger of these two axons is capable of producing ejps of differing characteristics in different muscle fibers. This appears to be a peripheral mechanism for modulation of motor control by firing frequency.

Two systems of sensory hairs were found which influence eyestalk responses. One set of hairs is located on the antennular basal segment and appears to function to allow the animal to differentiate between stimulation of the statocyst by movement of the whole body and stimulation of the statocyst by movement of the antennule, in which the statocyst is lodged. The second set of sensory hairs is located on the eyestalk and is believed to function to produce both compensatory eye movements and reciprocal inhibition of antagonistic eye muscles. Both these functions are achieved by a negative input from eyestalk hairs which nulls out the tonic statocyst input. A model is proposed to allow visually directed movements to escape this inhibition and execute movements in opposition to statocyst control.

III. INTRACELLULAR RECORDING FROM THE OCULOMOTOR SYSTEM IN THE CEREBRAL GANGLION OF CALLINECTES SAPIDUS

INTRODUCTION:

An introduction has been given to the work which has been done on the decapod oculomotor system to reveal some of the basic properties of the movement capabilities and the central mechanisms which control them. None of this work, however, can provide information on the specific cellular basis for the observed events. To accomplish this, it is necessary to leave the periphery and begin intracellular recording in the cerebral ganglion where the oculomotor integration takes place. The following section is an account of work with that aim. The only published work involving intracellular recording in the oculomotor system of the decapod is from a single motoneuron mediating the withdrawal reflex (Sandeman, 1967, 1969). The withdrawal reflex is a protective movement, much like the primate blink. There are several motoneurons responsible for the reflex and the largest of these was the subject of analysis of the synaptic link between the sensory input and the motoneuron response. The analysis involves the site of integration of synaptic inputs, the spike initiating zone, and the question of mono or poly-synaptic reflexes in one cell. The intracellular recordings published consist of summed synaptic activity recorded at a site distant from the site of synaptic impingement upon the cell and do not give much indication of the potential of the preparation for intracellular analysis. The cell body of the motoneuron was not recorded from since it could not be located. Since this work was done prior to the use of procion yellow, the dye used as a cell marker was Prussian Blue and the cell soma did not fill when the axon was injected. Although the system studied was the simplest reflex movement of the eye and limited to one motoneuron in that system, it is of interest to the following

work as the first intracellular recording from the oculomotor system in a decapod preparation.

The proposed approach of the following project is contained in two questions: (1) what is the location of the components of the system and (2) what can be recorded from them? A long range plan of approach to them is as follows: Where are the somata located? What is the course of the cell processes? Are the somata electrically active? (In invertebrate nervous systems, the somata (cell bodies) are often very large and electrically inactive). If the somata are electrically inactive, what sort of electrotonic activity can be recorded from them, that is, do they show electrotonic reflection of E.P.SP., I.P.S.P.s or spike activity from other parts of the cell? What are the sources of sensory input to these cells, i.e., visual, tactile, statocyst, etc.? What is the general visual receptive field of the motoneuron particularly with respect to directional movements? What are the differences between tonic and phasic motoneurons in cell size and sensory input sources? Are the tonic and phasic systems grouped separately anatomically? There are from 25 to 20 motoneurons controlling each eye. It is not intended to extend the analysis to every cell but rather to find representive tonic and phasic motoneurons for study. All interneurons encountered will be investigated by essentially the same criteria as motoneurons. The following work is the intial step in the above project and succeeds in answering some of the above questions. The data is the first set of recordings to yield good intracellular responses from cells mediating optokinetic responses and establishes the decapod preparation as a system for central analysis of the oculomotor system on a cellular basis.

MATERIALS AND METHODS

The animal preparation and recording was the same as given under Materials and Methods, Section I. In addition, the cerebral ganglion was desheathed and transilluminated to allow individual tracts and nerve cells to be seen with the aid of a Leitz dissection microscope.

Procion yellow for cell marking was used as a near saturated solution and cells filled either by the pressure injection method, electrophoretically (Stretton and Kravitz, 1968) or by the whole nerve iontophoretic technique (Iles and Mulloney, 1971). The latter technique, as modified under Materials and Methods, Section I, was used for the differential filling of the larger (and hence usually motor) axons in the optic and oculomotor nerves. The use of the method for the differential filling of the larger axons is possible because of the faster filling of the larger axons (perhaps due to their lower cytoplasmic resistance). If the dye passage in the large axons is observed and the current discontinued when the dye reaches the central ganglion, the distal portion of the nerve containing filled axons of all sizes can be cut off leaving only the large axons which have filled for a greater distance. By this means, the course of a mass of motor fibers can be followed in the central ganglion.

RESULTS

Upon visual inspection of the transilluminated ganglion, cell groupings could be seen in close proximity to the course of the oculomotor nerve in the central ganglion. These cells were chosen as the most likely choice for the cell bodies, or somata, of the oculomotor neurons. The following procedure was then followed to determine if the cell was part of the oculomotor system and, if so, whether it was a motoneuron or interneuron. The criteria for motoneurons

was as follows: (1) if the soma shows spiking activity, either spontaneously or in response to visual stimuli, does this activity appear as an extracellular spike in the optic or oculomotor nerve. (The recordable activity of the oculomotor nerve is composed almost entirely of motor fibers and the largest spikes in the optic nerve belong to the oculomotor system. Therefore, any large spike in either nerve is likely to be oculomotor). (2) if the cell doesn't fire as above, can it be made to fire by passing current through the cell and does this activity appear in the oculomotor or optic nerve. (3) is there an antidromic spike in the cell in response to stimulation of the optic or oculomotor nerve and (4) is there a one to one high frequency following between firing of the cell and activity recorded at the muscle. This latter criteria rules out the possibility of a synapse between the central cell body and the acitivity recorded at the peripheral nerve since the synapse would not follow the high frequencies of stimulation. If the cell was of interest by these criteria, it was marked by procion yellow and its location noted on a map of the ganglion.

MOTONEURONS

Recordings from the soma of motoneurons usually showed spike activity of a low level, below 20 mv, indicating electrotonic conduction into the soma of action potentials produced in an active spike initiating zone elsewhere in the cell. It was possible to correlate these small spikes with spikes in the oculomotor nerve and, in some cases, with activity in specific muscles. In addition, it was often possible to drive the cell by passage of current through the bridge circuit and record electrotonic spikes in the soma and corresponding spiking activity from the nerve (fig. 21). By this means the general plan of anatomical organization of the ganglion was revealed. There are at least three separate

Fig. 21 Electrotonic spikes recorded from the soma of two oculomotor neurons in cluster marked ● on ganglionic map. Upper traces, extracellular recording from the oculomotor nerve; lower traces, intracellular from cell soma. Intracellular activity in upper trace is evoked by passage of current through the cell. Intracellular activity in lower trace is antidromic spike following stimulation of the oculomotor nerve. Scale 10 mv, 2 msec.

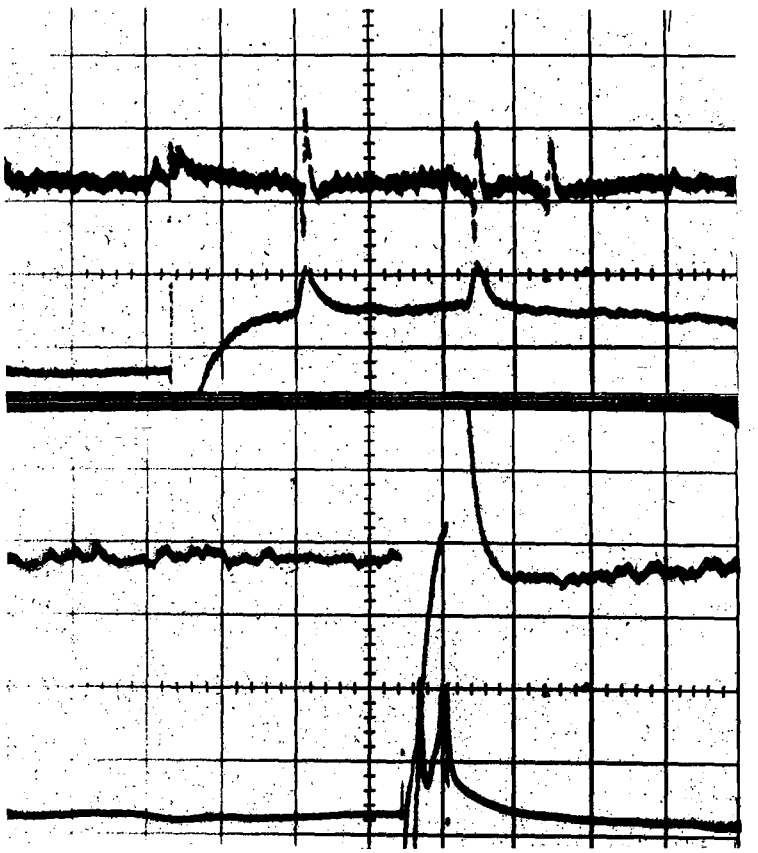
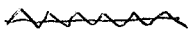


Fig. 22 Ganglionic map of oculomotor system constructed from preliminary recording in the cerebral ganglion of Callinectes.

a) motor axons in oculomotor nerve

b)  motor axons in optic nerve

c)  possible phasic oculomotor neurons

d)  tonic oculomotor neurons of optokinetic geotatic system

e)  interneurons in optokinetic geotatic system

f)  possible motoneurons for eyestalk rotation

g)  withdrawal motoneurons

i)  parabolic burster found under large withdrawal motoneurons

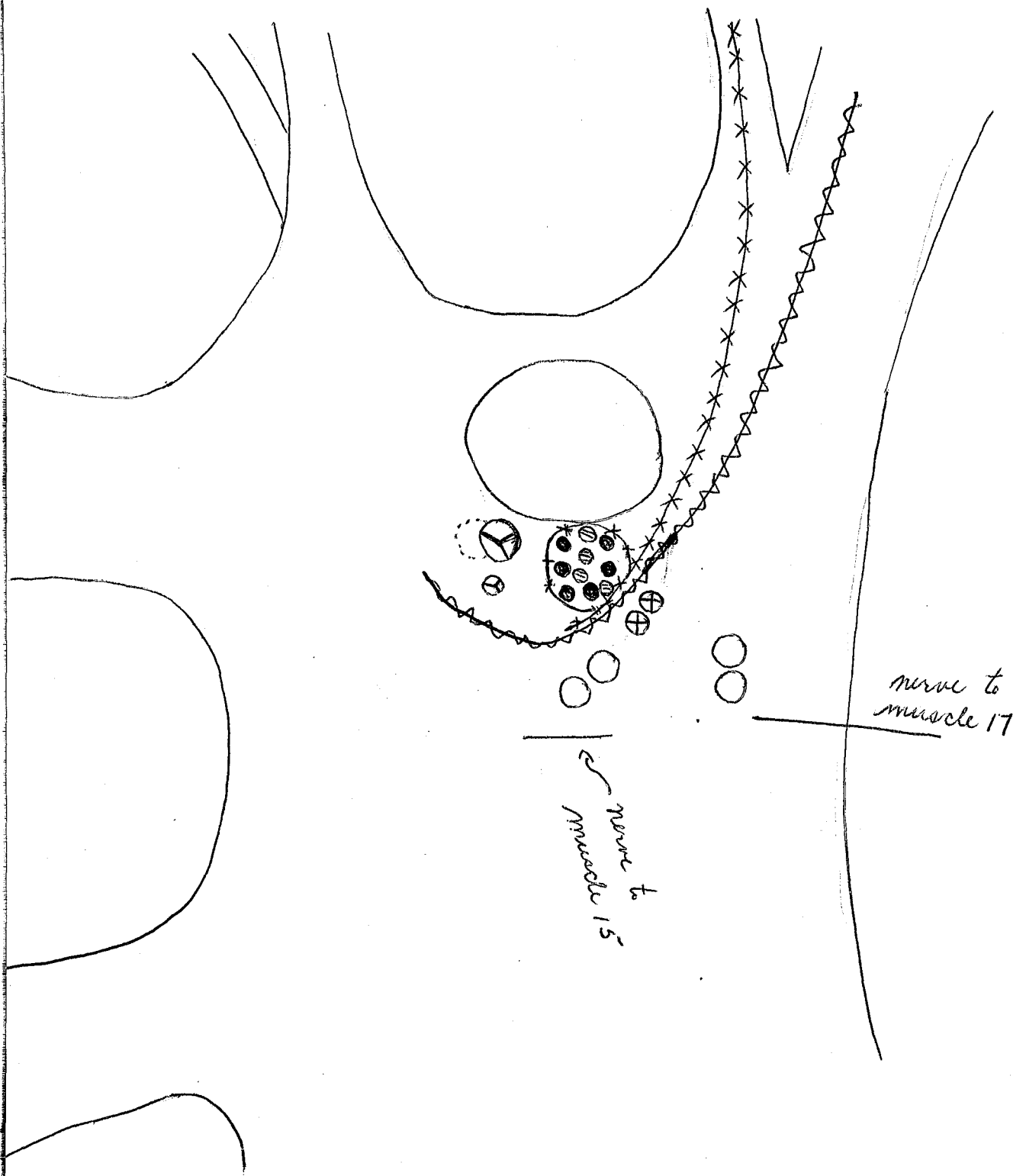

nerve to muscle 17
nerve to muscle 15

Fig. 23 Examples of recording from verified motoneurons. a. somas located in area of withdrawal motoneurons, marked ⊛ on ganglion map, lower trace is intracellular from soma, upper trace is extracellular from muscle 19a. b. soma located at ⊛ in ganglion map. Lower trace, intracellular from motoneuron soma; upper trace, extracellular from oculomotor nerve. c. soma located as in b above. Lower trace, extracellular or partial intracellular penetration from soma located near b above; upper trace, extracellular from oculomotor nerve. Scale 10 mv, 10 msec.

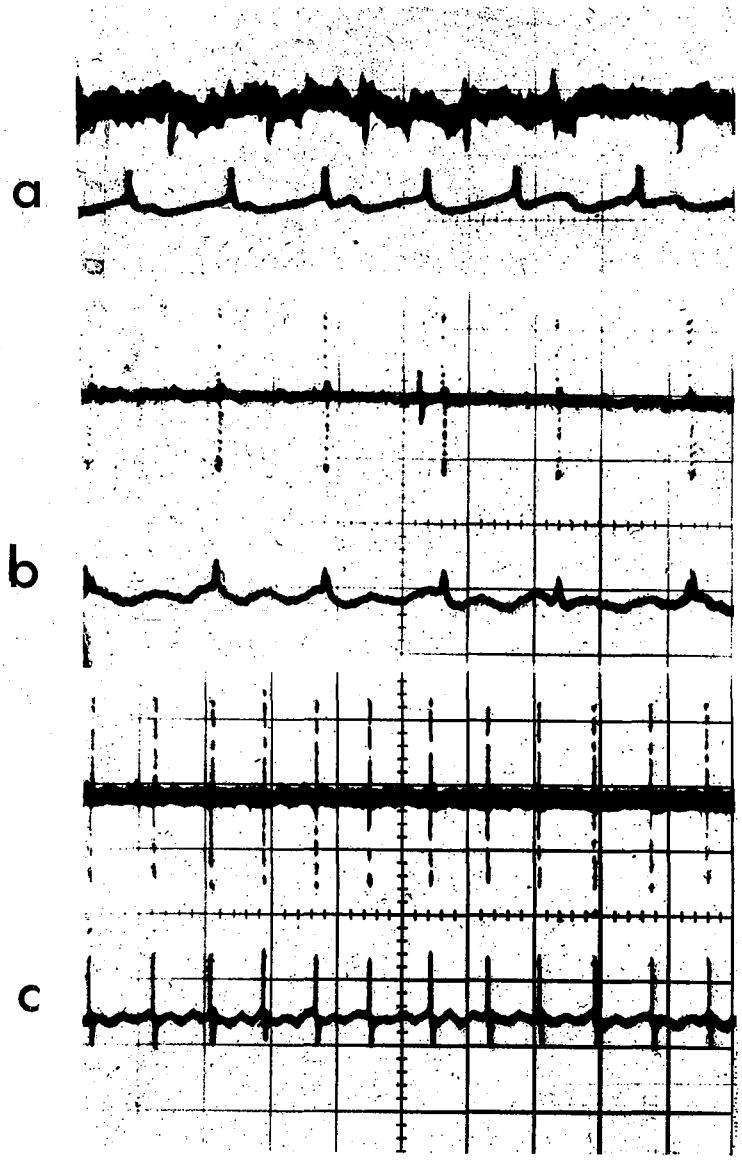

a
b
c

Fig. 24 Two cells of unknown function which burst, as shown, to a flash of light. Cells located at ⊕ on ganglionic map in area just outside the oculomotor cluster. Scale: 5 mv, 1 sec upper trace, 10 msec lower trace. The cells could be phasic oculomotor neuroms due to their location. The upper trace is probably an axon penetration (because of the large spike size) and the lower is from a known large soma.

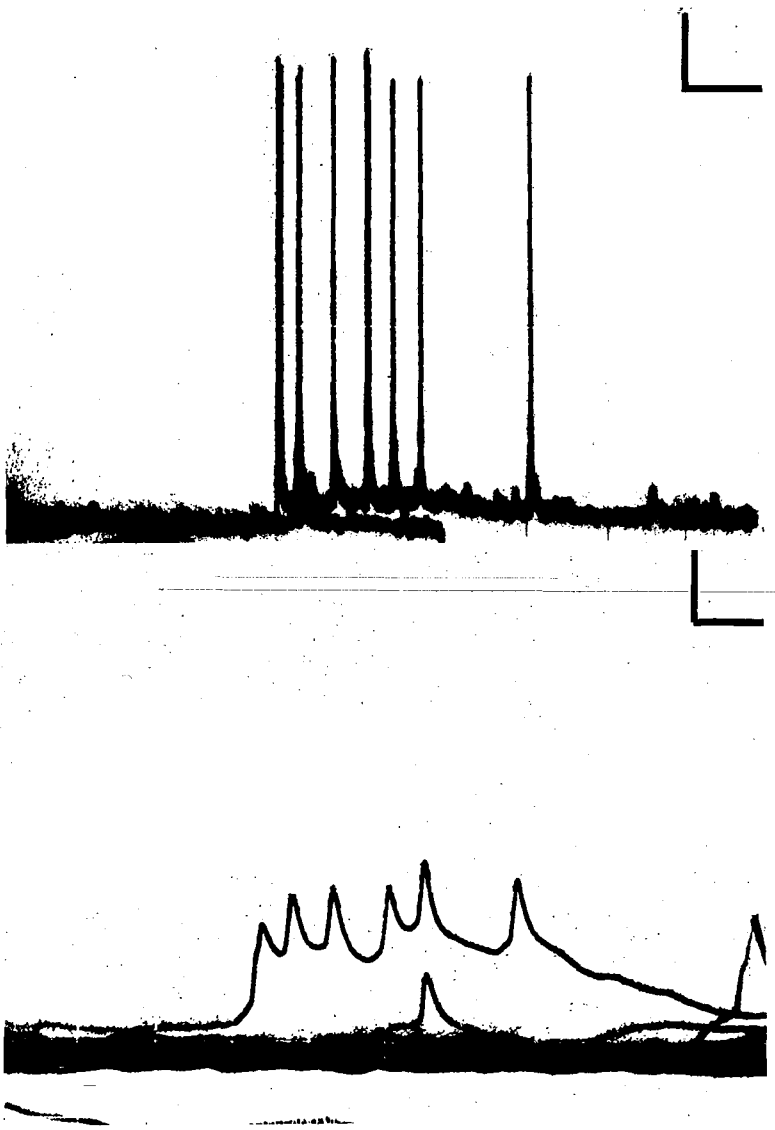


Fig. 25 Two soma recordings from the oculomotor cluster which could be tonic motoneurons. Verification was not made because extracellular channel was inoperative. Cells demonstrate prepotential foot seen in spontaneously active cells. Note activity before third and fourth spike in lower trace which, although greater than spike initiating level, fails to initiate spiking. Scales, 20 mv. upper trace, 10 mv. lower trace, 50 msec. both traces.

groupings of oculomotor neuron soma which appear to be anatomical divisions of the motoneurons according to function. The area outlined by a line and crosses in fig. 22 contains a tight cluster of medium size cell soma (30-70 microns) and associated fibers. This will be referred to as the optokinetic cluster since it contains motoneurons (marked ● on the map) which are believed to be tonic, and perhaps phasic, motoneurons of the optokinetic and geotatic system (see fig. 23 b, c). Included in this tight grouping are also interneurons (marked ⓘ on the map) which respond to stimuli of the optokinetic and geotatic system, i.e., response to movement in the visual field, stimulation of the statocysts, etc. (fig. 26-29). Below this is a loose group of somata of varying sizes, from a very large one of approximately 150 microns to smaller ones of 35 microns (marked ⊘ on the map). These are believed to be the somata of the eye withdrawal system. The cell of fig. 23 a comes from this area and is correlated with tonic activity in muscle 19a in the eyecup. Muscle 19 a is the major controller of eyecup withdrawal. The largest soma in this group is electrically silent and, on the basis of procion yellow injections, is believed to be the largest withdrawal motoneuron whose processes were the subject of intracellular recording by Sandeman (Sandeman, 1967, 1969). The procion yellow injection was seldom useful for more than marking cell bodies since the dye did not diffuse well into the processes but in the case of this large cell, it was possible to trace an axon leading to the optic tract where the large cell of the withdrawal motoneuron exits the ganglion.

The motoneurons responsible for rotation of the eyestalk exit the ganglion near the middle in the nerves to muscles 15 and 17. There are 5 or 6 large motoneurons and several small ones in this system. (Muscle 15 has been shown in the preceding section to have at most 3 large cells and 1 small one. The

supply to muscle 17 is unknown but must be about the same. On the basis of whole nerve filling of the nerve to muscle 15, it appears that the cell bodies of this muscle are the large ones which can be seen on the surface of the ganglion (marked 0 on the map).

INTERNEURONS

In the cluster of soma containing the tonic motoneurons of the optokinetic system, there were frequent recordings from cells which responded to optokinetic stimuli but which did not show spike activity going out the optic or oculomotor nerve. These cells were classified as interneurons because of the absence of correlation of their spike activity with action potentials in nerves exiting the ganglion. They were placed in the oculomotor system because of their location in the optokinetic cluster and because of their response to optokinetic stimuli. The cells varied widely in their activity pattern and very little information regarding their specific functions could be gained due to equipment limitations at the time. The records (fig. 25 to 29) are of interest because they illustrate the potential information content available from the abundant electrical activity of these cells.

The majority of the interneurons recorded from were located in the optokinetic cluster. These cells are marked on the ganglion map. There did not appear to be a separation of motor neurons and interneurons within this cluster; rather there is a tight interweaving of the cells into a compact sphere. An electron micrograph of a section taken through this cell cluster (fig. 30) shows tightly adjoining cells with very little interposing glial tissue and dark areas which may be nonsynaptic cell junctions. Running between the cell bodies can be seen cell processes, most of which are too small to be the

Fig. 26 Intracellular records from two cells in oculomotor cluster showing multiple spike heights. Second spike on upper trace is overshoot spike. Lower trace, similar activity of lower voltage. Time course of activity indicated active conduction in both cases. Notched spike activity in lower cell may represent successive spike initiation at several trigger zones. Scales: upper trace, 5 mv, 50 msec; lower trace 2.5 mv, 5 msec.

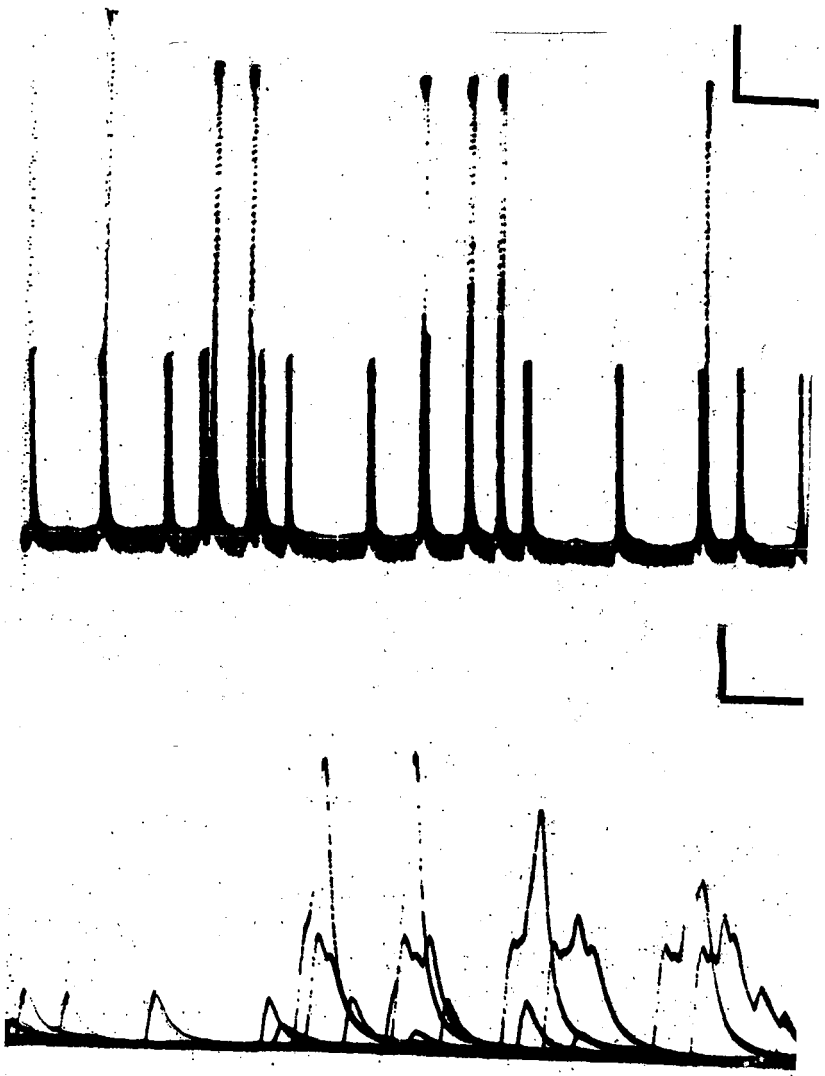


Fig. 27 An integrating interneuron found in the oculomotor cluster. Cell does not have spike activity in the motor nerves but shows synaptic input to electrical stimulation of the statocysts nerve in a, the optic nerve in b and the oculomotor nerve in c. Scale: 5 mv. 100 msec.

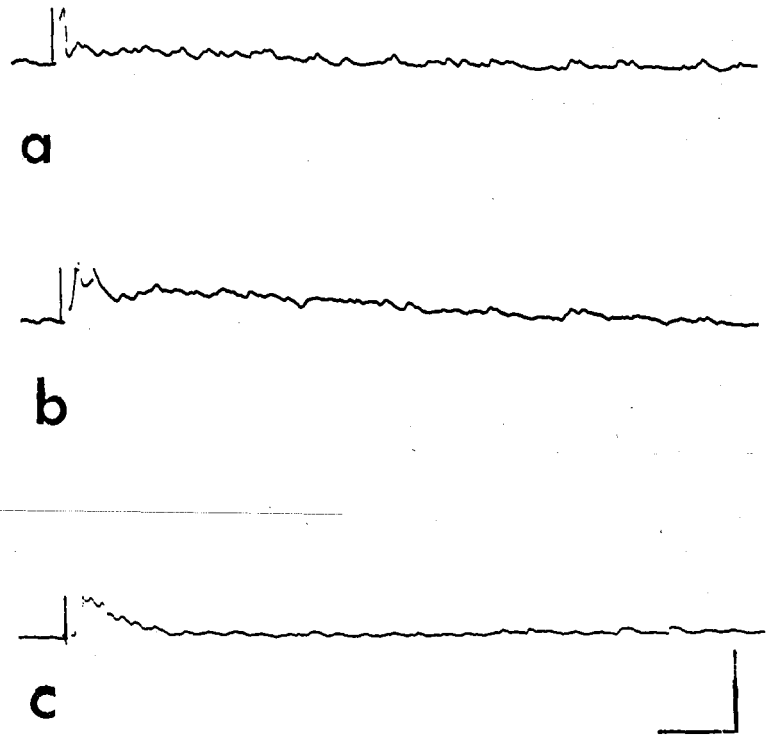

a
b
c

Fig. 28 Further example of large spiking cell with prepotential foot. This cell is judged to be an interneuron with oculomotor function, since it was found in the oculomotor cluster, marked ⓓ on the map, does not show correlation with spikes in the oculomotor nerve, upper trace, and showed an increase in firing rate to a moving stimulus. Scale: 10 mv, 50 msec.

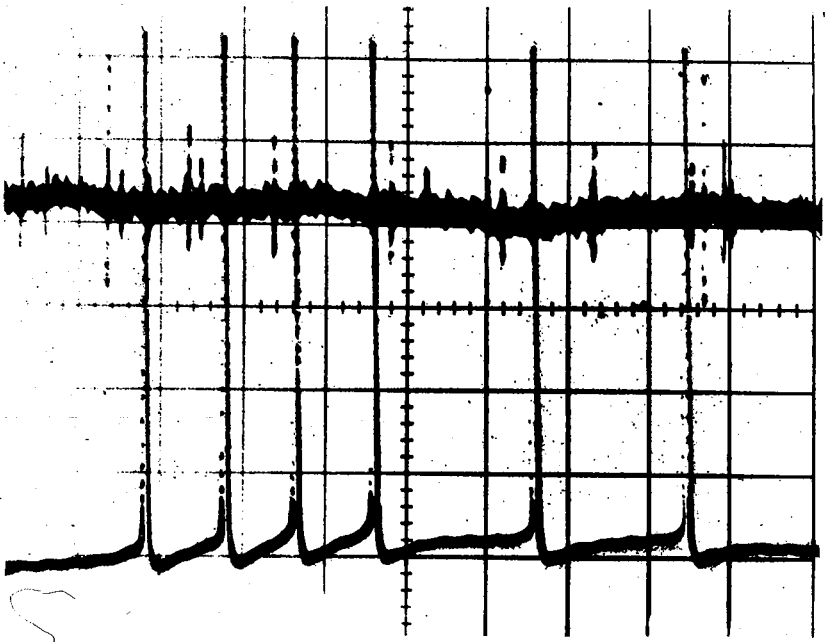


Fig. 29 Epsps and ipsps of an interneuron found in the oculomotor cluster. Upper four traces illustrate spontaneous epsps seen in cell, activity in lower traces is evoked by movement of pen light in visual field. Electrical stimulation of esophageal connective evoked spiking in the cell (not shown). Scales, 2.5 mv, 20 msec.

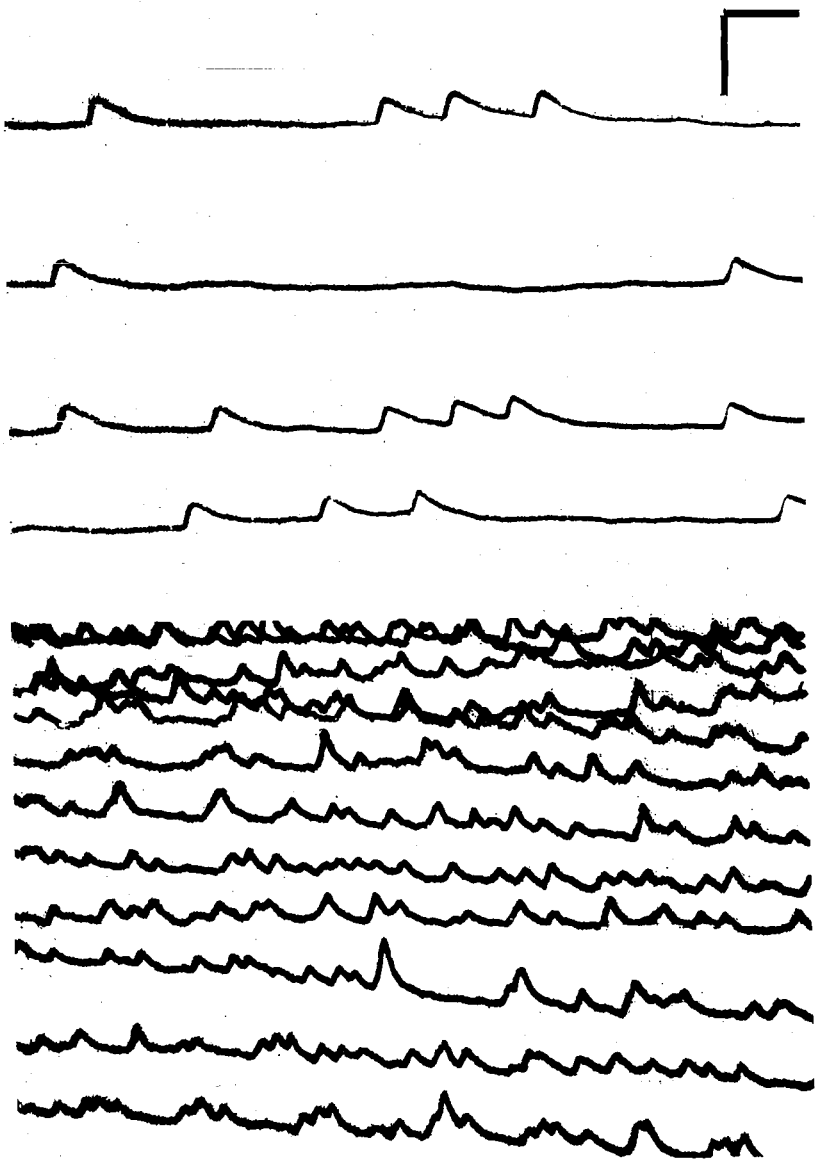


Fig. 30 Electron micrograph taken from the optokinetic cluster. Note:

a) sparce glial investment of cells and resultant tight adhesion of soma.

b) interdigitating small cell processes.
mag. 4 5,000 X, photo - courtesty of
Dr. Daniel Friend.

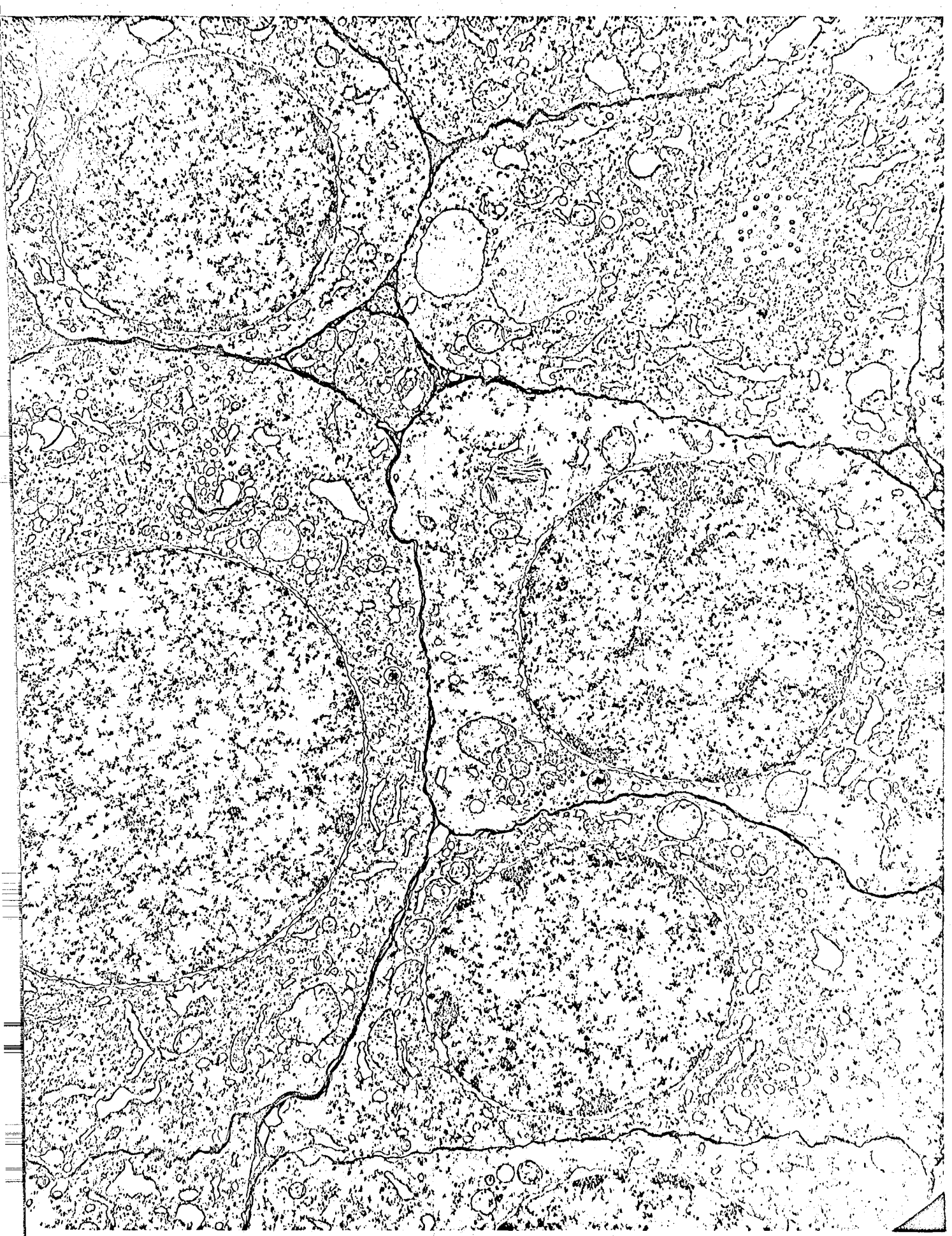
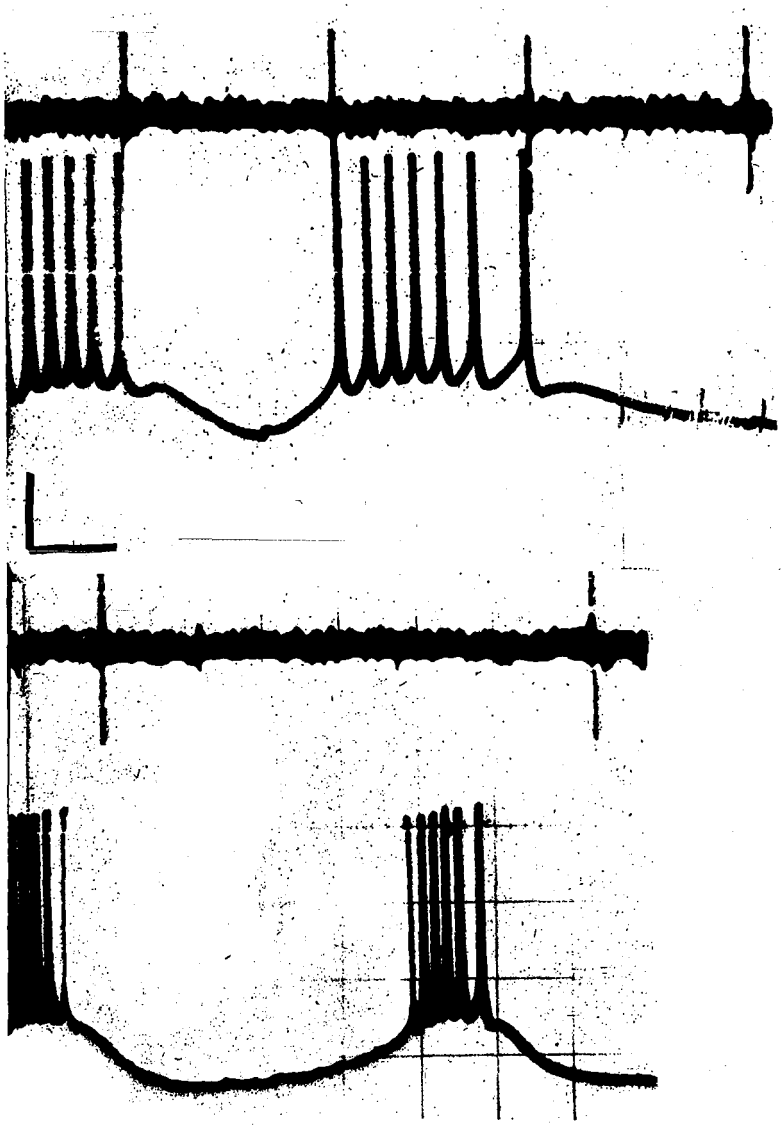


Fig. 31 Interneuron located with cells believed to be withdrawal motoneurons. The parabolic bursting form is characteristic of the cell. If bursting stopped, it could be evoked by the onset of a light near the preparation. The burst was often correlated with a spike in the oculomotor nerve, as seen in the upper trace but this was not always the case, as seen in the lower figure. Upper traces of the two records are extracellular from the oculomotor nerve, lower traces intracellular at location ( ) on ganglion map. Scales, 10 mv both traces; 100 msec upper trace, 200 msec. lower trace.



neurite-axon branch from the cell body and must represent dendritic processes involved in synaptic transmission in the cluster.

There were several points of interest to basic neurophysiology which were seen in these interneurons. In the first cell shown in fig. 26, up to five different spike sizes were seen. Three are illustrated in the upper figure, the second one being an overshoot spike. In the lower trace, on a faster time scale and at a lower amplitude, is another cell of the same type. The activity is probably electrotonic spread from another part of the cell, judging from the low amplitude response, and the notched form may represent successive spike initiation from different trigger zones.

The cell in fig. 27 is an excellent example of an integrating interneuron which is receiving inputs from several sensory systems, in this case, the visual information from the optic nerve, the geotatic from the statocysts and mechanoreceptors from the oculomotor nerve. In fig. 28 is seen another cell type which displays a prepotential foot typically seen in spontaneously active cells. The frequency of discharge could be increased by moving an optokinetic board in front of the preparation. However, even in the absence of any stimulus, this regular discharge with the prepotential foot is seen. The prepotential may represent a pacemaker potential or could be the summed synaptic input from another area of the cell, or a combination of the two. A classic record of epsps and ipsps is seen in fig. 29. The frequency of synaptic bombardment went from the low level of epsps seen in the upper trace to a dense input of both epsps and ipsps in the lower record when the cell was stimulated by a combination of a moving light and electrical stimulation of the esophageal connective. (The esophageal connective is an "alerting" input to the visual system).

The only interneuron which was encountered outside the optokinetic cluster which seemed to belong to the oculomotor system is that seen in fig. 31. This cell can be located repeatedly by advancing through the largest cell body of the withdrawal system. This form of parabolic bursting was usually seen, but if the bursting stopped, it could be reinstated by the onset or movement of a light near the preparation. It may represent some form of pacemaker for the withdrawal system (such a pacemaker has been speculated upon but not found Sandeman, 1967). The bursting pattern continued on one occasion for two hours and seemed to be correlated with a spike in the oculomotor system (see upper trace, fig. 31). Although exact correlation was not an invariable finding, an increase in the spike frequency in the oculomotor nerve appeared to accompany increased frequency of bursting. Since both tonic motoneurons of the withdrawal system and neurons of the optokinetic group are found in the oculomotor nerve, this cell could serve as an oscillator or pacemaker for either system if it is indeed linked to the spike in the oculomotor nerve.

DISCUSSION

The preceding records from the oculomotor system of Callinectes demonstrate the development of the cerebral ganglion into a preparation in which one can do intracellular recording while using the natural sensory stimuli of the oculomotor system. The aim was to have a preparation in which the location of the cellular components of the oculomotor system would be known and the relationship of the sensory units and interneurons to the motor units could be mapped on a cellular basis as has been done in several other invertebrate systems, for example, (Bentley, 1970; Otsuka, et al., 1967; Cohen and Jacklet, 1967). A substantial beginning has been made toward this end.

The technical aspects of the perfusion and dissection of the ganglion have been developed to achieve a preparation in which intracellular recording in the oculomotor system can be done while the animal is induced to make eye movements in response to movement in the environment or to tactile stimulation. A larger scale development of the work was limited by two factors. One was the equipment limitations in the early stage of the work. It is necessary to have numerous extracellular channels for investigating inputs and outputs of the cells being monitored intracellularly. The other is the need for a kymograph camera for continuous recording. After these were obtained, a limitation of the preparation appeared. The intracellular response of the animal decreased with the onset of winter. Callinectes migrates to deeper waters at this time and that may be involved in the phenomenon.

Several aspects of the oculomotor system have been established by the recording in this work. The main feature is the general anatomical organization of the oculomotor system within the ganglion. The functional division of the oculomotor system, the withdrawal system, the optokinetic-geotatic system and the system for eyestalk rotation appear to be separated anatomically within the ganglion. It is likely that each system has its own interneuronal network also. The integration necessary for the withdrawal system is quite different from that for eyestalk rotation or optokinetic-geotatic movements and though each system may receive input from the same sensory systems, the information will not be used in the same way. It is therefore unlikely that those interneurons grouped with the optokinetic group function for the other systems also.

Recording from interneurons in the oculomotor system are one of major stumbling blocks in work utilizing vertebrates. Recording from these cells in Callinectes appears excellent and is probably the main advantage of the use of the decapod for study of the oculomotor system.

In addition, the types of interneurons seen in this preparation should prove to be interesting as basic studies in neurophysiology. For example, the cells seen with multiple spike heights may prove to have dendritic spikes representing sensory inputs from different modalities, i.e., input from the statocysts, the visual and the mechanoreceptive systems. Active regenerative potentials were once thought to be found only in the axon, initial segment and soma but are now accepted phenomena in the dendritic system (Llinas, 1968). Another possibility is the presence of axonal branches of one motoneuron, a phenomena which has been seen before (Takeda and Kennedy, 1965). Alternatively, multiple spike heights have been thought to be spikes which failed to propagate past a frequency barrier caused by the narrowing of the axon (Mellon and Kennedy, 1964; Pabst and Kennedy, 1967; Sandeman, 1969). By stimulating the sensory inputs to the oculomotor system, it may be possible to link specific spikes with specific sensory inputs, thus establishing the spikes as dendritic.

The spontaneous bursting cell seen near the withdrawal somata merits study as a possible pacemaker for the spontaneous and regular withdrawal movements of the eye. It has been shown that the spontaneous firing of the large withdrawal neuron is not likely to be a property of the motoneuron and a separate pacemaker cell for this firing has been suggested (Sandeman, 1967). There are withdrawal motoneurons in the oculomotor nerve and the apparent correlation of the bursting cell and the spike in the oculomotor nerve could represent a coupling of the pacemaker cell with the withdrawal cell. The resolution of this and similar questions brought up in the course of the work on the ganglion appear to be within reach in a more comprehensive experimental approach.

SUMMARY

A preparation using the cerebral ganglion of the decapod, Callinectes sapidus, has been developed for study of central mechanisms of oculomotor control. The recordings from oculomotor neuron somata are attenuated electrotonic potentials but sufficient to establish the location of specific motorneurons within the ganglion. A variety of interneurons related to the oculomotor system have been recorded from and excellent cellular responses in the form of synaptic potentials, possible pacemaker potentials, single action potentials and cell bursting are obtainable. On the basis of the recording from motorneurons and interneurons, a preliminary anatomical map of the oculomotor system in the ganglion was begun. The preparation appears to be well suited as a model system in which to study central events in oculomotor control.

IV. COR FRONTALE

INTRODUCTION:

Auxilliary hearts are found throughout the arterial system of many invertebrates where they serve as booster pumps to maintain the blood pressure of peripheral areas of the body. An auxilliary heart, the cor frontale, is found interposed in the blood stream anterior to the cerebral system in many crustaceans. Although it was first described in the crayfish in 1916 (von Baumann) and later in other crustacea (Debaiseux, 1944; Demal, 1953), very little published work is available on the function of the cor frontale. It has been assumed by the above authors to be a mechanism for the regulation of blood flow to the cerebral ganglion. The muscles of the cor frontale in Callinectes sapidus were originally described with the eye musculature and termed the musculus oculi basalis posterior or muscle 16 (Cockran, 1935). Although muscle 16 arises from a common apodeme with the eyestalk muscles, the insertion of muscle 16 is on the carapace, rather than on the eyestalk, and they are, therefore, not functional in eye movement. The following work in Callinectes shows that muscle 16 in this animal is analogous to the cor frontale muscles of other decapods. The function of the cor frontale is redefined on the basis of data in Callinectes which suggests that the cor frontale may function, not as a heart adding propulsive force to the blood, but rather as a resistive element in the regulation of flow, acting more like an arteriole than a heart. In addition, the data suggests that the cor frontale is concerned with regulation of the blood flow to the peripheral oculomotor and visual system in the eye rather than regulation of flow to the cerebral ganglion.

MATERIALS AND METHODS

The animal preparation consisted of an isolated perfused section of the anterior carapace of the crab (see fig. 2). The dorsal artery was cannulated and perfused with a Callinectes saline (Perkins and Wright, 1969) buffered with Tes (Sigma Chemical Co.) adjusted to pH. 7.5. The "normal" perfusion pressure was that which had been previously determined to be optimal for intracellular recording in the cerebral ganglion. This pressure was maintained by a constant height of the perfusion bottle above the preparation. Alterations in the perfusion pressure could be made by changing the height of the bottle or by means of a valve in the supply tube. All electrophysiological recording was done with glass suction electrodes on the cor frontale muscle with the thin transparent wall of the sinus intervening between muscle and electrode. This was necessary to avoid dissection of the sinus which would have interfered with maintainance of the perfusion pressure.

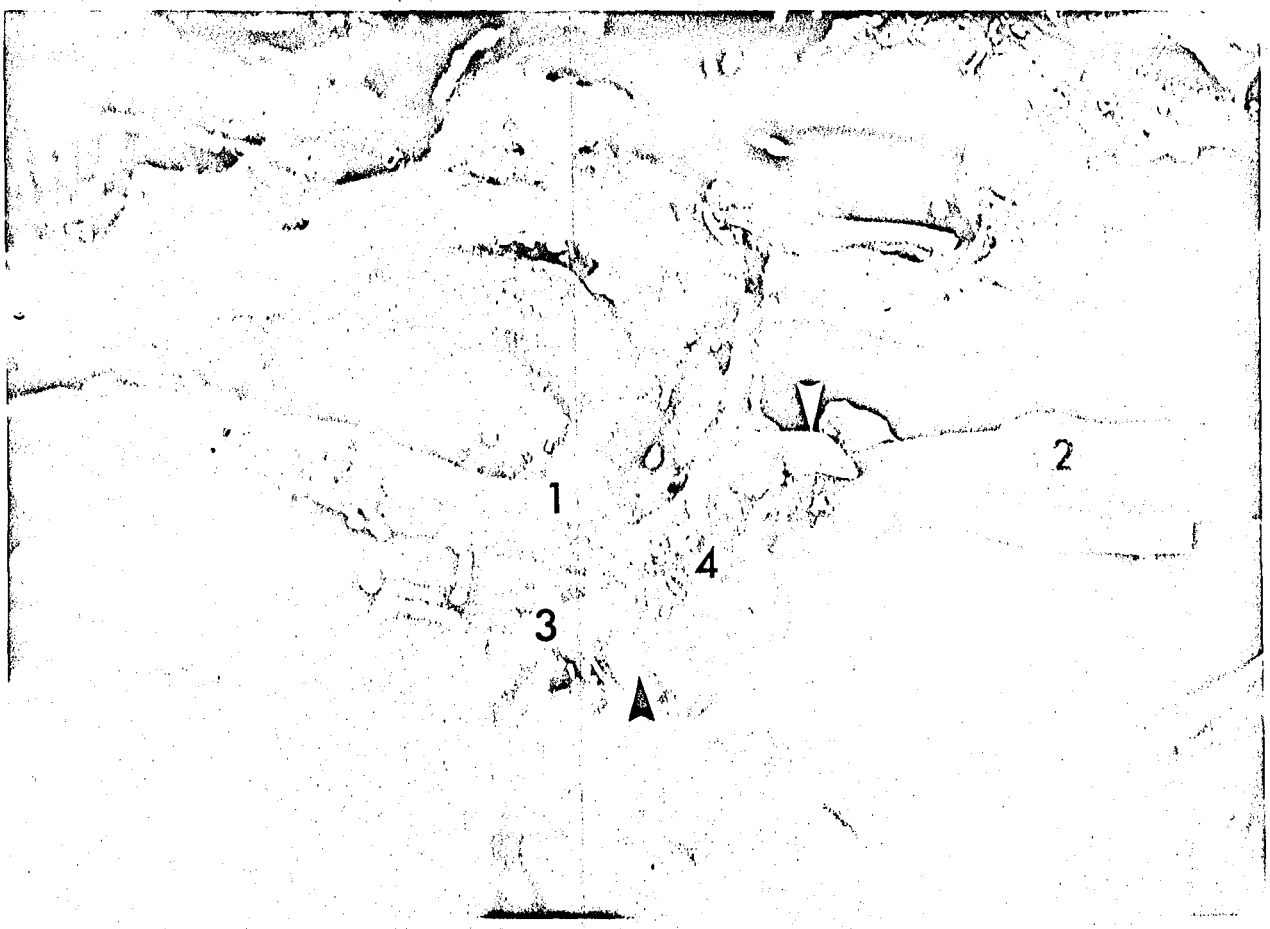
For electron microscopy, the muscle was clamped at rest length or in the contracted state and fixed in 2% paraformaldehyde 3.5% glutaraldehyde with 0.1 M Na phosphate buffer at pH 7.4.

RESULTS

ANATOMY:

The blood supply to the cerebral system is carried anteriorly from the main heart by the dorsal artery. This artery divides anteriorly into 2 main systems, the cor frontale sinus, from which the blood supply to the cerebral ganglion arises, and the two ophthalmic arteries, one to the right and one to the left side (fig.32). The cor frontale is a large dilation with the cerebral ganglion artery exiting from the floor by either one or two short stocky vessels. The

Fig. 32 Photograph of the cor frontale of Callinectes sapidus. (1) ophthalmic arteries (2) dorsal artery (3) cerebral artery (4) cor frontale sinus. The small arrows indicate the areas of insertion of the cor frontale muscle through the sinus. The majority of the muscle is located, when contracted, at the lower arrow. The tissue at the upper arrow is a tendonous extension of the cor frontale muscle. The system has been injected with liquid latex through the dorsal artery for visualization.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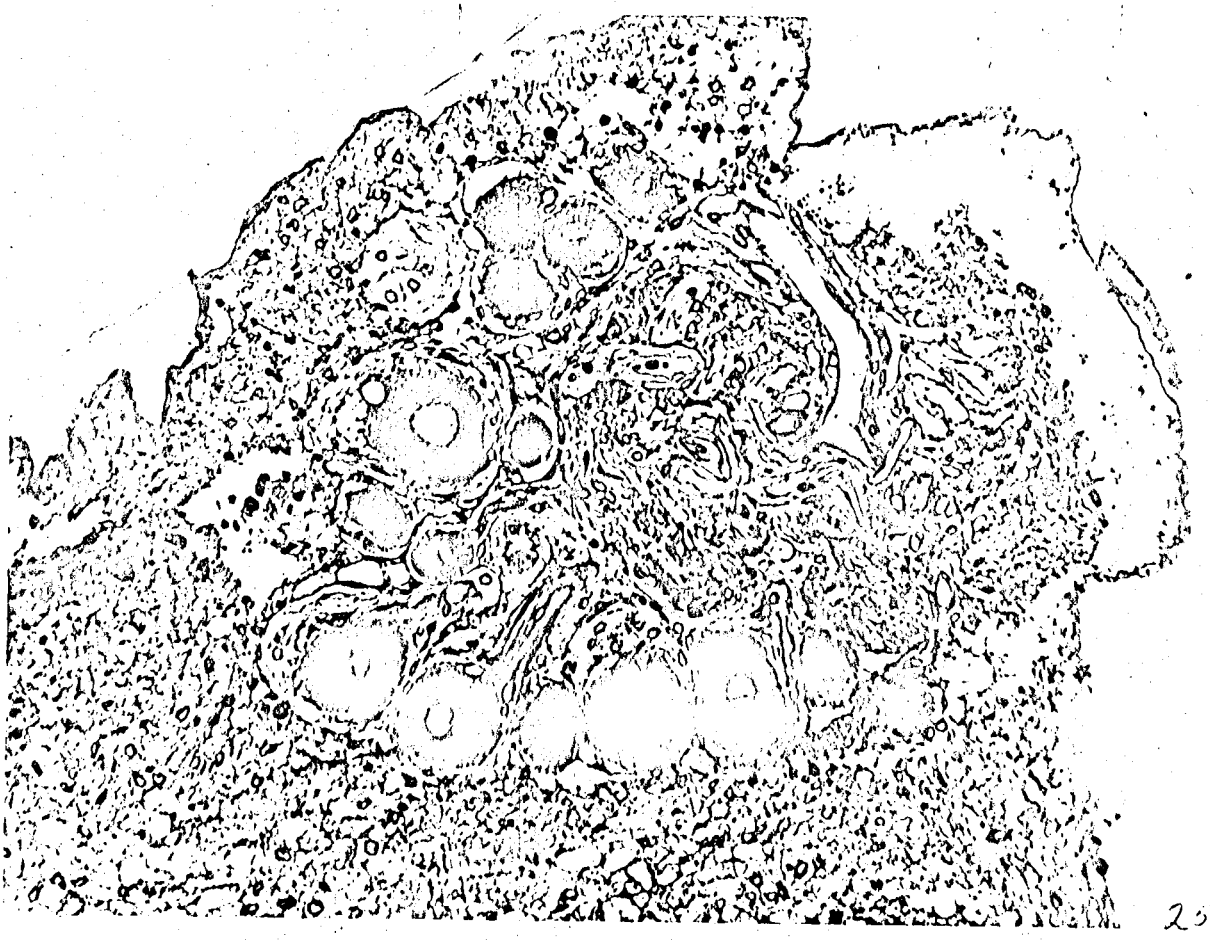
ophthalmic arteries are of approximately twice the diameter and ten times the length of the cerebral artery since they must extend to the eyes in the periphery where they supply the neural and muscular systems of the eyecup. There are four levels of neural integration in the visual system immediately behind the ommitidia, in addition to the nine pairs of ocular muscles, and blocking of blood flow to this area has been observed to result in cessation of visual response, even when the cells in the cerebral ganglion are still responsive to other modes of stimulation. (Responsiveness in the cerebral ganglion likewise does not survive the cessation of blood supply). The cor frontale is situated in such a position that it is able to control diversion of blood flow either through the cor frontale to the cerebral artery or to the ophthalmic arteries out to the eyes.

In Callinectes, the cor frontale consists of two thin muscle strips and a small ganglion and nerve running through a thin walled sinus (see fig. 1). The ventricular ganglion (terminology, von Baumann, 1916) arises between the two cor frontale muscle strips in the course of a nerve which arises from a branch from the right and left circumesophageal ganglion. The ganglion is from 0.5 to 0.75 millimeters in width and contains a maximum of 18 large neurons, the largest being around 90 microns, arranged around a central neuropile. (fig. 33). Several ganglia have been fixed, embedded in epon and sectioned for cell counts with the light microscope. The cell counts have varied with 18 being the maximum number seen in a preparation in which the alignment of the sectioning was optimal and the count therefore most reliable. The small size and the homogeneity of the cor frontale muscle make it unlikely that there are more than a few motorneurons for these muscles among the 18 cells in the ganglion. The remaining cells may be sensory neurons or interneurons of the cor frontale system or cell bodies

Fig. 33 Sections through the ventricular ganglion: note the large size of the somata, cell processes and neurites seen extending from the somata into the central neuropile. The large size of the processes in the central neuropile will facilitate intracellular recording here. Sections embedded in epon and stained with Toluidine blue. Magnification scale under figures.



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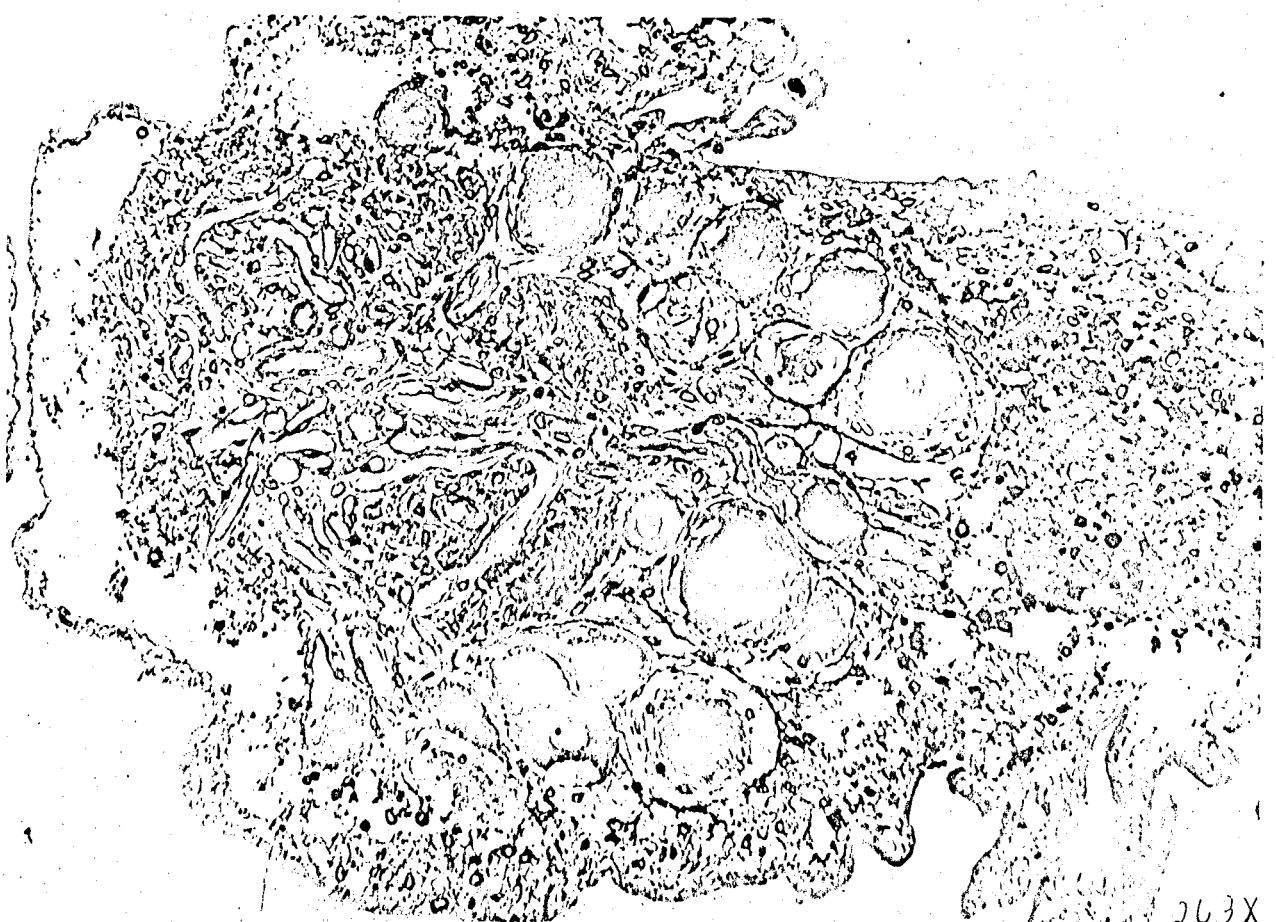
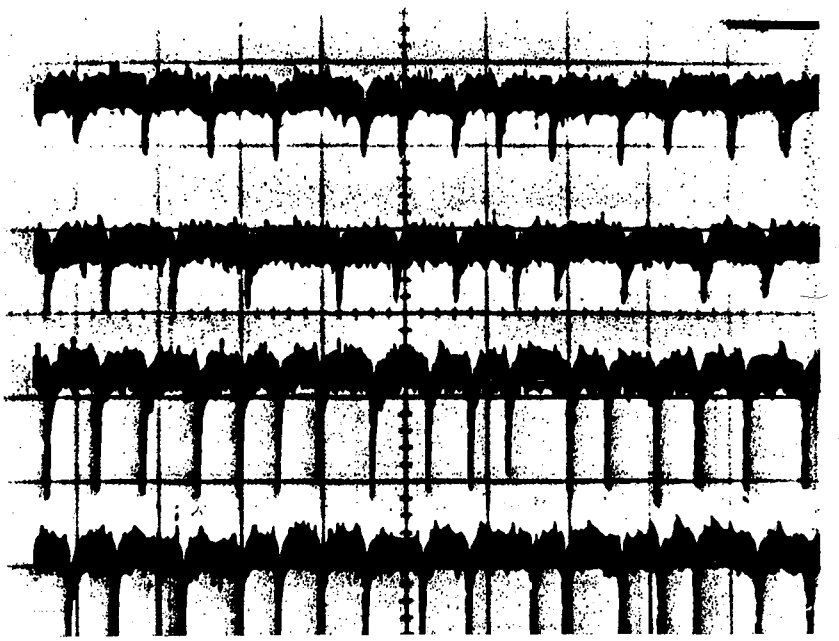


263X

whose processes continue along the dorsal nerve to the gastric muscles. Each muscle of the cor frontale receives a branch from the ganglion after which the main nerve curves dorsally to follow the median artery back to the region of the main heart. Branches can be traced to the visceral muscles but, due to the fineness of the branching, it could not be ascertained whether the branches extend to the main heart.

The muscles of the cor frontale are, in the uncontracted state, a pair of very compact thin strips with a glistening white appearance. At maximum contraction, the long thin strip reduces down to a third or less of the original length into a dense white bundle. This contracted state can be maintained for long periods of time. The whiteness of the muscle is usually associated with fast muscle contraction yet the contraction of the muscle appears to be slow and maintained. The electron microscopy which was done of the muscle does not give a clear picture in terms of the classical characteristics for differentiation of fast and slow fibers in crustaceans (Hoyle, 1967, Hoyle and McNeill, 1968). The sarcomere length varies from 3 to 6 microns and mitochondria are very small and sparse, characteristics which are associated with fast muscle fibers in crustacea. On the other hand, the endoplasmic reticulum in very poorly developed and irregular. Evidence of supercontraction (shortening of the sarcomeres so that the thick filaments extend through the Z band and no I band can be seen (Hoyle et al., 1965) is present in the muscle which was fixed in the contracted state. The Z band is very electron opaque and in the contracted state shows the discontinuities through which the thick filaments of adjacent sarcomeres interdigitate in supercontraction. The thick filaments appear to be in a loose hexagonal array but thin filaments rarely have any regulularity of arrangement. For this reason, it is difficult to give an exact figure for thick thin filament ratios although it would appear to be in excess of 1 to 10.

Fig. 34 Effects of decrease of perfusion pressure on the discharge of the muscles of the cor frontale. First trace shows extracellular recording from muscle when the perfusion pressure is at the optimally determined value. The perfusion is then cut off and the subsequent 3 records taken. Note facilitated muscle response with increased discharge. Scale: 100 msec.



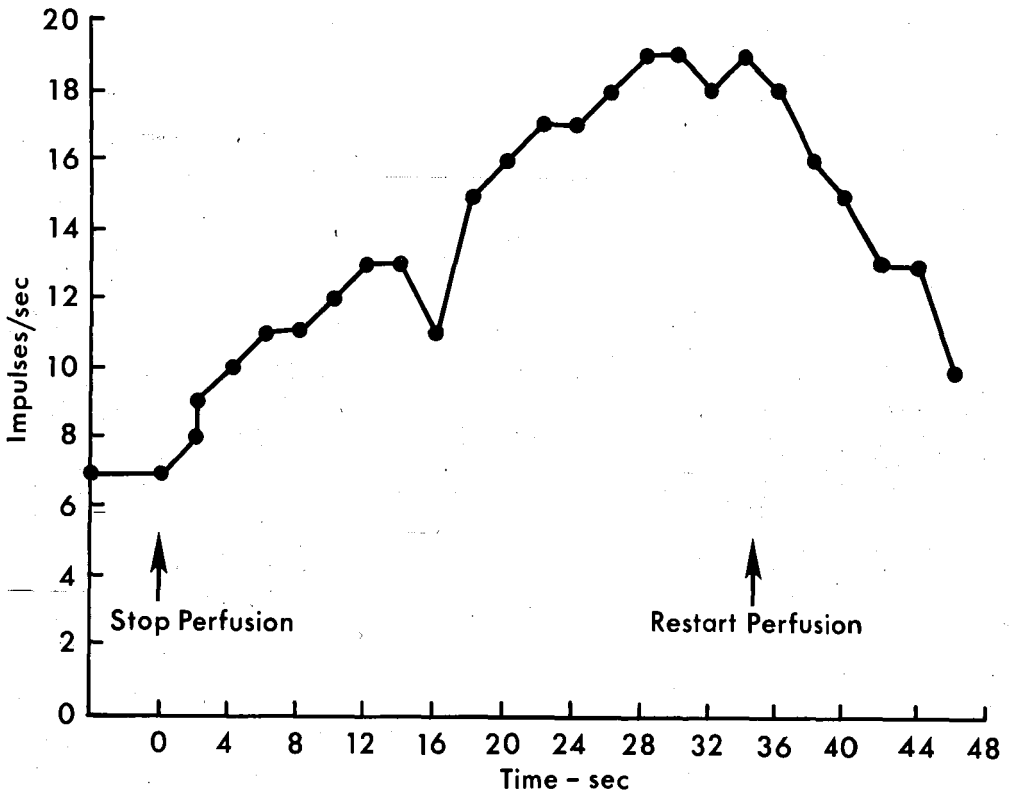
PHYSIOLOGY:

When recording extracellularly from the muscle when the perfusion pressure is "normal" a small muscle spike is recorded with an average frequency of 8 to 13 per second depending on the preparation. When the perfusion pressure is cut off by the valve in the perfusion supply tube, the frequency may rise to a maximum of 27 per second. The interspike interval is fairly constant in the "normal" state and decreases at a regular rate with decrease in perfusion (fig. 33). There is no bursting seen. The contraction of the muscle is that of a slow tonic muscle with no evidence of beating. With increase in firing rate, the muscle contracts in a slow sustained contraction down to a short bundle of 1/3 to 1/4 of the original length. Fig. 35 illustrates the response of the muscle firing rate to cessation of the perfusion. The firing rate of the muscle increases gradually with a very short latency, and rises, in this case, to a maximum of 20/sec. Activity which was recorded from nearby eyestalk muscles at the same time showed no change in firing rate (unless perfusion was shut off for several minutes in which case the activity in eyestalk muscles decreases due to deprivation of flow to the cerebral ganglion). In addition to an increase in firing rate a facilitation of the muscle activity is seen (see fig. 34). This increased muscle spike decreases gradually back to "normal" when flow is reinstated.

When the perfusion flow was not shut off but instead pressure decreased by lowering the perfusion bottle by 1 foot steps, the following changes in firing rate were recorded: at optimal height the firing rate was 96/sec; lowering the perfusion bottle one foot the rate decreased to 146/sec; lowering the bottle 2 feet reduced the firing rate to 164/sec and a final lowering of 9 inches, cutting off the flow entirely, resulted in a firing rate of 180/sec. The firing rate of the muscle thus appears to be proportioned to the perfusion pressure.

The time course of the response to cessation of perfusion is graphed in fig. 35. The absolute latency for response to cessation of perfusion could not be calculated by the methods used but is quite short, probably considerably less than one second. The response takes some time to reach the maximum value. In the case in fig. 35, the firing frequency appears to be reaching maximum around 28 - 34 seconds after cessation of perfusion. A similar slow decline of firing rate occurs after reinstatement of the perfusion.

Fig. 35 Illustration of the increase in discharge frequency of the muscles of the cor frontale with the cessation of perfusion through the cerebral system.


Impulses/sec
20
18
16
14
12
10
8
6
4
2
0
Stop Perfusion
Restart Perfusion
0
4
8
12
16
20
24
28
32
36
40
44
48
Time – sec

DISCUSSION

The major features of the anatomical and electrophysiological observations indicate that the cor frontale muscles are tonic muscles capable of slow sustained contraction. The contraction is not in the form associated with individual beats of a heart and the "heart" has never been observed to beat on any occasion. Rather, the contraction of the muscle is always a slow maintained shortening of the muscle which continues contraction down to a fraction of the original muscle length. This is consistent with the functioning of the muscle acting as a resistive element opening or narrowing the sinus at the entrance of the cerebral artery in much the same way that the muscular wall of an arteriole serves as a resistive element regulating flow through the arteriole. When the muscle contracts in response to decreased blood pressure in the cerebral system, it decreases the size of the sinus passage and increases resistance to flow to the cerebral ganglion. This is contradictory to the heart's assumed function of maintaining the pressure in the cerebral ganglion. It is possible that this may be the case; the heart is decreasing flow to the cerebral ganglion in order to divert it to the vessels supplying the optic and oculomotor system in the periphery. These vessels are of a very large diameter and must deliver blood a long distance out to the eyes where there is a large oculomotor apparatus and four levels of neural integration of the optic input which is vital to the animal. A central decrease in flow is more likely to give rise to a greater decrease in flow at the end of the long large diameter ophthalmic arteries in the periphery than it will in the short cerebral artery which is closer to the propulsive force of the main heart. The muscles of the cor frontale may then function to maintain pressure in the periphery at the expense of flow to the cerebral ganglion.

Without the input to the ganglion from the peripheral optic apparatus, the ganglion from the peripheral optic apparatus, the ganglion would be deprived of its major sensory system and maintenance of the function of this sensory input may be the highest priority in regulation of the cerebral blood flow.

The electron microscopy of the cor frontale muscles is, for the most part, consistent with a slow tonic function in crustacean muscle. There are two characteristics however, which are more typical of fast crustacean systems. These are the short sarcomere length and sparcity of mitochondria. The presence of these two features may be explained by the function of the muscle. Short sarcomeres are found not only in fast muscle fibers but also in muscles which are capable of supercontraction down to a fraction of their original length Hoyle, et al., 1965). The difference between the fast and slow fibers with short sarcomere length lies in the ratio and arrangement of thick and thin filaments. While the number of thin filaments in fast fibers is small and regular in arrangement, the thin filaments in the supercontracting slow fibers are more numerous and irregular in their arrangement. This may be a reflection of a basic relationship between the thin filaments and muscle function. While the fast muscle must perform work quickly against large resistances or in overcoming inertia, the supercontracting fibers do not perform work against great resistance. On the contrary, the supercontracting muscle of the cor frontale is closer to smooth muscle in performing a large amount of shortening with little work performed. The cor frontale muscle shortens, not against the resistance of a fixed structure such as the shell, but by stretching a tendon to which it is attached in a loose relation to the carapace. The short sarcomeres in the fast muscle may function, in conjunction with the regular thick-thin filament arrangement, to produce force quickly. The same short sarcomere length in association with a different

thick-thin filament arrangement in the supercontracting fiber may be associated with large changes in length with little work. The small work requirements may also explain the sparcity of mitochondria found in the slow supercontracting cor frontale muscles.

The sensory aspects of the cor frontale regulation of the cerebral blood pressure has not been explored in the present work. Two possibilities for sensory reception of the pressure change are stretch receptors or chemoreceptors. The short latency of response is more consistent with a stretch receptor in which decrease in pressure is signalled by an increase in firing rate of the receptor. However, extremely sensitive chemoreceptors monitoring oxygen decrease or carbon dioxide increase are also possibilities. Both receptor types exist in complex mammalian blood pressure regulating systems (White, 1972). The investigation of a pressure regulating system, from the sensory to the motor components, is possible in the simple system presented by the cor frontale and further study of this system may provide answers which will be applicable, not only to crustacean blood pressure regulation, but to general principles of pressure regulating systems in higher forms.

SUMMARY

The cor frontale is a cerebral blood pressure regulating system contained in an isolated unit composed of a small ganglion, two muscles and pressure transducing receptors. The function of this unit appears to be as a resistive element controlling flow in the manner of a vertebrate arteriole rather than as a heart. The system appears to function to protect not the cerbral ganglion but the peripheral neural and muscular components of the visual system.

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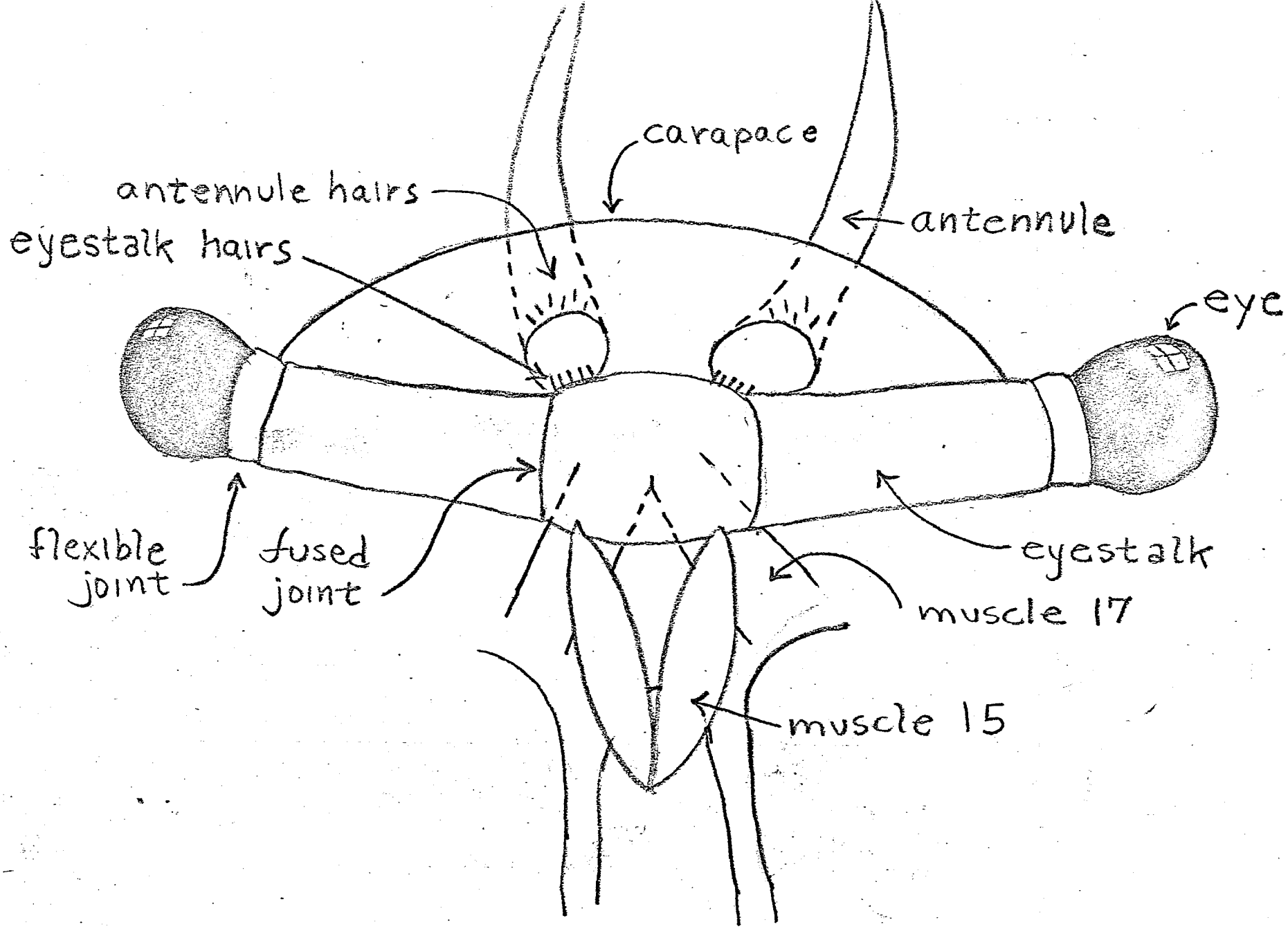
APPENDIX: LIST OF FIGURES

1. Schematic overview of the oculomotor and antennular system

2. Electron microscopy of the cor frontale muscles, longitudinal section 13, 200 X

3. Electron microscopy of the cor frontale muscles, transverse section 16,500 X

Schematic overview of the oculomotor and antennular system


carapace
antennule hairs
antennule
eyestalk hairs
eye
flexible joint
fused joint
eyestalk
muscle 17
muscle 15

Electron microscopy of the cor frontale muscles, longitudinal section 13,200 X

Electron microscopy of the cor frontale muscles, transverse section 16,500 X

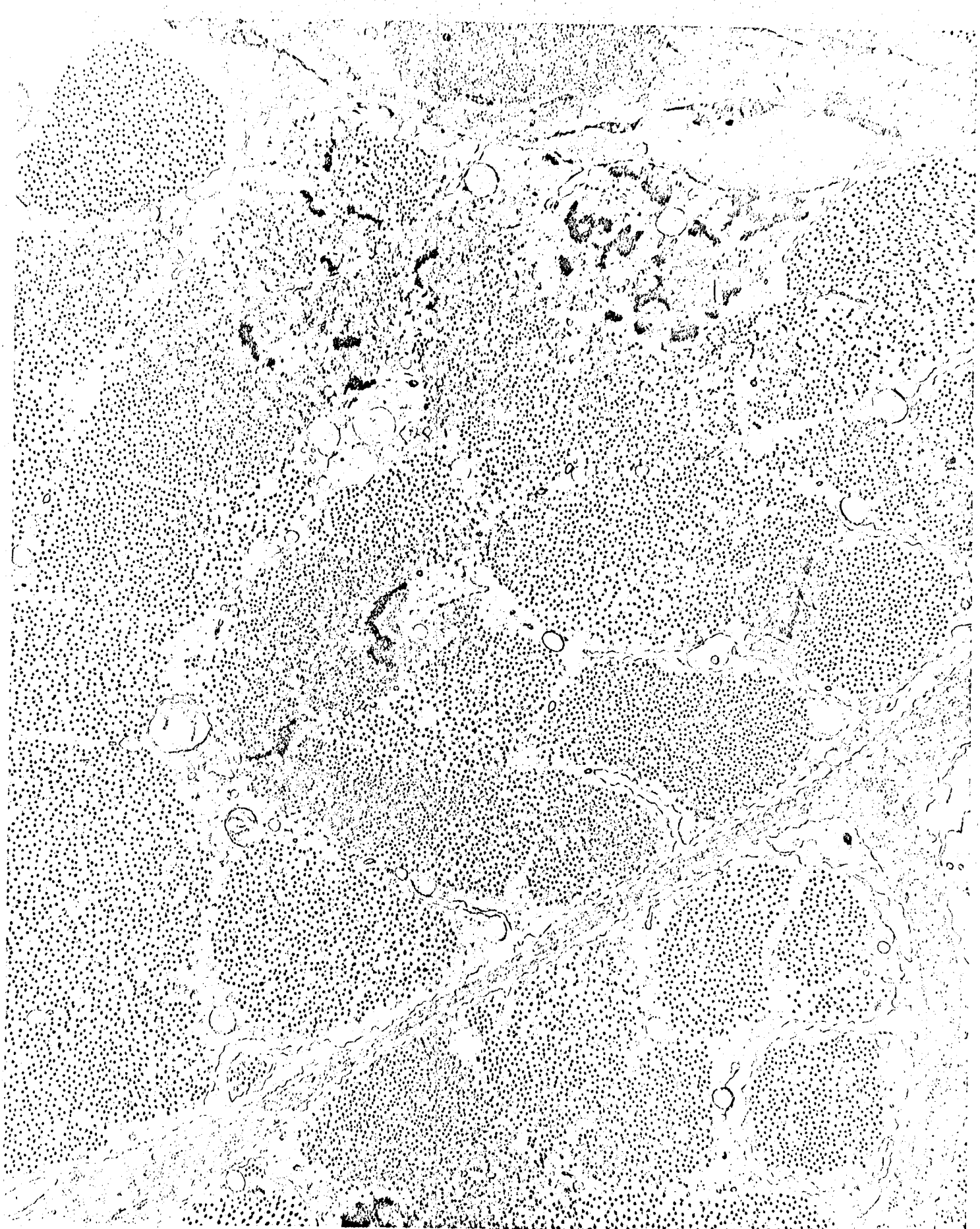


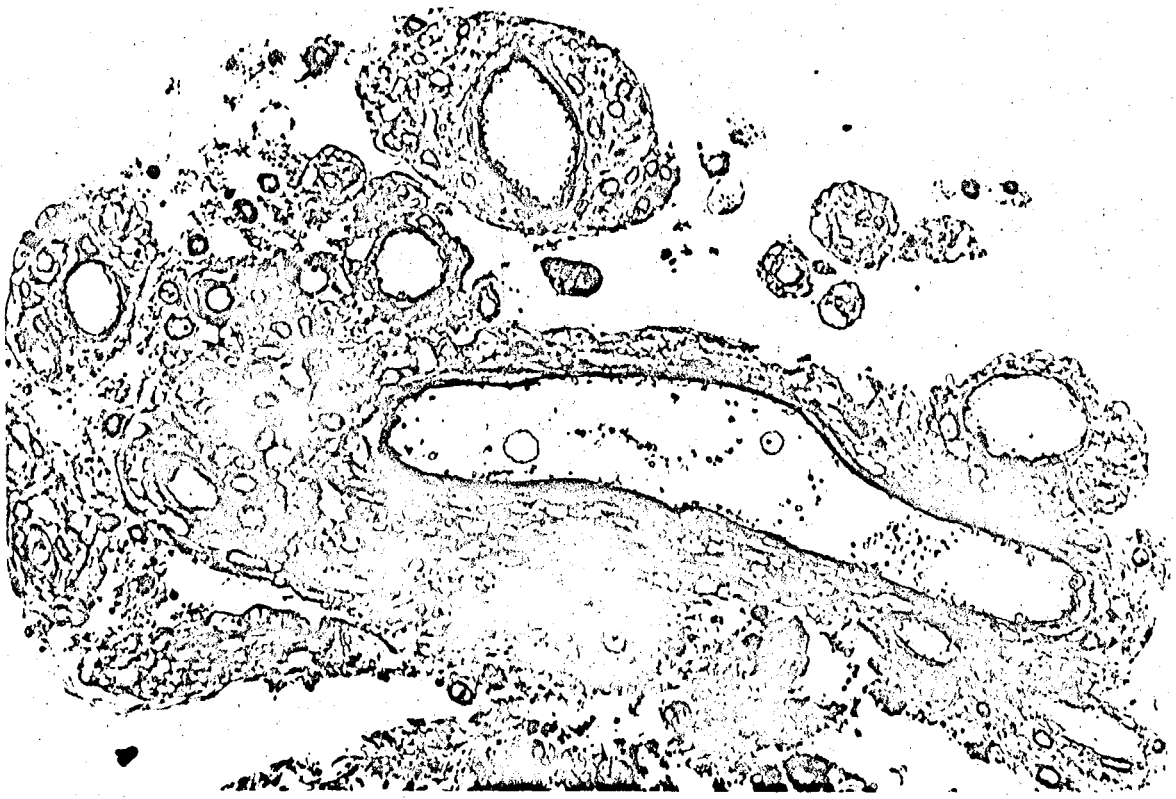
Fig. 9 Response of muscle 15 to pitch and roll. Extracellular recording from right muscle in intact animal. (a) animal horizontal. (b) 45° pitch downward. (c) 45° pitch upward. (d) 45° roll to left. (e) 45° roll to right. Time scale 100 msec. per division.

Fig. 10 Function of muscle 17 in pitch. Firing of muscle 17 recorded extracellularly from the muscle at varying degrees of pitch. (a) 15 degrees pitch upward. (b) level or resting position. (c) 10 degrees pitch forward. (d) 15 degrees pitch forward.

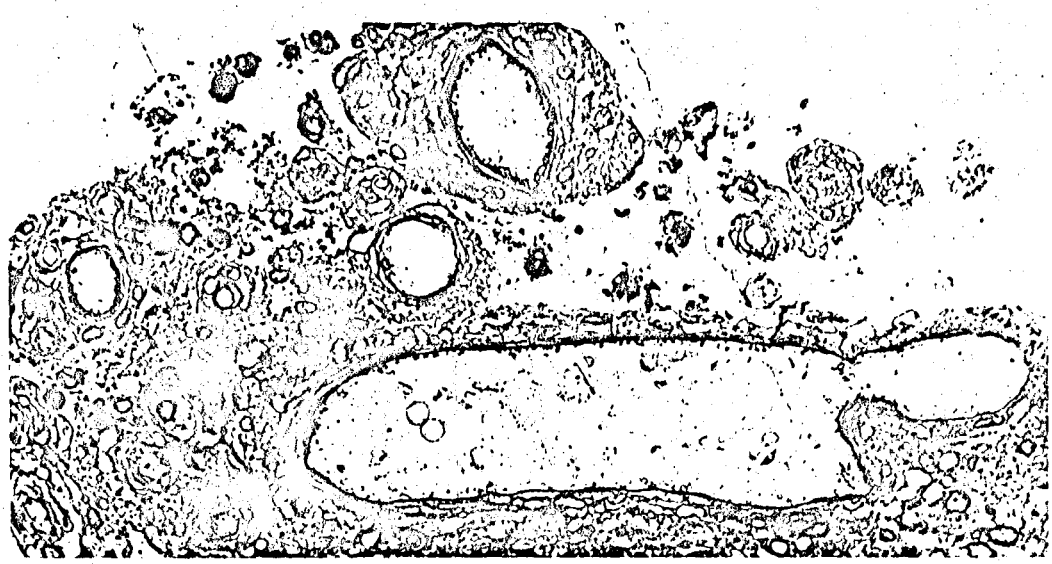
time scale: 0.5 sec.

Fig. 11 Simultaneous firing frequency of Muscle 17 and 15 to different degrees of pitch.

Pitch	Muscle 17 Firing Frequency	Muscle 15 Firing Frequency
level	7	1
5° forward	5	3
10° forward	4	7
15° forward	4	13
20° forward	3	11
level	11	1
5° backward	10	0
10° backward	12	0
15° backward	15	0
20° backward	21	0
4° forward	5	4



A



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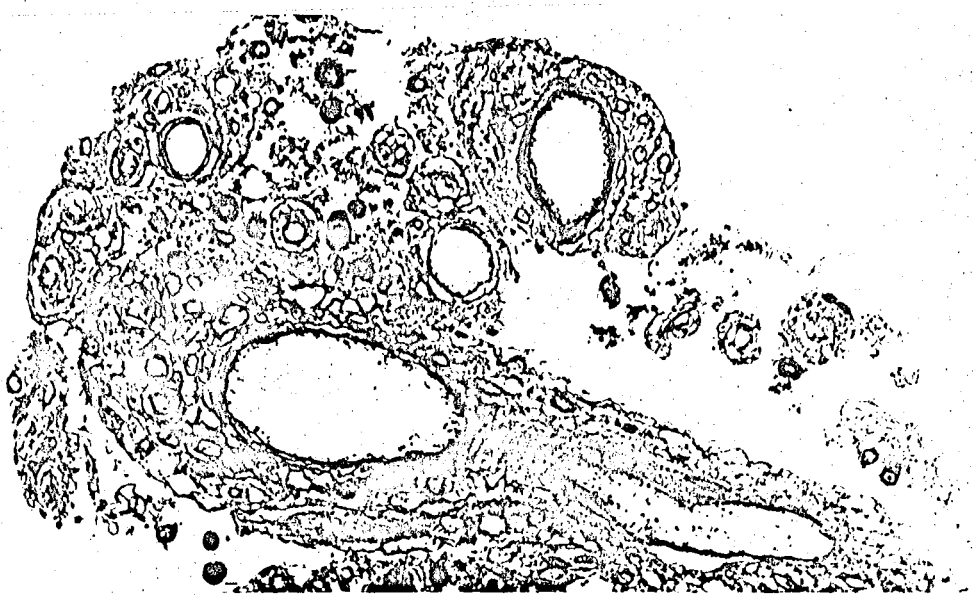


C

extracellular recording from the muscle one large and one small amplitude response with different frequencies of firing can be seen (fig. 13) indicating at least two motoneurons controlling the muscle response. The small amplitude e j p shows a regular tonic firing rate and superimposed on this at irregular intervals is a response of approximately 20 times greater amplitude and time course.

MEASUREMENT OF THE COMPENSATION OF THE EYESTALK FOR PITCH OF THE ANIMAL

Using the intact crab and a capacitative movement detection device (see Methods) the compensation of the eyestalk in response to pitch of the animal was measured. Pitch was restricted to values between 5 and 30°. Sine waves, ramp and step movements in the pitch plane were generated manually using the pitch table. The percentage compensation of movement of the eyestalk for movement of the body was typically under 10%. This is illustrated in fig. 14 in which the sine wave movement of the eyestalk, upper trace, varies between 0.9 degrees and 1.0 degrees in response to pitch of 10 degrees. The response to a step pitch shows an initial fast phase and a slower movement to a maintained value (fig. 15). The majority of the compensatory movement of the eyestalk to pitch showed between 8 and 10 percent compensation. There were a few exceptions to this however. In two preparations the responses showed 75 and 80% compensation. The response was consistent throughout the experiment showing the high compensation for all degrees of pitch. In addition, one preparation showed a high degree of tremor and instability. The eyestalk rotation is usually a smooth well-controlled movement. In this preparation, however, (see fig. 16) the response was larger than 10%, varied with each pitch of the animal and showed the illustrated tremor for the duration of the experiment (3-4 hours). These two exceptions to the

Fig. 13 The two types of excitatory junction potentials recorded extracellularly from muscle 17. The small response, seen as a notch, particularly prominent on the third large spike, is present as a tonic discharge in this muscle and probably corresponds to the small axons seen in fig. 12. The large signal is a phasic discharge which must correspond to the larger axon of the nerve to muscle 17.

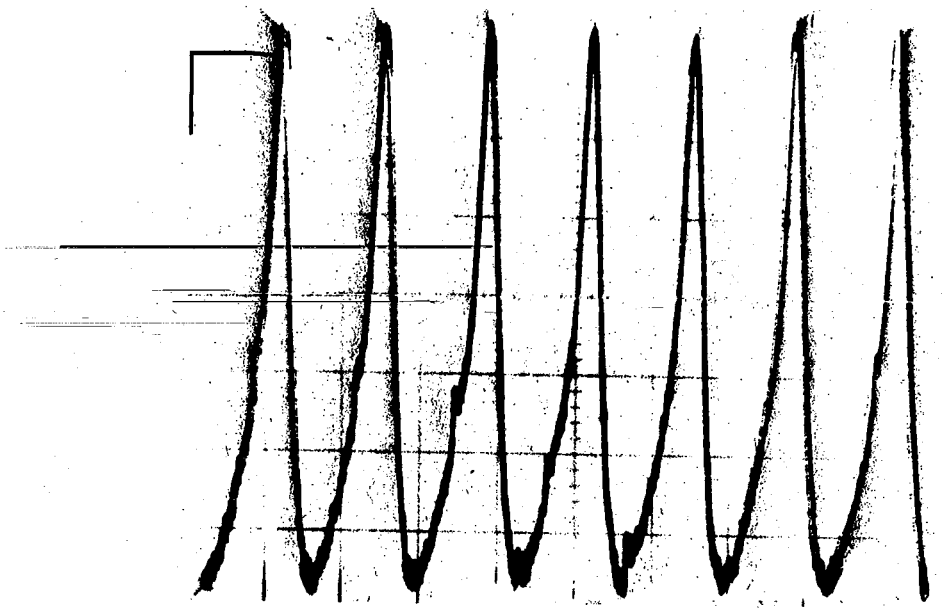


Fig. 14 Compensatory response of the eyestalk to quasi sine wave movements in forward pitch of the animal. Upper trace of each pair is the compensatory movement of the eystalk in response to forward pitch of the whole animal seen in the lower trace. Upper response in A is the normal response of approximately 10% compensation. B illustrates an infrequent recording of much greater compensation, in this case, approximately 70%. Scale: A, 1 degree, upper trace, for 10 degrees pitch in lower trace; lower set, 7 degrees upper trace and 10 degrees pitch in lower record, time 1 sec.

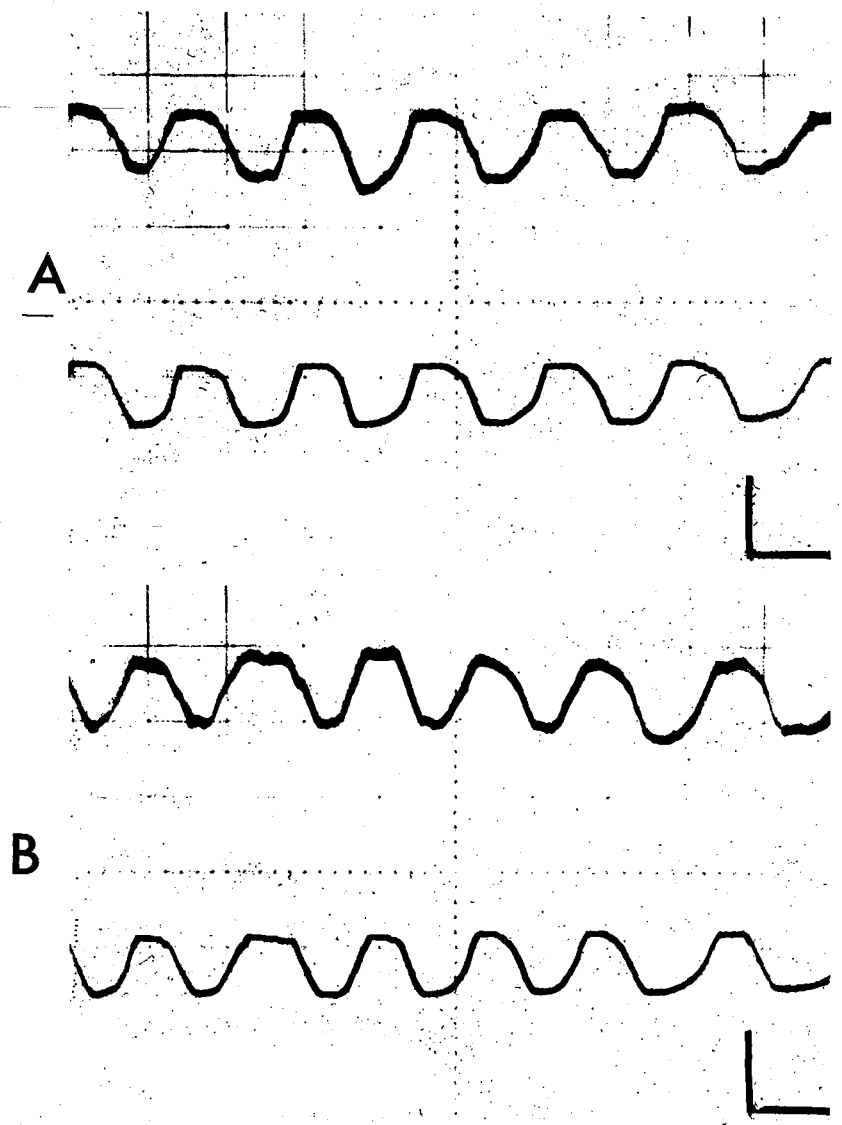

A
B

Fig. 15 Compensatory Response of the eyestalk to step movement in forward pitch of the animal. Response of eyestalk, upper trace; step movement of animal, lower trace. Scale: upper trace, 0.5 degrees; lower trace, 5 degrees; time, 1 second.

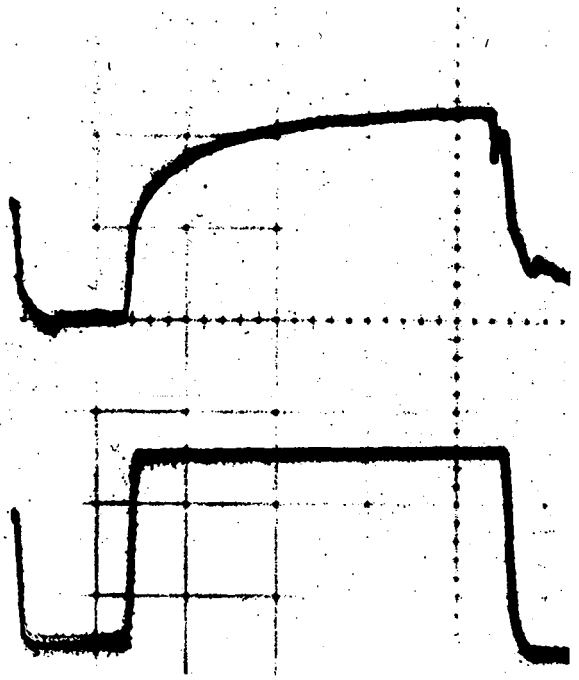
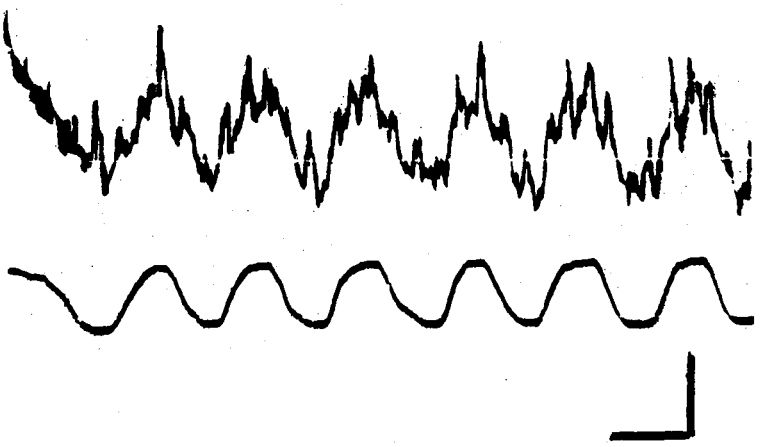


Fig. 16 Tremor and high compensation of eyestalk (upper trace) seen in response to pitch (lower trace) in one preparation. Scale: upper trace, 1.5 degrees; lower trace, 10^{o} degrees, time, 1 sec.

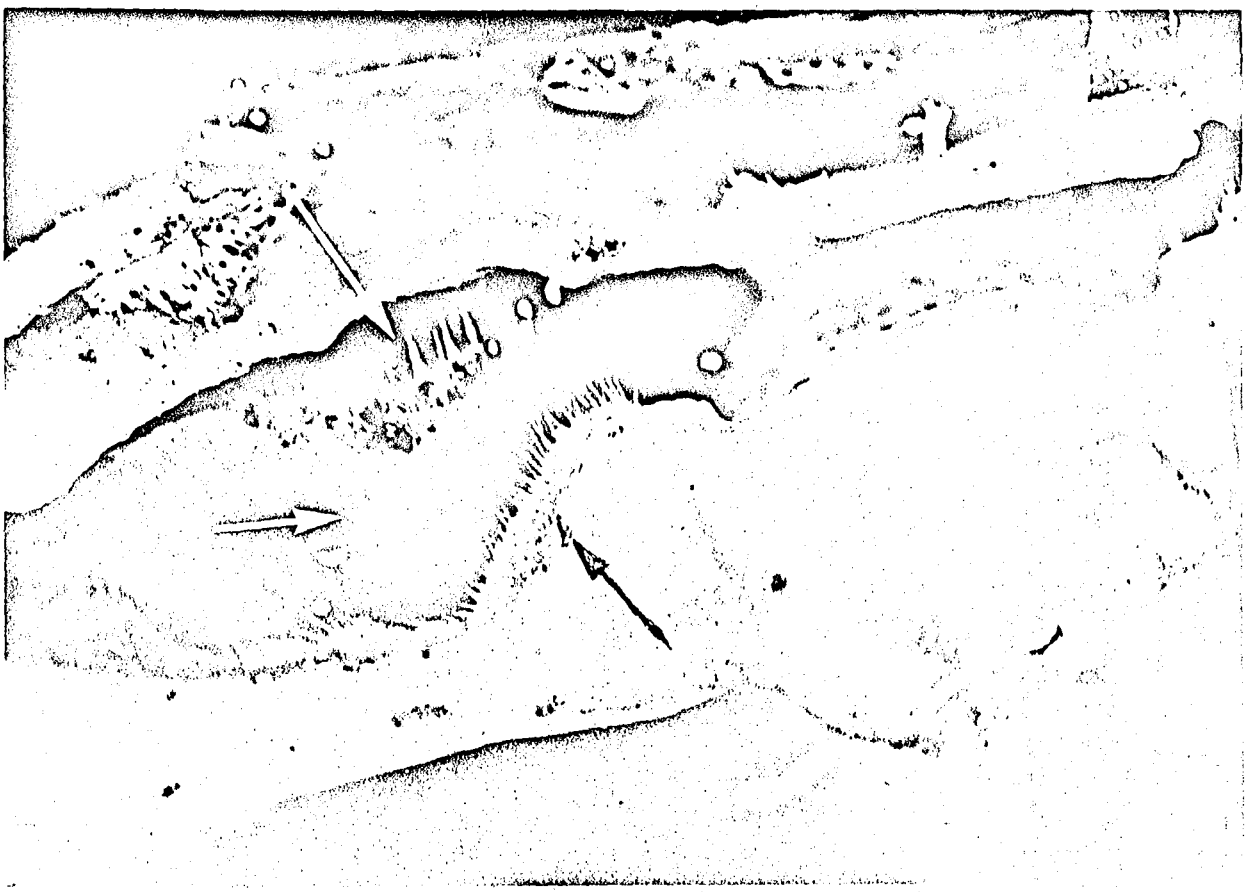


usual compensatory response are believed to be due to interference with a feedback mechanism (see discussion).

MECHANORECEPTIVE SENSORY HAIRS REGULATING MUSCLE 15 AND 17:

a. NEGATIVE FEEDBACK FROM ANTENNULAR SENSORY HAIR SYSTEM: Callinectes has a pair of small antennules which are constantly in motion. The statocysts are lodged in the basal segment of the antennules and move in common with antennule movement. On the dorsal surface of the basal segment are many sensory hairs (figs. 17 and 18). These hairs are long and thread-like and cover most of the surface of the basal segment, being especially numerous around the edges of the basal segment. The hairs extend upward to the under surface of the carapace over the antennule basal segment. The under surface of the carapace above the basal segment also has a number of these sensory hairs. When the animal moves the antennule, the sensory hairs are stimulated by the shearing movement of the hairs against the opposite surface. When the sensory hairs are displaced with a glass needle, an inhibition of activity is seen in both muscle 15 and 17 (fig. 19). The inhibition does not appear to show adaptation, being reproducible for repeated trials over a period of time. The same inhibition can be produced by stimulation of the hairs of the contralateral basal segment. If the sensory hairs are displaced by natural movements of the antennule which the animal is constantly making, this inhibition is not seen. Repeated stimulation of the sensory hairs evoked a movement response of the antennules, which did not cause inhibition of the muscles. The results of attempts to record the same inhibition seen in the eyestalk muscles from the nerves to the leg muscles were equivocal. Inhibition of certain fibers was sometimes seen but this result was not an invariable finding.

Fig. 17 Sensory hairs on eyestalk, (arrow, foreground) and antennular basal segment, (arrow, background). Eyestalk has been pulled away from the posterior aspect of the antennule against which it normally rotates. Note projection of antennular hairs toward overhanging carapace and basal segment of the antennule against which the eyestalk hairs rotate (horizontal arrow).



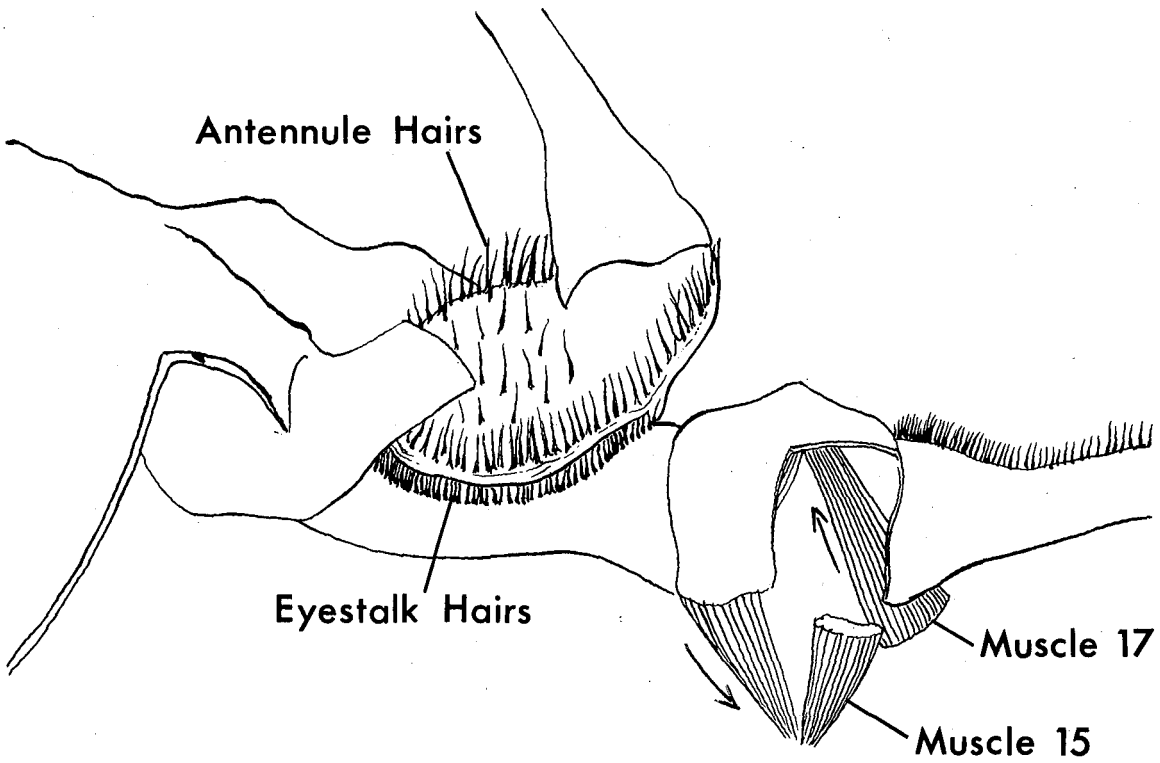
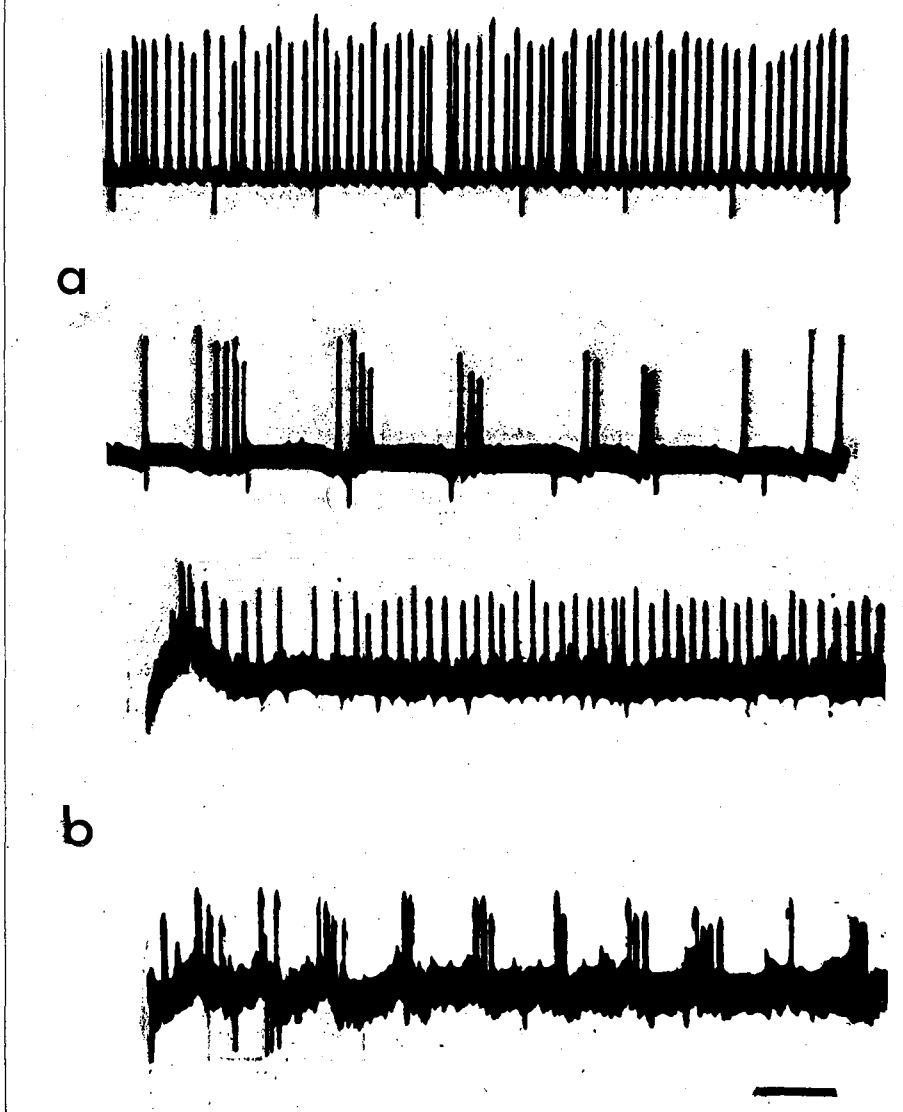


Fig. 18 . Antennule and Eyestalk of Callinectes sapidus illustrating sensory hair systems. Rotation of the eyestalk deflects eyestalk hairs against posterior portion of the antennule basal segment. Movement of the antennule deflects antennule hairs against the overlaying carapace.

Fig. 19 Inhibition of firing of muscle 15 and 17 following stimulation of antennular basal segment hairs. Upper traces in a and b, control level of firing: lower traces show inhibition following deflection of sensory hairs. a, muscle 15 ; b, muscle 17 . scale 0.5 sec.


a
b

To ascertain the behavioral function of the antennular hairs, the animal was tested behaviorally for several functions before and after removal of the hairs by cautery. The following functions were checked:

1. Compensatory eye movements
2. Movement of swimming appendages
3. Righting movements when the animal is inverted

The functions were tested with movement of the entire body and with movement of the antennules alone, since it was suspected that the hairs function to allow the animal to differentiate between the two means of stimulation of the statocysts.

After removal of the antennular hairs, compensatory eye movements to pitch were normal until the animal was completely turned over (180°). The normal animal in this position maintains the eyes approximately level with the carapace. The operated animal extends the eyes out and downward in exactly the same position (reversed by 180°) they would be extended if the animal were right side up. It appears that the animal is unable to tell whether it is upside down or right side up.

If the antennules were moved with forceps with sensory hairs intact, no eye movement was observed. After removal of the hairs, small movements of the eyes were seen in response to movement of the antennules. The movements were jerky and irregular and appeared to be opposite in direction to movement of the antennules.

Movement of the antennules with hairs intact has no influence on leg movement. After removal of the hairs, movement of the antennule resulted in a circular swimming motion by the periopod, the last leg of the crab, which is

adapted as a swimming paddle and also used in righting movements. When the operated animals were placed back in their retaining tank, swimming movements appeared normal. However, if the animal was turned over by 180° onto its back, it either did not attempt to right itself or attempted to right by pitch in the opposite direction than that seen in the normal animal. The normal animal rights itself by pitch in the anterior posterior direction, raising the frontal end up over the caudal end. The inverted operated animals which attempted to right did so by attempting pitch in the opposite direction using the rear periopod to raise the caudal end up to pitch over the frontal end.

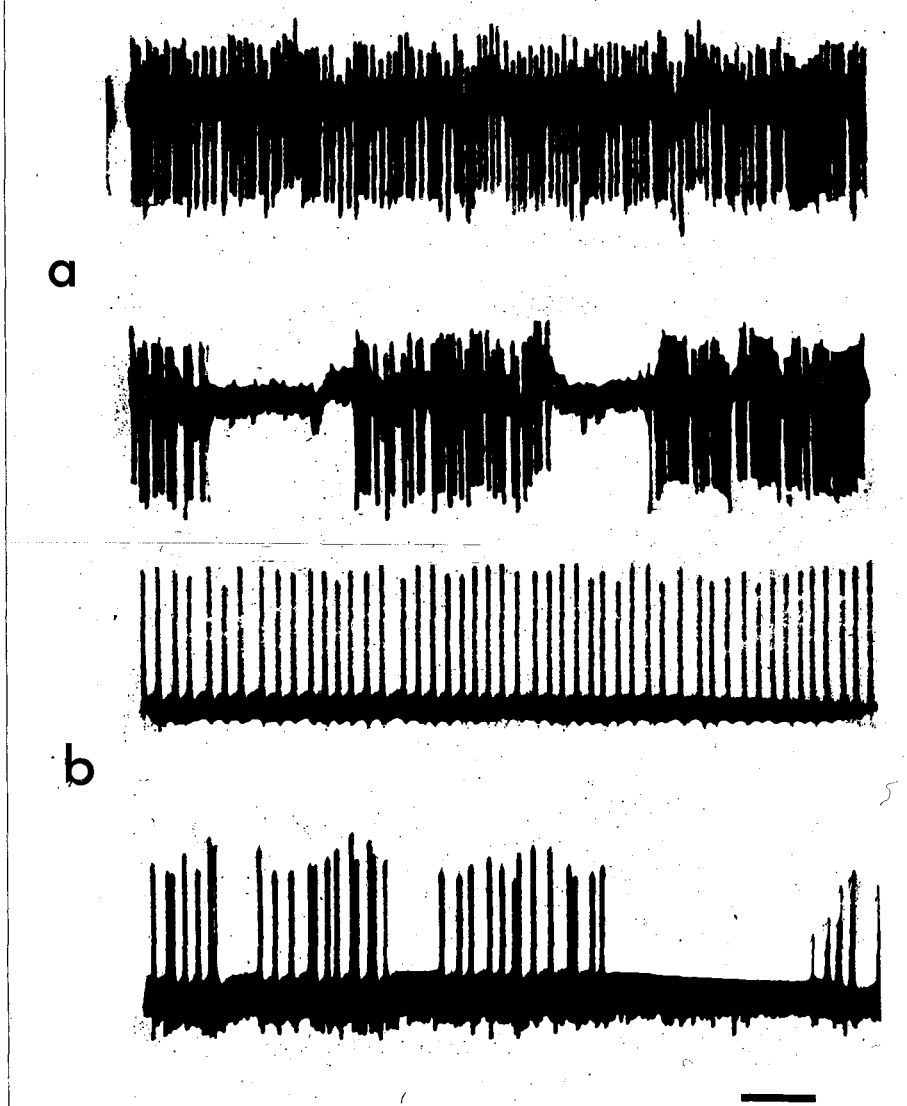
b. NEGATIVE FEEDBACK FROM EYESTALK SENSORY HAIRS:

On the anterior surface of the eyestalk which abuts the posterior surface of the basal segment of the antennule, there is a dense round patch of short bristle like sensory hairs (figs. 17 and 18). These hairs continue in a thin line down the eyestalk for about 1 cm. toward the eyecup, becoming more thread like. The hairs are so placed that a shearing force will be exerted on them as they pass the posterior surface of the basal segment of the antennule during rotation of the eyestalk. The influence of these hairs on the muscles which rotate the eyestalk was ascertained by recording from muscle 15 and 17 while displacing the hairs in several directions. A shearing force applied across the hairs, perpendicular to the direction of displacement by rotation of the eyestalk, was without effect. The tonic firing rate of the muscle, which has been shown above to be due to position sense input from the statocyst, continues without change. A displacement of the hairs in both direction of the dorso-ventral axis of the animal, the axis of displacement in rotation of the eyestalk, resulted in inhibition on the tonic discharge of muscle 15 (fig. 20). (The same effect seemed to be true for muscle 17 although, because of the inaccessibility of this muscle, the results were not as easily obtained.) The latency and duration of the inhibition is short. It was necessary to sweep past a row of hairs for the inhibition to be produced. No effect was seen to the stimulation of less than five hairs. The effect was, as for the antennule basal segment hairs, contralateral. Stimulation of the hairs of the opposite eyestalk produced inhibition of the same characteristics as stimulation of the ipsilateral hairs. With repeated stimulation of the hairs, the response was not as reproducible as for the antennular hairs. This was not adaptation or fatigue since the response decrement could still be seen an hour later when the response to antennular hairs was still

strong. The decrement may be due to damage to the sensory hairs. The hairs of the eyestalk are short, stiff and bristle like and strong displacement could result in damage to the base. Those of the antennule are thin threads which, being more pliable, are less liable to damage.

Using the whole animal on the tilt table, the effect of removal of the sensory hairs on the compensatory movements of the eyestalk was tested. A small hole was made in the carapace and the hairs on the eyestalk burned off with a small cautery. Movement of the eyestalk in response to pitch was tested as in previous experiments. The response to 10, 20 and 30 degree pitch of sine wave and step form was tested before and after removal of sensory hairs. There does not appear to be a significant difference in response after removal of the sensory hairs. The response is only slightly sluggish and more irregular, but the percent compensation does not appear to differ.

Fig. 20 Bidirectional sensitivity in the horizontal axis of eyestalk sensory hairs resulting in inhibition of firing of muscle 15. Upper traces, control firing level: lower traces, a. deflection of sensory hairs by passage of glass needle in an upward direction. b. deflection of sensory hairs by passage of glass needle down across the hairs. time scale: 0.5 sec.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DISCUSSION

MUSCLE FUNCTION AND FIBER COMPOSITION:

The movement of the eyestalk is under the control of two antagonistic pairs of muscles, muscle 15 and 17. Acting cooperatively, the 2 muscle pairs stabilize the eyestalk in a fixed position in space; acting antagonistically, muscle 15 rotates the eyestalk counter-clockwise around the eyestalk axis while muscle 17 controls rotation clockwise around the same axis. The majority of the data above was collected from work with muscle 15 but muscle 17 appears to operate in an analogous manner.

Muscle 15 is to be a complex muscle mediating movements originating from several sensory systems. The sensory drive appears to be differentially distributed to the motor axons to modulate the muscle output. The best example of this is seen in the characteristics of the intermediate axon which is part of a sensory to motor transduction of position sense input from the statocyst to the oculomotor system for unidirectional pitch responses. This one axon is responsible for maintaining the eyestalk in a constant position in space, probably by the influence of type I non-adapting position sense receptors in the statocyst. In addition, the same axon is under visual control although this does not appear to be a strong synaptic input as evidenced by the inability of the visual input to fire the muscle when the statocyst input is removed. In Callinectes, the non-adapting position sense receptors in the statocyst appear to keep the muscle at a constant low level of tension and the visual and acceleration input is superimposed on this.

Although four axons supply muscle 15, only two of them, the intermediate and the slow one, fired with consistancy. It is not likely that the silence of

the additional fibers was due to the dissected perfused preparation since, when the recording was done in a few preparations of the whole animal, these fibers were not seen. A more likely explanation is that the silent fibers fire only for specific situations which could not be duplicated under these experimental conditions. In the case of the fastest fiber, this is most easily seen. Callinectes is an extremely fast swimming crab. The movements on the tilt table used to induce responses in the intermediate axon may not have been of sufficient acceleration to excite the fast axon. If electrical stimulation of the statocyst nerve had been used, it may have evoked the firing of the larger axon. Among the optomotor fibers found in Carcinus (Wiersma and Fiore, 1971) there was a class of large fibers which could only be made to fire by fast rotation about the pitch axis, with an optimal speed of 90 degrees per second. Callinectes is a much faster swimming crab than Carcinus and it is to be expected that the acceleration factors in the oculomotor control would be corresponding greater. It is doubtful that the acceleration or velocity of movement used in these experiments would excite a fiber which was used in the maximum swimming activity of the animal.

The lack of firing of one of the two intermediate axons is not as easily explained. This fiber was labeled as a peripheral inhibitor in Podophthalmus on scanty evidence (Hoyle and McNeill, 1968). There was no evidence of peripheral inhibition seen in Callinectes. A more likely explanation is that what is seen anatomically as two axons peripherally is, in fact, branches of the same motoneuron which have split immediately after leaving the ganglion. This splitting is a common occurrence in axons as they near the muscle. Since the nerve is very short and the axons are of the same size, if they belong to the same motoneuron they may be recorded as one spike when the cell fired since the distance

traveled is not sufficient for differences in conduction velocity to register as two spikes. The splitting of the large axon to muscle 17 (fig. 12) is a good illustration of the danger of using peripheral axon counts in short nerves as indicative of the number of motoneurons centrally.

EXCITATORY JUNCTIONAL POTENTIAL DIFFERENCES:

The presence of ejps of two distinctly different amplitudes, time course and faciliation properties in response to the firing of one motoneuron has been described previously in other systems (Bittner, 1968, Atwood, Hoyle and Smyth, 1965, Wiersma, 1951). The ejp differences are attributed to differentiation in the nerve terminals rather than differences in the characteristic of the muscle membrane, e.g., the input resistance, properities of transmitter quanta, etc. (Bittner, 1968). Bittner was able to show, by recording the tension produced by a single muscle fiber, that equal depolarization of the two types of muscle fibers produced equal amounts of tension. The size of the ejp was shown to depend on the frequency of firing. As the firing frequency increased, the smaller ejp shows a greater facilitation and hence a greater tension is produced by the muscle. By this frequency control, one motoneuron is able to grade tension over a larger range than with a single type of ejp. In Callinectes, there are two distinctly different classes of ejps in separate muscle fibers in the firing of a single motoneuron (see figs. 4 & 5). It is possible to see, even at the limited frequency range in these experiments, that there is more facilitation in the smaller ejp than in the larger. One would expect, under conditions evoking high frequency discharge, that the smaller ejp would show a far greater increase in amplitude by facilitation than would be seen in the large ejp. By this means, it is possible by a peripheral mechanism at the level of the muscle to achieve, with frequency modulation, a greater tension range with use of a single motoneuron.

VISUAL CONTROL OF EYESTALK ROTATION:

The action of the eyestalk muscles is a forward and outward (or downward and outward in the case of muscle 17) extension and rotation of the eyecups. Optic input to the eyestalk muscles could serve two visual functions; to rotate the eyestalks in following movements in the pitch axis and to provide a stable base for the complex movements of the eyecup muscles in any axis. The response of muscle 15 to the variety of visual inputs (fig. 7) may indicate that the eyestalk is functioning as a stabilizer for the action of the nine eyecup muscles. The fact that visual input alone, when the statocyst input is cut, is not sufficient to cause firing of the muscle is an indication of the subsidiary function of visual control over eyestalk movements. Tonic input from the statocysts appears to be necessary to keep the motoneuron at a firing threshold and the visual input then results in an increase in the basal firing rate.

STATOCYST CONTROL OF EYESTALK MUSCLES:

The statocyst input to muscle 15 appears to have two components, a static position sense drive and a dynamic acceleration response. The static function is evidenced by a steady low firing frequency which is seen when the animal is in a resting position after all the inputs to the ganglion have been cut with the exception of those from the statocyst. If the animal is pitched forward and left in the new position, the steady firing continues but at a higher frequency, proportional to the degree of pitch (figs. 9 & 10). The dynamic response to acceleration is seen in an initial increase in firing rate which soon adapts to a maintained level. This division of position and acceleration responses is seen more clearly in the compensatory response of the eyestalk

to a step movement on the tilt table (fig. 15). The initial response is a rapid compensatory movement of the eyestalk bringing it close to the final position. A slower movement then brings the eyestalk to a final position which is maintained. The division of static and dynamic components of a compensatory response in the movement of the whole eye has been shown to be the result of activation of two types of receptors in the statocysts (Cohen and Dijkgraaf, 1960). Elimination of the thread hairs of the statocyst eliminates the response of the eye to acceleration, resulting in a compensatory movement which is composed of a slow approach to the final position. The slow compensatory response is controlled by the shorter statocyst hairs in contact with the statolith which mediate position sense.

There does not appear to be an appreciable contribution of the eyestalk in compensation for roll. This is consistent with the fusion of the middle cylinder with the eyestalks resulting in a rigid structure which does not allow movement of one eyestalk alone. Since the eyestalks appear to act as a unit, roll compensation must be carried out solely at the eyecup-eyestalk joint.

NEGATIVE FEEDBACK SYSTEMS INFLUENCING EYESTALK ROTATION:

a. Antennular basal segment system: All animals with geotatic receptor located in a moveable part of the body must be able to differentiate between stimulation of the receptor caused by movement of the whole body and that resulting from movement of the appendage in which the geotatic receptor is located. In man, this is a question of differentiating between stimulation of the vestibular system produced by movement of the whole body and that resulting from movement of the head alone. In crustaceans, the geotatic receptor is located in the basal segment of the antennule, which moves frequently. The central nervous system must have some knowledge of this movement of the antennule. This could be achieved by either efference copy or reafference. In efference copy, the central

nervous system would receive information about movement of the antennule by a signal which duplicated the motor impulses to the antennular muscles. In reafference, the feedback would consist of information from sensory receptors which signalled movement after it had been initiated.

An example of reafference which differentiates between body movement and movement of the appendage containing the geotatic receptor is the elastic strand found in the lobster, Panulirus argus (Schone and Schone, 1969, Schone, 1971). An elastic strand runs between the antennule and the muscle which raises the antennule so that when the antennule is lifted, the receptor is stretched. The function of the elastic strand was confirmed by recording eye movements to body and antennule movements before and after cutting the strand. The differentiation between the two movements is lost after cutting the elastic strand.

Reafference also appears to be used by Callinectes for the same differentiation. The sensory hairs which are found on the basal segment of the antennule in Callinectes feed back to the eyestalk system to null statocyst input to the eyestalk muscles. If the antennule alone moves stimulating the basal segment hairs and the statocyst, no change in the firing rate of the eyestalk muscles should be seen since the positive sign of the statocyst input and the negative sign of the antennule hairs will cancel. If the antennule hairs alone are stimulated, the negative sign of the input should result in an inhibition of the firing of the muscles. The latter is seen when recording from muscle 15 and 17 of the eyestalk. Inhibition of the tonic position sense statocyst input to the muscles is an invariable result of stimulation of the antennular hairs without concomittent movement of the statocysts.

The behavioral results of removing the antennular hairs agrees with the electrophysiological recording. After the antennular hairs are removed, the animal makes eye and periopod responses to antennular movement which could be the normal response to stimulation of the statocysts by body movement. The antennular sensory hair system thus appears to function to differentiate between stimulation of the statocyst by movement of the antennule and stimulation of the statocyst by body movement.

The response of the eyes when the animal is inverted does not appear to be related to the above differentiation mechanism. Rather, it may be the result of interference with a sensory mechanism which allows the animal to distinguish an upright from an inverted position. The statocyst is unable to signal inversion of the body because of the geometry of the receptors. However, the hairs on the under surface of the carapace above the antennular basal segment may be able to signal inversion of the body since they will be maximally stimulated by the weight of the antennular basal segment upon inversion. When the intact animal is inverted, the eyes are extended out and up, in line with the horizontal axis of the carapace. After the antennular and carapace hairs are removed and the animal is inverted, the animal extends the eyes outward and downward. This is exactly the same position to which the intact animal would extend the eyes if it were upright by 180 degrees. It appears from this that the animal is unable to tell that it is inverted by 180 degrees; it cannot tell up from down in its own body position. For this hypothesis to be so, it is necessary that the statocyst give the same response when the animal is upright or inverted. This is not impossible since the statolith position sense hairs are cemented into the statolith (Cohen, 1965) and the same hairs which are stimulated by a shearing movement in the upright position will be stimulated by the weight of the

statolith when the animal is inverted.

b. EYESTALK SENSORY HAIR SYSTEM:

The eyestalk in Callinectes rotates around the dorso-ventral axis by the action of two antagonistic pairs of muscles. In the course of this rotation, a group of sensory hairs on the eyestalk are stimulated and feed back onto the muscles as inhibition. The eyestalk muscles are under the influence of a tonic excitatory position sense input from the statocysts and the inhibition from the sensory hairs is modultaing this tonic excitatory drive. The two most obvious functions of the inhibition would be the production of compensatory eye movements or the reciprocal inhibition of antagonistic pairs of muscles. To produce compensatory eye movements, the inhibition must act upon the agonist muscle; to produce reciprocal inhibition, the inhibition must act upon the antagonist muscle.

From the experimental results it appears that the deflection of the hairs by movement of the eyestalk in either direction produces inhibition of both antagonist and agonist muscle. This appears to be paradoxical; the eyestalk should then be unable to move since its movement is inhibiting further movement. However, it is a question of the balance of excitatory and inhibitory inputs to each muscle to obtain the desired functional use of the eyestalk. It is then possible to produce both compensatory eye movements concomitantly with inhibition of the antagonist. This is achieved by the addition of an increased excitatory drive from the statocysts to one muscle. The sequency of events may be as follows. The animal is pitched forward, the statocysts send an excitatory drive to the agonist muscle to rotate the eyestalk in compensation for pitch. The eyestalk begins to rotate. This rotation deflects the sensory hairs on the eyestalk. The output of

the sensory hairs feeds back onto the agonist muscle and reduces its output thus producing the 10% compensation of the eyestalk instead of the 100% compensation seen in the legs in response to the same statocyst stimulation. At the same time, the output of the sensory hairs is being fed to the antagonist muscle as inhibition. This inhibition of the antagonist in rotation of the eyestalk may appear strange since it facilitates the action of the agonist at the same time that the action of the agonist is being limited by the same sensory receptor. However, the inhibition of the antagonist may function simply to make the movement of the agonist more efficient and smooth. As seen in fig. 9, 10 and 11, there is still some tonic excitatory drive to the antagonist muscle when the animal is in a stationary position of pitch. The reciprocal inhibition would function to decrease this excitation to the antagonist when the eyestalk began to move, making more efficient use of the agonist. If both agonist and antagonist receive equal amounts of inhibition from deflection of the sensory hairs, the increased statocyst drive to the agonist will produce the 10% compensatory movement.

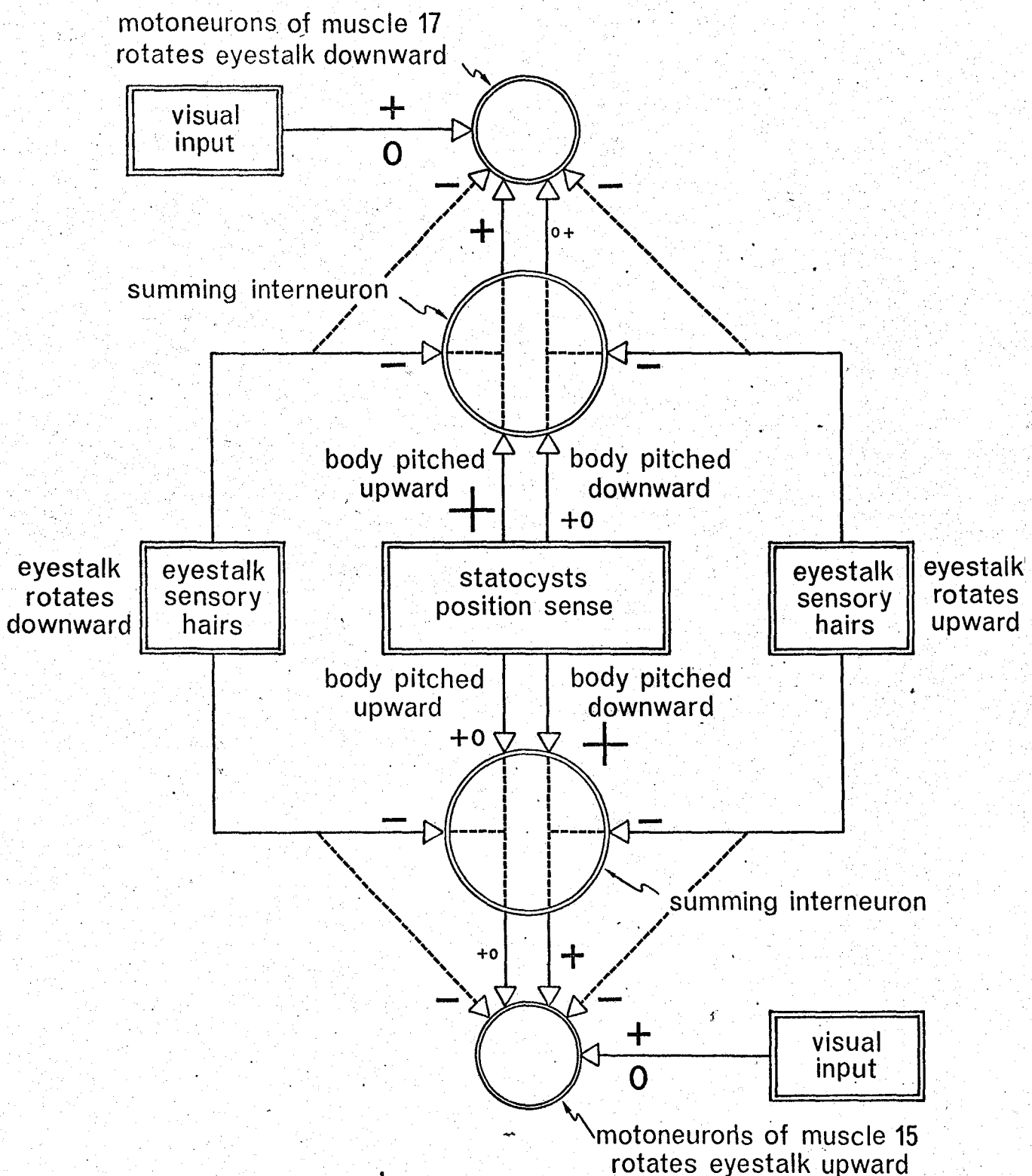
The production of compensatory eye movements by this mechanism requires only that 10% of the statocyst drive in the "on" direction escape inhibition. The inhibitory circuit limited to the eye answers the long standing question of how the same sensory receptor, the statocysts, could drive one reaction, the righting responses of the animal, to complete compensation and another reaction, eye movements, to such a small compensation. The second question asked of compensatory eye movements concerns their function. Why does the animal show incomplete compensation for pitch? This can be answered logically by considering the visual and statocyst information coming to the central nervous system about the animal's position in space. If the eyes were to compensate completely to the same degree as the body, the eyes, which can respond more quickly than the body,

would constantly be signalling information to the central nervous system that the body was in equilibrium while the statocysts would be signalling a disequilibrium. Having the eyes lag behind the body avoids conflict of visual and statocyst information as to body position.

There is an additional situation where the inhibition produced by the eyestalk sensory hairs may be used to null statocyst input. Such a situation occurs when the animal is pitched in one direction and may need to make a visually directed movement in the opposite direction. This would be the case when the animal is swimming in one direction of pitch and sees prey moving in the opposite direction of pitch. If the animal is able to make following movements with the eyes before it has changed the direction of pitch, the following movement of the eyes is opposite to the direction of pitch. Also, even in the resting position of the animal, in which the anterior end of the carapace is pitched 15 degrees above the horizontal, the visual input must overrule the tonic statocyst input in order for the animal to make a visual inspection of anything at a lower level than the eyes. Any visual guidance of the claws to prey on the sea floor must require some downward rotation of the eyestalk and this rotation is counter to the statocyst input. It is possible that the animal does not make use of the eyes for such movement but relies on the chemoreceptors on the claws and mouth parts. However, a model (fig. 20) can be devised whereby the animal is able to make visually directed movements which can both oppose the statocyst movement and escape the eyestalk inhibitory system. This can be done by inserting an interneuron between the statocyst and the motoneuron. The eyestalk inhibitory hairs feed into this interneuron. This interneuron accomplishes one purpose; it prevents the inhibitory input from the eyestalk hairs from feeding onto the

Fig. 20[1]

Model of the eyestalk feedback control system. The model can operate with or without the summing interneuron, depending upon the inclusion of the visual input. In the production of compensatory eye movements and reciprocal inhibition, the summing interneuron is not necessary and inhibition would act either directly upon the motoneuron or upon an interneuron. The interneuron is added to allow visually directed movements to escape the eyestalk sensory hair inhibition and to oppose statocyst directed movements. Plus and minus signs indicate excitation and inhibition, respectively. Scale of sign indicates the magnitude of the outputs. For further explanation see text.


motoneurons of muscle 17
rotates eyestalk downward
visual input
+
0
−
−
+
0+
summing interneuron
−
−
body pitched upward
body pitched downward
+
+0
eyestalk rotates downward
eyestalk sensory hairs
statocysts position sense
eyestalk sensory hairs
eyestalk rotates upward
body pitched upward
body pitched downward
+0
+
−
−
summing interneuron
+0
+
−
−
+
0
visual input
motoneurons of muscle 15
rotates eyestalk upward

motoneuron directly. Since the interneuron can signal only degrees of excitation, the excitatory visual input can drive the motoneuron and the inhibition from eyestalk rotation does not cancel the visual input but is felt only as a lessened excitation from statocyst drive. Since when the visual input is attempting to act antagonistically to the statocyst drive, the visually driven motoneuron is receiving less positive drive from the statocyst and will see only a decreased positivity from the interneuron, not inhibition. The opposing motoneuron which is the agonist for statocyst input will be under a decreased drive from the interneuron, due to inhibition from the eyestalk hairs and will also lack the excitatory drive from the visual input. In this way, the visual input may predominate and the eyes will be able to execute visually directed movements free from the inhibitory system and in opposition to statocyst drive.

The above oculomotor model can be tested in future experimental work by intracellular recording from the motoneurons of the eyestalk rotation system. The entire motor system appears to contain at the most seven motor neurons and perhaps as few as four. The resolution of the relationship between the motoneurons, the statocysts and the inhibitory sensory hairs may prove to have wider applications for oculomotor control systems in general. It is possible that the inhibitory control system in Callinectes may have a correlation with the inhibitory stretch reflex which feeds back onto the stretched muscle in cats (Back-y-Rita, in press). The extraocular muscles serve much as the same function for all animals and it would not be surprising to find similar control mechanisms throughout the animal world.

SUMMARY

The control system for stabilization and rotation of the eyestalk in Callinectes has been shown to involve two antagonistic pairs of muscles under visual and statocyst control. The primary sensory input to the muscles appears to be from the statocysts with both static position sense and dynamic acceleration components. The static position sense input has been shown to be proportional to the degree of pitch of the animal. Two motor axons are responsible for the tonic statocyst and visual control of the muscle. The larger of these two axons is capable of producing ejps of differing characteristics in different muscle fibers. This appears to be a peripheral mechanism for modulation of motor control by firing frequency.

Two systems of sensory hairs were found which influence eyestalk responses. One set of hairs is located on the antennular basal segment and appears to function to allow the animal to differentiate between stimulation of the statocyst by movement of the whole body and stimulation of the statocyst by movement of the antennule, in which the statocyst is lodged. The second set of sensory hairs is located on the eyestalk and is believed to function to produce both compensatory eye movements and reciprocal inhibition of antagonistic eye muscles. Both these functions are achieved by a negative input from eyestalk hairs which nulls out the tonic statocyst input. A model is proposed to allow visually directed movements to escape this inhibition and execute movements in opposition to statocyst control.

III. INTRACELLULAR RECORDING FROM THE OCULOMOTOR SYSTEM IN THE CEREBRAL GANGLION OF CALLINECTES SAPIDUS

INTRODUCTION:

An introduction has been given to the work which has been done on the decapod oculomotor system to reveal some of the basic properties of the movement capabilities and the central mechanisms which control them. None of this work, however, can provide information on the specific cellular basis for the observed events. To accomplish this, it is necessary to leave the periphery and begin intracellular recording in the cerebral ganglion where the oculomotor integration takes place. The following section is an account of work with that aim. The only published work involving intracellular recording in the oculomotor system of the decapod is from a single motoneuron mediating the withdrawal reflex (Sandeman, 1967, 1969). The withdrawal reflex is a protective movement, much like the primate blink. There are several motoneurons responsible for the reflex and the largest of these was the subject of analysis of the synaptic link between the sensory input and the motoneuron response. The analysis involves the site of integration of synaptic inputs, the spike initiating zone, and the question of mono or poly-synaptic reflexes in one cell. The intracellular recordings published consist of summed synaptic activity recorded at a site distant from the site of synaptic impingement upon the cell and do not give much indication of the potential of the preparation for intracellular analysis. The cell body of the motoneuron was not recorded from since it could not be located. Since this work was done prior to the use of procion yellow, the dye used as a cell marker was Prussian Blue and the cell soma did not fill when the axon was injected. Although the system studied was the simplest reflex movement of the eye and limited to one motoneuron in that system, it is of interest to the following

work as the first intracellular recording from the oculomotor system in a decapod preparation.

The proposed approach of the following project is contained in two questions: (1) what is the location of the components of the system and (2) what can be recorded from them? A long range plan of approach to them is as follows: Where are the somata located? What is the course of the cell processes? Are the somata electrically active? (In invertebrate nervous systems, the somata (cell bodies) are often very large and electrically inactive). If the somata are electrically inactive, what sort of electrotonic activity can be recorded from them, that is, do they show electrotonic reflection of E.P.SP., I.P.S.P.s or spike activity from other parts of the cell? What are the sources of sensory input to these cells, i.e., visual, tactile, statocyst, etc.? What is the general visual receptive field of the motoneuron particularly with respect to directional movements? What are the differences between tonic and phasic motoneurons in cell size and sensory input sources? Are the tonic and phasic systems grouped separately anatomically? There are from 25 to 20 motoneurons controlling each eye. It is not intended to extend the analysis to every cell but rather to find representive tonic and phasic motoneurons for study. All interneurons encountered will be investigated by essentially the same criteria as motoneurons. The following work is the intial step in the above project and succeeds in answering some of the above questions. The data is the first set of recordings to yield good intracellular responses from cells mediating optokinetic responses and establishes the decapod preparation as a system for central analysis of the oculomotor system on a cellular basis.

MATERIALS AND METHODS

The animal preparation and recording was the same as given under Materials and Methods, Section I. In addition, the cerebral ganglion was desheathed and transilluminated to allow individual tracts and nerve cells to be seen with the aid of a Leitz dissection microscope.

Procion yellow for cell marking was used as a near saturated solution and cells filled either by the pressure injection method, electrophoretically (Stretton and Kravitz, 1968) or by the whole nerve iontophoretic technique (Iles and Mulloney, 1971). The latter technique, as modified under Materials and Methods, Section I, was used for the differential filling of the larger (and hence usually motor) axons in the optic and oculomotor nerves. The use of the method for the differential filling of the larger axons is possible because of the faster filling of the larger axons (perhaps due to their lower cytoplasmic resistance). If the dye passage in the large axons is observed and the current discontinued when the dye reaches the central ganglion, the distal portion of the nerve containing filled axons of all sizes can be cut off leaving only the large axons which have filled for a greater distance. By this means, the course of a mass of motor fibers can be followed in the central ganglion.

RESULTS

Upon visual inspection of the transilluminated ganglion, cell groupings could be seen in close proximity to the course of the oculomotor nerve in the central ganglion. These cells were chosen as the most likely choice for the cell bodies, or somata, of the oculomotor neurons. The following procedure was then followed to determine if the cell was part of the oculomotor system and, if so, whether it was a motoneuron or interneuron. The criteria for motoneurons

was as follows: (1) if the soma shows spiking activity, either spontaneously or in response to visual stimuli, does this activity appear as an extracellular spike in the optic or oculomotor nerve. (The recordable activity of the oculomotor nerve is composed almost entirely of motor fibers and the largest spikes in the optic nerve belong to the oculomotor system. Therefore, any large spike in either nerve is likely to be oculomotor). (2) if the cell doesn't fire as above, can it be made to fire by passing current through the cell and does this activity appear in the oculomotor or optic nerve. (3) is there an antidromic spike in the cell in response to stimulation of the optic or oculomotor nerve and (4) is there a one to one high frequency following between firing of the cell and activity recorded at the muscle. This latter criteria rules out the possibility of a synapse between the central cell body and the acitivity recorded at the peripheral nerve since the synapse would not follow the high frequencies of stimulation. If the cell was of interest by these criteria, it was marked by procion yellow and its location noted on a map of the ganglion.

MOTONEURONS

Recordings from the soma of motoneurons usually showed spike activity of a low level, below 20 mv, indicating electrotonic conduction into the soma of action potentials produced in an active spike initiating zone elsewhere in the cell. It was possible to correlate these small spikes with spikes in the oculomotor nerve and, in some cases, with activity in specific muscles. In addition, it was often possible to drive the cell by passage of current through the bridge circuit and record electrotonic spikes in the soma and corresponding spiking activity from the nerve (fig. 21). By this means the general plan of anatomical organization of the ganglion was revealed. There are at least three separate

Fig. 21 Electrotonic spikes recorded from the soma of two oculomotor neurons in cluster marked ● on ganglionic map. Upper traces, extracellular recording from the oculomotor nerve; lower traces, intracellular from cell soma. Intracellular activity in upper trace is evoked by passage of current through the cell. Intracellular activity in lower trace is antidromic spike following stimulation of the oculomotor nerve. Scale 10 mv, 2 msec.

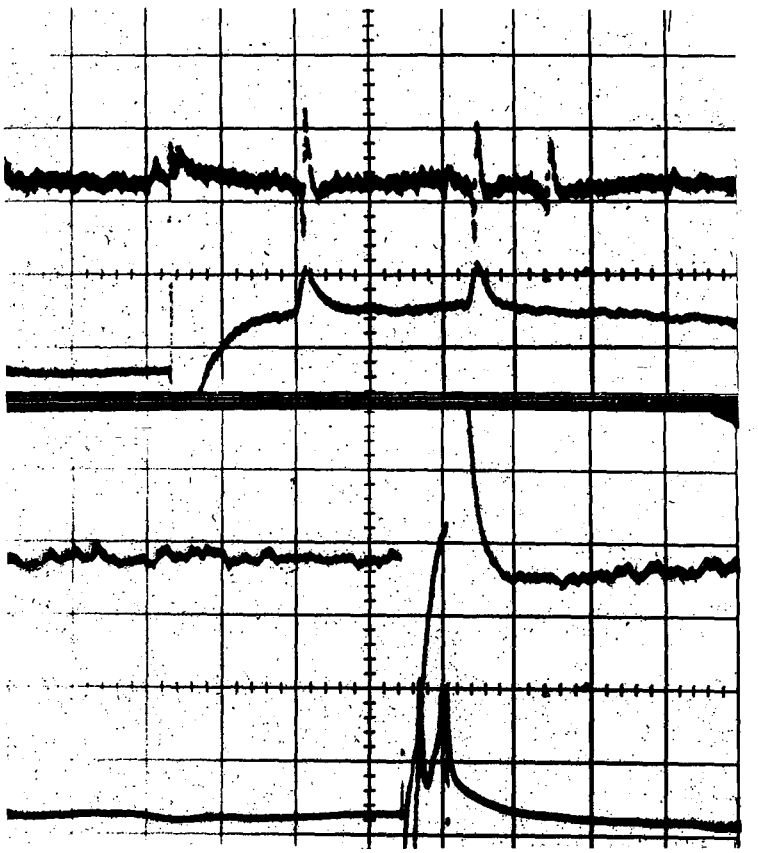
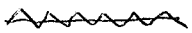


Fig. 22 Ganglionic map of oculomotor system constructed from preliminary recording in the cerebral ganglion of Callinectes.

a) motor axons in oculomotor nerve

b)  motor axons in optic nerve

c)  possible phasic oculomotor neurons

d)  tonic oculomotor neurons of optokinetic geotatic system

e)  interneurons in optokinetic geotatic system

f)  possible motoneurons for eyestalk rotation

g)  withdrawal motoneurons

i)  parabolic burster found under large withdrawal motoneurons

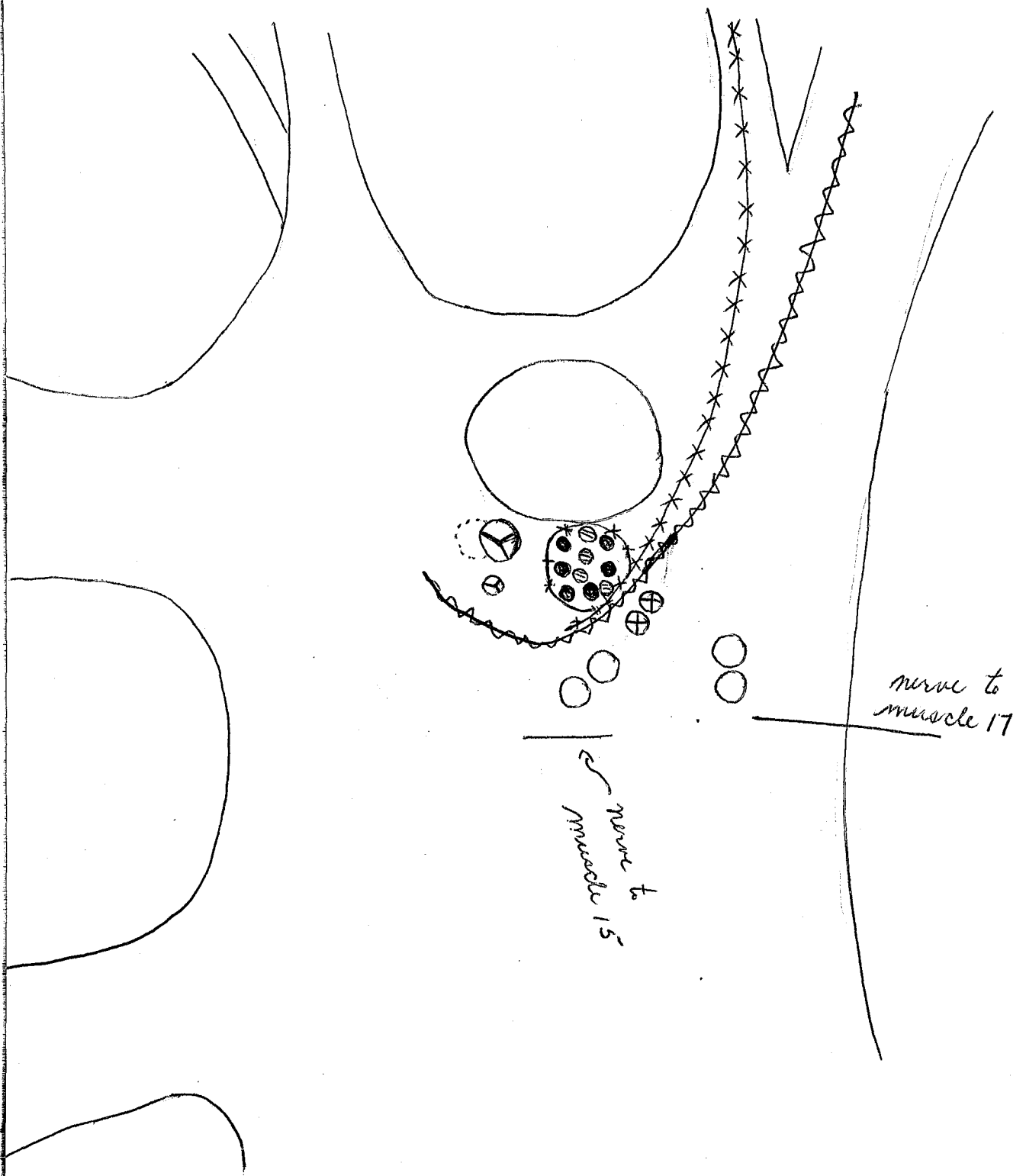

nerve to muscle 17
nerve to muscle 15

Fig. 23 Examples of recording from verified motoneurons. a. somas located in area of withdrawal motoneurons, marked ⊛ on ganglion map, lower trace is intracellular from soma, upper trace is extracellular from muscle 19a. b. soma located at ⊛ in ganglion map. Lower trace, intracellular from motoneuron soma; upper trace, extracellular from oculomotor nerve. c. soma located as in b above. Lower trace, extracellular or partial intracellular penetration from soma located near b above; upper trace, extracellular from oculomotor nerve. Scale 10 mv, 10 msec.

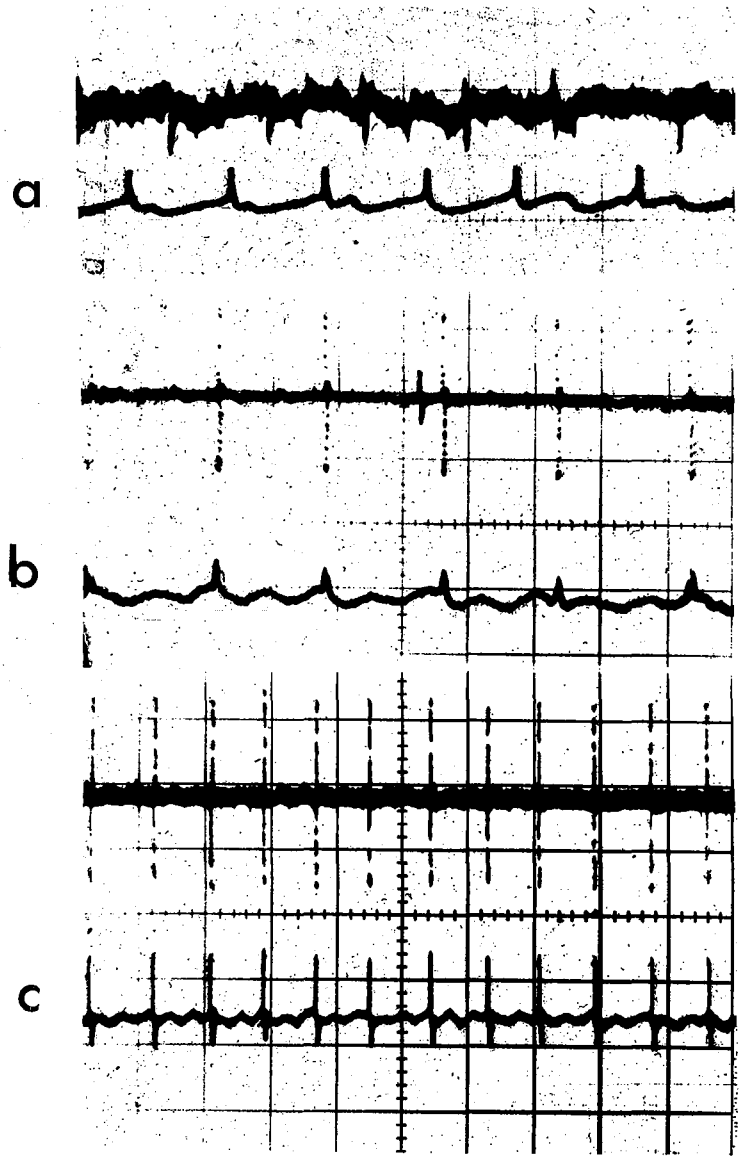

a
b
c

Fig. 24 Two cells of unknown function which burst, as shown, to a flash of light. Cells located at ⊕ on ganglionic map in area just outside the oculomotor cluster. Scale: 5 mv, 1 sec upper trace, 10 msec lower trace. The cells could be phasic oculomotor neuroms due to their location. The upper trace is probably an axon penetration (because of the large spike size) and the lower is from a known large soma.

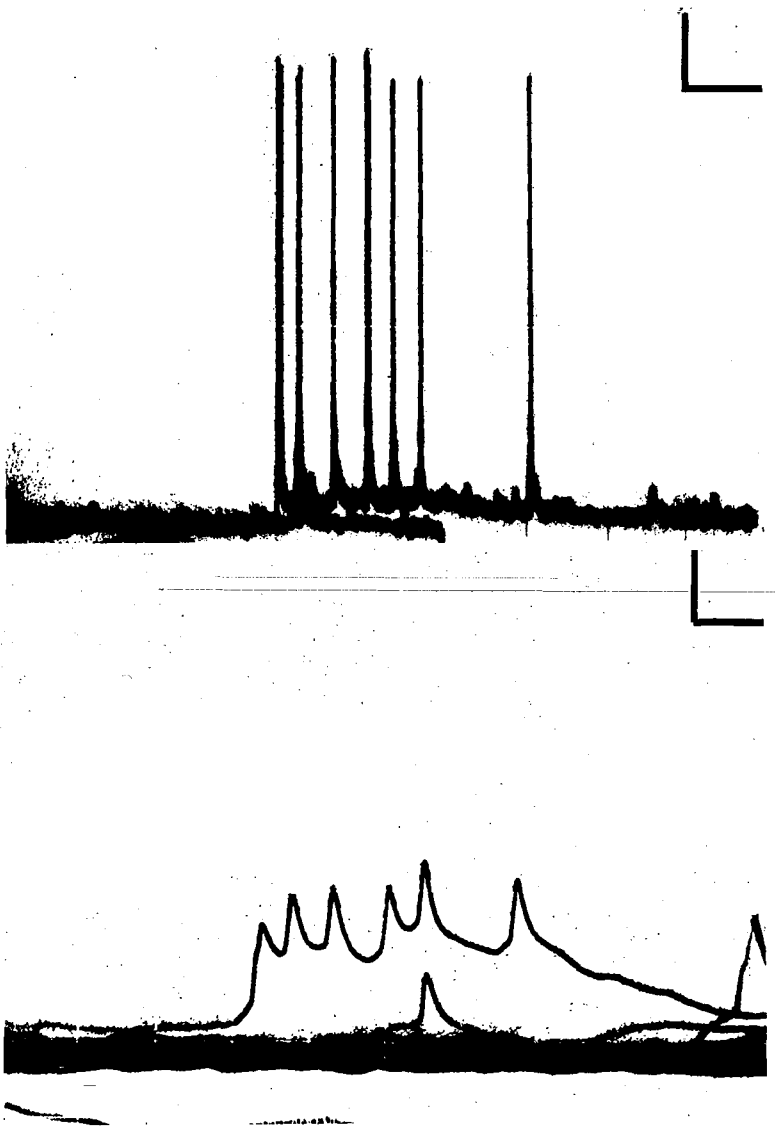


Fig. 25 Two soma recordings from the oculomotor cluster which could be tonic motoneurons. Verification was not made because extracellular channel was inoperative. Cells demonstrate prepotential foot seen in spontaneously active cells. Note activity before third and fourth spike in lower trace which, although greater than spike initiating level, fails to initiate spiking. Scales, 20 mv. upper trace, 10 mv. lower trace, 50 msec. both traces.

groupings of oculomotor neuron soma which appear to be anatomical divisions of the motoneurons according to function. The area outlined by a line and crosses in fig. 22 contains a tight cluster of medium size cell soma (30-70 microns) and associated fibers. This will be referred to as the optokinetic cluster since it contains motoneurons (marked ● on the map) which are believed to be tonic, and perhaps phasic, motoneurons of the optokinetic and geotatic system (see fig. 23 b, c). Included in this tight grouping are also interneurons (marked ⓘ on the map) which respond to stimuli of the optokinetic and geotatic system, i.e., response to movement in the visual field, stimulation of the statocysts, etc. (fig. 26-29). Below this is a loose group of somata of varying sizes, from a very large one of approximately 150 microns to smaller ones of 35 microns (marked ⊘ on the map). These are believed to be the somata of the eye withdrawal system. The cell of fig. 23 a comes from this area and is correlated with tonic activity in muscle 19a in the eyecup. Muscle 19 a is the major controller of eyecup withdrawal. The largest soma in this group is electrically silent and, on the basis of procion yellow injections, is believed to be the largest withdrawal motoneuron whose processes were the subject of intracellular recording by Sandeman (Sandeman, 1967, 1969). The procion yellow injection was seldom useful for more than marking cell bodies since the dye did not diffuse well into the processes but in the case of this large cell, it was possible to trace an axon leading to the optic tract where the large cell of the withdrawal motoneuron exits the ganglion.

The motoneurons responsible for rotation of the eyestalk exit the ganglion near the middle in the nerves to muscles 15 and 17. There are 5 or 6 large motoneurons and several small ones in this system. (Muscle 15 has been shown in the preceding section to have at most 3 large cells and 1 small one. The

supply to muscle 17 is unknown but must be about the same. On the basis of whole nerve filling of the nerve to muscle 15, it appears that the cell bodies of this muscle are the large ones which can be seen on the surface of the ganglion (marked 0 on the map).

INTERNEURONS

In the cluster of soma containing the tonic motoneurons of the optokinetic system, there were frequent recordings from cells which responded to optokinetic stimuli but which did not show spike activity going out the optic or oculomotor nerve. These cells were classified as interneurons because of the absence of correlation of their spike activity with action potentials in nerves exiting the ganglion. They were placed in the oculomotor system because of their location in the optokinetic cluster and because of their response to optokinetic stimuli. The cells varied widely in their activity pattern and very little information regarding their specific functions could be gained due to equipment limitations at the time. The records (fig. 25 to 29) are of interest because they illustrate the potential information content available from the abundant electrical activity of these cells.

The majority of the interneurons recorded from were located in the optokinetic cluster. These cells are marked on the ganglion map. There did not appear to be a separation of motor neurons and interneurons within this cluster; rather there is a tight interweaving of the cells into a compact sphere. An electron micrograph of a section taken through this cell cluster (fig. 30) shows tightly adjoining cells with very little interposing glial tissue and dark areas which may be nonsynaptic cell junctions. Running between the cell bodies can be seen cell processes, most of which are too small to be the

Fig. 26 Intracellular records from two cells in oculomotor cluster showing multiple spike heights. Second spike on upper trace is overshoot spike. Lower trace, similar activity of lower voltage. Time course of activity indicated active conduction in both cases. Notched spike activity in lower cell may represent successive spike initiation at several trigger zones. Scales: upper trace, 5 mv, 50 msec; lower trace 2.5 mv, 5 msec.

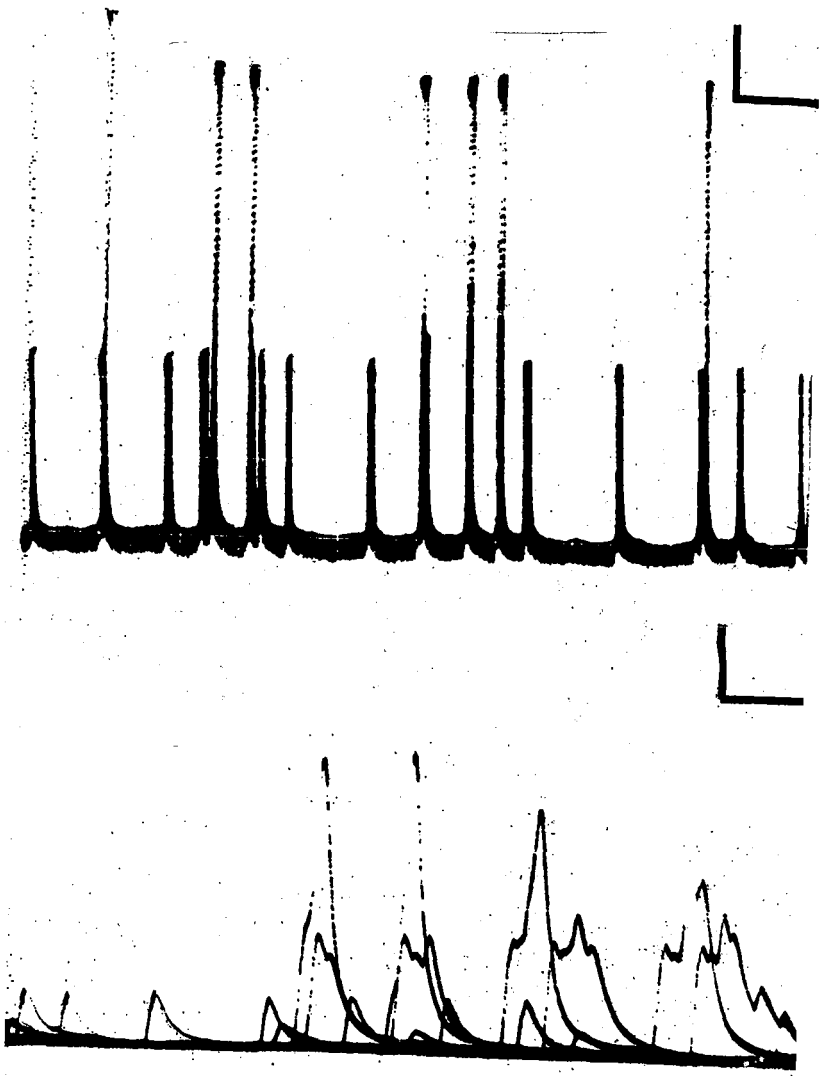


Fig. 27 An integrating interneuron found in the oculomotor cluster. Cell does not have spike activity in the motor nerves but shows synaptic input to electrical stimulation of the statocysts nerve in a, the optic nerve in b and the oculomotor nerve in c. Scale: 5 mv. 100 msec.

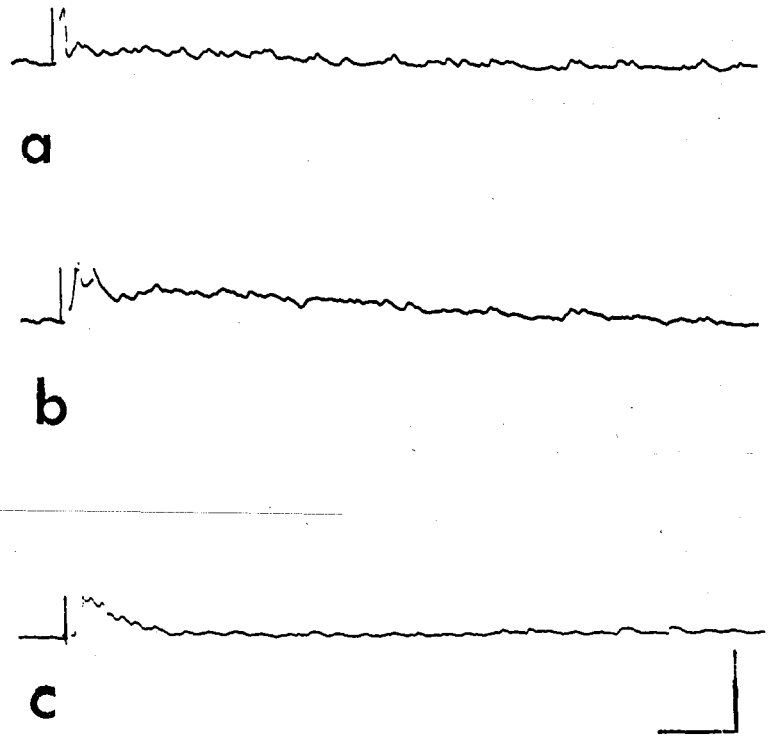

a
b
c

Fig. 28 Further example of large spiking cell with prepotential foot. This cell is judged to be an interneuron with oculomotor function, since it was found in the oculomotor cluster, marked ⓓ on the map, does not show correlation with spikes in the oculomotor nerve, upper trace, and showed an increase in firing rate to a moving stimulus. Scale: 10 mv, 50 msec.

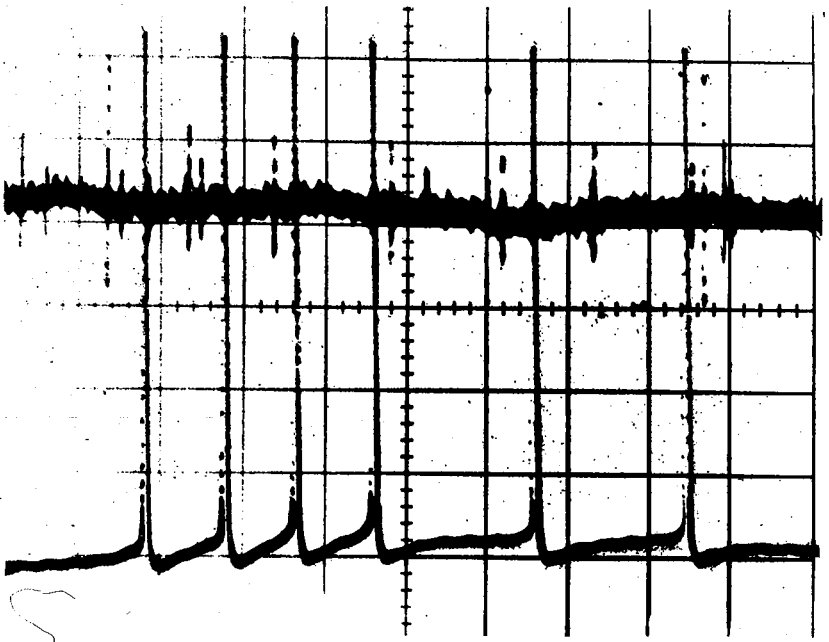


Fig. 29 Epsps and ipsps of an interneuron found in the oculomotor cluster. Upper four traces illustrate spontaneous epsps seen in cell, activity in lower traces is evoked by movement of pen light in visual field. Electrical stimulation of esophageal connective evoked spiking in the cell (not shown). Scales, 2.5 mv, 20 msec.

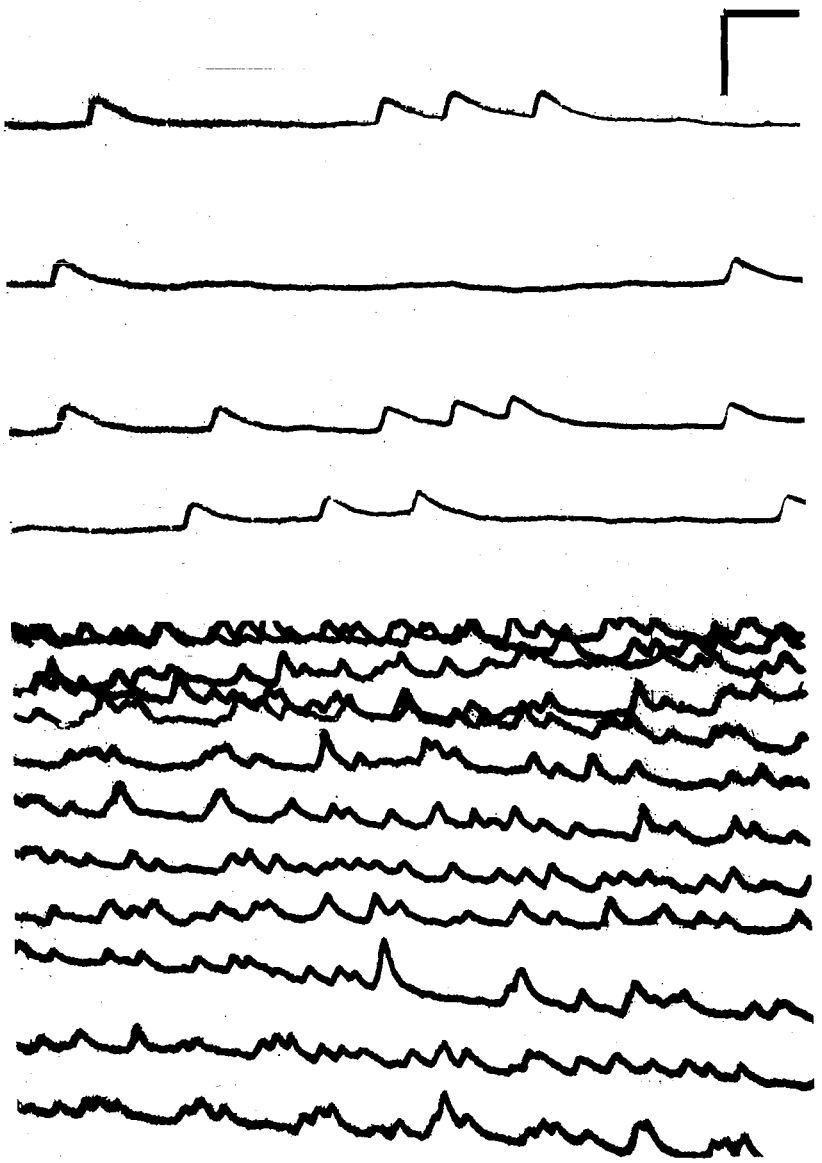


Fig. 30 Electron micrograph taken from the optokinetic cluster. Note:

a) sparce glial investment of cells and resultant tight adhesion of soma.

b) interdigitating small cell processes.
mag. 4 5,000 X, photo - courtesty of
Dr. Daniel Friend.

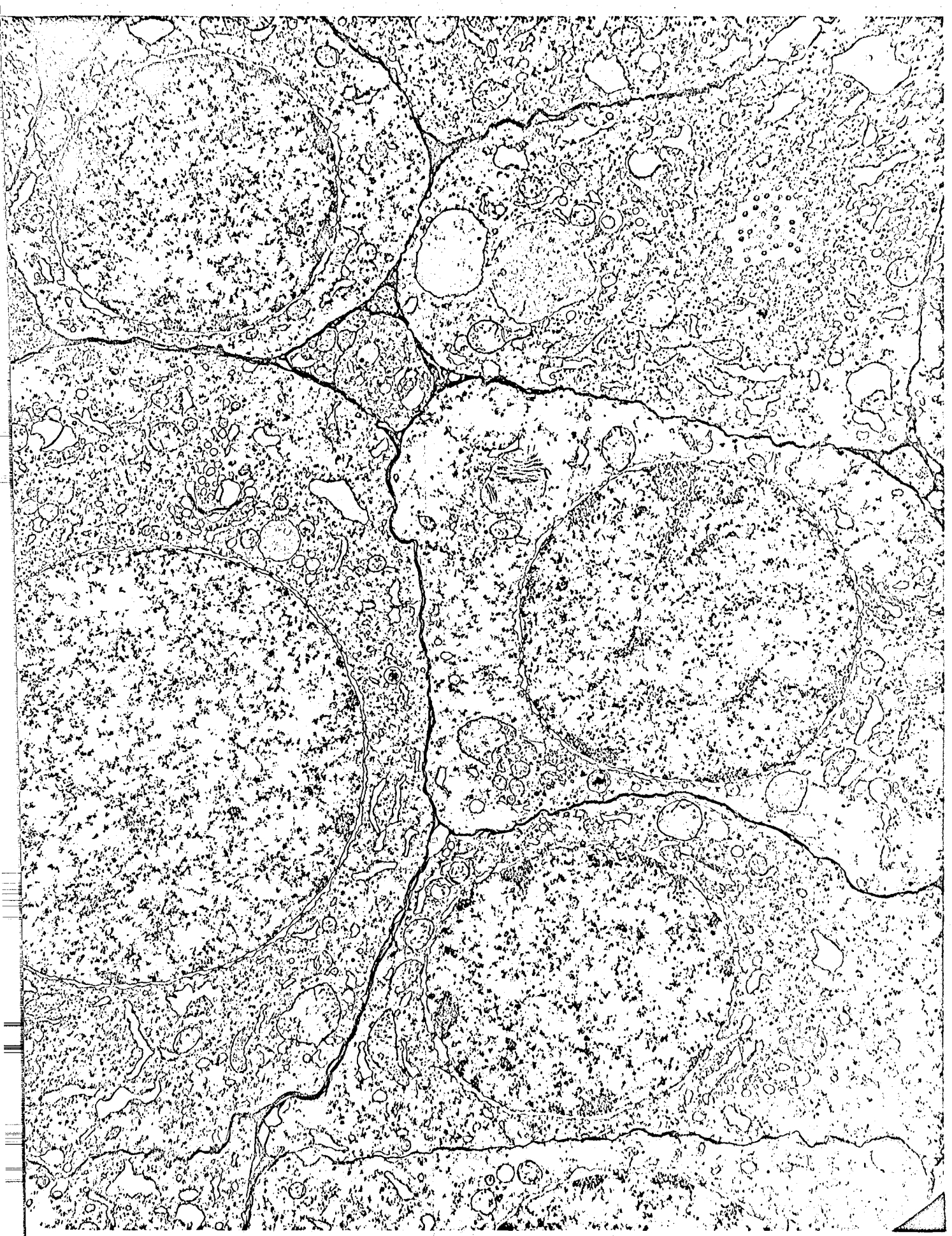
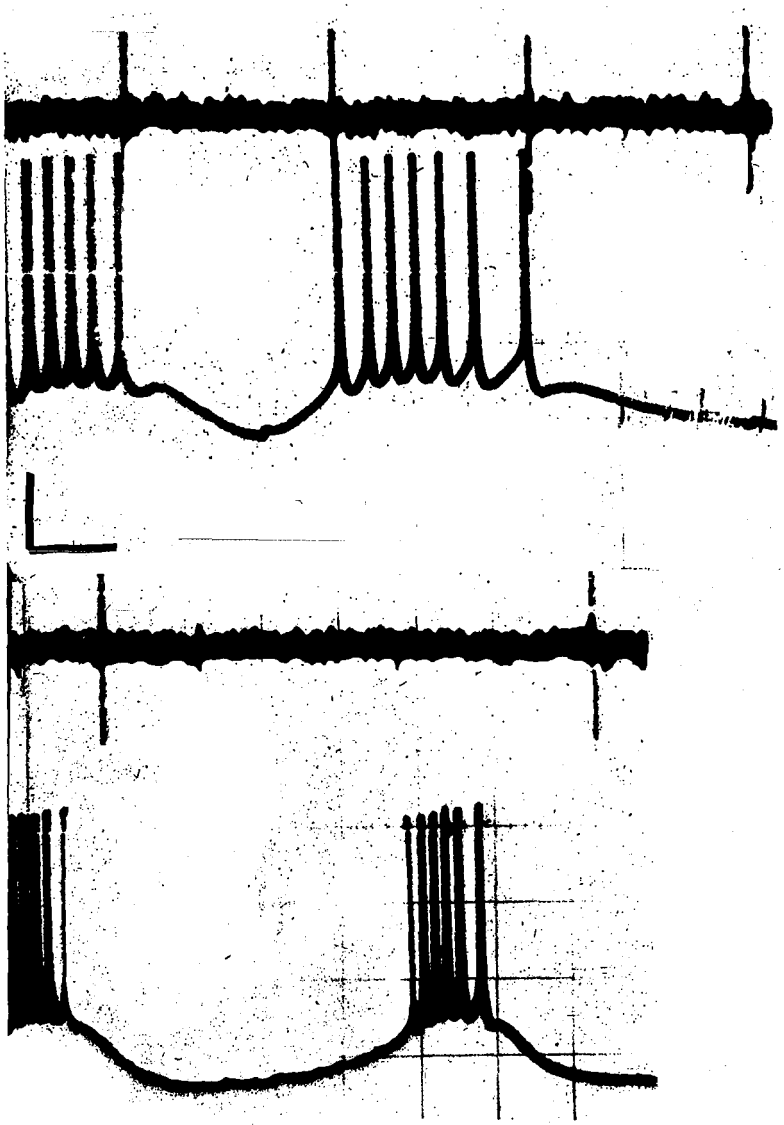


Fig. 31 Interneuron located with cells believed to be withdrawal motoneurons. The parabolic bursting form is characteristic of the cell. If bursting stopped, it could be evoked by the onset of a light near the preparation. The burst was often correlated with a spike in the oculomotor nerve, as seen in the upper trace but this was not always the case, as seen in the lower figure. Upper traces of the two records are extracellular from the oculomotor nerve, lower traces intracellular at location ( ) on ganglion map. Scales, 10 mv both traces; 100 msec upper trace, 200 msec. lower trace.



neurite-axon branch from the cell body and must represent dendritic processes involved in synaptic transmission in the cluster.

There were several points of interest to basic neurophysiology which were seen in these interneurons. In the first cell shown in fig. 26, up to five different spike sizes were seen. Three are illustrated in the upper figure, the second one being an overshoot spike. In the lower trace, on a faster time scale and at a lower amplitude, is another cell of the same type. The activity is probably electrotonic spread from another part of the cell, judging from the low amplitude response, and the notched form may represent successive spike initiation from different trigger zones.

The cell in fig. 27 is an excellent example of an integrating interneuron which is receiving inputs from several sensory systems, in this case, the visual information from the optic nerve, the geotatic from the statocysts and mechanoreceptors from the oculomotor nerve. In fig. 28 is seen another cell type which displays a prepotential foot typically seen in spontaneously active cells. The frequency of discharge could be increased by moving an optokinetic board in front of the preparation. However, even in the absence of any stimulus, this regular discharge with the prepotential foot is seen. The prepotential may represent a pacemaker potential or could be the summed synaptic input from another area of the cell, or a combination of the two. A classic record of epsps and ipsps is seen in fig. 29. The frequency of synaptic bombardment went from the low level of epsps seen in the upper trace to a dense input of both epsps and ipsps in the lower record when the cell was stimulated by a combination of a moving light and electrical stimulation of the esophageal connective. (The esophageal connective is an "alerting" input to the visual system).

The only interneuron which was encountered outside the optokinetic cluster which seemed to belong to the oculomotor system is that seen in fig. 31. This cell can be located repeatedly by advancing through the largest cell body of the withdrawal system. This form of parabolic bursting was usually seen, but if the bursting stopped, it could be reinstated by the onset or movement of a light near the preparation. It may represent some form of pacemaker for the withdrawal system (such a pacemaker has been speculated upon but not found Sandeman, 1967). The bursting pattern continued on one occasion for two hours and seemed to be correlated with a spike in the oculomotor system (see upper trace, fig. 31). Although exact correlation was not an invariable finding, an increase in the spike frequency in the oculomotor nerve appeared to accompany increased frequency of bursting. Since both tonic motoneurons of the withdrawal system and neurons of the optokinetic group are found in the oculomotor nerve, this cell could serve as an oscillator or pacemaker for either system if it is indeed linked to the spike in the oculomotor nerve.

DISCUSSION

The preceding records from the oculomotor system of Callinectes demonstrate the development of the cerebral ganglion into a preparation in which one can do intracellular recording while using the natural sensory stimuli of the oculomotor system. The aim was to have a preparation in which the location of the cellular components of the oculomotor system would be known and the relationship of the sensory units and interneurons to the motor units could be mapped on a cellular basis as has been done in several other invertebrate systems, for example, (Bentley, 1970; Otsuka, et al., 1967; Cohen and Jacklet, 1967). A substantial beginning has been made toward this end.

The technical aspects of the perfusion and dissection of the ganglion have been developed to achieve a preparation in which intracellular recording in the oculomotor system can be done while the animal is induced to make eye movements in response to movement in the environment or to tactile stimulation. A larger scale development of the work was limited by two factors. One was the equipment limitations in the early stage of the work. It is necessary to have numerous extracellular channels for investigating inputs and outputs of the cells being monitored intracellularly. The other is the need for a kymograph camera for continuous recording. After these were obtained, a limitation of the preparation appeared. The intracellular response of the animal decreased with the onset of winter. Callinectes migrates to deeper waters at this time and that may be involved in the phenomenon.

Several aspects of the oculomotor system have been established by the recording in this work. The main feature is the general anatomical organization of the oculomotor system within the ganglion. The functional division of the oculomotor system, the withdrawal system, the optokinetic-geotatic system and the system for eyestalk rotation appear to be separated anatomically within the ganglion. It is likely that each system has its own interneuronal network also. The integration necessary for the withdrawal system is quite different from that for eyestalk rotation or optokinetic-geotatic movements and though each system may receive input from the same sensory systems, the information will not be used in the same way. It is therefore unlikely that those interneurons grouped with the optokinetic group function for the other systems also.

Recording from interneurons in the oculomotor system are one of major stumbling blocks in work utilizing vertebrates. Recording from these cells in Callinectes appears excellent and is probably the main advantage of the use of the decapod for study of the oculomotor system.

In addition, the types of interneurons seen in this preparation should prove to be interesting as basic studies in neurophysiology. For example, the cells seen with multiple spike heights may prove to have dendritic spikes representing sensory inputs from different modalities, i.e., input from the statocysts, the visual and the mechanoreceptive systems. Active regenerative potentials were once thought to be found only in the axon, initial segment and soma but are now accepted phenomena in the dendritic system (Llinas, 1968). Another possibility is the presence of axonal branches of one motoneuron, a phenomena which has been seen before (Takeda and Kennedy, 1965). Alternatively, multiple spike heights have been thought to be spikes which failed to propagate past a frequency barrier caused by the narrowing of the axon (Mellon and Kennedy, 1964; Pabst and Kennedy, 1967; Sandeman, 1969). By stimulating the sensory inputs to the oculomotor system, it may be possible to link specific spikes with specific sensory inputs, thus establishing the spikes as dendritic.

The spontaneous bursting cell seen near the withdrawal somata merits study as a possible pacemaker for the spontaneous and regular withdrawal movements of the eye. It has been shown that the spontaneous firing of the large withdrawal neuron is not likely to be a property of the motoneuron and a separate pacemaker cell for this firing has been suggested (Sandeman, 1967). There are withdrawal motoneurons in the oculomotor nerve and the apparent correlation of the bursting cell and the spike in the oculomotor nerve could represent a coupling of the pacemaker cell with the withdrawal cell. The resolution of this and similar questions brought up in the course of the work on the ganglion appear to be within reach in a more comprehensive experimental approach.

SUMMARY

A preparation using the cerebral ganglion of the decapod, Callinectes sapidus, has been developed for study of central mechanisms of oculomotor control. The recordings from oculomotor neuron somata are attenuated electrotonic potentials but sufficient to establish the location of specific motorneurons within the ganglion. A variety of interneurons related to the oculomotor system have been recorded from and excellent cellular responses in the form of synaptic potentials, possible pacemaker potentials, single action potentials and cell bursting are obtainable. On the basis of the recording from motorneurons and interneurons, a preliminary anatomical map of the oculomotor system in the ganglion was begun. The preparation appears to be well suited as a model system in which to study central events in oculomotor control.

IV. COR FRONTALE

INTRODUCTION:

Auxilliary hearts are found throughout the arterial system of many invertebrates where they serve as booster pumps to maintain the blood pressure of peripheral areas of the body. An auxilliary heart, the cor frontale, is found interposed in the blood stream anterior to the cerebral system in many crustaceans. Although it was first described in the crayfish in 1916 (von Baumann) and later in other crustacea (Debaiseux, 1944; Demal, 1953), very little published work is available on the function of the cor frontale. It has been assumed by the above authors to be a mechanism for the regulation of blood flow to the cerebral ganglion. The muscles of the cor frontale in Callinectes sapidus were originally described with the eye musculature and termed the musculus oculi basalis posterior or muscle 16 (Cockran, 1935). Although muscle 16 arises from a common apodeme with the eyestalk muscles, the insertion of muscle 16 is on the carapace, rather than on the eyestalk, and they are, therefore, not functional in eye movement. The following work in Callinectes shows that muscle 16 in this animal is analogous to the cor frontale muscles of other decapods. The function of the cor frontale is redefined on the basis of data in Callinectes which suggests that the cor frontale may function, not as a heart adding propulsive force to the blood, but rather as a resistive element in the regulation of flow, acting more like an arteriole than a heart. In addition, the data suggests that the cor frontale is concerned with regulation of the blood flow to the peripheral oculomotor and visual system in the eye rather than regulation of flow to the cerebral ganglion.

MATERIALS AND METHODS

The animal preparation consisted of an isolated perfused section of the anterior carapace of the crab (see fig. 2). The dorsal artery was cannulated and perfused with a Callinectes saline (Perkins and Wright, 1969) buffered with Tes (Sigma Chemical Co.) adjusted to pH. 7.5. The "normal" perfusion pressure was that which had been previously determined to be optimal for intracellular recording in the cerebral ganglion. This pressure was maintained by a constant height of the perfusion bottle above the preparation. Alterations in the perfusion pressure could be made by changing the height of the bottle or by means of a valve in the supply tube. All electrophysiological recording was done with glass suction electrodes on the cor frontale muscle with the thin transparent wall of the sinus intervening between muscle and electrode. This was necessary to avoid dissection of the sinus which would have interfered with maintainance of the perfusion pressure.

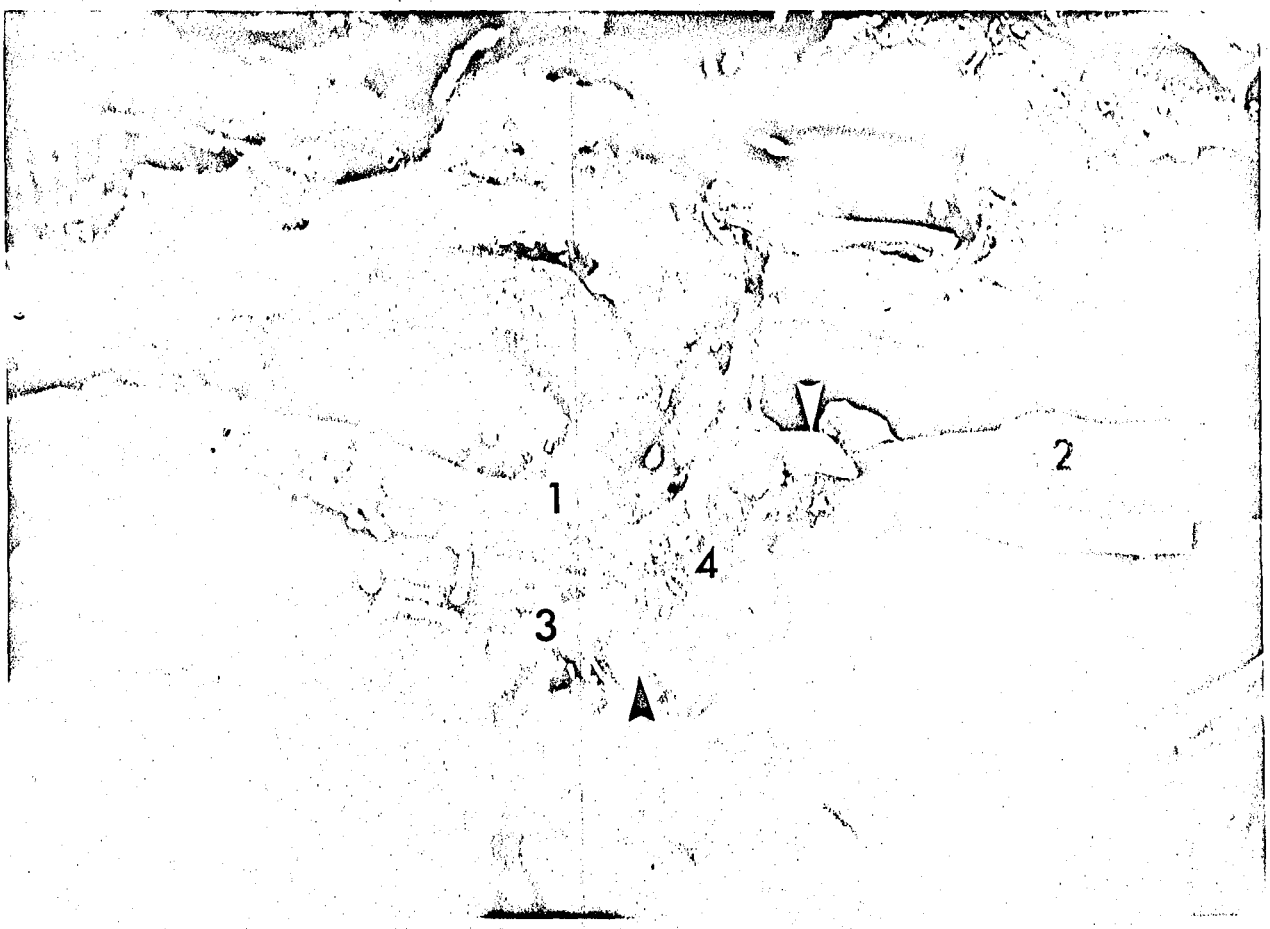
For electron microscopy, the muscle was clamped at rest length or in the contracted state and fixed in 2% paraformaldehyde 3.5% glutaraldehyde with 0.1 M Na phosphate buffer at pH 7.4.

RESULTS

ANATOMY:

The blood supply to the cerebral system is carried anteriorly from the main heart by the dorsal artery. This artery divides anteriorly into 2 main systems, the cor frontale sinus, from which the blood supply to the cerebral ganglion arises, and the two ophthalmic arteries, one to the right and one to the left side (fig.32). The cor frontale is a large dilation with the cerebral ganglion artery exiting from the floor by either one or two short stocky vessels. The

Fig. 32 Photograph of the cor frontale of Callinectes sapidus. (1) ophthalmic arteries (2) dorsal artery (3) cerebral artery (4) cor frontale sinus. The small arrows indicate the areas of insertion of the cor frontale muscle through the sinus. The majority of the muscle is located, when contracted, at the lower arrow. The tissue at the upper arrow is a tendonous extension of the cor frontale muscle. The system has been injected with liquid latex through the dorsal artery for visualization.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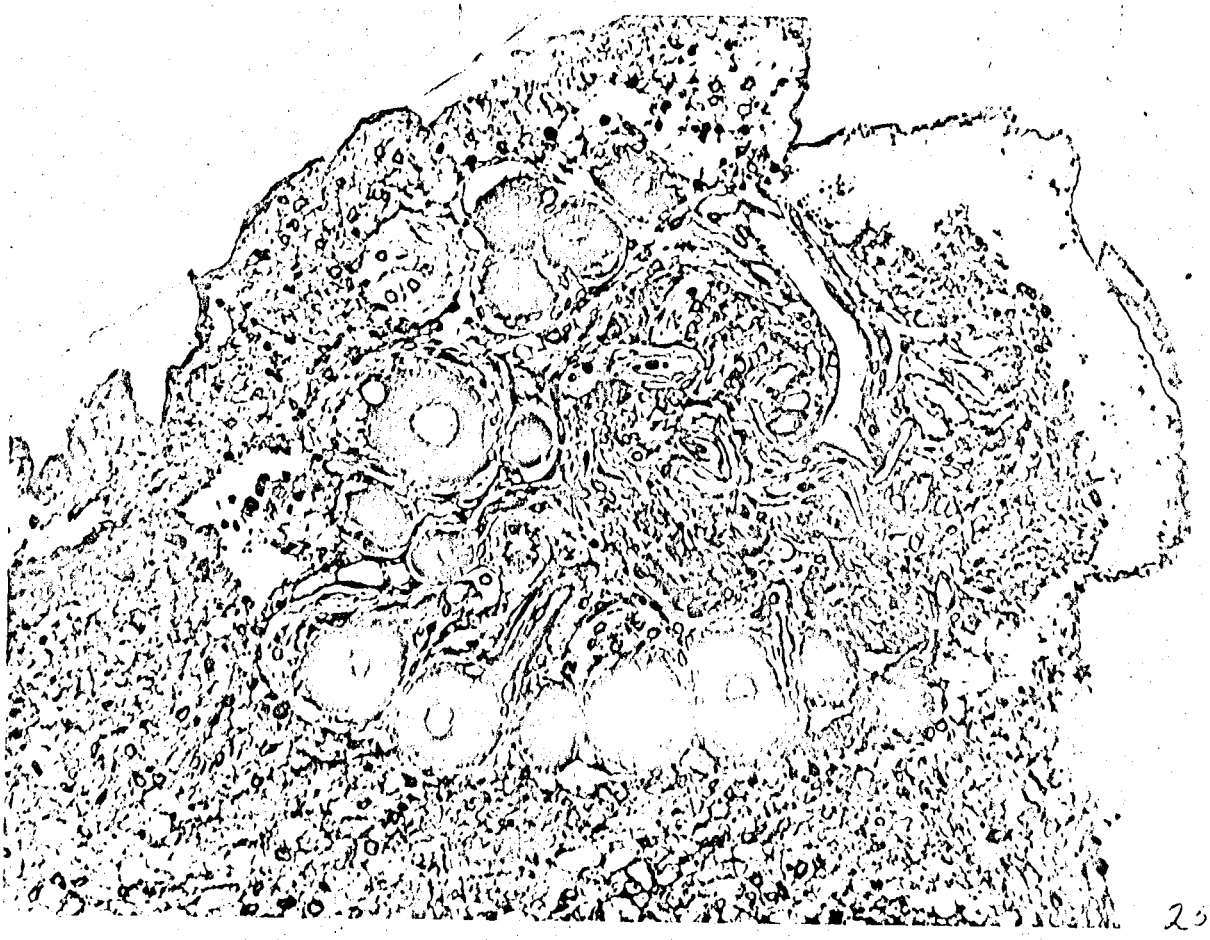
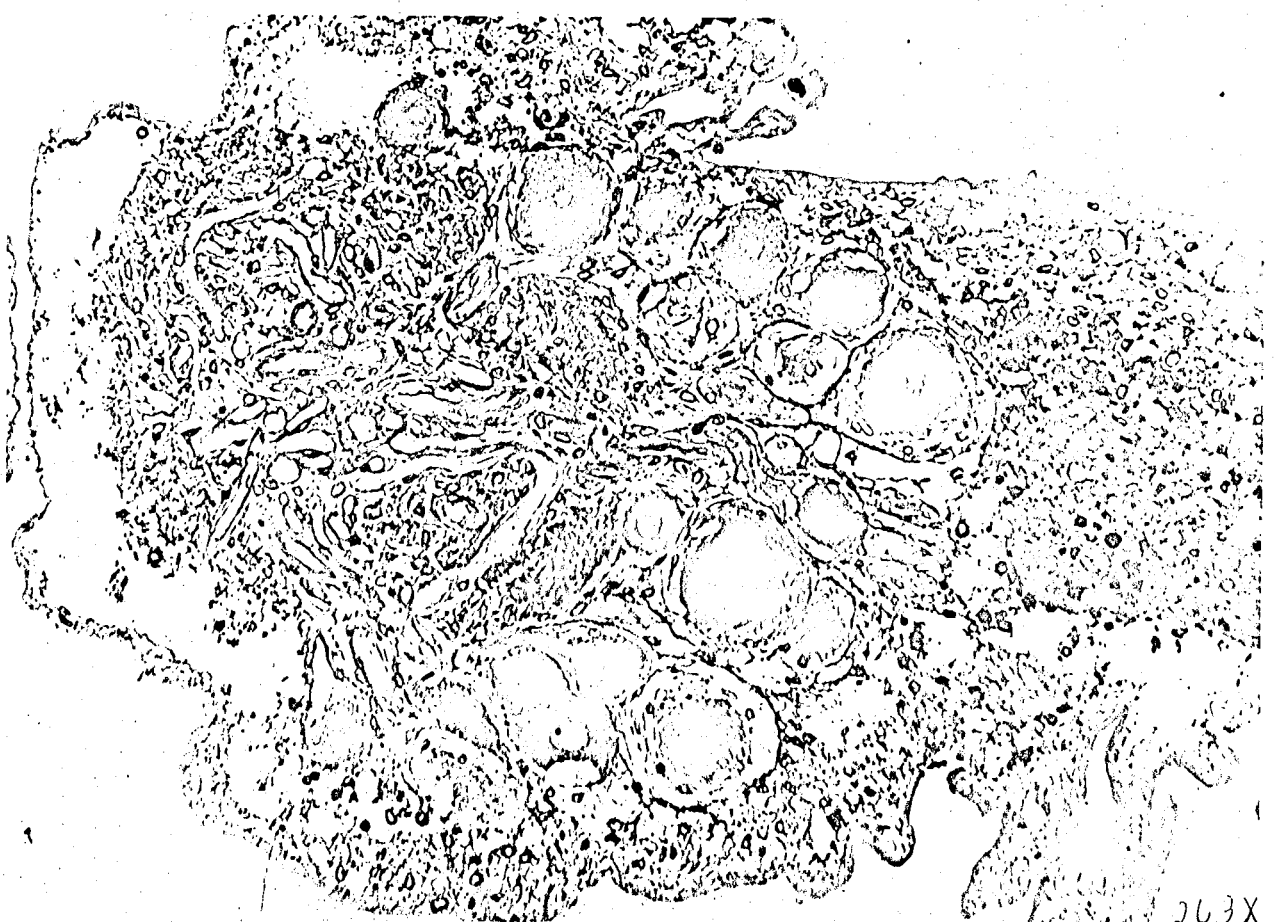
ophthalmic arteries are of approximately twice the diameter and ten times the length of the cerebral artery since they must extend to the eyes in the periphery where they supply the neural and muscular systems of the eyecup. There are four levels of neural integration in the visual system immediately behind the ommitidia, in addition to the nine pairs of ocular muscles, and blocking of blood flow to this area has been observed to result in cessation of visual response, even when the cells in the cerebral ganglion are still responsive to other modes of stimulation. (Responsiveness in the cerebral ganglion likewise does not survive the cessation of blood supply). The cor frontale is situated in such a position that it is able to control diversion of blood flow either through the cor frontale to the cerebral artery or to the ophthalmic arteries out to the eyes.

In Callinectes, the cor frontale consists of two thin muscle strips and a small ganglion and nerve running through a thin walled sinus (see fig. 1). The ventricular ganglion (terminology, von Baumann, 1916) arises between the two cor frontale muscle strips in the course of a nerve which arises from a branch from the right and left circumesophageal ganglion. The ganglion is from 0.5 to 0.75 millimeters in width and contains a maximum of 18 large neurons, the largest being around 90 microns, arranged around a central neuropile. (fig. 33). Several ganglia have been fixed, embedded in epon and sectioned for cell counts with the light microscope. The cell counts have varied with 18 being the maximum number seen in a preparation in which the alignment of the sectioning was optimal and the count therefore most reliable. The small size and the homogeneity of the cor frontale muscle make it unlikely that there are more than a few motorneurons for these muscles among the 18 cells in the ganglion. The remaining cells may be sensory neurons or interneurons of the cor frontale system or cell bodies

Fig. 33 Sections through the ventricular ganglion: note the large size of the somata, cell processes and neurites seen extending from the somata into the central neuropile. The large size of the processes in the central neuropile will facilitate intracellular recording here. Sections embedded in epon and stained with Toluidine blue. Magnification scale under figures.



256X

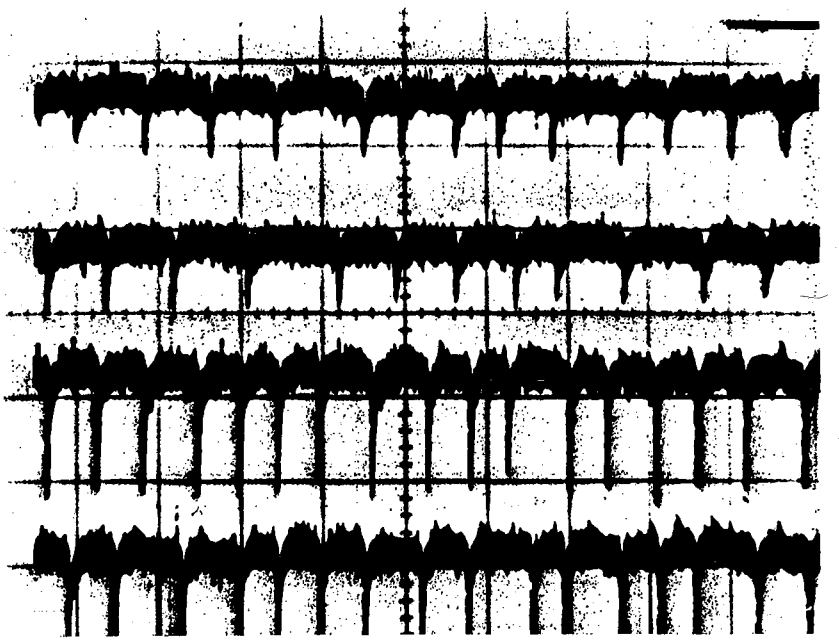


263X

whose processes continue along the dorsal nerve to the gastric muscles. Each muscle of the cor frontale receives a branch from the ganglion after which the main nerve curves dorsally to follow the median artery back to the region of the main heart. Branches can be traced to the visceral muscles but, due to the fineness of the branching, it could not be ascertained whether the branches extend to the main heart.

The muscles of the cor frontale are, in the uncontracted state, a pair of very compact thin strips with a glistening white appearance. At maximum contraction, the long thin strip reduces down to a third or less of the original length into a dense white bundle. This contracted state can be maintained for long periods of time. The whiteness of the muscle is usually associated with fast muscle contraction yet the contraction of the muscle appears to be slow and maintained. The electron microscopy which was done of the muscle does not give a clear picture in terms of the classical characteristics for differentiation of fast and slow fibers in crustaceans (Hoyle, 1967, Hoyle and McNeill, 1968). The sarcomere length varies from 3 to 6 microns and mitochondria are very small and sparse, characteristics which are associated with fast muscle fibers in crustacea. On the other hand, the endoplasmic reticulum in very poorly developed and irregular. Evidence of supercontraction (shortening of the sarcomeres so that the thick filaments extend through the Z band and no I band can be seen (Hoyle et al., 1965) is present in the muscle which was fixed in the contracted state. The Z band is very electron opaque and in the contracted state shows the discontinuities through which the thick filaments of adjacent sarcomeres interdigitate in supercontraction. The thick filaments appear to be in a loose hexagonal array but thin filaments rarely have any regulularity of arrangement. For this reason, it is difficult to give an exact figure for thick thin filament ratios although it would appear to be in excess of 1 to 10.

Fig. 34 Effects of decrease of perfusion pressure on the discharge of the muscles of the cor frontale. First trace shows extracellular recording from muscle when the perfusion pressure is at the optimally determined value. The perfusion is then cut off and the subsequent 3 records taken. Note facilitated muscle response with increased discharge. Scale: 100 msec.



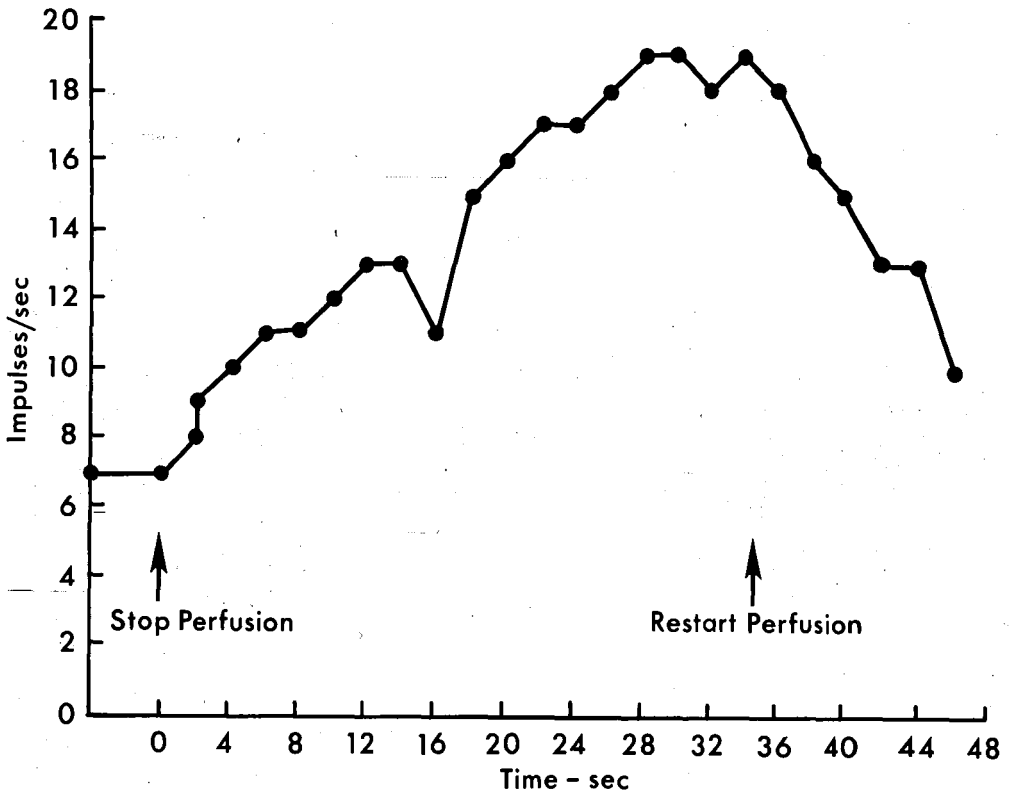
PHYSIOLOGY:

When recording extracellularly from the muscle when the perfusion pressure is "normal" a small muscle spike is recorded with an average frequency of 8 to 13 per second depending on the preparation. When the perfusion pressure is cut off by the valve in the perfusion supply tube, the frequency may rise to a maximum of 27 per second. The interspike interval is fairly constant in the "normal" state and decreases at a regular rate with decrease in perfusion (fig. 33). There is no bursting seen. The contraction of the muscle is that of a slow tonic muscle with no evidence of beating. With increase in firing rate, the muscle contracts in a slow sustained contraction down to a short bundle of 1/3 to 1/4 of the original length. Fig. 35 illustrates the response of the muscle firing rate to cessation of the perfusion. The firing rate of the muscle increases gradually with a very short latency, and rises, in this case, to a maximum of 20/sec. Activity which was recorded from nearby eyestalk muscles at the same time showed no change in firing rate (unless perfusion was shut off for several minutes in which case the activity in eyestalk muscles decreases due to deprivation of flow to the cerebral ganglion). In addition to an increase in firing rate a facilitation of the muscle activity is seen (see fig. 34). This increased muscle spike decreases gradually back to "normal" when flow is reinstated.

When the perfusion flow was not shut off but instead pressure decreased by lowering the perfusion bottle by 1 foot steps, the following changes in firing rate were recorded: at optimal height the firing rate was 96/sec; lowering the perfusion bottle one foot the rate decreased to 146/sec; lowering the bottle 2 feet reduced the firing rate to 164/sec and a final lowering of 9 inches, cutting off the flow entirely, resulted in a firing rate of 180/sec. The firing rate of the muscle thus appears to be proportioned to the perfusion pressure.

The time course of the response to cessation of perfusion is graphed in fig. 35. The absolute latency for response to cessation of perfusion could not be calculated by the methods used but is quite short, probably considerably less than one second. The response takes some time to reach the maximum value. In the case in fig. 35, the firing frequency appears to be reaching maximum around 28 - 34 seconds after cessation of perfusion. A similar slow decline of firing rate occurs after reinstatement of the perfusion.

Fig. 35 Illustration of the increase in discharge frequency of the muscles of the cor frontale with the cessation of perfusion through the cerebral system.


Impulses/sec
20
18
16
14
12
10
8
6
4
2
0
Stop Perfusion
Restart Perfusion
0
4
8
12
16
20
24
28
32
36
40
44
48
Time – sec

DISCUSSION

The major features of the anatomical and electrophysiological observations indicate that the cor frontale muscles are tonic muscles capable of slow sustained contraction. The contraction is not in the form associated with individual beats of a heart and the "heart" has never been observed to beat on any occasion. Rather, the contraction of the muscle is always a slow maintained shortening of the muscle which continues contraction down to a fraction of the original muscle length. This is consistent with the functioning of the muscle acting as a resistive element opening or narrowing the sinus at the entrance of the cerebral artery in much the same way that the muscular wall of an arteriole serves as a resistive element regulating flow through the arteriole. When the muscle contracts in response to decreased blood pressure in the cerebral system, it decreases the size of the sinus passage and increases resistance to flow to the cerebral ganglion. This is contradictory to the heart's assumed function of maintaining the pressure in the cerebral ganglion. It is possible that this may be the case; the heart is decreasing flow to the cerebral ganglion in order to divert it to the vessels supplying the optic and oculomotor system in the periphery. These vessels are of a very large diameter and must deliver blood a long distance out to the eyes where there is a large oculomotor apparatus and four levels of neural integration of the optic input which is vital to the animal. A central decrease in flow is more likely to give rise to a greater decrease in flow at the end of the long large diameter ophthalmic arteries in the periphery than it will in the short cerebral artery which is closer to the propulsive force of the main heart. The muscles of the cor frontale may then function to maintain pressure in the periphery at the expense of flow to the cerebral ganglion.

Without the input to the ganglion from the peripheral optic apparatus, the ganglion from the peripheral optic apparatus, the ganglion would be deprived of its major sensory system and maintenance of the function of this sensory input may be the highest priority in regulation of the cerebral blood flow.

The electron microscopy of the cor frontale muscles is, for the most part, consistent with a slow tonic function in crustacean muscle. There are two characteristics however, which are more typical of fast crustacean systems. These are the short sarcomere length and sparcity of mitochondria. The presence of these two features may be explained by the function of the muscle. Short sarcomeres are found not only in fast muscle fibers but also in muscles which are capable of supercontraction down to a fraction of their original length Hoyle, et al., 1965). The difference between the fast and slow fibers with short sarcomere length lies in the ratio and arrangement of thick and thin filaments. While the number of thin filaments in fast fibers is small and regular in arrangement, the thin filaments in the supercontracting slow fibers are more numerous and irregular in their arrangement. This may be a reflection of a basic relationship between the thin filaments and muscle function. While the fast muscle must perform work quickly against large resistances or in overcoming inertia, the supercontracting fibers do not perform work against great resistance. On the contrary, the supercontracting muscle of the cor frontale is closer to smooth muscle in performing a large amount of shortening with little work performed. The cor frontale muscle shortens, not against the resistance of a fixed structure such as the shell, but by stretching a tendon to which it is attached in a loose relation to the carapace. The short sarcomeres in the fast muscle may function, in conjunction with the regular thick-thin filament arrangement, to produce force quickly. The same short sarcomere length in association with a different

thick-thin filament arrangement in the supercontracting fiber may be associated with large changes in length with little work. The small work requirements may also explain the sparcity of mitochondria found in the slow supercontracting cor frontale muscles.

The sensory aspects of the cor frontale regulation of the cerebral blood pressure has not been explored in the present work. Two possibilities for sensory reception of the pressure change are stretch receptors or chemoreceptors. The short latency of response is more consistent with a stretch receptor in which decrease in pressure is signalled by an increase in firing rate of the receptor. However, extremely sensitive chemoreceptors monitoring oxygen decrease or carbon dioxide increase are also possibilities. Both receptor types exist in complex mammalian blood pressure regulating systems (White, 1972). The investigation of a pressure regulating system, from the sensory to the motor components, is possible in the simple system presented by the cor frontale and further study of this system may provide answers which will be applicable, not only to crustacean blood pressure regulation, but to general principles of pressure regulating systems in higher forms.

SUMMARY

The cor frontale is a cerebral blood pressure regulating system contained in an isolated unit composed of a small ganglion, two muscles and pressure transducing receptors. The function of this unit appears to be as a resistive element controlling flow in the manner of a vertebrate arteriole rather than as a heart. The system appears to function to protect not the cerbral ganglion but the peripheral neural and muscular components of the visual system.

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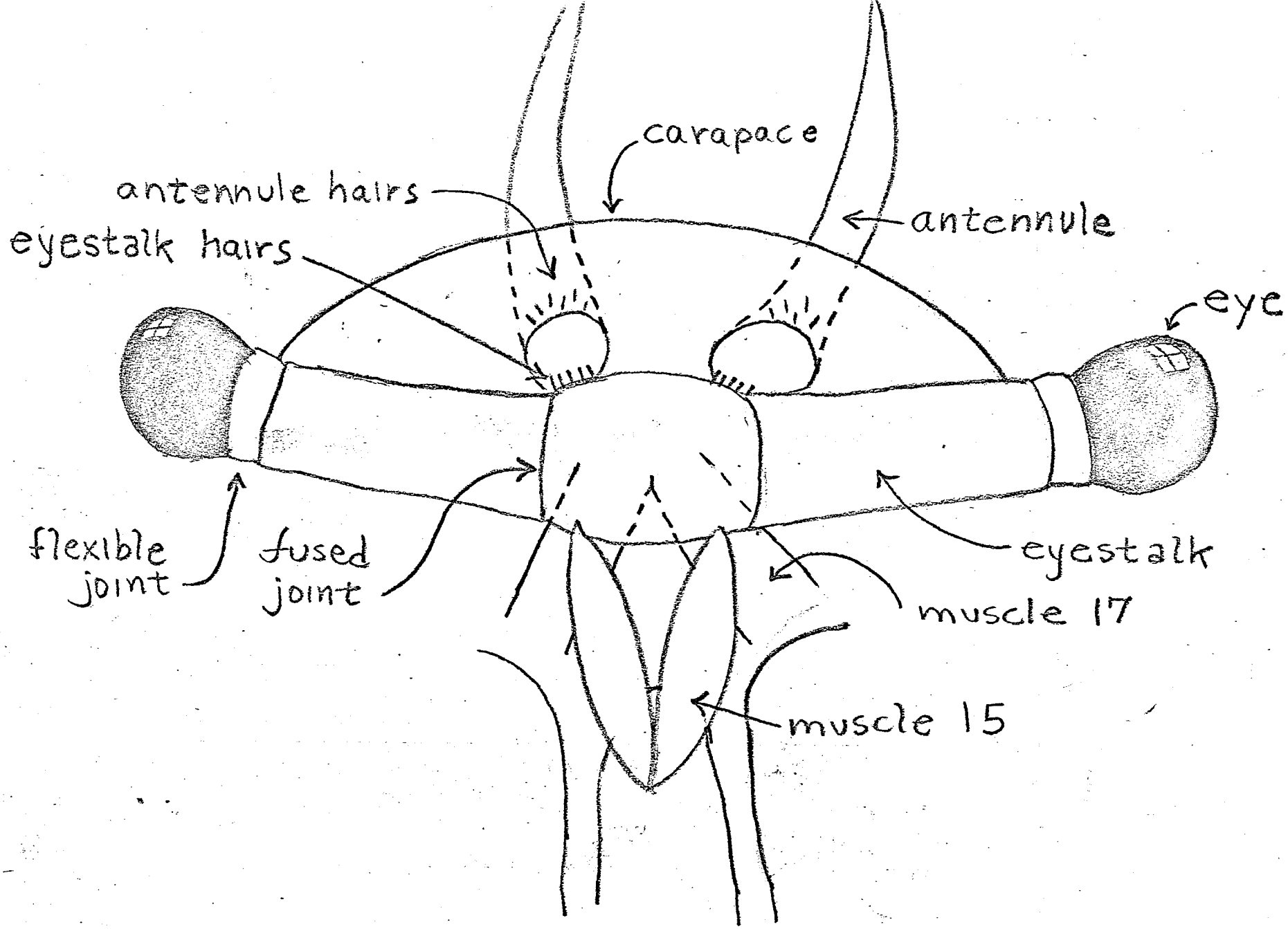
APPENDIX: LIST OF FIGURES

1. Schematic overview of the oculomotor and antennular system

2. Electron microscopy of the cor frontale muscles, longitudinal section 13, 200 X

3. Electron microscopy of the cor frontale muscles, transverse section 16,500 X

Schematic overview of the oculomotor and antennular system


carapace
antennule hairs
antennule
eyestalk hairs
eye
flexible joint
fused joint
eyestalk
muscle 17
muscle 15

Electron microscopy of the cor frontale muscles, longitudinal section 13,200 X

Electron microscopy of the cor frontale muscles, transverse section 16,500 X