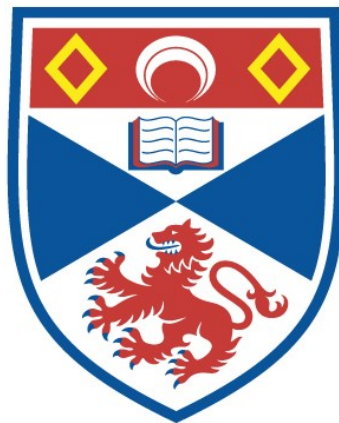


EYECUP MUSCLE ACTION OF THE CRAB CARCINUS
MAENAS

Malcolm Burrows

A Thesis Submitted for the Degree of PhD
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Eyecup muscle action of the crab *Carcinus maenas*.

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Thesis submitted for the degree of Doctor of Philosophy.

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I certify that Malcolm Burrows has fulfilled the conditions laid down in the regulations for a Degree of Doctor of Philosophy, under Ordinance No. 16 of the University Court of the University of St. Andrews and that he has accordingly qualified to submit this Thesis for the Degree of Doctor of Philosophy.

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S U M M A R Y

The muscular control of eyecup movements in the crab Carcinus maenas has been studied by both extracellular and intracellular recording from the nine eyecup muscles.

Each muscle involved in optokinetic movements is supplied by a fast and slow axon and each consists of a histologically mixed spectrum of fibres ranging from phasic (Fibrillenstruktur) through intermediate to tonic (Felderstruktur). Muscle 19a, which only participates in the withdrawal reflex consists of phasic fibres only. In general, a fast axon preferentially innervates the phasic fibres and a slow axon the tonic ones.

During optokinetic movements the muscles are activated by a complex motor output programme, which is different, not merely the reverse for movements in opposite directions. Both tonic and phasic muscle fibres are active but the latter are only active at greater amplitudes of stimulus movement. Tonic activity is responsible for maintaining the eyecup position in space and for low velocity, small amplitude movements. Phasic activity is recruited during large amplitude movements and is also responsible for fast movement and eyecup tremor.

The protective withdrawal reflex overrides any other eyecup movement and involves the firing of two axons in the optic tract, one supplying a group of two, the other a group of three muscles. One of the muscles involved in this eyecup withdrawal movement away from the mid-line is also active during horizontal optokinetic movements of the eyecup towards the mid-line. It is suggested that interpretation of eyecup muscle activity is more intelligible if the whole group of muscles, rather than the individual muscles themselves, is regarded as the functional unit.

I N T R O D U C T I O N

Several types of large eyecup movements are recognized in decapod crustaceans; following, in response to a movement within the visual field; compensatory, when the animal is tilted or rotated; protective withdrawal, when touched near the eyecup, and movements of central origin during walking. There is also a class of small eyecup movements, tremor, scanning, flicks and drift.

a) Compensatory movements.

A crab tilted about its pitch or roll axis compensates by movements of the eyecups, tending to stabilize them relative to the environment (Clarke 1896, Bethe 1897a). The eyecups partially maintain their position when the carapace is tilted through 100° in the pitch plane but only over 60° of tilt in the roll plane. The compensation is never complete but over a central range of 20° the change in eyecup angle is less than a tenth of the change in body angle (Horridge, 1966d). Similarly if the crab is rotated in a horizontal plane then the eyecups will move in the opposite direction, so tending to stabilize the visual field. If the rotation is continued, nystagmic movements occur, with the fast phase in the same direction as the rotation and the slow

phase in the opposite. Cessation of rotation may result in an "after nystagmus" (Dijkgraaf 1955). Bethe (1897b) observed that cutting the optic tract had no effect on these movements, whereas cutting the oculomotor nerve abolished them, except for some slight compensation during pitch tilt. This is almost certainly due to the rotation of the median plate by muscles not innervated by the oculomotor nerve. Although Bethe observed that these movements occur in a blinded animal, the sense organ responsible was in doubt at that time. Some believed (e.g. Hensen 1863) that the statocyst was a purely auditory organ, others that it was both auditory and static in function (e.g. Bethe 1897) while others (e.g. Kreidl 1893, Clark 1896) believed it to be purely static in function. Prentiss (1901) showed that the statocyst does not function as a true auditory organ, and is primarily an organ for maintaining equilibrium. Although statoliths were known in some decapod statocysts (e.g. Farre 1843) it was not until 1956 that Dijkgraaf (1956a) showed their presence in crabs. He showed (1955, 1956a & b) that the statocyst was both a static and dynamic detector, the thread hairs of which were responsible for detecting acceleration and the hook hairs position. Elimination of these hairs abolished eyecup movements produced respectively by rotation and by tilt. However, some compensatory movements can be

produced in animals which are both blind and without statocysts. These Dijkgraaf guesses are brought about by coxal proprioceptors stimulated by rotation of the body relative to the legs.

b) Byecup movements during walking.

The eyecup movements produced when a crab actively turns about a vertical axis are not affected by blinding, or by removal of the statocysts. Before moving in a particular direction the crab first swings its eyes in that direction and then swings them back and forth during movement. According to Bethe these movements do not have slow and fast components but Dijkgraaf has observed these. Dijkgraaf suggests that these movements are not elicited by sensory stimuli, but that they are primarily started from within the central nervous system in close co-ordination with the turning movements of the legs. These eye movements thus closely parallel those of a dogfish during swimming (Harris 1965) and those of some birds during walking (Dunlap & Mowrer 1931, Whiteside 1967). In all, compensation for body movements is not complete but in different degrees in each serves to stabilise the eyes on objects at visually important distances.

c) Protective Withdrawal of the eyecup.

Protective withdrawal of the eyecup was noted in 1860 by MacIntosh. "By a beautiful provision, the most delicate

part - the combined corneae - is almost wholly turned in towards the hard shell, when the eye is retracted; a row of hairs (sometimes absent), in addition, protects the little exposed portion, thus completely shielding it from external injury, while still admitting of useful vision. The force with which the eye is retained, when withdrawn, is very great, and it would seem that atmospheric pressure as well as muscular tension combined to keep it". Bethe (1897a) described a unilateral reflex withdrawal of the eyecup. This could most easily be elicited by mechanical stimulation of an area of carapace supplied by the tegumentary nerve. The areas of carapace innervated by the two tegumentary nerves are bounded posteriorly by the cervical grooves (terminology of Snodgrass 1952) and overlap slightly in the mid-line. Light stimulation of this area on one side causes withdrawal of the 1st antenna and eyecup of that side, while progressively stronger stimulation causes withdrawal of the 2nd antenna as well. Withdrawal of both eyecups is obtained by stimulation of the narrow area of overlap in the centre. Stimulation of any of the other brain nerves also results in a withdrawal movement of the eyecup on the side stimulated. There is no effect on the contralateral eyecup.

Cutting the optic tract was thought by Bethe (1897b) to have no effect on the withdrawal although he had shown

that motor axons were present. Sandeman (1964b) showed that section of the optic tract abolished the reflex but that section of the oculomotor nerve had little effect.

d) Following movements of the eyecup.

Of all eye movements, the following movements, induced by movement in the visual field, have been most extensively studied. Optomotor responses, as an indication that the movement has been detected, have been used in experiments on the perception of colour (e.g. von Buddenbrock & Friedrich 1933), acuity (e.g. Hecht & Wolf 1929), movement perception (e.g. Hassenstein 1951) and in memory experiments (Horridge & Shephard 1966).

The stimulus used in most experiments has been a rotating vertically striped drum. The responses produced are large and obvious and these factors tend to outweigh some of the serious disadvantages inherent in striped patterns on rotating drums. Drums can only move in one plane and cannot be switched instantaneously from one position to another. For precise experiments they are difficult to construct without subharmonics which can arise from faults in the pattern or uneven illumination. They have upper and lower edges which could produce spurious edge effects (Palka 1965) and centres which cannot be aligned with both eyes. They also stimulate

all exposed parts of the eye simultaneously, when many eyes are known to have different properties in different areas. Small lights as stimuli avoid these difficulties but the following movements obtained are small (Horridge 1966c). In my experiments striped drum stimuli have been the most useful because the responses produced are large.

Two types of responses of the animal to constant rotation of the drum can be distinguished; optomotor and optokinetic responses. In the former the animal moves its whole body continuously in the same or opposite direction as the movement. Optokinetic responses involve movement of only the eye, if movable, or the head, and are characterised by nystagmic movements. These consist of two phases; a slow forward phase in which the eyecup slowly follows the movement of the drum with an ever increasing lag, and a fast phase which returns the eyecup approximately to its original position. Bethe (1897b) has shown that section of the oculomotor nerve abolishes the following movements and this is confirmed by Sandeman (1964b). Unilateral section of the optic tract does not seriously affect these movements. Thus the principal motor axons mediating the following and compensatory movements run in the oculomotor nerve while those for the withdrawal are in the optic tract.

Electrophysiological studies

Patterns of motor impulses have been recorded in the oculomotor nerve of the crab Goniopsis (Waterman & Wiersma 1963) correlated with nystagmic movements of the eyecup. In Carcinus (Horridge & Sandeman 1964) two types of motor unit were found. One showed a progressive increase in frequency during the slow phase of nystagmus while the other gave a high frequency burst during the fast phase.

Afferent visual responses have also been studied by single unit recording from the optic tracts of a number of decapods (Waterman & Wiersma 1963) but more especially from the crab Podophthalmus (Waterman, Wiersma & Bush 1964) and in the crayfish (Wiersma 1966, Wiersma & Yamaguchi 1966). Several classes of unit are recognized. Sustaining units which alter their firing frequencies to changes in the intensity of illumination. For any given short period of time the firing of these units is constant but always decreases with length of exposure and may decay into a state where fluctuations occur. The units also respond to moving shadows and below a certain stimulus intensity may behave as "on-units". Other units respond solely to transient stimulation and thus may be considered true "on", "off" or "on-off" units. Movement sensitive units have

proved more difficult to analyse because of their erratic responses to repetition of the same stimulus. In crabs three types of movement sensitive units are recognized;

- 1) Units which give optimal responses to movements of about $0.01^{\circ}/\text{sec}$. and fire erratically to faster movements.
- 2) Units which respond to movements at about $1^{\circ}/\text{sec}$. and
- 3) Units which respond only to faster movements of $7-8^{\circ}/\text{sec}$.

A number of these units show differential sensitivity to the direction of movement and often the greater response is obtained when the target moves horizontally from lateral to medial. Still further units respond to dimming of lights and many respond to more than one type of visual stimulation, but it is a problem with all these experiments to know whether the correct testing stimulus has been used. Other interneurons have a mixed modality input responding to both visual and mechanoreceptive input.

All the units so far examined have receptive fields of greater than 10° so that their input must come from many ommatidia, but the equipment of the movement fibres in crabs seems to be more precise, and the field size presumably smaller than in the crayfish. This has a behavioural correlate in that crabs show a better optokinetic response than do crayfish.

The disorientation of the visual field during eyecup withdrawal has led to a postulated central adjustment of the

visual input (Wiersma & Bush 1963). Similar suppression of the visual input during rapid eye movements is known in vertebrates (e.g. Dodge 1900).

The eye movement control system

Awareness of the direction of the visual stimulus is indicated in shrimps, rock and squat lobsters, hermit crabs and many insects, by pointing movements of the antennae at the objects introduced into the visual field. If one eye of a hermit crab is blinded and the other eye is forcibly deflected into a new position by up to 90° , then no account is taken of this displacement, and the antennal pointing is correspondingly in error in the appropriate direction (Bullock & Horridge 1965).

Schöne (1952) showed that unilateral illumination of the shrimp Palaemonetes causes the eyes to compensate by turning towards the light. If a similar stimulus is applied to an eye fixed in an abnormal position then the contralateral blinded eye will show a compensatory movement dependent on the angle of light relative to the seeing eye and not to its angle relative to the body. The response depends on which ommatidia are illuminated and there is no evidence that the maintained forced movement is detected.

The normal pattern of motor impulses occurs in the oculomotor nerve associated with nystagmic movements, regardless of whether the blinded eyecup is free, fixed,

withdrawn or even absent altogether (Horridge & Sandeman 1964). In these experiments the optokinetic response is "driven" from the contralateral seeing eye. A blinded movable eyecup will execute the slow and fast phase movements in synchrony with the seeing "driving" eyecup. However fibres in the optic tract which convey mechanoreceptive and proprioceptive information from the ipsilateral (Waterman & Wiersma 1963) and contralateral (Bush, Wiersma & Waterman 1964) eye apparatus are known, but all experiments to date have failed to reveal the utilization of any such information in eyecup movements.

The movement of the eyecup in following the visual field reduces the apparent movement of that field and this is the only feedback loop. This loop can be opened by fixing the seeing eyecup and blinding the movable one. Because the receptor, the retina, is mounted on the effector, the eyecup, the real stimulus for movement is the difference between the drum speed and the eyecup speed, and this is called the slip speed (Horridge & Sandeman 1964). The following summary is taken from their analysis. This slip speed is linearly related to drum speed over a range of drum speeds from $0.001^\circ/\text{sec.}$ to $10^\circ/\text{sec.}$, but because the eyecup speed often exceeds the slip speed there is a need to postulate a "velocity amplifier" in the brain.

The gain of this amplifier, which is extremely variable but can be as large as 50, is defined as the ratio of eyecup speed to slip speed. The greater the gain the more closely will the eyecup movement approximate the drum movement and at infinite gain fixation will occur. The gain increases greatly at slower drum speeds and is maximal at speeds of $0.005^{\circ}/\text{sec}$. This means that the eye is most sensitive to movement at about the same speed as the sun or moon's movement across the sky. That the crab can detect the movement of the sun has been confirmed by direct test (Horridge 1966d). Another aspect of this sensitivity will be its effectiveness in stabilizing the eye over the longest possible periods of time. This is shown in memory experiments when a crab is clamped at the centre of a striped drum and after 30 secs. to 1 min. the lights are turned out and the drum is moved through a small angle in the dark. When the lights are switched on again the crab responds by moving its eyecups in such a way as to indicate that it correlates an impression of the former with the new position and moves its eyecups to the position of best correlation. Memory of small movements can last for up to 20-30 mins. (Horridge & Shephard 1966, Horridge 1966a).

Superimposed on the above movements are four kinds of small amplitude eyecup movements, 1, Tremor. 2, Saccades. 3, Scanning. 4, Drift. These also occur when the visual

field is stationary.

(1) When the crab is surrounded by a blank visual field the eyecup tremors at a frequency of 2-5c/sec. and with an amplitude of 0.05-0.2° peak to peak. The tremor movements, which are reduced in a contrasting visual field and abolished in the dark, are large enough to be visually significant and serve to sharpen visual images (Horridge 1966b).

(2) Spontaneous saccades or flicks also occur with much the same amplitude as the tremor movements but with a fast initial rise and a slow return phase (Horridge 1966e). The saccades appear to be ^{de}preetermined movements imposed centrally by a motor neuron carrying bursts of impulses.

(3) When a contrasting object is presented to a crab the eyecups often show scanning movements with an amplitude of 0.5-1° peak to peak but in Carcinus these may be partial withdrawal movements.

(4) In the dark, and when surrounded with a visual field with little contrast, the eyecups show irregular wandering movements and may drift away from their original position.

Despite the wealth of behavioural information about the ability of the eyecup to make extremely small, slow and accurately controlled movements, there is little physiological work to explain how such movements can be controlled by the peripheral neuromuscular system. The eyecup musculature of the American blue crab Callinectes sapidus was described

by Cochran (1935) and is similar to that in Carcinus. Each muscle is described as being innervated by a branch of both the optic tract and oculomotor nerve (Horridge and Sandeman 1964), but beyond this there is no information about the specific part played by each muscle in eyecup movements.

Crustacean muscle

Because the primary data to be presented is in the form of potentials from muscle fibres, and the results are inferences of nervous activity which cause these potentials, it is important to set out clearly the events at the neuromuscular junction in crustacea.

A great deal is now known of the peripheral organization of crustacean muscle. Each muscle is supplied by very few motor axons, usually a slow, fast and inhibitor axon which branch to innervate the fibres multiterminally and polyneuronally. However it cannot be assumed that all fibres in one muscle are similarly innervated when more than one axon is present (Furshpan 1955). There is at present little structural information about the neuromuscular junction, especially electron microscopic structure. The nerve fibres continue to branch over the surface of the muscle fibres, possibly becoming lost to view in grooves on the surface of the muscle fibre (van Harreveld 1939, Lavallard 1960, Peterson & Pepe 1961 a & b). Cohen (1963) has described two types of expanded structures seen in

conjunction with two efferent nerves supplying the accessory flexor muscle of the meropodite. One type is a 20-40 μ rectangular plaque while the other consists of a group of spheres in a cluster, called "en grappe" measuring 40 μ in its greatest diameter.

The presence of only a few motor axons means that gradation of contraction is of necessity more dependent on specific patterns of impulses in these axons and less on the recruitment of motor units. The study of crustacean muscle is thus especially concerned with the way in which graded contractions are achieved. Implicit in much of the early work is that contraction is a delicately graded event, but the influence of the "all-or-none" concept led later workers to explain the graded contraction in terms of the progressive recruitment, by facilitation, of individual muscle fibres each giving all-or-none twitches (e.g. Katz 1936). When the problem was examined with intracellular electrodes (Fatt & Katz 1953 a, b, c) it was found that stimulation of the slow and fast motor axons does not produce spike responses, but small electrical potentials which do not differ by more than a factor of 1.4 when measured at several points along the length and width of a fibre. The finding of these distributed junction potentials (an analogue of the term used for activity in the slow muscle fibres of the frog (Kuffler & Gerard 1947)) led to the idea that during

repetitive nerve stimulation a partial contraction occurs along the whole length of the fibre.

This postulate led to a comparative study of neuromuscular transmission in a variety of decapod muscles (Hoyle & Wiersma 1958, a, b & c) using the technique of stimulating single axons, recording the muscle fibre responses intracellularly and measuring the tension produced by the whole muscle. A wide variety of mechanical and electrical responses were found which not only varied from species to species, but even within a single muscle the responses recorded from individual fibres differed. Attempts to correlate the various junctional events in a single fibre with the overall tension of the muscle were not successful and led to the proposal of at least two transmitter substances, one for the 'slow' and one for the 'fast' axon. (cf. Wiersma 1961).

Atwood (1963) then re-examined the passive and dynamic properties of muscle fibres of the limbs and found they could be grouped into three main categories on the basis of their membrane properties. Type A fibres, which are a numerically small group, have membranes with short time constants and a high degree of electrical excitability, with a tendency to give spikes which can be propagated. Type B fibres have long time constants and respond only passively to depolarization. Type C fibres, the largest group, have

intermediate properties, with moderately long time constants and give a variety of graded responses on depolarization. He then showed (Atwood 1965) that type A fibres are innervated by the fast axons only while type B are innervated only by the slow ones. Type C fibres are innervated by both. On the basis of these findings he proposed that the membrane properties of the fibres are a significant factor in determining the fast and slow rates of contraction caused by the fast and slow axons. Most of the muscle responses could be explained without the need to postulate two excitatory transmitter substances.

Up to this time little was known of the histological structure of the muscle fibres probably because as Pantin (1956) said "It has long been tacitly assumed that 'muscle is muscle' wherever it is found among animals". Cohen (1963) showed that the fibres in the accessory flexor muscle of the meropodite are of two main types. In transverse sections one type has a punctate appearance of the fibrillar material, while the Z-bands, in longitudinal sections, are often broken across the width of the fibres and are spaced 2-3 μ apart. The other type has a clumped arrangement of its fibrillar material and a sarcomere length of 10-12 μ . The fibres thus show resemblances to the 'fibrillenstruktur' and 'felderstruktur' muscle fibres of vertebrates (Kruger 1949). However the differences between the two groups is probably one of

degree rather than kind, and the two probably represent the extremes of a wide spectrum of fibre types. Indeed many intermediate fibres are found.

In the distal head of the accessory flexor muscle of the meropodite Dorai Raj (1964) and Atwood & Dorai Raj (1964) were able to correlate the structure of the fibres with their electrical and mechanical properties.

Fibrillenstruktur fibres have high resting potentials with electrical properties typical of type A fibres and give twitch contractions. The felderstruktur fibres have smaller resting potentials with electrical properties typical of type B fibres, and give slow contractions. The intermediate fibres correspond with type C fibres. The complex responses in this muscle, innervated by a single motor axon can only be explained by different densities of innervation and different membrane properties of the fibres.

The development of techniques for recording the tension developed by a single muscle fibre and its intracellular activity (Orkand 1962, Atwood, Hoyle & Smyth 1965) has led to a more complete picture of the functioning of individual muscles. It has also led to the abandonment of some postulated mechanisms to explain previous inconsistent results. One of these has been the 'paradox' phenomenon (Wiersma & van Harreveld 1938, Hoyle & Wiersma 1958c).

Stimulation of the slow axon to the closer muscle of Randallia and Blepharipoda at 20/sec leads to appreciable tension development but only small electrical potentials. On the other hand stimulation of the fast axon at the same frequency produces large electrical responses but little tension. This finding has been a stumbling block to the acceptance of the idea that the contractile mechanism is coupled to the level of membrane depolarization, as suggested for vertebrate smooth muscle (Bülbring 1955) and for the slow skeletal system of the frog (Kuffler & Vaughan Williams 1953). The 'paradox' has been variously explained on the basis of a special direct effect of the slow transmitter on the coupling process (Hoyle & Wiersma 1958c) and by Falk & Fatt (1965) on the possible different time courses of release of the transmitters from the different axons in relation to the capacitance of the membrane and the T-system.

However, Atwood & Hoyle (1965), have shown a specialized group of muscle fibres in the 'paradox' muscles which give large jps to slow axon stimulation while the fast jps in these fibres are very small. It is the relatively small number of these fibres giving large slow jps together with their obscure distribution which has led to the paradox. There is now no bar to the idea of contraction being coupled to the level of membrane depolarization. That this is so can be determined by measuring the extent of applied

depolarization necessary to produce tension. This level, the E_c , varies in different fibres but jps are of sufficient amplitude to exceed this level. In vertebrates presumably the threshold for spike initiation is below the E_c level. Thus the contraction of a whole crustacean muscle will involve the progressive contraction of fibres as their E_c levels are exceeded.

The recording of tension produced by single fibres has also inevitably led to further categorization of fibre types on the basis of the following of tension upon electrical activity. Seven fibre types are now recognized ranging from spiking fibres giving only twitch contractions, through fibres giving graded electrical events and phasic or tonic contractions to passively responding fibres giving only tonic contractions. The properties of the fibres are also found to vary from crab to crab and are somewhat dependent on their moult cycle. Thus possible hormonal or other long term factors may affect the membrane properties, as in some vertebrate muscles (Strickholm 1966).

These findings have also led to an increasing tendency to explain the different types of contraction produced by the fast and slow axons, more on the different densities of innervation of fibres with different membrane properties and less on the release of two transmitter

substances. However, strychnine is found (Parnas & Atwood 1966) to cause rapid block of the fast system but to have little effect on the slow or inhibitory systems. Nerve and muscle remain excitable so the effect must be presynaptic or in the synaptic cleft. Although the possibility of different transmitter substances is again raised it is possible that the nerve endings of the two axons may be different with the slow and inhibitory endings protected by diffusion barriers.

Two actions of the inhibitory transmitter are at present recognized. The most widespread is an effect on the post-synaptic membrane which results in a shift of the membrane potential towards the chloride equilibrium potential (Boistel & Pott 1958). The second, which is found so far only in the claw opener muscle, occurs presynaptically causing a reduction in the amount of excitatory transmitter release and hence a reduced j.p. (Dudel & Kuffler 1961). Phasic muscle fibres differ in their sensitivity to inhibitory axon stimulation. Some are apparently not innervated by the inhibitor axon while in others the excitable membrane responses are more sensitive than the ejps. Tonic fibres are more densely innervated by the inhibitor axons and ^{con}sequently inhibitory stimulation is more effective in suppressing a slow contraction (Atwood 1965, Atwood, Parnas & Wiersma 1967).

By fixing muscle fibres which are undergoing graded contractions Hoyle (1966) has suggested that the fundamental unit for a graded contraction is a part of a sarcomere, or that part of a fibre supplied by a single element of the sarcoplasmic reticulum. These units have a range of thresholds and as the membrane potential is lowered units will be progressively recruited causing local contractions. In a muscle fibre say 2 cm long and 200 μ in diameter and with an average sarcomere length of 5 μ , there will be 4×10^8 units and a virtual infinite range for graded contractions.

Although there is now this detailed knowledge of the make-up of a crustacean muscle there is little work so far on how the animal uses these mechanisms at its disposal during normal movements. Kennedy & Takeda (1965 a & b) have studied the reflex control of the flexor muscles of the abdomen correlating it with the reflex connections, the discharge patterns of the motoneurons and the responses of the flexor muscles.

In the eyecup reflexes, where the behavioural responses are well known it would be interesting to know how these movements are brought about by the eyecup muscles. The preparation offers many advantages in that natural inputs in the form of readily controlled visual stimuli can be used, and the output in the form of the eyecup movement can also be measured. Because artificial stimulation is not used the

preparation should also reveal the normal motor output to the eyecup and the way in which the animal uses its peripheral neuromuscular system. The preparation has the disadvantages that the axons to the individual muscles are too small for direct recording of nerve impulses, and the tension produced by single muscle fibres cannot be measured.

M E T H O D S

Carcinus maenas, the common shore crab, has been used throughout the experimental programme. Male crabs with a carapace width of 6-8 cms. were predominantly chosen. Under experimental conditions they survive well out of water for several hours at room temperatures of 17-20°C.

ANATOMY

The musculature of the eyecup and eyestalk was examined in crabs which had been fixed for 12 hrs in Sea Water Bouin. This softens the exoskeleton and eases further dissection of the now coloured and hardened muscles. The sclerites were best observed in unfixed animals.

The muscles were fixed for histological sectioning by perfusing the eyecup with a solution of glutaraldehyde in sea water (20cc of 12% glutaraldehyde, 33cc of 0.2M sodium cacodylate and 47cc of sea water, buffered to pH 7.4). After half an hour the muscles were dissected out still attached to the exoskeleton and were post-fixed in a solution of 2% osmic acid in sea water. After Araldite embedding, $\frac{1}{2}$ μ transverse and longitudinal sections of each muscle were cut from the same block, and stained with toluidine blue. The pieces of exoskeleton enabled the muscle to be oriented, and it was possible to identify the same fibres in both T.S. and L.S.

Small lengths of the optic tract and oculomotor nerve were fixed in a 2% solution of osmic acid in sea water and embedded in Araldite. $\frac{1}{2}$ μ transverse sections were cut and stained with toluidine blue.

The nerve supply to the muscles was demonstrated by staining with methylene blue (Gurr's vital and fluorochrome). The muscles were exposed by removing part of the overlying exoskeleton with a high speed dental drill and the eyecup was immersed in sea water with a few drops of 0.5% methylene blue added. After 1-2 hrs, the eyecup was quickly dissected and then fixed in ammonium molybdate solution.

The blood supply to the eyecup was revealed by injecting the main vessels as they leave the heart with indian ink from which the shellac is removed (Gunther Wagner "Pelikan" C11 (1431a)).

ELECTROPHYSIOLOGY.

For electrophysiological recording the intact crab was rigidly held at the lateral edges of its carapace by a modified retort clamp. Flanges prevented the limbs from touching the eyecups. The crab was arranged so that its transverse axis was horizontal while its longitudinal axis was raised 15° above the horizontal at the anterior end. This position approximates that assumed by a resting crab.

Extracellular muscle potentials were recorded from the freely moving right eyecup, whose movements were also

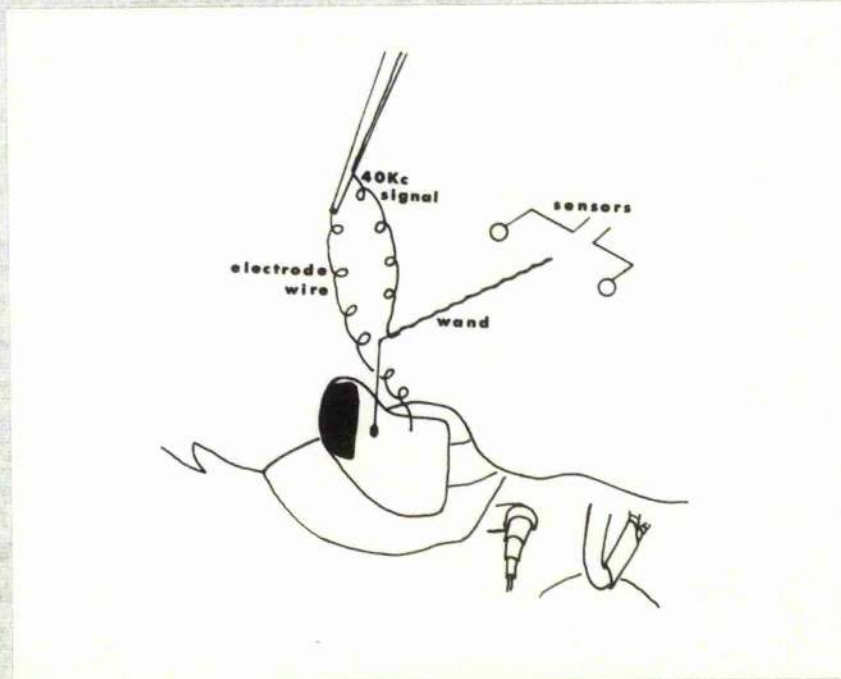


FIG. 1.

Arrangement of the recording electrode and the eyecup movement sensor. A coil of $50\ \mu$ silver wire carries the sine wave signal to the wand and along its length. The wand is fixed behind the cornea on the medial side of the eyecup and moves between two ball sensors over the crab's back. The electrode wire is also coiled so that eyecup movement is not impaired.

monitored at the same time. Holes were drilled with small entomological pins through the eyecup exoskeleton over the appropriate muscle. The end of a small coil of 50 μ silver wire, insulated but for the tip, was inserted through the hole and into the muscle. The electrode was rarely dislodged by eyecup movements and the free joint allowed the electrode to be moved to obtain optimal recording conditions. After the experiment the electrode wire was cut at its entry into the eyecup and the eyecup was removed and fixed for 12 hrs in sea-water Bouin. Dissection then revealed the location of the electrode tip. The indifferent electrode was placed in the crab's liver region. The muscle potentials recorded by this method were usually 100-200 μ V in amplitude. The signals were fed into conventional a-c coupled pre-amplifiers before display on an oscilloscope.

To measure the movements of the eyecup a 5 mg wand of 0.2 mm nylon twine was fixed with Eastman 910 adhesive to the medial side of the eyecup and extended over the back of the crab. A coil of 50 μ silver wire carried a stable 40 Kc 10v peak to peak sine wave signal to the wand and along its length (FIG 1). The wand moved between two metal balls placed over the crab's back, which picked up the signal and fed it to a differential a-c coupled amplifier. The output of this amplifier was then compared with the original 40 Kc signal and converted into a DC voltage signal

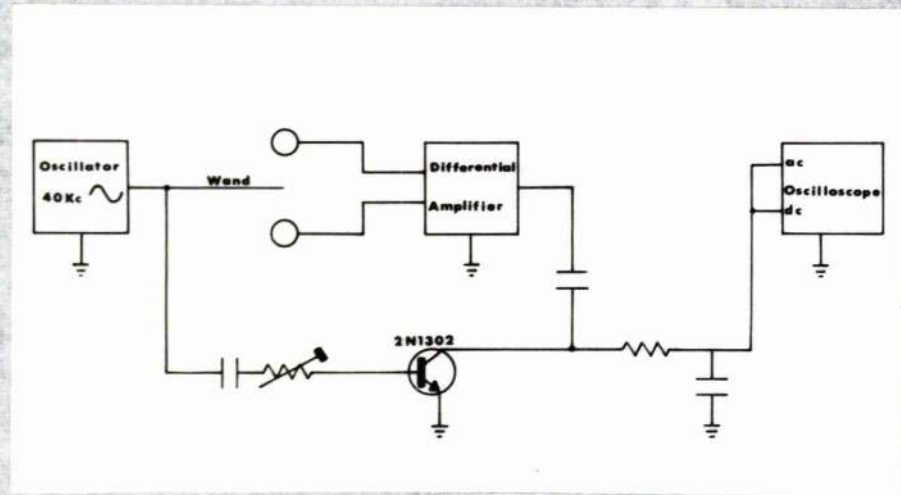


FIG. 2.

Circuit diagram (modified after Sandeman, unpublished) of the apparatus used to measure eyecup movements. A stable 40 Kc signal is fed to a wand on the crab's eyecup, and moves between two sensors. The amplified output of these sensors is then compared with the original signal and is converted into a DC output voltage. The DC component of the movement is displayed on an oscilloscope and a second trace is ac coupled at a higher amplification to reveal any irregularity of the movement.

in the circuit shown in FIG. 2. With a wand length of 2 cm. the system will measure eyecup movements linearly over a range of 25° and with a resolution of 0.01° . The frequency response is flat between 0.001 c.p.s. and 10 c.p.s. One oscilloscope channel was used to display the DC component of the movement while another was AC coupled at a higher amplification to reveal any unevenness of the movement.

After preparation for recording at bench level, the crab was lowered into the centre of 30 cm diameter and 25 cm high vertically black and white striped drum (FIG. 3). The stripes subtended an angle of 15° at the crab's eye and the base had a radial striped pattern. The whole drum was evenly illuminated by an overhead 75 w reflector bulb. Drum movements were controlled by a kymograph motor which gave smooth constant rotation in either direction at speeds ranging from $0.03^{\circ}/\text{sec.}$ to $5^{\circ}/\text{sec.}$ A clutch enabled the drum to be stopped or started abruptly.

To obtain small drum movements a linear pen recorder actuator was used. This solenoid was driven from a low-frequency waveform generator (Servomex L.F. 51) which provided a variety of waveforms of variable frequency and amplitude. The drum movements were here monitored by the system already described for monitoring eyecup movements.

For intracellular recording from the muscles the crab was rigidly held as before but the limbs were autotomized.

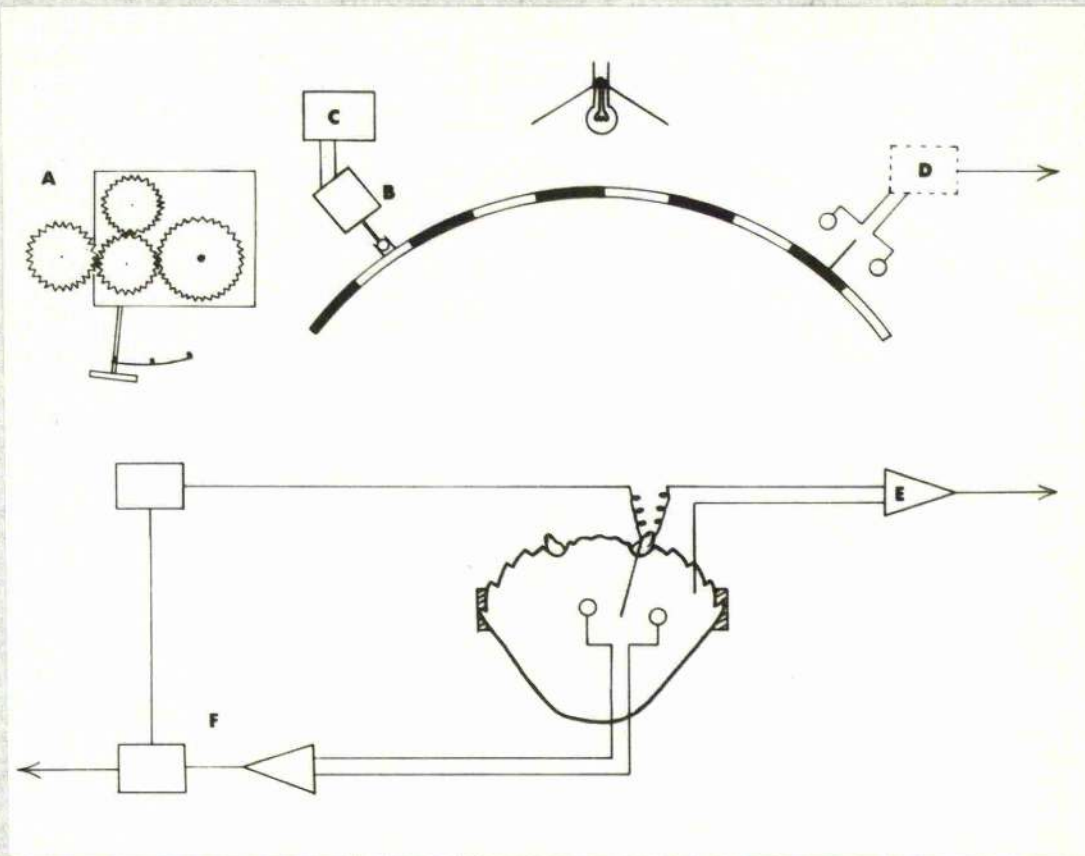


FIG. 3.

Apparatus used for extracellular recording from the eyecup muscles. The crab was rigidly held in the centre of a vertically black and white striped drum which could be rotated in either direction by a kymograph motor operating via a gearing arrangement (A). Smooth constant rotation with speeds from $0.03^{\circ}/S$ to $5^{\circ}/S$ were available. A linear pen writer actuator (B) driven from a low frequency generator (C) was used to obtain small drum movements which were monitored (D) by the system described for measuring eye movements. Muscle potentials were lead to a differential ac coupled amplifier (E) before display on an oscilloscope. Eyecup movements were also measured (F). The whole drum was evenly illuminated by an overhead bulb.

The right eyecup, from which most recordings were made, was firmly cemented in its socket with plaster of Paris and blinded by coating the cornea with quick drying black paint. The required muscle was exposed by paring away a small piece of the overlying eyecup exoskeleton with a high speed dental drill. Blood loss was not usually serious.

The intracellular activity of the muscle fibres was recorded with glass microelectrodes filled with 3M KCl and with a resistance of 10-30 M Ω . The muscle potentials were fed to a negative capacity 'Bak' pre-amplifier (Bak 1958) before display on an oscilloscope.

The seeing but fixed left eyecup was arranged to be at the centre of a semi-circular black and white striped drum with a radius of 7 cm. The stripes subtended an angle of 10° at the eye. Drum movements were controlled by the pen writer actuator and were also monitored.

For study of the withdrawal reflex, the crab was prepared as above and the tegumentary nerve was also exposed in the body of the crab. The nerve was hooked on to silver wire stimulating electrodes and single electrical pulses of variable duration and intensity were applied via a radio-frequency isolation unit. Nerve impulses in the optic tract, exposed near its exit from the brain were recorded with a 100 μ stainless steel wire electrode insulated but for the tip which could be moved over the tract to locate the required axons.

All responses were displayed on a Tektronix type 565 oscilloscope and were photographed with a Cossor oscillograph camera on Ilford NS6 recording paper.

R E S U L T SPart 1.Anatomy1. Eye assembly

The complete eye assembly of Carcinus is complex and consists of three skeletal elements moved by 13 pairs of muscles. Anterior to the brain is the median plate (middle cylinder) which is moved about the main body skeleton by 3 pairs of muscles. Attached to this plate are the elongated eyestalks which project laterally on either side. They are attached proximally to the main body skeleton by membranes along their anterior and posterior edges, and distally by a muscle which can rotate the eyestalk relative to main body skeleton. The eyestalks are completely enclosed by a fold of exoskeleton and in the intact crab only the eyecups, which attach to the proximal ends of the eyestalks, are visible protruding from their sockets.

The eyecups are approximately 6 mm long and 3 mm wide and deep at their base. The ventral surface, where the exoskeleton is thinner, is covered by a circlet of hairs and the corneal surface is wrapped around the distal rounded end.

2. Eyecup position.

When the transverse axis of the body is horizontal and the longitudinal axis is raised 10-20° above the horizontal

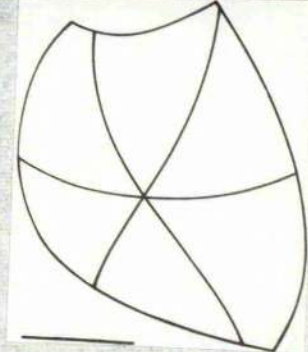
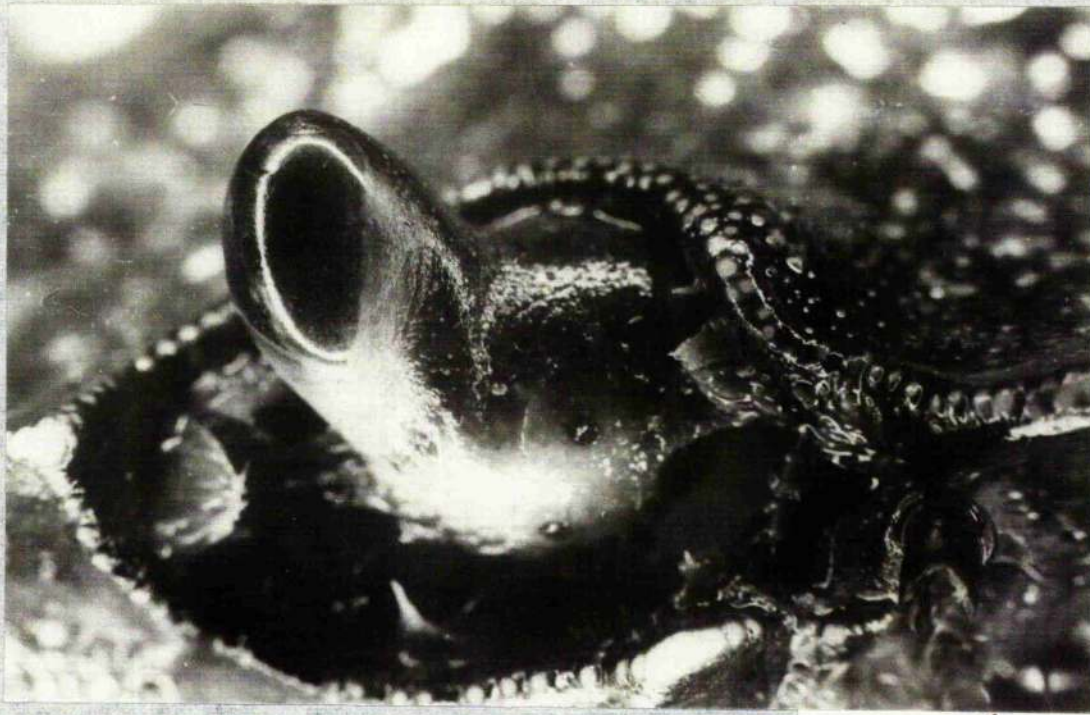


FIG. 4.

Photograph of the right eyecup of Carcinus, taken while the crab was sitting with its transverse axis horizontal and its longitudinal axis raised by 15° above the horizontal at the anterior end. In this position one of the three ommatidial rows is maintained horizontal (inset). The dark holes in the eyecup are places where extracellular recording electrodes were introduced into the muscles.

at the anterior end, the eyecups are held pointing forwards at an angle of $40-45^{\circ}$ to the longitudinal axis and at an angle of $40-45^{\circ}$ to the vertical. In this position one of the three ommatidial rows is maintained horizontal. (Fig. 4).

3. Eyestalk-eyecup joint.

Movement of the eyecup about the eyestalks is not restricted to one plane by fixed skeletal elements. A movement of 30° is possible in the horizontal plane, 70° in the vertical and a movement of 50° when the crab is rotated about its transverse axis - roll tilt.

The joint is flexible and does not act as a simple pivot. Superimposed photographs of the eyecup in successive positions as it was following a horizontal rotation of a striped drum, show that the "pivot" point is continually changing (Fig. 5). As the eyecup moves toward the mid-line the "pivot" point moves backwards from near the centre of the eyecup to the distal edge of the eyestalk. The joint is thus considerably more complex than most joints elsewhere in the body.

The membranes which attach the eyestalk to the body are continued and thickened around the eyestalk-eyecup joint. Near the main muscle attachments the membrane is further stiffened by three small sclerites which attach to the edge of the eyecup and tuck into the membrane.

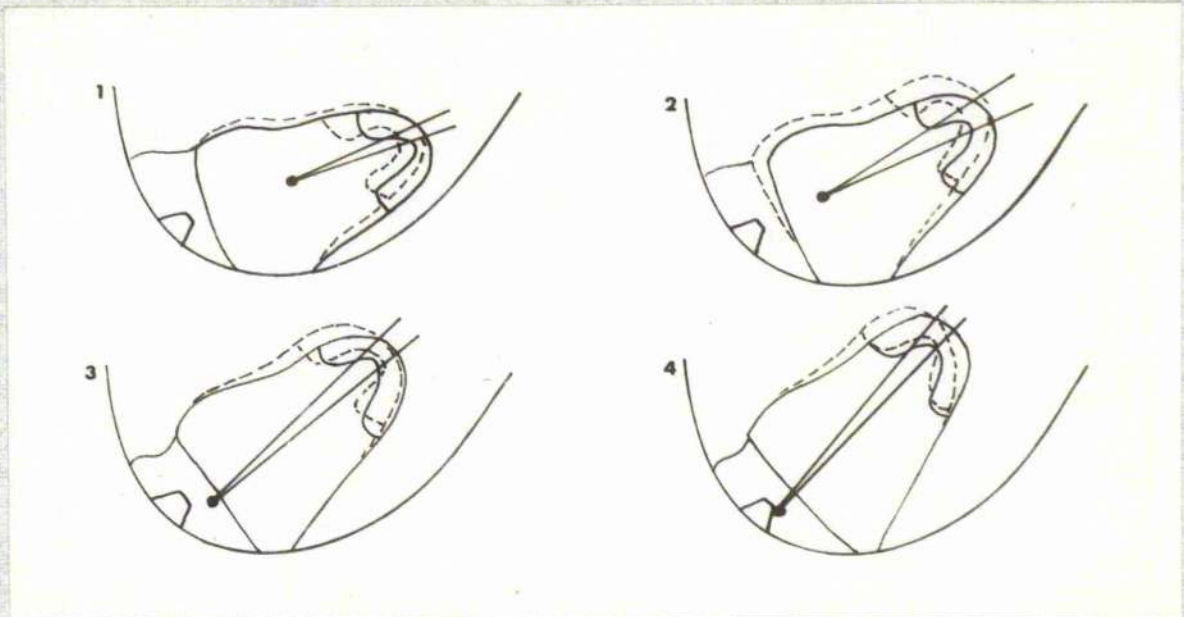


FIG. 5.

Tracings from photographs taken from vertically above the crab as the right eyecup was moving towards the mid-line. Several exposures, of the eyecup in successive positions, were made on the same frame, and the camera was not moved between frames. The "pivot" point of the eyecup is not constant, but continually changes during movement.

4. Eyecup musculature.

The nine eyecup muscles attach to internal projections of the eyestalk exoskeleton and their insertions on the eyecup are marked externally by a characteristically different texture of the surface exoskeleton. The musculature is similar to that described by Cochran (1935), for the American Blue Crab Callinectes sapidus. Her numbering of the muscles has been retained but her nomenclature has been abandoned as it implies function deduced, presumably, on anatomical findings. Because a muscle happens to be favourably placed to carry out a movement and even the fact that it may do so when electrically stimulated provides no guarantee that in the normal state the muscle actually does carry out this movement.

According to Cochran's terminology muscle 19, the oculi abductor and muscle 23, the oculi retractor medialis consist of two branches, while muscle 20, the oculi retractor dorsalis consists of three. Although the branches of these muscles are found to be distinct physiological units, their numbering is retained, implying that they have similar attachments rather than similar functions.

The arrangement of the musculature in Carcinus is

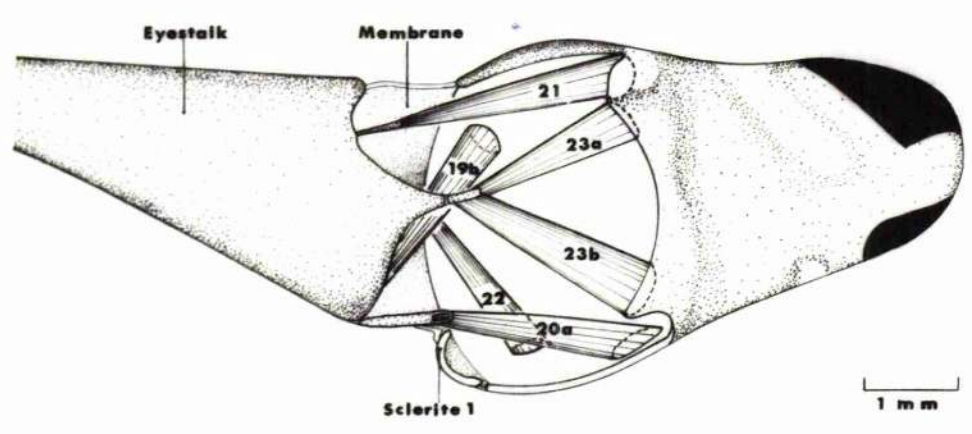
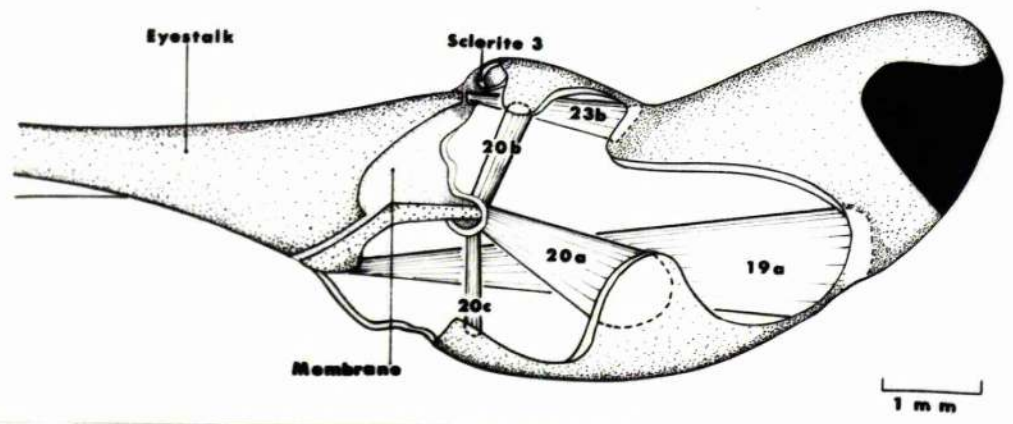
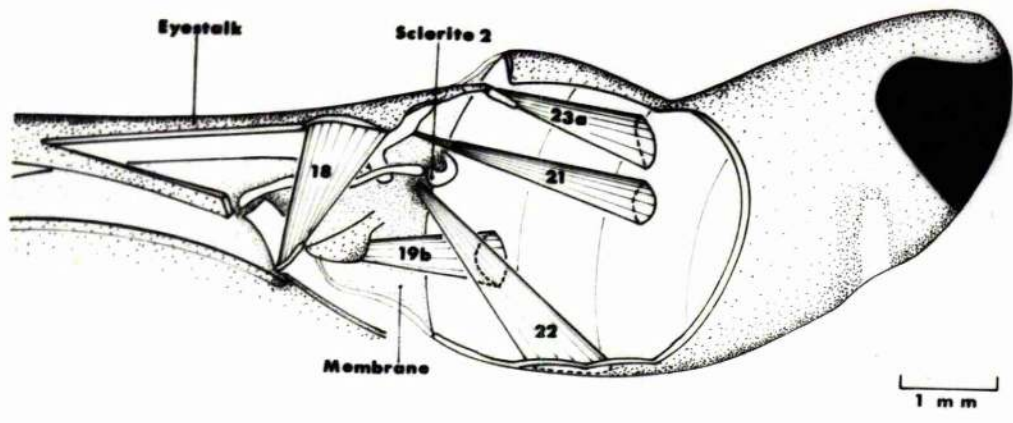


FIG. 6.

Diagrams to show the musculature of the eyecup and eyestalk in Carcinus. The ~~upper~~^{second} shows the right eyecup dissected from the lateral side, with only the lateral muscles revealed. The ~~second~~^{first} is a dissection from the same side but the lateral muscles are removed to show the deeper (medial) muscles. The third shows a dorsal view but here muscles 19a, 20a and 20b are omitted. The joint is surrounded by a continuation of the membrane which attaches the eyestalk to the main body skeleton, and this is stiffened by three sclerites near the attachment of the muscles to internal projections of the eyestalk exoskeleton.

shown in Fig. 6.

Muscle 19A which is the largest in the eyecup, originates at the base of a lateral projection of the eyestalk and inserts on the lateral wall of the eyecup just behind the cornea. In transverse section all the muscle fibres have a similar appearance. The fibrillar material of the fibres has a punctate appearance and is evenly distributed. The boundaries of the individual fibres are indistinct (Fig. 7) and fine membranes can be seen running into the blocks of fibres. This arrangement shows some resemblance to that in the deep extensor muscles in the abdomen of Procambarus (Parnas & Atwood 1966). In longitudinal section the fibres stain deeply with toluidine blue and the Z-bands are often broken across the width of a fibre and are spaced 3-4 μ apart. The fibres thus resemble the "Fibrillenstruktur" fibres described in vertebrates (Kruger 1949, Hess 1961) and in crustacea by Cohen (1963).

Muscle 19b originates beside 19a on the base of the same projection but runs medially and inserts on the ventral surface of the eyecup. The fibres are not uniform in appearance but the majority in T.S. have a clumped appearance of their fibrillar material. In longitudinal section the Z bands are spaced 10-12 μ apart and these fibres thus resemble "felderstruktur fibres". The remaining fibres have structures intermediate between the two types described and have a



Fig. 7.

Photograph of a transverse section of part of muscle 19a, stained with Mallory. The muscle fibrils are evenly distributed but the boundaries of individual fibres are indistinct. The muscle units are subdivided by fine membranes. Scale: 250 μ .

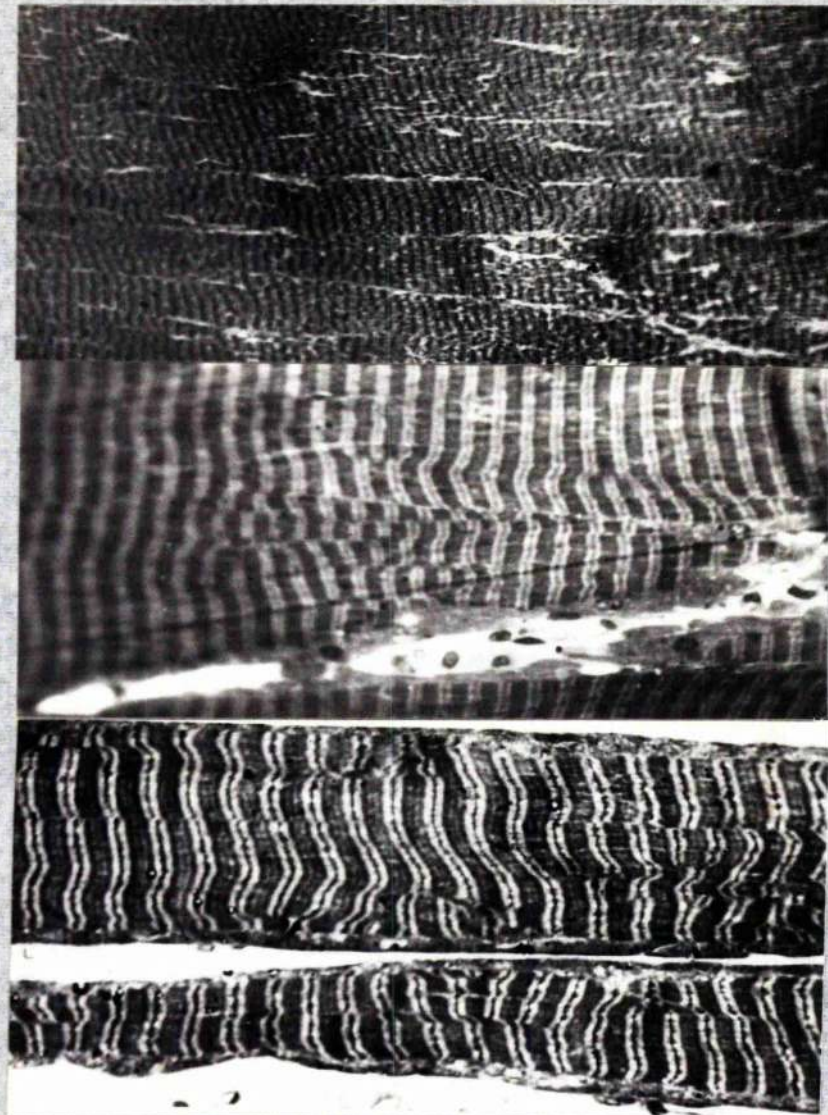


FIG. 8.

Longitudinal sections of three types of muscle fibres from various eyecup muscles, A. Fibrillenstruktur, B Intermediate and C Felderstruktur. Scale 100 μ

sarcomere length of 6-8 μ . Extreme fibrillenstruktur fibres are not found in this muscle. The three types of fibres, fibrillen, felderstruktur and intermediate are shown in Fig. 8. In general the differences between the fibres are more apparent in L.S. The marked differences previously described from paraffin sections have not been so apparent with the techniques used here.

Muscle 20a, attaches to the distal end of the lateral eyestalk projection and inserts on the lateral wall of the eyecup behind muscle 19a. The majority of the 25 or so fibres in this muscle have sarcomere lengths of either 3-4 μ or 10-12 μ , but a few have sarcomeres of intermediate length. The various fibre types are intermingled and are not separated into groups of the same kind.

Muscle 20b starts from the same attachment as 20a but runs vertically upwards to insert on the dorsal eyecup wall near its proximal edge. The muscle is small and is enclosed in a tight connective tissue sheath. Approximately 10 muscle fibres are present and of these only about 3 have sarcomere lengths of less than 4 μ ; the rest have a sarcomere length of 10-12 μ .

Muscle 20c runs vertically downwards from its attachment below 20a and 20b to insert on the ventral wall of the eyecup near its proximal edge. It is rather smaller than muscle 20b consisting of about 8 fibres, half of which have Fibrillen-

struktur and the other half Felderstruktur.

Sclerite 1 tucks into the membrane near the attachment of muscles 20a, b and c.

Muscle 21, a small compact muscle, arises from a tendon attached to the medial edge of the eyestalk and inserts in a depression on the medial side of the eyecup. Fibres with Fibrillen and Felderstruktur are intermingled but few show extreme Felderstruktur.

Muscle 22 runs diagonally across the eyecup from its attachment near to sclerite 2 and a skeletal bar to its insertion on the ventro-lateral wall of the eyecup. The skeletal bar runs in the joint membrane from the sclerite to which muscle 18 attaches. The muscle fibres are predominantly Felderstruktur with sarcomere lengths of 8-10 μ .

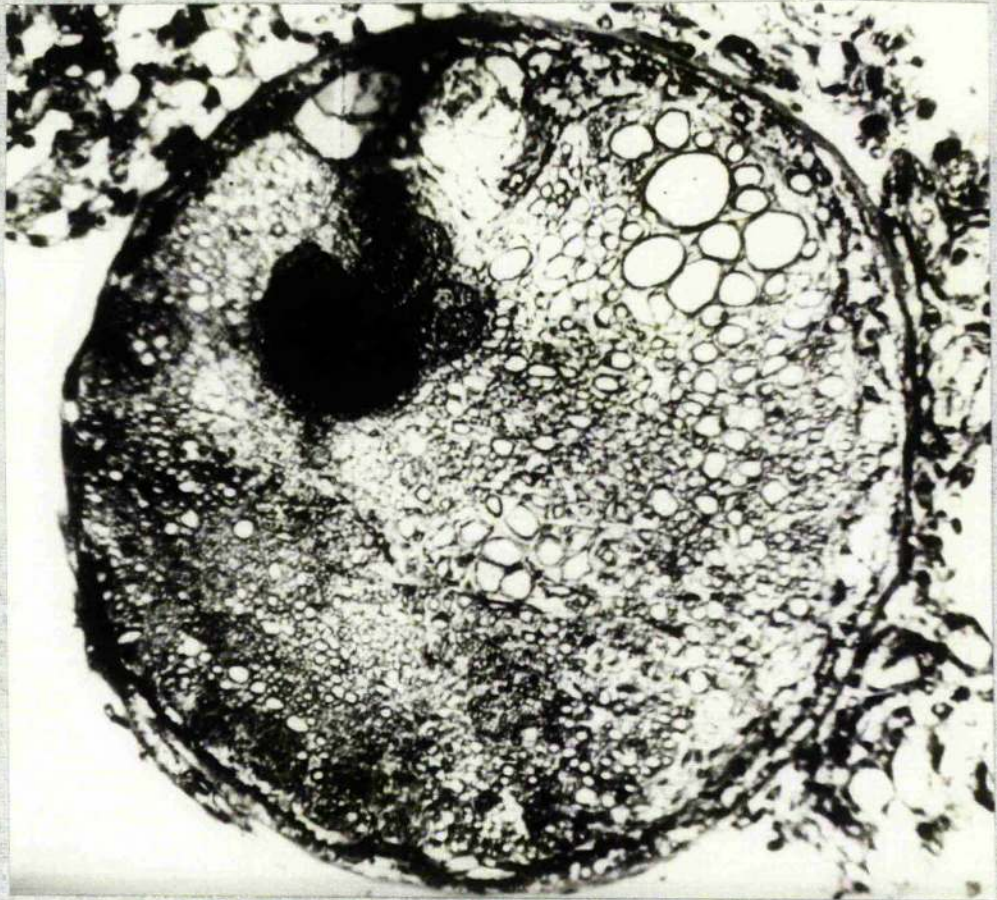
Muscle 23a attaches to the medial edge of a prominent jointed projection of the dorsal surface of the eyestalk and inserts above muscle 21 on the dorso-medial surface of the eyecup. Histologically the fibres are a mixed population with the felderstruktur fibres towards the dorsal surface.

Muscle 23b arises alongside muscle 23a and attaches to the lateral side of the dorsal eyecup surface. The joint membrane is stiffened at the attachment of these two muscles by the intucking of sclerite 3. The muscle fibres have sarcomere lengths of 6-12 μ but none with shorter lengths have been seen.

Muscle 18 is the only muscle present in the eyestalk. It attaches to a sclerite fixed to the main body skeleton and spreads out to insert on the dorsal wall of the eyestalk which it rotates relative to the main body skeleton. The muscle fibres are a mixed population of histological types.

5. Innervation of the muscles.

Each muscle in the eyecup is described by Horridge & Sandeman (1964) as being supplied by a branch of both the optic tract and oculomotor nerve. The latter branches before entering the eyestalk, and a smaller lower purely sensory branch ramifies in connective tissue and in the carapace around the eyestalk. The upper branch runs in the hollow of the eyestalk and at the level of muscle 18 branches profusely to supply the eyecup muscles. The optic tract branches at the same level and these branch then run to the eyecup muscles. The branches of both these nerve trunks are complex and interwoven within the eyecup, so that it has not been possible to build a complete picture, in terms of numbers of axons, of the muscle innervation. Methylene blue staining of the axons to muscle 20a has been the most successful. It receives two axons from the oculomotor and a large axon from the optic tract which also branches to supply muscle 19a. In transverse sections of muscle 19a a large 30-40 μ axon can be recognized.



100 μ

FIG. 9.

Photograph of a $\frac{1}{2}$ μ transverse section of the optic tract near its exit from the brain. The tract is surrounded by a thick sheath and a bundle of large, presumed motor axons are present on the dorsal surface. These axons are not present in sections of the tract taken near the optic lobes. The densely staining region is a bundle of small fibres, less than 1 μ in diameter. A blood vessel is present just above this region. The group of large diameter axons in the centre of the tract run straight to the optic lobes.

Similarly in transverse sections of the optic tract (Fig. 9) a large axon of this size can be seen in a group of large, presumed motor axons, on the dorsal surface. However the classification of axons into motor or sensory purely on the basis of their cross sectional diameter is unjustified. Axons in the oculomotor nerve have diameters up to 25 μ but none are as large as this axon. The group of large axons on the dorsal surface of the optic tract are not present in sections of the tract taken near the optic lobes. This lends some support to their being motor but they could equally spring from cuticular hair receptors in the eyecup. The optic tract also shows other features of interest. It is surrounded by a thick connective tissue sheath and in both T.S., and preparations when the blood system is injected with ink, a blood vessel can be seen in the proximal part of the tract. Close to this is a densely staining region surrounded by a sheath which consists of numerous axons with diameters of less than 1 μ . Nunnemacher (1965) has described similar regions in the optic tracts of other decapods and concludes from axon counts that this region contains 91% of the total number of axons. The group of large axons in the centre of the tract run straight to the optic lobes. Electron micrographs of the tract near the optic lobes show neuropile areas, emphasizing that this is merely an elongated tract

connecting two brain regions and not a peripheral nerve.

Obvious proprioceptive structures, although sought in methylene blue studies and in serial transverse and longitudinal sections of the eyecup have not been found. However bipolar cells, of unknown function, but typical of many arthropodial membranes have been described in the eyecup eyestalk joint membrane (Sandeman 1964a).

6. Blood supply to the eyecup.

The eyecup is supplied by branches of two blood vessels. The optic artery which runs along the anterior edge of the eyestalk supplies the optic lobes (Sandeman 1967a) but also branches to supply muscles 20a b and c, 23a and b and muscle 21. The oculomotor artery runs along the posterior edge of the eyestalk and supplies the rest of the musculature but also muscle 21 as well. This muscle thus has an extremely dense invasion by blood vessels and the significance of this will be discussed in later sections.

Part 2.The Eyecup movement

Continuous horizontal rotation of a striped drum around the crab induces nystagmic movements of the eyecups. This optokinetic nystagmus consists of two phases; a slow forward phase during which the eyecups follow the direction of drum rotation but with an ever increasing lag, and a fast return phase - flick back - which returns the eyecups approximately to their original position. The response involves the movement of the two eyecups in opposite directions relative to the mid-line. Fig. 10 shows the movement of the right eyecup with the slow phase away from (a) and towards (b) the mid-line over a range of drum speeds. Only the horizontal component of movement is measured and the slight vertical displacement of the eyecup which occurs toward the end of a slow phase is not.

1. Slow Phase

The eye speed during a slow phase is not uniform. After a flick back the eye initially moves faster than the drum but then slows down to a relatively uniform speed, which is slower than the drum speed, over the middle part of the traverse. Towards the end of the slow phase the eye speed is further reduced and may often cease altogether before the next flick back occurs. The flick back in the

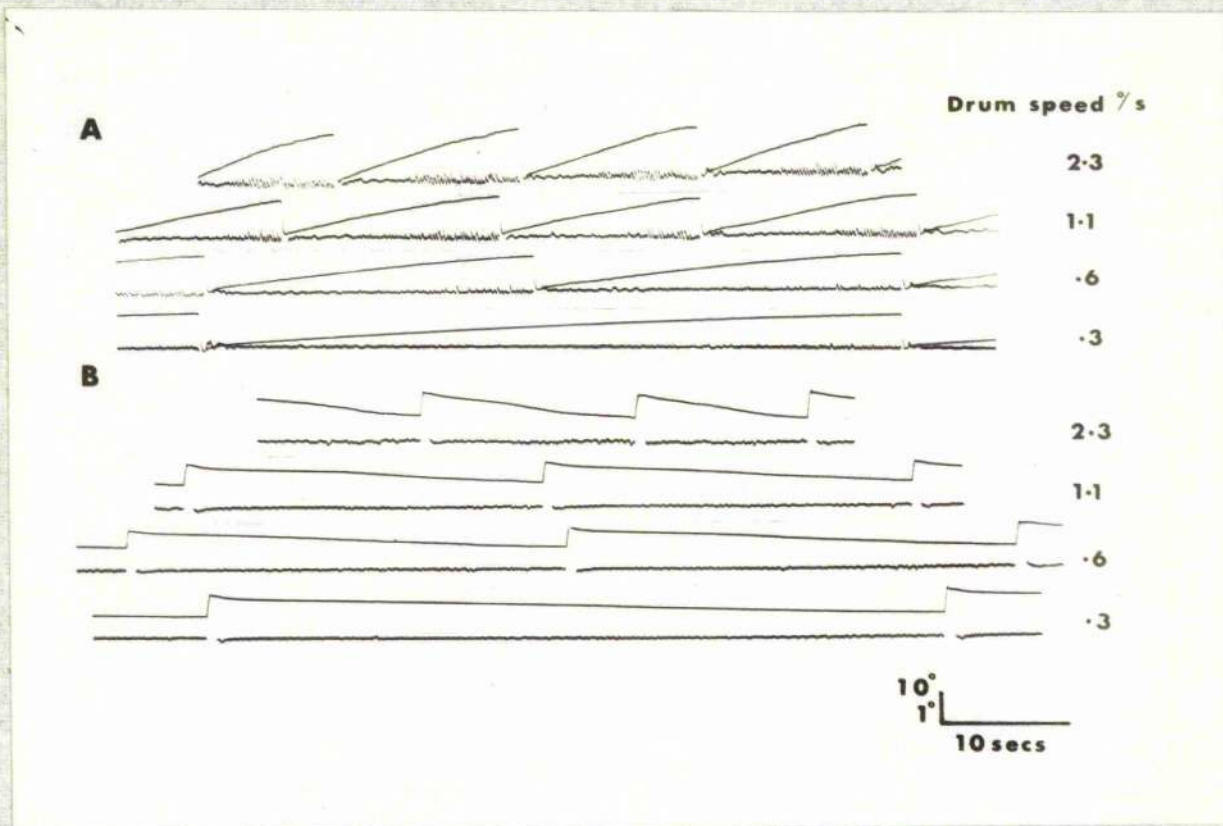


FIG. 10.

Movements of the right eyecup of the same crab recorded over a range of drum speeds. In (A) the eyecup is following a clockwise rotation of the drum and is thus moving away from the mid-line. In (B) the drum movement is reversed so that the slow phase is now towards the mid line. The upper trace represents the DC component of the movement. The eye speed is not uniform during a slow phase, but that during the fast phase is. The amplitude of the response is not related to drum speed, but the eye speed during the slow phase is. The lower trace shows the eyecup movement ac coupled and amplified still further to reveal tremor. This increases greatly in both frequency and amplitude during the slow phase, but is always less at lower drum speeds and when the eye moves toward the mid-line.

two eyecups is not necessarily synchronous (Barnes 1967). The relative stimulus to the eye - the difference between drum speed and eye speed - is thus not constant throughout the slow phase. Uneven movements of the eyecup, usually called eye tremor, are similarly not uniform throughout the response. Initially the tremor is of a low frequency and amplitude, but part way through increases both in frequency and amplitude. The tremor can vary from 2-5 c/s and from 0.05° - 0.2° peak to peak in amplitude, but is always of smaller amplitude when the eyecup moves toward the mid-line, and at lower drum speeds.

The extent of the slow phase can vary from 2-20 $^{\circ}$ and its time course for the same drum speed shows similar variation even in successive nystagmic movements by the same crab. The amplitude of the movement is not dependent on the drum speed. Such variability precludes a more exact description of the response.

2. Fast Phase

The eye speed during the fast phase is relatively uniform but is slightly decelerated towards the end. The eye speed during the slow phase is directly related to drum speed but there is no relation between the eye speed in the slow phase and that in the fast phase. The eye speed during the fast phase is constant over the range of drum speeds used and hence is independent of that in the

slow phase. This differs from the situation in the rabbit where Koike (1959) has shown a correlation between the speeds in the two phases.

3. Mechanics of horizontal movement.

Some measure of the mechanical characteristics of the eyestalk - eyecup joint during imposed horizontal movements is necessary for the later interpretation of impulse frequency in relation to eyecup position. Measurements were made on anaesthetized, blind and normal crabs. Animals were blinded by coating both corneas with black paint, while the seeing animals were surrounded by a black and white, vertically striped drum. Small weights were hung on the eyecup by means of a pulley system and the resulting angular deflexion of the eyecup was measured with the movement detector system previously described. A plot of eyecup position resulting from the applied load is shown in Fig. 11. Over 15° of movement in the centre of the orbit the relationship is linear, but towards the extremities resistance to movement greatly increases. In the anaesthetized crab a torque of 0.006 deg./Kdyne-cm. is needed to move the eyecup in the centre of the orbit. In the blind crab the torque needed rises to 0.02 deg./Kdyne-cm and is greatest at 0.04 deg/Kdyne-cm in the seeing eye. In the anaesthetized crab the electrical activity in the

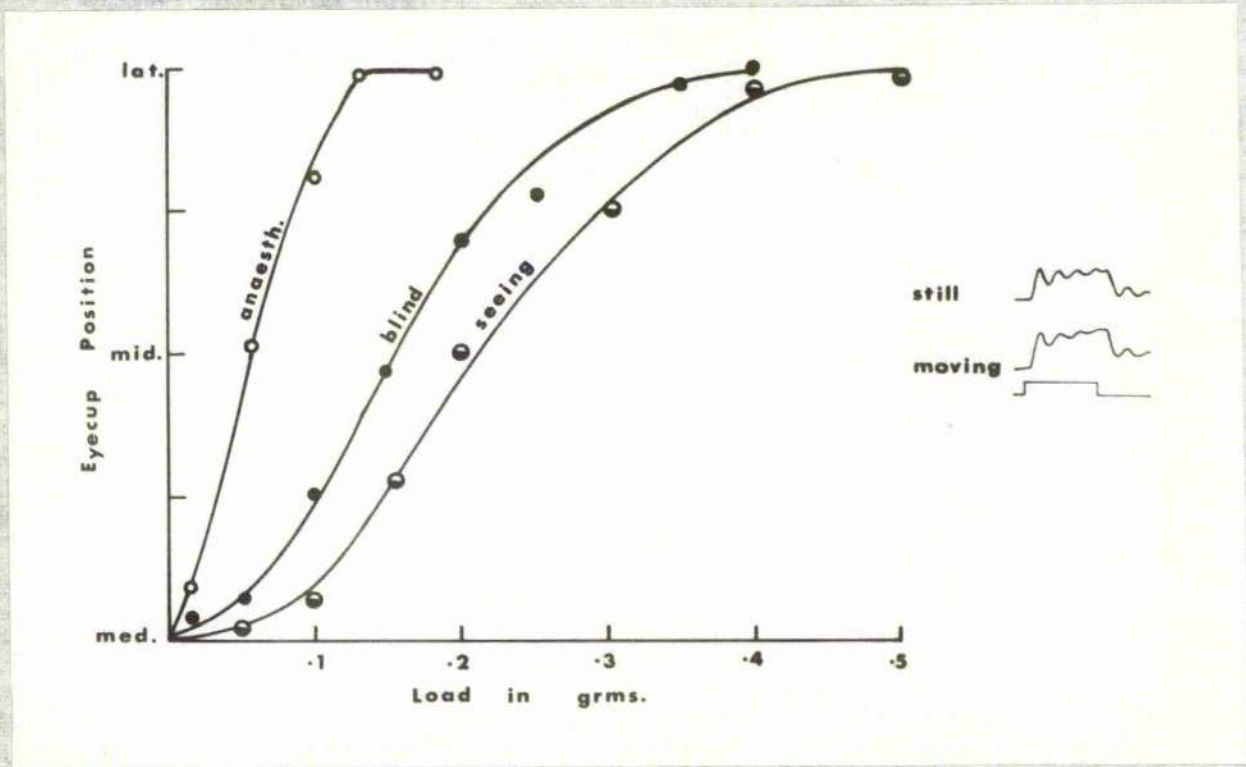


FIG.11.

Graphs of load imposed on the eyecup and the resulting deflection of the eyecup in anaesthetized, blind and normal crabs. The curve for the anaesthetized crabs represents the torque necessary to overcome the passive elements of the joint. The difference between the curves for the seeing and blind crabs represents the effectiveness of visual feedback. The inset shows the effect of applying a weak electromagnetic pulse to the eyecup while stationary and while moving through the same point as a comparison of dynamic with equilibrium opposing forces. Forward resistance is the same in both.

muscles is abolished so that the torque measured represents that which is necessary to overcome the passive resistance of the joint and muscles. In the blind crab background activity is present in the muscles and subsequently torque rises. The difference between the curves for blind and seeing crabs represents the effectiveness of visual feedback. This method of mechanically imposing a movement on the eyecup in front of a stationary visual field could be an effective way of measuring visual feedback.

The inset in Fig. 11 shows a measure of the forward resistance of the eyecup while stationary and while moving through the same point. A light metal wand was attached to the eyecup whose movements were monitored as before. The DC output of this movement detector system triggered an electromagnet when the eyecup reached a certain point, and a sudden pull of 200 ms duration was then applied to the eyecup. An optokinetic movement of the eyecup was induced by rotating a striped drum around the crab and the imposed pull was in the same direction as the eyecup movement. The strength of the pull was adjusted to be just above threshold. The rise time of the resulting eyecup movement in the stationary and in the moving eyecup is the same, indicating that resistance does not increase during following movements. From this it is reasonable to

conclude that a higher muscle tension, caused by higher frequencies of excitation of some muscles at least, necessarily accompanies a greater angular movement of the eyecup.

Muscle activity during optokinetic nystagmus

Description of muscle activity during horizontal optokinetic movements will be divided into two parts : 1) Clockwise rotation of the drum in which the slow phase movement of the right eyecup is away from the mid-line and its fast phase towards the mid-line; 2) Anti-clockwise drum rotation where the eyecup movements are reversed. All recordings were made from the right eyecup.

Two types of responses are recorded from each muscle block. The first is a steady tonic activity present both when the eyecup is stationary and when moving. This could be caused by the discharge of what is usually called a 'slow' axon. The second is a bursty or phasic activity usually present only when the eyecup is moving. This could be caused by the discharge of a 'fast' axon. There is thus at least a dual innervation of each muscle, and the fact that the two responses are often recorded from distinct and separate areas of each muscle, suggests that the two axons innervate separate groups of muscle fibres.

The optokinetic nystagmus recorded in different crabs varies considerably, both in time course, and in the frequency of activity recorded from the various eyecup muscles. The results presented are thus averages of very many responses recorded from some 200 crabs.

1) Clockwise drum rotationMuscle 20a.

Tonic activity is present in this muscle while the eyecup is stationary, and the frequency of this depends upon the horizontal position of the eyecup in space. During a slow forward phase of nystagmus the regularly occurring muscle potentials increase in frequency to reach a maximum of 50/sec. about mid-way through the full swing of the eyecup (Fig. 12). This frequency is then maintained until 50 msec. before the next fast phase when the activity is centrally inhibited and suppressed for the duration of this fast phase. Immediately after the fast phase the muscle potentials resume at three quarters of their maximum height and gradually increase to full amplitude as the frequency again builds up during the next slow phase.

Phasic activity in this muscle is usually not recorded in the absence of any known stimuli but sometimes 'spontaneous' muscle potentials at very low frequency are recorded. During a slow phase of nystagmus activity does not begin immediately after the fast phase but only when tonic activity has already reached a high level [FIG 13]. The muscle potentials are initially barely visible above noise but grow in amplitude throughout the slow phase. This amplitude increase is not caused by the contraction of the muscle pulling

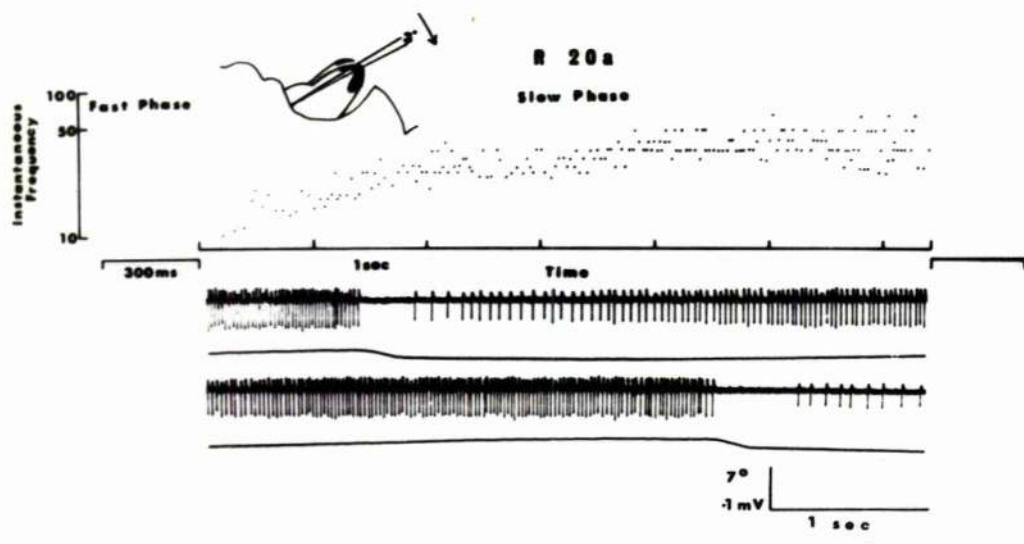


FIG. 12.

Tonic activity recorded from muscle 20a during a slow phase of nystagmus away from the mid-line and a fast return phase toward the mid-line. The graph shows the relationship between instantaneous muscle potential frequency (the reciprocal of the interval between one potential and the next) plotted on a logarithmic scale, and the instant of time to which this refers. Each frequency measurement is referred to the instant of time half way between the two potentials (cf. Pringle & Wilson 1952). The time scale for the fast phase is expanded and the inset shows the extent and direction of the slow phase.

The lower traces show the extracellular muscle potential record from which the graph has been plotted, together with the simultaneously recorded eye movement.

Activity in other muscles is similarly presented.

The muscle potentials here show a gradual increase in frequency with little growth in amplitude in the slow phase but are centrally inhibited during the fast phase only to resume at the start of the next slow phase.

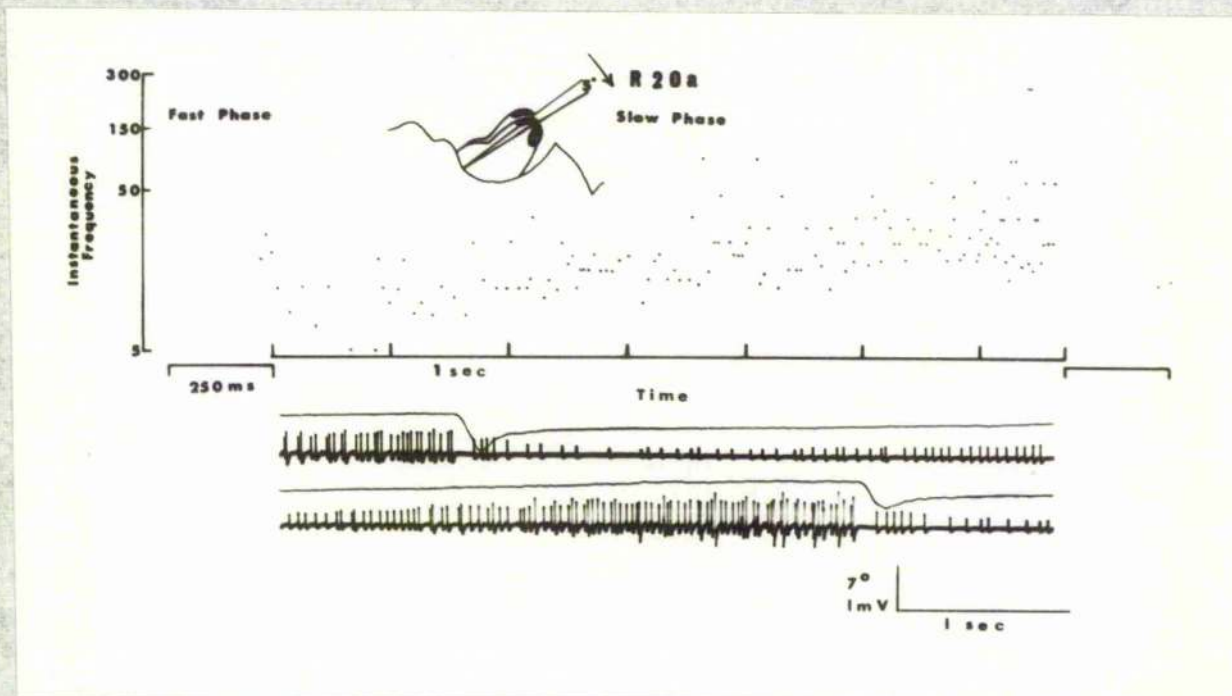


FIG. 13.

Phasic activity of muscle 20a. The increase in the frequency of muscle potentials is here more uneven and their growth in amplitude by neuromuscular facilitation is more marked than that shown by the tonic muscle potentials. The potentials are only recorded when tonic activity has already reached a high level and activity is again centrally inhibited during the fast phase. The group of large muscle potentials which occur after the fast phase are associated with the initial spurt in the movement of the eyecup. These potentials then antifacilitate before increasing once again during the next slow phase.

the electrode closer to the active fibres but is due to neuromuscular facilitation. The amplitude of the muscle potentials shows a regular dependence on previous activity (Harmont and Wiersma 1938) and the extent of facilitation is dependent both on the length of this activity and its frequency. A closely spaced pair of nerve impulses results in a conspicuous augmentation of the second muscle potential and there is a positive relationship between the interval between two potentials and the increase in size of the second.

The muscle potentials also show a gradual but erratic increase in frequency, but the maximum frequency of 100/sec. is not reached until near the end of the slow phase. Like the tonic activity, the phasic activity is centrally inhibited just before and throughout the fast phase.

After the fast phase a group of muscle potentials may occur which are approximately half the amplitude of the last ones of the preceding slow phase but are considerably larger than the ones of the next slow phase which follow after a short interval. This group of muscle potentials are associated with the initial rapid movement of the eyecup. They then anti-facilitate down to noise level before once again facilitating and increasing in frequency during the next slow phase. The eyecup movement similarly decreases in speed after the initial spurt. At high frequencies there is a tendency for the muscle potentials to occur in groups and in pairs (FIG 14), indicating

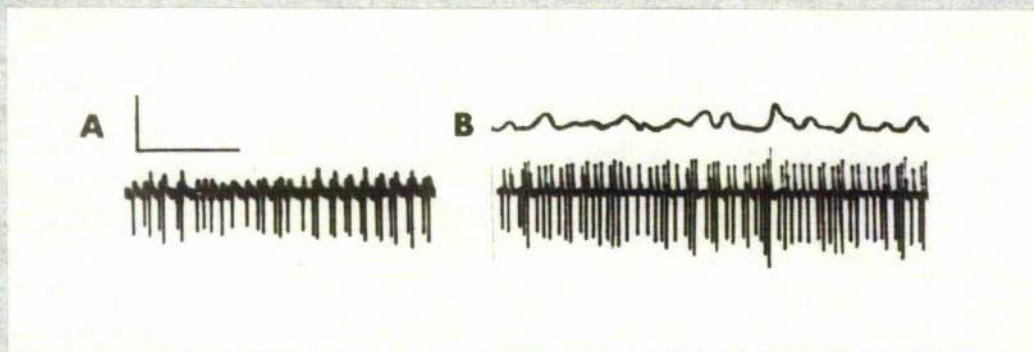


FIG. 14. Phasic activity recorded in muscle 20a during a slow phase of nystagmus. (A), pairing of the muscle potentials; (B), grouping of potentials associated with tremor movements of the eyecup (upper trace). Scale 1mV and 0.4° ; time $\frac{1}{2}$ sec.

a temporal patterning of the 'fast' motor output to this muscle. The pattern is not regular but consists of an irregular train containing an unusually high number of closely spaced groups and doublets. This tendency of the muscle potentials to occur in groups shows itself in an increase in scatter in the instantaneous frequency curve at the higher frequencies. Directly associated with the groups and pairs of muscle potentials are uneven tremor movements of the eyecup [FIG 14]. In fig. 10 the tremor during a slow phase shows a gradual increase in both frequency and amplitude. This increase is apparently caused by the gradual increase in the irregular frequency of phasic activity in this muscle.

Muscle 22

The tonic activity in muscle 22 is rather uneven as shown by the large amount of scatter in the early part of the instantaneous frequency curve [FIG 15]. As the eyecup moves through the slow phase the frequency of the muscle potentials increases slightly from 10-20/sec. to 20-5/sec. At the higher frequency there is less scatter in the instantaneous frequency curve. Activity is contrally inhibited just before the fast phase but is suppressed for only the first 100 ms of the 500 ms long fast phase. During the latter two thirds of the fast phase a group of muscle potentials occur at a frequency of 25-50/sec. which is higher than the frequency achieved during the slow phase. The muscle potentials show little amplitude change due to facilitation.

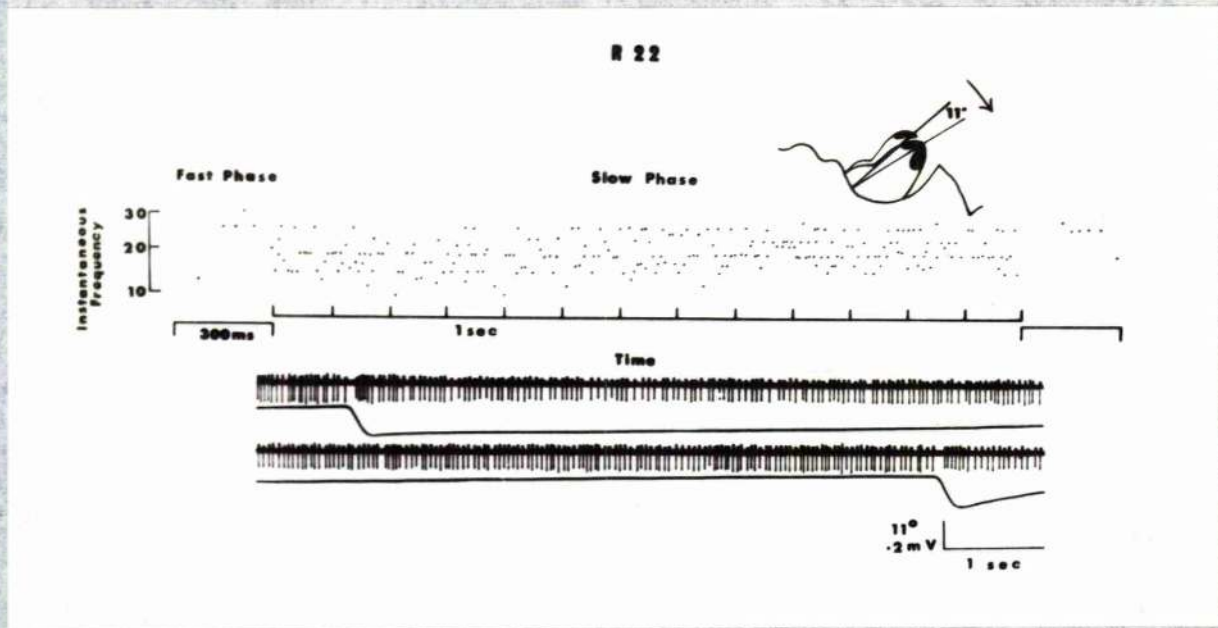


FIG. 15.

Tonic activity of muscle 22. The muscle potentials show little facilitation but gradually increase in frequency during the slow phase only to be centrally inhibited at the start of the fast phase. They resume at a higher frequency during the last $\frac{2}{3}$ of the fast phase. There is a greater scatter in the instantaneous frequency curve at the lower firing frequencies.

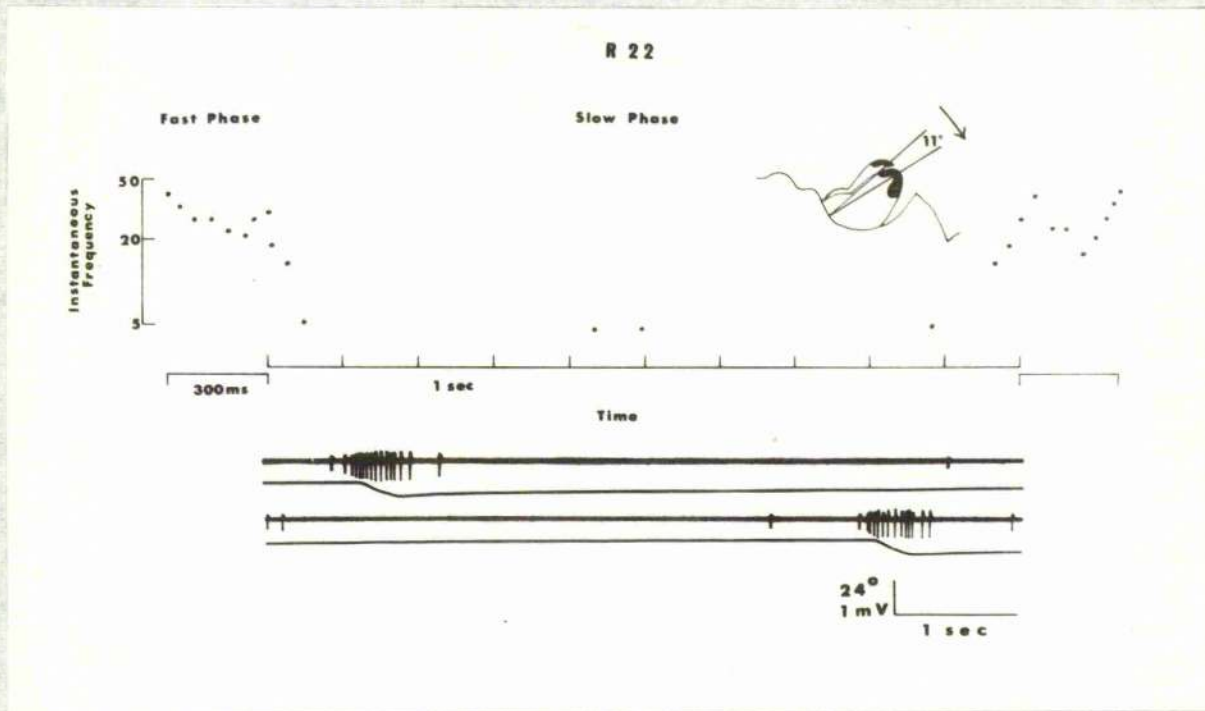


FIG. 16.

Phasic activity in muscle 22 during the slow phase is limited to a few potentials at a low frequency. 100 ms before the start of the fast phase a burst of facilitating potentials occurs which reach a peak frequency of 50/sec. and decline slowly during the first 200 ms of the next slow phase.

During the greater part of the slow phase, phasic activity in muscle 22 is limited to a few muscle potentials at a low frequency. However about 200 ms before the fast phase a burst of muscle potentials is recorded which increase in frequency and also in amplitude [FIG 16]. The burst, which approaches a peak frequency of 50/sec. lasts for the duration of the fast phase and the antifacilitating muscle potentials may then persist for the first 200 ms of the next slow phase.

Muscle 23a

Tonic activity in muscle 23a is more regular than that in muscle 22, as shown by the small scatter in the instantaneous frequency curve. Throughout the slow phase the muscle potentials decline in frequency, from 30/sec. to 15/sec. [FIG 17]. Activity is not centrally inhibited during the fast phase but increases in amplitude and in frequency to reach a peak of 60/sec. The frequency then declines gradually once more during the next slow phase.

Phasic activity in this muscle is completely absent during the slow phase but at the beginning and at the end of the fast phase a short burst of muscle potentials which show much facilitation is recorded. The bursts shown in FIG 18 consist of only two potentials but the number is variable and the two bursts may merge into one. The initial frequency of 20-30/sec. is always the higher.

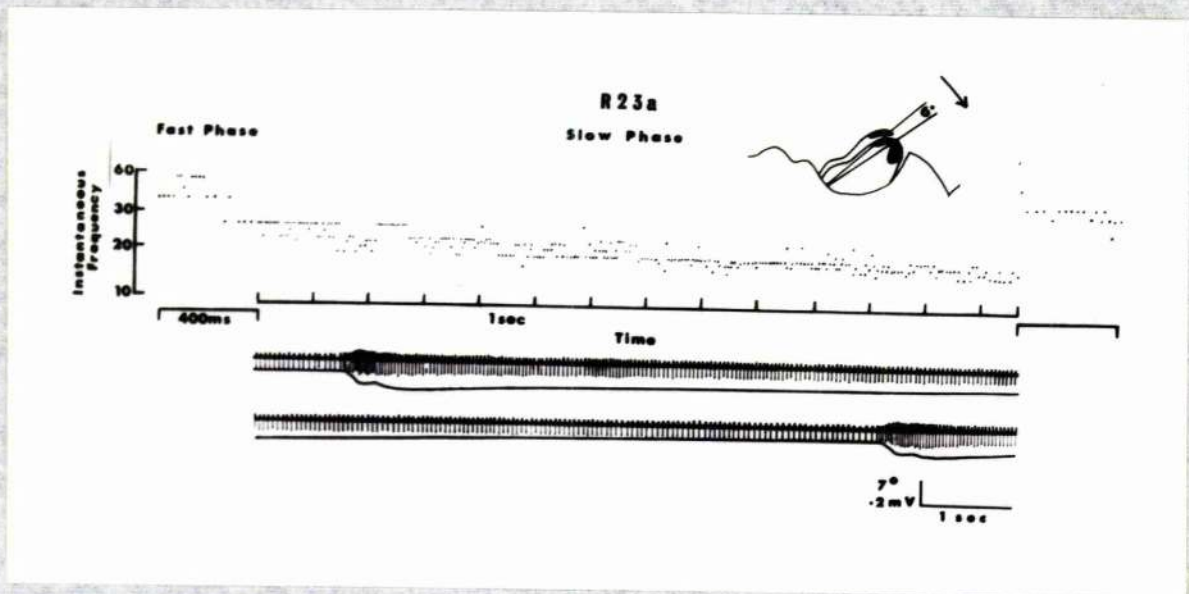


FIG. 17.

Regular tonic activity of a declining frequency recorded in muscle 23a. There is little scatter in the instantaneous frequency curve. Activity during the fast phase is not inhibited but is greatly increased.

The eyecup movement during the fast phase occurs in two jumps, a less common occurrence than the normal single jump.

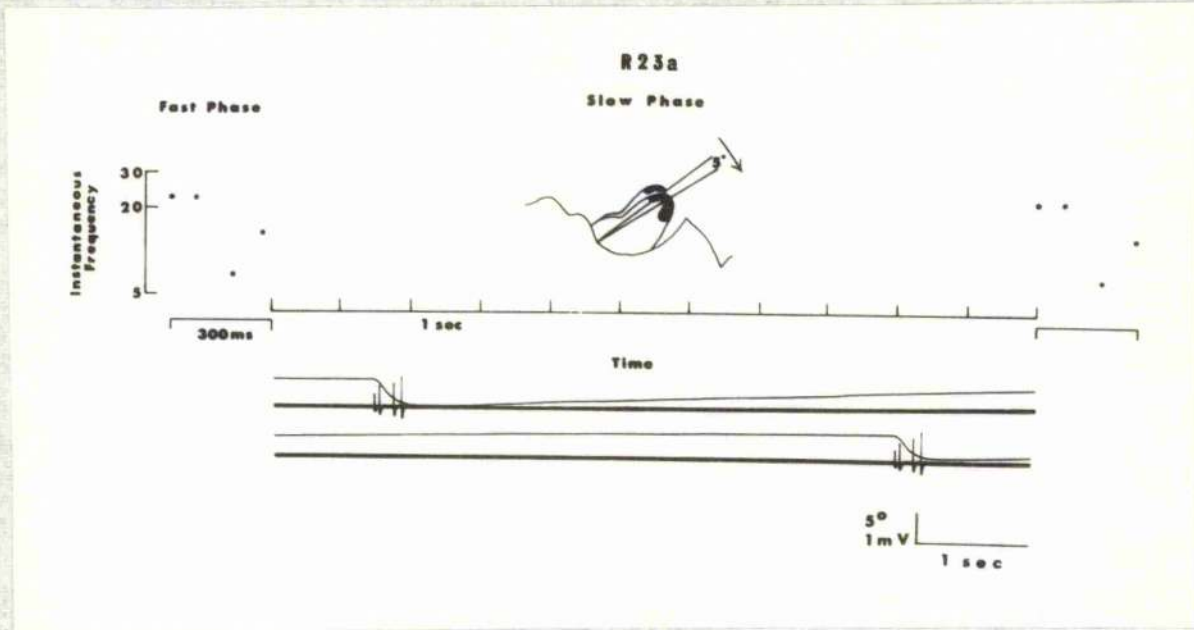


FIG. 18.

Phasic activity in muscle 23a is limited to two short bursts of muscle potentials during the fast phase. The potentials show much facilitation but the form of the bursts is variable and they may often merge into one.

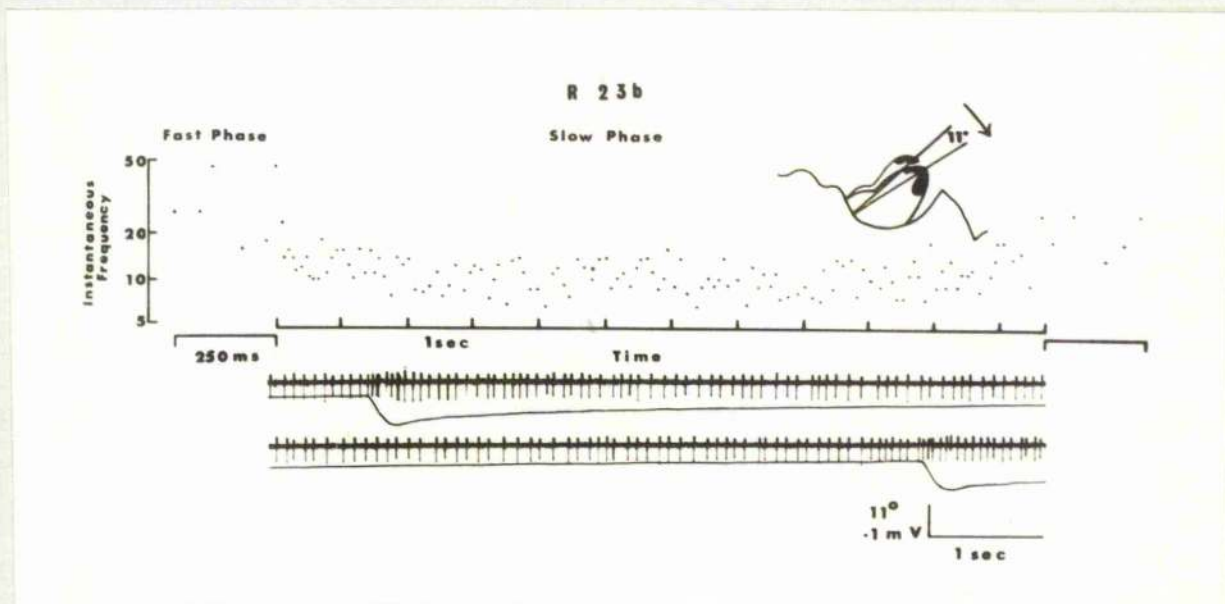


FIG. 19.

No trends are apparent in the tonic activity of muscle 23b during a slow phase. The low frequency is associated with much scatter in the instantaneous frequency curve and there is a suggestion of an oscillation with a period of 1 sec. During the fast phase the frequency is raised by the addition of a few potentials.

muscle 23b

No trends are apparent in the frequency profile of tonic activity of muscle 23b during a slow phase (FIG 19). Activity is rather irregular with a frequency of 10/sec. and there is a good deal of scatter in the instantaneous frequency curve sometimes with a faint suggestion of an oscillation with a period of 1.5 sec. During the fast phase a few muscle potentials are interjected raising the frequency to 2-50/sec. Activity is thus continuous throughout the whole cycle.

Phasic activity is usually absent in this muscle during clockwise rotation of the drum but occasionally a few potentials at a very low frequency may occur.

muscle 19b

Like that in muscle 23b, tonic activity in muscle 19b occurs throughout the cycle (FIG 20). During the slow phase the frequency declines very gradually from 20/sec. to 15/sec. with a corresponding increase in scatter in the instantaneous frequency curve. At the onset of the fast phase the frequency rises to 30/sec. and stays at that frequency for the duration of the fast phase. It then declines again during the course of the next slow phase.

Although it was possible to record tonic activity in this muscle by itself it was never possible to record phasic activity without recording the tonic activity as well (FIG 21).

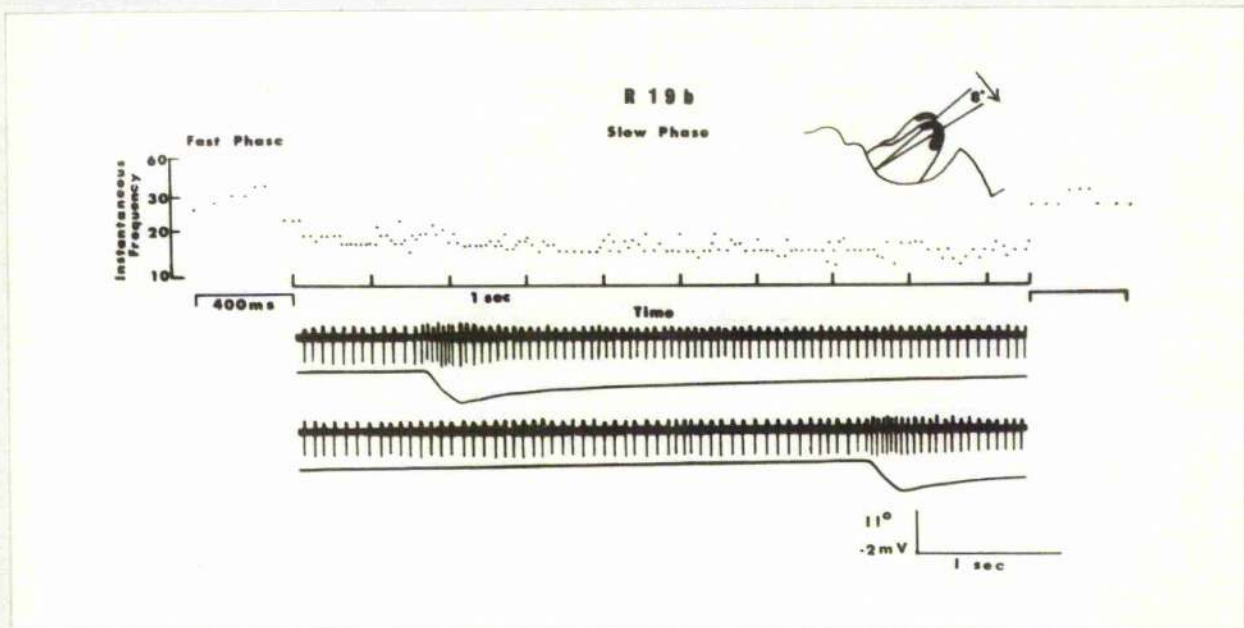


FIG. 20.

Tonic activity is present in muscle 19b during the whole optokinetic cycle. During the slow phase the frequency declines gradually from 20-15/sec. but during the fast phase the frequency is raised to 30/sec. Again there is increased scatter in the instantaneous frequency curve at the lower frequencies.

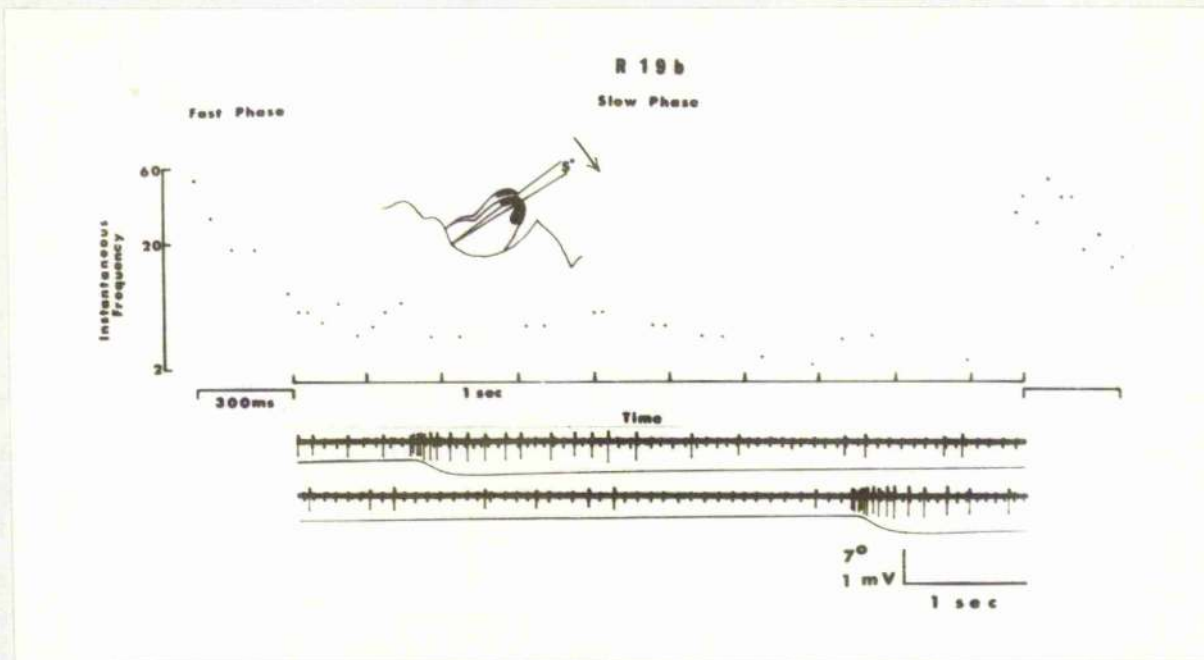


FIG. 21.

A burst of phasic activity in muscle 19b. precedes the onset of the fast phase. The frequency falls rapidly during the fast phase but persists at an ever declining frequency during the slow phase. The muscle potentials show little facilitation and tonic activity (the small potentials) is always recorded together with phasic activity.

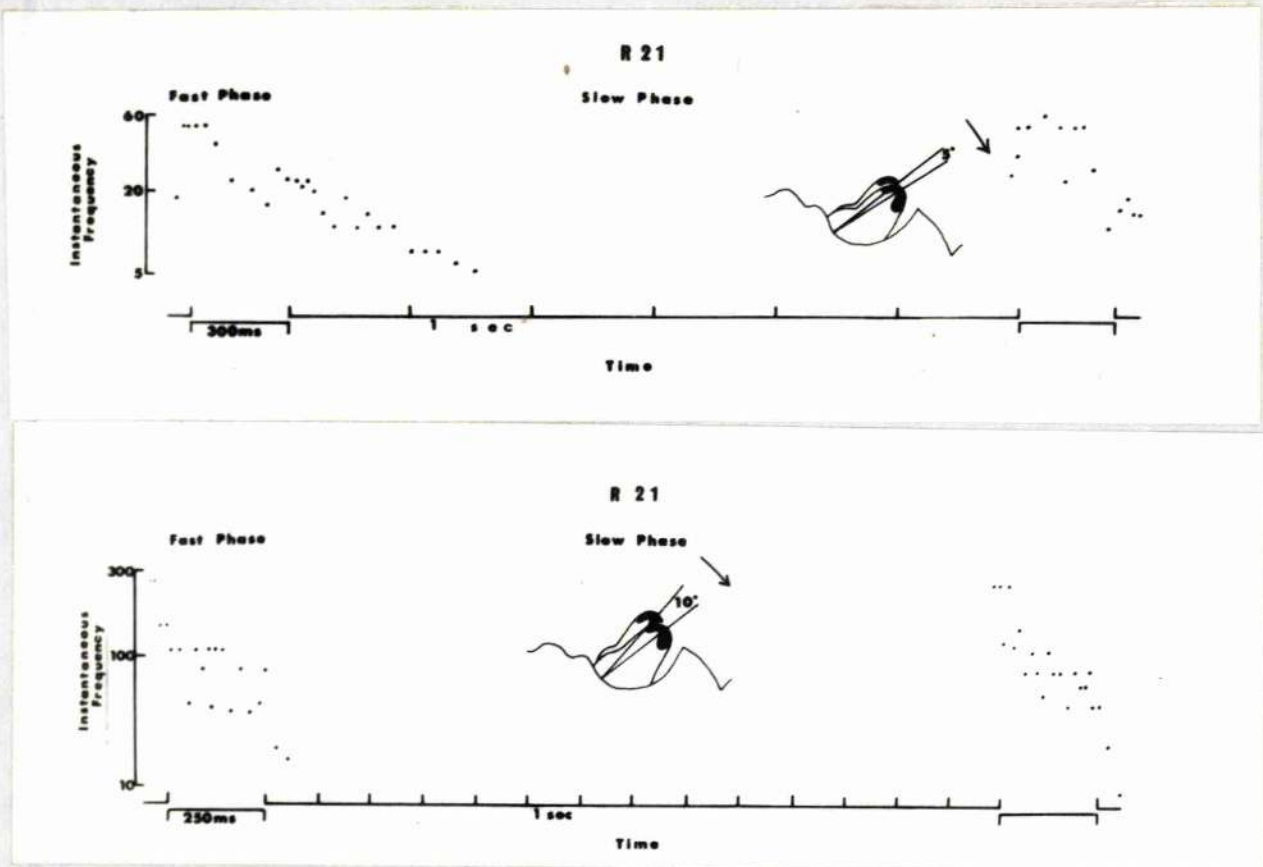


FIG. 22.

Tonic (upper) and phasic (lower) responses recorded from muscle 21. Tonic activity begins at the onset of the fast phase and reaches a frequency of 60/sec. It then declines slowly during the remainder of the fast phase and the first quarter of the next slow phase. Likewise phasic activity begins at the onset of the fast phase but reaches a higher frequency which declines abruptly at the end of the phase, and is absent during the next slow phase.

100 ms before the onset of the fast phase a burst of phasic muscle potentials occurs. The peak frequency of 60/sec. is reached just before the start of the fast phase of movement and then activity declines rapidly during the fast phase but continues at an ever declining frequency during the next slow phase. Unlike the phasic muscle potentials recorded in the other muscles, the ones here show little amplitude change due to facilitation.

muscle 21

Muscle 21 is not active during the slow phase movement away from the mid-line, but is extremely active during the fast return phase (FIG 22). At the onset of the fast phase a burst of tonic activity occurs at a frequency of 50/sec. This activity declines slowly during the remainder of the fast phase and during the first quarter of the next slow phase. Tonic activity is then completely absent until the start of the next fast phase. The term "tonic" used here in describing this activity will be justified when a slow phase toward the mid line is considered.

Phasic activity begins 50-100 ms before the start of the fast phase and occurs at frequencies which initially reach 200 c/s. The activity is maintained at 100 c/s but then declines rapidly before the end of the fast phase. No phasic activity is recorded during the slow forward movement.

Muscle 19a

Many penetrations of muscle 19a, both with extracellular

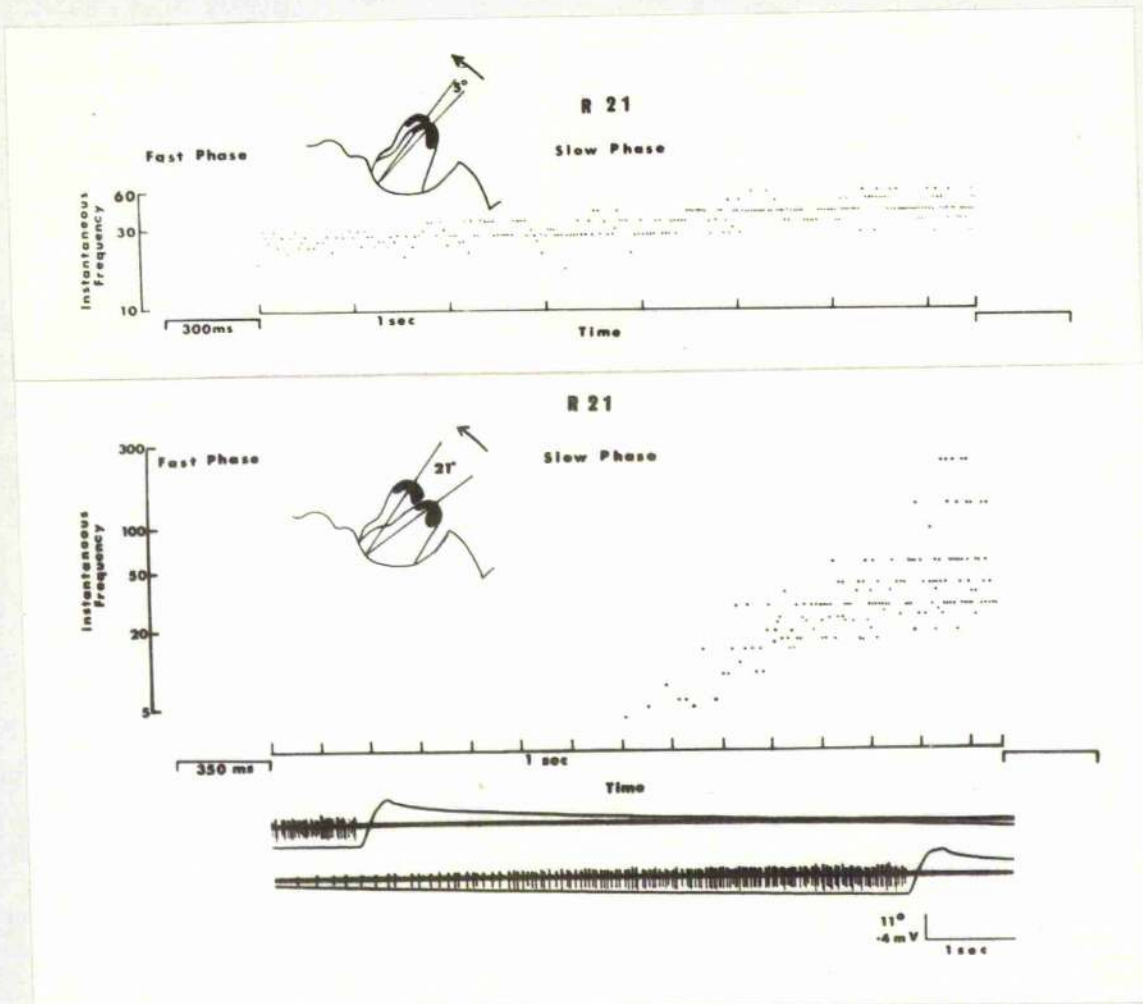


FIG. 23.

Tonic (upper) and phasic (lower) activity recorded from muscle 21 during a slow forward phase toward the mid-line and a fast return phase away from the mid-line. During the slow phase the tonic potentials show an even increase in frequency and are centrally inhibited during the fast phase. Phasic activity begins only when the eye movement is half completed and shows an erratic increase in frequency up to 200c/sec. Scatter in the instantaneous frequency curve for phasic activity increases at the high frequencies.

and intracellular electrodes have failed to reveal any electrical activity during the slow and fast phase movements.

2) Anti-clockwise drum rotation

The same muscles as before are involved in these movements in the opposite direction, but the motor pattern is not merely reversed but involves a completely different pattern.

Muscle 21

Tonic activity in muscle 21 is present in a stationary eyecup and its frequency, like that in muscle 20a, depends on the horizontal position of the eyecup in space. During a slow phase of nystagmus the activity increases from 20-40/sec. (Fig. 23). At the start of the fast phase, the activity is centrally inhibited and suppressed for the duration of the phase. Afterwards the muscle potentials resume at their previous amplitude and facilitation is not evident.

Phasic activity begins only when the tonic activity has reached a high frequency and when more than half of the eyecup movement has occurred. The start of this phasic activity is correlated with the increase in eyecup tremor (FIG 10). The muscle potentials show much facilitation and an uneven increase in frequency to a peak of 200 c/s before being centrally inhibited at the start of the next fast phase. As for the phasic activity of muscle 20a during the opposite movement, the muscle potentials tend to occur in groups and

doublets. A patterned output to this muscle is thus also indicated. The scatter in the instantaneous frequency curve shows an increase as the frequency of firing increases. This is in direct contrast with the curves for tonic activity which show an increased scatter as the firing frequency decreases. Directly associated with the groups of muscle potentials are small tremor movements of the eye cup.

Muscle 19b

Tonic activity in muscle 19b occurs at the relatively uniform frequency of 15-20/sec. but increases slightly towards the end of the slow phase (FIG 24). The activity is centrally inhibited just prior to and for the first 100 ms of the fast phase. The muscle potentials then resume at their previous frequency and amplitude increase due to facilitation is not evident.

Phasic activity in this muscle increases gradually in frequency during the slow phase to reach a peak frequency of 20/sec. mid-way through the traverse (FIG 25). This frequency is then maintained until the start of the fast phase when activity is centrally inhibited. The first muscle potential of the slow phase occurs $\frac{1}{2}$ sec. after the completion of the fast phase and is already $\frac{2}{3}$ of its final amplitude, so that further increase in amplitude is small.

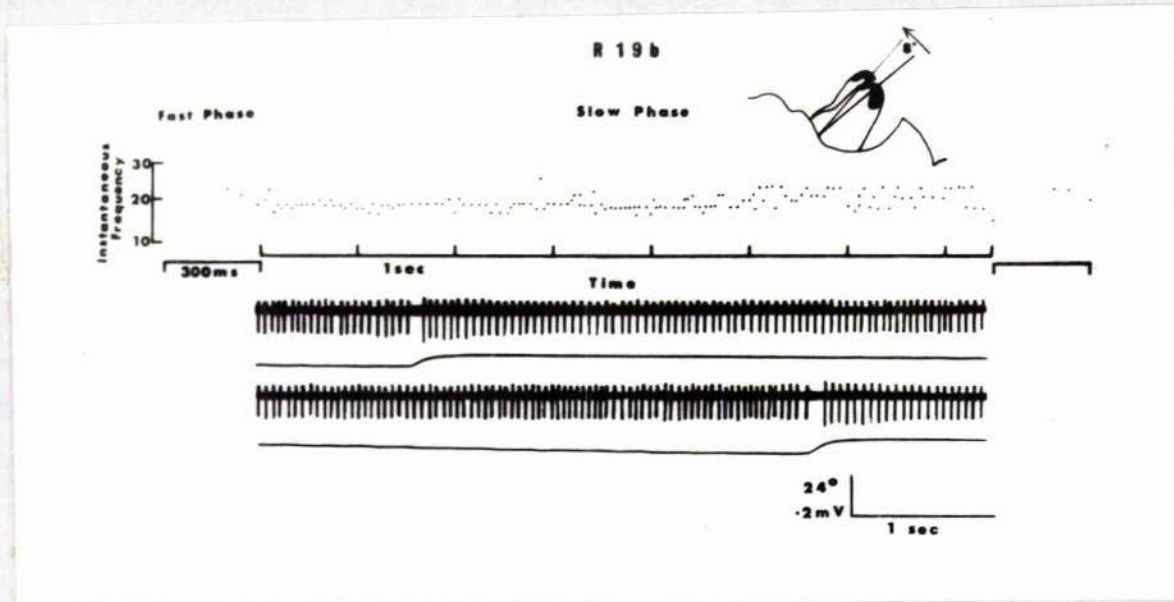


FIG. 24.

During most of the slow phase tonic activity in muscle 19b occurs uniformly at 15-20/sec., but towards the end may increase slightly. Activity is then centrally inhibited for the first third of the fast phase but then resumes as before.

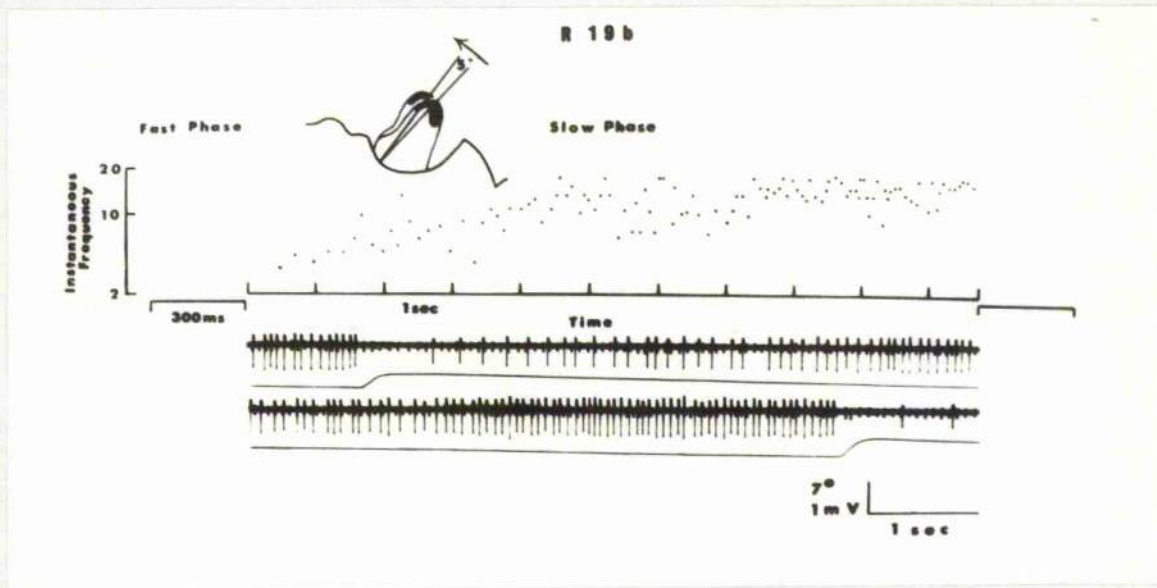


FIG. 25.

The phasic activity in muscle 19b shows a steady increase in frequency as the slow phase precedes but the maximum frequency of about 20/sec is reached half way and is then maintained until the end. Central inhibition of the activity occurs just before the onset of the fast phase.

The small unit on the muscle potential trace is the tonic activity.

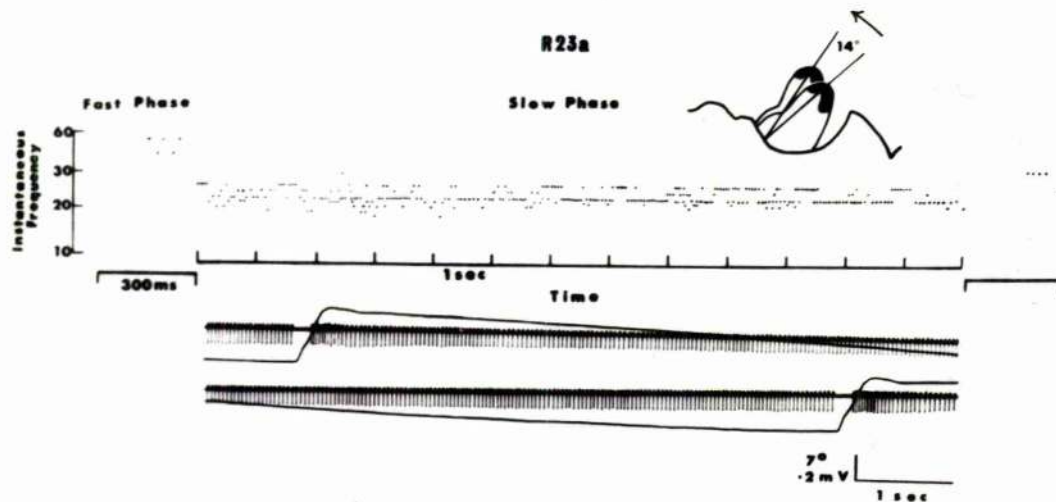


FIG. 26.

Tonic activity in muscle 23a is maintained at a relatively constant frequency of 20-25/sec throughout the slow phase. Activity is centrally inhibited for the first half of the fast phase, but increases in frequency during the second. The instantaneous frequency curve shows little scatter and facilitation of the muscle potentials is not evident.

Muscle 23a

No trends are apparent in the frequency profile of the tonic activity in muscle 23a (FIG 26). Throughout the slow phase a frequency of 20-25/sec. is maintained with little scatter in the instantaneous frequency curve. Once again the activity is centrally inhibited 100 ms before the start of the fast phase and is suppressed for 150 ms of this phase. During the latter half of the fast phase the tonic frequency is raised to 50/sec. but this then declines rapidly to 20/sec. during the next slow phase.

Phasic activity in this muscle is usually completely absent during both the slow and fast phases though occasionally a few muscle potentials occurring at low frequency are recorded.

Muscle 23b

The tonic activity in muscle 23b again shows no obvious trend in frequency (FIG 27). Throughout the slow phase the activity occurs at the low frequency of 10-15/sec. with much scatter in the instantaneous frequency curve. During the first half of the fast phase activity is centrally inhibited but shows a slight increase in frequency during the second.

Phasic activity is absent during the slow phase but at the end of the fast phase a burst of rapidly facilitating muscle potentials occurs (FIG 28).

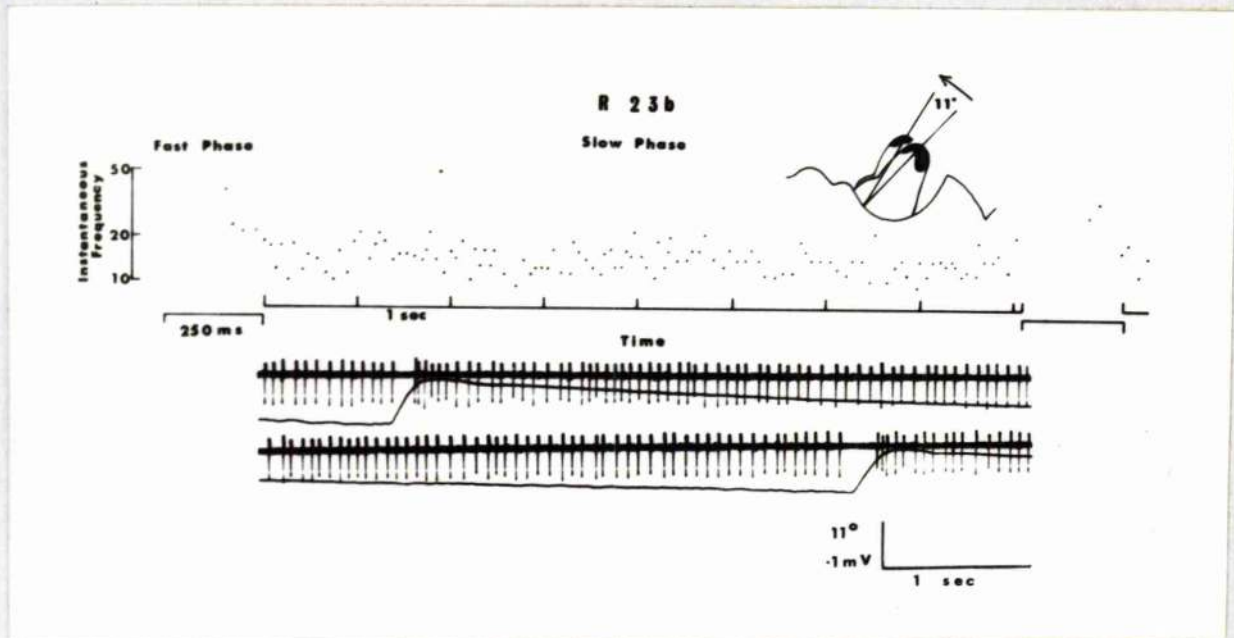


FIG. 27.

Tonic activity in muscle 23b occurs at a low, uneven frequency throughout the slow phase. As in this muscle's activity in movements in the opposition direction there is a faint suggestion of an oscillation. Activity is centrally inhibited during the first half of the fast phase, but resumes at a raised frequency during the second.

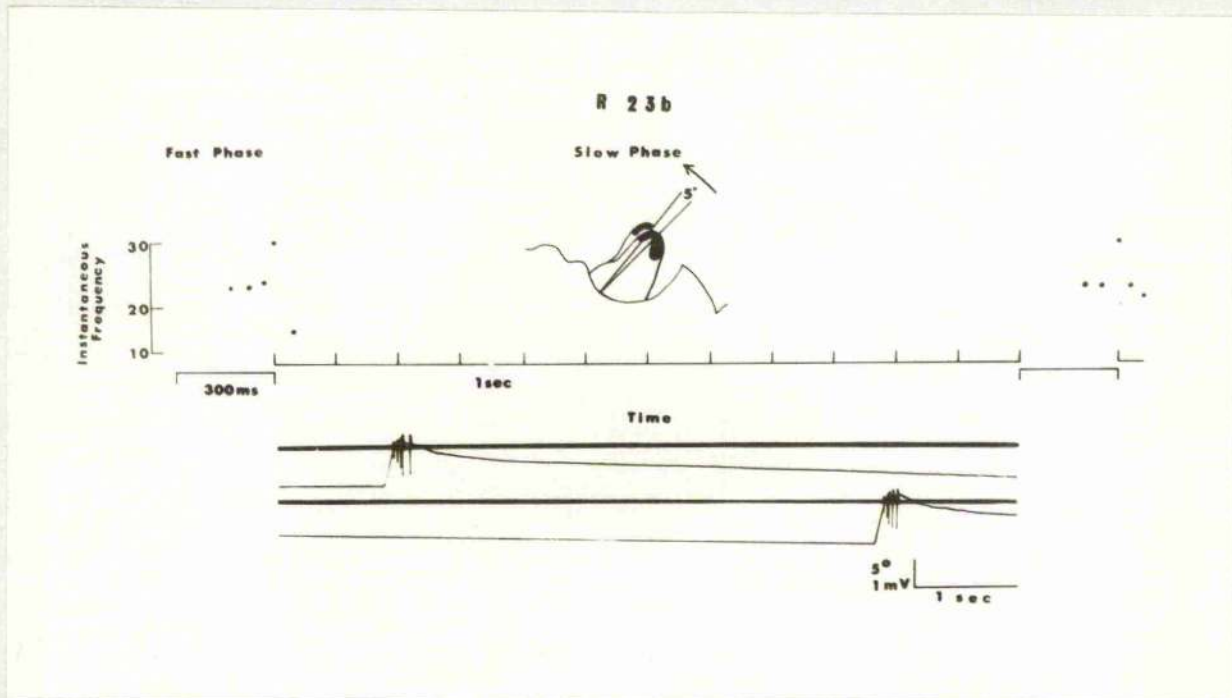


FIG. 28.

Phasic activity in muscle 23b is limited to a short burst of usually 5 muscle potentials at a low frequency which occurs towards the end of the fast phase movement. The burst cannot be responsible for bringing about the fast phase movement and it is suggested that it may serve to decelerate this movement.

The burst usually consists of five muscle potentials at a frequency of 20-30 c/s and lasts for only 200 ms. It occurs only when the fast phase movement is almost complete and cannot thus be responsible for bringing about the movement. No resultant eye-cup movement can be correlated with this activity and it is possible that the fibres are contracting in order to oppose the fast phase movement and hence decelerate it.

Muscle 22

As for the last two muscles described, the tonic activity of muscle 22 shows no trends in its frequency during the slow phase (FIG 29). The activity is uneven and varies in frequency between 10 and 20/sec. However, during the fast phase the activity is not inhibited but is considerably raised in frequency to 50/sec. This then declines slowly during the next slow phase.

Phasic activity during the slow phase is limited to the occurrence of a few muscle potentials at a low frequency (FIG 30). During the fast phase, however, there is a burst of a few facilitating muscle potentials at a frequency of about 20 c/s. The burst again occurs only after the fast phase movement has been initiated. Thus while assisting the movement this muscle cannot cause the onset of the movement.

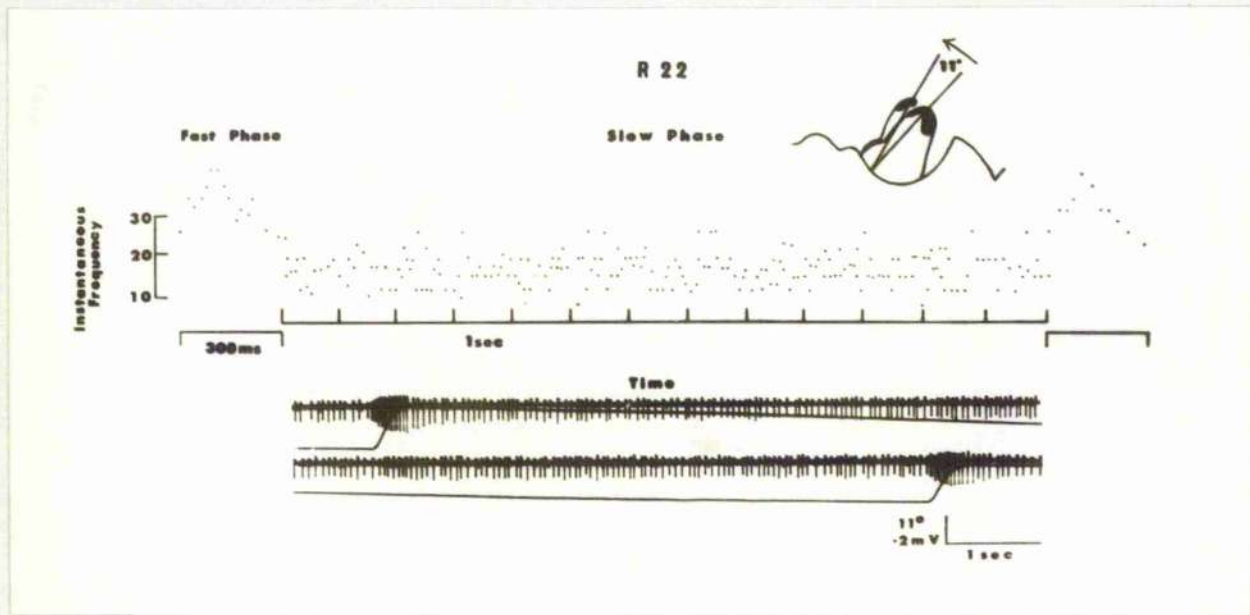


FIG. 29.

Tonic activity in muscle 22 occurs at a rather uneven frequency during the slow phase and no trend is apparent in the frequency profile. Activity is not inhibited during the fast phase but is increased both in frequency and amplitude.

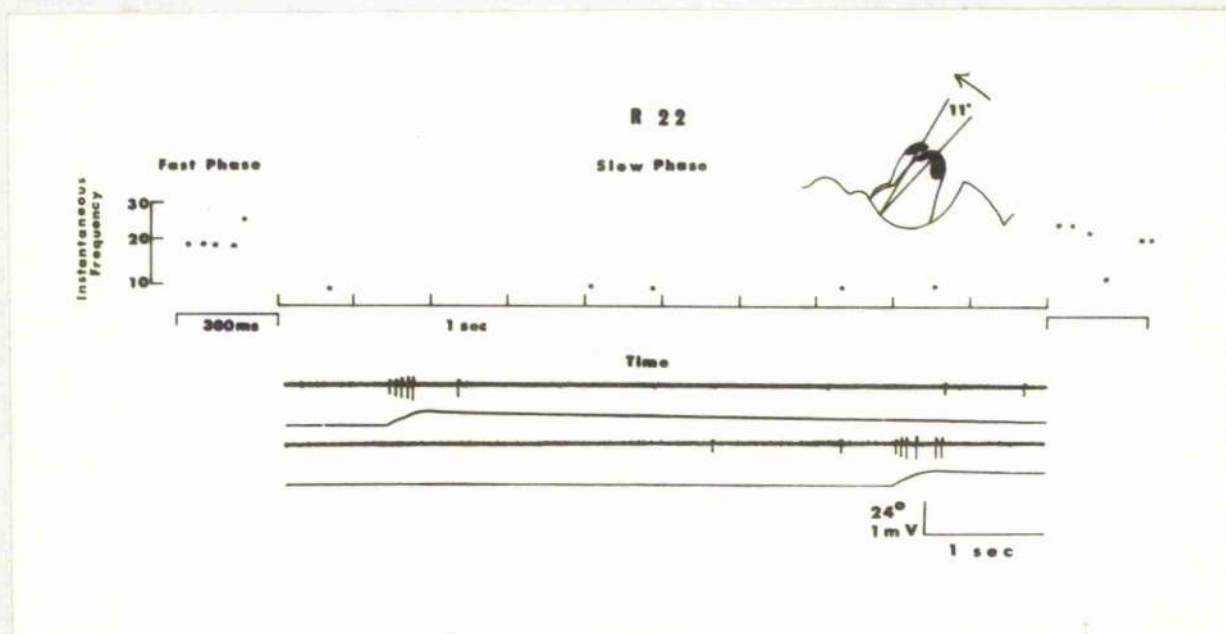


FIG. 30.

Phasic activity in muscle 22 is limited during the slow phase to the occurrence of a few potentials at a very low frequency. During the fast phase, however, there is a low frequency burst at 20 c/s of a few facilitating muscle potentials. The burst occurs only after the fast phase movement has been initiated.

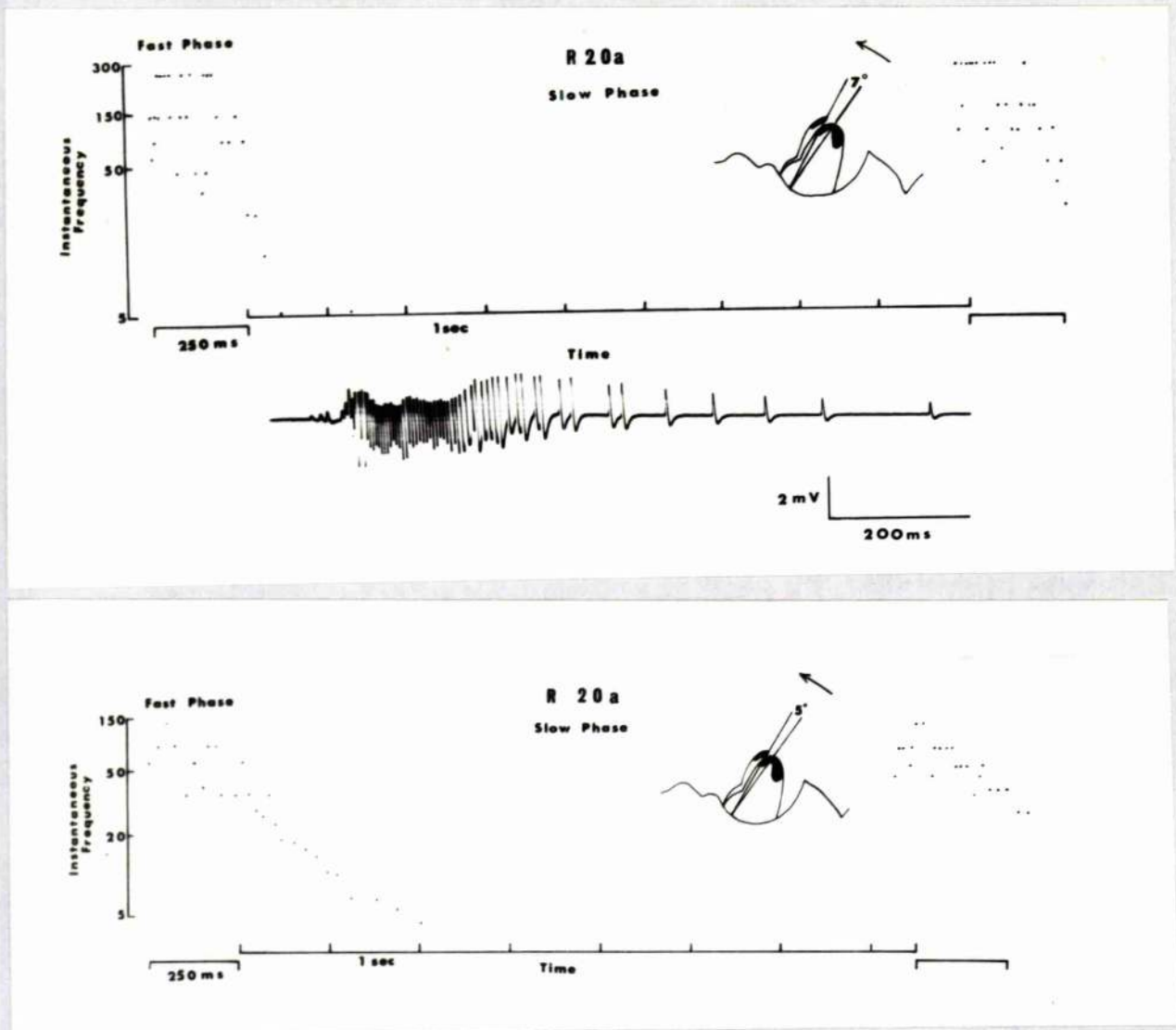


FIG. 31.

Both phasic (upper) and tonic (lower) activity in muscle 20a is confined to the fast phase. Tonic activity begins just before the start of the fast phase and reaches a peak frequency of 50-100 c/sec during it, but then declines slowly during the next slow phase. Phasic activity (a typical burst is shown) reaches a peak frequency of 200 c/sec but then declines more abruptly.

muscle 20a

Activity in muscle 20a closely mirrors that of muscle 21 during opposite movements. The muscle is not active during the slow phase but is extremely active during the fast return phase (FIG 31).

Tonic activity begins just before the start of the fast phase and reaches a peak frequency of 50-100 c/s during this phase. The frequency then declines slowly during the first quarter of the next slow phase.

Phasic activity begins about 50 msec before the start of the fast phase movement and reaches a peak frequency of 200 c/s during this phase. The activity then abruptly declines.

Muscle 19a is again inactive in both the slow and fast phases.

Muscles 20b and 20c proved very difficult to record from with extracellular wire electrodes. The muscles are tiny, consisting of 8-10 fibres, and are closely opposed to other eyecup muscles. Thus although electrodes could be located in these muscles and activity sometimes recorded, there was always the possibility that this activity was merely spreading from the other muscles. However, it was possible to record from these muscles with intracellular electrodes but this technique involves clamping the eyecup, precluding simultaneous recording of eyecup movements. It is possible

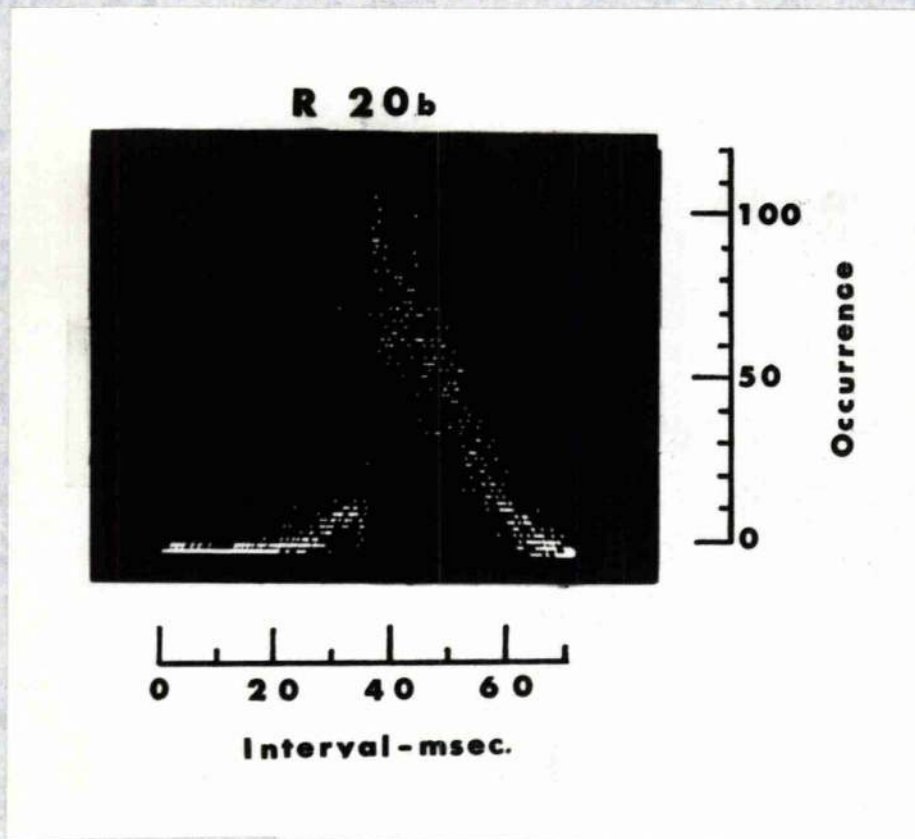


FIG. 32.

Interval histogram of the tonic activity in muscle 20b while the drum was stationary. The activity is irregular, even when the drum is stationary and changes little during horizontal optokinetic movements of the eyecup.

The histogram is plotted on a Biomac Special purpose computer (Data Labs. htd.).

to relate activity in these muscles with inferred eyecup movement by recording simultaneously from another muscle whose activity has previously been linked with eyecup movement. Phasic activity is absent in both 20b and 20c during nystagmic movements, but tonic activity is present. The activity is rather irregular in both and occurs at a frequency of 10-20/sec. throughout the cycle. It is apparently unrelated to the horizontal eyecup movement during a nystagmus. The irregularity of the activity is shown in an interval histogram (FIG 32) of the tonic activity of muscle 20b recorded when the striped drum was stationary.

The activity of all the muscles during clockwise and anticlockwise drum movements are summarized in FIGS 33 and 34.

During a slow phase away from the mid-line only muscle 20a shows a steady increase in activity but during movements towards the mid-line, two muscles, 19b and 21 show an increase. Muscles 20a and 21 show similar though by no means the same activity during movements in opposing directions. It is usual to call muscles which perform such opposite movements "antagonists". However the word "antagonist" implying active opposition, gives the incorrect impression that during movement of the eyecup in one direction, the muscles that move it in the other oppose this movement. This is not so, and the muscle not directly involved in movement relaxes. This is also true for most human muscle systems

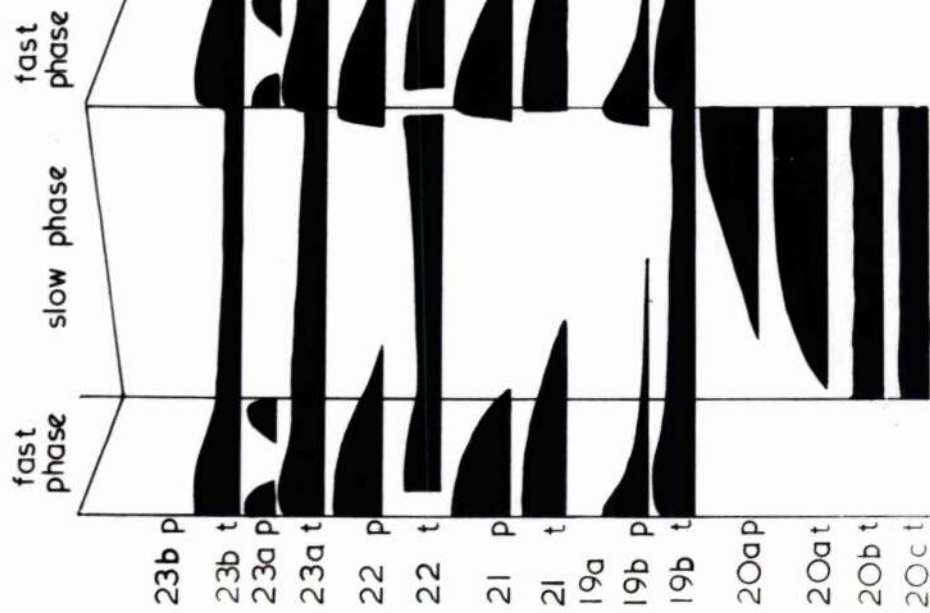


fig 33

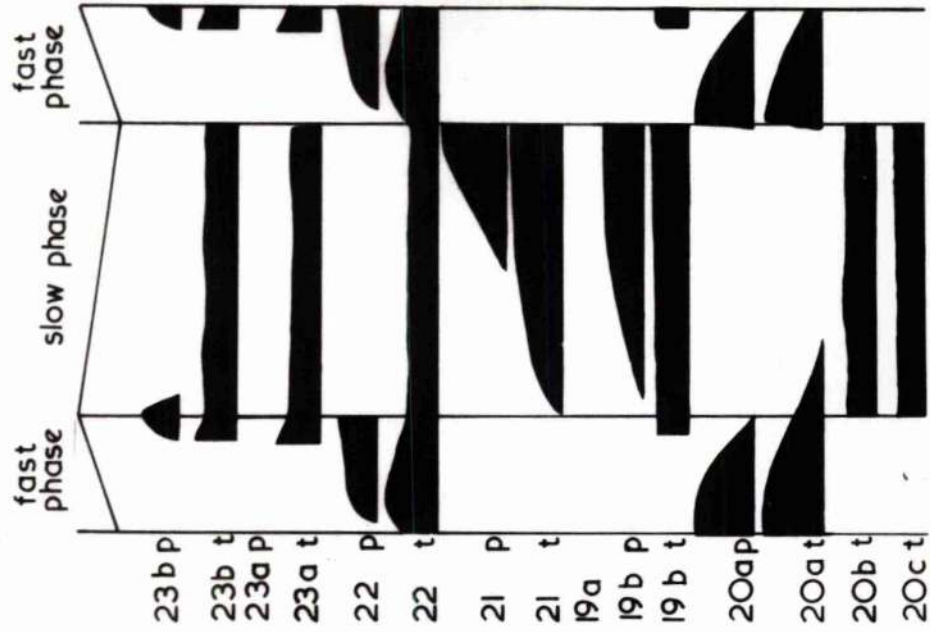
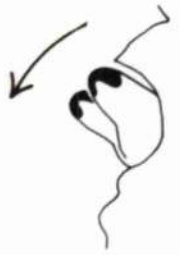


fig 34

FIG. 23 & 24.

Summaries of eye-cup muscle activity during a slow phase movement away from (fig. 23) and towards (fig. 24) the mid-line and fast return phases in the opposite directions, in response to horizontal movements of the drum. The frequency of muscle activity is represented vertically on an approximate logarithmic scale. The time scale of the fast phase is expanded relative to that for the slow phase.

examined (Basmajian 1962). It would perhaps be better to drop the term antagonist, at least for eyecup muscles, and replace it by synergist.

Phasic activity in the other muscles that is 22, 23a and b is limited to bursts of low frequency during the fast phases or a few potentials at very low frequency during the slow phases. The activity occurs only after the fast phase movement has been initiated and thus cannot be the prime cause of the movement. Phasic activity in muscle 23b occurs at the end of the fast phase and may be responsible for decelerating the movement.

Of the tonic activity in these muscles only that in muscle 22 is centrally inhibited during a fast phase, following a slow phase away from the mid-line. However, a fast phase in the opposite direction involves central inhibition of all other tonic activity but not that in muscle 22.

Apparently the effect of muscle activity on eyecup movement depends on the position of the eyecup at that moment. For example, during the slow phases, the tonic activity in muscles 23a and 22 show slight changes in frequency depending on the direction of movement. However it is not possible to detect long term changes of frequency in muscles 19b, 20b and c and 23b. This does not mean that the muscles do not

contribute to the movement of the eyecup. The eyestalk-eyecup joint is not a simple one and the pivot point is continually changing. Thus the activity which does not appear to change may contribute to the movement after the eyecup has moved a certain distance. Also the activity may be all that is needed to prevent torsion during a horizontal movement. It is thus not possible to ascribe a function to one muscle at any one time.

Muscle 19a is the only eyecup muscle which is completely inactive in all optokinetic movements but it is active during eyecup withdrawal.

3) Patterning of the motor output

The instantaneous frequency curves for phasic activity in muscles 20a and 21 show a greater scatter at the higher frequencies (FIGS 13, 23). On the other hand the curves for tonic activity show a greater scatter at the lower frequencies (e.g. FIG 27). Because the curves are plotted on a logarithmic scale the scatter at the low frequencies is emphasized while that at the high frequencies is more cramped. To overcome this difficulty the responses of muscle 21 are treated statistically, but ordinary statistical analysis can only be usefully interpreted if it is assumed that the records show no trends. By definition this is impossible for the phasic activity and so it is necessary to compromise between

samples so short that the number of data points is inadequate, and those so long, that long term changes of state are reflected in an appreciable drift in the variables being measured. Phasic activity during slow phase movements of the eyecup was thus divided into one second intervals and results averaged over many repetitions of the response. Long runs of tonic activity are possible, as each horizontal eyecup position is associated with a particular frequency of tonic activity in this muscle. The eyecup could thus be induced to move and held at certain positions by moving a striped drum through a small angle and then holding it at the new position. Even so there tends to be a long term drift of the eyecup back to a "preferred" position and records with such trends were rejected.

A quantitative measure of the spread of interval changes with a changed mean is given by plots of standard deviation from the mean against the mean itself. FIG 35 shows such a plot for the interval distributions of tonic activity in muscle 21. The points lie around a straight line indicating a regular relation between mean interval and the standard deviation from that interval. Thus low frequency activity is associated with more scatter, as in most nerve impulse trains examined (e.g. Werner and Mountcastle 1963, Bierderman-Thorson 1968). However the plot for phasic activity (FIG 36) shows a departure from this, in that the standard

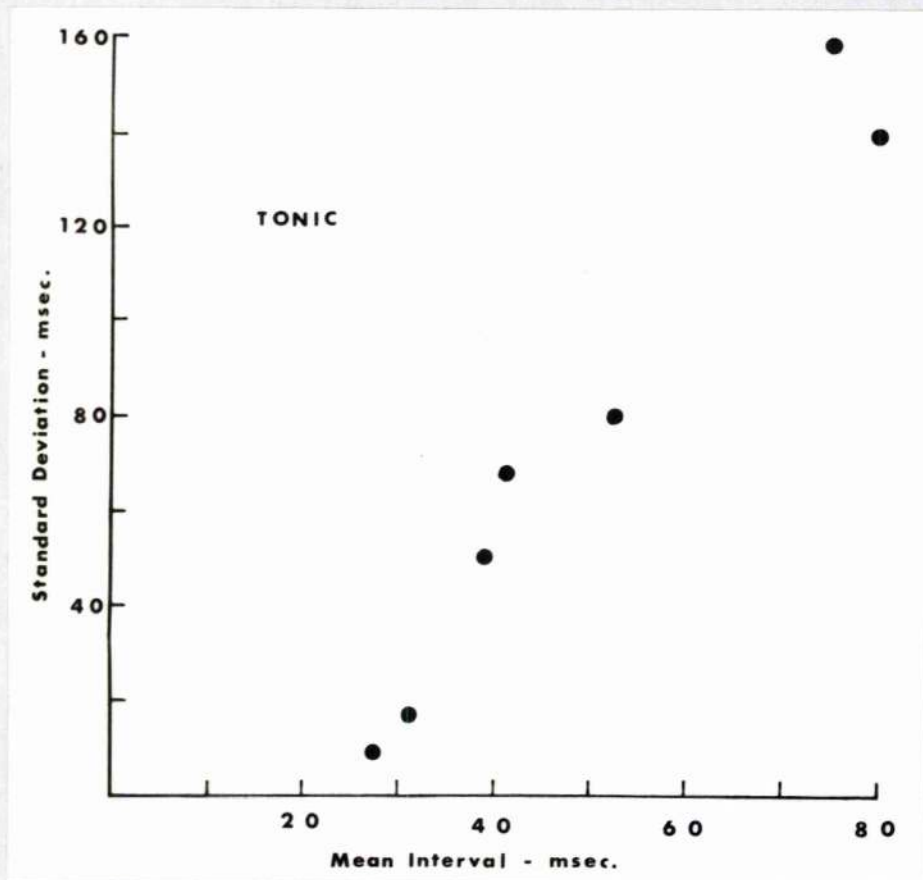


FIG. 35.

Plot of standard deviation versus mean interval for the tonic activity in muscle 21 at various eyecup positions. The increased scatter at low frequencies is typical for most trains of nerve impulses.

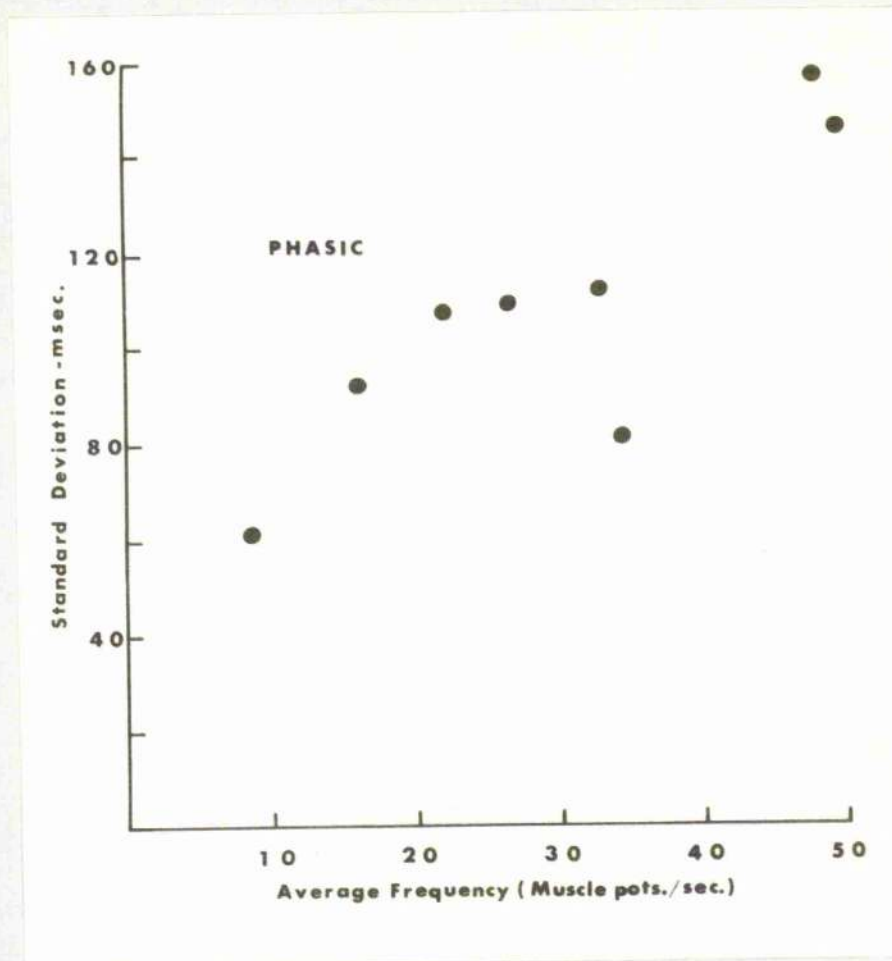


FIG. 36.

Plot of standard deviation versus mean frequency for phasic activity in muscle 21. Here scatter increases with increasing frequency which is the reverse of the situation for the tonic activity. It is the clustering of the muscle potentials at the high frequency which is responsible for the increased variance.

deviation increases with frequency and not with interval. Examination of the actual records (Fig. 23) shows that this increased variability at high frequencies does not simply represent increased randomness but reflects the addition of a new class of shorter intervals due to the grouping of the potentials. The significance of this patterned output will be dealt with in the discussion.

Small signal analysis

The two types of activity, called tonic and phasic, recorded during optokinetic movements are indicative of a dual innervation of each muscle. This is clearly seen in muscle 21 where both types of activity show an increase during a slow phase toward the mid-line, but with different relative time courses and frequencies (Fig. 23). In a further attempt to distinguish the properties of these two motor outputs, the drum was oscillated at small amplitudes and at various frequencies. Such analysis has several advantages (Thorson 1966a). 1) Because small signals are used, many of the non-linear mechanisms comprising the visual reflex may respond linearly over the limited range. 2) Low frequency noise in the input pattern is reduced if the amplitude of the pattern movement is small. 3) The response elicited is more consistent than that produced by constant-velocity drum rotation.

Both eyecups were cemented in their sockets and muscle potentials were recorded from muscle R20a with extracellular leads. Drum movements, which were monitored, of 0.01-10 c/s and from 0.03° - 0.43° peak to peak were used.

At drum oscillations of 0.1 c/s with an amplitude of 0.15° p.p. tonic activity shows a phase lead of 20° over

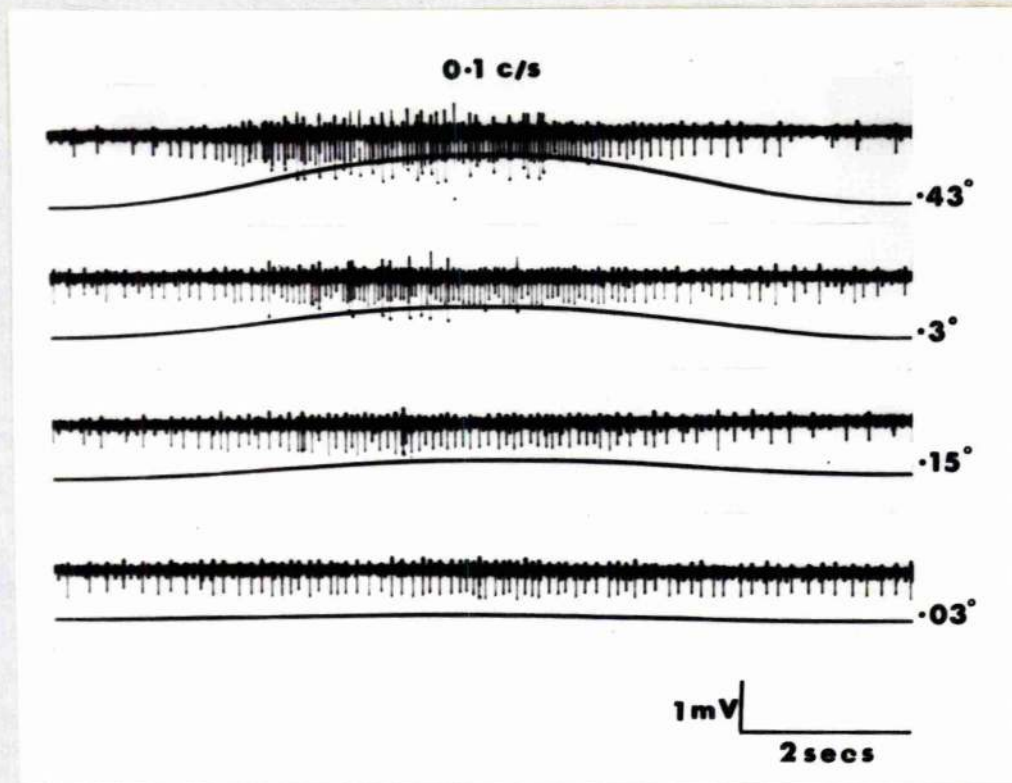


FIG. 37. Tonic and phasic activity recorded in muscle R20a during sinusoidal drum movements (lower trace) at a frequency of 0.1 c/sec and at the peak to peak amplitudes shown. The peak frequency of tonic activity shows a phase lead of 20° over drum position and phasic activity (respected) gradually disappears as the amplitude of drum movement is lowered.

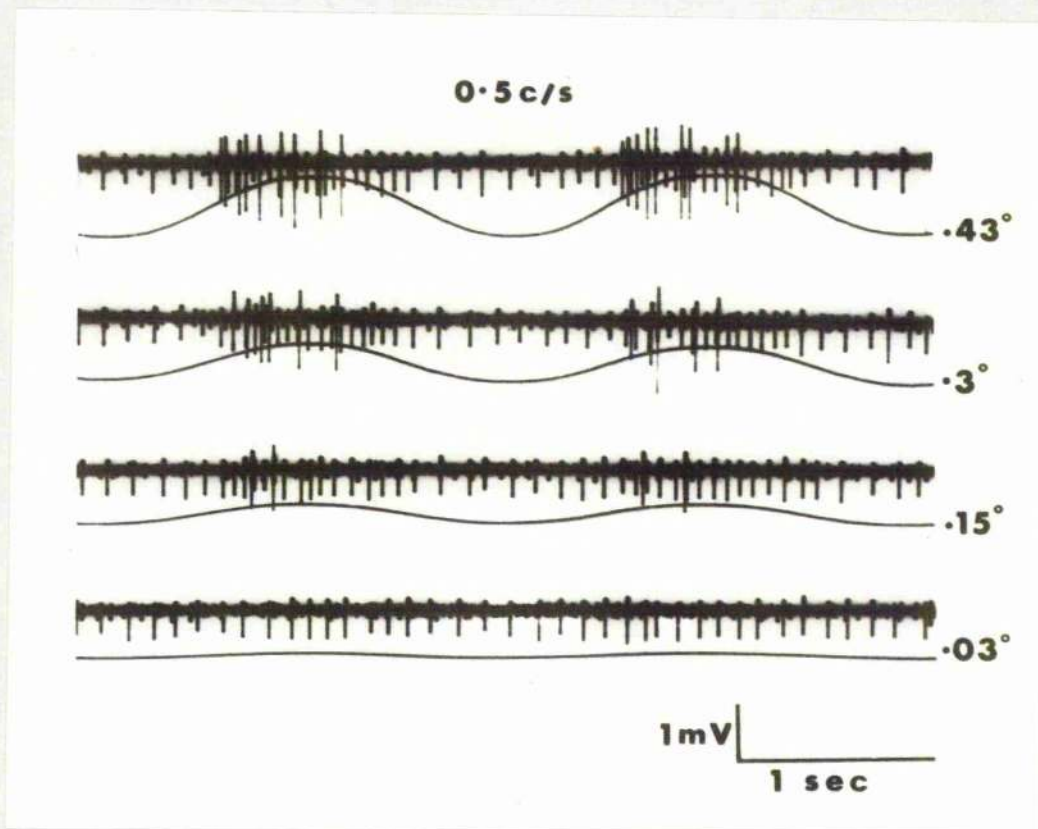


FIG. 38.

Tonic and phasic activity in muscle R20a during sinusoidal drum movements (lower trace) at a frequency of 0.5 c/sec and at the peak to peak amplitudes shown. The peak frequency of tonic activity is now in phase with drum position and phasic activity again falls out at the low amplitudes of drum movement.

drum position (Fig. 37) but at 0.5 c/s tonic activity is in phase with drum position (FIG 38). At higher drum oscillation frequencies a phase lag develops, which is 20° at 1 c/s but then increases rapidly to 160° at 5 c/s. This relationship is similar to that found in the locust (Thorson 1966b) and in the crab Pachygrapsus (Sandeman, unpublished) where head and eyecup torque respectively were measured. Here maximum torque exerted by the eyecup corresponds to the peak of tonic activity. Electrical activity associated with drum movement can be recorded at drum oscillations of 12 c/s, but movement of a free eyecup falls out at frequencies of 5 c/s. A limitation of the movement response at these frequencies is therefore a relatively slow rate of muscle tension development, as in the cockroach leg (Wilson 1965), and the associated mechanical movement of the joint. However a sufficiently high frequency of impulses necessary to produce the power for movement may not be achieved.

The main interest here in these experiments centres on the ability to separate the two types of activity. Phasic activity is directly related to the amplitude of the drum movement and as this amplitude is lowered so the amount of phasic activity decreases. At amplitudes below 0.03° pp phasic activity is absent altogether but tonic activity can still be related to drum position. If an electromechanical

transducer or isometric lever is attached to a blinded, driven eyecup from which the recording is made, then torque can still be recorded when only tonic activity is present. The maximum torque exerted by the eyecup corresponds to the peak frequency of tonic activity. This is true even at the larger drum amplitudes where the tonic and phasic activities show different phase relationships. Thus it is possible to separate out the tonic motor output and demonstrate directly that it contributes to eyecup torque during movement. The phasic motor output cannot be separated by these experiments and always occurs when a certain amplitude of drum movement has occurred. It thus seems useful to think of the motor neurons to a particular muscle as being part of a motor neuron pool in which the fast motor axons have a higher threshold for excitation than do the slow axons (Hoyle 1964).

The phasic activity cannot be directly shown to contribute to eyecup torque. However, the groups of phasic muscle potentials in muscles 20a and 21 are associated with small tremor movements of the eyecup. Similarly the fast phase of nystagmus is brought about predominantly by phasic activity. Thus both types of activity contribute to eyecup torque, but the phasic activity is recruited only for larger amplitude movements.

The tonic activity is also responsible for locating the eyecup in space. By moving the drum through small angles and holding it at the new position, it is possible to record

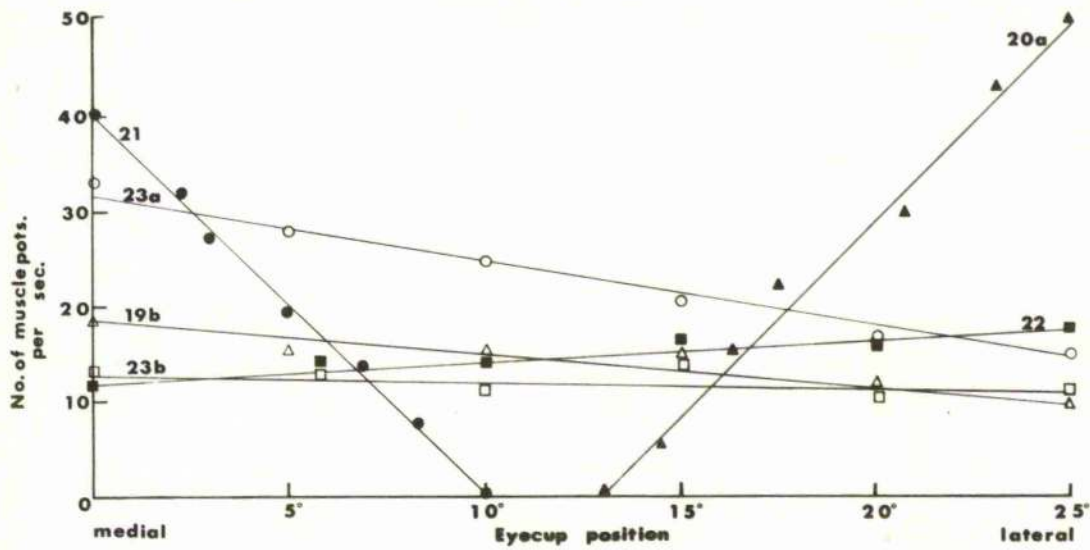


FIG. 39.

Plot of the tonic activity in the eyecup muscles against the position of the eyecup in the horizontal plane. The eyecup is induced to move by small movements of a striped drum. The average frequency of the tonic activity at that particular eyecup position is then measured. Tonic activity in muscle 19a is absent and that in 20b and c, which could only be recorded intracellularly, and so could not be directly linked with eyecup position, is omitted. In fact activity in both these muscles changes little from an average frequency of 10-15/sec. Muscles 20a and 21 show the greatest change in frequency during horizontal movements and the 'preferred' position of the eyecup is in the centre of the orbit where the firing of these two muscles is minimal.

the maintained tonic activity in each muscle at various eyecup positions. FIG 39 shows a plot of eyecup position against the frequency of tonic activity in the eyecup muscles. Activity is absent in 19a and that in 20k and c which was recorded intracellularly could not be directly related to eyecup position and is thus omitted. In the horizontal plane the tonic activity in muscles 20a and 21 show the greatest changes in frequencies while that in the other muscles shows little change in this plane of movement. The position of the eyecup in space is thus the resultant of activity in eight of the nine eyecup muscles. The "preferred" position of the eye seems to be about the centre of the orbit where activity in muscles 20a and 21 is minimal.

Phasic activity is present only in muscle 21 when the visual field is stationary. (FIG 40). When surrounded by a contrasting visual field phasic activity in this muscle occurs in bursts which are associated with tremor movements of the eyecup. The bursts occur at a frequency of 2-3 c/s and consist of 2-10 muscle potentials at frequencies approaching 100 c/s. When the lights are turned out the bursts are abolished. The muscle potentials which occur when the lights go out cause a drift of the eye not seen in the a-c coupled movement trace of fig. 40. The drift is restored by the burst of potentials when the lights are again turned on. There is

MUSCLE R 21

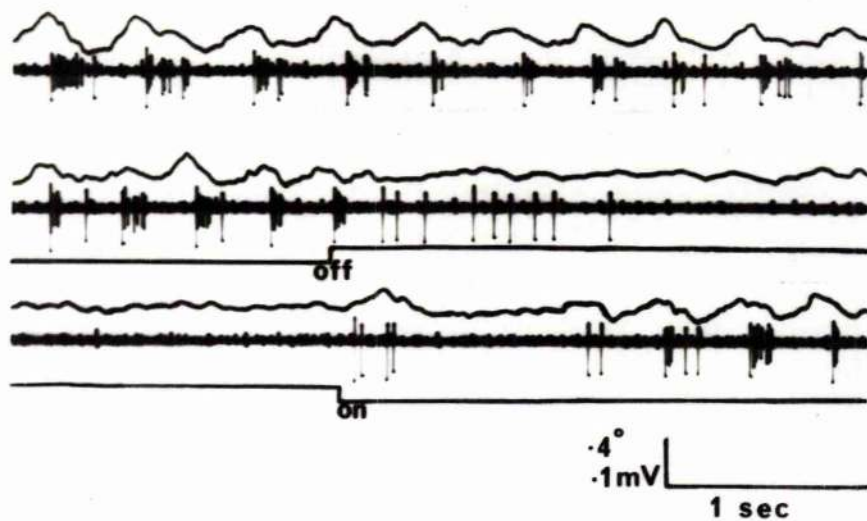


FIG. 40.

Bursts of phasic activity recorded from muscle R21 while the crab was surrounded by a stationary black and white striped drum. Associated with this activity are tremor movements of the eyecup, shown a-c coupled on the upper trace. When the lights are turned out the eye drifts (not shown on the movement trace as it a-c coupled) and the bursts are abolished only to resume as before when the contrasts are reilluminated.

then a pause of up to one second before the bursting is resumed as before. The eye tremor resulting from this feedback arc varies in amplitude from $0.01-0.2^{\circ}$ peak to peak.

In the absence of any similar activity in the other eyecup muscles it must be presumed that muscle 21, in producing eyecup tremor, is working against the passive elastic properties of the joint and the other muscles. However the similar time constants for the movements in both directions makes it difficult to accept this idea.

Correlated with the phasic activity in muscle 21 when the visual field is stationary is the fact that this muscle receives the densest blood supply of any eyecup muscle. It is thus well suited for applying tremor movements to the eyecup.

Intracellular muscle activity

When recording extracellularly it was often noted that the tonic and phasic activity could be recorded from different areas of a muscle. For example, tonic activity in muscle 23b was recorded on the surface but phasic activity was recorded if the electrode was pushed deeper. This is suggestive that the two motor axons, which each muscle apparently receives, supply predominantly separate groups of fibres within each muscle. Other differences were also noted. The amplitude of the tonic activity was always smaller and showed little facilitation. On the other hand phasic activity was of a greater amplitude with much facilitation.

To investigate this further different fibres within the eyecup muscles were explored with intracellular electrodes in a fixed, blinded eyecup, driven by the contralateral fixed eyecup. The seeing eyecup was at the centre of a semi-circular black and white striped drum. Extensive exploration was limited to muscle 20a. Histologically this muscle consists of fibres with 'Felderstruktur' and 'Fibrillenstruktur' and during optokinetic movements two types of activity are recorded from it.

On the basis of their innervation fibres can be grouped into three main classes; types A fibres, phasic,

which are supplied only by the fast axon; type B, tonic, which are supplied only by the slow axon and type C, intermediate, which are supplied by both axons. These types corresponds to those described for the leg muscles (Atwood 1963). FIGS 41, 42, 43 show activity in each of these three types recorded from the same muscle during an optokinetic response.

In the fibres supplied only by the fast axon (FIG 41), no junction potentials are recorded where the drum is stationary. When a certain amplitude of drum movement has occurred jps are recorded which then facilitate with some summation at the higher frequencies. Graded active responses may occur at these frequencies but not spike responses.

Activity is then inhibited centrally at the start of the fast phase. Following the fast phase are a group of jps approximately half the size of the ones of the previous slow phase but larger than the initial ones of the next slow phase. The activity thus exactly corresponds to that recorded in a freely moving eyecup, so that in this context proprioceptive information is disregarded. As the slip speed is constant throughout, because the seeing eye is clamped, the onset of the phasic activity cannot depend on a sudden increase in slip speed. The evidence from intracellular recording therefore shows that the onset of this activity is governed by a completely central mechanism which is dependent

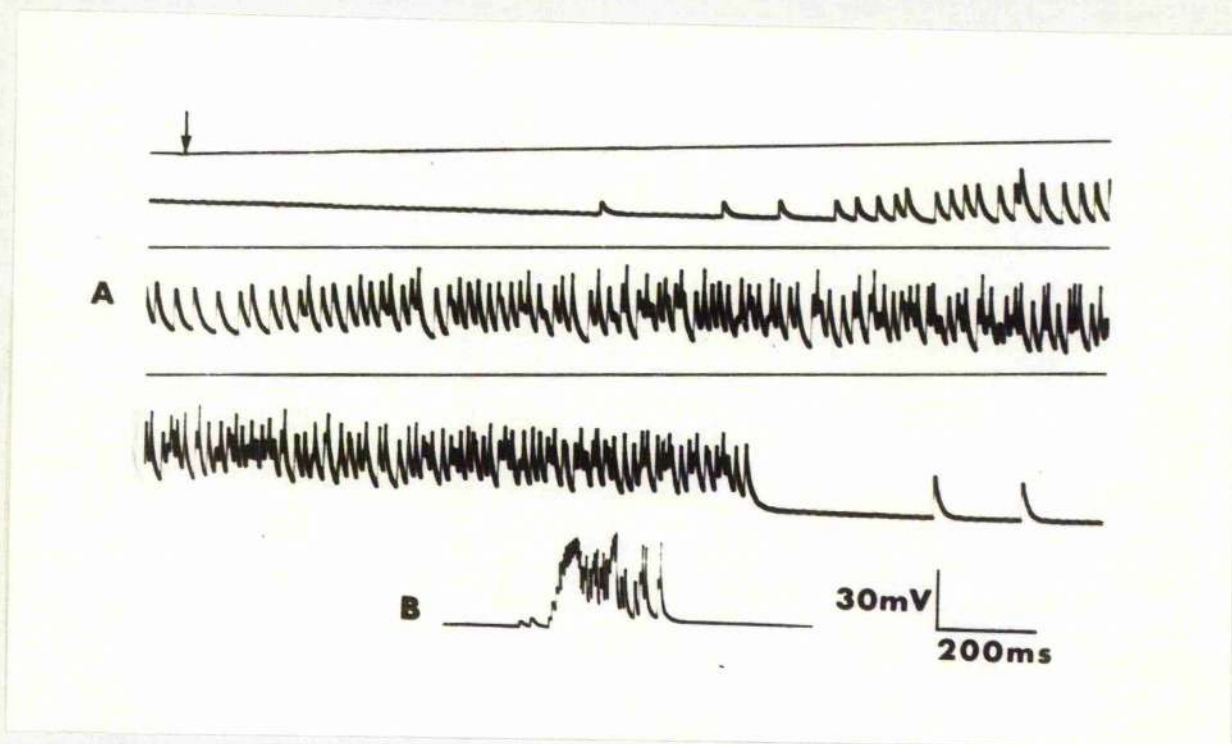


FIG. 41.

Intracellular recording from a phasic fibre of muscle R20a during optokinetic stimulation. In (A) activity is not present when the drum is stationary but during clockwise movement (upper trace) the jps show a gradual increase in frequency with much facilitation, and at the high frequencies active responses may occur. Activity is centrally inhibited during the fast phase. In (B) the drum is moved in the opposite direction and activity is now restricted to the fast phase. The jps rapidly facilitate and summate to reach a depolarization plateau from which active responses may occur.

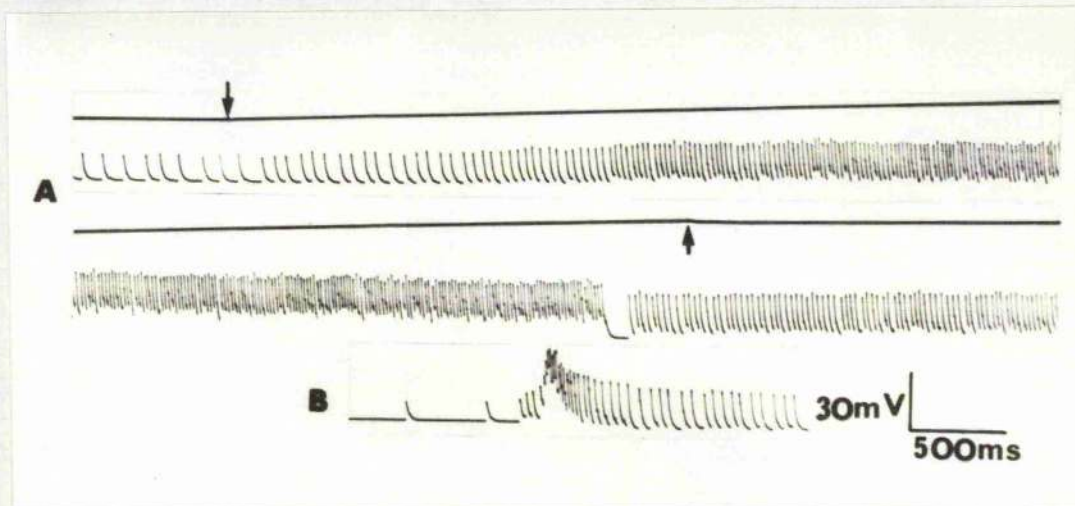


FIG. 42.

Intracellular activity from a tonic muscle fibre in muscle R20a during optokinetic stimulation. Jps are present when the drum is stationary (upper trace) and when it is moved clockwise the jps show a gradual increase in frequency with some summation but little facilitation. Activity is again centrally inhibited during the fast phase. In (B) the drum is moved anti-clockwise and activity is largely restricted to the fast phase. The jps summate rapidly, with little facilitation, to reach a depolarization plateau which declines more slowly than that in the phasic fibres.

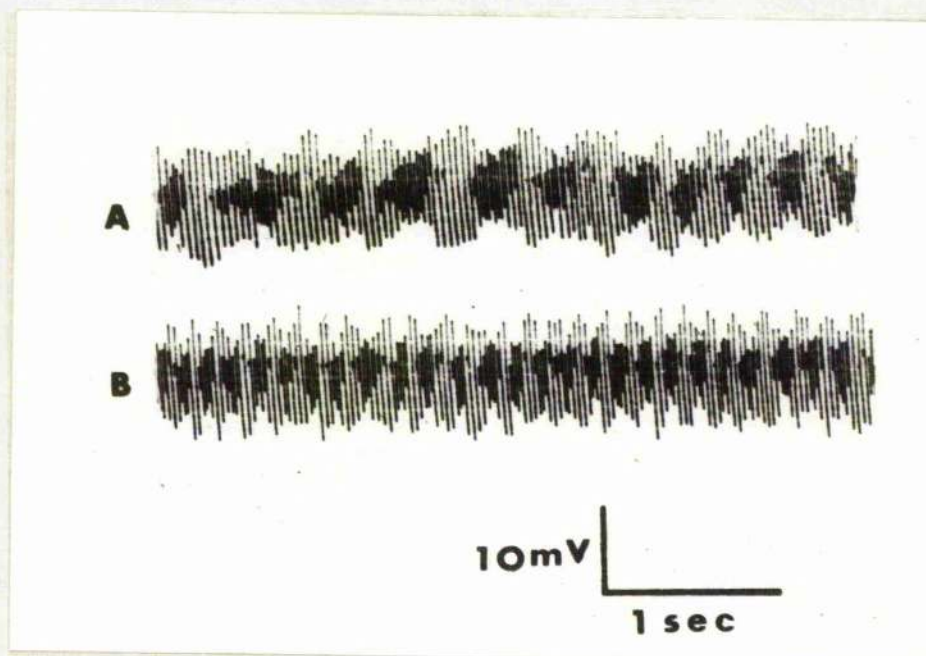


FIG. 43.

Intracellular activity in an intermediate fibre of muscle R20a showing the oscillations which are set up during optokinetic stimulation by the different firing frequencies of the slow and fast axons. In (A) the drum has just started to move clockwise and the beat frequency is 2-3c/sec. but as the movement continues (B) the beat frequency increases to 7c/sec.

solely on the amplitude of the visual input.

A nystagmus recorded from a type B muscle fibre is shown in Fig. 42. Jps are present when the drum is stationary and as the drum moves they show a gradual increase in frequency with some summation but little facilitation. During the fast phase activity is centrally inhibited. There is no evidence from extracellular or intracellular recording for the existence of peripheral inhibition.

When the drum is moved in the opposite direction the fibres are active only during the fast phase. The jps in fibres of type A facilitate rapidly and summate to reach a depolarization plateau from which active responses may occur. Jps in muscle fibres of type B facilitate a little and summate to reach a depolarization plateau which then declines more slowly.

The interaction of the two axons supplying the intermediate type C muscle fibres causes "beats" to occur (FIG 43) because the two axons are firing at different frequencies. At low amplitudes of drum movement these oscillations occur at 2-3 c/s but as the amplitude of movement increases so does the frequency of oscillation and reaches a frequency of 7 c/s.

The decussation of the slow and fast axons to different muscle fibres is again clearly seen during small

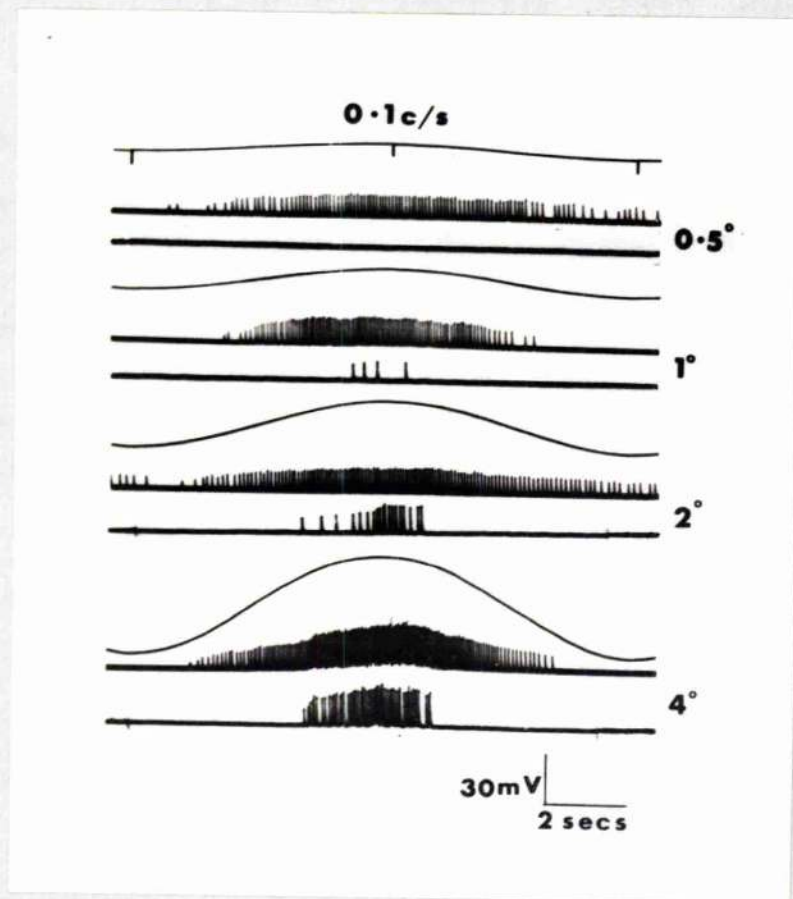


FIG. 44.

Response of two fibres in muscle R20a to sinusoidal drum movements (upper trace—clockwise is upwards). Phasic activity (lower trace) is absent at low amplitudes of drum movement but is recruited at the higher amplitudes. Tonic activity (middle trace) occurs at all amplitudes of drum movement.

drum oscillations (FIG 44). At the low amplitudes of drum oscillation only tonic activity is present but as the drum amplitude is raised not only is this activity increased but activity in the phasic fibres also occurs.

The phasic muscle fibres have a resting potential of between 65 and 80 mV. The jps occurring in these fibres are of large amplitude and amplitudes of 20-25 mV are frequently seen. They have a fast rise time and a time constant of decay of 20-50 msec. They show much facilitation and at high frequencies active membrane responses may occur though the second of a closely spaced pair of potentials is usually a large jp. without the initial rapid repolarization of an active response.

Tonic fibres usually have a smaller resting potential of 50-65 mV. The jps are usually of smaller amplitude but are frequently 5-15 mV. They have a slower rise time and a longer time constant of decay which can vary from 50-200 ms. They show little facilitation and at high frequencies the amplitude of the individual jps may actually decrease as a depolarization plateau is reached.

Fibres of type C show properties intermediate with those described above. The jps produced by the slow and fast axons vary considerably from fibre to fibre. In some, the slow jp is larger than the fast jp but this is often reversed. At high firing frequencies the fibres show the typical oscillations already described.

Reflex eyecup withdrawal

Withdrawal of the eyecup into its socket overrides any other concurrent eyecup movement. The response is unilateral and is most easily elicited by mechanical stimulation of an area of carapace supplied by the tegumentary nerve, though stimulation of the other ipsilateral brain nerves is also effective. The motor pathway mediating the response, unlike that for optokinetic movements runs in the optic tract (Sandeman 1964b), but the number of axons involved and their peripheral connexions is unknown. Horridge and Sandeman (1964) recorded a high frequency burst of impulses with single units approaching a frequency of 500 c/s from the optic tract which was correlated with a withdrawal of the unrestrained eyecup. The normal pattern of motor impulses associated with such a withdrawal can be recorded from the optic tract when the eyecup is clamped, withdrawn or even absent altogether. As in optokinetic movements it seems that proprioceptors are of no importance. The crab was thus prepared as described previously for intracellular recording. The reflex withdrawal was elicited by stimulating the tegumentary nerve. Single 0.1 ms shocks were usually sufficient to elicit the full pattern of impulses in the optic tract, but the central threshold below which no response could be obtained varies in different crabs and rises with repetition.

During a withdrawal a train of efferent impulses of two different amplitudes is recorded from the optic tract. This pattern cannot be further broken down, even in fatigued preparations, to the firing of more than two axons. The larger amplitude spike is correlated with jps recorded simultaneously in muscle 19a, while the smaller spike is correlated with jps in eyestalk muscle 18 (FIG 45). These two axons also branch to other eyecup muscles and jps in muscle 19a are in phase with those in a small number of fibres on the dorso-lateral surface of muscle 20a. (FIG 45). Similarly jps in muscle 18 are in phase with those in muscles 21 and 20b. The jps of muscles 19a and 18 are never in phase. Despite many penetrations of fibres in the remaining eyecup muscles, phasic activity associated with eyecup withdrawal has not been found, but until it is certain that all the fibres have been penetrated, the possible involvement of these muscles in the withdrawal cannot be completely excluded. Thus two axons and five muscles are involved in the withdrawal; the axon with the large amplitude spike innervates a group of two muscles, 19a and 20a, while the axon with the smaller spike innervates a group of three muscles 18, 20b and 21. The latency of 10-20 ms between stimulation of the tegumentary nerve and the appearance of the first jp is the same for both muscle groups. However, the form of the response in the two groups differs. The

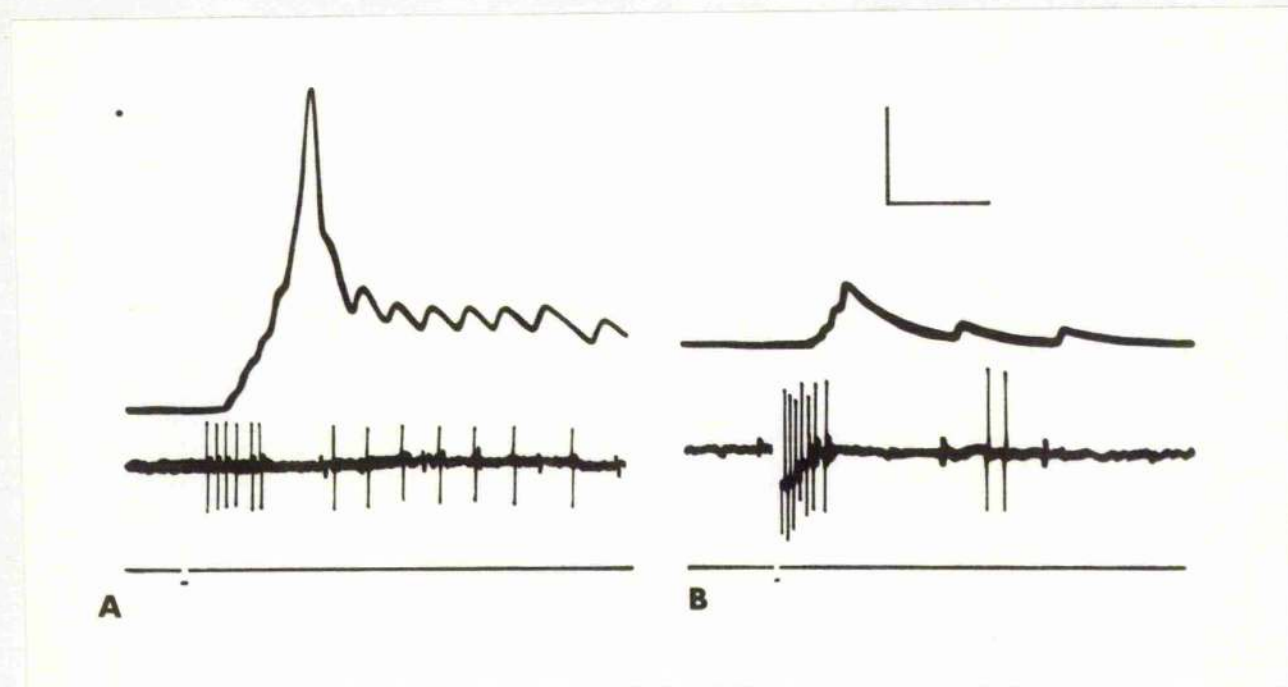


FIG. 45.

Activity recorded from the optic tract (middle trace) simultaneously with that in muscle 19a (A) and muscle 18 (B), to a single shock applied to the tegumentary nerve (lower trace). Jps in 19a are in phase with the large spike and those of 18 with the smaller spike. The dot in the upper left hand corner represents the zero membrane voltage for muscle 19a. Records are from different preparations. Scale: voltage 20 mV, time 40 ms.

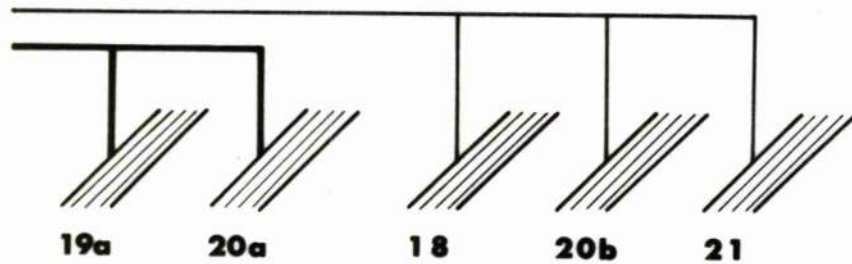
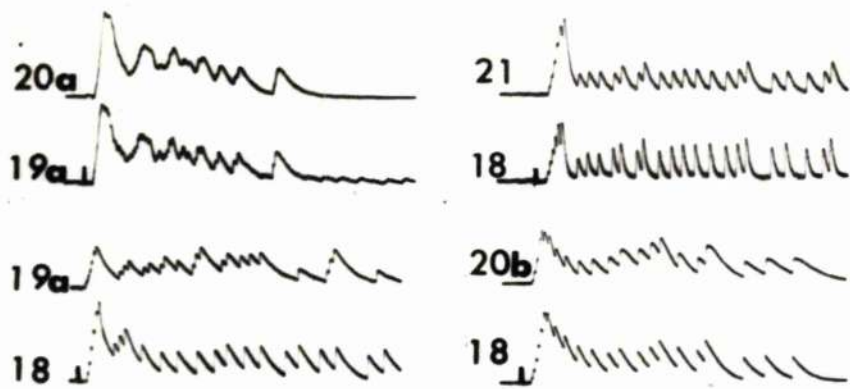


FIG. 46.

Simultaneous recordings from pairs of muscles in different preparations during eyecup withdrawal. The jps of 19a and 20a are in phase but those of 19a and 18 are not. However 18 is in phase with 21 and with 20b, indicating the presence of two axons, one supplying 19a and 20a the other 18, 20b and 21. Scale: voltage 20mV, time 100 ms.

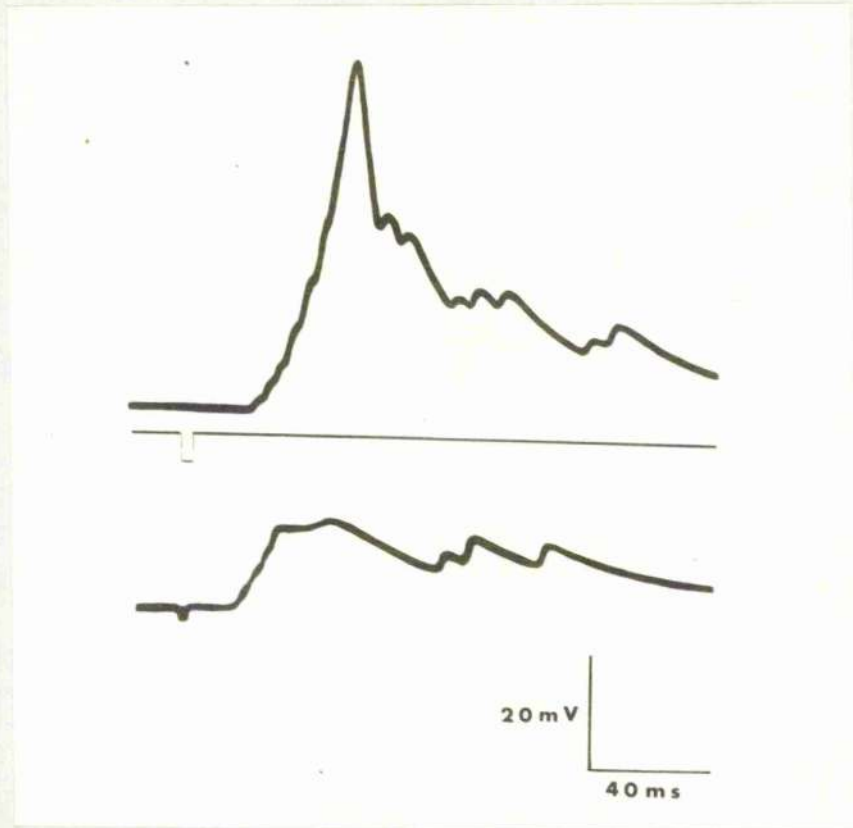


FIG. 47.

Successive responses of muscle 19a to the same stimulus applied to the tegumentary nerve. In the first an active membrane response occurs but in the second the summated jps do not reach a sufficient depolarization level for the active response to occur.

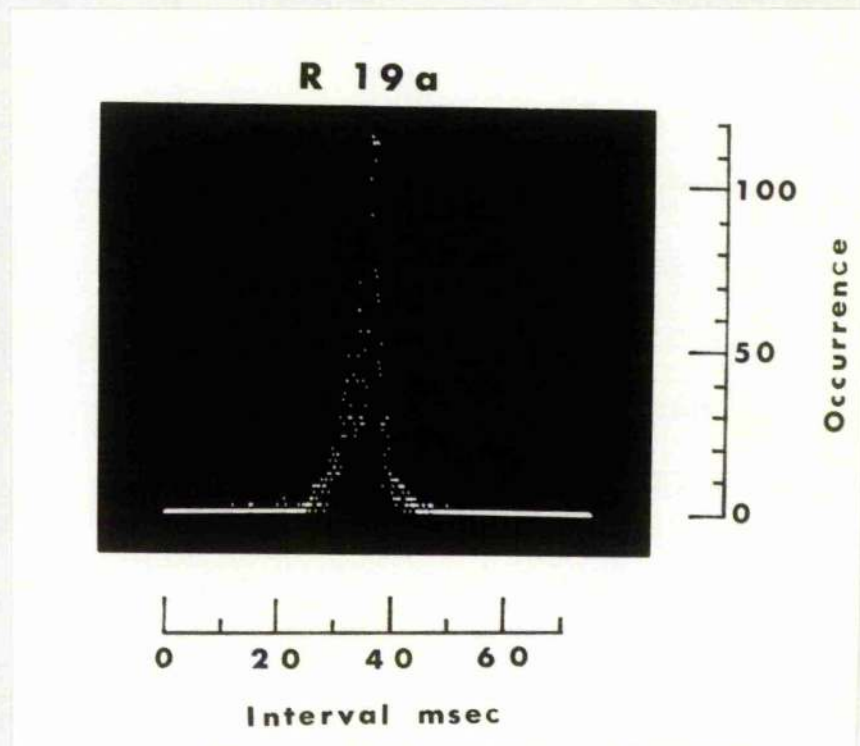


FIG. 48.

An interval histogram of the tonic activity in muscle 19a when the eyecup is withdrawn. The histogram is slightly skewed about a mode of 35 m sec, but the regularity of firing is indicated by the small standard deviation. Compare with fig. 32.

jps in muscles 19a and 20a facilitate and summate rapidly and graded active responses occur which sometimes overshoot zero. Fig. 47 shows two successive responses recorded from muscle 19a. In the first an active response occurs which just reaches zero, but in the second the summated jps do not reach a sufficient depolarization level for the active responses to occur. The whole response lasts for 50-500 ms depending on the preparation. The jps in the second muscle group also facilitate and summate but active responses are absent. The response is usually of a lower frequency and longer lasting than that in the first muscle group.

Muscle 19a is the only muscle from which no electrical activity can be recorded during optokinetic movements. It is also histologically uniform in that all fibres have a sarcomere length of 3-4 μ . During a withdrawal all the fibres penetrated in muscle 19a shows similar responses, differing only in their density of innervation by a slow axon from the oculomotor nerve. In an unrestrained eyecup tonic activity, caused by this axon is present only when the eyecup is held in a withdrawn position. Fig 48 shows an interval histogram, with a mode at 35 msec, of the activity in muscle 19a caused by this axon. Presumably its function is to retain the eyecup in its socket, once withdrawn. Sandeman (1964b) showed that the maintenance of withdrawal

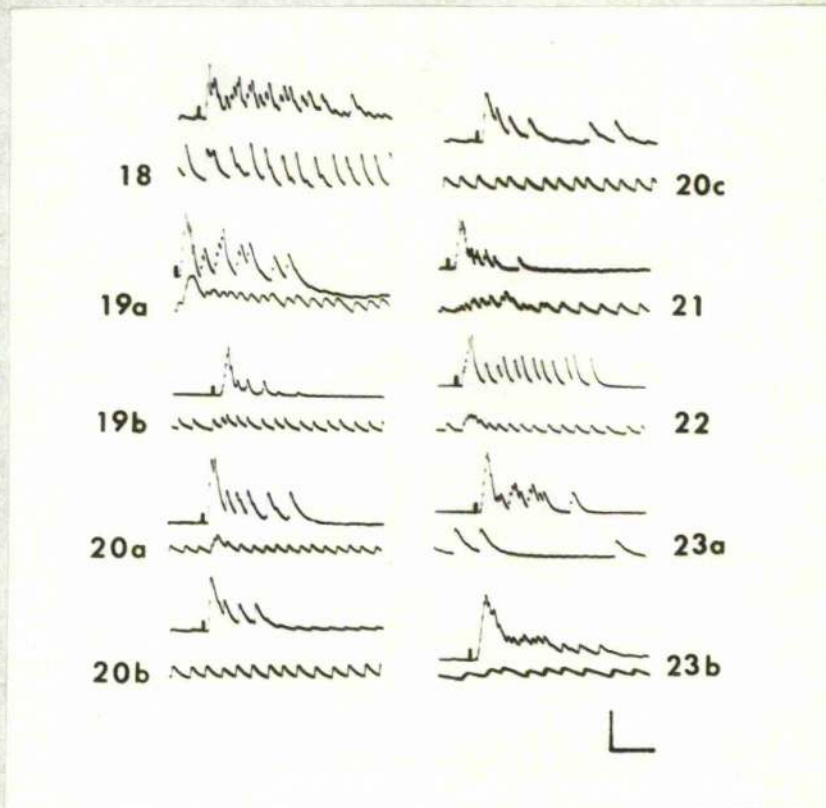


FIG. 49.

Records from different preparations showing the effect on tonic activity of a reflex withdrawal. Tonic activity in 19b and 22 is recorded with phasic withdrawal activity in muscle 21 while that in the remaining muscles is recorded with muscle 19a. In some muscles the tonic activity is speeded (e.g. 20a and 21) but in others only a single jp is interjected (e.g. 19b). Activity in 20b and 20c is unaffected while that in 23a is centrally inhibited. No activity is inhibited peripherally. Note the different densities of innervation of 19a by the slow axon responsible for holding the eyecup in its socket. Scale; voltage 20 mV time 100 ms.

depends on the oculomotor nerve, while the actual withdrawal movement depends on the optic tract.

All the fibres in the other muscles involved in withdrawal are not innervated by the withdrawal axons. In muscles 18, 20a and 21 only those fibres which respond phasically in optokinetic movements are innervated, but in muscle 20b some tonic fibres are also innervated.

During a withdrawal the tonic activity which normally locates the eyecup in space is modified (FIG 49). None of the activity is inhibited peripherally and only that in muscle 23a is inhibited centrally. The remaining activity is presumably overridden by the strong phasic action of the withdrawal muscles. After the withdrawal the activity continues as before regardless of whether the eyecup is extended or retained in its socket.

When one statocyst is removed in a preparation whose other sensory input is kept at a constant low level, withdrawal of the ipsilateral eyecup occurs "spontaneously" about every 13 seconds (Sandeman 1967b). This "spontaneity" indicates the presence of a pacemaker in the brain usually counteracted by normal sensory input. FIG 50 shows the periodic bursts of activity recorded in muscle 19a in such a preparation.

Up to 500 ms before such a spontaneous withdrawal the tonic activity in all the eyecup muscles is centrally

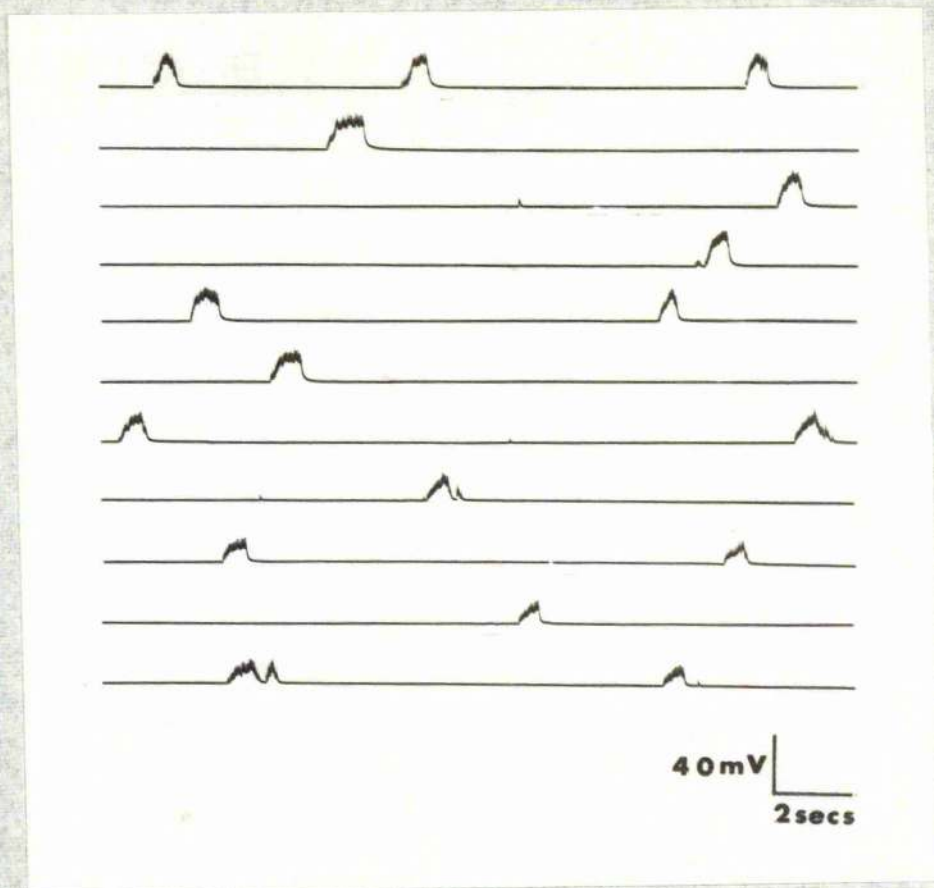


FIG. 50.

A statocyst has been removed from this crab which then shows 'spontaneous' withdrawals of the ipsilateral eyecup. Intracellular activity in muscle 19a shows periodic bursts, associated with these withdrawals, at intervals of about 13 secs.

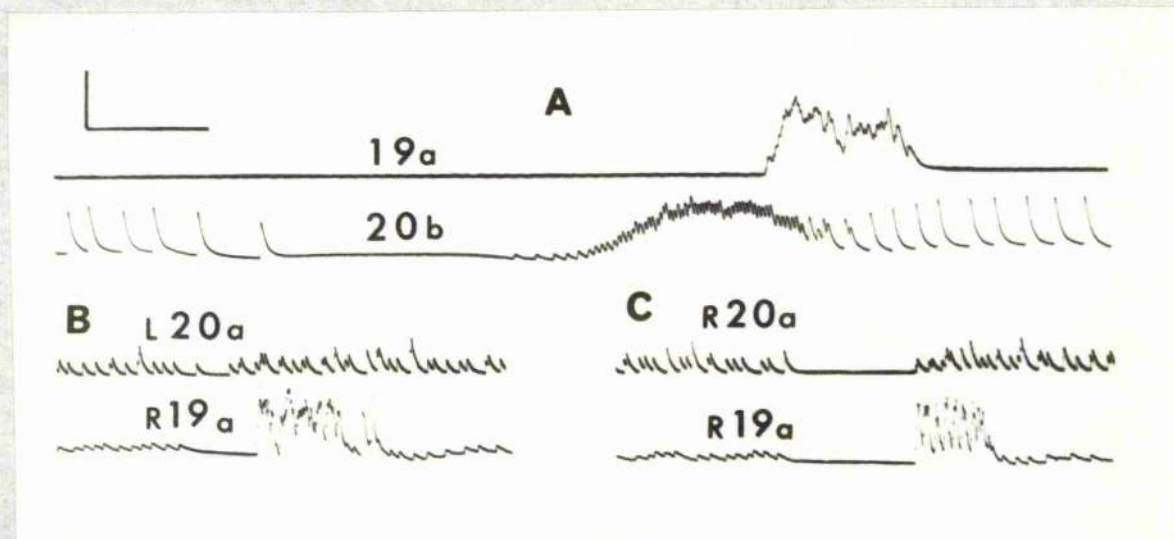


FIG. 51. (A) Recordings from muscle 19a and 20b during a 'spontaneous' withdrawal showing the preceding inhibition of tonic activity followed by a burst of activity in muscle 20b then 19a. (B) shows the effect on contralateral tonic activity in muscle L 20a compared with that in the ipsilateral eyecup (C) during a 'spontaneous' withdrawal. The lower trace shows the concurrent activity in muscle R 19a. Scale voltage 20 mV, time in (A) 250 ms in (B, C) 500 ms.

inhibited (FIG 51) and there is also a slight slowing of the tonic activity in the contralateral eyecup. Thus although the behavioural output is unilateral there is some nervous interaction between the two sides. Following the inhibitory period jps are recorded in muscles 18, 20b and 21, that is the group supplied by the axon with the small spike. These jps increase in frequency, summate and also facilitate slightly to reach a depolarization plateau which may last for up to 500 ms. During this plateau, a high frequency burst of rapidly facilitating and summing jps with some active responses occurs in muscles 19a and 20a (FIG 51), that is the group supplied by the axon with the large spike.

Although the firing sequence of the two axons in a "spontaneous" withdrawal differs from that in a reflex withdrawal, the overall movement of the eyecup is a rapid withdrawal in both.

The axon supplying muscles 19a and 20a is probably the large 30-40 μ diameter axon on the dorsal surface of the optic tract. The large spike easily recorded from the optic tract suggests a large axon, and this spike can still be recorded from a small bundle of dorsal fibres split from the optic tract. Activity in such a bundle is absent when the large axon is not present. In transverse sections of muscle 19a an axon of similar diameter can be seen and there is only one axon of comparable size in both the optic tract

and oculomotor nerve. Moreover a large axon can be traced in methylene blue preparations leaving the optic tract and branching to muscles 19a and 20a. All this is circumstantial evidence, however, and direct proof must await marking of the axon after intracellular recording from it.

Eyecup extension.

Extension of the eyecup again after withdrawal returns the eyecup to its previous position in space before the withdrawal. It is achieved by bursts of slowly rising frequency in both muscles 23a and 23b (FIG 52). The amount of activity depends precisely on the extent of withdrawal (FIG 53) so that the eyecup is returned to its previous position. This fine control of the movement cannot be the result of proprioceptive monitoring as the same results are obtained when eyecup movement is prevented. It is probably that 'memory' of the previous position is important in perfecting the final position.

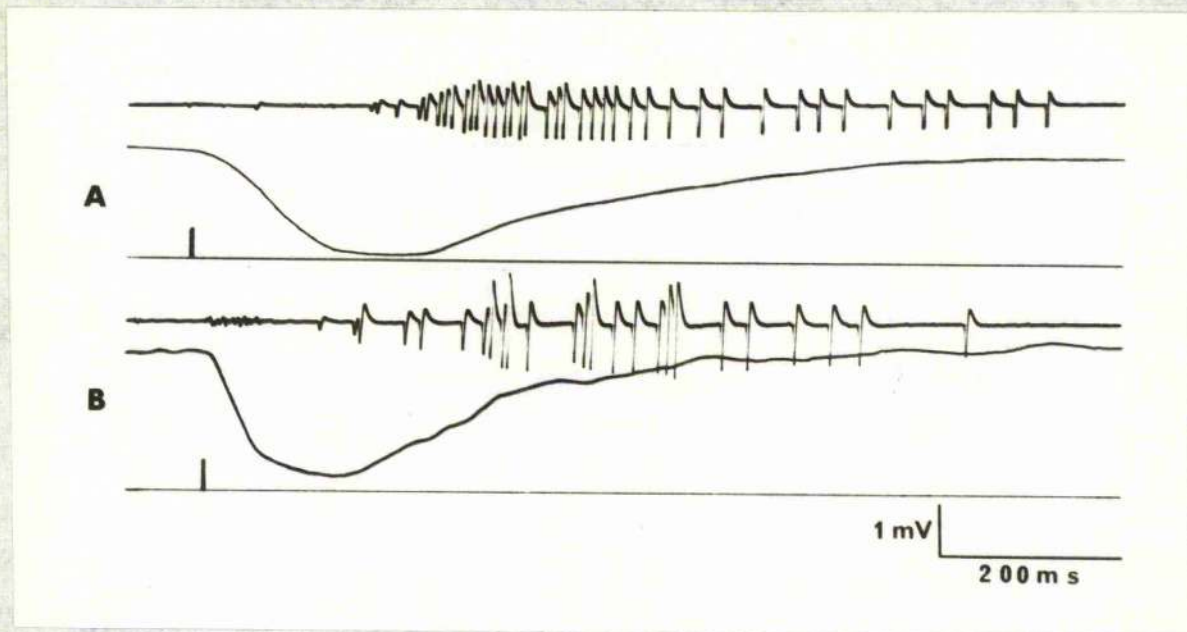


FIG. 52.

Eyecup extension. The eyecup has been induced to retract by a light touch to the carapace (lower trace) and extracellular muscle potentials bringing about extension are recorded from muscles 23a (A) and 23b (B), together with the resulting movement of the unrestrained eyecup. Records are from different crabs.

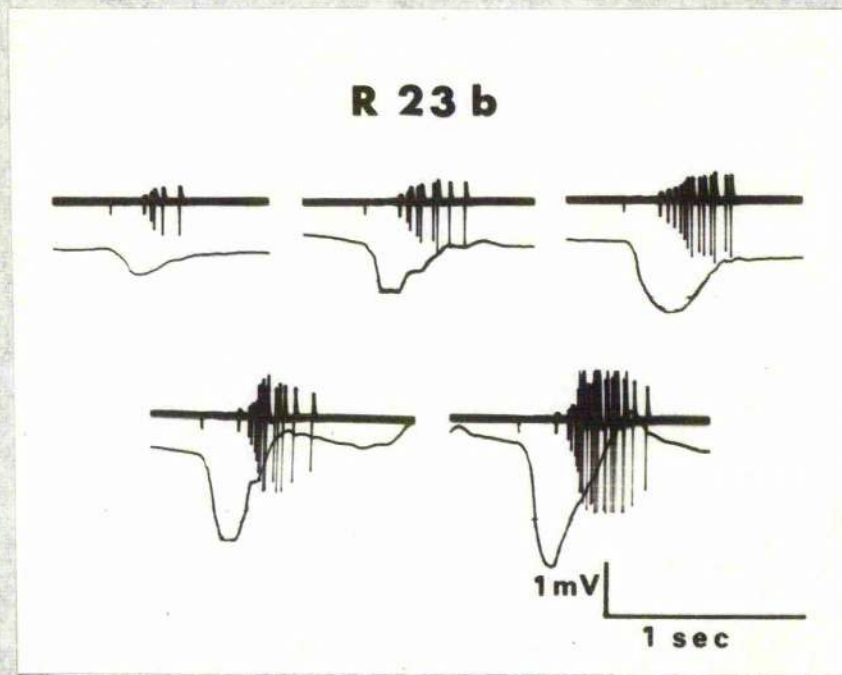


FIG. 53.

Successive withdrawals of increasing amplitude are induced by increased mechanical stimulation of the carapace and eyecup movement is recorded together with activity in muscle 23b, responsible for extending the eye. Although the movement is not controlled by proprioceptive feedback, the amount of activity in the extensor muscles is directly linked to the amplitude of the withdrawal movement. The response is the same if the eyecup is clamped. The eyecup will thus be returned approximately to its original position.

DISCUSSION

1. Muscle structure and function

The neuromuscular system of the eye-cap is complex. This complexity exists at several levels; in the direction and attachment of the muscle blocks, in the structure of the muscle fibres, which is probably related to contraction speed, in their membrane properties and in the detailed pattern of innervation of the various fibres.

Each of the muscles involved in optokinetic movements consists of fibres of two basic structural types, which somewhat resemble the Felderstruktur and Fibrillenstruktur of vertebrate muscle fibres, but intermediate types are also present. Each muscle is also supplied by a slow and a fast axon and there is no evidence for inhibitory axons. The two axons to each muscle tend to supply separate groups of muscle fibres but with some overlap. From each muscle it is possible to record, both intracellularly and extracellularly, two types of electrical activity associated with the firing of the slow and fast axons; these have been called tonic and phasic responses. Thus histologically two basic types of muscle fibre are recognized in the electrical records, usually from distinct groups of muscle fibres. Because the various types of muscle fibres are intermingled and not separated into groups, as in the accessory flexor muscle of the meropodite of the leg.

(Dorai Raj & Cohen, 1964), correlation of the structural with the functional observations must await the marking of individual fibres after electrical recording, and their subsequent histological identification. Present evidence suggests that the fast axons predominantly supply the phasic fibres while the slow axons supply the tonic ones. In muscle 23a tonic activity is only recorded from the superficial fibres which are felderstruktur fibres. In muscle 19b, where no extreme fibrillenstruktur fibres are present, the phasic activity differs from that in other muscles. The jps have a slower rise time, a longer time constant of decay and show little facilitation. This could be caused by the fast axon supplying intermediate type muscle fibres.

The presence of two neuromotor systems closely parallels the situation in the extraocular muscles of vertebrates. Measurement of the mechanical properties of the human globe (Robinson, 1964, 1965) and eye movements, have led to the incorporation of two systems, one responsible for saccadic, the other for smooth pursuit movements, in possible models of the eye control system (e.g. Fender, 1964). Under complete inhibition of muscular activity the globe promptly recentres near to its primary position. For each pattern of motor output to the muscles there is only one position for the eye. The dynamics of eye movement from one position to another depends upon which of the two systems,

the saccadic or smooth pursuit, is activated. Robinson (1965) offers a possible explanation of this in terms of a myotactic reflex which causes net muscle tension to be a function of eye position. This will express itself as an equivalent viscoelastance in the mechanics of the globe, which will be present in saccadic but not in smooth pursuit movement. While this may be one factor, the finding of Hess 1958, Hess and Pilar 1963 that two distinct types of muscle fibre are present in the extraocular muscles of the cat, offers an alternative explanation that the two movements might be mediated by muscle fibres with differing mechanical properties. Histologically the muscle fibres show Fibrillen and Felderstruktur with the latter concentrated in the outer layers (Kato 1938). The fibrillenstruktur fibres probably have only a single end plate and in vitro recording from them shows propagated spike activity with a twitch contraction (Hess and Pilar 1963). The felderstruktur fibres have multiple endings with distributed jps and give a slow contraction. Matyushkin (1961, 1964) has called the 'twitch' fibres 'phasic' and the 'slow' fibres 'tonic' and has shown by anodal block studies that the phasic fibres are innervated by large axons and the tonic fibres by small axons. Thus the extraocular muscles have two types of motor unit. In vivo recordings from cat extraocular muscles (Bach-y-Rita & Ito 1966) has shown that both types of fibres are capable of

producing overshoot spike activity but that they differ in impulse conduction velocity, ranges of membrane potential, frequencies of stimulation necessary to produce a tetanus and in the conduction velocity of the axons innervating them. The "slow multinnervated twitch fibres" have a lower fusion frequency than "fast fibres", so are well suited for tonic activity which may include smooth pursuit movements, as well as maintenance of eye position. However, the correlation between the two types of movement observed and the two types of muscle activity has not been made in eyes in situ.

In the crab, by recording the eye movement and the electrical activity in the muscles at the same time, it has been possible to correlate the two. The technique offers the further advantage in that a normal visual input is used, so that the motor output will be that generated by the animal itself. In a stationary eye only the tonic fibres, innervated by the slow axon, are active. Each particular motor output pattern to the muscles corresponds to only one eyecup position. In other words each eyecup position is the resultant of a specific activity in 8 of the 9 muscles. Small signal analysis of the eyecup movement shows that small amplitude following movements are also brought ^{about} by increased activity in the tonic system. For larger amplitudes of movement the phasic system is recruited. This is the situation in a slow forward phase of nystagmus. Rapid movements, such as the fast phase of nystagmus are brought about primarily by the phasic system,

which is also responsible for the tremor movements of the eyecup. The saccadic flicks are also probably brought about by bursts in either of the two axons responsible for eyecup withdrawal. Thus the tonic system is responsible for maintenance of eyecup position and slow, small amplitude following movements, while the phasic system is recruited at larger amplitudes of following movements and is responsible for rapid eyecup movements.

The functional need for such diverse peripheral neuromuscular mechanisms lies in the need to produce such extreme types of contraction. Mammalian eye muscles have small motor units, usually of 4-6 muscle fibres (Whitteridge 1960) but the crab eyecup muscles probably receive only two motor axons. Gradation of contraction must thus depend more on the frequency coding in these axons and the varied responses of the muscle fibres and less on the recruitment of motor units.

Thus the diversity of crab muscle fibres compensates for the paucity of the motor axons and provides a wide range of peripheral tension control.

2. Optokinetic following movements

Optokinetic movements of the eyecup are brought about by a complex motor output programme, which is different and not merely reversed for opposite directions of movement. During horizontal movements, muscles 20a and 21 show the greatest changes in activity but it is not possible to

assign a particular function to one muscle. This is particularly true for muscles such as 23b with tonic activity showing no obvious trends during the slow forward phase of nystagmus. Because the eyestalk-eyecup joint is flexible it is possible that a muscle may contribute to a movement when the eyecup has moved a certain distance, without any detectable change in its electrical activity. Thus although it is possible to deduce muscle action with certainty from electromyography it is not possible to state firmly the function of the muscle at that moment.

During optokinetic movements both eyes move together, but in opposite directions relative to the mid-line, so that each eyecup receives a different motor output programme. The optokinetic movements of a blinded driven eyecup demonstrate the precise neural compensation for any non-linearities in the effector system. This has led to the proposal of a "central motor neurone driver" (Horridge and Sandeman 1964) responsible for controlling the output to both eyecups. But under certain stimulation the two eyecups can move in opposite directions relative to each other and the fast phase nystagmus movements of both eyes are not synchronous (Barnes, 1967). Although the concept of a central driver neuron requires modification, the eyecup movements are certainly controlled by a precise central

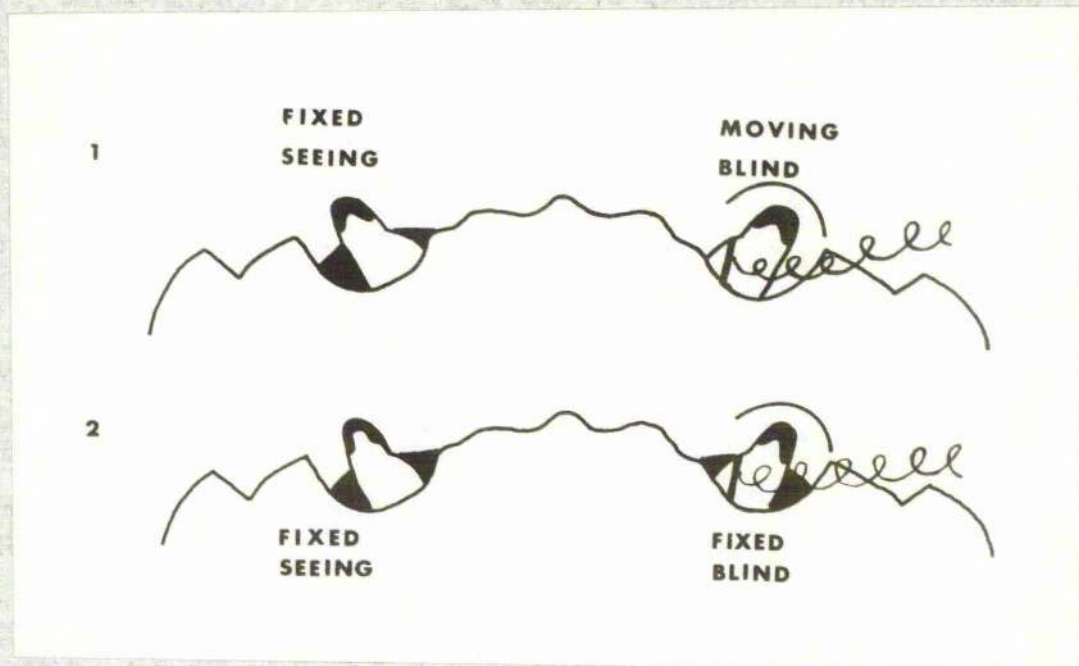


FIG. 54.

Experimental arrangement to test if use is made of any possible proprioceptive information from a moving eyecup. The left seeing eyecup is cemented in its socket and the right eyecup from which recordings are made is blinded. In the first experiment the right eye is allowed to move freely but in the second it too is fixed. The muscle activity and hence the frequency of the nystagmic movements is the same in both experiments.

mechanism with feedback only from the visual mismatch between eyecup and stimulus movement.

These experiments confirm previous findings that proprioceptors are not used in the control of eyecup movements. This parallels the situation in vertebrates where although muscle spindles are often present in the extraocular muscles, their information is apparently disregarded in eye movements.

Similarly the change from a slow to a fast phase of nystagmus seems not to be controlled by proprioceptive feedback. This is clearly shown in the experiment indicated in FIG 54. The seeing left eyecup is firmly cemented in its socket and the right eyecup from which muscle activity is recorded is blinded. In the first experiment the right eyecup is allowed to move freely but in the second it too is firmly cemented into its socket. In both experiments the same pattern, in both time course and form, of muscle activity is recorded. If tension receptors were triggering the fast phase, then nystagmus movements in the second experiment should occur at a higher frequency as tension will be achieved more quickly.

The position reached by the eyecup also does not determine the onset of the fast phase.

A blinded driven eyecup can be stopped mechanically at any position in its path without changing the nystagmus frequency.

It also seems unlikely that the onset of the fast phase depends on some measure of the visual input to the eye. Horridge and Sandeman (1954) argued as follows. At high drum speeds ($1-3^{\circ}/\text{sec.}$) the eyecups of unilaterally blinded crabs fail to keep up with the drum movement and the lag increases to a much greater extent than in normal crabs. Increasing the drum speed increases the delay before the onset of the fast phase in both normal and unilaterally blinded crabs but the fast phase still occurs at approximately the same eyecup deflexion. A measure of the total number of stripes which have slipped past the eye is thus not the stimulus which produces the fast phase.

However the fact that unilaterally blinded crabs show a greater lag and hence a lower frequency of nystagmic movements than normal crabs, may merely reflect the fact that under binocular stimulation the two eyes do not contribute equally to the response. That this is so is revealed in two types of experiments. In rabbits Ter Braak, 1936, and, Fukuda and Tokita 1957 have shown that in monocular optokinetic stimulation vigorous nystagmic movements are evoked only when the stimulus pattern passes from the temporal to nasal edge of the eye; movement in the opposite or "null" direction produces few nystagmic movements. The same is true in the crab though nystagmic movements can be produced by stimulation in the null direction, but at greatly reduced frequency. Also during a clockwise movement of the visual field

the fast phase of the left eyecup precedes that of the right and conversely for anti-clockwise drum movements (Barnes 1967).

The unequal contribution of the two eyes also manifests itself in rabbits when central nystagmus elicited by stimulation of an optic pathway is combined with a synergistic optokinetic response evoked from one eye only, (Gutman et al 1963, Bergmann et al 1964). For reinforcement of the central nystagmus, optokinetic stimulation of the eyes in the "preferred" direction is much superior to excitation in the "null" direction (Bergmann et al 1963). Thus by rotating a striped drum in a given direction left and right eyes are not activated to the same extent. In the rabbit (Barlow, Hill, and Levick 1964), have demonstrated retinal ganglion cells which have a directional selectivity to a light spot moving across the retina. Units from the same part of the retina do not have the same axis of preferential response. Fibres in the optic tract of the crab Podophthalmus also respond to unidirectional movement (Waterman et al 1964). In many units the greater response was obtained when a target moved horizontally from the lateral to the medial edge of the eye. This may represent bias in the sampling and may not necessarily indicate the actual number of different types of units, but it does suggest a possible explanation of the above phenomena.

Another line of evidence against the control of the fast phase by some measure of the visual input comes from memory experiments (Horridge 1936a). One eye is fixed but seeing and the other is blinded but allowed to move freely. The crab is surrounded by a striped drum, a small movement of which is made during a dark period. When the light is turned on again the blinded eye moves in the same direction as the new drum position. With this arrangement the angle moved by the eyecup is greater than the drum angle, so that the eyecup can be induced to undergo a nystagmus. Since there is no ^{actual} ~~relative~~ drum movement during the eyecup movement and since the nystagmus can be arranged to occur at any time during the response, the onset of the fast phase cannot be controlled by the visual input.

Although the form and direction of the visual input greatly affects the onset of the fast phase there are apparently no clues in the stimulus which could directly trigger the fast phase. Many factors seem to indicate that the onset of the fast phase is caused by a trigger-like mechanism which becomes active when the motor output to the muscles has reached a certain, critical level dependent on the amplitude of the stimulus movement. The contraction of the muscles during the slow phase is due to an increase in the frequency of discharge of the slow motor axons and the recruitment of the fast motor axons. The relaxation of these

muscles, which is not due to peripheral inhibition and the contraction of these bringing about the fast phase is very rapid. Also the duration of the fast phase varies within narrow limits. With these facts in mind it is possible to put forward many neuronal networks which could explain how the continuous input in the form of a constantly rotating drum is turned into a discontinuous nystagmic output (cf. Lorente de No 1958), but the salient feature of any mechanism seems to be a switch mechanism triggered by some measure of the motor output.

If a measure of the tonic output is the trigger then theoretically it should be possible to reset the zero of the mechanism by changing the tonic motor output frequency. The tonic output to muscles 20a and 21 would seem the most likely candidates. Experimentally the idea fails as forcibly moving a blinded eye about its orbit does not change the motor output frequency, there being no proprioceptive feedback. Similarly tilting the crab does not alter the tonic output to muscles 20a and 21; the other muscles compensate for the tilt and horizontal nystagmic movements continue as before.

5. Patterning of the motor output

The fast motor output to muscles 20a and 21 show a temporal patterning at high frequencies. Patterning is

rare in the animal kingdom but has been demonstrated in some crustacean leg muscles (e.g. Wiersma and Adams, 1950 and Ripley and Wiersma, 1953). They showed that the tension developed by certain crustacean muscles is influenced not only by the average frequency of the nerve impulses but also by the spacing or pattern of the impulses. For example, when the excitatory axon to the crayfish claw opener muscle was stimulated at 12/sec., more tension resulted when the shocks were delivered in pairs, than when the same number of shocks were delivered with equal intervals between them. Such examples were cited as pattern sensitive neuromuscular junctions. Wilson and Davis (1965) recorded opener muscle activity during reflex claw opening and demonstrated that the normal motor output to the muscle is temporally patterned. This patterning showed up in the form of doublets and clusters of potentials which caused an increase in the coefficient of variance at the high frequencies. They presumed that the patterning led to an enhancement of tension.

The patterning of the fast motor output to muscles 20a and 21 is not regular but consists of an unusually large number of pairs and closely spaced groups of jps. In plots of standard deviation from the mean against the mean, tonic activity shows the usual increase in scatter

at low frequencies, but phasic activity shows an increased scatter at the high frequencies. This is caused by the introduction of a separate interval class due to the clustering of the jps. These clusters of jps can be directly associated with the small tremor movements of the eyecup, thus indicating - if it is justifiable to extrapolate from the tension produced by a group of muscle fibres to movement of the whole eyecup - an increase in tension. Thus it seems that the animal is able to make use of the pattern sensitivity of its myoneural junction by generating a suitably patterned motor output. Wilson and Davis (1965) have suggested that the relative variance increase at the high frequencies may be adaptively related to the need for rapid adjustment of tension and that the use of two sequence parameters, average frequency and variance, narrows the frequency spectrum needed for a given range and sensitivity of control. Here the variance may have a further value in producing eyecup tremor which is known to improve vision (Horridge 1966b).

4. Eyecup withdrawal

The rapid reflex withdrawal of the eyecup overrides any other concurrent eye movement. The tonic activity of only one muscle is centrally inhibited while the remaining activity is presumably overridden by the strong phasic action of the withdrawal muscles. After the withdrawal the eye may return to its former position or remain withdrawn. The visual field is

apparently not disoriented during the rapid withdrawal movement. Either the movement is too rapid to stimulate movement receptors or vision is centrally suppressed. The latter is found to be true during rapid eye movements of vertebrates, when the suppression starts before the actual movement begins (e.g. Zuber and Stark 1966).

The movement is brought about by the firing of two motor axons, which run in the optic tract. One supplies a group of two muscles, the other a group of three. In a reflex withdrawal all five muscles are fired synchronously but in a 'spontaneous' withdrawal the firing sequence is different, yet the resultant movement is similar. Muscle 19a which is inactive in following movements participates in the withdrawal. The remaining four muscles are active in both withdrawal and in following movements. For example muscle 21 together with a specific motor output to the other muscles is most active in a horizontal following movement towards the mid-line. However in a withdrawal the same muscle fibres in 21, this time together with muscles 18, 19a, 20a and 20b cause a withdrawal movement downward and away from the mid-line. It is thus possible for one muscle, in conjunction with different patterns of activity in other muscles to be involved in opposite movements.

A similar situation occurs in the thoracic muscles of some grasshoppers where the anatomical connexions are such that muscles which are antagonists with respect to the

wings in flight are synergists with respect to the legs in walking and vice versa (Wilson 1962).

In these situations it becomes more apparent that the action of a muscle is solely to shorten or to resist lengthening. The movement that occurs depends on the arrangement of the joint and the other forces applied to it. Interpretation of eyecup muscle activity is therefore intelligible only if groups of muscles rather than the individual muscles themselves are regarded as the functional units.

5. Function of movable eyes

The functions of stalked eyes are numerous. Their underlying function does not lie in their ability to perform optokinetic movements, but in their ability to modify the visual input to the eye of a freely moving animal (Horridge 1966f). If the eye is allowed to move freely and tremor, as in ourselves, these small movements result in an improved performance of the visual system. Also in the absence of any proprioceptive control of eye movements, a stalked eye allows free movement whereby the visual feedback loop can be closed. This obviates the need for a precise central control yet allows accurate movements to be made. This is well shown by the crab's ability to measure the sun's speed. Once the eye is allowed to move freely then the optokinetic response becomes a by-product of the system necessary for stabilization of the eye.

If the crab is to use its ability to detect polarized light (Shaw 1966) or its ability to measure the sun's speed then it is essential that the eye be stabilised relative to some fixed parameter of the environment. Compensatory and optokinetic movements of the eye provide such stabilization.

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