

EXCITATION AND INHIBITION OF THE REFLEX EYE WITHDRAWAL OF THE CRAB *CARCINUS*

BY D. C. SANDEMAN

*Gatty Marine Laboratory and Department of Natural History,
University of St Andrews*

(Received 4 January 1967)

INTRODUCTION

The eye-withdrawal reflex of crabs was described for *Carcinus* by Bethe (1897*a*) who showed that mechanical stimulation of the anterior portion of the carapace, the eye-cups, antennae or antennules, or sudden illumination of the eye, would elicit the quick retraction of the eye into its socket. The eye withdrawal is unilateral except following mechanical stimulation of a narrow zone in the centre of the carapace when both eyes may retract. The motor neurons which bring about the withdrawal of the eye lie in the optic tract (Sandeman, 1964) while the sensory fibres from mechanoreceptors on the eyecup and on the carapace run in the oculomotor and tegumentary nerves. The reflex is quite distinct from, but superimposed upon, the eyecup movements produced by other neurons in the oculomotor nerve (Horridge & Sandeman, 1964).

This paper reports on the excitation and inhibition of one of the motor neurons effecting the eye withdrawal.

MATERIAL AND METHODS

The common shore crab, *Carcinus maenas*, was used in all experiments. Isolated brains were prepared for electrophysiological studies by dissecting the anterior portion of the carapace away from the rest of the body leaving the eyes, brain and statocysts intact. The oesophageal connectives and part of the tegumentary nerves are the only nerves of the brain which are interrupted by the dissection. The brain was perfused through the cerebral artery (Sandeman, 1967) with *Carcinus* saline (Pantin, 1948). Saline was also allowed to flow over the outside of the brain and associated nerve bundles. The preparations give consistent recordings for up to 6 hr.

Electrical recordings were taken from the central end of the peripherally cut optic tract with a polyethylene suction electrode. Whole or split nerve branches from the brain were stimulated through suction electrodes enclosing their cut ends.

The path of the retractor axon in the brain was mapped by recording from the surface of the de-sheathed brain with a coarse saline-filled glass micropipette of approximately 100 μ diameter. Selected areas in the brain were stimulated through the same glass electrodes, the indifferent electrode being a large silver wire placed close to the brain between the two cut oesophageal connectives.

Nerve impulses were recorded with conventional a.c.-coupled amplifiers and displayed on an oscilloscope. Stimuli were delivered by Tektronix square-wave generators.

RESULTS

Behavioural observations

Five to ten minutes after a statocyst of *Carcinus* has been removed the animal begins to quickly withdraw and extend the eye on the damaged side about once every 10 sec. This effect of statocyst removal is apparent only if the animal is allowed to settle quietly, and it has not been reported in previous studies on statocyst function (Bethe, 1897*b*, Dijkgraaf, 1956). The 'blinking', which is infrequently seen in undamaged animals, persists for several hours but can be temporarily prevented by making the crab walk about. Mechanical stimulation of the eye or carapace still induces eye withdrawal in the normal way. Similar repetitive withdrawal of the eyes follows section of the oesophageal connectives, and in all cases of unilateral damage the blinking is confined mainly to the damaged side. If both connectives are cut or both statocysts removed, both eyes blink but are not synchronized.

Electrophysiology

A burst of characteristically large impulses can be recorded at intervals from the optic tract of isolated preparations. These spikes occur spontaneously approximately every 13 sec. and are routinely 10 times larger than any other discharges in the nerve bundle. That they are from one of the motor neurons responsible for eye withdrawal is supported by some evidence. (1) The burst is of the same size, duration and frequency as those recorded from the optic tracts of intact animals during eye withdrawal (Horridge & Sandeman, 1964). (2) Simultaneous records taken from the optic tract and eye muscles during eye withdrawal show nerve and muscle potentials to be coincident (Burrows and Sandeman, unpublished observation). (3) The impulses never lose amplitude or break down into smaller units even in deteriorated preparations. Apparently they stem from a single axon essential for eye withdrawal but there is evidence (Burrows, 1967) that at least one other axon is involved in the reflex.

The number of impulses in a spontaneous burst varies from 80 to 180 but the frequency pattern of the burst does not change with the burst length. Initially about 250 impulses/sec., it reaches a peak of about 400 impulses/sec. after 10–20 msec., and then gradually slows down to approximately 200 impulses/sec. before stopping abruptly (Fig. 2*a*). A short burst reaches the peak frequency in the same time as the long burst but has a shorter tail. The interburst interval can be as short as 2 sec. or longer than 50 sec. but is more often between 6 and 20 sec. A histogram of 420 consecutive interburst intervals shows the characteristic variation in one preparation (Fig. 1). The mode lies at 7 sec. and the distribution is asymmetrical. There was no appreciable trend over the 420 burst period.

In some preparations a second smaller unit appeared which responded in approximately the same way as the large motor neuron. It gave spontaneous bursts of impulses and was excited by tegumentary nerve stimulation (Fig. 2*c, e, f*). The spontaneous bursts of the two neurons can occur separately or simultaneously. When both bursts occur together the smaller unit usually starts its burst about 200–500 msec. before the larger and often continues to fire for about 200 msec. after the large neuron has stopped. The response of the smaller unit was not explored but the properties of

the large motor neuron are described below under the headings of excitation, inhibition and spontaneity.

Excitation

Oculomotor and tegumentary nerves. A single electric shock of 0.1 msec. duration to the whole oculomotor or tegumentary nerve can produce the entire burst in the optic tract. However, it is found that the responsiveness of the motor neuron fluctuates in isolated preparations. A shock to the oculomotor or tegumentary nerve just after the spontaneous burst will produce only one or two spikes, whereas if the stimulus precedes the spontaneous burst by a few seconds the entire burst appears. Between these two extremes there is a rough gradation of the number of motor spikes following the stimulus. Latencies are usually 5–7 msec. for oculomotor stimulation and 10–20 msec. for tegumentary stimulation. In both cases increased intensity of stimulation tends to decrease the latencies (Fig. 2*b, c, d, e, and f*).

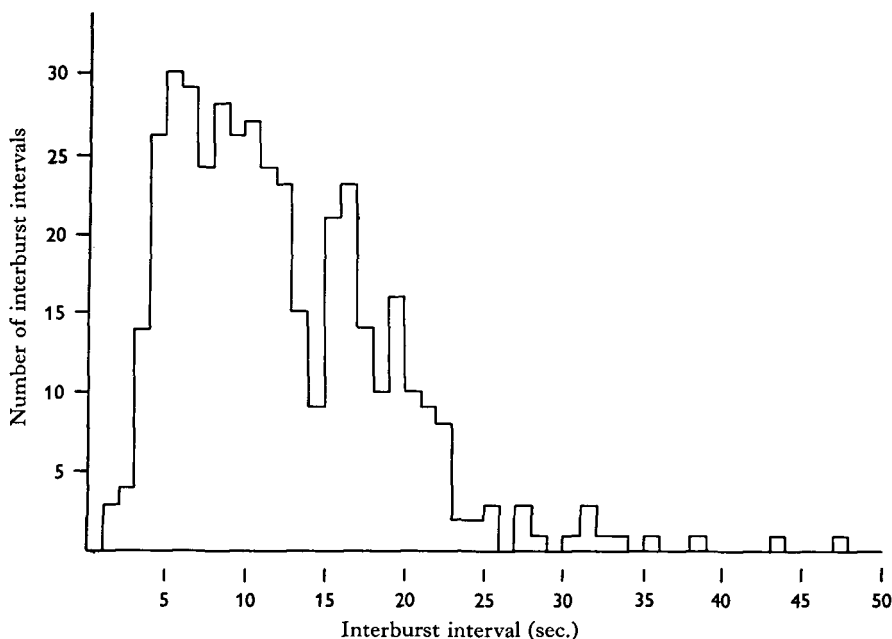


Fig. 1. The variation of the interburst intervals. The histogram is compiled from 420 consecutive interburst intervals taken from one preparation with a mode of 7 sec.

A pause of 10–20 msec. usually follows the first two or three spikes when the entire burst is produced by oculomotor or tegumentary stimulation, as if the initial excitation of the motor neuron is followed by the excitation of a mechanism causing the long burst. Such gaps in the spike train are rarely seen in spontaneously generated bursts.

Dual shocks to either the tegumentary or oculomotor nerves appear to be more effective than single shocks, and the entire burst is elicited sooner in the cycle. Similar summation occurs with the simultaneous stimulation of the tegumentary and oculomotor nerves. However, the continually changing responsiveness of the motor neuron and the variation in the interval between the bursts made careful controls impractical.

Direct stimulation of the motor neuron. De-sheathing the brain does not prevent the motor neuron from firing spontaneously and its course was easily mapped by moving the tip of a large ($100\ \mu$) micropipette back and forth over the brain surface and monitoring the spike amplitude. The motor axon passes posteriorly from the optic tract and skirts the optic and oculomotor neuropiles. Its actively spiking portion apparently ends within the tegumentary and antennary neuropile area (Fig. 3). The characteristic burst of spikes is confined to this path through the brain and no clearly

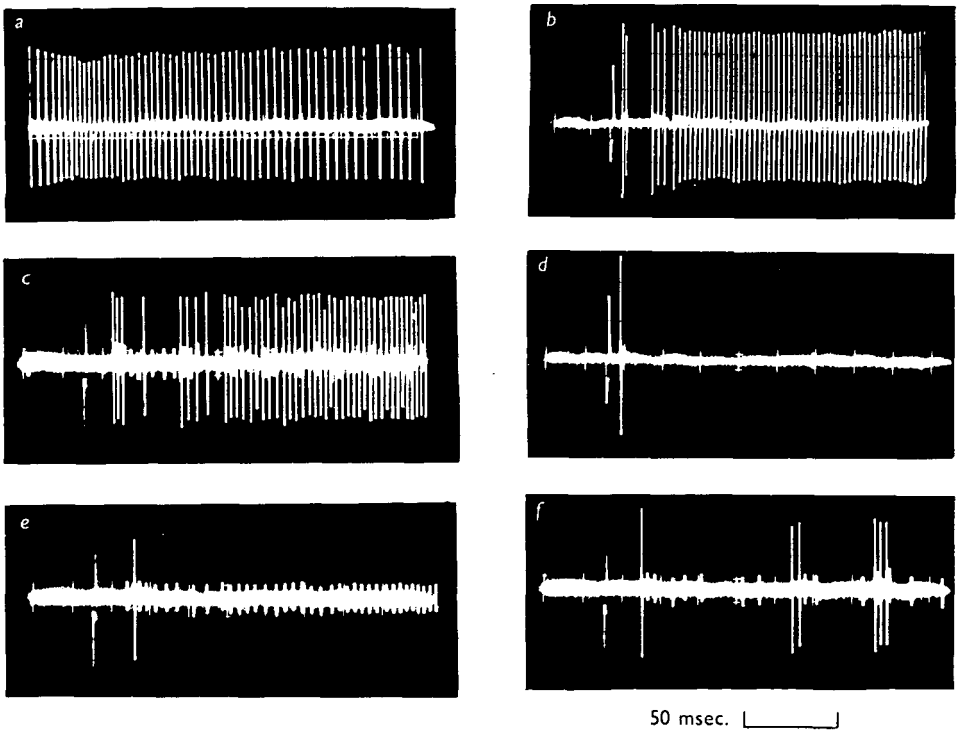


Fig. 2. (a) A spontaneous burst in the optic tract. The peak frequency of 400 impulses/sec. is attained after 30 msec. and is followed by a gradual decrease in impulse frequency. The burst stops abruptly after the last spike shown. (b) Stimulation of the central stump of the oculomotor nerve a few seconds before the burst was due. A gap of 12 msec. follows the first two impulses in the spike train. (c) Similar stimulation of the tegumentary nerve, showing a longer latency between stimulus and initial spike and a greater gap between the initial spikes and the long burst. (d and e) Stimulation of the oculomotor and tegumentary nerves 2 sec. after the spontaneous burst. Only one large spike follows the stimulus in each case. (f) A stimulus to the tegumentary nerve between spontaneous bursts produces a variable number of impulses after the initial spike. The smaller unit in (c), (e) and (f) appears in some preparations and is sensitive to tegumentary stimulation. It is thought to be the response of one of the motor neurons controlling the muscle attached between the eyestalk and the main body skeleton.

correlated antecedent activity has yet been recorded. Nothing has been found of a possible separate pacemaker. However, a single spike in the giant neurons of the crayfish abdominal cord can elicit a high-frequency train of action potentials in an associated motor axon (Wiersma, 1952). If such a system is driving the eye-withdrawal motor neuron, the initial trigger impulse would be easily missed in extracellular recordings.

The recorded amplitudes of the motor-neuron impulses are consistently greater when the recording electrode is directly over the end of the actively spiking portion of the axon in the tegumentary-antennary neuropile. This difference in amplitude may be solely due to the fortuitously close application of the electrode tip to the nerve axon at this point, but the marked change of amplitude following a $50\ \mu$ movement of the electrode in any direction prompted further investigation. Inspection of this area reveals no obvious cell bodies.

Electrical stimulation of the above region with a cathodal square pulse produces a burst of impulses in the axon, recorded in the optic tract. The repetitive activity is maintained for the duration of the stimulus (Fig. 4*a*). Direct stimulation applied at other points along the axon path is never so selective nor does it produce spike trains with such low stimulus intensities as a pulse applied at this 'excitatory' point. The time lapse between direct stimulation in the brain and the arrival of the spikes in the optic tract is about 2 msec. and it is therefore thought that there are no intervening synapses.

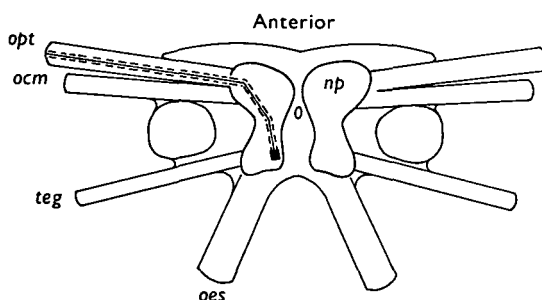


Fig. 3. The approximate course of the motor axon causing eye withdrawal on the left side, mapped by recording from the surface of the de-sheathed brain with a large micropipette. Only the optic tract (*opt*), oculomotor (*ocm*), tegumentary (*teg*) and oesophageal (*oes*) nerve bundles are shown. Delineated areas in the brain show the approximate extent of the neuropile areas (*np*) crossed by the fibre. The small square at the proximal end of the axon marks the 'excitatory' point within the tegumentary/antennary neuropile.

The repetitive responses of isolated crab axons to applied d.c. potentials have been carefully analysed and classified by Hodgkin (1948) and further by Chapman (1966). The motor neuron causing the eye withdrawal belongs to Hodgkin's class 2 in that (1) its frequency of discharge does not follow precisely the intensity of the stimulus; (2) the interval between the spikes in the train bears little or no relation to the response time, and (3) two consecutive stimuli reveal a short period of supernormality lasting from about 2 until about 10 msec. after the impulse.

Stimulation at the 'excitatory' point during the spontaneous discharge has an effect which depends upon the polarity of the stimulus. A 10 msec. cathodal pulse 75 msec. after the start of the burst causes a transient increase in the impulse frequency during the stimulus. After the stimulus the spike train may slow down or, with higher stimulus intensities, lose several spikes (Fig. 4*b, c*). Generally the normal rate of discharge is resumed after about 20 msec., and the overall duration of the burst is not affected. Anodal current pulses applied during the spontaneous burst block the spike discharge and are followed by a transient increase in frequency (Fig. 4*d*). Like cathodal stimulation the after-effect lasts for only about 20 msec.

before the normal spike frequency is resumed. The overall duration of the burst is unchanged by anodal stimulation unless very high stimulus intensities are used, in which case the normal burst is cut short.

Intense direct stimulation of the motor neuron will always produce a train of spikes, but the fluctuation in responsiveness seen in experiments where the oculomotor and tegumentary nerves were stimulated is still observable. This is most clearly brought out by applying a train of low intensity 1 msec. current pulses to the excitatory point in the brain. If the frequency of the stimulus train is 100 pulses/sec., several single nerve impulses which are synchronised with the stimulus precede and follow the spontaneous burst. This result could be expected if the membrane beneath the stimulus electrode were becoming depolarized just before the spontaneous burst. Spikes after the burst are explained by a residual depolarization, not great enough to maintain it.

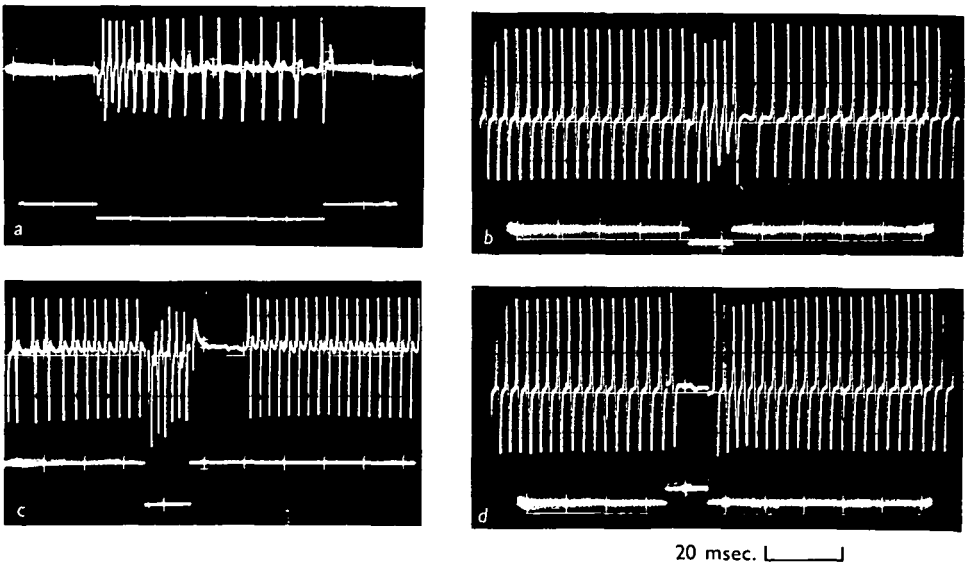


Fig. 4. Direct stimulation of the motor neuron with a large micropipette at the 'excitatory' point within the brain. Records are from the optic tract. (a) The response to a 60 msec. cathodal pulse. The train of spikes, firing initially at 500 impulses/sec., decreases rapidly to half this frequency but fires repetitively for the duration of the stimulus. (b and c) Cathodal pulses of different intensities applied to the 'excitatory' point 100 msec. after the start of the spontaneous burst. In (b) the frequency of firing is raised from 330 to 450 impulses/sec. during the stimulus and this is followed by a decrease in frequency (200 impulses/sec.) which lasts for 20 msec. before the train resumes the initial discharge rate. There is no gap in the spike train. In (c) strong cathodal stimulation raises the spike frequency to 500 impulses/sec., but the stimulus is followed by a 15 msec. break before the original frequency is resumed. (d) Anodal stimulation of the 'excitatory' point blocks the spike train during the stimulus and is followed by an increase in frequency (330-400 impulses/sec.) lasting 20 msec.

The above results suggest that the stimulus pulses are acting directly on the axon of the eye-withdrawal motor neuron and perhaps at the spike-initiating locus. The strongest evidence for the latter is provided by direct cathodal stimulation during the spontaneous burst. An increase in the firing frequency of the neuron follows moderate stimulation but there is no gap in the spike train. If the stimulus were having its

effect further out along the axon the extra pulses generated would occlude those in the spike train which had been centrally generated. Such a gap does occur after strong stimulation when presumably a greater length of the axon is driven repetitive, and antidromically travelling spikes occlude those stemming from the central generating point.

The sensitivity of certain areas of nerve cells to cathodal stimulation has been previously demonstrated in crustacean abdominal ganglia (Biederman, 1964), and shown to correspond with the spike-initiating regions in receptors (Edwards, 1954; Terzuolo & Bullock, 1956; Murray, 1956, 1962) and in spinal cord motor neurons (Barron & Matthews 1938).

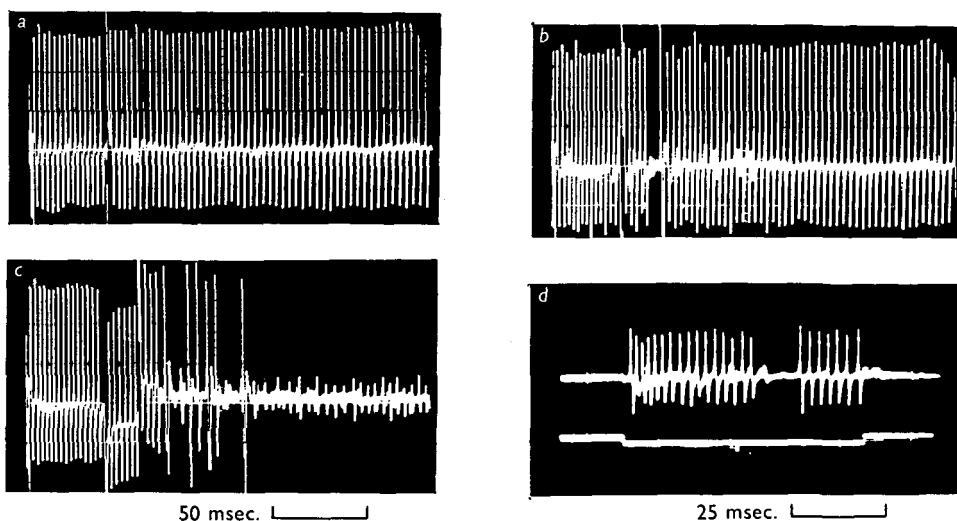


Fig. 5. Inhibition of the repetitive response by stimulation of the ipsilateral oesophageal connective. (a) A 1 msec. stimulus given during the spontaneous burst slows down the spike train after a 12 msec. latency. (b) An increase in the stimulus intensity causes spikes to drop out of the train. (c) Longer duration pulses (20 msec.) stop the burst. In (c) the spikes are displaced by the stimulus artifact. (d) Simultaneous stimulation of the motor axon and the connective. The spike train is generated by a 70 msec. cathodal pulse (lower trace) and the connective receives a 1 msec. pulse 30 msec. after the beginning of the spike train. This causes a 15 msec. interruption in the spike train after a latency of 6 msec. In (a), (b) and (c) the onset of the stimulus is marked by the stimulus artifact and in (d) the sweep speed is twice that of the other records.

Inhibition

The intervals between the spontaneous bursts can be considerably lengthened by stimulating the inside half of the ipsilateral oesophageal connective with a train of 1 msec. pulses. The inhibitory effect adapts and subsequent increase in stimulus intensity and frequency does not necessarily restore it, implying that the inhibition is acting through a synaptic pathway capable of fatigue and is not caused by a direct electrotonic effect of the stimulus on the motor neuron.

A single 1 msec. pulse applied to the connective during a spontaneous burst will slow down the spike train, or cause spikes to drop out, after a latency of about 10 msec. (Fig. 5a, b). Increasing the duration of the stimulus pulse will completely abolish the train (Fig. 5c). The long latency and lasting effect of the inhibition both support the concept that the inhibition is neurally mediated.

The inhibitory effect also spreads to the motor neuron's 'excitatory' point within the train. This is seen when a continuous train of spikes is generated in the motor axon with a long cathodal pulse to the excitatory point, and a single 1 msec. stimulus is applied simultaneously to the connective. 5-7 msec. after stimulation of the connective, spikes in the generated train drop out for periods of up to 15 msec. (Fig. 5*d*).

Spontaneity

The spontaneous firing of the neuron in whole animals and in isolated brains is one of the most interesting aspects of the preparation. The motor neuron is probably not its own pacemaker because statistical tests show no correlation between the burst

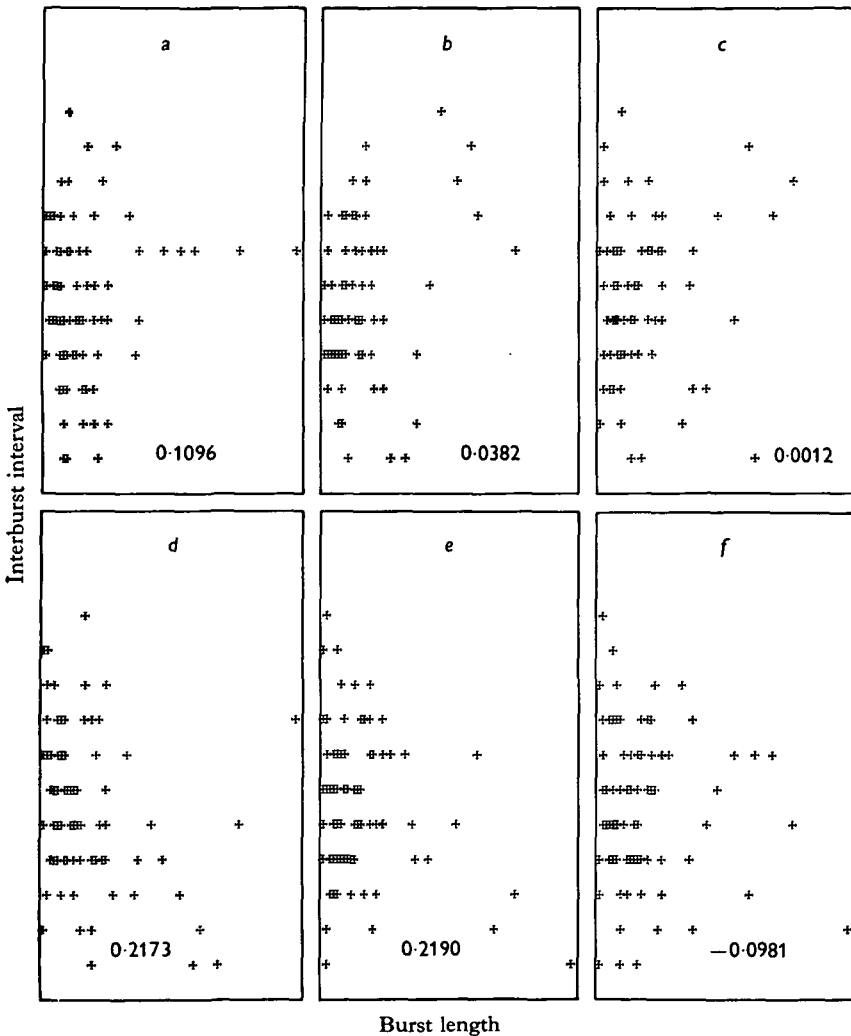


Fig. 6. Scatter diagrams showing the correlation between the burst length and the three preceding and three following interburst intervals. (a) is the relationship between the burst and the third interburst interval before the burst; (b) the second before; and (c) the interval immediately before; (d) is the first interval after the burst, (e) the second and (f) the third. The grouping of the points in the scatter diagrams and the computed correlation coefficients shown for each diagram indicate no significant correlation between the burst length and the interburst intervals.

length and any one of the three preceding or three following interburst intervals (Fig. 6).

The pace-maker rhythm can be temporarily accelerated by stimulating the oculomotor and tegumentary nerves with a continuous train of low-intensity 1 msec. pulses at 100 msec. intervals. The pauses between the spontaneous bursts are reduced initially to about one-third of their previous length, but the effect adapts after only one or two bursts with tegumentary stimulation and after about ten bursts with oculomotor stimulation. In both cases an increase in the stimulus intensity will cause a further slight acceleration of the rhythm before re-adaptation.

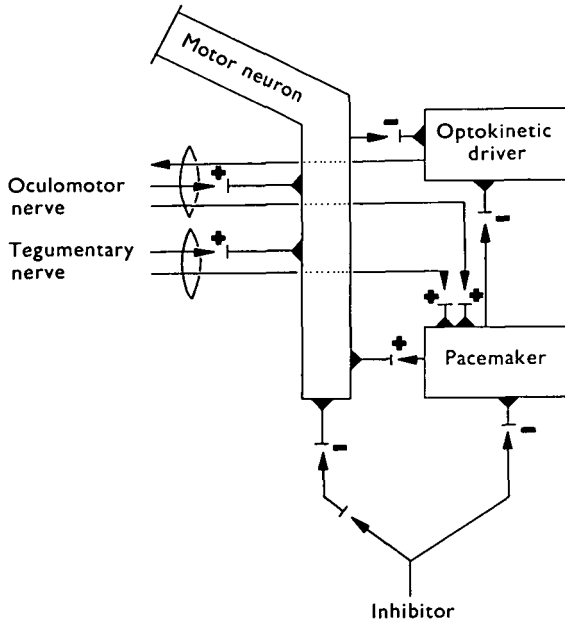


Fig. 7. A flow diagram summarizing the inhibitory and excitatory inputs to the motor neuron described. Arrows mark the direction of impulse travel and excitation or inhibition is marked as a plus or a minus at the different junctions.

The inhibitory effect of the neuron or neurons in the oesophageal connective has already been mentioned in conjunction with direct stimulation of the motor axon, but it is clear that the pacemaker is also affected. The spontaneous bursts can be prevented for 3–4 min., and after the removal of the stimulus to the connective the inhibition is generally followed by a period of acceleration lasting for about 50 msec.

The inhibition of the pacemaker was investigated further by perfusing pharmacological agents into the brain through the cerebral artery. Gamma-amino-butyric acid at a concentration of 10^{-7} g./ml. prevents the spontaneous bursts but its action is blocked by picrotoxin at the same concentration. However, neuronally induced inhibition of the spontaneous bursts is not blocked by picrotoxin at this concentration. The immediate inference is that the neuronal inhibition is not mediated by a picrotoxin-blockable transmitter, but it is also possible that the picrotoxin is not reaching the appropriate site. Pharmacological agents supposed to facilitate or block excitatory pathways suffer from the same criticism and useful experiments depend on being able to impale the axon at the site of action of the transmitter substance.

DISCUSSION

Extracellular recordings from whole nerve bundles and gross stimulation of relatively large areas of the brain are clumsy methods and do not allow more than a tentative interpretation of the action of the large motor neuron within the reflex withdrawal system. Nevertheless, the results of the experiments have been summarised in the flow diagram in Fig. 7. This represents only one possibility of how the components are arranged.

The recorded bursts in the optic tract are from a single motor axon and not several because (1) the spike amplitude decreases slightly with increase in frequency, (2) there is an all-or-none response to direct stimulation over a wide range of stimulus frequencies, (3) there is never a stepwise amplitude change in deteriorating or fatiguing preparations.

The tegumentary and oculomotor nerves are the two explored excitatory inputs. Because they have different latencies they have been shown in the scheme to have separate contacts with the motor neuron, but unequal inter-neuron chains between themselves and the motor neuron are not ruled out. In any event the synaptic contacts are probably spread over arborizations of the motor neuron if the usual pattern of crustacean nervous systems prevails (Kennedy, 1966).

The burst produced by stimulation of the oculomotor or tegumentary nerve has a characteristic pause after the first two or three spikes in the train. This pause may be accounted for if the initial two or three spikes were initiated in the axon separately and distally to the long burst. The first few spikes in the long burst would then be occluded by impulses travelling antidromically from the distal synaptic site, and hence a gap appears in the peripherally recorded spike train. This would also explain the absence of a pause in spontaneous bursts where the axon is activated at only one synaptic site.

The argument for an extrinsic pacemaker rests on the lack of correlation between burst length and interburst intervals. The results, however, do not separate the actions of the burst generator from those of the pacemaker because excitatory pathways which produce bursts also have longer-term acceleratory effects. Similarly inhibitory neurons in the oesophageal connective affect both the burst pattern and the interburst interval. The action of the inhibitor is relatively long-lasting (15 msec.) following a single 1 msec. shock to the connective, and in the scheme a multiplicative stage between the inhibitor and the motor neuron is incorporated to account for this.

Other connexions of the motor neuron can be deduced from previous work. The discharge of the axon is known to cause a central inhibition of some motor fibres controlling compensatory eye movements (Horridge & Sandeman, 1964). Also it has been shown by Burrows (1967) that inhibition of the motor output to some eye muscles precedes a spontaneous burst of the motor neuron by some 500 msec. These two results have been represented in Fig. 7 as a direct inhibitory coupling between the motor axon and the 'optokinetic driver' for one side, and a separate inhibitory link from the pacemaker to the 'optokinetic driver'. The true central organization of the system may be answered by intracellular recordings from its various components within the brain. This aspect is at present being pursued.

The eye-withdrawal reflex is clearly protective. Not only does it quickly secure the eye from possible physical injury, but the very rapid withdrawal would help to sweep detritus and settling parasites out of the eye socket. That this second function is not

always fulfilled is exemplified by some animals which are found with the eye sockets filled with small bivalve molluscs.

SUMMARY

1. Damage to the statocysts or section of the oesophageal connectives of *Carcinus* causes repeated 'spontaneous' eye withdrawals or 'blinking' on the damaged side.

2. When the eyes and brain are isolated from the body, repetitive blinking persists and concomitant bursts of large impulses appear in a single motor axon in the optic tract. The length of these bursts varies from 80 to 180 impulses and the interburst intervals from 5 to 60 sec. There is no obvious correlation between burst length and interburst interval.

3. The bursts are inhibited by stimulating the inside half of the ipsilateral oesophageal connective or initiated by stimulation of the oculomotor and tegumentary nerves. If stimulated with a continuous train of pulses these pathways also cause an increase or decrease in the interburst intervals.

4. The actively spiking portion of the eye-withdrawal motor neuron extends into the brain at least as far as the tegumentary/antennary neuropile. Here it is particularly sensitive to cathodal stimulation, yielding trains of spikes to maintained d.c. stimulation. This point is considered to be near the spike initiating locus for the bursts.

I thank Dr A. J. Cole of the St Andrews Computing Laboratory for his assistance with the statistical computations and Dr G. A. Horridge for his criticism of the manuscript.

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