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INTRASEGMENTAL PROPRIOCEPTIVE INFLUENCES ON THE PERIOD OF THE SWIMMERET RHYTHM IN CRAYFISH

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SUMMARY

When the swimmerets of decapods beat, they do so because the muscles of each swimmeret are driven by a series of periodic bursts of impulses in its motor neurones. We investigated the effects of proprioceptive feedback on the period of this motor pattern by interfering with the movement of particular swimmerets. In different experiments, we observed three different kinds of results during interference with a swimmeret. Either the period decreased, or it did not change, or bursting was inhibited altogether. These different results are discussed in terms of the connectivity of different command fibres.

INTRODUCTION

Hughes & Wiersma (1960) showed that swimmeret movement in crayfish is driven by a centrally generated motor pattern. Each of the swimmerets is driven by alternating bursts of impulses in antagonistic populations of motor neurones. This basic motor pattern can be elicited from isolated, deafferented chains of abdominal ganglia by electrically stimulating command fibres in the CNS, and the patterns generated by these isolated chains of ganglia have the same periods and phase relations as those which occur normally. Sensory feedback is not necessary for the production of the basic motor pattern, but does contribute to the motor output. Proprioceptors at the different joints of the swimmeret respond to bending, and setae on the swimmeret's more distal parts respond to movements of the water (Hughes & Wiersma, 1960). Ikeda & Wiersma (1964) noticed that although the phasing and period of the motor patterns were similar in intact and deafferented abdomens, the detailed structure of the bursts changed in deafferented preparations. Davis (1969*b*) showed that reflexes mediated by proprioceptors and sensory setae reinforce the power stroke and help to initiate the return stroke. If a swimmeret is held experimentally in a position which conflicts with the activity of the endogenous central oscillators, the amplitude of the motor neurone burst is affected, but the period of the rhythm

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remains constant (Davis, 1973). Strong deformation of the soft cuticle at the posterio base of a swimmeret will inhibit bursting in a subset of the motor neurones which innervate that swimmeret, but does not otherwise affect the motor pattern (Stein, 1970).

From these data it appeared that swimmeret reflexes are incapable of contributing to the periodicity of the motor pattern (Davis, 1973). However, swimmerets perform a wide variety of functions and it seems unlikely that the effects of sensory input on the motor pattern would always be so limited. In most behaviours produced by central pattern generators, sensory feedback plays a critical role in adapting the animal's movements to the requirements imposed by the immediate environment (Wilson, 1968). For this reason we decided to repeat some of these earlier experiments to gain more insight into the role of afferent information in the generation of this pattern. We find three different results of interfering with swimmeret movement during periodic beating driven by command fibres.

MATERIALS AND METHODS

Crayfish, *Procambarus clarkii* (Girard), were used in all experiments. The abdomen was separated from the body and bathed in van Harreveld's solution buffered with Tris-maleate. The saline was aerated before use and 2 mM glucose was added at the start of the experiment. Throughout the experiment the saline was cooled to 12–16 °C. The extensor and flexor musculature was dissected away dorsally to expose the ventral nerve cord. The abdomen was then pinned in a Sylgard-lined dish which allowed free movement of the swimmerets.

Recording and stimulation were done with suction electrodes or with pin electrodes. The recorded signals were amplified and filmed directly from the oscilloscope or recorded on tape for later filming. Recording electrodes were placed on anterior and posterior nerve bundles of the first root of the ganglion to record the alternating power and return-stroke motor pattern of the swimmeret. To stimulate the command fibres that drive the swimmeret motor neurones the nerve cord was desheathed in a region directly anterior to the ganglion and small bundles of axons were tested for command fibres. Glass needles were used to separate the nerve bundles of the connectives.

In all experiments the search for swimmeret command fibres was a slow process. Interneurones that inhibit the motor pattern run alongside some of the command fibres in the connective (Wiersma, personal communication; Wiersma & Ikeda, 1964). Stimulation of these inhibitory fibres may result in the misleading inhibition of the swimmeret motor pattern. For this reason bundles in the connective were split as finely as possible for stimulation. Nerve bundles were drawn into the suction electrode and stimulated over a wide range of frequencies and intensities. Stimulation at 45–60 Hz commonly elicited vigorous bursting. Once a command fibre was located and a regular motor pattern was produced we interfered with the swimmeret's movement by blocking with a bent pin held in its path. The swimmeret was held back in the fully retracted position that occurs at the end of a power stroke for several bursts, then allowed to move freely. After several more strokes the swimmeret was

held forward in the protracted position that occurs at the end of a return stroke. The forward position was also maintained for several bursts, then released. We tried to hold the swimmerets tonically, without varying periodically the position or strength of the block to movement.

RESULTS

The three command interneurones that lie in the most lateral regions of each connective were easiest to find and stimulate; all our experiments were done with these fibres. They probably correspond to Wiersma & Ikeda's interneurones A, B and C (1964). Our methods did not permit confident identification of the same command interneurone from one animal to the next and did not allow us to differentiate between the three possible interneurones in each animal. Since the swimmerets were intact and free to move, we identified bursts of impulses recorded from branches of the first root as power-stroke or return-stroke bursts by correlating their occurrence with the movement of the swimmeret. We report here only the results of experiments in which the swimmerets beat vigorously and regularly.

Once a command fibre had been isolated satisfactorily, we began a prolonged train of stimuli while recording the motor output to a chosen swimmeret. After several bursts with similar periods had occurred, we manually placed a small insect pin in the path of the beating swimmeret to hold it retracted for several burst periods, then released it. After the bursts had stabilized again, we used the pin to hold the swimmeret maximally protracted for several bursts, and then released it. This procedure allowed us to define a normal or control period – the average time from the start of one burst to the start of the next burst – and to measure the changes in the periods of bursts which occurred while the swimmeret was held. In non-beating preparations, strong retraction by this method caused a tonic discharge in several small units in the first root. This discharge was graded with the extent of retraction, and continued if the first root was cut proximal to the recording electrode, so we take it to be the afferent input to the ganglion from the coxal proprioceptors (Hughes & Wiersma, 1961; Davis, 1969*b*, 1973). When we performed this test in different experiments we found three classes of results.

No change in period

In six experiments, retraction and protraction of the swimmeret during rhythmic swimmeret movement had no effect on the burst period. Fig. 1 illustrates one of these experiments. Here, bursts in return-stroke motor neurones were recorded from the left first root of the 3rd abdominal ganglion while stimulating a command fibre in the left connective. Burst duration increased after release of the retracted swimmeret. In the other experiments, burst duration remained relatively constant throughout the experiment. In two experiments we stimulated the command fibre at two frequencies (28 and 45 Hz) to see if the proprioceptive effects were sensitive to the intensity of the command; we obtained the same negative results at both stimulus frequencies.

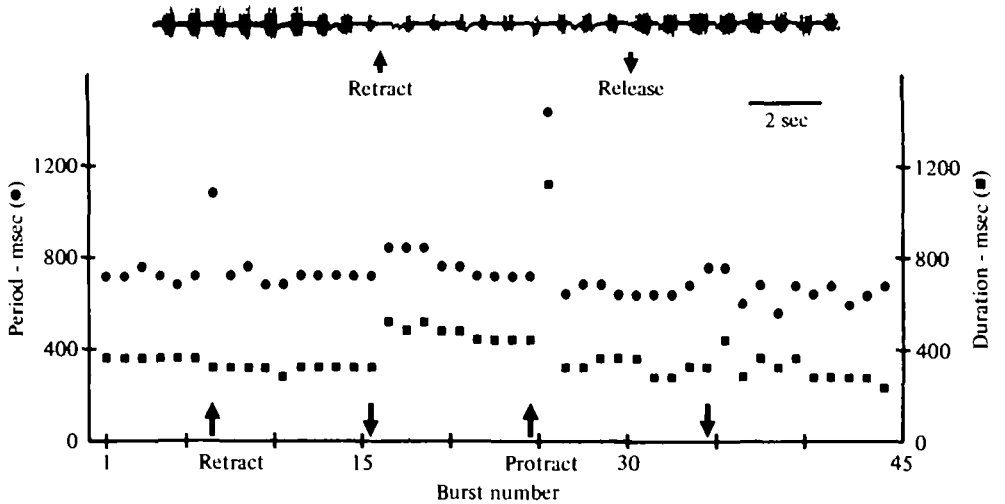

Fig. 1. The oscilloscope trace shows bursts of the return-stroke motor neurones resulting from electrical stimulation of a command fibre. This motor pattern was recorded from the first root of the third ganglion and drives the return-stroke phase of swimmeret movement. The plotted data show no change in burst period when the swimmeret was held in retracted or protracted positions. Duration increased upon release of the retracted swimmeret. Arrows mark the beginning and end of mechanical interference with swimmeret movement. ●, period; ■, duration.

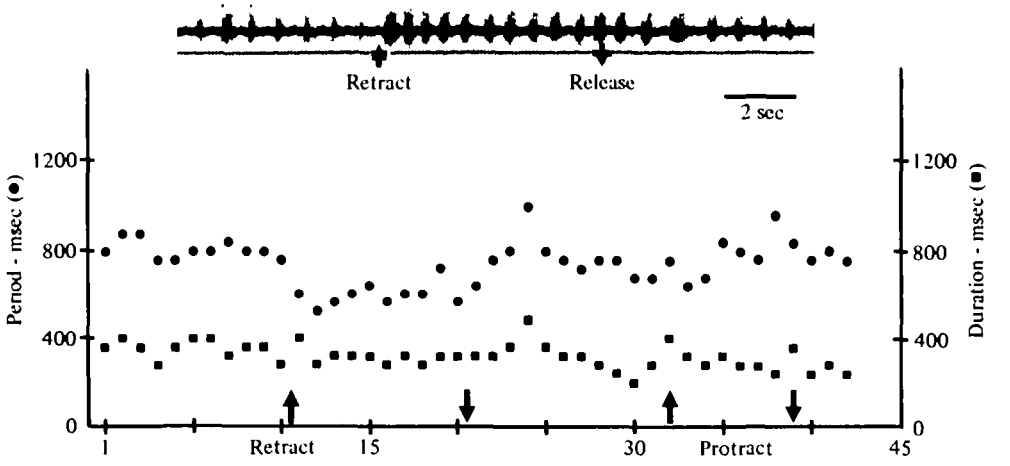

Fig. 2. Same type of preparation and format as Fig. 1, but in this case holding the swimmeret back in the retracted position reduced the burst period of the motor pattern. The protracted position did not alter the burst period. Burst duration was not affected by retraction or protraction of the swimmeret. ●, period; ■, duration.

Decrease in period

In six experiments, retraction of the swimmeret caused a decrease in the period of the rhythmic motor pattern. Fig. 2 illustrates the data on return-stroke bursts recorded from the left 1st root of the 3rd abdominal ganglion. The command fibre was stimulated in the left connective. Other experiments that showed a decrease in burst period were performed on swimmerets of the 4th and 5th ganglia. Bu

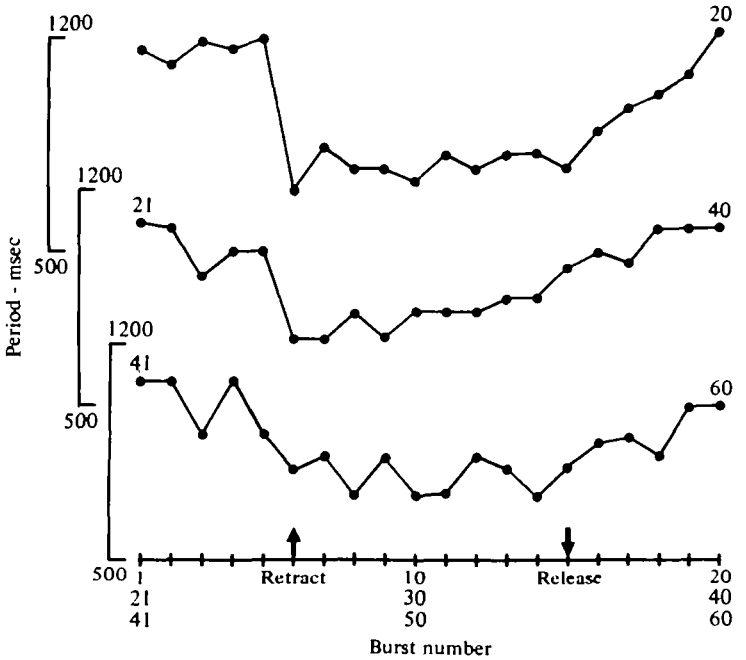

Fig. 3. The periods of a continuous train of bursts in swimmeret motor neurones during which the right swimmeret of the fifth ganglion was retracted three times. Ten bursts occurred between each retraction; successive retractions are superimposed to permit comparison of the periods of corresponding bursts. The periods of successive bursts are connected in this figure to make clear the sequence in which they occurred.

duration remained relatively constant throughout these experiments. In two experiments we retracted the swimmeret repeatedly to see if these manipulations produced reproducible effects; Fig. 3 shows the results of repeated blocks of the right swimmeret of the 5th segment. In this experiment, the command fibre was stimulated at 17 Hz, and the swimmeret beat freely in the interval between each test. In both experiments period decreased each time the swimmeret was held back.

Inhibition of bursting

Three experiments showed complete inhibition of bursts during retraction and protraction of the swimmeret. In these experiments, signals were recorded from the left first root of the third abdominal ganglion while stimulating a command fibre in the left connective. The oscilloscope trace in Fig. 4 shows the inhibition of power-stroke bursts during mechanical interference with swimmeret movement.

We did not observe other responses to mechanical interference with a swimmeret in any experiment in which stimulation of a command fibre elicited a stable period of beating. In experiments in which period was unstable, we could not distinguish changes in period caused by interference with movement from changes caused by other uncontrolled variables. We did observe several times that retraction increased the period of beating, but this increase was not neatly reproducible. Repeated tests in any one preparation caused both increases in period and complete inhibition.

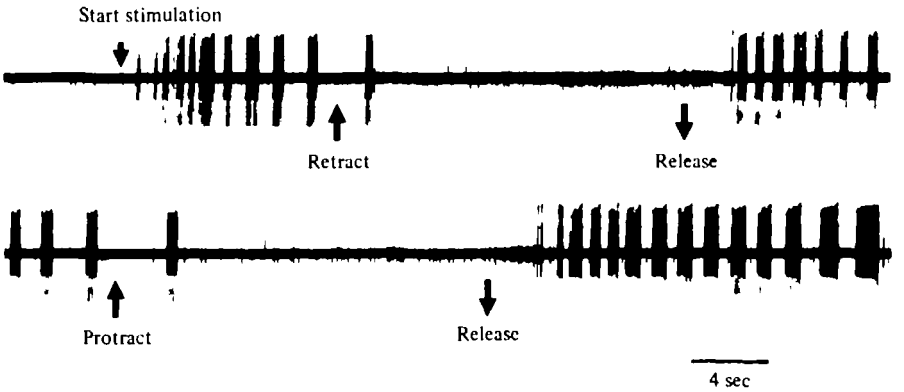

Fig. 4. The oscilloscope traces are continuous and show the bursts of the motor neurons driving the powerstroke phase of swimmeret movement. The experimental format was the same as that of Figs. 1 and 2. Arrows mark retraction, protraction and release phases of mechanical interference with the swimmeret movement. Burst activity is completely stopped when the swimmeret is retracted and protracted. Normal bursting activity returns when the swimmeret is allowed to move freely.

DISCUSSION

The three distinct results we have observed are reproducible, and do not seem to intergrade. The decrease in period during maintained retraction is novel in this system, but not unexpected since interference with periodic movements of limbs in other animals will alter the period of their centrally generated motor patterns – for example, stepping in cats (Duysens, 1977) and cockroaches (Wong & Pearson, 1976). These manipulations of the swimmeret will elicit resistance reflexes from inactive swimmerets (Davis, 1969*b*), and would be predicted if, under some conditions, afferent signals excite the central oscillator in their own hemisegment.

The cases where we saw no effect on period confirm Davis's (1969*b*) finding in *Homarus* that neither retraction nor protraction altered period, although they had major excitatory effects on impulse frequency during each burst in excitatory motor neurones. The cases where bursting in powerstroke neurones stopped during retraction is consistent with Stein's observation (1970) in *Procambrus* that 'pushing the cuticle of the stump of the swimmeret so that the stump was in the maximally retracted position' could inhibit powerstroke excitors, return-stroke peripheral inhibitors and the medial ascending co-ordinating interneurones. We suspect that pushing the cuticle hard enough to retract the swimmeret would not only activate sensory afferents in the cuticle (Pabst & Kennedy, 1967) but also the coxal proprioceptors.

Several factors could have contributed to our finding more than one result, and to the failure of other workers to observe a decrease in period. Perhaps because it is difficult to position precisely a hand-held pin, we were not applying the same stimulus in different trials. We do not think so because repeated tests of the same swimmeret in any one preparation gave consistent results (Fig. 3). Perhaps since several joint receptors occur at different swimmeret joints (Hughes & Wiersma, 1960) and axons from setae on the rami also excite motor neurones (Davis, 1969*b*, 1973), we were actually stimulating a variable mixture of receptors, and this variability is the source of the different responses. Again, we do not think so because repeated trials

any one preparation gave consistent results; if we stimulated a significantly different set of receptors each time we blocked the swimmeret, we should not have observed consistent results.

A referee suggested another source of variability: that the proprioceptive input might be relatively weak, and can only influence period when the command is weak. The experiments in which we saw the same effects at two different frequencies of stimulation of the command fibres make this possibility unlikely. We think the significant variable is the command fibre which was isolated for stimulation. We could not be sure which of the three command fibres in the lateral region of the connective we isolated in each case, but it is very likely that we isolated only one in each experiment since two of the three run near inhibitory fibres (Wiersma & Ikeda, 1964) and we split the bundles of axons very finely to eliminate these inhibitors. The three distinct results suggest to us the hypothesis that each command fibre controls the access of the central oscillator to sensory information and each does so in a distinctive way. According to this idea, the experiments in which we, and Davis, saw no effect of retraction on period, were ones in which we were stimulating a command fibre which blocks the flow of impulses from the proprioceptors to the central oscillator. In the experiments in which we, and Stein, saw an effect, we were stimulating a different command fibre.

The mechanism by which command fibres might gate the flow of information is unknown, but two alternatives merit attention; the command fibres might inhibit directly the terminals of a selected set of sensory afferents by a presynaptic mechanism (Kennedy, Calabrese & Wine, 1974), or each might activate a different set of corollary discharge interneurones which themselves inhibit some of the terminals of these afferent fibres (Wine, 1977; Krasne & Wine, 1977). The behavioural significance of these different results is also unknown. Davis & Kennedy (1972) argue that each command fibre can drive only part of the range of beat frequencies which the crayfish uses, so the animal uses more than one pair in concert to obtain its full range. An alternative view is that each pair of command fibres is responsible for a different behaviour, and that it gates the influx of proprioceptive information in a pattern appropriate for that behaviour.

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REFERENCES

DAVIS, W. J. (1969). Reflex organization in the swimmeret system of the lobster. I. Intrasegmental reflexes. *J. exp. Biol.* **51**, 547–564.

DAVIS, W. J. (1973). Neuronal organization and ontogeny in the lobster swimmeret system. *Adv. behav. Biol.* **7**, 437–455.

DAVIS, W. J. & KENNEDY, D. (1972). Command interneurones controlling swimmeret movements in the lobster. II. Interaction of effects on motor neurons. *J. Neurophysiol.* **35**, 13–19.

DUYSENS, J. (1977). Reflex control of locomotion as revealed by stimulation of cutaneous afferents in spontaneously walking premammillary cats. *J. Neurophysiol.* **40**, 737–751.

HUGHES, G. M. & WIERSMA, C. A. G. (1960). The coordination of swimmeret movements in the crayfish, *Procambarus clarkii*. *J. exp. Biol.* **37**, 657–670.

IKEDA, K. & WIERSMA, C. A. G. (1964). Autogenic rhythmicity in the abdominal ganglion of the crayfish: the control of swimmeret movements. *Comp. Biochem. Physiol.* **12**, 107–115.

KENNEDY, D., CALABRESE, R. L. & WINE, J. J. (1974). Presynaptic inhibition: primary afferent depolarization in crayfish neurons. *Science, N.Y.* **186**, 451–454.

KRASNE, F. B. & WINE, J. J. (1977). Control of crayfish escape behavior. In *Identified Neurons and Behavior of Arthropods* (ed. G. Hoyle), pp. 275–292. Plenum.

PABST, H. & KENNEDY, D. (1967). Cutaneous mechanoreceptors influencing motor output in the crayfish abdomen. *Z. vergl. Physiol.* **57**, 190–208.

STEIN, P. S. G. (1970). A neurophysiological study of two systems of coupled oscillators in crayfish. Doctoral thesis, Stanford University.

STEIN, P. S. G. (1971). Intersegmental coordination of swimmeret motor neuron activity in the crayfish. *J. Neurophysiol.* **34**, 310–318.

WIERSMA, C. A. G. & HUGHES, G. M. (1961). On the functional anatomy of neuronal units in the abdominal cord of the crayfish, *Procambrus clarkii*. *J. comp. Neurol.* **116**, 209–228.

WIERSMA, C. A. G. & IKEDA, K. (1964). Interneurons commanding swimmeret movements in the crayfish, *Procambrus clarkii* (Girard). *Comp. Biochem. Physiol.* **12**, 509–525.

WILSON, D. M. (1968). Inherent asymmetry and reflex modulation of the locust flight motor pattern. *J. exp. Biol.* **48**, 631–642.

WINE, J. J. (1977). Neuronal organization of crayfish escape behaviour: Inhibition of giant motoneurons via a disynaptic pathway from other motorneurons. *J. Neurophysiol.* **40**, 1078–1097.

WONG, R. K. S. & PEARSON, K. G. (1976). Properties of the trochanteral hair plate and its function in the control of walking in the cockroach. *J. exp. Biol.* **64**, 233–250.