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Mechanisms for the Snapping Behavior of Two Alpheid Shrimp, *Alpheus californiensis* and *Alpheus heterochelis*

Roy E. Ritzmann

Department of Biology, University of Virginia, Charlottesville, Virginia, USA

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Summary. 1. In the snapping movement of *Alpheus californiensis* the opener muscle of the propus is first contracted to cock the dactyl of the snapping cheliped, then the closer muscle is excited by a high frequency train of impulses. This train continues until the force holding together disks on the dactyl and propus is overcome. At this point the dactyl snaps closed and the closer excitatory motoneuron becomes silent.

2. A reflex is demonstrated whereby the opener muscle is excited upon passive opening of the dactyl. This is opposite to typical passive limb reflexes and may be important in setting the cocking mechanism.

3. All of the characteristics of snapping can be found in the small pincer cheliped with the exception of the long duration high frequency train of impulses in the closer muscle.

4. Another alpheid shrimp, *Alpheus heterochelis*, uses a completely different mechanism to hold the dactyl open while the closer builds tension. Here the closer apodeme is lifted over the pivot point around which the dactyl closes. In this way the dactyl is locked until a second closer muscle contracts to pull the closer apodeme down.

Introduction

Shrimp of the family Alpheidae have one cheliped which is greatly enlarged and can be closed with remarkable force. The dactyl of the large cheliped possesses a plunger which fits into a socket on the propus. Rapid closure of the cheliped causes a jet of water to be expelled from the socket. At the same time, a loud snap is produced by the dactyl hitting the propus. Previous investigators of alpheid snapping (Johnson *et al.*, 1947; Knowlton and Moulton, 1963; Hazlett and Winn, 1962; Nolan and Salmon, 1970) have shown that this behavior is used by the animals in defensive and aggressive activities. Most investigators agree that the significant component of the snapping behavior is the jet of water that is expelled from the socket on the propus, rather than the snapping sound.

While the snapping behavior has been studied in detail, the mechanism responsible for snapping has not. Suggestions have been made to explain the snap (Courtier, 1899; Verrill, 1922; Johnson *et al.*, 1947; Knowlton and Moulton, 1963), but very little experimental evidence has been presented to support any of these hypotheses.

Several types of arthropod have developed unusually rapid or forceful movements by employing exoskeletal modifications. Examples of these include the mantid shrimp strike mechanism (Burrows, 1969), flea jumping (Rothschild *et al.*, 1972; Bennet-Clark and Lucey, 1967) and locust jumping (Brown, 1967; Heitler, 1974). In addition to exoskeletal modifications, rapid movements have also been acquired by neuromuscular specializations. An example of this is in the very fast

muscle used during lobster antennular vibration (Mendelson, 1969). Finally some arthropods have employed both exoskeletal and neuromuscular modifications, such as the flight adaptations of several insects, in which a click stop mechanism has been developed at the articulation of the wings and the thorax along with the highly specialized asynchronous flight muscle (Pringle, 1957).

In the case of the shrimp *Alpheus californiensis*, extremely well-matched disks located on the dactyl and propus stick to each other probably by cohesive forces of the layer of water between the surfaces of the two disks and, thereby, prevent the dactyl from closing while the closer muscle builds tension (Ritzmann, 1973). When a large amount of force is developed in the closer muscle the force holding the disks together is overcome and the dactyl snaps closed. The fact that the disks play an important role in the snapping behavior of this species does not eliminate the possibility that neurophysiological modifications also are necessary for snapping in this species or in other species of snapping shrimp. The following study was performed to determine what, if any, neurophysiological modifications are important in the snapping behavior.

The main part of the study was done with two species from the Pacific Coast of North America *Alpheus californiensis* and *A. dentipes*. No differences were found in the snapping mechanism of these two species and, therefore, reference will be made only to *A. californiensis*. A comparative study was performed with an Atlantic Coast species, *A. heterochelis*.

Materials and Methods

Alpheus californiensis and *A. dentipes* were obtained from Pacific Bio-Marine Supply Company, Venice, California and were maintained in an artificial sea water tank at 12–16° C. Specimens of *A. heterochelis* were obtained from Jack von Montfrans of Boca Raton, Florida, and were kept at room temperature. In all cases the saline used was an artificial sea water having the following composition: NaCl, 423 mM; KCl, 9.00 mM; $CaCl_2$, 9.27 mM; $MgCl_2$, 22.94 mM; $MgSO_4$, 25.50 mM; $NaHCO_3$, 2.15 mM.

Suction electrodes were used for stimulation and extracellular recording from nerves. Successful recording and stimulation were achieved only when the cut end of a nerve was sucked into the suction electrode tip, never with the electrode attached to the side of the nerve, or with hook electrodes in oil.

Extracellular electromyograms (EMGs) were recorded with 85 μ copper wire electrodes insulated except at the tips, which were inserted into the muscle of the propus through small holes in the cheliped exoskeleton. Intracellular muscle recordings were made with glass micropipette electrodes filled with 2.5 M KCl and having resistances of 15–35 megohms. The electrodes were suspended on a silver wire to allow a greater degree of movement for recordings during snapping movements. All of the electrical records were amplified and displayed by conventional methods.

An oscillator transducer (Sandeman, 1968) was used for monitoring the dactyl movements of *A. californiensis*. To determine the timing of the snap of *A. heterochelis* a microphone was placed near the animal. This gave an electrical signal when the animal snapped its dactyl closed.

Cross sections of nerves were prepared in the following manner. The nerves were dissected out of live animals and were fixed for three hours in cold fixative consisting of two parts 2% potassium permanganate dissolved in unbuffered artificial sea water and one part 0.05 M cacodylate buffer. The final pH was 7.4. The tissue was dehydrated in an ethanol series, embedded in epon and cut into 10^{-1} micron sections. These sections were stained with a saturated solution of uranyl acetate in 50% acetone for two minutes and counterstained in an aqueous solution of lead citrate for one minute (Reynolds, 1963). The tissue was studied in an

Hitachi HU-11E electron microscope. Fixation was unnecessary in the preparation for scanning electron microscopy, because only the hard exoskeleton was observed. The chelae were coated with gold before being studied.

Results

I. Organization of the Motor Nerve Supply

There are two main nerves running through the cheliped, which will be referred to as the dorsal and ventral nerves (Fig. 1). The dorsal nerve can be followed along the dorsal border of the merus, through the carpus and along the dorsal edge of the propus to the opener muscle. From this point it passes between the opener and closer muscles and into the dactyl. All along the course of this nerve branches are found which lead to the overlying epidermis and presumably contain axons of cuticular sensory receptors. Branches also pass into the opener muscle.

The ventral nerve is approximately ten times larger in diameter than the dorsal nerve. It is made up of two bundles of equal size which usually can be separated in the merus. As the ventral nerve enters the propus, these two bundles separate. The ventrolateral bundle can be followed along the lateral side of the propus and innervates the hairs on the cuticle in this area. At about the level of the articulation of the propus and dactyl, this nerve bifurcates. One branch continues around the socket into which the plunger on the dactyl fits and innervates the distal portion of the propus. The other branch passes dorsally into the dactyl.

The ventromedial bundle of the ventral nerve goes through the closer muscle to the medial side of the propus and passes along this surface for approximately 5 mm. It then branches several times to supply the hairs in this area. Branches to the closer muscle can also be seen along the course of this nerve.

The neuromuscular organization of the propus was studied by two methods: intracellular recording of muscle fibers in intact animals during snapping and in

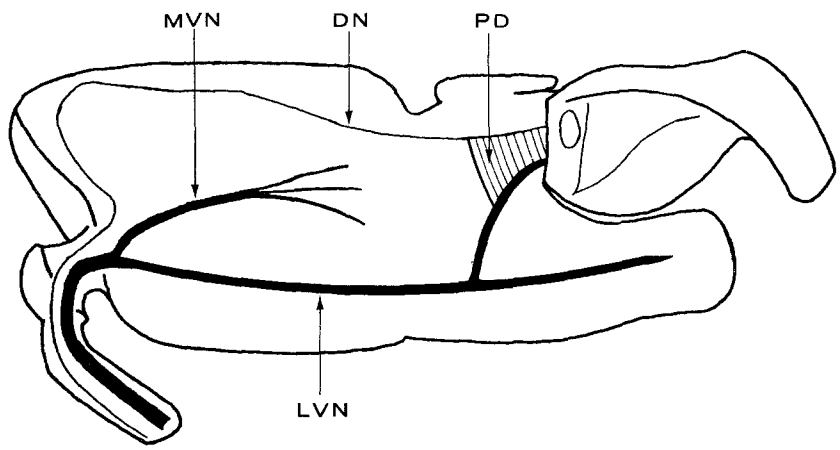

Fig. 1. Diagram of the nervous system of the snapping cheliped. *MVN*, medial bundle of the ventral nerve; *LVN*, lateral bundle of the ventral nerve; *DN*, dorsal nerve; *P-D*, propus-dactyl chordotonal organ. The opener muscle is located dorsal to the dorsal nerve. The rest of the propus contains the closer muscle

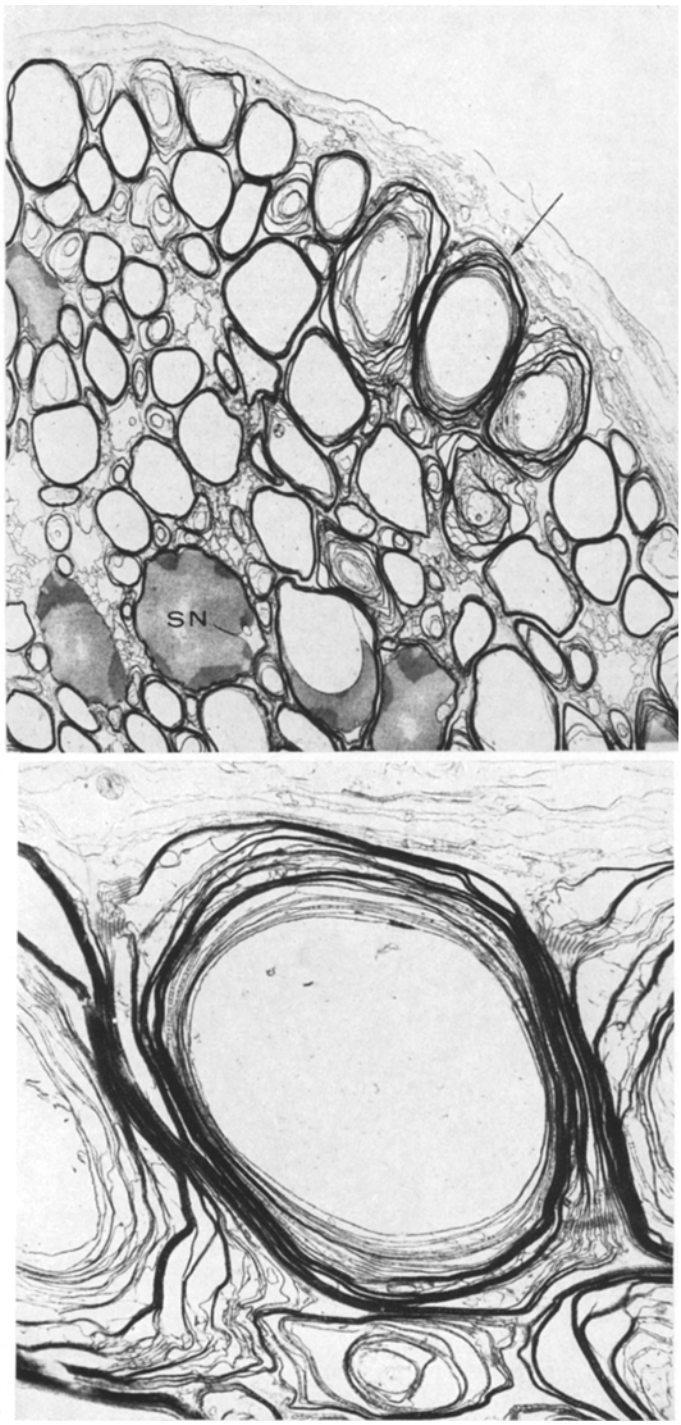

Fig. 2. (A) Cross section of the dorsal nerve showing axons surrounded by myelinated sheaths. *SN*, sheath nucleus. Magnification $\times 1500$. (B) Higher magnification electron micrograph of the axon in (A) which is labeled with an arrow. Magnification: $\times 6562.5$

isolated chelae during stimulation of nerves located in the merus. It would have been desirable to record from the motor nerves while recording muscle potentials in order to be certain about the physiological identification of axons. However, all attempts to record from nerves *en passant* failed, even though the same nerve could be recorded from by sucking the cut ends into a suction electrode. This may have been because the axons are very small, and are surrounded by myelinated sheaths, as can be seen in cross sections of the nerves (Fig. 2). Myelination has been reported in other shrimp (Heuser and Doggenweiler, 1966; Kusano, 1966). Kusano (1966) reported that the myelin sheath around the giant fibers in the ventral nerve cord of the shrimp *Penaeus japonicus* has a high electrical resistance. He further demonstrated that a microelectrode could detect action potentials in the axon when it was positioned between the axon and the myelin sheath, but not when it was placed outside the sheath.

Intracellular recording from muscle fibers in isolated chelae indicated that the opener muscle receives no innervation via the ventral nerves but does receive one excitatory axon from the dorsal nerve (Fig. 3). In recording from thirty fibers no case was found where the excitatory junction potential (EJP) of an opener muscle fiber suddenly increased to a higher amplitude as the stimulus to the dorsal nerve was increased in amplitude or duration. Such an increase would indicate that the threshold of excitation for a second excitatory axon had been reached, although there is a possibility that this could result from the blockage of an inhibitory axon as well. Neither were there distinctly different sized EJPs seen in the records from opener muscles of intact animals (Figs. 4 and 5). While this suggests that only a single motor axon innervates the opener muscle, there is other evidence which casts doubt on this conclusion. Recordings were made from the proximal stump of the dorsal nerve while reflex output to the opener muscle was elicited. This reflex activity will be discussed later. In these records three sizes of action potentials were seen (Fig. 6C). The largest and the intermediate size potentials were always paired in a doublet, with an interval of 5 msec or less. The smallest potentials seemed to have no temporal relationship with these two. Several possible explanations for these results will be presented in the discussion.

The motor axons to the closer muscle are in the medial bundle of the ventral nerve. The closer is innervated by one inhibitory axon and at least one excitatory

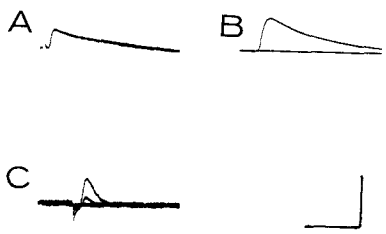

Fig. 3A—C. Intracellular recordings from muscles of an isolated cheliped. The nerves were stimulated in the merus with increasing stimulus strength. (A) EJP from an opener muscle fiber during stimulation of the dorsal nerve. (B) EJP from a closer muscle fiber during stimulation of the ventral nerve. (C) Extracellular current recording from the closer muscle showing two thresholds to stimulation. Calibrations: (A and B) 20 mV, 50 msec; (C) 2 mV, 50 msec

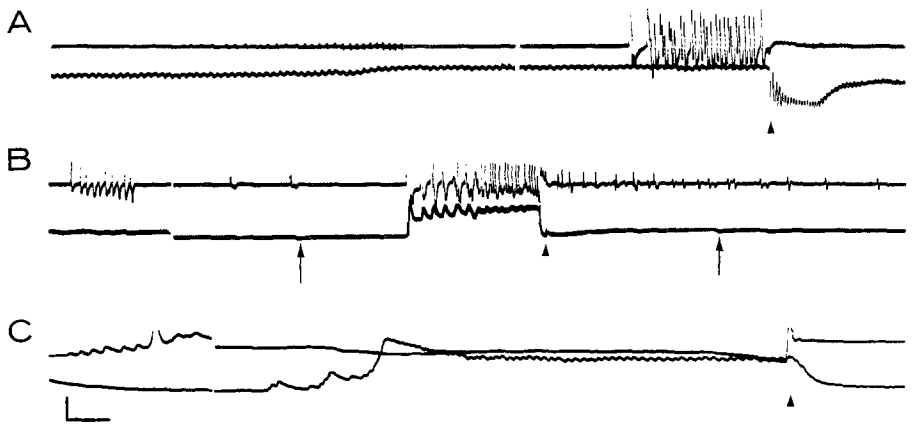

Fig. 4. (A) Top trace is an extracellular recording from the muscles of the propus of a cheliped on an intact animal. Bottom trace is output from a transducer attached to the dactyl. Up on the transducer record indicates opening of the dactyl. The snap occurs at the sharp downward deflection indicated by an arrowhead. The small potentials in the upper trace are presumably from the opener muscle, while the large potentials are from the closer. (B) Top trace is an extracellular recording from the muscles of the propus during opening and a snap. Potentials from both the opener and closer muscles are seen in this trace. Bottom trace is a simultaneous intracellular recording from a closer muscle fiber. The snap occurs after the train of EJPs in the closer muscle fiber as indicated by an arrowhead. This was determined by comparing the extracellular trace with extracellular recordings, such as in (A), of several other fibers in which a transducer was used to monitor movement. The burst of potentials at the beginning of the upper trace are from the opener muscle. Note the IJPs (arrows) in the closer recording before and after the snap. (C) Simultaneous recordings from opener (top trace) and closer (bottom trace) muscle fibers during a snap. The snap occurs at the arrowhead. Note that the opener is silent during the train of EJPs in the closer which immediately precedes the snap. In all cases several hundred milliseconds have been removed between opening and closing. Calibrations: (A) 100 msec; (B) 25 mV, 200 msec; (C) 25 mV, 100 msec

axon. The inhibitor was identified in the records from intact animals, where hyperpolarizing inhibitory junction potentials (IJPs) could be seen in records taken from closer muscle fibers during activation of the opener muscle (Figs. 4B and 5C). EJPs could be consistently recorded in isolated cheliped preparations by stimulating the medial bundle of the ventral nerve in the merus, as well as in intact animal recordings. One hundred fibers were recorded from intracellularly in isolated chelae, and none showed an increase in amplitude as stimulus strength was increased to the ventral nerve (Fig. 3B). One extracellular current recording did show a second amplitude (Fig. 3C). The smaller of these potentials could be from a depolarizing inhibitory potential or a second excitor. If only one excitor exists, this would be contrary to the organization in all other crustaceans that have been studied, which have two excitors to the closer muscle of the propus (Wiersma and Ripley, 1952). The solution to this problem may be that the axons innervate different populations of fibers so that recording from few if any fibers would show EJPs from both excitors. Another possibility is that one of the axons innervates only a few muscle fibers.

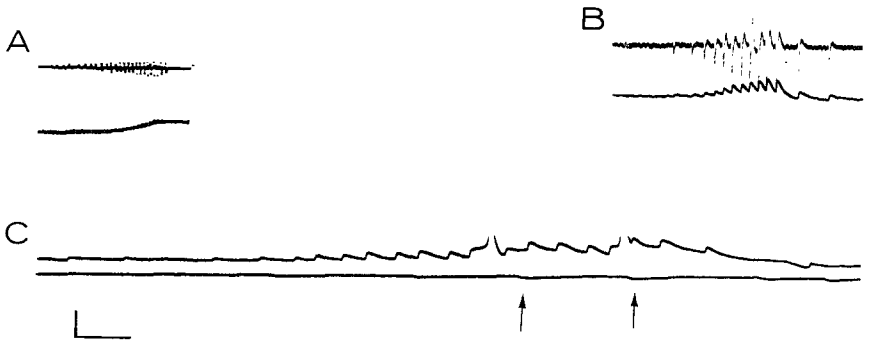

Fig. 5. (A) Top trace is an extracellular recording from the muscles of the propus of a cheliped on an intact animal during passive opening of the dactyl. Bottom trace is output from a transducer attached to the dactyl. Up on the transducer record indicates opening of the dactyl. The sharp rise in the transducer record occurs as the dactyl moves away from the probe that is used to lift the dactyl. (B) Top trace is an extracellular recording of the passive opening reflex. Bottom trace is a simultaneous intracellular recording in an opener muscle fiber. (C) Simultaneous intracellular recordings from opener (top trace) and closer (bottom trace) muscle fibers during the passive opening reflex. The two breaks in the opener record are the result of action potentials in the opener muscle fiber which cause the oscilloscope beam to go off the screen. Arrows indicate IJPs in the closer record. Calibrations: (A) 250 msec; (B) 25 mV, 200 msec; (C) 25 mV, 100 msec

II. Neuromuscular and Reflex Physiology of the Snap

The snapping behavior begins with a contraction of the opener muscle, bringing the dactyl into the open or cocked position. This is followed by excitation of the closer muscle (Fig. 4). When the dactyl is cocked, the two dactyl disks meet and hold the dactyl open until the closer muscle tension can overcome the forces holding the disks together (Ritzmann, 1973). A series of EJPs can be recorded in the closer prior to the snap. Initially these EJPs occur as large temporally summated potentials, but as the train continues, the individual EJPs separate. The instantaneous frequency of this train is initially up to 100/sec and decreases to 50/sec at the end of the train. The duration of the train is highly variable. However, it is always terminated at the time that a snap occurs, or when the dactyl moves out of the cocked position. The one exception to this occurred in an animal that was obviously fatigued. In this case after the dactyl was cocked, several short abortive trains of impulses were initiated before a long train finally started. The long train continued until the dactyl snapped closed except in the last trial that was observed. At this point the closer could no longer develop enough tension to overcome the force holding the disks together. After about 200 msec the train of EJPs stopped for a short period and then started again. This was repeated five times before the dactyl was manually released from the cocked position. When this was done the train stopped and did not start again. Trains of EJPs are seen only in the closer muscle fibers when the dactyl is cocked. When the dactyl is not in the cocked position the closer muscle is silent.

Simultaneous intracellular recordings from the opener and closer muscles show the relative timing of activity in these two muscles (Fig. 4C). The closer does not

show any excitatory activity to general tactile stimulation, while the opener is excited whenever the animal is disturbed. Occasionally IJPs could be seen in the closer muscle at the same time that EJPs were observed in the opener. During a snap sequence, the latency between opener and closer activity varies from 100 msec to several seconds. Once the snap train is initiated in the closer, the opener is silent, but it will often be excited immediately after the snap. This would suggest that there is reciprocal inhibition between the closer and opener excitatory neurons in the CNS.

The opener muscle can also be reflexly excited by passively opening the dactyl (Fig. 5). If the dactyl is opened with a probe, at a point about 30° from the fully cocked position a train of rapidly facilitating EJPs is seen in the opener muscle fibers. At the same time, IJPs are generated in the closer muscle. The resulting opener muscle contraction moves the dactyl away from the probe, and brings the dactyl to the fully cocked position. Passive opening reflexes are well-known in crustaceans (Bush, 1962a, b, 1963; Wilson and Davis, 1965), but they are usually resistance reflexes. That is, a passive movement in one direction results in contraction of the muscle that would bring the segment back to its original position. In the present case the reflex excites the muscle which moves the segment in the same direction as the passive movement, thus enhancing the movement. A positive reflex of this type would be useful in assuring that the disks on the propus and dactyl meet with sufficient force to make them hold together.

The existence of a positive opening reflex, and the fact that the EJPs in the closer muscle stop at exactly the same time that the dactyl is uncocked, suggest that a proprioceptor provides information about the position of the dactyl, and that such information is important to the snapping behavior. When the dactyl is passively moved, nerve impulses, presumably of sensory origin, can be seen in the distal stump of the cut ventral nerve (Fig. 6). This activity could provide the necessary proprioceptive information.

There are two possible sources of this activity. Surrounding the disks are located several long cuticular hairs. Also, many smaller hairs can be seen all along the dactyl and on the plunger. Activity can be recorded in the ventral nerve when any of these hairs is deflected. As the dactyl is moved into the cocked position, the hairs are deflected, and the resulting activity could be used to detect the position of the dactyl. However, the opener reflex remains unchanged when the exoskeleton of the propus is removed from the area in which the hairs are located, and the dactyl is cut down to a stub. These operations remove all of the hairs that are normally deflected when the dactyl is moved.

Another candidate for the source of this information is a stretch receptor which spans the propus-dactyl joint. These receptors are common in crustacean limbs (Burke, 1954; Whitear, 1962) and have been implicated in various resistance reflexes (Bush, 1962a, b; 1963). In crabs the receptor at the P-D joint is an elastic strand innervated by bipolar sensory neurons which respond to stretch of the strand when the joint is moved (Wiersma and Boettiger, 1959; Hartman and Boettiger, 1967). Hartman and Boettiger (1967) have demonstrated that a phasically active population of neurons is involved in sensing joint movement. Another group of tonically active neurons senses joint position.

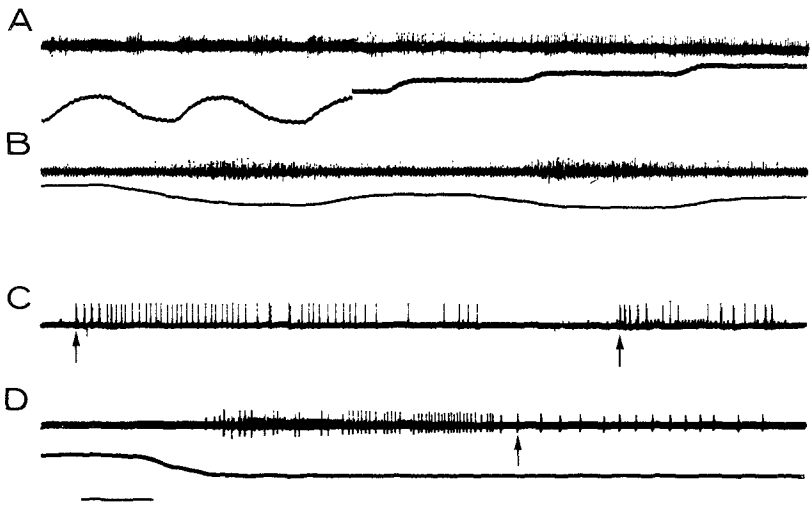

Fig. 6. (A) Top trace is a recording from the distal stump of the ventral nerve while the dactyl is being moved manually. Bottom trace is from a transducer monitoring dactyl movement. Up on the transducer record represents opening of the dactyl. (B) Top trace is a recording from the distal stump of the ventral nerve while the P-D organ is being moved with a probe. Bottom trace is from a transducer monitoring movement of the probe. (C) Activity in the proximal stump of the dorsal nerve in response to passive opening of the dactyl. (D) Top trace is activity in the proximal stump of a dorsal nerve in response to movement of the P-D organ with a probe. Bottom trace is from a transducer monitoring movement of the probe. Doublets of action potentials in (C) and (D) are indicated by arrows. Calibration: (A, B, and D) 400 msec; (C) 200 msec

The P-D organ in *A. californiensis* is not a strand, but a flat sheet resembling the M-Cl and C-Pl organs found in crabs (Whitear, 1962). It lies between the opener and closer muscles and spans the propus-dactyl joint (Fig. 1). Bipolar neurons could be seen in the sheet after staining with methylene blue, and action potentials could be recorded in the ventral nerve when the sheet was moved with a probe (Fig. 6B). This probe consisted of a fine glass needle fastened to a larger rod which was then held by a micromanipulator so that the probe could be moved manually. The probe was carefully positioned on the end of the sheet of the *in situ* P-D organ with just enough pressure so that lateral movement of the probe stretched the sheet but did not do any obvious damage. The position of the probe on the sheet was sufficiently far away from the cuticular hairs surrounding the disks to avoid accidental deflection of these hairs. If the function of this organ is the same as in crabs, the tonic fibers could relay position information when the dactyl is cocked and thereby provide the sensory information necessary for gating the snap train. Phasic movement receptors also could be used during the passive opener reflex.

A conclusive demonstration of the involvement of the P-D organ in the opener reflex would require moving the P-D organ with a probe while recording from the opener muscle. However, the opener muscle must be removed to expose the P-D organ. Nevertheless, action potentials can be recorded from the proximal end of the

cut dorsal nerve in response to passive opening of the dactyl (Fig. 6C). Since attempts to record from the dorsal nerve *en passant* failed, it was not possible to confirm that these potentials originated in the opener motor axons. However, the only motor axons that have been found in the dorsal nerve are those going to the opener muscle, and the timing of these action potentials is similar to that of the EJPs elicited in the opener muscle during passive dactyl opening. Thus, it can be assumed that the activity in the proximal end of the dorsal nerve is from the motor axons of the opener muscle. The same activity can be seen in the dorsal nerve when the P-D organ is moved with a probe as described above (Fig. 6D). Therefore, the P-D organ is presumably the structure that provides proprioceptive information, at least for the passive opening reflex.

III. The Pincer Cheliped

The cheliped on the contralateral side of the same segment as the snapper is a small pincer. The pincer cheliped resembles those of other crustaceans and is used by the shrimp in much the same way that other crustaceans use their chelae. When a large snapping cheliped is removed, the pincer develops into a snapper in two or three molts and is then used in the snapping behavior (Wilson, 1903). A new pincer grows at the site of the old snapper. Because the pincer has this ability to transform from a normal cheliped into a snapping appendage, a comparative study of the motor organization in the two chelae seemed valuable.

The external anatomy of the pincer is very different from that of the snapper in that the dactyl of the former is more elongated and lacks the plunger and socket of the latter. Disks are present on the dactyl and propus that are about one fourth the size of the disks on the snapper cheliped. While the propus disk looks like a miniature of the disk found on the snapper, the dactyl disk is not well-formed at all (Fig. 7). The position of the major nerves and muscles in the pincer is similar to that found in the snapper.

The neuromuscular organization of the pincer was studied by stimulating the nerves in the merus and recording intracellularly in the muscles of the propus. This yielded much the same results as in the snapper cheliped (Fig. 8). One excitor innervates the opener muscle. Most of the closer fibers also had only one excitor, but some did have two. The closer fibers had a tendency to spike when excited with a train of impulses, unlike the homologous fibers of the snapper (Fig. 9).

Intracellular muscle recordings from intact animals showed that, as in the snapper, the opener muscle of the pincer can be reflexly excited by passive opening of the dactyl (Fig. 10A). The closer can be excited by touching hairs along the inside rim of the dactyl or by holding the dactyl open while perturbing the animal (Fig. 10B). The latter case mimics a snap, but the burst that is seen in the closer muscle is not exactly the same as that in the snapper closer during a snap. The EJPs of the pincer are not initially grouped together into compound muscle potentials as in the snapper. Also, in spite of the fact that the dactyl remains pinned open, the burst is relatively brief. In the snapper the closer continues to be excited as long as the dactyl is cocked. This would suggest that a certain amount of CNS reorganization is necessary to produce the pattern of the snap train when the pincer develops into a snapper.

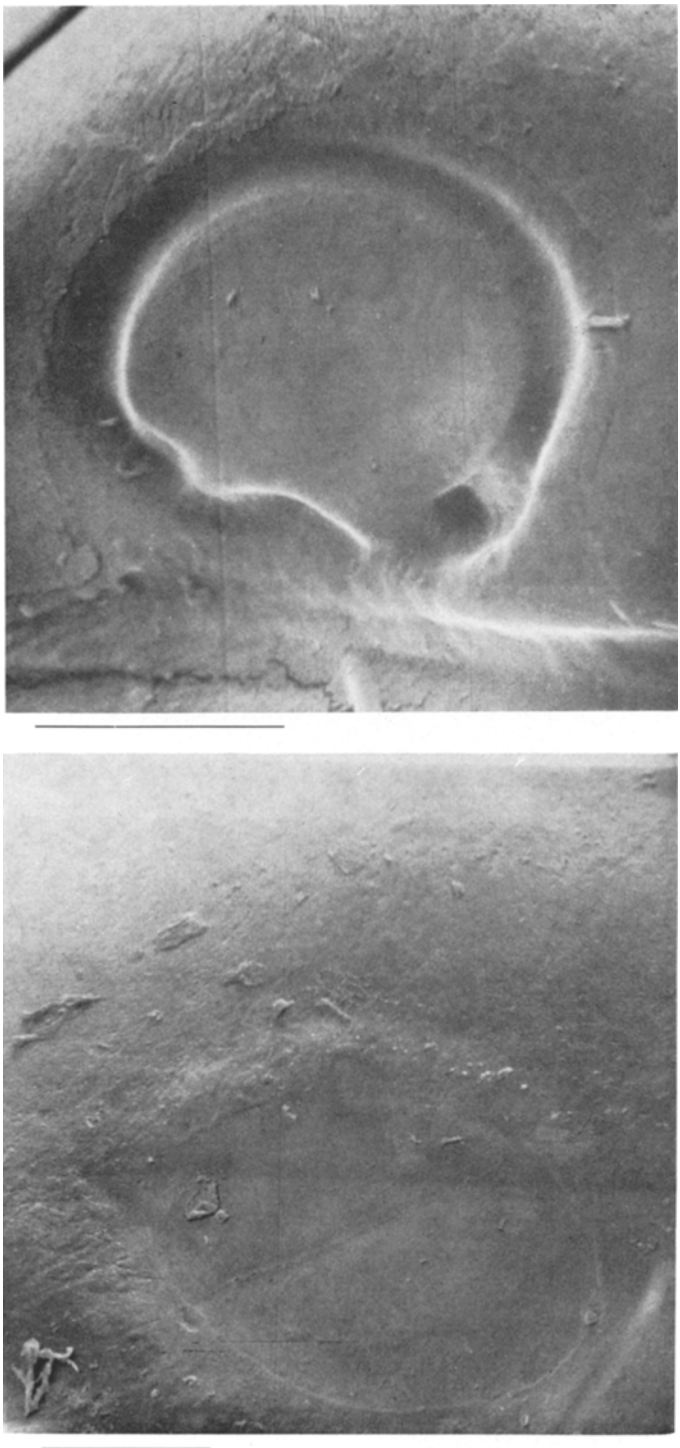

Fig. 7A and B. Scanning electron micrographs of the disks found on the pincer cheliped. (A) Propus disks. (B) Dactyl disk. Calibration represents 100 μ

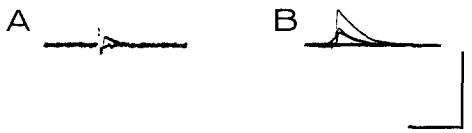

Fig. 8A and B. Intracellular recording from muscle fibers in an isolated pincer cheliped. The nerves were stimulated in the merus with increasing stimulus strength, to determine the number of axons innervating the two muscles. (A) An EJP in an opener muscle fiber resulting from dorsal nerve stimulation. (B) Recording from a closer muscle fiber showing two amplitudes of EJPs as stimulus intensity to the ventral nerve is increased. The small increase in the lower amplitude was due to facilitation. The slow rise in potential which precedes the largest response is probably artifactual. Calibrations: (A) 10 mV, 50 msec; (B) 5 mV, 50 msec

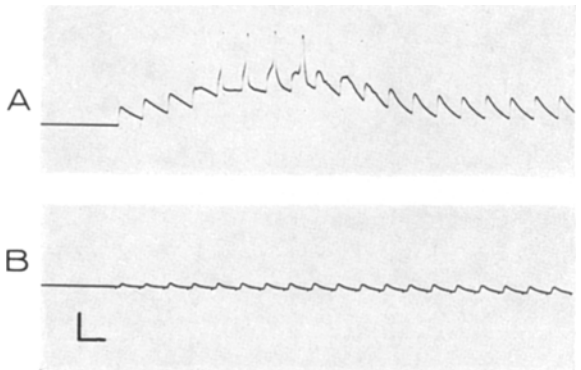

Fig. 9. (A) Intracellular recording from a closer muscle fiber of an isolated pincer cheliped while the ventral nerve in the merus is being stimulated at 25 imp/sec. (B) Same recording situation as in (A), but from a closer muscle fiber of a snapper cheliped. Calibration: (A) 1 mV, 50 msec; (B) 5 mV, 50 msec

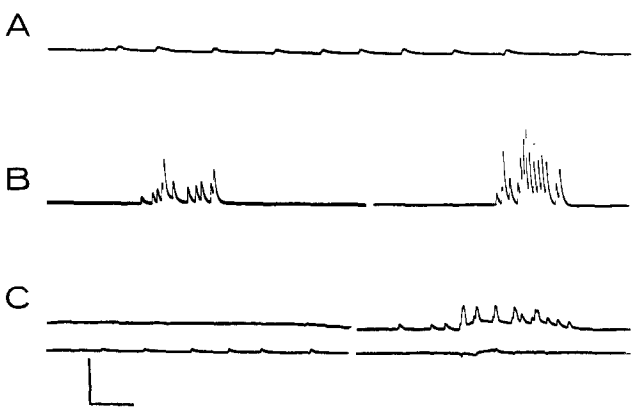

Fig. 10. (A) Intracellular recording from an opener muscle fiber of the pincer cheliped on an intact animal during passive opening of the dactyl. (B) Intracellular recording from a closer muscle fiber of a pincer cheliped on an intact animal. The first burst was elicited by touching hairs on the inner rim of the dactyl. The second burst was elicited by perturbing the animal while the dactyl was held in an extreme open position. (C) Simultaneous intracellular recordings from closer (top trace) and opener (bottom trace) muscle fibers during passive dactyl opening and closing elicited by touching dactyl hairs. There is a break of several hundred milliseconds between the opening and closing responses. Calibrations: (A) 100 mV, 100 msec; (B) 25 mV, 100 msec; (C) top 50 mV, 100 msec, bottom 25 mV, 100 msec

IV. Comparison with Alpheus heterochelis

A comparative study of snapping was done with another shrimp, *Alpheus heterochelis*, which lives along the southeastern coast of North America. Its behavior in general, and more specifically its snapping behavior is much the same as that of *A. californiensis*. The snapping cheliped is somewhat different morphologically in that the orientation of the dactyl is not lateral, as is the case with *A. californiensis*. Instead it is more similar to the typical crustacean cheliped, except for the presence of the plunger and socket on the dactyl and propus, respectively. Disks are present, but they are much smaller than the ones on the snapper cheliped of *A. californiensis*. While the disks can be made to stick together, only a small force is needed to separate them. There is certainly not enough force provided by these disks to counteract the tension produced in the closer muscle. Furthermore, scratching these disks does not affect the shrimp's ability to snap, which suggests that they do not serve the same function as the disks on the snapping cheliped of *A. californiensis*. This means that a different mechanism must be operating in *A. heterochelis* to hold the dactyl open while the closer muscle builds tension.

When *A. heterochelis* opens its dactyl to snap, the angle which the dactyl makes with the propus is about 100°. In *A. californiensis* this angle is about 80°. As the dactyl of *A. heterochelis* moves into this extreme open position, the insertion of the closer muscle apodeme is pulled up and out of the propus. In the final position the apodeme insertion is directly in front of the articulation between the dactyl and the propus, and the apodeme passes through the axis of the dactyl-propus articulation (Fig. 11). The dactyl-propus articulation is the pivot point around which the dactyl must rotate if it is to close. However, because the closer apodeme insertion is now directly in front of this pivot point, pulling on the closer apodeme, as the closer normally does, will not cause the dactyl to close. Instead it will simply pull the dactyl back against the propus. This means that the dactyl is essentially locked open.

There is a small projection on the closer apodeme which extends ventrally when the dactyl is open, and a triangular slip of muscle inserts into this part of the apodeme (Fig. 11 A). If this muscle (Cl_2) contracts after the main closer (Cl_1) has built up tension, it would unlock the main closer, allowing it to release the tension it had built up and causing the dactyl to snap closed.

If this mechanism is operating, Cl_1 and Cl_2 must contract sequentially. This would mean that they are under the control of different excitatory axons. That this is the case can be demonstrated by stimulating the ventral nerve in the merus and recording from Cl_1 and Cl_2 with separate electrodes. As can be seen in Fig. 12, the two muscles are innervated by different axons, although the possibility that additional shared axons exist cannot be eliminated.

To determine if this is indeed the mechanism for snapping in *A. heterochelis*, recordings were made during snapping in the opener muscle and Cl_1 simultaneously and in Cl_1 and Cl_2 simultaneously (Fig. 13). The frequency of the EJPs in the opener increases to 80 sec 300 msec before the dactyl reaches the end of its opening movement. This may be the result of the positive opening reflex, which can be demonstrated in this species also. The increase in frequency could result in a tetanus in the opener muscle which would keep the closer apodeme in the locked

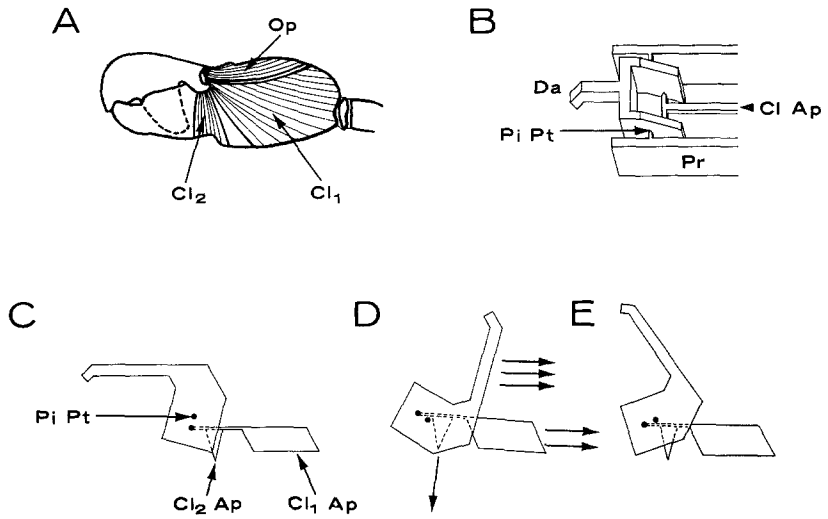

Fig. 11 A—E. Diagram showing the mechanism of snapping in *A. heterochelis*. (A) Diagram of the cheliped musculature. *Op*, opener muscle; Cl_1, main closer muscle; Cl_2, triangular slip of muscle that is separate from Cl_1 but inserts into the same apodeme as Cl_1. (B) Diagram of the articulation of the dactyl and propus and the insertion of the closer apodeme into the dactyl. Because of the structure of the apodeme insertion, the apodeme can move through the pivot point of the dactyl and propus. *Cl Ap*, Closer apodeme; *Da* dactyl; *Pr* propus; *Pi Pt*, pivot point around which the dactyl rotates. (C) Diagram showing the position of the closer apodeme insertion when the dactyl is closed. Cl_1 *Ap*, Part of the closer apodeme into which Cl_1 inserts. Cl_2 *Ap*, Part of the closer apodeme into which Cl_2 inserts. *Pi Pt*, Pivot point around which the dactyl rotates. (D) Position of the closer apodeme when the dactyl is cocked open for a snap. The insertion of the closer apodeme is now above the pivot point, so that as Cl_1 contracts to move the apodeme in the direction of the double arrows, the dactyl moves backward (as indicated by the triple arrows) rather than rotating closed. This means that Cl_1 is locked until Cl_2 contracts moving the apodeme down (as indicated by the single arrow). (E) Position of the apodeme after Cl_2 contracts. Cl_1 is now able to rotate the dactyl closed. Since Cl_1 builds tension while it is locked open, the unlocking of the apodeme by Cl_2 results in a rapid forceful closing movement

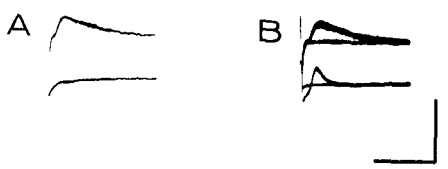

Fig. 12 A and B. Recording in an isolated snapping cheliped of *A. heterochelis* during increasing stimulus strength to the ventral nerve in the merus. Top trace is an intracellular recording from a muscle fiber in Cl_2. Bottom trace is a simultaneous extracellular recording from Cl_1. (A) An EJP is only seen in Cl_2. Increasing the stimulus strength in (B) results in the initiation of EJPs in both muscles. Calibration: 10 mV, 50 msec

position for several hundred milliseconds. After the dactyl is opened, and the closer apodeme is locked, Cl_1 is excited. The excitation to the closer muscle is similar to that of *A. californiensis* except that the duration of the train is more constant

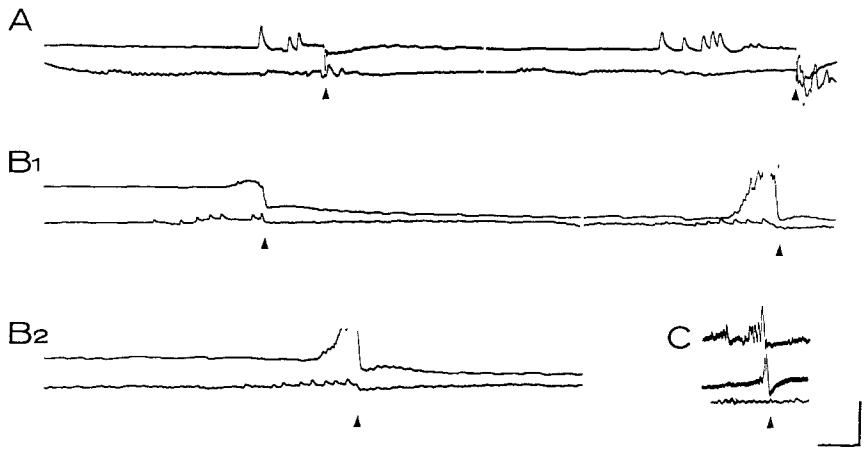

Fig. 13. (A) Simultaneous intracellular recordings from muscle fibers in Cl_1 (top trace) and the opener (bottom trace) during snapping. Note the small rapid opener activity before the initiation of the closer EJPs, and the IJPs in the closer trace after the first snap. (B) Simultaneous intracellular recordings from muscle fibers in Cl_2 (top trace) and Cl_1 (bottom trace) during snapping to show the relative timing of excitation between the two muscles. EJPs are not seen in Cl_2 until 100–150 msec after the initiation of EJPs in Cl_1. IJPs are seen in Cl_2 before and after each snap. In the second snap an IJP is seen in the Cl_2 record while the EJPs are beginning to be initiated in Cl_1. After the first snap there are also IJPs in Cl_1. Several hundred milliseconds were removed between each snap. (C) Extracellular record from Cl_1 (top trace), Cl_2 (middle trace) and output from a microphone placed near the animal (bottom trace). An electrical signal from the microphone occurs at the arrow. This signal was identifiable on the oscilloscope when the tape record was played, but was obscured in photographs of the records. In all cases the snap occurred at the arrow. Calibration: (A) Top trace 50 mV, 100 msec; bottom trace 25 mV, 100 msec. (B) 50 mV, 100 msec. (C) 250 msec

for *A. heterochelis*. EJPs are not seen in Cl_2 until about 100–150 msec after the beginning of the excitation to Cl_1, which is what would be predicted if Cl_2 were used to unlock Cl_1. Activity in both Cl_1 and Cl_2 stops when the snap occurs. Immediately after the snap IJPs can occasionally be seen in both Cl_1 and Cl_2. When IJPs are seen in both muscles, they are synchronous. This would suggest that while different excitatory axons innervate Cl_1 and Cl_2, the inhibitory axon is shared. The timing of the electrical activity in the muscles, along with the mechanics of the closer apodeme, demonstrate that the snap does occur as a result of unlocking the mechanism described above.

Discussion

I. Innervation of the Opener Muscle

An inconsistency occurs in the data concerning the innervation of the opener muscle. The experiments in which records from the opener muscle of isolated chelae were obtained during electrical stimulation of the dorsal nerve in the merus indicated that only one axon innervates the opener. However, recordings from the dorsal nerve during the passive opener reflex contain three sizes of action potentials. The smallest potentials do not have any relationship to excitatory activity in the

opener muscle. They possibly occur in an inhibitory axon which, when excited, results in IJPs which could not be detected in the recordings. The other two sizes of potentials occurred in doublets having the same pattern and timing as EJPs in the opener muscle. It is assumed that one of the potential classes represents activity in the excitor leading to the opener muscle. The origin of the other potential is unknown, but several possibilities are given below.

The question is essentially whether there are two or three axons innervating the opener muscle. If there are two axons, the doublets could be explained as two potentials from the same excitatory axon which occur so close together that the second potential is initiated in the refractory period of the first, thus making the second potential smaller. If there are three axons the second potential could be either a second inhibitory potential, which would not necessarily be detected in the intracellular recordings, or a second excitatory axon which does not innervate the same muscle fibers as the first. Either of these possibilities could explain the appearance of only one EJP in the isolated cheliped recordings.

Proof of any of these hypotheses would require simultaneous recordings from the dorsal nerve and the opener muscle. This could not be done because of the difficulty in recording *en passant* from the nerve. Methylene blue staining has been used in crustacean preparations to determine the number of axons in nerve branches, but this test also gave unsatisfactory results, again probably due to the myelination around the axons of the nerve. Wiersma and Ripley (1952) also reported difficulty in using this technique on the bandana shrimp, *Stenopus hispidus*. Nevertheless, in a few successful preparations, they did report that three axons were present in some of the branches going to the opener muscle. Thus, at the present time the origin of the doublet remains in question.

II. The Snapping Appendage

The two species of snapping shrimp that were studied have both developed effective snapping appendages but by employing different mechanisms. Nevertheless, the essential elements for a snapping appendage are the same in both species. The modifications necessary to make a snapping appendage from a typical crustacean cheliped are 1. the ability to quickly and forcefully contract the opener muscle so that the dactyl can be moved into the cocked position, 2. some cocking mechanism to delay the movement of the dactyl while the closer muscle is contracting, 3. the generation of intense activity in the closer muscle after the dactyl is cocked.

In both *A. californiensis* and *A. heterochelis* quick opening of the dactyl is assured by the positive opener reflex. This reflex results in an increase of the activity in the opener muscle and an inhibition of the closer. In *A. californiensis* the rapid opening of the dactyl assures that the disks will meet with enough force to make them hold together, while in *A. heterochelis* it brings the dactyl into the extreme open position necessary for locking the closer muscle apodeme.

The actual cocking mechanisms of the two species are the most dissimilar elements of their snapping appendages. In *A. californiensis* the dactyl is simply held to the propus by cohesive forces of the layer of water between the specialized exoskeletal disks until the closer muscle can build enough tension to overcome

the forces holding the disks together. However *A. heterochelis* has a complicated arrangement of the closer apodeme and the dactyl-propus articulation. This arrangement is such that when the dactyl is in the cocked position the closer apodeme is locked and cannot move the dactyl. The result, as with *A. californiensis*, is that the closer can build tension while the dactyl is prevented from moving. However, unlike the mechanism of *A. californiensis* in which the closer muscle simply contracts until it overcomes the cocking mechanism, here the dactyl must be actively released from the cocked position. Furthermore, this release must occur after the closer muscle has built sufficient tension to snap the dactyl closed and before it contracts so much that damage is done to its insertions. Active release is accomplished by the small slip of muscle (Cl_2) which contracts and unlocks the closer apodeme at the correct time, so that the dactyl is snapped closed.

In both species, it is important that, after the cocking mechanism has been set and the dactyl is held open, a high frequency train of EJPs is generated in the closer muscle, so that the muscle can develop the tension needed to snap the dactyl closed. This train of impulses in the closer of *A. californiensis* and the main closer of *A. heterochelis* is again similar. It is simply a long high frequency train of EJPs that stops at the time that the snap occurs.

The fact that the train of EJPs in the closer muscle, which results in snapping, occurs only when the dactyl is in the cocked position and that it always stops immediately after the snap occurs, suggests that the closer excitor is under some control from proprioceptive activity monitoring the position of the dactyl. Such activity may be provided by the P-D organ. The latency between opening and the initiation of the snap train is extremely variable. This means that it is unlikely that the closer excitor is reflexly activated by the P-D organ. Nevertheless, the activity from the P-D organ could provide a gate to the closer activity by lowering the threshold for initiation of a train. It would then act permissively, allowing the train to occur, rather than generating it directly. While the dactyl is cocked, the activity from the P-D organ would lower the threshold for closer activity so that when other information (e.g. visual, tactile or chemosensory) indicated that snapping should occur, the gate would be open and a train of impulses would be initiated in the closer excitor. These impulses would continue until the snap occurred and the dactyl moved out of the cocked position. The proprioceptive activity would then cease, and the threshold for the generation of the snap train would return to a high level, resulting in cessation of activity in the closer excitor. The threshold would remain at this high level until the dactyl was again cocked, thus assuring that intense trains of impulses only occur in the closer excitor at the proper time, when the dactyl is cocked for a snap.

To prove conclusively that such a gate exists would require recordings from neurons in the central nervous system, but there is considerable circumstantial evidence to suggest that a gate is present. In addition to the temporal relations of the snap train mentioned above, in a fatigued animal abortive trains were seen when the dactyl was cocked. These looked like what would be predicted if the threshold for generation of the train had been lowered to the level needed to allow a train to be generated, but not below this level. Furthermore, when the train was

initiated the muscle could not overcome the force holding the disks together. As a result trains were generated that stopped and started several times until the dactyl was uncocked manually. When the dactyl was uncocked, the trains stopped and did not start again. This reinforces the evidence that trains of impluses in the excitor can only occur when the dactyl is cocked open.

The positive opener reflex is found in the pincer cheliped. However, even if the pincer dactyl is held open as though it were cocked, the pattern of activity in the closer muscle is not the same as that seen in the snapping cheliped during a snap. Activity in the closer of the pincer cheliped does not last until the restrained dactyl is released. Instead the burst is similar to the burst of activity elicited by simply touching hairs on the inner rim of the dactyl. Since the positive opener reflex is seen in the pincer cheliped, the alteration in the closer activity is the only neuromotor change that must take place, at least in *A. californiensis*, for the pincer to develop into a large cheliped with the ability to snap. Indeed, some very large specimens of this species have been seen to snap their pincer cheliped. The great amount of force supplied by the increase in the duration of the activity of the closer muscle of the snapper was probably not necessary, since the disks of the pincer were still considerably smaller than the disks of the snapper cheliped.

In both species that were studied the snapping behavior could hypothetically be described in the following way. Any disturbance results in a low level of excitation in the opener muscle. If the dactyl is opened far enough, the stretch of the P-D organ will initiate the positive opener reflex. This will increase the activity to the opener muscle, causing it to cock the dactyl rapidly. With the dactyl in this position, the gate to the closer excitatory motoneuron is opened so that when more information indicating that a snap should occur is processed in the central nervous system, a high frequency train of impulses is initiated in the closer excitor. This train will continue until the cocking mechanism is either overcome or turned off, and the dactyl is snapped closed. When the snap does occur the closer excitor gate is closed and the train of impulses in the excitor stops.

While the cocking mechanisms found in *A. californiensis* and *A. heterochelis* are very different, one can speculate on an evolutionary relationship. Since the cheliped of *A. heterochelis* is more typical in general form than the twisted cheliped of *A. californiensis*, one could assume that the type of snapping appendage found in *A. heterochelis* represents an earlier form. The small disks on the cheliped of *A. heterochelis* are similar in shape to those of *A. californiensis* but are too small to function in a cocking mechanism. Perhaps the disks of *A. heterochelis* merely serve as pads between the dactyl and propus, preventing damage when the dactyl is opened rapidly and hits the propus. Another possibility is that they serve to stabilize the dactyl while it is locked in the open position. In any case these small disks may eventually have evolved into the large disks that are found on *A. californiensis*. When they became large, the disks could stick together well enough to counteract the force generated by the closer muscle for several hundred milliseconds. This would mean that the complicated neuromuscular and articular systems of *A. heterochelis* would no longer be required. The same movement could be made with the much simpler system of *A. californiensis*.

It is interesting that two species of the same genus of alpheid shrimp should employ such different mechanisms to perform the same behavior. Considering the large number of species of snapping shrimp, it would not be surprising to find that even more mechanisms for snapping have been developed. Indeed, a mechanism suggested by Knowlton and Moulton (1963) for snapping in two shrimp of the genus *Synalpheus* may represent yet another mechanism. In this case protuberances on the propus and dactyl slide over each other and can hold the dactyl open while the closer muscle builds tension. Here again the unique element appears to be the cocking mechanism.

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References

Bennet-Clark, H. C., Lucey, E. C. A.: The jump of the flea: a study of the energetics and a model of the mechanism. J. exp. Biol. **47**, 59–76 (1967)

Brown, R. H. J.: Mechanism of locust jumping. Nature (Lond.) **214**, 939 (1967)

Burke, W.: An organ for proprioception in the legs of *Carcinus maenas* (L.). J. exp. Biol. **39**, 89–105 (1954)

Burrows, M.: The mechanics and neural control of the prey capture strike in the mantid shrimps *Squilla* and *Hemisquilla*. Z. vergl. Physiol. **62**, 361–381 (1969)

Bush, B. M. H.: Peripheral reflex inhibition in the claw of the crab, *Carcinus maenas* (L.). J. exp. Biol. **39**, 71–88 (1962a)

Bush, B. M. H.: Proprioceptive reflexes in the legs of *Carcinus maenas* (L.). J. exp. Biol. **39**, 89–105 (1962b)

Bush, B. M. H.: A comparative study of certain limb reflexes in decapod crustaceans. Comp. Biochem. Physiol. **10**, 273–290 (1963)

Coutiere, M. H.: Les "Alpheidae", morphologie externe et interne, formes larvaires, binomie. Ann. Sci. Nat. Zool. et Pal., Ser. **8, 9**, 1–599 (1899)

Hartman, H. B., Boettiger, E. G.: The functional organization of the propus-dactyl organ in *Cancer irroratus* Say. Comp. Biochem. Physiol. **22**, 651–663 (1967)

Hazlett, B. A., Winn, H. E.: Sound production and associated behavior of Bermuda crustaceans (*Panulirus*, *Gonodactylus*, *Alpheus*, and *Synalpheus*) Crustaceana **4**, 25–38 (1962)

Heitler, W. J.: The locust jump: specialisations of the metathoracic femoral-tibial joint. J. comp. Physiol. **89**, 93–104 (1974)

Heuser, J. E., Doggenweiler, C. F.: The fine structural organization of nerve fibers, sheaths, and glial cells in the prawn, *Palaemonetes vulgaris*. J. Cell Biol. **30**, 381–403 (1966)

Johnson, M. W., Everest, F. A., Young, R. W.: The role of snapping shrimp (*Crangon* and *Synalpheus*) in the production of underwater noise in the sea. Biol. Bull. **93**, 22–128 (1947)

Knowlton, R. E., Moulton, J. M.: Sound production in the snapping shrimps *Alpheus* (*Crangon*) and *Synalpheus*. Biol. Bull. **125**, 311–331 (1963)

Kusano, K.: Electrical activity and structural correlate of giant nerve fibers in *Karuma* shrimp. J. cell. Physiol. **68**, 361–384 (1966)

Mendelson, M.: Electrical and mechanical characteristics of a very fast lobster muscle. J. Cell Biol. **42**, 548–563 (1969)

Nolan, B. A., Salmon, M.: The behavior and ecology of snapping shrimp (Crustacea: *Alpheus heterochelis* and *Alpheus normanni*). Forma et Function **2**, 289–335 (1970)

Pringle, J. W. S.: Insect flight. London: Cambridge University Press 1957

Reynolds, E. S.: The use of lead citrate at high pH as an electronopaque stain in electron microscopy. J. Cell Biol. **17**, 208–212 (1963)

Ritzmann, R.: Snapping behavior of the shrimp *Alpheus californiensis*. Science **181**, 459–460 (1973)

Rothschild, M., Schlein, Y., Parker, K., Sternberg, S.: Jump of the oriental flea *Xenopsylla cheopis* (Roth). Nature (Lond.) **239**, 45–48 (1972)

Sandeman, D. C.: A sensitive position measuring device for biological systems. Comp. Biochem. Physiol. **24**, 635–638 (1968)

Verrill, A. E.: Decapod crustacea of Bermuda. Part II: Macroura. Trans. Conn. Acad. Arts Sci. **26**, 1–179 (1922)

Whitear, M.: The fine structure of crustacean proprioceptors I. The chordotonal organs in the legs of the shore crab, *Carcinus maenas*. Phil. Trans. B **245**, 291–325 (1962)

Wiersma, C. A. G., Boettiger, E. G.: Unidirectional movement fibers from a proprioceptive organ of the crab. *Carcinus maenas*. J. exp. Biol. **36**, 102–112 (1959)

Wiersma, C. A. G., Ripley, S. H.: Innervation patterns of crustacean limbs. Physiol. Comp. Oecol. **2**, 391–405 (1952)

Wilson, D. M., Davis, W. J.: Nerve impulse patterns and reflex control in the motor system of the crayfish claw. J. exp. Biol. **43**, 193–210 (1965)

Wilson, E. B.: Notes on the reversal of asymmetry in the regeneration of the chelae in *Alpheus heterochelis*. Biol. Bull. **4**, 197–210 (1903)

Roy E. Ritzmann
Division of Biological Sciences
Section of Neurobiology and Behavior
Cornell University
Ithaca, New York 14850, USA