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PATTERNS OF ACTIVITY IN THE ANTENNULAR MOTONEURONES OF THE HERMIT CRAB *PAGURUS ALASKENSIS* (BENEDICT)

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SUMMARY

1. Using electromyogram recordings from the antennular muscles of intact animals and recordings from the antennular nerves of partially dissected preparations, the patterns of activity in specific antennular motoneurones have been described during antennular flicking and antennular withdrawal.

2. The slow extensor motoneurone A30S is active during flicking in addition to the phasic component of the antennular motor system (A30F, A31F and A32F).

3. The flexion phase of a flick is the result of a burst of variable duration and number of spikes within flexor motoneurones A31F and A32F.

4. The extension phase of a flick is the result of a burst of variable duration and number of spikes in extensor motoneurones A30F and A30S.

5. Extension–withdrawal and slow flexion–withdrawal reflexes, tonic flexion withdrawal and maintained flexion at the MS–DS joint usually result from activity in part of the tonic component of the antennular motor system: moto-neurones A30S, A31S and A32S.

6. Fast flexion–withdrawal reflexes result from a burst of spikes in motoneurone A31F-S which constitutes the phaso-tonic component of the antennular motor system.

7. During high-frequency activity ([illegible]–60/sec), reciprocity exists between the slow flexor motoneurones A31S and [illegible]32S and slow extensor motoneurone A30S.

INTRODUCTION

In crustaceans, the antennules are generally considered of primary importance in chemoreception. Little has been known about the form or possible functions of the antennular activities, but recent studies of the hermit crab *Pagurus alaskensis* (Snow, 1973*a*), and the lobster *Panulirus argus* (Maynard & Dingle, 1963), have shown that four types of activities may be defined: flicking, wiping, pointing or rotation, and withdrawal. It has been proposed (Snow, 1973*a*) that, in the hermit crab, flicking, wiping and rotation may be related to the chemoreceptive process, while withdrawal may be important in avoiding potentially noxious stimuli.

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In the hermit crab, movements at the medial segment–distal segment joint, and the distal segment–outer flagellum joint, are controlled by five muscles which are differentially innervated by seven motoneurones (see Fig. 10 in Snow, 1973*b*). The objectives of the present work were to record the patterns of activity in the antennular motoneurones during specific antennular activities in intact, although partially restrained, crabs, and to interpret these patterns in the light of the proposed functions of the activities (Snow, 1973*a*). These objectives have been achieved for antennular flicking and the various forms of antennular withdrawal.

MATERIALS AND METHODS

Collection and maintenance of *Pagurus alaskensis* (Benedict) is described elsewhere (Snow, 1973*b*). Data presented here are based on recordings from 100 large (body length *ca.* 8 cm) crabs. Motoneuronal activity in intact animals was monitored by recording electromyograms in the antennular muscles and these data were supplemented by recordings made directly from the antennular nerves in partially dissected preparations.

In order to record electromyograms from the antennules of intact animals, crabs were removed from their shells and held ventral side up in a Plexiglass chamber which was continuously supplied with running sea water (10–12 °C). To enable the antennules to be viewed from above the preparation, the distal segments of the endopodites of the 3rd maxillipeds were tied together with thread and both appendages were drawn towards the abdomen and secured in this extended position. The antennae were also secured in an extended position by pinning the antennal flagella to the bottom of the chamber. In this condition the base of the antennules is about 2–3 mm above the bottom of the chamber. To facilitate implantation of the electrodes, a block of Sylgard 184 Encapsulating Resin (Dow Corning), $4 \times 8 \times 20$ mm, was pinned underneath the antennules. The antennules were stapled to this block and the electrodes implanted. The antennules could then be freed and the block removed.

Each myogram electrode consisted of two 15–20 cm lengths of 50 μm insulated copper wire. These lengths were twisted around one another and painted with Insul-X. Each wire was connected to one differential input of a Tektronix 122 preamplifier. Prior to each recording the distal end of each electrode was cut squarely to ensure that only the tips of the component wires were exposed.

The optimum placement of an electrode for recording from each muscle is shown in Fig. 1. A small hole was poked in the semi-transparent exoskeleton and the tip of an electrode was inserted into the relevant muscle. The electrodes seldom appeared, on the basis of visual inspection, to impede any of the antennular activities.

In the presence of water currents the antennules were flicked frequently and showed the various types of antennular withdrawal (see Snow, 1973*a*). The frequency of flicking could be increased by pipetting a little distilled water over the dactylopodites or into the inlet of the experimental chamber, or by initiating additional water currents in the chamber by alternately squeezing and releasing the inlet hose. When the endopodites of the 3rd maxillipeds were released, many animals showed antennular wiping. For short periods of time it was possible to record from freely moving animals.

Although electrodes were rarely dislodged from the antennules of partially restrained

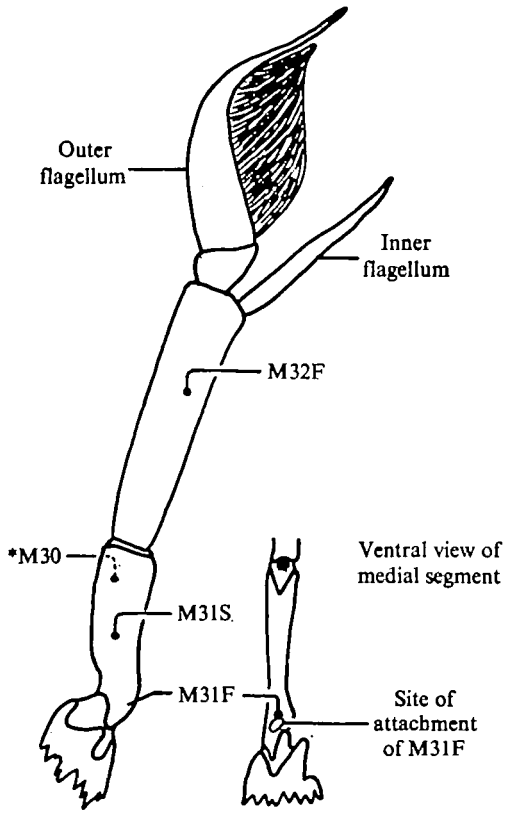

Fig. 1. Mesial view of the left antennule and ventral view of the medial segment, showing the sites of placement of the myogram electrodes. The myogram electrode for muscle 30 (see *M30) is introduced through the exoskeleton of the lateral side of the medial segment.

animals, small displacements of their tips often resulted in changes in the wave-form of the record. Readjustment of the electrode position usually restored the clarity of individual EJPs as well as improving the signal/noise ratio.

Partially dissected preparations had the abdomen ligatured just anterior to the columella muscle. The abdomen was then excised posterior to a ligature. Autotomy of the legs and claws was induced and most of the remaining cephalic and thoracic appendages were then excised. Animals were secured ventral side up in oxygenated *Cancer pagurus* saline (Pantin, 1948) and the medial antennular segment was secured to a Sylgard block placed under the antennules. The proximal segment (with the statocyst) of the antennule was dissected away and recordings were made directly from the antennular nerves using fine suction electrodes.

RESULTS

(1) *Identification of the motoneurones active during specific antennular activities*

Electromyogram recordings in extensor muscle 30 (M30) usually revealed large non-facilitating EJPs or initially small, facilitating EJPs. In about 65 % of the animals tested clear recordings of both types of EJP could not be obtained from a single

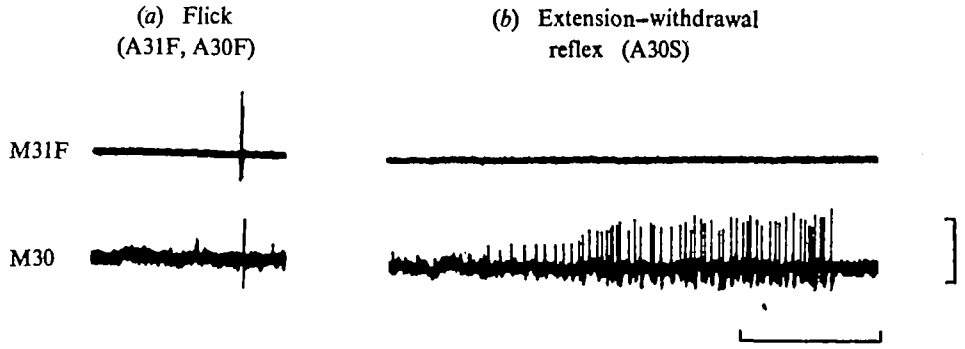

Fig. 2. Simultaneous electromyogram recordings in flexor muscle 31F and extensor muscle 30 of the medial segment. (*a*) Recording during a flick. (*b*) Recording during an extension–withdrawal reflex elicited by light mechanical stimulation of the inner flagellum. Motoneurones considered to be responsible for the electromyogram patterns are shown in parentheses at the top of records (*a*) and (*b*). Scale: 1000 msec, top traces 100 μV, bottom traces 50 μV.

electrode placement, yet small shifts of the electrode tip resulted in a change in the type of EJP recorded. This is in agreement with the finding that the fibres in one portion of M30 are innervated by a single motoneurone while the fibres in the other portion are innervated by a different motoneurone (Snow, 1973*b*).

The facilitating EJPs occurred at highest frequency during extension movements at the medial segment—distal segment (MS–DS) joint (Fig. 2*b*) or when this joint was being held in a fully extended position. A burst of 1–4 large, non-facilitating EJPs occurred only during antennular flicks (Figs. 2*a*, 5*a*). The facilitating EJPs almost certainly result from spikes in the slow extensor motoneurone A30S which innervates the slow fibres of M30. Similarly, the non-facilitating EJPs almost certainly result from spikes in the fast extensor motoneurone A30F which innervates the fast fibres of M30 (Snow, 1973*b*).

It was thus initially concluded that only the fast extensor motoneurone A30F was active during antennular flicking and only the slow extensor motoneurone A30S was active during the extension withdrawal reflex. In animals where only the EJPs of motoneurone A30S were being recorded it was sometimes possible to distinguish a burst of facilitating EJPs in M30 during a flick. These EJPs varied in size depending on the frequency of tonic activity in motoneurone A30S, being almost indistinguishable from the noise at low frequencies. This suggested that the slow extensor motoneurone A30S might also be active during a flick. To test this a fine suction electrode was used to make recordings from the extensor motor nerve (nerve 2a). Nerve 2a contains only the two axons of the extensor motoneurones A30F and A30S (Snow, 1973*b*). In the proximal antennular segment these are about the same diameter and in only 20% of the preparations could the spike size be used to distinguish activity in motoneurone A30F from activity in motoneurone A30S. When this was possible one unit was often tonically active with a low (5–10/sec) mean frequency (Fig. 3*a*) while there was a burst of activity in both units during a flick (Fig. 3*b*). The tonically active unit is considered to be the slow extensor motoneurone A30S because single spikes did not elicit large twitches in M30. Large twitches in M30 could be visually observed through the antennular exoskeleton following single spikes in the other unit. The other unit

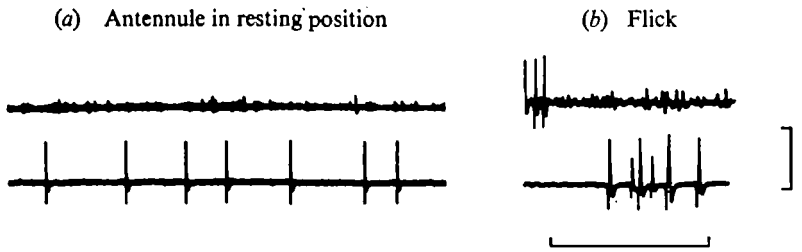

Fig. 3. Recording from antennular nerves 2 and 2a in a partially dissected preparation. (*a*) Recording of tonic activity in motoneurone A30S (lower trace). (*b*) Recording during antennular flicking showing the activity in flexor motoneurones A31F and A32F (top trace) and activity in extensor motoneurones A30S and A30F (bottom trace). Large spikes in the bottom trace are those in motoneurone A30S. Scale: 100 μV; (*a*) 100 msec; (*b*) 40 msec.

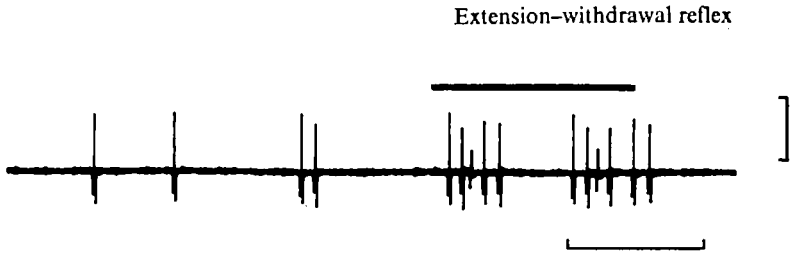

Fig. 4. Recording from extensor motor nerve (nerve 2a) during strong mechanical stimulation (bar) of the inner flagellum. The slow extensor motoneurone A30S is considered to be responsible for the large spikes as these were present prior to any stimulation. The fast extensor motoneurone A30F is considered to be responsible for the smaller spikes during the stimulation period. Scale: 100 μV, 100 msec.

is thus considered to be the fast extensor motoneurone A30F. Both extensor motoneurones are therefore active during a flick although slow extension movements at the MS–DS joint usually resulted only from activity in the slow motoneurone A30S. Strong mechanical stimulation of the inner flagellum elicited a more rapid extension movement at the MS–DS joint and this was often accompanied by a few spikes in the fast extensor motoneurone A30F in addition to increased activity in the slow extensor motoneurone A30S (Fig. 4).

Electromyogram recordings from M30 during flicking probably did not at once reveal the involvement of motoneurone A30S because of the very small size of its non-facilitated EJPs. Similarly, the involvement of motoneurone A30F in extension withdrawal reflexes was probably masked in electromyogram recordings because of the large size of the facilitated EJPs of extensor motoneurone A30S.

The synchronous recording of electromyograms in muscles 31F and 31S (M31F and M31S) revealed the presence of three axons innervating muscle group 31. During flicking there was a burst of usually 1–7 large, non-facilitating EJPs in M31F but no temporally related activity in M31S (Fig. 5(*a*), Table 3). The size of the EJPs usually decreased throughout these bursts but increased if an unusually long inter-EJP interval occurred (Fig. 7). In contrast, during slow or tonic flexion at the MS–DS joint, there was a train of initially small, facilitating EJPs in M31S but no activity in M31F (Fig. 6*a*). The non-facilitating EJPs in M31F almost certainly represent spikes

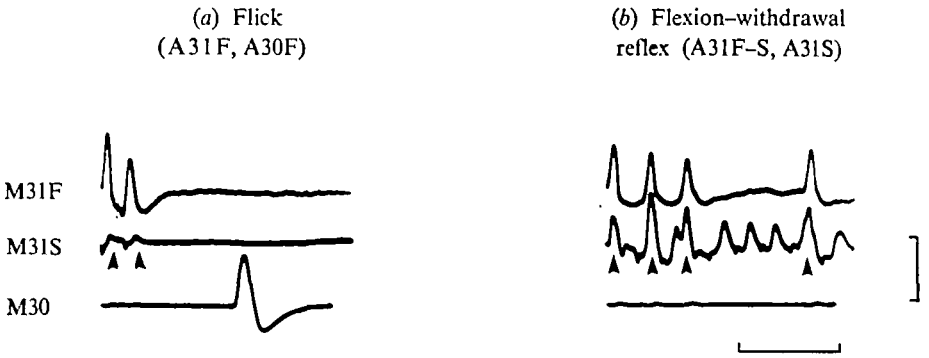

Fig. 5. Simultaneous electromyogram recordings in flexor muscles 31F and 31S and extensor muscle 30. (*a*) Recording during a flick. Arrows mark pick-up of spikes in muscle 31F by the electrode in muscle 31S. The EJPs of the slow extensor motoneuron e A30S in muscle 30 were not discernible with the electrode placement used. (*b*) Recording during a flexion-withdrawal reflex. Arrows mark times of occurrence of spikes in motoneurone A31F-S. Motoneurones considered to be responsible for the electromyogram patterns are shown in parentheses at the top of records (*a*) and (*b*). Scale: 100 μV, 20 msec.

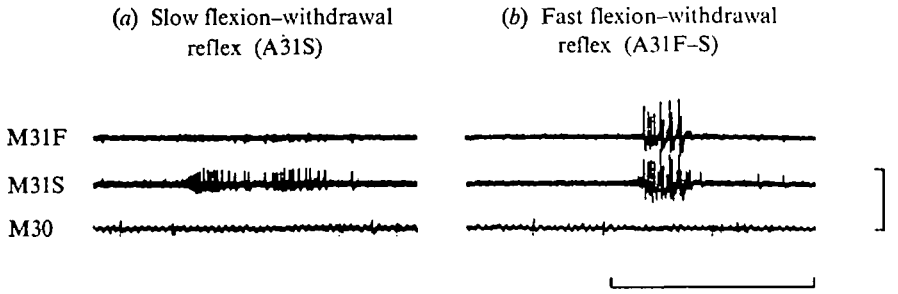

Fig. 6. Simultaneous electromyogram recordings in flexor muscles 31F and 31S and extensor muscle 30. (*a*) Recording during a slow flexion-withdrawal reflex. (*b*) Recording during a fast flexion-withdrawal reflex. Motoneurones considered to be responsible for the electromyogram patterns are shown in parentheses at the top of records (*a*) and (*b*). Scale: 200 μV, 1000 msec.

in motoneurone A31F, whereas the facilitating EJPs in M31S can easily be attributed to spikes in motoneurone A31S.

During rapid, large flexions at the MS–DS joint, a burst of large, non-facilitating EJPs occurred in both M31F and M31S. Each EJP in M31F occurred synchronously with an EJP in M31S (Figs. 5*b*, 6*b*). This muscle activity is almost certainly the result of spikes in motoneurone A31F-S which innervates both M31F and M31S (Snow, 1973*b*).

A single electrode was used to record from muscle group 32. During flicking, a burst of usually 1–5 large, non-facilitating EJPs was observed (Fig. 7, Table 3). In two preparations initially small, facilitating EJPs were recorded during slow flexion at the distal segment–outer flagellum (DS–OF) joint (Fig. 8). The small, facilitating EJPs were only observed when the recording electrode was placed within the distal one-third of the distal segment, and are thus considered to be the result of spikes in motoneurone A32S which innervates the tiny slow muscle M32S. In contrast, the large, non-facilitating EJPs are considered to be the result of spikes in motoneurone A32F which innervates fast muscle M32F (Snow, 1973*b*).

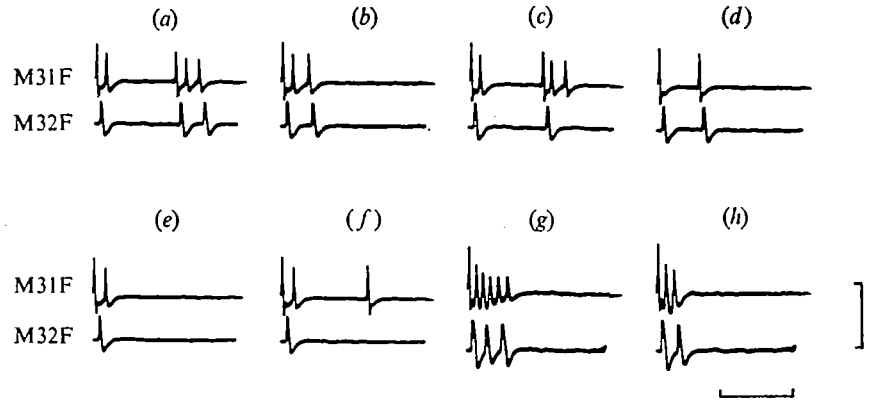

Fig. 7. Simultaneous electromyogram recordings in flexor muscles 31F and 32F during single flicks in a single crab. The activity in each muscle during each flick is considered to represent a single burst. Note the extreme variation in the number of EJPs/burst, the burst lengths and the inter-EJP intervals. Note also the occurrence of bursts with many EJPs but no long inter-EJP intervals (*g*) and the occurrence of a very long inter-EJP interval in muscle 31F which is not followed by an EJP in muscle 32F (*f*). Scale: 200 μV, 50 msec.

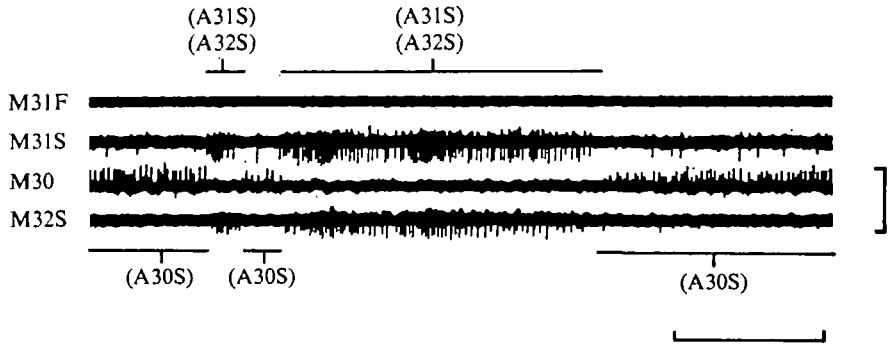

Fig. 8. Simultaneous electromyogram recordings in flexor muscles 31F, 31S and 32S and extensor muscle 30. During the periods of electrical activity in muscles 31S and 32S there was slow flexion at the MS–DS and DS–OF joints. During the periods of electrical activity in muscle 30 there was extension at these joints. The motoneurones considered to be responsible for various portions of the recording are shown in parentheses. Scale: 50 μV, 100 msec.

In conclusion, it seems that the phasic components of the antennular motor system (motor units 30F, 31F and 32F) are active during flicking, as is the slow extensor motor unit 30S (cf. Snow, 1973*b*). Slow or tonic flexion at the MS–DS and DS–OF joints results from activity in the slow flexor motor units 31S and 32S. In contrast, movements at the MS–DS joint during extension withdrawal reflexes result from activity in the slow extensor motor unit (30S) or sometimes in both the fast (30F) and slow (30S) extensor motor units (cf. Snow, 1973*b*). Powerful and rapid flexion movements at the MS–DS joint reflects activity in the phaso-tonic component of the antennular motor system (motor unit 31F-S).

Because of electrode displacements clear records were not obtained during antennular wiping. Nevertheless, some incomplete recordings suggest that motor units 30F, 31F and 32F are not involved in wiping. Motor unit 31F-S may be active during a wipe, in addition to motor units 30S, 31S and 32S (cf. Snow, 1973*b*).

Table 1. (*a*) *Delay between the first EJP in the flexor muscle 31F and the first EJP in the flexor muscle 32F during flicking*

Preparation	Mean delay ± standard deviation	No. of observations	Coefficient of variation
1	4·13 ± 0·376	48	9·1 %
2	3·08 ± 0·806	16	26·7 %
3	3·74 ± 0·138	12	3·7 %
4	3·64 ± 0·191	39	5·2 %
5	3·76 ± 0·252	70	6·7 %
6	2·30 ± 0·368	20	16·0 %
7	2·87 ± 0·230	52	8·0 %
8	2·75 ± 0·225	35	8·2 %

Table 1. (*b*) *Delay between the first spike in the flexor motoneurone A31F and the first spike in the flexor motoneurone A32F during flicking; flexor activity recorded from nerve 2 in the proximal segment*

Preparation	Mean delay ± standard deviation	No. of observations	Coefficient of variation
1	1·49 ± 0·067	15	4·5 %
2	2·32 ± 0·098	35	4·2 %
3	2·15 ± 0·064	27	3·1 %
4	2·57 ± 0·130	23	5·1 %

(2) *Motoneuronal activity during antennular flicking*

It was rarely possible to achieve stable, clear electromyogram recordings of activity in M31F, M32F and M30 during flicking. The usual procedure was to record in M31F and M32F, or M31F and M30, and to make comparisons between preparations.

Within any animal there is often considerable variation in the structure of the bursts in motoneurones A31F, A32F, A30F and A30S during flicking. In general, however, a flick usually results from a burst of two or three spikes in the flexor motoneurone A31F. At the level of the proximal antennular segment the first spike in A31F precedes the first spike in A32F by 1·4–2·6 msec. Usually only 1 or 2 spikes occur in the flexor motoneurone A32F. From 20 to 40 msec after the first spike in motoneurone A31F a burst of usually 1–2 spikes occurs in the fast extensor motoneurone A30F. In addition, from 20–40 msec after the first spike in motoneurone A31F there is a burst of usually 2–3 spikes in the slow extensor motoneurone A30S.

A marked feature of the flexor activity during antennular flicking is the invariance, within any animal, of the delay between activity in flexor motoneurones A31F and A32F (Table 1 *a*, *b*). During the recording of electromyograms the first sign of a flick is a burst of 1–7 EJPs in M31F (Fig. 7). From 2·3 to 4·2 msec after the first EJP in M31F a burst of usually 1–5 EJPs was always observed in M32F (Fig. 7; Tables 1 *a*, 3). Only some of this delay is due to the conduction time in motoneurone A32F between M31F and M32F, since extracellular recordings from nerve 2 in the proximal segment show that during a flick the first spike in motoneurone A31F precedes the first spike in motoneurone A32F by 1·4–2·6 msec (Table 1 *b*).

From 20 to 40 msec after the first EJP in M31F a burst of 1–4 non-facilitating EJPs is usually recorded in M30 (Tables 2 *a*, 4 *a*). The delay between the first EJP in M31F and the first EJP in M32F is more constant than the delay between the first EJP in

Table 2. (*a*) *Delay between the first spike in the flexor motoneurones A31F and the first spike in the fast extensor motoneurone A30F during flicking*

Preparation	Mean delay ± standard deviation	No. of observations	Coefficient of variation
1	29·5 ± 4·08	56	13·8 %
2	33·0 ± 4·89	43	14·8 %
3	28·1 ± 2·55	100	9·1 %
4	25·7 ± 4·38	36	17·0 %
5	21·4 ± 0·94	10	4·4 %
6	32·8 ± 1·16	10	3·5 %
7	36·1 ± 2·12	10	5·9 %
8	37·3 ± 2·01	10	5·4 %

Table 2. (*b*) *Delay between the first spike in flexor motoneurone A31F and the first spike in the slow extensor motoneurone A30S during flicking*

Preparation	Mean delay ± standard deviation	No. of observations	Coefficient of variation
1	25·9 ± 3·95	17	15·3 %
2	35·1 ± 7·83	10	22·3 %
3	28·7 ± 4·67	25	16·3 %
4	24·6 ± 2·28	15	9·3 %

Table 2(*a*) is derived from electromyogram recordings while Table 2(*b*) is derived from recordings made directly from antennular nerves 2 and 2a in the proximal segment.

M31F and the first non-facilitating EJP in M30 (cf. standard deviations in Tables 1*a*, 2*a*).

Direct recordings from the extension motor nerve (nerve 2a) showed that a burst of 1–6 spikes in motoneurone A30S occurs 20–40 msec after the first spike in motoneurone A31F (Tables 2*b*, 4*b*). Once again this delay is more variable than the delay between bursts in flexor motoneurones A31F and A32F (cf. standard deviations in Tables 1*b* and 2*b*).

The number of spikes per flick in flexor motoneurones A31F and A32F or extensor motoneurones A30F and A30S was highly variable in some preparations but more constant in other preparations (Tables 3, 4*a*, *b*). There were never more and usually less spikes in flexor motoneurones A32F than in flexor motoneurone A31F (Table 3). The most frequently occurring number of spikes in the flexor motoneurones varied between different animals and during long (over 10 h) recording sessions from a single animal (Table 3).

There were usually more spikes in the slow extensor motoneurone A30S than in the fast extensor motoneurone A30F (Fig. 3*b*, Table 4*a*, *b*). In most, but not all preparations the first spike in the slow extensor motoneurone A30S preceded the first spike in the fast extensor motoneurone A30F. No clear relationship between the number of spikes in either extensor motoneurone and the number of spikes in either flexor motoneurone was evident. Furthermore, the delay between the first spike in flexor motoneurone A31F and the first spike in either extensor motoneurone did not bear any relationship to the number of flexor spikes.

During flicking in different animals the most frequent inter-spike interval in flexor motoneurone A31F was in the range of 3·3–5·5 msec. This is less than the most

Table 3. *Patterns of activity in flexor motoneurones A31F and A32F during antennular flicking*

	No. of A31F spikes/no. of A32F spikes																
Animal	1/1	2/1	2/2	3/1	3/2	3/3	4/2	4/3	4/4	5/2	5/3	5/4	6/3	6/4	6/5	7/3	7/4	Total
1	2	2	28	—	1	2	—	—	—	—	—	—	—	—	—	—	—	35
2	8	25	38	—	12	—	—	—	—	—	—	—	—	—	—	—	—	83
2*	—	2	2	—	57	—	26	—	—	2	2	—	5	—	—	—	—	96
3	9	18	20	—	10	—	2	1	—	—	—	—	—	—	—	—	—	60
4	—	2	—	—	45	2	3	—	—	—	12	1	2	—	1	1	1	70
5	2	17	4	4	57	—	1	1	—	—	—	—	—	—	—	—	—	86
6	1	2	1	—	6	—	29	2	—	—	7	1	4	—	—	—	—	54
7	7	53	2	5	5	—	11	—	—	4	—	—	—	—	—	—	1	88
8	—	2	10	—	36	11	1	23	4	—	8	3	—	2	—	—	—	100

* Recordings from animal 2, taken 20 h after first set of recordings.

Table 4. (*a*) *Patterns of activity in the flexor motoneurone A31F and the fast extensor motoneurone A30F during flicking*

	No. of A31F spikes/no. of A30F spikes																	
Animal	1/0	2/0	3/0	4/0	1/1	2/1	3/1	4/1	2/2	3/2	4/2	5/2	2/3	3/3	4/3	3/4	4/4	5/4	Total
1	1	12	2	1	4	115	36	4	24	21	6	2	—	9	2	—	1	2	243
2	—	4	—	—	2	25	1	—	21	1	—	—	2	—	—	—	—	—	60
3	62	52	5	—	14	16	6	—	—	—	3	—	—	—	—	—	—	—	155
4	—	—	—	—	—	55	6	—	27	10	3	—	—	—	—	—	—	—	101
5	—	—	—	—	—	26	—	—	4	2	—	—	—	—	—	—	—	—	33
6	5	26	1	1	2	53	15	11	3	1	2	2	—	2	—	1	1	—	126

Table 4. (*b*) *Patterns of activity in the flexor motoneurone A31F and the slow extensor motoneurone A30S during flicking*

	No. of A31F spikes/no. of A30S spikes																
Animal	1/0	1/1	1/2	1/3	2/1	2/2	2/3	2/4	2/5	2/6	3/1	3/2	3/3	4/2	4/3	4/4	Total
1	—	1	—	—	—	3	1	1	—	—	1	2	2	1	3	1	14
2	—	7	1	—	2	1	—	—	—	—	—	—	—	—	—	—	11
3	1	4	10	2	—	2	—	1	—	—	—	—	—	—	—	—	20
4	—	—	—	1	1	5	5	—	1	1	—	—	—	—	—	—	14

Table 4(*a*) is derived from electromyogram recordings while Table 4(*b*) is derived from recordings made directly from the antennular nerves 2 and 2a in the proximal segment.

frequent inter-spike interval in flexor motoneurone A32F, which usually lay between 5·0 and 8·0 msec. When there was more than one spike in the fast extensor motoneurone A30F the most frequent inter-spike interval lay between 5·0 and 11·0 msec. During flicking the most frequent inter-spike interval in the slow extensor motoneurone A30S was between 7 and 15 msec. It should be noted, however, that in a few preparations the most frequent intervals in some or all of the motoneurones A31F, A32F, A30F and A30S lay outside these ranges.

Bursts of 4–6 spikes in motoneurone A31F usually contained one or more inter-spike intervals of more than 6·0 msec. Occasionally these longer inter-spike intervals also occurred in A31F bursts of as few as 3 and sometimes only 2 spikes. Following a

long interval the first spike in motoneurone A31F usually preceded 1 or 2 spikes in motoneurone A32F, the first of which occurs with a delay approximately equal (within 0·2 msec) to the delay between the first spikes in motoneurones A31F and A32F on initiation of a flick. Thus a long inter-spike interval in motoneurone A31F was often reflected by a long inter-spike interval in motoneurone A32F (compare Figs. 7*d* and 7*g*). It should be noted that in a few preparations flexor bursts of 3–6 spikes which were not interrupted by a long inter-spike interval (e.g. Fig. 7*g*) were the most frequently occurring type.

During some flicks a single spike in motoneurone A31F occurred following a long inter-spike interval without being accompanied by a spike in motoneurone A32F. Such spikes may occur in the middle of a burst in motoneurone A31F but are more frequently the last spike in the burst. In the latter case such a spike has been seen to follow an interval of up to 50 msec (Fig. 7*f*).

Rarely, long intervals in the bursts in motoneurone A31F result in an overlap of the flexor bursts with the bursts in the fast and slow extensor motoneurones. This would be expected to result in synchronous tension in antagonistic muscles during a flick.

(3) *Motoneuronal activity during antennular withdrawal*

Bending of the tip of the outer flagellum towards the aesthetasc hairs or pipetting distilled water directly over the aesthetasc hairs elicited a burst of 1–16 spikes in the flexor motoneurone A31F-S (Figs. 5*b*, 6*b*). The mean intra-burst frequency of motoneurone A31F-S [(no. of EJPs-1)/burst duration] was highly variable but could reach 150/sec. In two freely moving animals such activity was seen each time the tip of the outer flagellum touched the side of the small observation chamber. When this response was recorded the antennule was rapidly flexed at the MS–DS joint (cf. fast flexion–withdrawal reflex, Snow, 1973*a*) and any tonic activity in the extensor motoneurone A30S was abolished.

Bursts in motoneurone A31F-S often overlapped with activity in motoneurones A31S (Fig. 5*b*) and A32S. When this occurred, extension at the MS–DS joint was usually delayed for several seconds, due presumably to tonic tension in M31S, resulting from continued activity in motoneurone A31S.

High-frequency (15–60/sec) activity in motoneurones A31S and A32S (Fig. 8) and a slow flexion–withdrawal reflex (Snow, 1973*a*) could be elicited by touching the sides or dorsal surface of the outer flagellum once with a glass rod or pipetting distilled water into the sea water near the aesthetasc hairs. Prolonged stimulation of the aesthetasc hairs with distilled water or repetitive mechanical stimulation often resulted in high-frequency activity in motoneurone A31S being maintained for a period of minutes, even in the absence of further stimulation. Limited observations suggest that the high-frequency activity in motoneurone A32S, elicited by these stimuli, is maintained for less time. During activity in motoneurone A32S the posture of the antennule was similar to that described as tonic flexion withdrawal (Snow, 1973*a*).

Touching the inner flagellum or stroking the endopodites of the 3rd maxillipeds with a glass rod elicited an extension–withdrawal reflex (Snow, 1973*a*) and high frequency (15–60/sec) activity in motoneurone A30S (Figs. 2*b*, 9*b*). As mentioned above, direct recordings from the extensor motor nerve (nerve 2a, Snow, 1973*b*) in

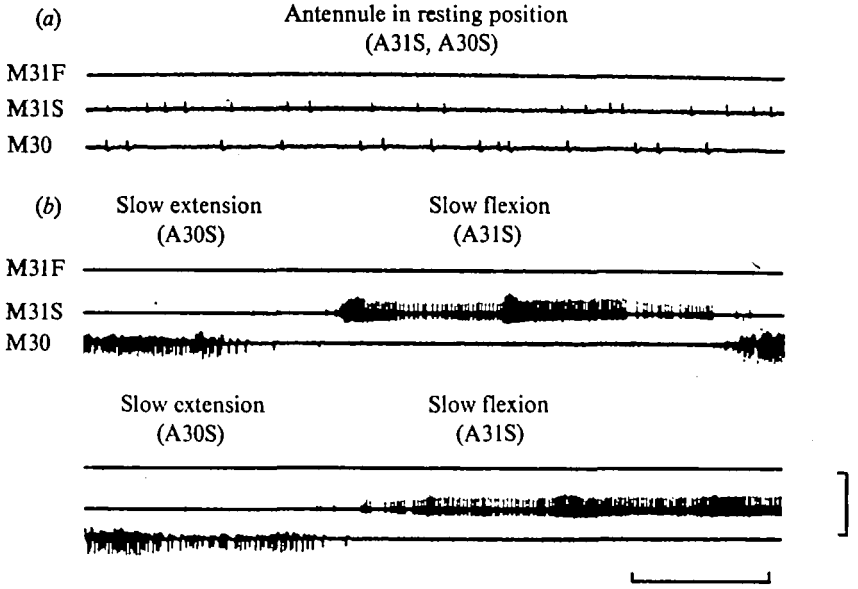

Fig. 9. Electromyogram recordings in flexor muscles 31F and 31S and extensor muscle 30. (*a*) Low frequency of small EJPs in antagonistic muscles 31S and 30. (*b*) Alternate stimulation of the endopodites of the 3rd maxillipeds and the outer flagellum to produce extension, then slow flexion, then extension and then slow flexion movements at the MS–DS joint. Upper three traces in (*b*) are continuous with the lower three traces. Note the inhibition of high-frequency activity in one muscle before the initiation of activity in its antagonist. The motoneurones considered to be responsible for various portions of the recording are shown in parentheses. Scale: 100 μV, (*a*) 500 msec; (*b*) 1000 msec.

partially dissected preparations showed that intense mechanical stimulation of the inner flagellum elicited several spikes in the fast extensor motoneurone A30F in addition to increased activity in the slow extensor motoneurone A30S (Fig. 4).

In a few preparations a low frequency (mean: 7·4/sec) of small EJPs could be simultaneously recorded in M31S and M30 (Fig. 9*a*). During this activity the antennule was maintained in its resting position. Appropriate stimulation of the outer flagellum elicited an abolition of the small EJPs in M30, then an increase in the frequency and facilitation of the EJPs in M31S, and flexion at the MS–DS joint. Stroking the endopodites of the 3rd maxillipeds or the inner flagellum during this response abolished the activity in M31S and then elicited a high-frequency train of facilitating EJPs in M30 and extension at the MS–DS joint (Fig. 9*b*). Stimulation of the outer flagellum during this response abolished the activity in M30 and then re-elicited a high frequency of facilitating EJPs in M31S.

Clearly these recordings represent activity in motoneurones A31S and A30S but they cannot be considered as evidence for reciprocal inhibition between these motoneurones. Firstly, the high-frequency activity in one motoneurone usually followed abolition of activity in the other motoneurone. Secondly, triggering the oscilloscope on the small EJPs in M30 or M31S, during low-frequency activity in both these muscles, did not show any period following or during an EJP in one muscle when EJPs in the other muscle were rare. Thirdly, preliminary analysis of the simultaneous low-frequency activity in motor units 31S and 30S using cross-correlation and

auto-correlation techniques, did not produce any evidence for direct inhibitory coupling between motoneurones A31S and A30S.

In conclusion, it seems that inputs which elicit slow flexion at the MS–DS and DS–OF joints first inhibit slow extensor motoneurone A30S, and that inputs which elicit extension at the MS–DS joint first inhibit the slow flexor motoneurones A31S and A32S.

(4) *Overlap of activity between phasic and tonic motor units*

Activity in phasic motor units 30F, 31F and 32F often overlaps with activity in tonic motor units 30S, 31S and 32S. When flicking occurs during high-frequency activity in the slow flexor motoneurone A31S there is often no activity in the extensor motoneurones A30F and A30S. When motoneurone A31S is firing at frequencies above 15/sec the antennule is tonically flexed at the MS–DS joint. While recording flexor and extensor activity during flicking, occasionally a series of flicks would occur which were not correlated with activity in the extensor motoneurones A30F and A30S. Visual examination showed that the MS–DS joint was being maintained fully flexed during these anomalous patterns. Flicking usually did not occur during high-frequency activity in the slow flexor motoneurone A32S but it must be stressed that only two preparations yielded short recordings from this unit.

DISCUSSION

(1) *Functional significance of motoneuronal activity during antennular flicking*

An analysis of the movements occurring during a flick showed that flexion at the MS–DS joint preceded flexion at the DS–OF joint by about 2·5 msec, a lag which compares favourably with the lag between electrical activity in muscle 31F and muscle 32F (Snow, 1973*a*). Furthermore, it was shown that flexion at the MS–DS joint ceased for about 5 msec following the initiation of flexion at the DS–OF joint. This interruption was considered to result from water-resistance forces generated by flexion at the DS–OF joint (Snow, 1973*a*). Electromyogram recordings from muscle 31F support this suggestion by showing that there is no consistent interruption of activity that might explain the interruption of flexion at the MS–DS joint. It is thus possible that the major function of activity in flexor motoneurone A31F is to prevent extension at the MS–DS joint during the rapid flexion at the DS–OF joint. Prevention of MS–DS joint extension could ensure that a burst in flexor motoneurone A32F would cause a strong movement of the outer flagellum through the water to provide adequate exchange of water trapped around the chemoreceptive aesthetasc hairs, i.e. a fresh sample of the chemicals in the crab's immediate environment (Snow, 1973*a*). The patterns of activity in the fast flexor motoneurones might thus have evolved in response to the selective advantage of sampling dissolved chemicals.

The length and number of spikes in the flexor bursts is highly variable in many animals. Assuming that longer flexor bursts result in an increase in the amplitude and duration of flexion at the MS–DS and DS–OF joints, then an extension of the above hypothesis is that longer flexor bursts also result in a more complete exchange of water around the aesthetasc hairs.

Very long flexor bursts often result in an overlap between bursts in flexor motoneurone A31F and the fast and slow extensor motoneurones. The difficulty of

suggesting a function for synchronous tension development in these antagonistic antennular muscles during flicking leads one to suggest that the longest bursts in motoneurone A31F may be a functionally non-significant result of the mechanisms underlying the generation of bursts in the flexor motoneurones.

(2) *Neuronal control of antennular withdrawal*

Powerful flexion at the MS–DS joint is always correlated with a burst of spikes in motoneurone A31F-S. This movement has been classified as a fast flexion–withdrawal reflex and is considered to be a protective response to immediately noxious stimuli (Snow, 1973*a*).

Slow flexion–withdrawal reflexes are the result of activity in the slow flexor motoneurones A31S and A32S, while extension–withdrawal reflexes are the result of activity in slow extensor motoneurone A30S and sometimes fast extensor motoneurone A30F. Sometimes there was an overlap of activity in motoneurones A31S and A32S with activity in motoneurone A31F-S. Whether the resultant movements are classified as a fast or a slow flexion–withdrawal reflex probably depends on the relative burst lengths and intra-burst frequencies in A31F-S and A32S. It seems best to consider that flexion–withdrawal reflexes are graded in intensity, from those which result from a train of spikes in motoneurones A31S and A32S just sufficient to cause flexion at the MS–DS and DS–OF joints, to those which result solely from a high-frequency burst of spikes in motoneurone A31F-S causing the powerful flexion at the MS–DS joint characteristic of the fast flexion–withdrawal reflex (Snow, 1973*a*). Similarly, extension–withdrawal reflexes could be considered to be graded in intensity from those which result from a small increment in activity in the slow extensor motoneurone A30S to those which result from a high-frequency train of spikes in motoneurone A30S plus several spikes in the fast extensor motoneurone A30F.

Sometimes low-frequency activity (mean: 7·4/sec) was recorded simultaneously in antagonistic motoneurones A30S and A31S. It is possible that this activity is important in maintaining slight tension in muscles 31S and 30 which could stabilize the MS–DS joint against passive flexions or extensions induced by fluctuations in water currents (see Snow, 1973*b*). This would allow more controlled positioning of the antennules in the crab's surroundings.

Within the postural control system of the crayfish abdomen, Kennedy, Evoy, Dane & Hanawalt (1967) have shown that activity in specific command interneurones initiates and maintains specific abdominal postures. Furthermore, the frequency of spikes in single motoneurones may be controlled by the frequency of spikes in a single command element (Evoy & Kennedy, 1967). Within the tonic component of the antennular motor system (motor units 30S, 31S and 32S) of the hermit crab, four discrete output patterns may be recognized. Thus one might propose that the tonic motor units are controlled by as few as four interneuronal command elements. Threshold excitation of one command element could result in an extension–withdrawal reflex by exciting motoneurone A30S while inhibiting motoneurones A31S and A32S. Another command element could result in tonic flexion at the MS–DS joint by exciting motoneurone A31S after inhibiting motoneurone A30S. Another could result in tonic low-frequency activity in motoneurones A31S and A30S which may play a role in stabilizing the MS–DS joint to water-current fluctuations. Finally, still

another could result in excitation of both motoneurone A31S and motoneurone A32S after inhibition of motoneurone A30S. The duration of activity in this last element could determine whether tonic flexion withdrawal follows a slow flexion–withdrawal reflex. In all cases the level of activity in the command interneurones could control the level of activity in the relevant motoneurones and thus be used to grade the velocity of the movements or the amount of postural change.

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