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ABDOMINAL MOTONEURONE RESPONSES ELICITED BY FLEXION OF A CRAYFISH LEG

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SUMMARY

Flexion of the mero-carpal joint of the leg of a partially dissected *Procambarus* initiated an intersegmental reflex in the superficial extensor (SEMNs) and flexor (SFMNs) abdominal motoneurones. When flexion induced a full extension of the abdomen, as in intact crayfish, there was a discharge of the 5 SEMN exciters and the SFMN peripheral inhibitor throughout the extension. The extension antagonists – the SEMN peripheral inhibitor and the five SFMN exciters – discharged one or two spikes immediately following the stimulus, which suggests that more than one interganglionic interneurone must be excited to activate the neuronal pathway which mediates the abdominal extension.

INTRODUCTION

In the crayfish *Procambarus clarkii*, stimuli applied to the cephalic and/or thoracic appendages evoke complex reflexes that include postural extension of the abdomen. Postural abdominal extension is initiated by the visual (Glantz, 1974) and tactile stimuli (Tsukada, 1974) that induce defence reactions and the proprioceptive stimuli that elicit equilibrium reactions (Larimer & Eggleston, 1971; Page, 1975*b*). Abdominal extension is produced by the superficial extensor muscle (SEMs) that span the dorsal aspect of each half abdominal segment (Page, 1981). The SEMs and their antagonists the superficial flexor muscles (SFMs) are each innervated by five excitatory motoneurones and a peripheral inhibitor (Fields, Evoy & Kennedy, 1967; Kennedy & Takeda, 1965). During extension, there is excitation of those motoneurones that are extension synergists (SEMN exciters and SFMN inhibitor) and inhibition of the extension antagonists (SEMN inhibitor and SFMN exciters) (Evoy & Kennedy, 1967; Fields *et al.* 1967; Larimer & Eggleston, 1971; Sokolove, 1973; Page, 1975*a*; Williams & Larimer, 1980; 1981).

In the present investigation, activity in the motoneurones of partially dissected preparations was recorded following flexion of the mero-carpal (M–C) joint of the walking leg. The activities were categorized according to the observed abdominal responses, which ranged from full abdominal extension, as in the intact animal (Page,

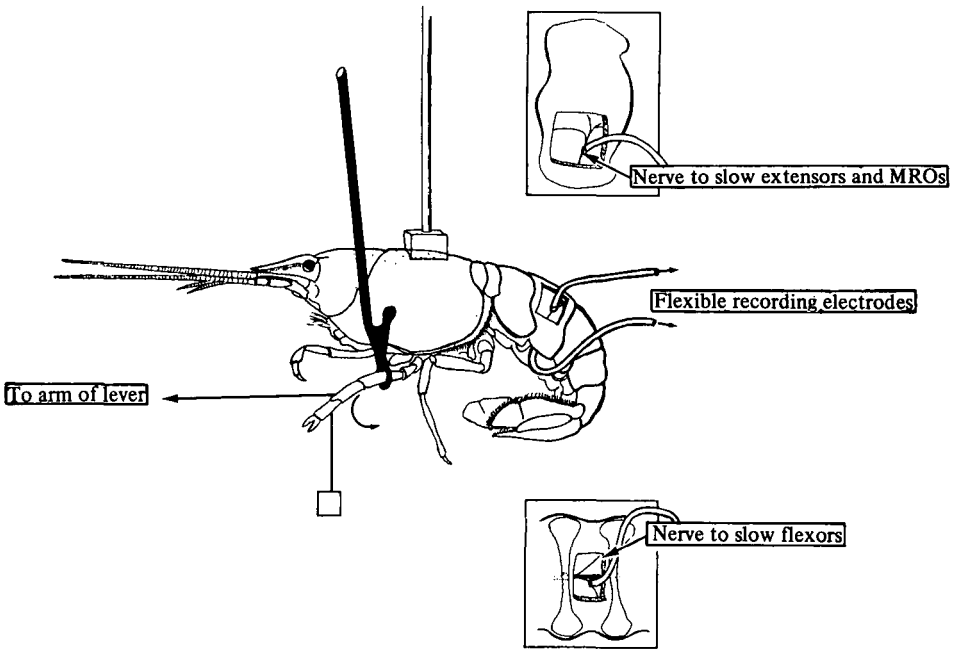

Fig. 1. Preparation for recording SEMN and SFMN unit activity from crayfish suspended via a lucite block and brass rod. The animal was submerged in chilled saline following perfusion. The merophodite of the 2nd leg was clamped in a horizontal position. The mero-capal (M–C) joint was held extended via a string attached to a lever. A 5 g weight attached to the carpus flexed the M–C joint upon release of the lever. Access to SEMNs and SFMNs was made through windows cut in the dorsal and ventral cuticle respectively (insects). Adapted from Page & Sokolove (1973).

1981), to flexion. A preliminary report of some of these results has been published (Page & Jones, 1982).

METHODS

The Preparation All crayfish (*Procambarus clarkii* from Monterey Bay Hydroculture Farms, Soquel, California) were prepared and tested for responsiveness to M–C flexion as previously described (Page, 1981). Several weeks before an experiment adult crayfish were prepared by removal of all legs except the second and fifth pairs and by attaching a plastic mount to the centre of the dorsal thoracic carapace. Before each experiment, the eyes of the crayfish were covered with black petroleum jelly and the resonse to M–C flexion was observed. Only animals which responded with a strong abdominal extension (about 95 % of those tested) were used in these experiments. The crayfish was perfused with saline (van Harreveld, 1936) to prevent obscuration of the nerves which innervate the SEM and SFM. The animal was gradually cooled to 18 °C and immersed in 18 °C saline while 18 °C saline was introduced through a catheter into the thoracico-coxal joint of the chela. The perfusate left through a small hole in the dorsal cuticle of the 5th abdominal segment.

Recording A small window was cut in the cuticle of the first and/or second segments to expose either the dorsal nerve or the superficial branch of the 3rd root.

Table 1.

Response	% SEMN trials	% SFMN trials
Full extension	45	16
Delayed extension	21	5
Weak extension	15	33
No abdominal movement	18	43
Flexion	1	3

N is 280 for total trials, 167 for SEMN trials and 113 for SFMN trials.

Flexible Tygon suction electrodes (Page & Sokolove, 1972) were attached to either the dorsal nerve, to record SEMN activity, or to the superficial branch of the third root, to monitor SFMN activity (Fig. 1). The superficial branch of the 3rd root was visualized for electrode placement by positioning a mirror beneath the abdomen. Potentials were amplified with a high gain differential amplifier, displayed on an oscilloscope and stored with an Ampex SP700 FM tape recorder. Permanent records were obtained by replaying the tapes on the oscilloscope and filming with a kymograph camera.

Stimulation A mechanical release system was used to flex the M–C joint of one of the second legs (Fig. 1) (Page, 1981).

Unit Analysis Identification of each efferent was based upon its relative impulse amplitude, pattern of discharge and correlation with the abdominal movement noted on the voice channel of the tape (Kennedy & Takeda, 1965; Fields *et al.* 1967). Impulses produced by the largest exciters (E6 and F6) and the peripheral inhibitors (E5 and F5) were easily identified as were those of the thick accessory nerve and the sensory neurone (SR_1) which innervates the tonic receptor muscle of the muscle receptor organ. Identification of SR_1 and the accessory nerve spikes was based upon the stretch sensitivity of SR_1 and the suppression of SR_1 discharge by accessory nerve activity. Although the spikes of the medium size SEMNs (E3 and E4) and SFMNs (F3 and F4) were often similar in size and therefore difficult to identify separately, they were readily distinguished from the larger units (numbers 5 and 6) and the smaller units (numbers 1 and 2). The small SEMN and SFMN units (E1, E2, F1 and F2) were each easily identified except when the very small size of the E1 spikes made accurate counts impossible. Activities of all motoneurones were readily identified in about 50 percent of the recordings. The data presented in the results are based upon these recordings.

Comparisons of motoneurone responses were based upon histograms constructed by normalizing the responses of several animals to the MCF stimulus. The Wilcoxon matched-pairs signed rank test was used to measure the statistical significance of differences between histograms. All differences between the histograms mentioned in the text were significant at the 1% level.

RESULTS

Abdominal Movement Responses Abdominal movements in response to M–C flexion were examined in 280 trials upon 22 animals. The responses depended upon the condition of the experimental preparation. Animals whose dorsal nerves were

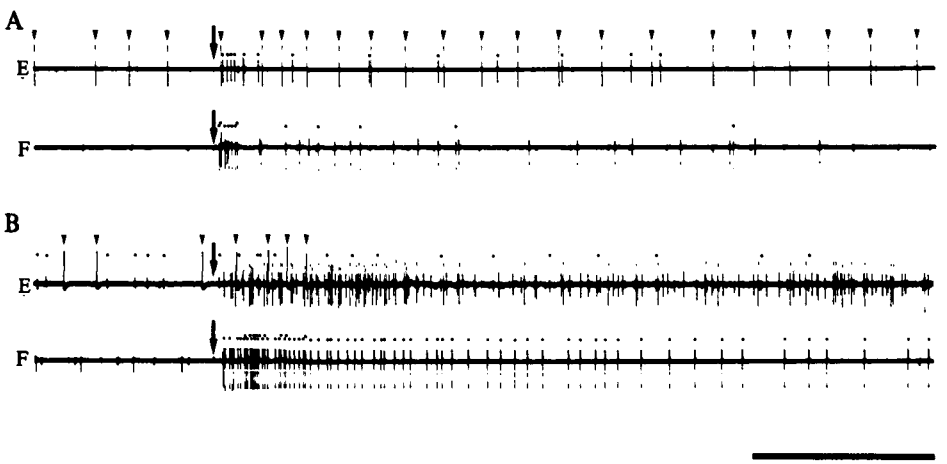

Fig. 2. SEMN and SFMN responses to M–C flexion. In A and B upper traces are recordings from 2nd segment SEMNs, lower traces are from 1st segment SFMNs. Each trace is from a different animal. Stimulus applied at arrow. (A) No abdominal movement involved a brief burst of SEMN and SFMN peripheral inhibitors as well as weak discharge of the SEMN and SFMN exciters. SR_1 activity reflects the maintenance of a flexed abdominal posture throughout the response. (B) Full extension consists of a vigorous discharge of the excitatory SEMNs and SFMN inhibitor. SR_1 discharge stops when abdominal extension begins. E_5 and F_5 spikes are identified by dots; SR_1 impulses by triangles. SEMN and SFMN exciters are not marked. Time mark is 1 s.

exposed generated full extensions in 45% of the trials while those with exposed superficial 3rd roots produced full extensions in only 16% of the MCF trials. The responses were divided into five groups (Table 1): (1) full extension of the abdomen (latency of less than 1 s) to a fully extended position at or above the horizontal plane; (2) delayed extension–movement of the abdomen to a fully extended position (latency of 2 to 10 s); (3) weak extension–extension insufficient to move the abdomen to a fully extended position; (4) no abdominal movement; (5) flexion.

Abdominal Motoneurone Responses The SEMNs and SFMNs always discharged when the M–C joint was flexed. The minimal response was a burst in the SEMN and SFMN inhibitors and medium sized exciters (Fig. 2A). This brief motoneurone response was not accompanied by an abdominal movement. Full extension was characterized by a vigorous sustained discharge of the SEMN exciters and the SFMN inhibitor which followed a short early burst of mixed (excitatory and inhibitory) SEMN and SFMN activity (Fig. 2B). Efferent activity accompanying delayed extensions was similar except that the early period of mixed activity was prolonged for 2–10 s. Weak extensions were accompanied by a lower level of discharge in the SEMN exciters and SFMN inhibitor and an increase in SFMN exciter activity.

Full extension was initiated by a burst in the SEMN exciters that was usually accompanied by weak discharge of the SEMN inhibitor (Figs. 2B and 3). The latencies for initiation of SEMN exciter discharge ranged from 20 to 98 ms with a mean of 53·6 (S.E. = 6.3) ms in segment 1 (17 responses in 4 animals) and from 26 to 105 ms with a mean of 58·5 (S.E. = 3·7) ms in segment 2 (38 responses in 4 animals). The order of activation of the excitatory SEMNs differed in the first (S_1) and second

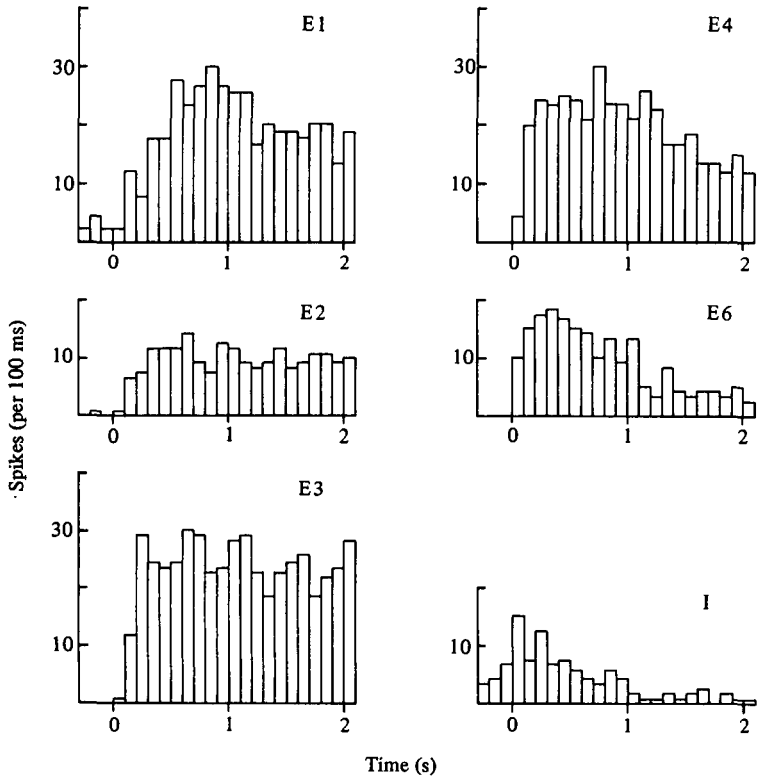

Fig. 3. Histograms of 2nd segment SEMN activity during a full extension. Stimulus at $T = 0$. Each 100 ms bin consists of the sum of spikes occurring in a total of 12 trials from three animals. The histograms are normalized so that $N = 10$.

(S_2) segments (Table 2). Excitatory SEMN activity was initiated by a spike in the largest exciter (E6) in 71% of S_1 responses and 18% of the S_2 responses. The remainder of the S_2 excitatory SEMN responses (82%) as well as 22% of the Si exciter responses began with a medium sized exciter impulse (E3 or E4). In 7% of the S_1 responses the small exciters (E_1 and E_2) fired the initial SEMN exciter spikes.

The SR_1 discharged during the initial moments of 40% of the full extensions (Fig. 2B). This SR_1 activity resulted from the flexed posture that was often maintained by the abdomen before MCF. SR_1 discharge ceased shortly after the initiation of SEMN exciter activity. During full extension the initial impulse of the SEMN inhibitor often preceded the beginning of SEMN exciter activity (in 41% of S_1 and 63% of S_2 full extensions). However, since the inhibitor was usually active before M–C flexion (Fig. 2B and 3), it is possible that some of the initial E5 spikes resulted from a continuation of activity rather than having been elicted by flexion.

As indicated in Fig. 3, discharge of the excitatory SEMNs increased over the 200–500 ms period following the stimulus. Activity of the small and medium sized exciters continued for the duration of the response albeit at a slowly decreasing rate. The two largest exciters (E4 and E6) adapted more rapidly, although they discharged an

Table 2.

	sE	mE	lE	I	N
		Full extension			
SEMNs S_1	123±19	125±17	60±9	55±19	17
SEMNs S_2	149±7	75±4	116±7	61±8	38
SFMNs S_1	65±16	51±5	84±7	58±1	15
		No abdominal movement			
SEMNs S_1	208±17	68±7	131±37	37±4	10
SEMNs S_2	121±25	130±5	102±33	64±6	12
SFMNs S_1	78±19	47±7	60±7	55±14	12

Latency for initial spike following MCF stimulus. Latencies in ms with standard errors. SEMN S_1 recordings from 3 animals, SEMN S_2 from 4 animals and SFMN S_1 from 3 animals. sE = small exciters; mE = medium exciters; lE = large exciters; I = peripheral inhibitor. S_1 = first segment; S_2 = second segment.

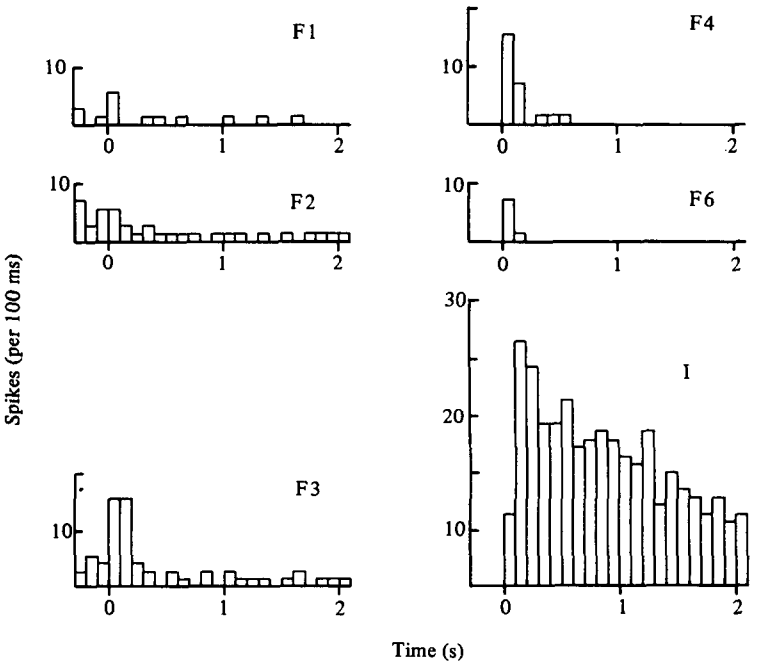

Fig. 4. Histograms of 1st segment SFMN activity during a full extension. Stimulus at T = 0. Each 100 ms bin consists of the sum of spikes occurring in a total of seven trials from two animals. The histograms are normalized so that N = 10.

occasional spike throughout the remainder of the extension. The inhibitory SEMN produced a brief burst of spikes immediately after M–C flexion which was followed by suppression of inhibitory activity (Fig. 3).

Comparison of SEMN responses recorded simultaneously from S_1 and S_2 in 3 preparations revealed consistent differences in the levels of activity of four of the SEMNs. When compared with S_2 responses, E3 and the inhibitor had significantly (Wilcoxon test) lower discharge rates in S_1 while the E2 and E6 responses in S_1 were always larger than those recorded from S_2.

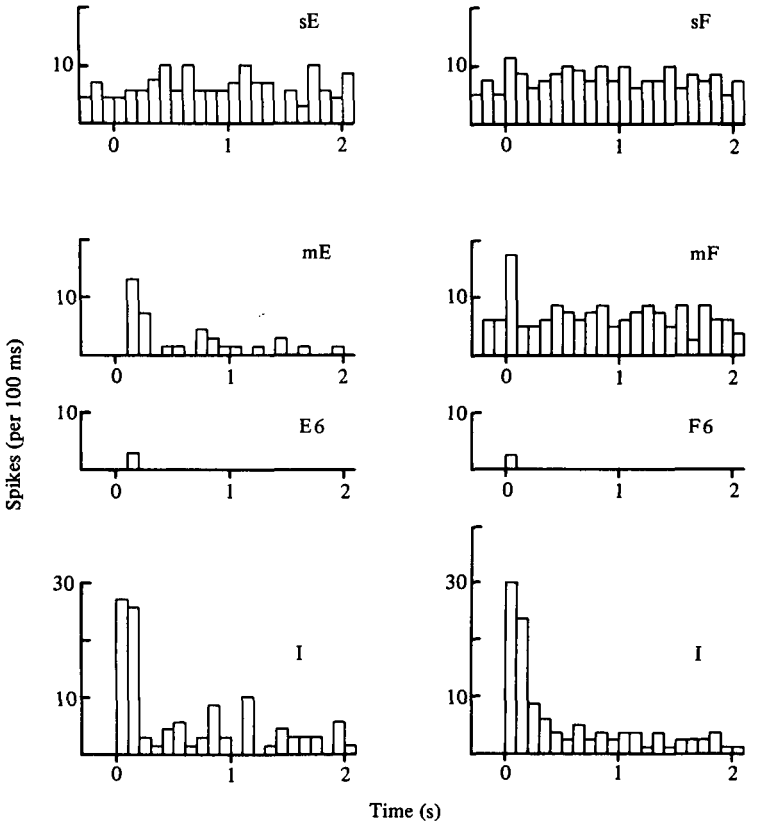

Fig. 5. Histograms of the 2nd segment SEMN and 1st segment SFMN activities that occur during responses that did not produce abdominal movement. Stimulus at $T = 0$. For the SEMN histograms each 100 ms bin consists of the sum of spikes occurring in a total of seven trials in a single animal. The SFMN histograms were constructed from eight trials in two animals. The histograms are normalized so that $N = 10$.

Flexion was suppressed during full extension by the discharge of the SFMN inhibitor (Figs. 2B and 4). In the first segment the inhibitory SFMN response had a mean latency of 58 (S.E. = 1·4) ms (Table 2) and reached a maximum discharge 100–300 ms following stimulation. The large and medium SFMN exciters discharged briefly during the initial 100–200 ms of the response (Figs. 2B and 4). In many instances SFMN inhibitor activity was preceded by the firing of one or more exciter impulses. The large exciters (F4 and F6) were suppressed for the remainder of the response while the smaller exciters (F1–F3) continue to discharge spikes at a very low rate in about half of the responses (Fig. 4).

In 28% of the M–C flexion trials motoneurone activities did not produce any abdominal movement. These no-movement responses were characterized by strong discharge of the SEMN and SFMN inhibitors and a low level of activity in the SEMN and SFMN exciters (Figs. 2A and 5). There was an overall lack of reciprocity between the SEMNs and the SFMNs; SEMN and SFMN antagonists had similar patterns of activity (compare E6 and F6, both inhibitors, etc. in Figs. 2A and 5). During the

non-movement response there was typically an initial 300–400 ms period of mixed discharge of both the SEMN and SFMN exciters and inhibitors. Following this initial period there was a suppression of activity in the largest motoneurones (E6 and F6). In contrast there was little diminution in the activity of the smallest SEMNs and SFMNs. The medium SEMN and SFMN exciters continued to fire impulses for the remainder of the response.

The latencies for the initiation of SEMN and SFMN activities associated with the no-movement response exhibited considerable variability (Table 2), however in most instances activity began in the inhibitory SEMN and SFMN. More than half (57%) of the SFMN responses began with the discharge of the inhibitor while the small and medium sized SFMN exciters initiated 20 and 33 % respectively of the responses (15 responses from 3 animals).

DISCUSSION

Flexion of the M–C joint was shown to have an excitatory effect on each of the SEMNs and SFMNs. In many trials this motoneurone discharge did not produce any abdominal movement, apparently because it was accompanied by excitation of both peripheral inhibitors. When full extensions were produced, there was strong excitation of the extensor synergists (SEMN exciters and SFMN inhibitor) and suppression of the extensor antagonists (SEMN inhibitor and SFMN exciters).

The SFMN activities involved in the full extensions produced by M–C flexion resemble those initiated by other stimuli (Larimer & Eggleston, 1971; Williams & Larimer, 1981). The SEMN responses also appear to be similar to those recorded in response to a platform drop (Sokolove, 1974).

The neuronal patheways which mediate abdominal extension responses are not known. In highly dissected preparations postural abdominal extension can be initiated by stimulation of interneurones ('command fibres') present in the nerve cord connectives. When compared with the motor activity produced by command fibre stimulation (Evoy & Kennedy, 1967; Fields *et al.* 1967; Page, 1975*a*) motoneurone discharges during full extensions are characterized by shorter latencies and less complete reciprocity. Latencies for extensions evoked by command fibre stimulation in the circumoesophageal connectives range between 0·5 and 2 s (Page, 1975*a*; 1978) while responses to M/C flexion begin within 100 ms. These differences suggest that reflex extension is not produced by the discharge of a single command interneurone.

Recent analyses of interganglionic interneurones which mediate crayfish escape responses have emphasized the importance of parallel interneuronal pathways formed by both command and intersegmental neurones in the generation of complex motor responses (Kramer, Krasne & Wine, 1981). The motor effects produced by the simultaneous stimulation of more than one command fibre vary according to the identity of the individual command neurone. When two extension command axions are stimulated, their motor effects sum (Williams & Larimer, 1980). Similar experiments with extension and flexion command fibres have produced an initial discharge of the SEMNs and SFMNs which was followed by a rhythmic alteration of antagonistic motoneurone activity (Sokolove & Tatton, 1975). Therefore, initiation of a full

abdominal extension by M–C flexion may require the excitation of several extension command fibres as well as other parallel interganglionic interneurones. The lack of movement observed in some trials could reflect a shift in reflex pathways which reduces extension command fibre excitation while increasing the activation of flexion command and other parallel interganglionic interneurones (Kennedy, Evoy & Fields, 1966).

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