

EFFERENT REGULATION OF THE ABDOMINAL STRETCH RECEPTORS OF THE CRAYFISH

By O. B. ILYINSKY,* D. L. SPIVACHENKO† AND E. I. SHTIRBU†

* *Laboratory of General Physiology Reception, I.P. Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad,*

† *Laboratory of Neurophysiology, Institute of Zoology, Academy of Sciences of Moldavian SSR, Kishinev*

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SUMMARY

1. Efferent regulation of the activities of the slowly adapting and fast adapting stretch receptors (the MRO_1 and MRO_2 , respectively) of *Astacus fluviatilis* was studied.

2. It was shown that the receptors activity is under the efferent control of two central inhibitory neurones: the main inhibitory neurone (MIN) and the accessory neurone (AIN). Their activities were determined by the degree of discharge from the receptors.

3. There was close interaction between the MRO_1 and the MIN. The activity of the MIN was determined by the activity of the MRO_1 to a higher degree than vice versa; the activity of the MIN was influenced not only by the MRO_1 impulses, but by the cessation of those impulses as well (silent periods). The MRO_1 could inhibit or excite the MIN, whereas the MIN could inhibit the activity of the MRO_1 .

4. Interaction between the MRO_1 and the MIN could occur during background activity of the MRO_1 or of the MIN. In both cases the activity of the initially non-active neurone appeared only after stimulation had been applied to the MRO_1 .

5. Often each impulse of the MRO_1 resulted in a MIN discharge of from 1 to 361 impulses. Sometimes the responses appeared after burst-discharges, induced by mechanical stimulation, of the otherwise inactive MRO_1 .

6. During interaction between the MRO_1 and the MIN, the neurone commencing activity had a higher discharge frequency, if only by 1-2 imp./sec, than the one firing previously.

7. Under the influence of MIN impulses the response of the MRO_1 to stimulation changes from slowly adapting to fast-adapting.

8. The MIN has a slight inhibitory effect on the activity of the MRO_2 which is most clearly seen in the course of background activity of the MRO_2 . The MRO_2 , however, does not exert much influence on the activity of the MIN.

9. The AIN had much less effect than the MIN on receptors, and the receptors did not naturally evoke AIN impulses. There is, therefore, apparently a more complicated connexion between the receptors and the AIN than between the receptors and the MIN.

INTRODUCTION

The properties of mechanoreceptors have been the subject of many recent reviews (Kuffler, 1960; Matthews, 1964, 1972; Ilyinsky, 1967, 1972; Catton, 1970; Granit, 1970; Flock, 1971; Loewenstein, 1971; Ottoson & Shepherd, 1971). The stretch receptors of the crayfish have been one of the main objects of investigation, because they are very convenient for the study, not only of the primary processes of reception, but also of the efferent regulation of their activity. In spite of growing interest in the latter phenomenon (Eckert, 1961*a, b*; Fields, 1966; Fields, Evoy & Kennedy, 1967; Jansen, Njå & Walløe, 1970*a, b*; Jansen, Njå, Ormstad, Walløe & 1971*a, b, c*) many aspects of the problem are unresolved. The present study describes some features of efferent regulation in the activity of stretch receptors.

MATERIALS AND METHODS

Experiments were performed on the freshwater crayfish (*Astacus fluviatilis*), either upon the intact nervous system or upon the abdominal nerve chain, after the connexion to the thorax had been severed. The forelegs were removed, and the abdomen was slightly curved by a special clamp and fastened to a stand. The abdomen was then dissected from the dorsal side to expose the receptor muscles and the dorsal nerve. The crayfish was immersed in van Harrevald's solution (van Harrevald, 1936) of the following content: NaCl, 12; KCl, 0.4; CaCl₂, 1.5; MgCl₂, 0.25 g/l. The solution was buffered to pH 7.2–7.4 with 0.01 M tris-buffer and saturated with oxygen before use. The temperature was 16–18 °C. Experiments typically lasted 3–4 h.

Receptor muscles of the 2nd–4th abdominal segments were held in a pair of forceps and connected to the output of a stimulator. The stimulator was as described by Spivachenko & Maximchuk (1968), and allowed the application of stimuli of various amplitudes, shapes and frequencies. Potentials of the intact dorsal nerve branch, connected to receptors and ganglion, were recorded with a thin platinum electrode: the grounded electrode was put in the bath containing the crayfish. The nerve was lifted from the solution into liquid paraffin for recording activity. Amplification was by means of a capacity-coupled preamplifier (UBP-02) and nerve impulses were displayed upon an oscilloscope, where they were photographed.

RESULTS

Identification of nerve response. Various morphological and electrophysiological investigations (Alexandrowicz, 1951, 1967; Burgen, Kuffler, 1957; Eckert, 1961*a*; Jansen *et al.* 1971*a*) have shown that the nerve-branch connected to the abdominal stretch receptors consists of (1) two axons of slowly adapting and fast adapting stretch-receptors (called the MRO₁ and the MRO₂ respectively), (2) three efferent fibres of inhibitory neurons (thick, thin and very thin), (3) motor fibres of receptor muscles, and (4) nerve fibres of surrounding dorsal muscles.

In the activity of an intact nerve the following impulses were observed (see Figs. 1 and 2):

(1) Impulses of high amplitude, which quickly disappeared in the course of a long lasting stretch of moderate strength, and were absent during the background activity

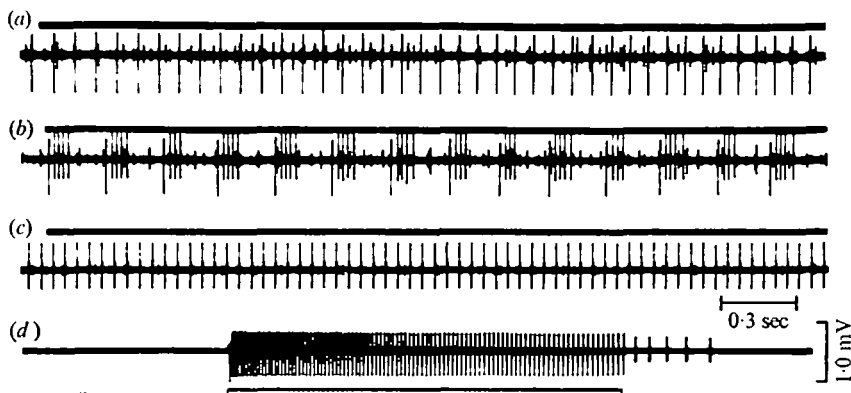


Fig. 1. Different patterns of activity of the MRO_1 and the MIN. Low-amplitude impulses in (a-c) are due to the activity of nerve fibres connected with dorsal muscles. (a) Rhythmic (16 imp./sec) spontaneous activity of the MRO_1 . (b) Low-frequency (4 imp./sec) spontaneous activity of the MRO_1 and accompanying burst-impulses of the MIN. (c) High-frequency (19 imp./sec) spontaneous activity. (d) A burst-discharge of the MRO_1 followed by responses of the MIN. The MRO_1 discharge was in response to \square -like mechanical stimuli (interval indicated on bottom trace) applied to the receptor.

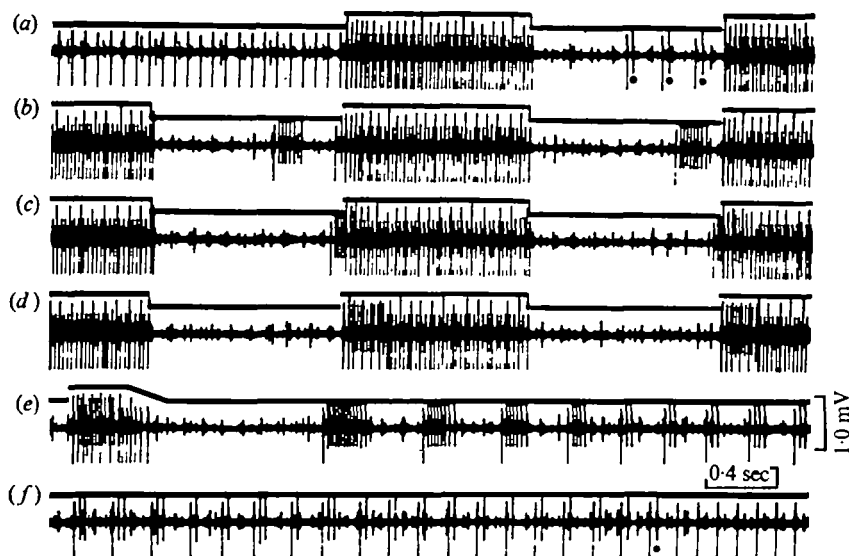


Fig. 2. Interaction between response of the MIN impulse of the MRO_1 and the MRO_2 in the course of background activity of the MRO_1 . Top traces indicate the period of the mechanical stimulus. (a-e) Successive records of impulses of the MRO_1 and the MRO_2 in response to rhythmic stimulation (1 Hz), applied to receptor neurones. (e, f) The recovering activity of the MRO_1 after the end of rhythmic stimulation. MRO_2 spikes are observed only in the course of stimulation. Impulses of the MIN, marked by dots (a), occur only after the 'silent period' in the activity of the MRO_1 (a-f). The moment of cessation of the MIN activity is marked by a dot (f).

of the nerve. These impulses appeared also in response to selective stimulation of the MRO_2 and so this activity may be considered to be due to this sensory neurone.

(2) Impulses of an amplitude 1.5-2 times smaller than type 1, often observed as spontaneous background activity. This neurone slowly adapted during stimulation.

The impulses were observed during the selective stimulation of the MRO_1 and are apparently a result of the activity of this sensory neurone.

(3) Impulses of a nearly equal or a bit lower amplitude than type 2, but with spikes of inverse phase. These impulses (and also the impulses of types 4 and 5) disappeared after the dorsal nerve had been cut and receptors were separated from the centre, which shows their efferent nature. The responses seemed to appear in the thick efferent fibre: the central neurone can be termed the main inhibitory neurone (the MIN). It is interesting to note that under certain conditions the MIN responded by continuously rhythmical impulses, which could fully inhibit the activity of the MRO_1 .

(4) Quite irregular impulses of a low amplitude, which sometimes hardly rose above noise-level, in response to discharges from receptors. These impulses were not so regular as the MIN impulses and showed an essentially lower inhibitory effect on receptors; so they may be considered to result from the activity of the thin efferent fibre. The central unit of this fibre may be termed the accessory inhibitory neurone (the AIN).

(5) Low-amplitude impulses, which usually did not show any noticeable effect on the activity of either the receptor or the central neurone; these impulses may be considered to result from the activity of nerve fibres connected with surrounding dorsal muscles.

Our identification of the dorsal nerve impulses is in accord with the data of other authors (Burgin & Kuffler, 1957; Eckert, 1961*a*; Fields *et al.* 1967; Jansen *et al.* 1970*a*). However, we could not show which of the two thin fibres were active under the applied stimulation. Perhaps we recorded impulses of the thin fibre which has the greater effect on receptors (Jansen *et al.* 1971*a*).

Interactions between activities of the MRO_1 and the MIN. It has been shown that a close interaction exists between central and receptor units. This interaction can be determined by functional features of the neurones. The closest interaction was observed between the MRO_1 and the MIN. There are two main possibilities of interaction between the MRO_1 and the MIN: (1) during the background resting activity of the MRO_1 (when there was no activity in the MIN), and (2) in the course of the resting rhythmical activity of the MIN (when the activity of the MRO_1 was absent) (Fig. 1*a, c*).

There exist two more cases of interaction between the MRO_1 and the MIN. First, responses of the MIN to each spontaneous impulse of the MRO_1 could be regularly observed; the duration of the responses could vary over a wide range (Fig. 1*b*). The changing activity of both neurones could be easily explained by the two main types of interaction between the MRO_1 and the MIN proposed above. Secondly, an inactive neurone commenced activity only when the receptors were stimulated: impulses of the MIN were sometimes observed after the inactive MRO_1 responded to stimulation of the receptors (Fig. 1*d*).

Figs. 2 and 3 represent the typical appearance of MIN responses during considerable background activity of the MRO_1 . Impulses of the MIN were initially absent. This condition could last for a long time. When the stimulation was applied to the receptors the activity of the MRO_1 increased and impulses of the MRO_2 appeared. When the stimulation was off, there was a 'silent period' in the activity of the MRO_1 , as a result of post-tetanic hyperpolarization of the receptors and also of the appearance of the hyperpolarization phase of the receptor potential (Eyzaguirre & Kuffler, 1955; Florey,

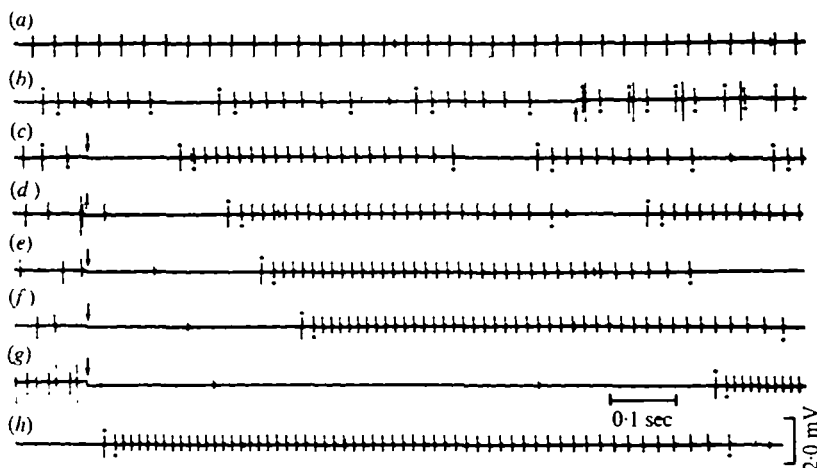


Fig. 3. Increasing discharge duration of the MIN in the course of rhythmical stimulation of receptors. \square -like stimulus is indicated by a small deflexion of the trace: an arrow from below indicates the start, and vice versa. The beginning and the end of a burst-discharge of the MIN are marked by dots from below; spontaneous impulses of the MRO_1 are marked by dots from above.

(a) Spontaneous rhythmical activity of the MRO_1 . (b) Burst-discharges of the MIN appear in response to rhythmical stimulation of receptors. Between the MRO_1 and the MIN a rhythm of 1:1 is established; MRO_1 spikes (high amplitude) appear but do not exert influence on the activity of the MIN. (c-f) Increased duration of the silent period after rhythmical stimuli of equal amplitudes were applied to the MRO_1 , and the increased duration of the MIN burst-discharges in response to the very first impulse of the MRO_1 . (g) Stimulation amplitude is 3 times as much as in (b-f); only the beginning of the MIN response is shown (to the right). The whole of the MIN response is shown (h). Low-amplitude responses are due to the activity of nerve fibres connected to dorsal muscles.

1956; Nakajima & Onodera, 1969). The duration of the 'silent period' depends, *ceteris paribus*, on the initial receptor excitability, which can be evaluated from the impulse frequency of the MRO_1 . If the frequency of the background receptor activity increases, the 'silent period' duration shortens and vice versa. Recovery of the MRO_1 activity occurred after the 'silent period' was over and if the pause lasted for a sufficiently long time there appeared some responses of the MIN to arising impulses of the MRO_1 . If the stimulation was moderate, there appeared only one afferent impulse to each efferent signal (Fig. 2a). This 1:1 rhythm remained until the frequency of the afferent signals gradually increased and, at a certain moment, efferent impulses disappeared. Upon cessation of the MIN activity, the discharge frequency of the MRO_1 significantly increased (Fig. 2f). Under intensified stimulation or when the excitability of the MRO_1 lowered, for instance as a result of the MIN's activity, the duration of the silent period increased and each impulse of the MRO_1 evoked the appearance of several spikes of the MIN: rhythms of 1:2, 1:3, 1:4, etc., could be observed (Fig. 3b). Such a rhythm was not constant; when the impulse frequency of the MRO_1 became higher and approached its initial value, the burst-discharge duration of the MIN shortened and a full cessation of the efferent regulation was observed.

The duration of the silent period could also increase during rhythmical stimulation of the receptor. In our experiments we used the frequency of 1 imp/sec, which was not higher than the frequency of tail movements of a swimming crayfish. Under the applied stimulation the progressive prolongation of silent periods was accompanied by

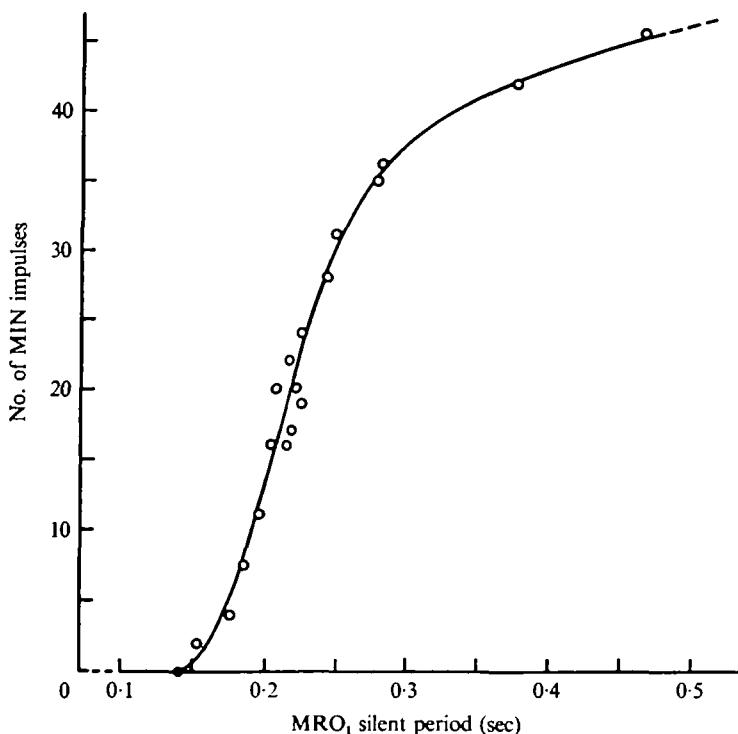


Fig. 4. Relationship between the duration of the MRO₁ silent period after stimulation and the number of impulses in the following MIN discharge.

an increase in the number of MIN impulses in response to the very first spike of the MRO₁ (Figs. 3c-g, 4).

If the silent period lasted for a relatively long time, the following stimulus superimposed on an efferent burst-discharge which appeared after the previous stimulation. Moreover, the MIN responses fully depressed the MRO₁ activity and lowered the MRO₂ impulse frequency (Fig. 2c). When the silent period duration was equal to the interval between stimuli, the activity remained constant: the very first response of the MRO₁ to the stimulus resulted in the appearance of the MIN activity. In its turn the central neurone produced spikes, which inhibited the activity of the MRO₁. If the stimulus duration was relatively long, the activity of the MRO₁ appeared again after the end of the MIN discharge; the silent period could be observed, and then the whole process began again (Fig. 2d). When the rhythmical stimulation was over, there appeared the above-mentioned burst-discharge conditions of the MIN, but in each of the new burst-discharges the number of impulses decreased and the rhythm was at first 1:2, then 1:1 and at last the activity of the MIN was blocked completely (Fig. 2e, f).

The changing MIN responses appeared quite naturally in the course of recovering activity of the MRO₁. Fig. 5 (values obtained from data presented in Fig. 2) displays indicators of the excitability of the MIN. The highest number of impulses and the lowest latency of the MIN were observed at the beginning of the stimulation, when intervals between discharges of the MRO₁ were the longest. Then, while the inter-

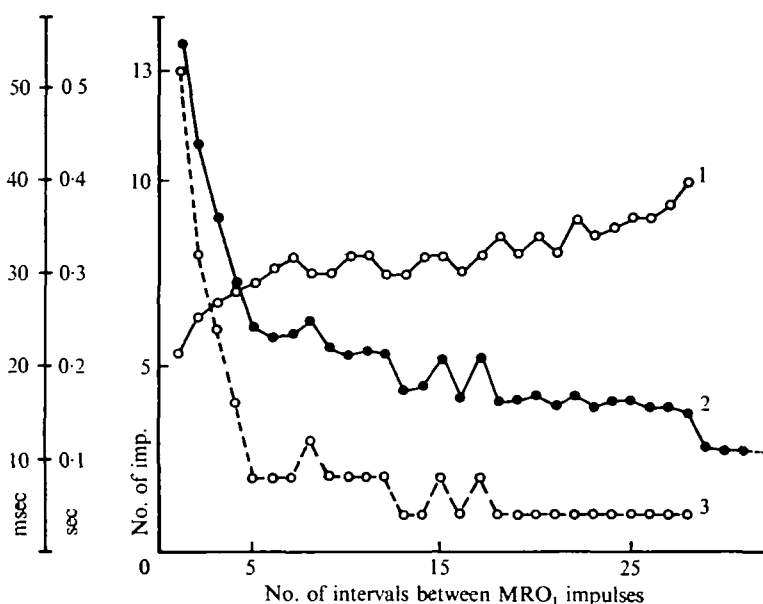


Fig. 5. Changes in nerve activity after the receptor was stimulated. (1) Latency (msec) of burst-discharges of the MIN. (2) Duration (sec) of intervals between impulses of the MRO₁. (3) No. of impulses in bursts of the MIN.

spike duration of the MRO₁ shortened, the number of impulses in the MIN response decreased and the latency increased. This condition lasted until the MIN responses vanished completely. It is noticeable that even a slight change in interval between MRO₁ spikes produced a corresponding change in the number of impulses in the MIN discharge.

After-effects of the MIN discharges. MIN impulses instantaneously depress the activity of the MRO₁. An inhibitory after-effect was also produced, but it was feebly marked and appeared only as a gradually changing excitability of the MRO₁ under the influence of a long-lasting discharge of the MIN. This was most clearly seen with the continuously high rhythmic activity of the MRO₁ during the recovery of excitability of the sensory neurone, after the end of the MIN discharge.

At the beginning of the experiment (Figs. 6, 7) responses of the MIN appeared only after the silent period was over. The duration of the silent period gradually increased in the course of rhythmical stimulation. The response of the MIN to the very first impulse of the MRO₁ increased simultaneously. However, the interval between the end of the MIN discharge and the appearance of the following impulse of the MRO₁ remained unchanged (82–86 msec) in all cases (Figs. 6a, 7A). It is necessary to note that this interval was considerably longer than the interval between impulses (45 msec) of the spontaneously active MRO₁ before the stimulation was applied to it. This difference in the duration of the interval is due to the change in excitability of the MRO₁, which did not recover completely after the silent period and so the rhythm of the receptor activity was very slow. But when the receptor activity approximated to its initial level, intervals between inhibitory impulses and impulses of the MRO₁ were nearly equal to intervals between impulses of the MRO₁.

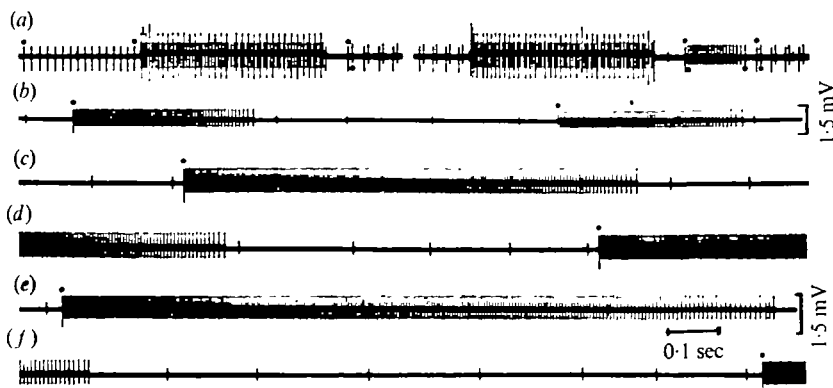


Fig. 6. Influence of MIN impulses on MRO_1 excitability. The beginning (a), the middle (b-d) and the end (e-f) of an experiment that lasted for 2 h. The trace is deflected to indicate stimulation. Low-amplitude impulses are due to the activity of nerve fibres connected with dorsal muscles. In (c-f) amplification was increased.

(a, left) The beginning of rhythmical stimulation of receptors. Background activity is due to the MRO_1 . Upon stimulation MRO_2 impulses appeared. Some MRO_2 impulses are blocked by impulses of the AIN. Each MRO_1 impulse (dots above) evokes one MIN impulse (dot below) after stimulation has been removed. (a, right) The first MRO_1 impulse evokes a burst-discharge of the MIN after the stimulus (the 12th). The rhythm between the MRO_1 and the MIN then reverts to 1:1.

The interval between the end of the first discharge of the MIN and the appearance of the second impulse of the MRO_1 does not depend on the number of impulses in the MIN response. (b, c, e) Powerful burst-discharges of the MIN. (b, d, f) Corresponding intervals between discharges of the MIN. In (d+f) only the end of the previous, and the beginning of the following, discharges are shown.

The inhibitory impulses of the MIN thus interrupted natural changes in the excitability of the MRO_1 , but had no direct after-effect upon the activity of the sensory neurone. So, when the discharge of the MIN ceased, the activity of the MRO_1 remained the same as at the beginning of the inhibitory burst-discharge.

In the middle of this experiment (Fig. 6b-d) and at the end of it (Fig. 6e-f) the excitability of the MRO_1 gradually decreased and the frequency of spontaneously following discharges of the MIN considerably decreased. As the impulse frequency of the MRO_1 decreased, the discharge duration of the MIN increased. Responses appeared to each afferent impulse, but there was no close relationship between the discharge rate of the MIN and the time of appearance of the following impulse of the MRO_1 (Fig. 7B-D). For instance, after MIN discharges with quite different durations of 4800 and 6400 msec, the succeeding impulses of the MRO_1 were at similar intervals to each other, being 4400 and 4080 msec, respectively. On the other hand, the decreasing impulse frequency of the MRO_1 (i.e. the decreasing excitability of the receptor) and the increasing discharge duration of the MIN bore a closer relationship. Thus, in the middle of the experiment, when the discharge duration of the MIN was about 3000 msec, the average duration of the 'resting period' (i.e. the interval between the end of the MIN discharge and succeeding impulse of the MRO_1) was about 2600 msec. At the end of the experiment the average discharge duration of the MIN was about 5300 msec, and the resting period lasted about 4300 msec (Fig. 6f). Therefore, although there is no direct connexion between the discharge duration of the MIN and the successive changing of the impulse frequency of the MRO_1 , there is a connexion between changing excitabilities of the MIN and MRO_1 .

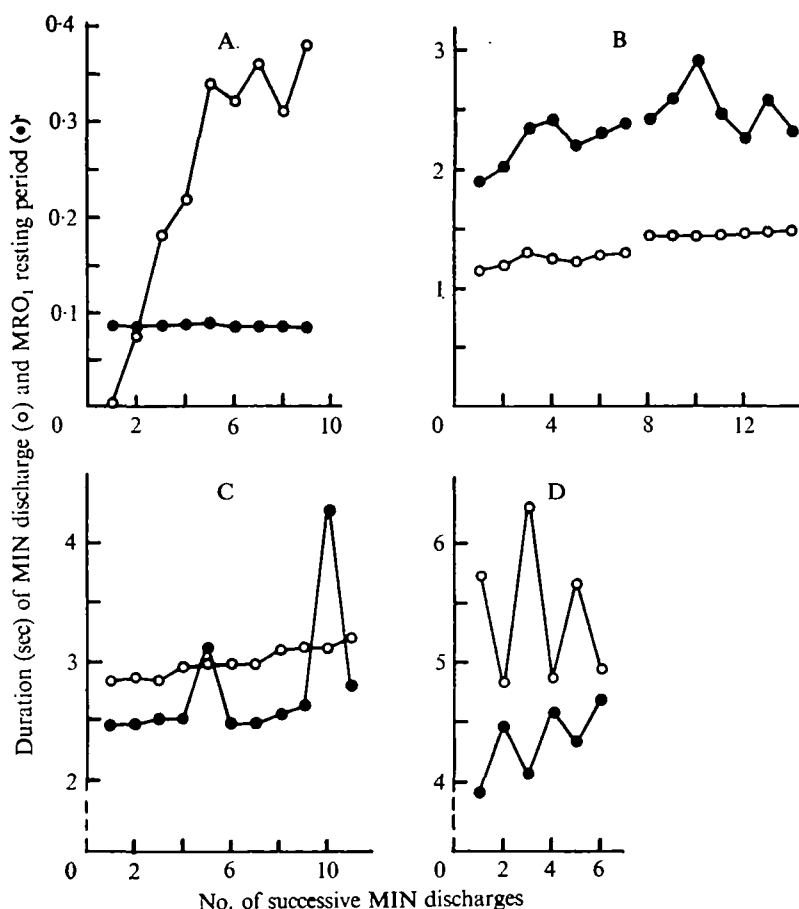


Fig. 7. Duration of MIN discharge and of the following 'resting period' in MRO_1 activity. Data was obtained from the experiment shown in Fig. 6. Discontinuity in (B) is due to discontinuity in recording. (A) Start of application of stimuli at 1 Hz to the spontaneously active MRO_1 . MIN responses appeared after the silent period in MRO_1 activity (see Fig. 6a). (B-D) Different periods in the activity of neurones. The MIN responded by burst-discharges of increasing duration (see Fig. 6b-f).

Interaction between the MRO_2 and the MIN. Unlike the MRO_1 , the MRO_2 had no noticeable effect upon the MIN. When MRO_2 impulses appeared during MIN activity (Figs. 2, 3b, 8), the rhythm of the activity remained unchanged. The MIN, however, had some slight inhibitory effect on the MRO_2 . This effect was most clearly observed in the course of long-lasting activity of the MRO_2 and during continuous discharge of the MIN. It can be clearly seen in Fig. 2(b-e) and in Fig. 8; the latter shows that the rhythmical background activity of the MRO_2 is gradually inhibited by successive burst-discharges of the MIN (the initial high-frequency part of the discharges produced the most effect).

Excitation of the MIN after the cessation of activity of the MRO_1 during its non-active state. Sometimes, discharges of the MIN appeared after stimuli had been applied to the MRO_1 during its non-active state (Fig. 1d). In the course of repeated rhythmical

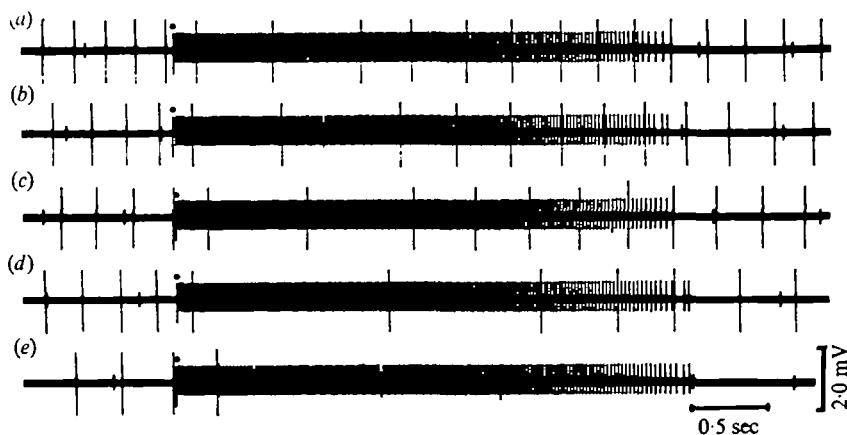


Fig. 8. Influence of successive long-lasting MIN discharges upon MRO_2 activity. Each response of the MRO_1 (dots above) evokes a long-lasting high-frequency discharge of the MIN. (a-e) Gradually increasing inhibition of the spontaneous rhythmic activity of the MRO_2 (largest spikes) by the MIN burst-discharges.

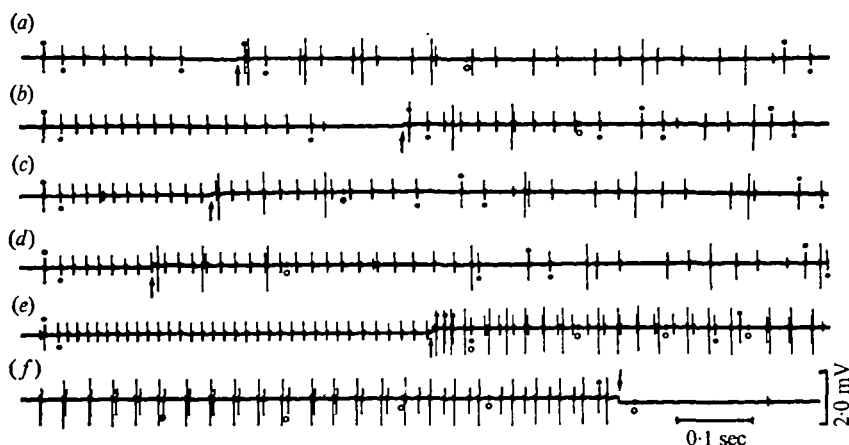


Fig. 9. Excitation of receptors in the course of rhythmic discharges of the MIN.

(a-e) MRO_1 spikes (dots above) evoke burst or single discharges of the MIN. Dots below indicate the first and the last impulses of burst-discharges of the MIN. During receptor stimulation the dots mark only single spikes. MRO_2 spikes have the highest amplitude, AIN spikes (open circles) the lowest. Arrow from below indicates start of stimulation, and vice versa.

(a-d) Responses of the MRO_1 and the MRO_2 to the beginning of \square -like mechanical stimuli of equal amplitudes. (e-f) The beginning (e) and the end (f) of a stimulus with an amplitude 3 times greater than in (a-d).

stimulation of the receptor the discharge value of the MIN progressively increased and latency decreased.

Excitation of the MRO_1 during resting burst-discharge activity of the MIN. Sometimes, relatively constant burst-discharge conditions of the efferent neurone were observed; at each impulse from the receptor the MIN responded with about 3 or 4 impulses (Fig. 1 b). In some experiments bursts were much greater and could assume enormous proportions (Figs. 6, 8). The discharge frequency decreased exponentially. In one case we observed a discharge with a duration of 6.40 sec, which consisted of 361 impulses

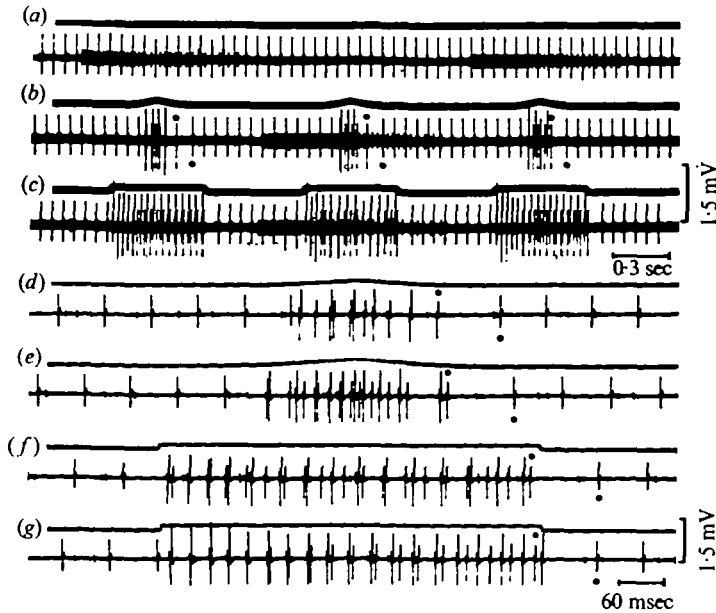


Fig. 10. Some features of receptor activity in the course of background activity of the MIN.

In (b-g), the largest impulses are MRO_1 spikes. In (b, d-g), last impulses of the MRO_1 in response to stimuli are marked by dots from above. First impulses of the MIN after the end of stimulation are marked by dots from below. Top trace shows mechanical stimuli of various types.

(a) Background MIN activity and spontaneous burst-discharges in nerve fibres connected with dorsal muscles. (b, c) The response of receptor neurones to repeated stimuli of various shapes. The activity of the MIN is immediately inhibited upon the discharges of receptor neurones. In (b, c, g) at the beginning of stimulation the increased amplitude of impulses is due to the compounding of impulses of both receptors.

And all this enormous efferent discharge appeared in response to only one afferent impulse!

Receptor stimulation during different periods of MIN activity revealed some features of the relationship between central and peripheral units (Figs. 9-11). In Fig. 9(a) can be seen the effect of applying stimulation immediately prior to the regularly appearing burst-discharges of the MIN. It is clear that if the first impulse of the MRO_1 appeared 'suddenly' to the MIN it evoked only a single spike and not the usual burst-discharge of the efferent neurone. Following impulses of the MRO_1 also evoked the appearance of only one spike of the MIN. After the period of stimulation, the first impulse of the MRO_1 evoked a MIN discharge that lasted longer than the discharge before stimulation was applied to the receptor. Subsequent stimuli, which caused more and more prolonged silent periods, resulted in the appearance of more and more powerful MIN discharges. If the stimulation was applied to receptors immediately prior to the appearance of the regular MRO_1 impulse, the first spike of the MRO_1 evoked the appearance of MIN burst-impulses (Fig. 9b). During MIN activity, MRO_1 responses were inhibited. After a MIN burst-discharge, the MRO_1 responses appeared again, and each impulse of the MRO_1 produced only one MIN spike. So, if the moderate stimulation was applied, not in the interval between burst-discharges, but during the discharge, the MRO_1 did not respond until the cessation of the MIN responses

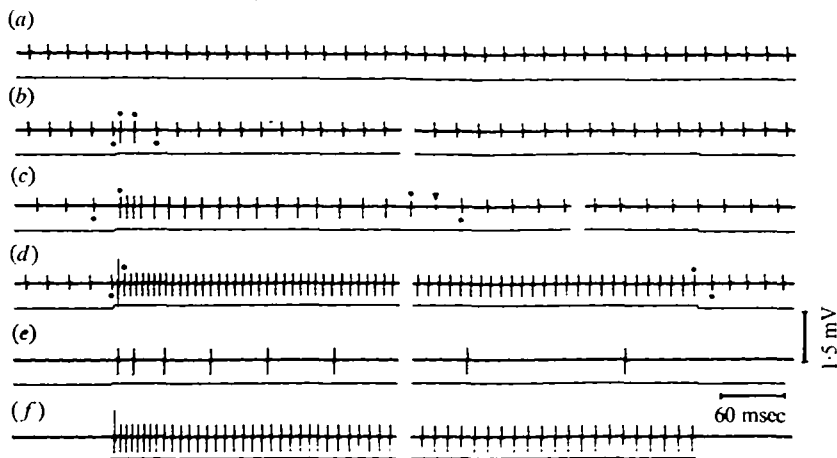


Fig. 11. Responses of the MRO_1 to stimulation in the course of background activity of the MIN. The bottom trace indicates stimulation. In (b-d) the first impulse of the MIN (before stimulation is applied to receptors), and the last (after the cessation of the MRO_1 activity), are marked by dots from below; the first and the last impulses of the MRO_1 in response to stimulation are marked by dots from above. The change between MRO_1 and MIN impulses is marked by an arrow in (c). In (d, f) MRO_1 spikes are the largest.

(a) Background activity of the MIN. (b-d) Responses of receptors to stimulation in the course of rhythmical background activity of the MIN; stimulation amplitude was increased from (b) to (d). (e-f) Impulses of receptors after efferent fibres had been cut. Stimulation amplitude is the same in (e) as in (b), and in (f) as in (d).

(Fig. 9c, d). When the MIN activity was over, the MRO_1 began to respond; each response produced one MIN impulse.

The burst-discharge of the MIN could, however, be interrupted by MRO_1 impulses if a relatively high level of stimulation was applied to the receptor. Fig. 9(e) shows the beginning of such stimulation: three impulses of the MRO_1 appeared simultaneously with the MIN discharge. MIN responses then ceased briefly and reappeared after the MRO_1 activity.

In Fig. 9 it can be clearly seen that the MRO_2 impulses, which appeared as a result of the receptor stimulation, did not influence the activity of the MIN and were not influenced by this neurone. Single impulses of the MRO_2 were, however, blocked when the accessory inhibitory neurone (the AIN) was excited.

Excitation of the MRO_1 at the background of rhythmical impulses of the MIN. In order to determine how MRO_1 impulses could inhibit the MIN, stimulation was applied to the MRO_1 during the background activity of the MIN (Figs. 10, 11). The appearance of the MRO_1 responses, and accordingly the cessation of MIN activity, took place only when the discharge frequency of the MRO_1 exceeded the impulse frequency of the MIN (if only by 1-2 imp./sec). The duration of the MRO_1 response also depended on the initial impulse frequency of the MIN and the degree of stimulation (Fig. 11). Repeated bursts of rhythmical (1 Hz) stimulation decreased the duration of the response (Fig. 12A): the response of the MRO_1 turned from the tonic to the phasic. The response could be converted back to the tonic by lowering the stimulation frequency and increasing the stimulus intensity. Naturally, the tonic response of the MRO_1 remained constant if the efferent regulation of the MIN was eliminated (for example, by cutting the central part of the dorsal nerve; Fig. 11e, f). It is important to note that

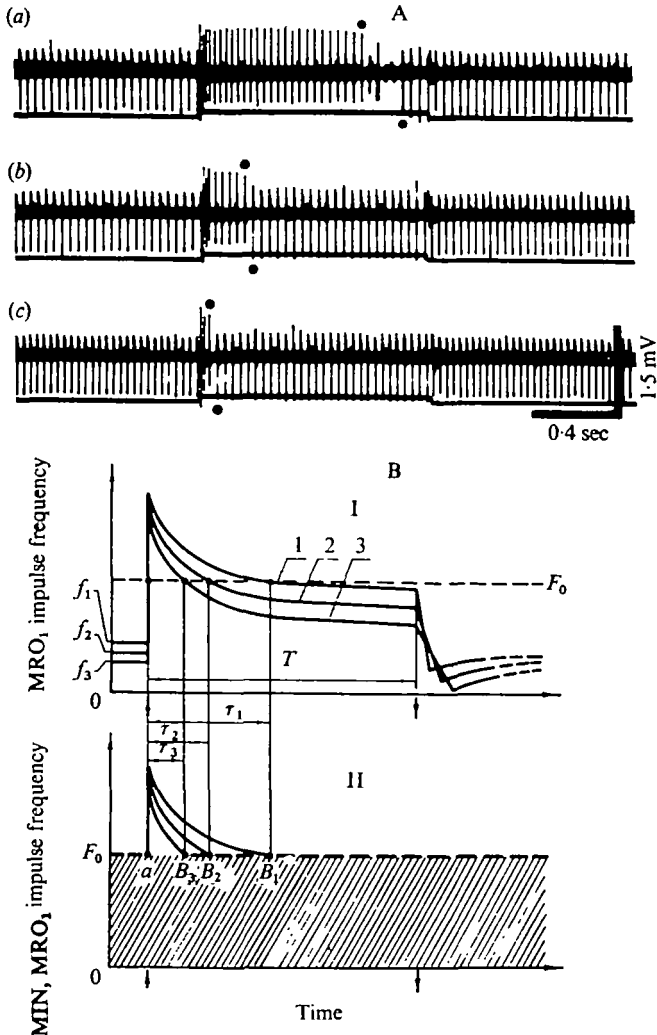


Fig. 12. Adaptation process of the MRO₁.

(A) Changing responses of the MRO₁ to mechanical stimulation in the course of background activity of the MIN. The bottom trace indicates mechanical stimulation. The top trace displays nerve activity and is of inverted polarity to that in Figs. 1-11. The last response of the MRO₁ to stimulation is marked by a dot from above, the first impulse of the MIN after the end of MRO₁ discharges is marked by a dot from below. (a, b, c) Responses of the MRO₁ to rhythmical stimulation (1 Hz).

(B) Adaptation of the MRO₁ without (I) and during (II) background activity of the MIN. The beginning and the end of a stimulus with duration T are marked by arrows. 1, 2, 3, Changing discharge frequency curves of the MRO₁ in response to the first, the second and the third stimuli. MRO₁ predominates over MIN at a . Dominance is reversed in successive stimulations at B_1 , B_2 , B_3 (time of adaptation, τ_1 , τ_2 , τ_3 , respectively).

the shortening of the MRO₁ response was accompanied by a decreased discharge frequency, and in all cases the cessation of the MRO₁ response occurred only after the impulse frequency of the MRO₁ had become lower than the impulse frequency of the MIN. In comparison, the rhythmical activity of the MIN (when stimulation was applied to the MRO₁) was interrupted only when the discharge frequency of the MRO₁ became higher than that of the MIN.

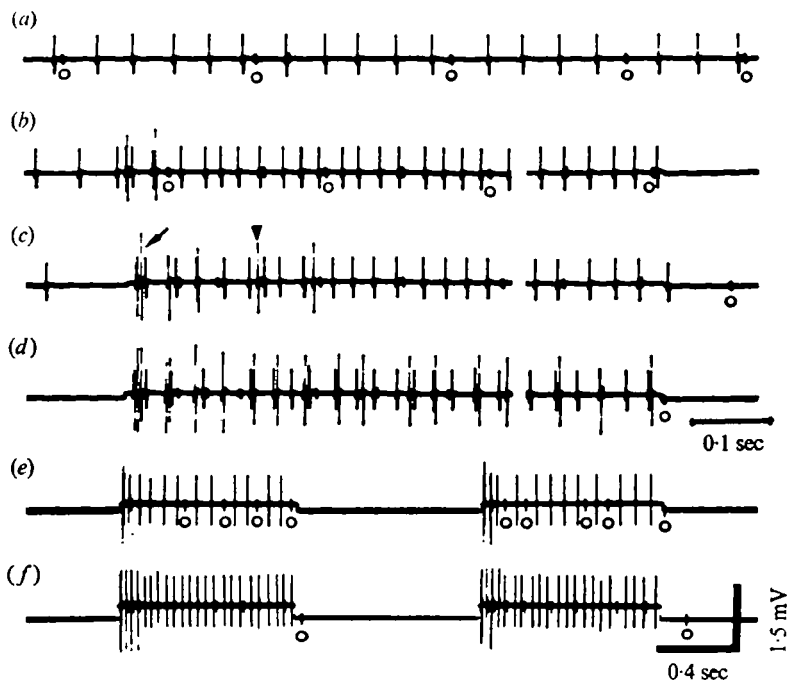


Fig. 13. Influence of the AIN on activities of the MRO_1 and the MRO_2 . Stimulation is indicated by a deflexion of the trace, which is of inverted polarity to that in Figs. 1-11. Spontaneous activity of the MRO_1 was inhibited in the course of rhythmical stimulation in (d-f). Some impulses of the AIN are marked by open circles. High-amplitude impulses are MRO_2 spikes: one is marked by the wide arrow on (c). Simultaneous occurrences of impulses is marked by oblique arrow.

(a) Alteration of the spontaneous activity of the MRO_1 by impulses of the AIN. (b-d) Responses of the MRO_1 and the MRO_2 to successive \square -like stimuli of increasing amplitudes. Only the beginning and the end of stimulation are shown. (e-f) The same as in (b-d) but with a compressed time-scale. Stimulation amplitude was increased in (f).

The disappearance of MRO_1 responses during stimulation (Figs. 11, 12A) could occur in two ways. First, when impulses of the MRO_1 disappeared immediately, and impulses of the MIN appeared in replacement (Figs. 11b, 12A, B, C). Secondly, during a transitional period when both neurones either did not respond at all, or there appeared incomplete spikes (Figs. 11c, 12A, a). The appearance of incomplete spikes and the disappearance of impulses from both neurones is apparently due to the fact that the MRO_1 and the MIN had the same discharge frequency: when impulses from the two neurones were recorded simultaneously the result was (by virtue of the similar size but converse phase of impulses) an illusion of the absence of responses.

Spontaneous switching from MRO_1 activity to MIN activity and vice versa was sometimes observed. Cessation of the rhythmical activity of one neurone occurred suddenly, and it was immediately replaced by activity of the other, at a somewhat higher frequency. Impulse frequencies of the MRO_1 and the MIN were so close to one another during long periods of time that this alteration gave the appearance of a reciprocal 'tuning' of both neurones.

Influence of impulses of the AIN on receptor activity. The activity of stretch receptors changed not only under the influence of MIN impulses, but also under the influen

of the accessory inhibitory neurone (the AIN). The AIN responses were observed, as a rule, during application of the stimulus to receptors, i.e. during the increased activity of the MRO_1 and the MRO_2 (Figs. 6*a*, 9, 13). They were also sometimes observed shortly after the end of the stimulus (Figs. 9*f*, 13*c, f*). Finally, impulses of the AIN could be traced in the course of spontaneous MRO_1 activity (Fig. 13*a*).

Each impulse of the AIN appeared quite irregularly and showed roughly equal inhibitory effects on the activity of the MRO_1 and the MRO_2 . Whether single impulses were fully blocked or delayed was determined by the difference in time between the appearance of impulse of the AIN and the receptors (Figs. 9, 13).

Occurrence of the AIN impulses basically depended on MRO_1 activity. However, in contrast to the MIN, the reflex responses of the AIN were not so exactly timed in relation to the MRO_1 impulses. Therefore, the AIN impulses were recorded either preceding, following or even coinciding with the MRO_1 and the MRO_2 responses. Though the discharge frequency of the AIN increased with increasing activity of receptors, its inhibitory effect was lowered. This was offset by the increasing excitability of receptors; the possibility of coincidence in time of impulses of the AIN and the receptors, which resulted in the highest inhibitory effect, was lowered (compare *b, c*, and *d, e* and *f* on Fig. 13).

In spite of the, on the whole, irregular appearance of the AIN impulses, some regularity in the reflex activity of this neurone could be marked. Thus, impulses appeared in response to 3–5 impulses of receptors (the MRO_2 , Fig. 9; or the MRO_1 , Fig. 13*a–c*). Also a peculiarity in the activity of the AIN was the frequent occurrence of a single impulse when the stimulus applied to the receptors was switched off. Sometimes this impulse appeared with a delay of as much as about 50 msec (Figs. 6*a, 9f*, 13*c, f*).

Activity of nerve fibres connected with dorsal muscles. In practically all experiments there occurred discharges in the activity of the dorsal nerve which did not influence the activity of central and peripheral neurones. It could be supposed that these discharges were the result of the activity of nerve fibres connected to dorsal muscles. Nevertheless in rare cases when the activity was of a burst-like type it had some inhibitory effect on responses of the MRO_2 (Fig. 10*b, c*). Fig. 10 shows the coincidence of MRO_2 impulses with the low-amplitude discharge, which resulted either in blocking of the MRO_2 impulse (*b*) or in lowering of the frequency of the receptor (*c*). Responses of the MIN and MRO_1 during the discharge did not change at all (*a–c*). The nature of this influence upon the activity of the MRO_2 remains obscure.

DISCUSSION

It was shown that the activity of the crayfish abdominal stretch receptors is under the effective control of central neurones. These neurones show only a slight effect on the activity of the MRO_2 and are practically not influenced by this receptor. There is, however, a close reflex interaction between the MRO_1 and the AIN. A relationship, to a lesser degree, was found between the MRO_1 and the AIN. The MIN showed a greater effect than the AIN on the activity of receptors, which is in accord with the observations by Jansen (1971*b*).

Naturally, the MIN activity depends not only on the activity of ipsilateral stretch

receptors, but also on the other sensory inputs, for instance, the contralateral stretch receptors as shown by Jansen *et al.* (1970b). These authors demonstrated the close interaction between the MIN and the MRO_1 , whereas the AIN activity depends apparently on the other contralateral inputs. Results obtained in the present study accord with these data.

In the interaction between MRO_1 and the MIN, the MIN showed only an inhibitory effect, while the MRO_1 exerted influence in two ways. MRO_1 impulses could either excite the MIN or decrease, even to vanishing point, the activity of the MIN. Full inhibition of the MIN occurred if the impulse frequency of the MRO_1 was rather high; it reappeared only after the pause in the activity of the MRO_1 (the silent period). It is yet to be resolved if the origin of the reflex arc between the MIN and the MRO_1 is monosynaptic (Jansen *et al.* 1970b) or polysynaptic (Eckert, 1961a). Therefore it is unknown if the dual influence of the MRO_1 on the MIN is direct, or indirect with the help of interneurones. The high latency (14–41 msec) and the ability of the MIN to respond to a long-lasting discharge to only one afferent impulse suggests that there is polysynaptic connexion between the MRO_1 and the MIN. On the other hand some experiments by Wachtel & Kandel (1967) demonstrated a monosynaptic reflex arc with a long synaptic delay in invertebrates, and it is difficult to deny the possibility of there being, in crayfish ganglia, efferent neurones possessing monosynaptic afferent connexions which respond to a long-lasting rhythmical discharge. It should also be taken into account that a single neurone of the invertebrate central nervous system can have either excitatory or inhibitory effect, depending only on the stimulus frequency (Wachtel & Kandel, 1967). In addition, the efficient interaction of the neurones observed in our experiments is an argument in favour of the presence of monosynaptic connections. Finally, morphological data (Czvilineva, 1970) shows that the crayfish ganglion connected with abdominal stretch receptors, as a rule contains a monosynaptic reflex arc without interneurones.

Even if some features in the interaction between the MRO_1 and the MIN are still obscure, more is known about the switching between activity of these neurones. A neurone only commenced activity during, or instead of, the activity of another having a higher impulse frequency.

The role of impulse frequency in the interaction between the MRO_1 and the MIN can be understood in the light of the adaptation process (Fig. 12B). As soon as the first stimulus of a rhythmical stimulation was applied to the receptor (Fig. 12B, a), the impulse frequency of the MRO_1 quickly increased (curve 1, the initial frequency f_1) and afterwards gradually decreased. When the stimulus ceased it became even lower, and then gradually grew to its initial value. Further stimuli evoked practically the same response, but if the interval between stimuli was made shorter than the period required for full recovery of activity of the receptor, then each new stimulus was applied before discharges of the MRO_1 had increased to their initial value ($f_1 > f_2 > f_3$).

During background activity of the MIN, with a discharge frequency F_0 , the adaptation process of the MRO_1 differed (Fig. 12B, II). The MIN would 'cut' lower and lower parts of the MRO_1 activity curves during rhythmical stimulation (the level of F_0 is shown with a dotted line in diagram I), leaving smaller and smaller periods during which the impulse frequency of the MRO_1 exceeded the impulse frequency of the MIN. This interaction between discharge frequencies could be shown at variou

moments during the background activity of a neurone. It is possible that such an apparently simple yet effective mechanism of interaction between neurones can play an important role in the normal activity of the nervous system.

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