J. exp. Biol. (1976), 64, 251–266
With 9 figures
Printed in Great Britain

CONNEXIONS BETWEEN HAIR-PLATE AFFERENTS AND MOTONEURONES IN THE COCKROACH LEG

BY K. G. PEARSON, R. K. S. WONG* AND C. R. FOURTNER†
Department of Physiology, University of Alberta, Edmonton, Alberta, Canada

(*Received* 28 *July* 1975)

SUMMARY

1. The trochanteral hair-plate afferents in the metathoracic leg of the cockroach, *Periplaneta americana*, were stimulated electrically and at the same time intracellular recordings were made from either motoneurones, interneurones or afferent terminals within the metathoracic ganglion.

2. Activity in the hair-plate afferents evoked short latency excitatory postsynaptic potentials (EPSPs) in femur extensor motoneurones and inhibitory postsynaptic potentials (IPSPs) in femur flexor motoneurones. The latency of the IPSPs was on average 1·8 ms longer than the latency of the EPSPs.

3. Intracellular recordings from terminal branches of the hair-plate afferents showed that the delay between the peak of the afferent terminal spike and the beginning of the EPSPs is about 0·4 ms. This finding, together with the observations that the amplitude of the EPSPs is increased by the passage of hyperpolarizing current and decreased following high-frequency stimulation, indicates that the EPSPs are evoked via monosynaptic chemical synaptic junctions.

4. The observations of the long latency of the IPSPs, the need for a number of afferents to be simultaneously active for them to be evoked and the occasional variability in latency, all indicate that the IPSPs are evoked via a disynaptic pathway.

5. These findings provide information on the synaptic mechanisms underlying the reflexes from the trochanteral hair plate to femur flexor and extensor motoneurones identified in a previous investigation (Wong & Pearson, 1976).

INTRODUCTION

The recent use of intracellular recording techniques in arthropod nervous systems has led to the identification of monosynaptic excitatory pathways from sensory afferents onto motoneurones in the locust flight system (Burrows, 1975) and crab eye-movement system (Sandeman, 1969; Silvey, 1974). With the lack of studies in the majority of other arthropod systems the extent of the existence of monosynaptic reflex pathways cannot yet be assessed, and it is certainly premature to propose a general rule that most reflexes are via interneurones (Burrows & Horridge, 1974). In the previous paper (Wong & Pearson, 1976) we demonstrated, using extracellular recording techniques, a short-latency excitatory connexion from trochanteral

* Present address: Division of Ophthalmology, Stanford University Medical Center, Stanford, California 94303.
† Present address: Department of Biology, State University of New York, Buffalo, New York.

plate afferents to the slow coxal depressor motoneurone (D_8) in the cockroach metathoracic leg. The results reported in this paper show that this connexion is monosynaptic and transmission across it is mediated chemically.

The only detailed intracellular study of an inhibitory reflex pathway in arthropods is that by Burrows (1975) on the locust flight system, where he showed that action potentials in wing stretch receptor afferents evoke fairly short-latency (4–6 ms) inhibitory postsynaptic potentials, IPSPs, in wing elevator motoneurones. He concluded that this inhibitory connexion is probably monosynaptic. However, from the published data this conclusion cannot be fully accepted since the possibility that the IPSPs were elicited via a polysynaptic pathway containing non-spiking interneurones was not tested and excluded. The importance of attempting to demonstrate monosynaptic inhibitory connexions from sensory afferents to motoneurones is that until very recently (Reinking *et al.* 1975) it has been generally believed all first-order afferents produce excitatory postsynaptic potentials in the neurones on which they terminate (Eccles, 1964, p. 209). Moreover, it is important to determine whether single sensory afferents can have a direct excitatory effect on some neurones and a direct inhibitory effect on others, as has been found for some interneurones in molluscs (Kandel *et al.* 1967). Previously we have shown (Wong & Pearson, 1976) that activity in the trochanteral hair-plate afferents produces a short latency inhibition of tonic activity in leg flexor motoneurones of the cockroach. One aim of the present study was to determine whether this inhibitory effect was mediated monosynaptically or polysynaptically. Although our data does not exclude the possibility of a monosynaptic inhibitory connexion, we conclude that it is more probable that IPSPs elicited in flexor motoneurones, and some unidentified interneurones, in response to activity in trochanteral hair-plate afferents are produced via a pathway containing a non-spiking inhibitory interneurone.

MATERIALS AND METHODS

Experiments were performed on male and female cockroaches, *Periplaneta americana*. The experimental arrangement is shown in Fig. 1. The animal was mounted in wax ventral side up on a cork board. In most preparations the animal's left metathoracic leg was rotated to expose the dorsal surface of the coxa, and the nerve 6Br4 to the left posterior coxal levator muscles (Pearson & Bergman, 1969) was cut close to the levator muscles and retracted medially. This allowed recordings to be made from the motor axons in this nerve with the ventral surface of the coxa uppermost (Fig. 1). In other preparations nerve 6Br4 was left intact.

The left leg was held rigidly ventral side up in wax with the femur extended to expose the trochanteral hair plate (t.h.p.). The cuticle above the metathoracic ganglion was removed together with the underlying fat deposits. The exposed ganglion was kept constantly moist by the periodic application of cockroach saline (215 mM sodium chloride, 3·1 mM potassium chloride, 1·8 mM calcium chloride, 2·0 mM sodium bicarbonate, 0·1 mM sodium phosphate monobasic). A stainless steel platform (*p*) was positioned under the ganglion and staples (*s*) placed around the anterior and posterior connectives. This procedure held the ganglion reasonably firm. Extracellular recording electrodes were placed either on nerve 6Br4 or in the coxal levator

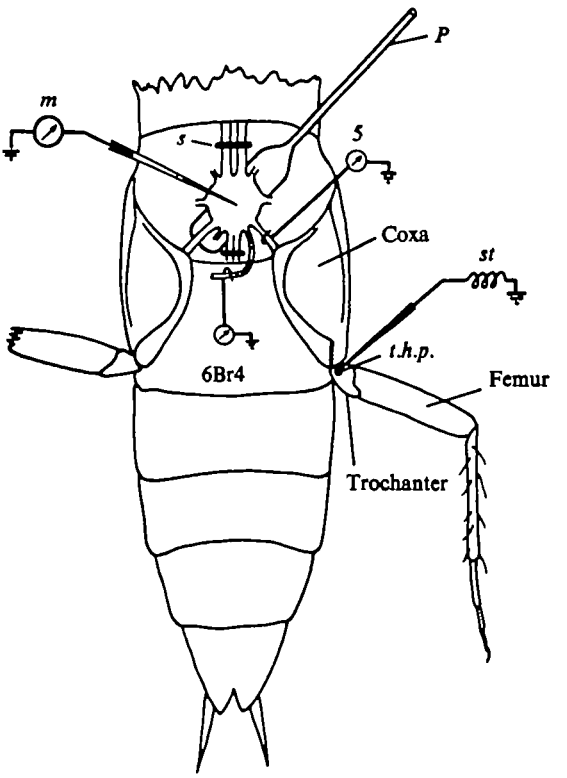

Fig. 1. Schematic diagram showing the experimental preparation and the arrangement of recording and stimulating electrodes. The animal was fixed ventral side up on a cork board and a stainless steel plate, *p*, placed under the exposed metathoracic ganglion. Staples, *s*, were placed around the anterior and posterior connectives to hold the ganglion firmly on the plate. Monopolar extracellular recording electrodes were placed on nerve 5 and nerve 6Br4 (the latter being positioned after dissecting nerve 6Br4 from the dorsal side of the coxa). The trochanteral hair plate, *t.h.p.*, was exposed by extending the femur and removing a cuticular flange at the distal end of the coxa. A fine stimulating electrode, *st*, was placed on the hair plate. The ganglion was penetrated by an intracellular recording microelectrode, *m*, on the side ipsilateral to the stimulating electrode.

muscles to monitor the activity in the flexor motor axons to the posterior coxal levator muscles. Recording electrodes were also placed on nerve 5 to monitor the afferent input from the hair plate and activity in the slow motoneurone, D_8, to the coxal depressor muscles. The hair-plate afferents were excited by the application of 0·05 ms current pulses through a sharp insect pin to the base of the hairs in the hair plate. By carefully placing the stimulating electrode and finely adjusting the current, single afferents from the hair plate could be excited. Synchronous activation of more than one afferent was achieved by using larger stimulus currents (see Wong & Pearson, 1976).

Intracellular recording. The technique for recording intracellularly from neurones in the metathoracic ganglion was the same as that described by Pearson & Fourtner (1975) and hence will not be repeated here. However, two additional procedures were required when penetrations were made from the ventral side of the ganglion. Firstly, to prevent drying of the ganglion, a small wax ring was placed around the

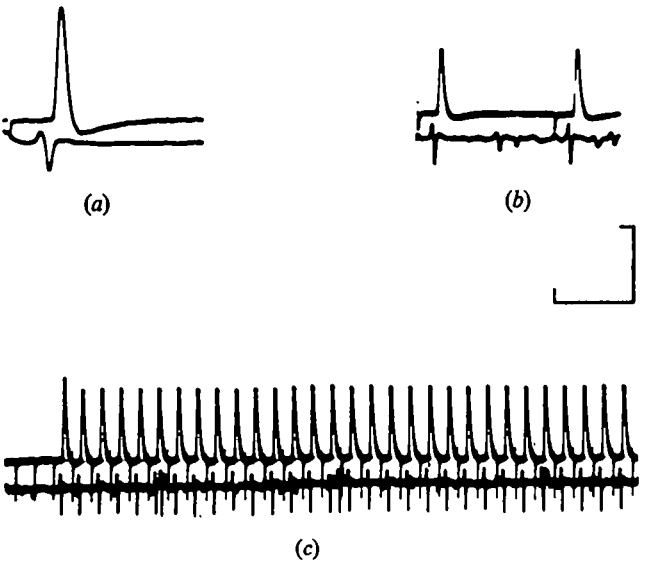

Fig. 2. Intracellular recordings from terminal branches of trochanteral hair-plate afferents. Top traces, intracellular records; bottom traces, nerve 5 records. (*a*) The extracellular potential recorded from the axon of a single afferent (bottom) is followed shortly by an action potential in one of its terminal branches (top). Note that there is no prepotential preceding the terminal spike. (*b*) and (*c*) are records from another preparation showing that the terminal spikes follow 1:1 high-frequency stimulation. In (*c*) the stimulating frequency was 200/s. Calibrations: terminal spike amplitude, 20 mV; time, (*a*) 4 ms, (*b*) 8 ms, (*c*) 20 ms.

ganglion and saline retained with it. Secondly, since the ventral connective tissue sheath is tougher than the sheath on the dorsal surface, the sheath was softened by treating the ganglion with a 0·1% pronase-in-saline solution for 10 min, then thoroughly washed with saline.

RESULTS

A. *Afferent terminal spikes*

On six occasions microelectrode penetration of the lateral region of the ganglion close to nerve trunk 3 resulted in impalement of an intraganglionic terminal branch of a hair-plate afferent (Fig. 2). The criteria we used for identifying a record from the terminal branch of a hair plate afferent were: (1) the action potentials elicited in them by stimulation of the hair-plate were of short duration ($\sim$1·5 ms) without a depolarizing propotential (Fig. 2*a*), (2) the spikes followed the action potentials recorded extracellularly from the afferent axon in nerve 5 with a constant short latency and at high frequencies (Fig. 2*c*), and (3) the lack of any spontaneous synaptic activity. One further reason for being confident that these recordings were from the terminal branches of the afferents is that anatomical studies using the cobalt staining technique have shown the terminal branches of the hair-plate afferents to be located in the region of the ganglion from which these recordings were made (unpublished). The amplitude of the afferent terminal spikes was within the range of 20–40 mV and the peak was never positive. The characteristics of these spikes are similar to those of spikes recorded from sensory afferent terminals in other animals (Kennedy, Calabrese & Wine, 1974; Koketsu, 1956).

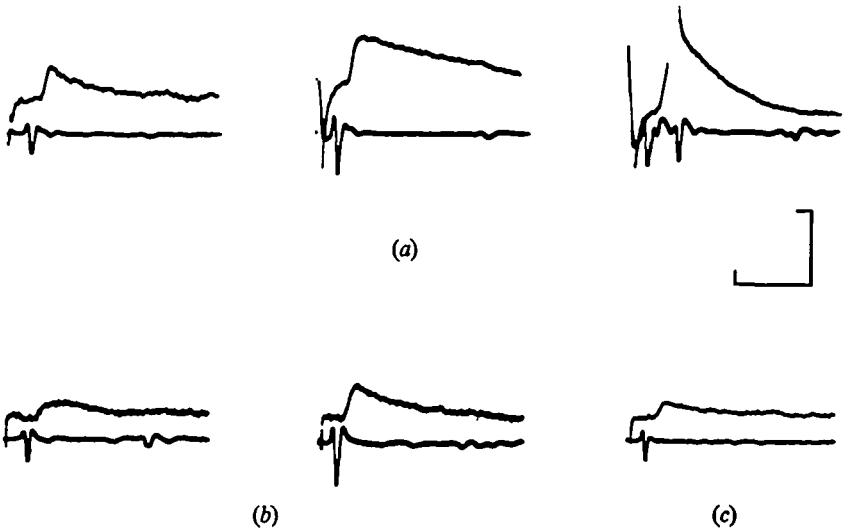

Fig. 3. EPSPs recorded in extensor motoneurones (*a* and *b*) and a non-spiking interneurone (*c*) in response to electrical stimulation of trochanteral hair-plate afferents. Top traces, intracellular records; bottom traces, nerve 5 records. (*a*) EPSPs in motoneurone D_s; left, EPSP in response to activity in a single afferent; middle, increase in the amplitude of the EPSP upon synchronous activation of a number of afferents; right, a large EPSP elicits an action potential in motoneurone D_s (this action potential can be seen as the second spike in the extracellular record from nerve 5). (*b*) EPSPs in a fast extensor motoneurone: left, EPSP in response to activity in a single afferent; right, increase in amplitude of the EPSP upon synchronous activation of a number of afferents. Calibration: amplitude EPSPs, 4 mV; time, 8 ms.

The latency of the afferent terminal spike following the axon spike recorded from nerve 5 was in the range of 0·5–0·8 ms in different preparations (Fig. 4). The latency was measured from the negative peak of the triphasic extracellular record to the positive peak of the afferent terminal spike. Both these points could be accurately identified in our records. Since the negative peak of the triphasic record from nerve 5 corresponds to the time when the peak of the action potential is passing the recording site (Stein & Pearson, 1971), this measurement of latency allowed us to calculate the average conduction velocity in the afferents. The distance between the point of recording on nerve 5 and the site of recording within the ganglion was approximately 2 mm, thus the average conduction velocity of the action potentials in the hair-plate afferents was between 2·5 and 4 $m.s^{-1}$. This estimate of the range of conduction velocities of the action potentials in the hair-plate afferents is very close to that (3·5–5 $m.s^{-1}$) reported in the previous paper (Wong & Pearson, 1976).

Having obtained recordings from the terminal branches of the hair-plate afferents, we could then more accurately determine the times of transmission of information from these afferents to other neurones within the ganglion.

B. *Excitatory postsynaptic potentials*

Occurrence. Electrical stimulation of the hair-plate afferents evoked an excitatory postsynaptic potential, EPSP, in at least two motoneurones giving extension movements of the femur and a number of unidentified non-spiking interneurones (Fig. 3;

see Pearson & Fourtner (1975) for the criteria used for classifying a neurone as non-spiking interneurone). The two extensor motoneurones found to be excited by the hair-plate afferents were (1) the slow motoneurone to the coxal depressor muscles, motoneurone D_s, and (2) a fast motoneurone to the main coxal depressor muscle with its axon in nerve 4.

To record from the neurites of motoneurone D_s, microelectrode penetrations of the neuropil were made slightly posterior to lateral nerve trunk 3 and from 150 to 250 μm from the midline. In a previous study it has been shown that a number of prominent neurites of this motoneurone are located in this region (Pearson & Fourtner, 1975). Since motoneurone D_s is the only motoneurone with an axon in nerve 5 to be readily activated by hair plate stimulation, the criterion used for establishing that recordings were made intracellularly from this motoneurone was that stimulation of the hair-plate afferents evoked a short latency spike in the impaled neurone and this spike was correlated 1:1 with spikes recorded from a single motor axon in nerve 5 (the axon of motoneurone D_s is in nerve 5). To confirm that this criterion alone is sufficient for identifying motoneurone D_s, extracellular recordings were made from the coxal depressor muscle in which the excitatory junctional potentials evoked in these muscles by motoneurone D_s can be unambiguously identified (Pearson, 1972). A fast extensor motoneurone with an axon in nerve 4 was identified by the 1:1 correspondence of intracellular spikes with phasic extension movements of the femur and the absence of a spike in the extracellular record from nerve 5. Since there are a number of fast axons in nerve 4 to the coxal depressor muscle, we cannot be certain that the same fast extensor was penetrated in different preparations. No attempt was made to identify, physiologically or anatomically, the non-spiking neurones from which EPSPs were recorded. Since these interneurones were encountered in different areas of the dorsolateral region of the ganglion, it is probable that a significant number of interneurones receive excitatory input from the hair-plate afferents.

An important observation was that in repeated penetrations in the same preparation different neurones were encountered in which the same afferent fibre produced EPSPs. This observation demonstrates that single hair-plate afferents can simultaneously excite both extensor motoneurones and certain non-spiking interneurones.

Amplitude, duration and latency. The evoked EPSPs in the slow and fast extensor motoneurones and the unidentified interneurones were very similar as regards their amplitude, time course and latency. Consequently, in the following description of these characteristics of the EPSPs, we will not always attempt to make a distinction between the EPSPs recorded in the different neurones.

The EPSPs produced by a single afferent had an amplitude of between 1·0 and 2·0 mV. There was no obvious correlation between the amplitude of the spikes recorded extracellularly on nerve 5 from single afferents (which gives an indication of the diameter of the afferent; Pearson, Stein & Malhotra, 1970) and the EPSP amplitude. Stimulation of more than one hair-plate afferent led to a larger EPSP due to the summation of the individual EPSPs (Fig. 3). An EPSP of more than a few millivolts usually initiated an action potential in motoneurone D_s (Fig. 3), while synchronous activation of many hair-plate afferents only rarely initiated a spike in the fast extensor motoneurone.

The duration of the EPSPs was between 10 and 20 ms and the rise time approxi-

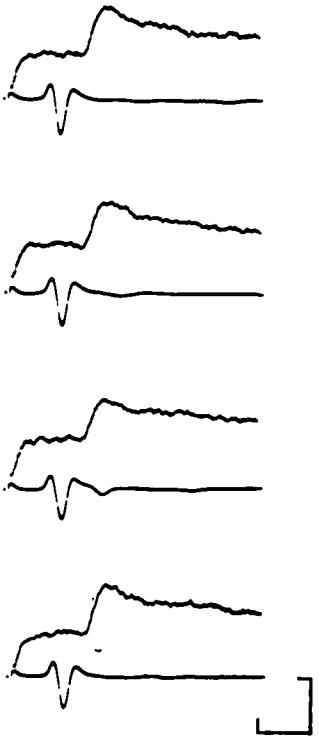

Fig. 4

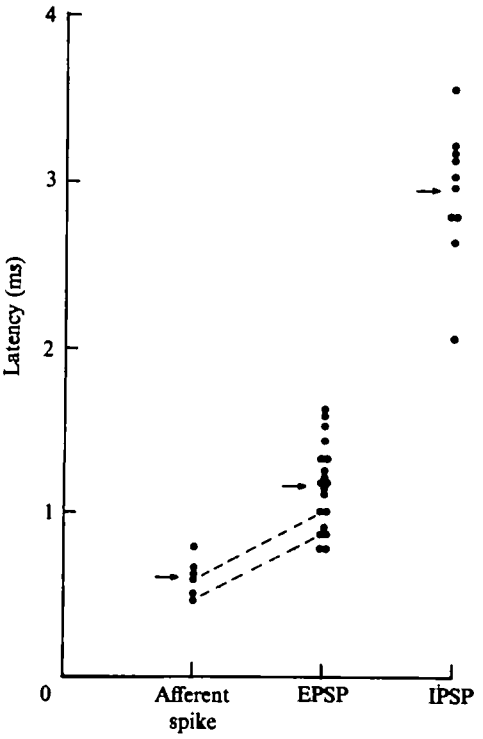

Fig. 5

Fig. 4. Records showing the constant latency of the EPSPs elicited in motoneurone D_s (top traces) by activity in a single hair-plate afferent. Bottom traces, nerve 5 record of afferent activity. The four sets of records shown in this figure are from four consecutive stimulations of the hair-plate afferent. Calibration: amplitude EPSPs, 2 mV; time, 2 ms.

Fig. 5. Diagram showing the latencies of the afferent terminal spikes, the EPSPs and the IPSPs measured for all preparations. The latencies were measured from the negative peak of the extracellular record of afferent activity in nerve 5 to the peak of the afferent terminal spikes and to the beginning of the postsynaptic potentials. The arrow to the left of each group of data points shows the mean latency for that group. The two dotted lines connect the data points obtained in two preparations in which an EPSP was first recorded in response to activity in a single afferent then on a subsequent penetration of the ganglion the terminal of the same afferent was impaled. In both cases the delay between the peak of the terminal spike and the beginning of the EPSP was 0·4 ms.

mately 2 ms. The time course of these EPSPs is similar to that observed in other neurones of the cockroach (Callec *et al.* 1971).

The latency of the EPSPs measured from the negative peak of the triphasic potential recorded from the afferent axon in nerve 5 was extremely constant for repeated stimulation in a single preparation (Fig. 4), and within the range of 0·9 and 1·6 ms in different preparations (Fig. 5). Table 1 lists the mean value of the latency measured in the extensor motoneurones and the non-spiking interneurones in different preparations. From this it can be seen that for all these neurones the mean latency was very similar and slightly greater than 1 ms. Allowing for the conduction time of the afferent impulses into the ganglion (which is approximately 0·6 ms; see Fig. 5) the transmission time from the afferent terminal spike to the initiation of the EPSPs is clearly less than 1 ms. In two preparations we were able to measure this delay directly,

Table 1. *Mean latencies in milliseconds of the afferent terminal spikes, EPSPs and IPSPs*

(The number of observations is shown in parentheses.)

Afferent terminal spikes	EPSPs		IPSPs	
0·62 (6)	Motoneurone D_s	1·24 (4)	Flexor motoneurones	2·81 (3)
	Fast extensor	1·02 (2)	Other	3·00 (7)
	Other	1·17 (15)		
	Total	1·17 (21)	Total	2·95 (10)

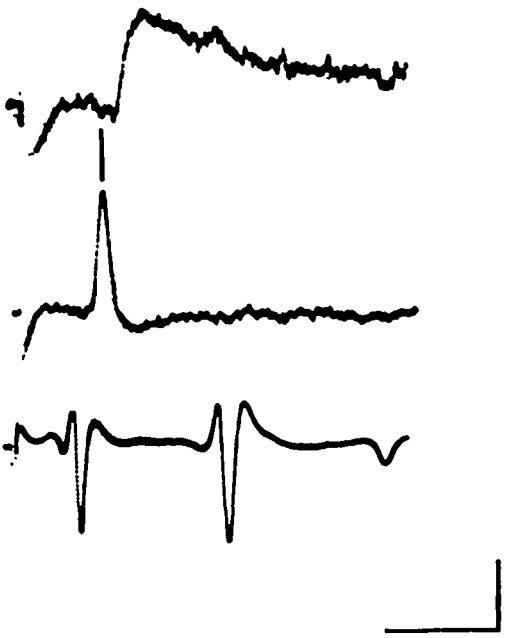

Fig. 6. Records showing the delay between an afferent terminal spike (middle trace) and the beginning of an EPSP in a fast extensor motoneurone (top trace). The bottom trace is the nerve 5 record. The EPSP was evoked by an action potential in a single afferent and the terminal spike recorded in the same afferent on a subsequent penetration of the ganglion. The extracellular afferent spikes recorded from nerve 5 in the two sets of original records were aligned to show the delay between the peak of the terminal spike (indicated by the short vertical line under the top record) and the beginning of the EPSP. Only one nerve 5 record is shown in this figure. The second spike in the nerve 5 record is from motoneurone D_s. Calibrations: EPSP amplitude, 2 mV; terminal spike amplitude, 10 mV; time, 4 ms.

since we were fortunate enough first to record the EPSP elicited by a spike in a single afferent and then on a subsequent penetration of the ganglion to record intracellularly from the terminal branch of the same afferent (Fig. 6). The latency between the peak of the afferent terminal spike and the beginning of the EPSP in both preparations was 0·4 ms. This small but significant value of the central transmission delay indicates that the EPSPs are elicited via a monosynaptic connexion from the hair-plate afferents and that transmission across this synapse is chemically mediated. A further demonstration that there is an appreciable delay between the afferent terminal spike and the initiation of the EPSPs is seen by comparing the latencies of these two events in all preparations in which they were recorded (Fig. 5). The mean value of the peak of the terminal spike was 0·62 ms, while the mean value of the latency to the beginning

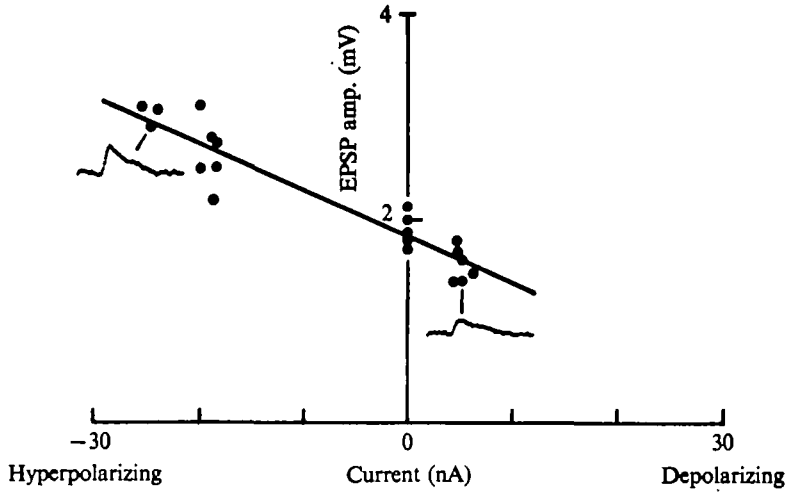

Fig. 7. Effect of hyperpolarizing and depolarizing currents on the amplitude of the EPSPs evoked by stimulation of the trochanteral hair-plate afferents. Fast extensor motoneurone.

of the EPSP was 1·17 ms. These observations argue against the possibility that transmission between the hair-plate afferents and central neurones is via an electrical synaptic junction.

Chemical transmission. The significant delay (approximately 0·4 ms) between the peak of the spike in the terminal branches of the hair plate afferents and the beginning of the EPSPs indicates that transmission is chemically mediated. A second indication for chemically mediated transmission is that the duration of the EPSPs (10–20 ms) is similar to the duration of other chemically evoked EPSPs in arthropods (Burrows, 1975; Callec *et al.* 1971; Zucker, 1972) and significantly longer than the duration of electrically evoked EPSPs (Zucker, 1972).

Two further tests for demonstrating chemical transmission were carried out, namely the studying of the effects of (1) hyperpolarizing currents in the postsynaptic neurone on the amplitude of the EPSPs, and (2) bathing the ganglion in a solution containing a high concentration of magnesium. Fig. 7 shows that the passage of hyperpolarizing currents increases the amplitude of the EPSP, as is expected if transmission is mediated chemically. We were unable to study the effects of large depolarizing currents in motoneurones since the generation of action potentials by the depolarizing current obscured the evoked EPSPs. In non-spiking interneurones depolarizing currents decreased the EPSP amplitude but in none of these neurones could sufficient current be passed to reverse the EPSP. By itself, the observation of an increased EPSP amplitude by a hyperpolarizing current does not conclusively prove that transmission is via a chemical transmitter since the imposed currents could alter the passive membrane resistance (Nicholls & Purves, 1970). None the less, this finding, together with the significant synaptic delay and long duration of the EPSP, strongly argues in favour of chemically mediated transmission.

A conventional technique to demonstrate chemical transmission is to increase the external magnesium ion concentration, which has been shown at many synapses to block the release of transmitter. However, we were unable to demonstrate this effect

on transmission from the hair-plate afferents. Bathing the ganglion in a solution containing a high concentration of magnesium (50 mM) for periods up to 45 min did not block the reflex activation of motoneurone D_s by the hair-plate afferents nor the production of short-latency EPSPs by these afferents. Desheathing the ganglion in this high-magnesium solution also did not lead to a block of transmission. The ability of high magnesium solutions to prevent the generation of EPSPs does not exclude chemical transmission, for it is possible that there is a marked diffusion barrier between the exterior of the ganglion and the synaptic sites within the neuropile. In some other animals chemically mediated synaptic transmission is also known to be unaffected by high-magnesium solutions (Zucker, 1972). Burrows (1975) was also unable to completely block the production of EPSPs in locust flight motoneurones with high magnesium but he did observe a significant decrease in EPSP amplitude over a period of 45 min. If a similar decrease in amplitude occurred in our experiments we would not have detected it since we were unable to record for long periods of time from the same neurone and the amplitudes of the EPSPs were variable from neurone to neurone, and even in the same neurone on maintained penetration.

A common characteristic of chemical synapses but not electrical synapses is a facilitation and/or depression of EPSP amplitude following repetitive stimulation (Bennett, 1974). For a wide range of stimulation frequencies we never observed any facilitation in EPSP amplitude but with high frequency stimulation there was a progressive diminution in amplitude. Stimulation at 100/s for 1 s decreased the amplitude of the EPSPs evoked immediately following the stimulus train to about 75% of the initial value.

C. *Inhibitory postsynaptic potentials*

Occurrence. In the previous paper it was shown that the slow flexor motoneurones to the posterior coxal levator muscles were inhibited by stimulating the hair plate (Wong & Pearson, 1976). On three occasions we penetrated the neurites of slow motoneurones to these muscles and observed an inhibitory postsynaptic potential in response to electrical stimulation of the hair plate (Fig. 8). We were unable to individually identify the flexor motoneurones from which we recorded, but since the region of the ganglion we penetrated contains the main neurites of the motoneurones labelled as 5 and 6 (Pearson & Fourtner, 1975), it is probable that on at least one occasion we penetrated one of these neurones. IPSPs were also recorded from unidentified non-spiking interneurones in regions close to main neurites of the flexor motoneurones (deep within the lateral edge of the ganglion approximately level with nerve 3). Since the amplitudes, durations and latencies of the IPSPs in the motoneurones and the non-spiking interneurones were similar, we shall not distinguish between the neurones in the following description of the characteristics of the IPSPs.

Amplitude, duration and latency. The amplitude of the IPSPs depended upon the number of active afferent fibres. Only rarely was an IPSP produced by activation of a single afferent. On most occasions a number of afferents had to be synchronously activated to evoke an IPSP. With strong stimulation of the hair plate the peak

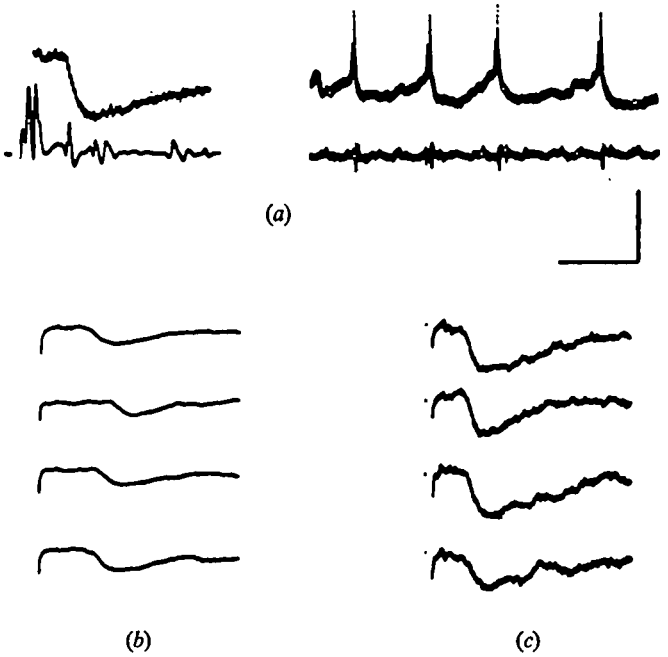

Fig. 8. IPSPs recorded from flexor motoneurones (*a* and *b*) and a non-spiking interneurone (*c*) evoked by stimulation of the trochanteral hair-plate afferents. (*a*) Left, IPSP in flexor motoneurone, the bottom trace is the nerve 5 record; right, recording in same motoneurone showing 1:1 correspondence between intracellularly recorded spikes (top) and extracellularly recorded junctional potentials from the posterior coxal levator muscle (bottom trace). (*b*) A series of IPSPs recorded in a flexor motoneurone on repeated stimulation of the hair-plate afferents showing variations in latency. (*c*) A series of IPSPs recorded in a non-spiking interneurone on repeated hair-plate stimulation showing almost no variation in latency. Calibrations: amplitude, (*a*) and (*c*) 4 mV, (*b*) 8 mV; time, (*a*) left, (*b*) and (*c*) 8 ms, (*a*) right 50 ms.

amplitude of the IPSPs could be as high as -5 mV. The durations of the IPSPs were within the range of 15–30 ms and thus slightly longer than the duration of the EPSPs.

The latencies of the IPSPs were in the range of 2·5–4 ms and on average about 1·8 ms longer than the mean latency of the EPSPs occurring in other neurones (Table 1; Fig. 5). The variation in latency of the IPSPs in any one neurone was usually (7 out of 10) very small for repeated stimulation of the same group of afferents and may be regarded as constant (Fig. 8*c*). This variability was not noticeably different from that of the EPSPs described above (Fig. 4). By contrast, in one motoneurone and two interneurones the latency of the IPSPs showed considerable variation with repeated stimulation of the same afferent fibres (Fig. 8*b*). The mean values of the variable latency IPSPs were similar to those of the constant latency IPSPs and the range of variation was between 2·5 and 4 ms. Because the neurones in which we recorded IPSPs were not identified, it was not possible to determine whether the individual neurones in which the latency of the IPSP was variable differed from those in which the latency was constant. Clearly this is an important point since the pathway for eliciting the constant latency IPSP may differ from the pathway eliciting the variable latency IPSP. We did observe, however, that both motoneurones and interneurones could have constant and variable latency IPSPs.

Both constant and variable latency IPSPs could be elicited 1:1 by stimulation of

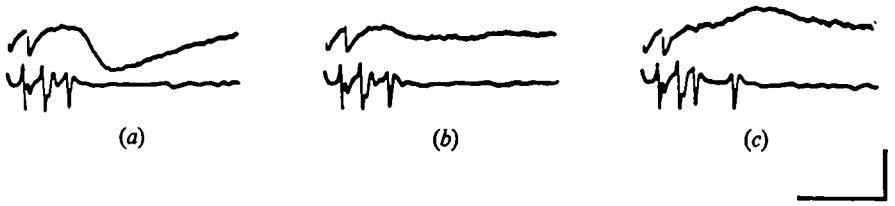

Fig. 9. Reversal of IPSP in a non-spiking interneurone by the passage of a hyperpolarizing current. To elicit the IPSP the hair-plate afferents were stimulated by two pulses separated by 2 ms. No IPSP was elicited by single pulse stimulation. Top traces, intracellular records; bottom traces, nerve 5 records. (*a*) Current = 0 nA; (*b*) current, 7·5 nA; (*c*) current, 14 nA. Calibrations: IPSP amplitude, 4 mV; time, 8 ms.

the hair-plate afferents at frequencies up to 60/s. Beyond this frequency fusion and diminution in amplitude of the IPSPs prevented us determining whether they continued to be elicited 1:1. In two pulse stimulation experiments two IPSPs could be elicited with the interpulse interval as short as 5 ms. Thus the inhibitory synaptic link appears to be very secure.

Chemical transmission. Injection of small hyperpolarizing currents diminished the amplitude of the IPSPs while large hyperpolarizing currents reverse the IPSPs (Fig. 9). The reversal of the IPSPs demonstrates that these are generated by a conductance change with an equilibrium potential more negative than the resting membrane potential. Thus we conclude that the IPSPs are produced in the conventional manner by an inhibitory chemical transmitter and not by the removal of a tonic excitatory input to the neurones. No further tests for chemical transmission such as measuring postsynaptic conductance changes, studying the effects of high magnesium and of chloride ion injection were conducted.

DISCUSSION

A. *Excitatory postsynaptic potentials*

In the previous study (Wong & Pearson, 1976) we described an excitatory reflex pathway in the cockroach metathoracic leg from the trochanteral hair-plate afferents to the slow motoneurone producing extension movements of the femur in a walking animal (motoneurone D_8). Some data in that study indicated that transmission in this reflex pathway could be monosynaptic. One of the main conclusions of the present investigation is that the trochanteral hair-plate afferents do monosynaptically connect to motoneurone D_8, as well as to at least one other extensor motoneurone and to a number of unidentified interneurones, and that transmission at these junctions is mediated by a chemical transmitter.

The conclusion that the EPSPs are elicited monosynaptically follows from the observation of a short constant delay ($\sim$0·4 ms) between the peak of the afferent terminal spike and the beginning of the EPSPs. This latency is too short for the EPSPs to be generated via a disynaptic pathway with transmission at each synapse being chemically mediated, and too long for the EPSPs to be produced monosynaptically via an electrical junction. Thus the EPSPs must be produced either monosynaptically via a chemically transmitting junction, or disynaptically with either the first

or both junctions being electrical. Since no postsynaptic potentials were recorded with latencies as short as the afferent terminal spikes (Fig. 5) the possibility of the hair-plate afferents making electrical synaptic connexions on any neurones within the ganglion must be considered unlikely. However, it is conceivable that we simply failed to record from the intercalated neurones either because of their small size or location in regions of the ganglion other than those penetrated. Despite this reservation the simplest explanation of our data is that the EPSPs are elicited monosynaptically via a chemically transmitting synapse. In addition to a short synaptic delay, further evidence for chemical transmission is the increase in amplitude of the EPSPs on the passage of a hyperpolarizing current (Fig. 7), and the diminution of the EPSP amplitude following high-frequency stimulation of the hair-plate afferents. The failure of high concentrations of magnesium ions to abolish the EPSPs does not exclude chemical synaptic transmission for it is possible that there is a barrier which prevents the diffusion of magnesium ions to presynaptic sites within the neuropile. Chemical synaptic transmission at some junctions in the crayfish abdominal ganglia are also resistant to elevated concentrations of magnesium ions (Zucker, 1972), as is chemical transmission at some junctions in the mollusc *Aplysia* (Tauc, Epstein & Mallard, 1965).

An interesting observation in this study was that in none of the many hundreds of neurones we penetrated did we record long-latency (>2 ms) EPSPs in response to stimulation of the hair-plate afferents. There are two possible explanations for this fact: firstly, that the afferent volley may not have been sufficiently large to produce large EPSPs or action potentials in second-order interneurones which in turn give rise to long latency EPSPs in third-order interneurones, or secondly, that all the interneurones excited by the hair-plate afferents are inhibitory. At present we cannot decide between these two possibilities.

B. *Inhibitory postsynaptic potentials*

The main question to be considered in this part of the discussion is whether or not the inhibitory postsynaptic potentials recorded in flexor motoneurones and some unidentified non-spiking interneurones are monosynaptically elicited by spikes in the hair-plate afferents. The importance of this question is that at present there is no convincing example of inhibitory afferents in any arthropod system. The IPSPs occurring in elevator motoneurones of the locust flight system in response to activity in wing stretch receptor afferents may be monosynaptically elicited (Burrows, 1975), but the possibility that this inhibitory pathway is di- or polysynaptic has not been excluded.

The long latency of the IPSPs following the spike in the afferent terminals (Fig. 5) is the first indication that the connexion is not monosynaptic. The value of this latency, 1·5–3 ms, is significantly greater than the value for synaptic delay conventionally taken to be about 0·5 ms. However, it may be that the synaptic delay at central inhibitory synapses in the cockroach is larger than the delay at synapses in other animals. Long-latency monosynaptic excitatory connexions have been described in the leech (Nicholls & Purves, 1970) while the latency at some inhibitory synapses in molluscs can be as high as 3 ms (Kandel *et al.* 1967). Another possible reason for the long latency of the IPSPs could be that conduction along the inhibitory terminal

branches may be slower than that along branches giving excitation. If this was so then the inhibitory branches would presumably be very small and not allow microelectrode penetration. This would then explain our failure to record the long-latency afferent terminal spikes expected in the inhibitory afferent terminals. Since we have never recorded any long-latency extracellular field potentials from the afferent terminals, it seems unlikely that the conduction velocity in the terminals leading to the IPSPs is slow.

More compelling reasons for believing that the IPSPs are not monosynaptically elicited are that usually more than one afferent fibre is required to produce an IPSP and that there is a variability in IPSP latencies in some neurones. Neither of these two effects can be readily explained if the connexion is monosynaptic. It is conceivable that the IPSPs elicited by a spike in a single afferent are too small to be detected above the noise, while variability in the latency may be due to unusually high variability in the stochastic processes underlying chemical transmission at the inhibitory synapses. However, both these effects, as well as the long latency of the IPSPs, are far more readily explained by assuming that the IPSPs are elicited via a disynaptic pathway involving a single intercalated inhibitory interneurone. The need for a synchronous afferent volley to elicit the IPSPs is then explained by the need for summation of EPSPs in the inhibitory interneurone either to produce an action potential or to depolarize the interneurone to a level where inhibitory transmitter is released in a graded manner. The variability in latency would then be due to variation in the time for the membrane potential to reach this threshold level as a result of spontaneous fluctuations in membrane potential which are known to occur in some interneurones (Pearson & Fourtner, 1975). Thus we can conclude that it is more probable that the IPSPs are produced disynaptically. It remains to consider whether action potentials in the inhibitory interneurone are required for transmitter release.

The observation that the variability in the latency of the IPSPs is usually very small (Fig. 8*c*) immediately suggests that graded depolarization in the inhibitory interneurone is responsible for the production of the IPSPs. When recordings were made from neurones in which spikes were elicited by stimulation of the hair-plate afferents the variability in the latency of the spikes was always larger than the variation in latency of the IPSPs (for example, see Fig. 7, in Wong & Pearson (1976) and compared with Figs. 8*b*, *c*). Thus the action potentials in none of the spiking neurones from which we recorded could have produced the IPSPs. Of course it is conceivable that the IPSPs were elicited by action potentials in interneurones from which we failed to record. However, since we now know that there are many non-spiking interneurones in the cockroach ganglion and no spiking neurones have yet been found to influence activity in flexor motoneurones (Pearson & Fourtner, 1975), the simplest explanation for our data is that the IPSPs are produced by the graded depolarizations in non-spiking interneurones. Some of the non-spiking interneurones found to be monosynaptically excited by hair-plate afferents could obviously be the interneurones in the inhibitory pathways to the flexor motoneurones and some non-spiking interneurones. We know that at least three non-spiking interneurones can inhibit activity in the flexor motoneurones (Pearson & Fourtner, 1975), but we have been unable to determine clearly whether any of these interneurones are monosynaptically excited by the hair-plate afferents.

3. *Comparison with other systems*

The organization and properties of the reflex pathways from the hair-plate afferents to the femur flexor and extensor motoneurones described in this paper are similar to those in the locust flight system described by Burrows (1975). In both systems the primary afferent fibres give monosynaptic, chemically mediated, excitation of one group of motoneurones and inhibition of the antagonistic group of motoneurones. The latency of the IPSPs is from 1·5 to 3 ms later than the EPSPs in both systems and it is therefore probable that the IPSPs are elicited via a non-spiking inhibitory interneurone. Furthermore, in both systems activity in the group of motoneurones receiving the inhibition causes movements which excite the sensory afferents. Thus the reflex pathways are organized so as to terminate the movements in one direction and promote the initiation of the opposite movement. The possible functional advantages of organizing the reflex pathways in this manner have been discussed elsewhere (Burrows, 1975; Wong & Pearson 1976).

Monosynaptic reflex pathways onto motoneurones are well known in vertebrates but have only recently been identified in invertebrates. Burrows (1975) has clearly demonstrated a monosynaptic excitatory reflex pathway in the locust from wing stretch receptor afferents to wing depressor motoneurone, and in the crab Sandeman (1969) has described a monosynaptic excitatory connexion from head hair receptors to motoneurones giving eye withdrawal movements. Our finding of the monosynaptic connexion from trochanteral hair receptor afferents to femur extensor motoneurones is thus a third example of a monosynaptic reflex pathway in arthropods. The existence of these pathways together with the finding of monosynaptic reflex pathways in the leech (Nicholls & Purves, 1970) and the mollusc *Aplysia* (Castellucci *et al.* 1970) clearly indicates that monosynaptic excitatory connexions of sensory afferents on to motoneurones may be widespread in invertebrates.

REFERENCES

Bennett, M. V. L. (1974). Flexibility and rigidity in electronically coupled systems. In *Synaptic Transmission and Neuronal Interaction* (ed. M. V. L. Bennett), pp. 153–78. New York: Raven Press.

Burrows, M. (1975). Monosynaptic connexions between wing stretch receptors and flight motoneurones of the locust. *J. exp. Biol.* **62**, 189–219.

Burrows, M. & Horridge, G. A. (1974). The organization of inputs to motoneurons of the locust metathoracic leg. *Phil. Trans. R. Soc.* B **269**, 49–94.

Callec, J. J., Guillet, J. C., Pichon, Y. & Boistel, J. (1971). Further studies on synaptic transmission in insects. II. Relations between sensory information and its synaptic integration at the level of a single giant axon in the cockroach. *J. exp. Biol.* **55**, 123–50.

Castellucci, V., Pinsker, H., Kupferman, L. & Kandel, E. R. (1970). Neuronal mechanisms of habituation and dishabituation of the gill withdrawal reflex in *Aplysia*. *Science, N.Y.* **167**, 1745–8.

Eccles, J. C. (1964). *The Physiology of Synapses*. Berlin: Springer-Verlag.

Kandel, E. R., Frazier, W. T., Waziri, R. & Coggeshall, R. E. (1967). Direct and common connections among the identified neurons in the abdominal ganglion of *Aplysia*. *J. Neurophysiol.* **30**, 1352–76.

Kennedy, D., Calabrese, R. L. & Wine, J. J. (1974). Presynaptic inhibition: primary afferent depolarization in crayfish neurons. *Science, N.Y.* **186**, 451–4.

Koketsu, K. (1956). Intracellular potential changes of primary afferent nerve fibers in spinal cords of cats. *J. Neurophysiol.* **19**, 375–92.

Nicholls, J. G. & Purves, D. (1970). Monosynaptic chemical and electrical connections between sensory and motor cells in the central nervous system of the leech. *J. Physiol.* **209**, 647–67.

Pearson, K. G. (1972). Central programming and reflex control of walking in the cockroach. *J. exp. Biol.* **56**, 173–93.

PEARSON, K. G. & BERGMAN, S. J. (1969). Common inhibitory motoneurones in insects. *J. exp. Biol.* **50**, 445–71.

PEARSON, K. G. & FOURTNER, C. R. (1975). Nonspiking interneurons in walking system of the cockroach. *J. Neurophysiol* **38**, 33–52.

PEARSON, K. G., STEIN, R. B. & MALHOTRA, S. K. (1970). Properties of action potentials from insect motor nerve fibres. *J. exp. Biol.* **53**, 299–316.

REINKING, R. M., STAUFFER, E. K., STUART, D. G., TAYLOR, A. & WATT, D. G. D. (1975). The inhibitory effects of muscle spindle primary afferents investigated by the afferent triggered averaging method. *J. Physiol.* **248**, 20 *P*.

SANDEMAN, D. C. (1969). The synaptic link between the sensory and motoneurones in the eye-withdrawal reflex of the crab. *J. exp. Biol.* **50**, 87–98.

SILVEY, G. E. (1974). Integration at a sensory-motor synapse in the oculomotor system of the crab *Scylla serrata*. *Am. Zool.* **14**, 1273, Abs. 246.

STEIN, R. B. & PEARSON, K. G. (1971). Predicted amplitude and form of action potentials recorded from unmyelinated nerve fibres. *J. theoret. Biol.* **32**, 539–558.

TAUC, L., EPSTEIN, R. & MALLARD, A. (1965). Action des ions Mg^{++} et Ca^{++} sur les potentiels postsynaptiques unitaires chez l'*Aplysie*. *J. Physiol., Paris* **55**, 284.

WONG, R. K. S. & PEARSON, K. G. (1976). Properties of the trochanteral hair plate and its function in the control of walking in the cockroach. *J. exp. Biol.* **64**, 233–249.

ZUCKER, R. S. (1972). Crayfish escape behavior and central synapses. I. Neural circuit exciting lateral giant fiber. *J. Neurophysiol.* **35**, 599–620.