J. Exp. Biol. (1970), **52**, 313–324
With 8 text-figures
Printed in Great Britain

GIANT FIBRE AND SMALL FIBRE PATHWAYS INVOLVED IN THE EVASIVE RESPONSE OF THE COCKROACH, *PERIPLANETA AMERICANA*

BY D. DAGAN AND I. PARNAS
Department of Zoology, Hebrew University, Jerusalem

(*Received* 9 *September* 1969)

INTRODUCTION

Using air movements as stimuli to the cerci Roeder (1948) studied the functional pathways and central connexions involved in the consequent evasive response of the cockroach. The major pathways were thought to involve cercal afferent fibres which converge to excite giant axons originating in the last abdominal ganglion, A_6 (Pumphrey & Rawdon-Smith, 1939; Roeder, 1948; Farley & Milburn, 1969). These giant axons are known to ascend the nerve cord and were thought to synapse with leg motoneurones. Roeder (1948) calculated that the latency between cercal nerve activation and first leg movement should be 19·8 msec. However, it was found that the actual latency is much longer, of the order of 28–90 msec. (Roeder, 1948). The difference between calculated and averaged measured values of response times for startle has been explained as lability of connexions to, and temporal summation on, motoneurones in the thoracic ganglia (Roeder, 1948; Hughes, 1965).

Stimulation of the cercal nerve (CN) initiates activity also in an unknown number of small ascending fibres (Roeder, 1948). It will be shown that these small fibres and not the giant axons, activate the leg motoneurones. Furthermore, it will be shown that the giant axons have no excitatory inputs to the leg motoneurones. The slower conduction velocity of the small axons partly explains the failure of measured and calculated startle latencies to tally. The question arises then: What are the functions of the giant axons and do they play any role in the evasive response? It is suggested that the giant fibre system has a different function in evasive behaviour than control of leg movements.

MATERIALS AND METHODS

Adult male cockroaches *Periplaneta americana* L. were used. The animals were pinned down dorsal side up and two longitudinal incisions made from the cerci to the head. Gentle removal of dorsal tergites, intestine and fat exposed the entire nerve cord. Care was taken to avoid damage to the thoracic trachea. Nerves (N_3, N_4, N_5, N_6) of the metathoracic ganglion innervating the leg muscles were carefully excised from the leg and cut distally to abolish sensory input. The nomenclature of Pipa, Cook & Richards (1959) is used.

The experimental arrangement is represented in Fig. 1:

$St_{(1-3)}$ indicate copper hook electrodes, insulated to the tip, used for extracellular stimulation.

R_1 is a copper hook electrode, insulated to the tip, used for extracellular recording.

R_2 is a suction electrode used for recordings from leg nerves.

R_4 is a pointed stainless steel electrode sharpened electrolytically and insulated to the tip for recordings from the base of the antenna. St_3: R_3 indicates a glass micro-electrode (filled with 2 M-Na citrate, 10–20 Ω) used alternatingly as a recording and as a stimulating electrode. Extracellular recording electrodes were connected via Tektronix low-level 122 amplifiers to a Tektronix 502 A oscilloscope. Intracellular recording was monitored on the CRO via a Bioelectric NF_1 high-impedance amplifier. For stimulation Grass stimulators were used connected via Devices Limited Mark IV stimulus-isolation units.

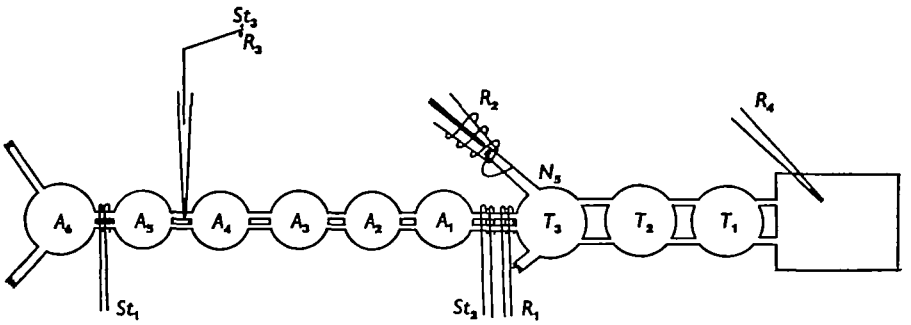

Fig. 1. Generalized scheme of the experimental arrangement. Thoracic ganglia marked T_1–T_3, abdominal ganglia A_1–A_6. St_1, St_2, copper wire hook electrodes for stimulation. R_1, hook electrode for recording. R_2, suction electrode for recording from leg nerves. R_4, pointed stainless-steel electrode to record at base of antenna. St_3: R_3, micro-electrode for recording and stimulating.

The preparation was moistened with a bathing solution described by Yamasaki & Narahashi (1960) which contains Na^+ 159·6 mM; K^+ 3·1 mM; Ca^{2+} 1·8 mM; Cl^- 160·1 mM; $H_2PO_4^-$ 0·2 mM and HPO_4^{2-} 1·8 mM. Nicotine sulphate (Hopkins and Williams Ltd.) was dissolved in this solution when applied topically. To facilitate micro-electrode penetration the cord sheath was treated with a 2% pronase solution for 2 min. (Willows, 1967; Parnas, Spira, Werman & Bergmann, 1969).

Dissected nerve cords were fixed in a 1:3 diluted Bouin's fluid with Ca^{2+} added for isotonicity (Farley & Milburn, 1969), embedded in paraffin, sectioned serially at 7 μ and stained with haematoxylin-eosin.

RESULTS

Ascending abdominal fibres that induce firing in the leg nerve N_5 were identified using five different methods: (1) Comparison of thresholds; (2) conduction velocity; (3) degeneration of fibres having somata in the last abdominal ganglion; (4) conduction block by nicotine, and (5) intracellular stimulation of a single giant axon.

1. *Comparison of thresholds*

Currents of increasing intensity applied to the abdominal connectives at A_5–A_6 gradually raise the number of evoked responses in the abdominal cord recorded at A_1–T_3 (Fig. 2). The first spikes to appear at the lowest threshold were always those of the giant fibres (Fig. 2*a*, *b*, lower beam) as ascertained by conduction velocity (mean $6 \pm 1{\cdot}5$ m./sec.) and the large size of the spikes. It is clear that with such a stimulus strength that activated only the abdominal giant fibre population no responses were observed in the leg nerve, N_5 (Fig. 2*a*, upper beam). However, when the stimulus strength was increased further and activation of smaller fibres with higher thresholds

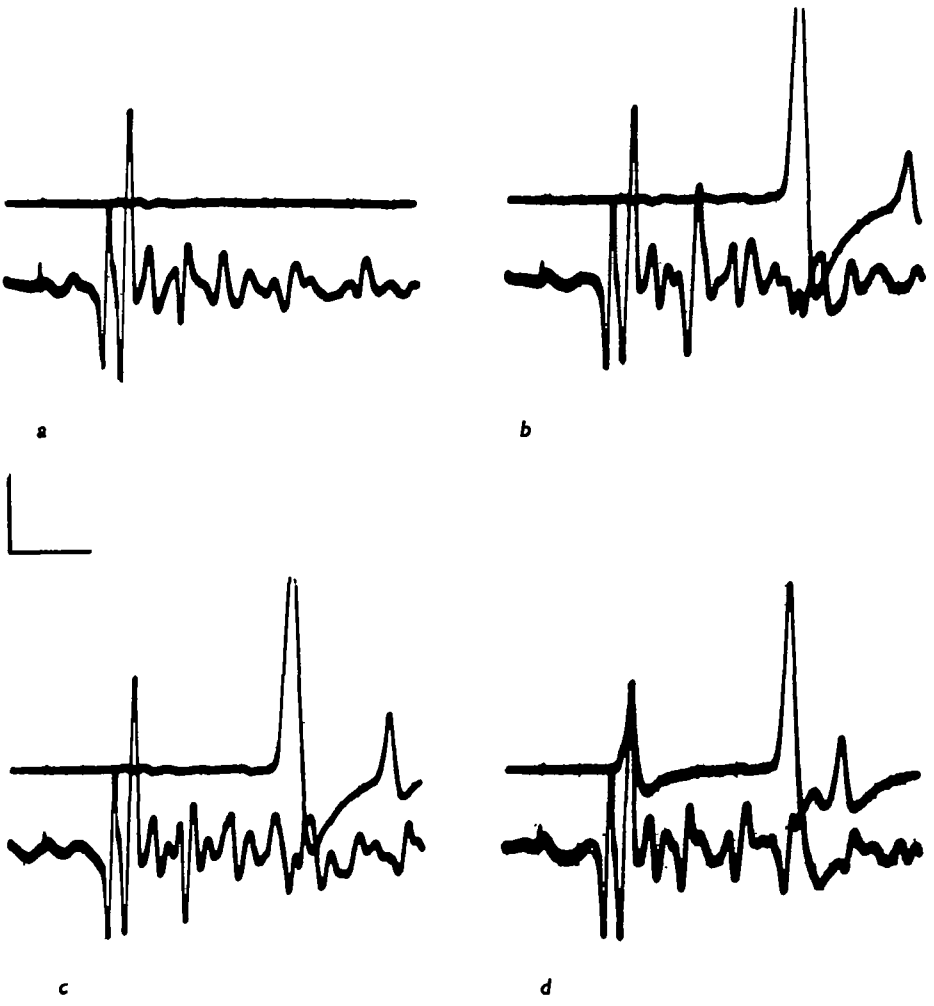

Fig. 2. Evoked potentials recorded from abdominal cord (lower trace) and leg nerve N_5 (upper trace). (*a*) At low strength of stimulation, note giant fibre responses in abdomen but no responses in leg nerve. (*b–d*) Gradual increase of stimulus strength; an evoked response is observed in N_5 together with potentials of small fibres in abdomen. In (*d*), a spontaneous response is observed before the evoked one in N_5. Calibration: 0·4 mV., 5 msec.

occurred, a synchronized response was evoked in N_5 (Fig. 2*b*, *c*, *d*). Increasing strength of stimulation produced firing of more and more units in N_5. However, distinct abdominal connective fibres could not be correlated by this method with specific units of N_5.

2. Conduction velocity

To evaluate the conduction velocity of the abdominal fibres which activate the leg nerve N_5 (Fig. 3), stimulating electrodes were placed at both A_5–A_6 and A_1–T_3, and the evoked responses were recorded at two places, one at the caudal base of ganglion T_3

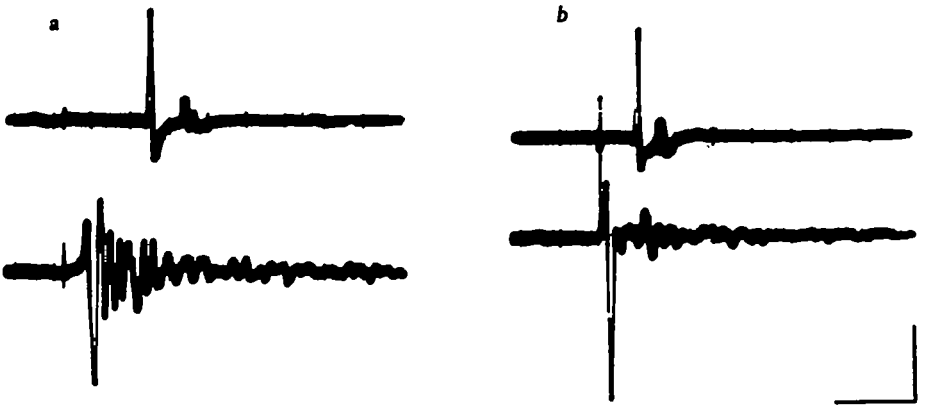

Fig. 3. Conduction velocity of the abdominal pathway inducing the response in N_5. Upper trace, response from N_5; lower trace, response from T_3–A_1. (*a*) Responses to stimulation at A_5–A_6. (*b*) Responses to stimulation at T_3–A_1. Distance between the two stimulating electrodes 12 mm., difference in delays for response in N_5, 6 msec.; conduction velocity of abdominal pathway inducing the response in N_5, 2 m./sec. Calibration 0·2 mV., 10 msec.

and the second on N_5. Stimulation of A_5–A_6 and T_3–A_1 produced responses at N_5 with latencies of 10 and 4 msec., respectively. The difference, 6 msec., therefore must represent the conduction time in the N_5 activating pathways in the abdomen. Assuming a continuous pathway from A_6 to T_3, these results give a calculated conduction velocity of 2 m./sec., the distance between the two stimulating electrodes being 12 mm. in this case. The conduction velocity in six different preparations ranged from 1·5 to 3·5 m./sec. Note that the fastest giant fibre spike traverses the abdominal cord in 2 msec., or at a conduction velocity of 6 m./sec., which is twice as fast as that of the smaller abdominal fibres initiating activity in the leg nerve N_5. Even the slowest giant fibres having a conduction velocity of 4·5 m./sec. propagate a spike in 2·7 msec. up to ganglion T_3.

A faster conduction velocity than 3·5 m./sec. can be assumed if the N_5 activating pathway includes synapses in the abdominal ganglia. However, the faster conducting giant axons are known to be continuous throughout the abdominal cord. Thus, this assumption already excludes the possibility that the giant axons are responsible for N_5 activation. These results provide further evidence that N_5 activation is induced by a pathway other than that of the giant fibres.

3. *Degeneration of giant fibres*

The nerve cord was transected between the fifth and sixth abdominal ganglia to cause degeneration of the giant fibres and any other nerves whose somata are located in A_6 (Hess, 1958; Farley & Milburn, 1969; Spira, Parnas & Bergmann, 1969*b*). The nerve cord was exposed 30–40 days after the transection, and the same experiment as described in the previous section (Fig. 3) was carried out, except for placement of the lower stimulating electrode at A_4–A_5.

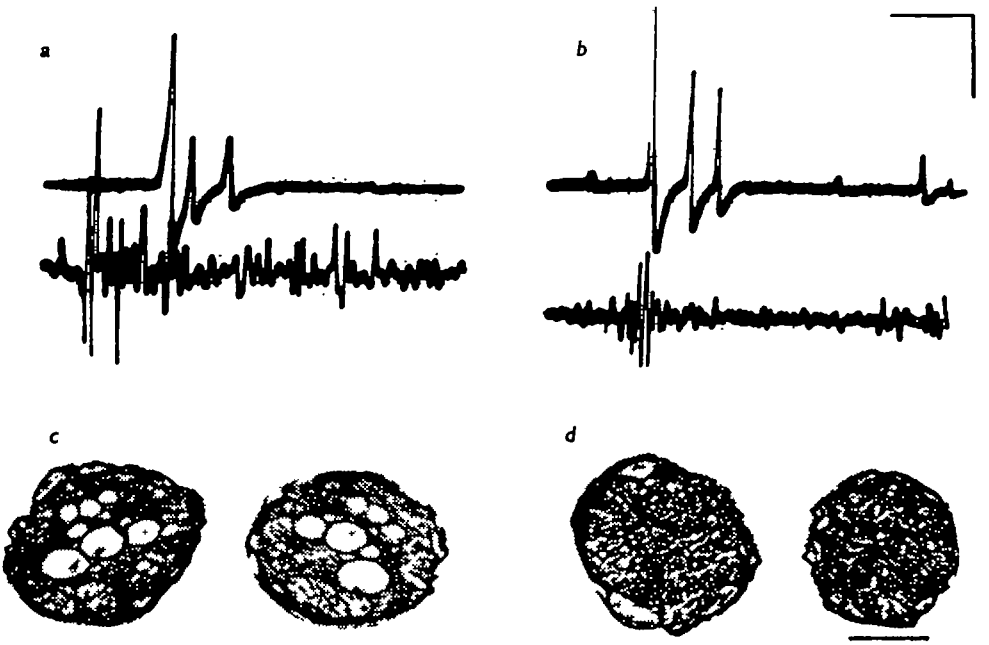

Fig. 4. Evoked potentials recorded in N_5 (upper trace) and abdominal cord (lower trace) in normal cords (*a*) and in cords with degenerated giant fibres (*b*). Note response in N_5 even when giant-fibre responses are completely absent in (*b*). Cross-sections of control and degenerated cords from which the recordings were made are shown in (*c*) and (*d*). Calibration: 0·2 mV., *a*, 10 msec., *b*, 20 msec., *c*, *d*, 100 μ.

As can be seen in Fig. 4*b*, no giant fibre responses appear in the abdominal cord recording (lower trace), while at the same time evoked activity was recorded at N_5 (upper trace) which did not appear substantially different from the normal evoked activity (Fig. 4*a*). Conduction velocity of the pathway in the abdomen responsible for N_5 activity was found to vary in six experiments between 2 and 3·5 m./sec. At the termination of the experiment the cord was fixed, sectioned and stained; the histological cross-sections showed complete degeneration of the giant fibres (Fig. 4*d*).

4. *Block by nicotine*

Nicotine in low concentrations blocks synaptic transmission in the C.N.S. of the cockroach (Roeder, 1948) and axonal conduction in the fine axons (Spira *et al.* 1969*a*). Only high doses of nicotine and longer exposure (20–30 min.) block conduction in the abdominal giant fibres (Spira *et al.* 1969*a*). It is therefore expected that low doses of

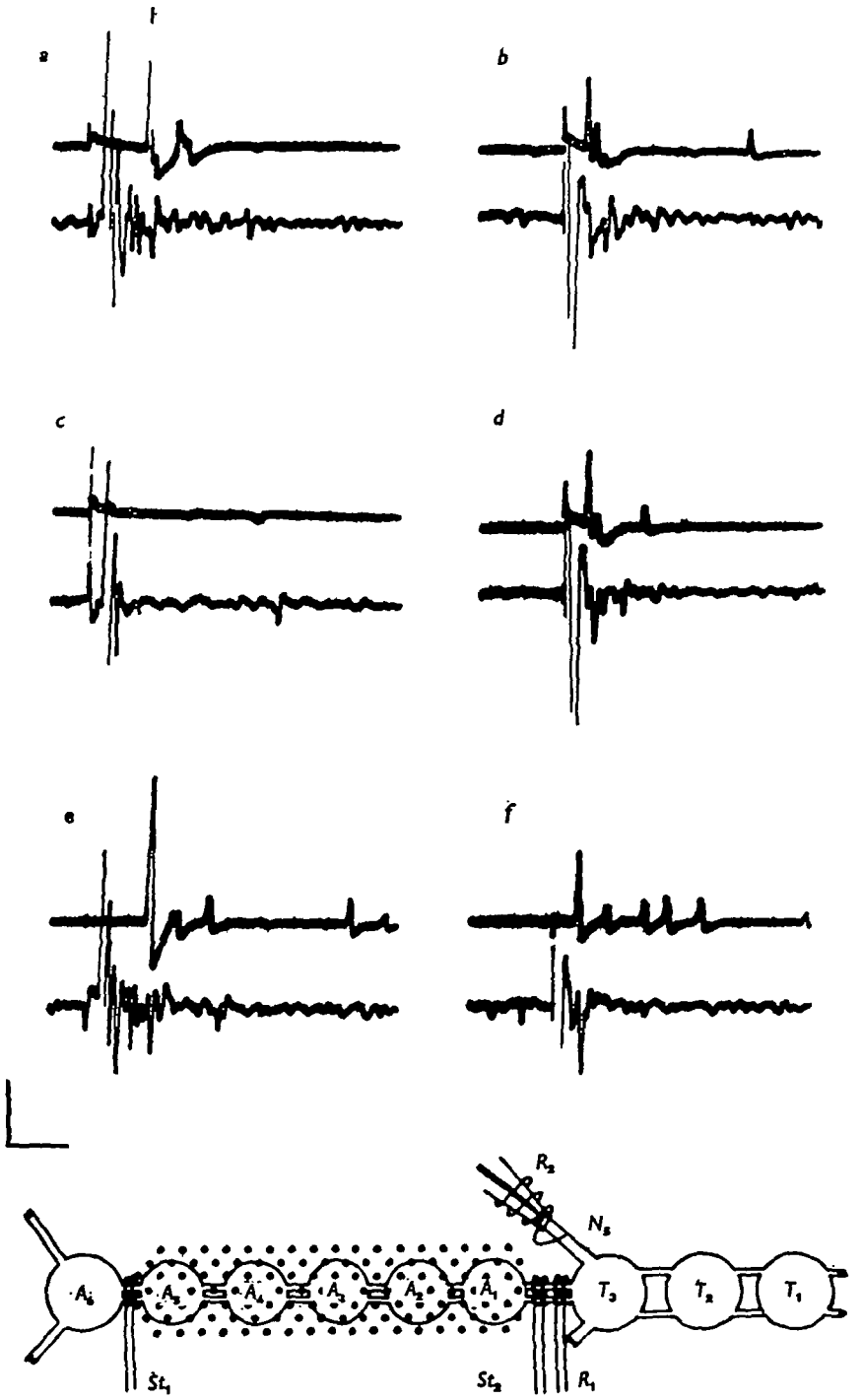

Fig. 5. Evoked potentials recorded from N_5 (upper trace) and abdomen (lower trace) to stimulation at A_5–A_6 (left column) and T_3–A_1 (right column) in normal and nicotine-treated cords. Nicotine 10^{-5} mg./ml. was topically applied to ganglia A_4–A_1 between stimulating electrodes, dotted area in scheme.

(*a*, *b*) Control: Note differences in delay of N_5 response in (*a*) and (*b*). (*c*, *d*) 3 min. after 10^{-5} mg./ml. nicotine; note block of small axon responses in abdominal recording and lack of an N_5 response to stimulation caudally to the blocked region. (*e*, *f*) Recovery after washings. Calibration: 1 mV., 10 msec.

nicotine would block conduction in small abdominal fibres and thus eliminate induced activity of N_5, while the giant axons should show normal activity. Indeed this was the case. Fig. 5 *a*, *b* show N_5 and abdominal potentials (upper and lower traces respectively) induced by stimulation at A_5–A_6 and A_1–T_3. Nicotine (10^{-5} mg./ml.) was then topically applied to each of the ganglia A_5–A_1. After 3 min. of incubation the N_5 responses to A_5–A_6 stimulation (Fig. 5 *c*) were blocked while an identical response of the control (Fig. 5 *b*) was obtained when A_1–T_3 was stimulated (Fig. 5 *d*). Note that the giant fibre response recorded at A_1–T_3 to stimulation at both A_5–A_6 and A_1–T_3 remained unaltered (Fig. 5 *c*, *d*, lower beams), but the difference associated with the loss of the N_5 response in Fig. 5 *c* is the absence of small slow spikes in the abdominal response. After several washings a recovery of the N_5 response was observed (Fig. 5 *e*). These results further indicate that giant axons are not excitors of N_5 motoneurones and that the latter are activated by smaller axons.

Recordings from nerves N_3, N_4, N_6

The same experiments as described for the major leg nerve N_5 were conducted on the other leg nerves N_3, N_4, N_6. Essentially the same results were obtained for these nerves. Namely, in no case did stimulation of abdominal giant fibres cause activation of any of the leg nerves. It appears that motor axons of these nerves are activated by a similar pathway to that activating N_5.

5. *Intracellular stimulation of single giant axons*

Attempts were made to activate giant axons by intracellular stimulation. For these experiments only the anterior part of the abdominal nerve cord between the metathoracic ganglion and the fourth abdominal ganglion was exposed leaving the thoracic region intact. The animal was firmly secured with its legs and antennae free to move. The experimental arrangement included two pairs of extracellular electrodes; one for stimulation at A_5–A_4 and the other for recording at T_3–A_1. A small portion of the nerve cord was treated briefly with 2% pronase (Parnas *et al.* 1969) to make possible penetration with a glass micro-electrode. After penetration of a giant axon with the micro-electrode, manifested by recording a resting potential of 65–70 mV. and intracellular recording of an evoked action potential by the micro-electrode to extracellular stimulation at A_5–A_6, the micro-electrode was used for stimulation. An extracellular recording of a single intracellularly evoked potential at T_3–A_1 of proper latency for giants was obtained, as was also a steady resting membrane potential of the giant axon after the stimulation period, indicating mechanical stability.

Rapid leg movements were observed following extracellular stimulation by a single pulse, while no such movements occurred in response to intracellular giant-fibre stimulation either with single pulses or at stimulation rates of up to 200/sec. for durations up to one second. After the intracellular stimulation the cord was again stimulated externally and repetitive movement of legs indicated that the lack of response to micro-electrode stimulation was not due to fatigue. Repetition of this experiment using different penetration angles to activate different giant fibres in turn never caused any observable movements of the leg. In other experiments the N_5 nerve

of the metathoracic ganglion was exposed and its activity was recorded. In no case did intracellular activation of a giant axon cause the N_5 nerve to fire.

The results of these experiments show that stimulation of a single giant axon is insufficient for leg-movement activation. Convergence of several giant fibres on leg motoneurones likewise cannot account for such an activation, since the results discussed above rule out this possibility.

Recordings from the base of the antenna

Preliminary behavioural experiments showed that normal cockroaches direct their antennae forward following a cercal stimulus and prior to their escape. When the animals meet obstacles in their path they usually change direction or stop running. Similar experiments were conducted on animals 30 to 40 days after disconnecting the giant-fibre pathways from their somata in ganglion A_6. After the experiment total

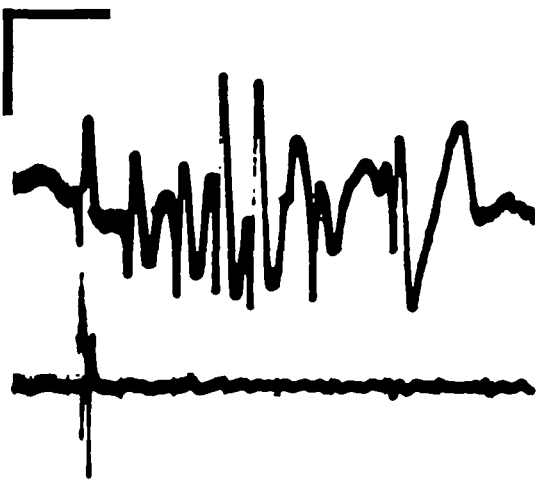

Fig. 6. Evoked potentials recorded at the base of an antenna (upper trace) and at T_3–A_1 (lower trace) to a single intracellular stimulus of an abdominal giant fibre. Calibration: 0·4 mV., 10 msec.

degeneration of giant fibres was ascertained histologically. In such animals gentle tactile stimulation rostrally to the cut cord induced an evasive response. However, in these animals, the antennae were not thrown forward and, more noticeable, the animals ran into obstacles.

This behaviour led us to assume that the giant pathways are connected with a co-ordination–orientation system whose activity is manifested among other ways by forward movement of the antennae. Indeed, when recording electrodes were inserted at the base of an antenna, evoked electromyographic responses could be recorded to intracellular activation of a giant fibre (Fig. 6).

Often a single pulse to the giants was not sufficient to evoke a response at the base of the antenna, and a short burst of stimuli to the abdominal giants (Fig. 7, lower trace) was necessary to cause repetitive firing at the base of the antenna lasting for several seconds. Note that the first response at the base of the antenna started after

ten stimuli. Furthermore, activation of single giant axons with intracellular stimulating electrodes induced responses at the base of the antenna.

To be effective the forward thrust of the antennae must be a pre-evasion response. To check this point, simultaneous recordings were made from the leg nerve (N_5) of the metathoracic ganglion and from the base of the ipsilateral antenna (Fig. 8). In

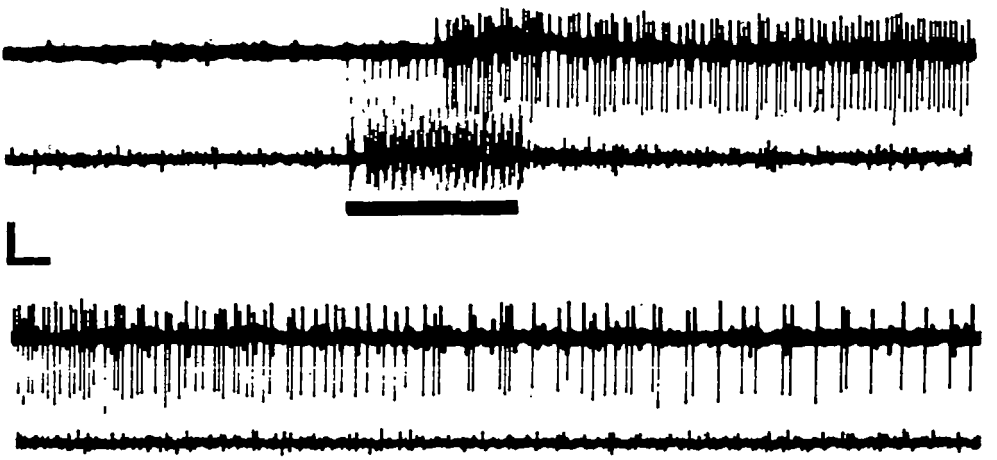

Fig. 7. Repetitive firing recorded at the base of an antenna (upper trace) to a short train of pulses at A_3–A_4. Duration of stimulus is marked by a horizontal bar. Note the responses of the abdominal giant fibres (lower trace) and that the firing at the base of the antenna starts only after ten stimuli and persists long after cessation of stimulation. The lower pair of recordings follows immediately the upper pair. Calibration: upper beam 200 μV., lower beam 40 μV., 50 msec.

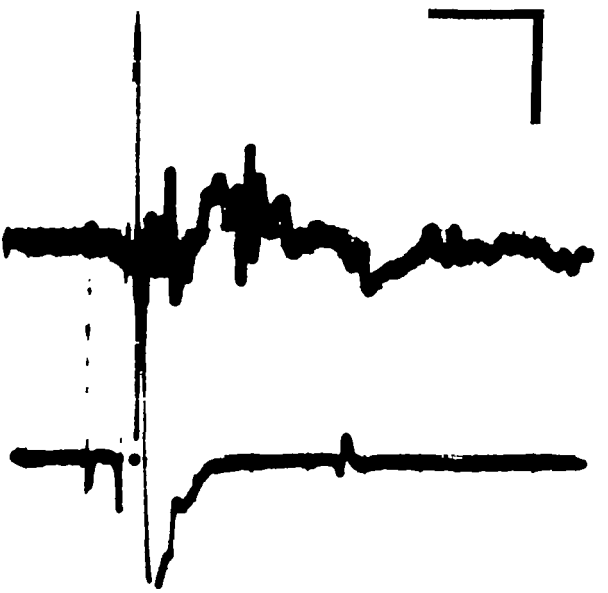

Fig. 8. Evoked potentials recorded at the base of an antenna (upper trace) and at N_5 (lower trace) to stimulation at A_3–A_4. First response from the antenna is marked by a dot. Note that the responses at N_5 and at the antenna appear with the same delay. Calibration: 1 mV., 10 msec.

spite of the distance being twice as long from the point of stimulation at A_5–A_6 to the head, simultaneous responses were recorded at both leg and head recording points. If a latency of 5–8 msec. is added for transmission from N_5 to the leg muscles (Roeder, 1948; Hughes, 1965) the movement of the antennae is well in the pre-evasive period.

The same experiments were conducted with animals whose antennae were cut at the base to eliminate sensory input. Again stimulation of the giant axons evoked activity recorded at the cut base of the antenna.

DISCUSSION

The results of the present study are in conflict with the assumption of Roeder (1948) that the abdominal giant fibres ascend the cord and excite the leg motoneurones. It seems that the cercal nerves also activate a slower conducting pathway with a conduction velocity of 1·5–2 m./sec. responsible for excitation of leg motoneurones. This conclusion receives further support from Roeder's behavioural experiments where a marked difference was found between calculated and measured startle latency. Certainly, the use of smaller conduction velocities in the A_6 to T_3 pathway reduces the discrepancies. Using Roeder's figures for startle times and the other components of the pathway, conduction velocities of 1–2 m./sec. give very good agreement; the extremely fast and extremely slow response times in his data do not. It is of interest that Cook (1951) found no cercal 'evasion response' in *Locusta* while successfully recording giant fibre activity in the abdomen.

In longitudinal sections of the cockroach metathoracic ganglion Farley & Milburn (1969) showed lateral branches of the giant axons which they interpreted as collaterals to leg motoneurones. It is difficult to explain these structures in view of our evidence and also since we have failed to demonstrate any sensory input at thoracic ganglia to the giants. These branches may also have other functions such as activation of interneurones, other extra appendage motoneurones or a 'clear all stations' function as suggested by Parnas *et al.* (1969).

The results of this study show that the giant fibres by themselves do not serve as excitors of the leg motoneurones, but they may pass on information conditioning these motoneurones for the following excitatory impulses relayed by the slower conducting pathway. Intracellular recordings from these motoneurones may provide an answer to this problem.

Since the giant axons do not appear to activate leg movements during escape, their true function remains to be shown. In the crayfish, Roberts (1968) has suggested that the giant fibres must 'terminate all other actions or postures and ensure that no further, competing reactions occur during the escape response'. Indeed, antennae grooming motions are arrested before evasion ensues in a normal cockroach. Furthermore, Farley & Milburn (1969) and Spira *et al.* (1969*a*, *b*) have shown that giant fibres ascend without interruption to the suboesophageal ganglion. Moreover, Dingle & Caldwell (1967) have reported brief bursts of spikes in multimodal interneurones in the protocerebrum to gentle touches or brief displacements of the cerci. Since this activity may be mediated earlier than leg movements according to our data, it could well be connected with the complicated organization of an escape response.

From histological observations it is evident that the giant fibres taper off as they

ascend through the thorax and reach the head as thin axons (Roeder, 1948; Farley & Milburn, 1969; Spira *et al.* 1969*b*). This gradual decrease in the diameter is accompanied by a decrease in conduction velocity, which may be important in providing a proper timing interval between cerebral activation and movements in the escape reflex.

It is postulated that a general alarm or arousal system is triggered by ascending giant-fibre impulses. This arousal system may include a command interneurone (Evoy & Kennedy, 1967) or a system of interneurones generating fast stereotyped behaviour such as a forward thrust of antennae, lifting of palpi and other mouth appendages, while inhibiting other conflicting actions.

SUMMARY

1. Giant fibres were found *not* to activate leg motoneurones during evasion.

2. A pathway of small axons having a conduction velocity of 1·5–3·5 m./sec. was found to govern leg activation during escape.

3. This pathway remains functional after giant-fibre degeneration after the giant axons have been severed from their somata.

4. Movements of the antennae were found to be activated by the giant fibres simultaneously or slightly earlier than movements of the legs.

5. It is suggested that a general alarm system is activated by the giant fibres concomitantly with activation of the leg motoneurones by a slower conducting pathway.

We wish to thank Dr R. Werman and Dr M. E. Spira for their helpful suggestions during the course of this work and for critical reading of the manuscript, and Miss I. Harari for technical assistance.

REFERENCES

Cook, P. M. (1951). Observations on giant fibres of the nervous system of *Locusta migratoria*. *Quart. J. Micr. Sci.* **92**, 297–305.

Dingle, H. & Caldwell, R. L. (1967). Multimodal interneurones in cockroach cerebrum. *Nature, Lond.* **215**, 63–4.

Evoy, W. H. & Kennedy, D. (1967). The central nervous organization underlying control of antagonistic muscles in the crayfish. I. Types of command fibres. *J. Exp. Zool.* **165**, 223–38.

Farley, R. D. & Milburn, N. S. (1969). Structure and function of the giant fibre system in the cockroach, *Periplaneta americana*. *J. Insect. Physiol.* **15**, 457–76.

Hess, A. (1958). Experimental anatomical studies of pathways in the severed central nerve cord of the cockroach. *J. Morph.* **103**, 479–502.

Hughes, G. M. (1965). Neuronal pathways in the insect central nervous system. In *The Physiology of the Insect Central Nervous System* (Papers from the 12th International Congress of Entomology held in London, 1964). Edited: Treherne, J. E., and Beament, J. W. L. London and New York: Academic Press.

Parnas, I., Spira, M. E., Werman, R. & Bergmann, F. (1969). Non-homogenous conduction in giant axons of the nerve cord of *Periplaneta americana*. *J. exp. Biol.* **50**, 635–49.

Pipa, R. L., Cook, E. F. & Richards, A. G. (1959). Studies on the hexapod nervous system. II. The histology of the thoracic ganglia of the adult cockroach, *Periplaneta americana* (L). *J. Comp. Neurol.* **113**, 401–23.

Pumphrey, R. J. & Rawdon-Smith, A. F. (1937). Synaptic transmission of nerve impulses through the last abdominal ganglion of the cockroach. *Proc. R. Soc.* B **122**, 106–18.

Roberts, A. (1968). Recurrent inhibition in the giant fibre system of the crayfish and its effect on the excitability of the escape response. *J. exp. Biol.* **48**, 545–67.

Roeder, K. D. (1948). Organization of the ascending giant fibre system in the cockroach *Periplaneta americana*. *J. exp. Biol.* **108**, 243–62.

SPIRA, M. E., PARNAS, I. & BERGMANN, F. (1969*a*). Organization of the giant axons of the cockroach *Periplaneta americana*. *J. exp. Biol.* **50**, 615–27.

SPIRA, M. E., PARNAS, I. & BERGMANN, F. (1969*b*). Histological and electrophysiological studies on the giant axons of the cockroach *Periplaneta americana*. *J. exp. Biol.* **50**, 629–34.

WILLOWS, A. O. D. (1967). Behavioural acts elicited by stimulation of single identifiable cells. *Science, N.Y.* **157**, 3788.

YAMASAKI, T. C. & NARAHASHI, T. (1960). Synaptic transmission in the last abdominal ganglion of the cockroach. *J. Insect Physiol.* **4**, 1–13.