A New Efferent Pathway in the Cockroach CNS

GUNTHRIE and TINDALL[1] refer that in some specimens of *Periplaneta americana* 'there appear to be motor branches' as part of the cercal nerve (NXI) which is predominantly a sensory nerve. By consulting the recent literature we were unable to find further work which would substantiate this finding. The present note is intended to report the results of experiments which prove the existence of a new efferent pathway in the cockroach CNS.

Adult male *Periplaneta americana*, 15–30 days old, bred under standard conditions, were dissected and the nerve cords were isolated by cutting the head and severing all peripheral nerves at the level of the cord ganglia. Both the intact cercal nerves and the cerci were removed together with the cord. A buffered saline[2] was employed. The cord was suspended on a series of silver-silver chloride electrodes mounted in a plastic chamber which was covered with glass slides to prevent drying of the preparation. A layer of saline at the bottom of the chamber provided adequate humidity. A couple of flying electrodes of the same type, moved by means of a Narishiga micromanipulator were placed at desired distances from the ganglia so as to measure conduction at known distances. The various positions of electrodes are summarized in Figure 1. All experiments were carried out at 24–26°C. Recordings were made with a 2A61 Tektronix Differential amplifier and 564 Tektronix Storage oscillograph. A laboratory-built stimulator provided voltage pulses.

The efferent pathway runs from the 1 a.g. (abdominal ganglia) to the cercal nerves. The fibers do not originate from the 3 t.g. (thoracic ganglia) nor from the ganglia cephalad to that because no response is obtained by stimulating either the connectives 2–3 t.g. or any one of the lateral nerves originating from 3 t.g.

The average conduction times and synaptic-like delays (Table) were obtained by stimulating the connectives at various positions and recording at R. The responses at R following stimulation at S_{13} and S_3 are shown in Figure 2. The conduction velocity for the different connectives appears to be rather uniform, averaging 3.93 ± 0.10 m/sec, which corresponds to an average axonal diameter of 20 µm, as derived from velocities of conduction obtained by different workers[3]. The average synaptic-like delays are significantly higher at 1, 2 and 6 a.g., the reason for this being still to be determined. The response of recordings across each abdominal ganglion increases in amplitude from threshold (1.5 V) to maximal voltage (3.5 V) thus indicating spatial summation.

Response induced at any of the positions from S_3 to S_{13}, at R disappears if tetanic shock is given or if 10^{-4} *M* eserine is applied at any one of the abdominal ganglia, and reappears after washing. In operated cockroaches kept alive for 30 days after the connectives had been severed at different levels from S_4 to S_{12}, the response was in all cases maintained at R only if stimulation was applied beyond the abdominal ganglion immediately caudal to the severed connectives, and not if the connectives caudal stumps were stimulated, thus suggesting degeneration of the neurone involved. Therefore, by removing the 5 a.g., no response is obtained at the cercal nerves 30 days after operation. These experiments, added to those demonstrating the presence of synaptic-like delays across the abdominal ganglia, prove that these efferent fibers are interrupted at each a.g. by at least one synaptic junction.

With a maximal intensity of 3.5 V and 0.2 msec duration, a steady response is maintained at R, for at least 1 h

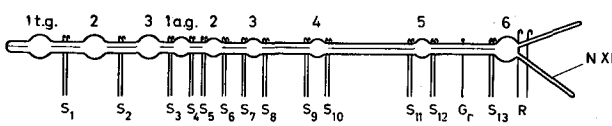

Fig. 1. Cockroach nerve cord (schematic): 1–3 t.g., thoracic ganglia; 1–6 ag.., abdominal ganglia. S_1 to S_{13}, position of stimulating electrodes on connectives; R, recording electrodes on cercal nerves; Gr, ground electrode.

[1] D. N. GUNTHRIE and A. R. TINDALL, *The Biology of the Cockroach* (E. Arnold, London, England 1968).

[2] K. D. ROEDER and E. A. WEIANT, *Methods of Testing Chemicals on Insects* (Ed. H. S. SHEPARD; Burgess, Minneapolis, USA 1958).

[3] T. NARAHASHI, Adv. Insect Physiol. *1*, 175 (1963).

a) Length of connectives, times of conduction and velocity in connective efferent fibers at various levels and b) delays across abdominal ganglia

a)

	1–2 a.g.	2–3 a.g.	3–4 a.g.	4–5 a.g.	5–6 a.g.	
Length[a] of connectives (mm) m	1.0	1.5	2.5	4.5	3.0	
Time of conduction (m/sec) $m \pm s_m$	0.25 ± 0.020	0.40 ± 0.063	0.69 ± 0.033	1.20 ± 0.20	0.77 ± 0.024	
Velocity of conduction (m/sec) $m \pm s_m$	4.10 ± 0.30	4.19 ± 0.71	3.63 ± 0.18	3.76 ± 0.07	3.91 ± 0.14	Average of $m \pm s_m$ 3.93 ± 0.10

b)

	1 a.g.	2 a.g.	3 a.g.	4 a.g.	5 a.g.	6 a.g.
Delay across abdominal ganglia (m/sec) $m \pm s_m$	1.30 ± 0.18	1.20 ± 0.016	0.75 ± 0.021	0.65 ± 0.21	0.70 ± 0.014	1.25 ± 0.022

[a] Fixed distances between electrodes

period, if stimulation is kept at S_9 at a frequency not superior to 0.1 c/sec. At a frequency of 1 c/sec, however, the response is unstable and disappears in 10 min, at 10 c/sec in 3–4 min and at 100 c/sec in a few seconds. The same applies for the analogous junctions of the other abdominal ganglia.

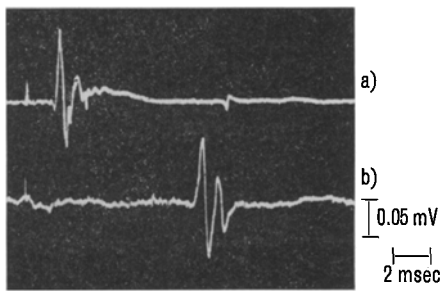

Fig. 2. Response at R following stimulation at S_{13} (a) and S_3 (b). See Figure 1.

The function of this efferent pathway is still to be investigated. A detailed account of this work will be published elsewhere.

Riassunto. Viene descritta una nuova via efferente della catena ganglionare di *Periplaneta americana*, che origina nel 1° ganglio addominale, raggiunge i nervi cercali (NXI) ed è interrotta da sinapsi in ciascuno dei sei gangli addominali. Vengono riferiti la velocità di conduzione nei connettivi ed i tempi di trasmissione a livello dei gangli.

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The Effect of Picrotoxin and Strychnine upon Inhibition of Fusimotor Neuron Discharges Caused by Cutaneous Fiber Stimulation

It was reported by Hunt and Paintal and by Koizumi et al.[1] that some spontaneously firing fusimotor neurons are inhibited for up to several hundred milliseconds following stimulation of various peripheral nerves: most evident with cutaneous nerve stimulation. The present experiments have been designed to observe the effects of picrotoxin and strychnine upon the fusimotor inhibition, since the actions of these two convulsants upon synaptic transmission in the spinal cord appear to be different[2].

Experiments were performed on 16 cats. Under ether anesthesia both carotid arteries were ligated and the trachea was cannulated. The lumbosacral cord was exposed by laminectomy in the usual manner and the L_7 or S_1 ventral root was cut intradurally at its exit from the dural cavity. After the spinal cord was transected at the atlanto-occipital membrane ether was discontinued and respiration was maintained artificially. The central cut end of dissected ipsilateral sural nerve was mounted on silver stimulating electrodes. The L_7 or S_1 ventral root was split into fine filaments containing only one spontaneously firing fusimotor fiber and these filaments were mounted on a pair of silver recording electrodes. Criteria for identification of γ motoneuron is described elsewhere[3].

After the animals were immobilized by i.v. injection of gallamine triethiodide (Gallamine, Teisan, Co.), a saline solution of picrotoxin (0.2 mg/ml) or strychnine nitrate (0.02 mg/ml) was slowly injected in 1 min into the radial vein. The drug was applied at least 3 h after the cessation of ether anesthesia.

Results. In preliminary experiments on 7 units, it was found that the inhibitory effects were strongest when the sural nerve was stimulated at around 7 to 10 times the threshold of the largest fibers. Thereafter, the observation was made at about 10 times threshold where the strength was sufficient to produce clear inhibitory effect, the duration of the rectangular pulses being 0.05 msec and the frequency 0.3 per sec.

One representative example of the effect of picrotoxin on a spontaneously firing fusiomotor neuron is shown in Figure 1A. The firing intervals became more irregular at the dose of 0.4 mg/kg (Figure 1Ab), the number of spikes

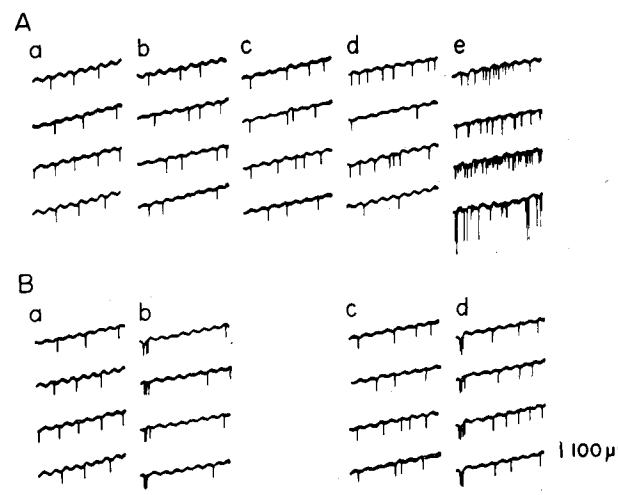

Fig. 1. A) The effect of picrotoxin upon spontaneous firing of a fusimotor neurone. a, control records; b, c, d, e, after injection of picrotoxin at the doses of 0.4, 0.6, 0.8 and 1.0 mg/kg, respectively. At 1.0 mg/kg convulsive discharges of both large and small neurones appeared (e). B) The effect of picrotoxin on inhibition of the fusimotor neurone shown in A. a, b, before injection of picrotoxin; a, spontaneous discharge; b, the spontaneous discharges ceased for about 134 msec on stimulation of sural nerve, stimuli being applied at the beginning of the sweeps; c, d, after picrotoxin injection of 0.6 mg/kg; d, the period of inhibition was shortened to about 74 msec. One sweep represents 180 msec.

[1] C. C. Hunt and A. S. Paintal, J. Physiol., Lond. *143*, 195 (1958). – K. Koizumi, J. Ushiyama and C. McC. Brooks, Am. J. Physiol. *200*, 694 (1961).

[2] D. R. Curtis, in *Structure and Function of Inhibitory Neuronal Mechanisms* (Ed. C. von Euler, S. Skoglund and U. Soderberg; Pergamon Press, New York 1968), p. 429.

[3] M. Kato and J. Tanji, Brain Res. *30*, 385 (1971).