Hoyle (1974) showed that the myogenic rhythm in locust proximal extensor tibiae muscle could be inhibited by a DUM neuron. Hoyle *et al.* (1974) examined the mode of action of this 'identifiable' DUM neuron (DUMETi³). More recent studies have examined the distribution of biogenic amines (*eg.*, octopamine) in DUM neurons and mushroom bodies of the CNS (locust & cockroach, Evans, 1978a; Dymond & Evans, 1979); specific reinnervation of coxal depressor muscles by T3 DUM neuron(s) (cockroach, Denburg & Barker, 1982); and a re-examination of branch patterns of metathoracic DUM neurons (cockroach, Arikawa *et al.*, 1984).

Cockroach and locust T3 DUM neurons have many physiological and morphological properties in common. The somata of T3 DUM neurons in both animals produce large, overshooting action potentials (60-110 mV) usually of 2-5 ms duration followed by a long duration after-hyperpolarization (30-50 ms; Crossman *et al.*, 1971; Goodman & Heitler, 1979). The somata may be spontaneously active (0.2-0.5 Hz - higher in locust DUMETi than cockroach DUM neurons). Alternatively, an AP can usually be evoked by direct stimulation of the soma or by stimulating a peripheral nerve root that contains an axon of the impaled DUM neuron. (The latter method of stimulation results in an antidromic AP which conducts back to the soma and also down the contralateral DUM axon (Crossman *et al.*, 1972; Evans & O'Shea, 1978).

³ DUMETi is a DUM neuron in the metathoracic ganglion of the locust. Its sole peripheral target is the extensor tibiae muscle of the hindlimb.

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EFFECTS OF DDT ON
CENTRAL NERVOUS SYSTEM ACTIVITY IN THE COCKROACH,
Periplaneta americana (L.)

by

ANDREW JAMES ELIA, B.Sc., M.Sc.

A thesis submitted to the Faculty of
Graduate Studies and Research in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

Department of Biology
Carleton University
Ottawa, Ontario
CANADA

May 29, 1989

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submitted by

ANDREW JAMES ELIA, B.Sc., M.Sc.

in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

Chairman, Department of Biology

Thesis Supervisor

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Carleton University

June 30, 1989.

ABSTRACT

1. The effects of an LD50 96 h dose of *p,p'*-DDT on activity in the nervous system of the cockroach, *Periplaneta americana* (L.), were investigated using extracellular and intracellular recording techniques. Nerve activity was evoked by air currents directed at the cerci or by electrical stimulation of the abdominal ventral nerve cord (AVNC).
2. Use was made of both semi-intact and isolated nerve preparations to investigate: (1) if activity in the CNS of sublethally poisoned animals could be related to behaviour at various times after injection of DDT, and (2) if 'filtering' of DDT-induced extraneous activity in the CNS might occur. Recruitment of a dorsal unpaired median (DUM) neuron during stimulation for the escape response was also investigated.
3. 24 h after DDT treatment, the behavioural signs of poisoning had declined but DDT-induced repetitive firing in the AVNC continued.
4. Localized perfusion of the 5th abdominal ganglion with 4×10^{-5} M DDT produced repetitive firing in the AVNC but not in metathoracic motor nerves 5r1 or 6Br4. This suggested that 'filtering' of the DDT-induced extraneous activity could occur in the metathoracic ganglion.

5. The role that dorsal unpaired median (DUM) neurons might play in 'filtering' extraneous activity in the metathoracic ganglion was investigated.

6. Axon patterns of DUM neurons in the metathoracic ganglion were revealed by iontophoresis of Lucifer Yellow into DUM somata. Physiological and morphological evidence showed that only one DUM neuron (DUM5r1) sends an axon into the metathoracic coxal depressor muscle motor nerve (5r1).

7. Following stimulation of the cerci or AVNC, activation of cockroach DUM5r1 never preceded recruitment of motor neurons. Therefore, this evidence does not support the Orchestration Hypothesis proposed by Hoyle (1985b) for DUM neurons of locust.

8. It is suggested that DUM5r1 is not directly involved with 'filtering' extraneous, DDT-induced activity in the metathoracic ganglion of the cockroach.

ACKNOWLEDGEMENTS

I would like to thank the "Ottawa-Carleton Resource Centre for Biological Microscopy" at Carleton University for use of their darkroom and a fluorescent microscope. I am particularly grateful for the personal attention provided by Dr. Nathalie Chaly. I would also like to formally acknowledge the assistance of Mr. L. Speers (Dept. of Biology) and Carleton University Computing Services (Dr. B. Eastwood) for their help in preparing the statistical analyses of the data.

Drs. C. A. Barlow, S. L. Jacobson and M. McCully kindly provided lab space and/or equipment during my research. C.A.B. also translated parts of Nicklaus (1966) from German. Joan Mallett gave up many a lunch hour to help in the preparation of Figure 21. Her technical expertise (and Dr. McCully's indulgence) were very much appreciated. Gabriela Rushton helped with Figures 3 & 5 and Appendix 1. I thank her for her efforts and the quality results produced, despite given very little time. Dr. B. A. Pappas introduced me to "Sigmaplot", his computer imaging system, and some of the finer points of ice hockey (viz., elbowing, holding, boarding). C. A. Wilson's technical assistance, his preparation of various pieces of apparatus, troubleshooting and friendship were indispensable. Thanks Cliff.

I would be remiss if I failed to mention several other persons who have been prominent over the last few years.

Thanks to three women who have kept me on the straight and narrow (Jacquie Boudreau, Linda Langille and especially Sandy Spence). JoAnne Whittingham kindly provided space in 'her' lab. Ian Babcock cut through red tape for me on many occasions. Thanks to Dr. H. G. Merriam for his indulgence and belief. And J.L. and J.S., invest some of those big bucks in golf lessons.

And special thanks to that mysterious man (C.A.B.), who's usually in at work by the first 'farrow spart', but as elusive as *Canis lupus*.

Three colleagues - Borys Brezden, Peter Canney and Alex Juhasz - have made life in and around the lab interesting, enlightening, unpredictable, and quite enjoyable.

My thesis Advisors, Drs. S. L. Jacobson and C. E. Morris, survived too many committee meetings. Their help over the years and 'open door policy' were appreciated. A special note of thanks to Dr. Jacobson, whose equipment I borrowed and ear I 'bent' on many occasions.

My thesis Supervisor, Dr. D. R. Gardner, has generously provided support during the tenure of this thesis. His help and patience over the years have been instrumental in the completion of this thesis. My sincere thanks.

And finally, I'd like to thank my Parents for their unrelenting support.

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GLOSSARY OF SUPPLIERS

31 Gauge Needle

Popper and Sons, Inc., New Hyde Park, New York, 11040
U.S.A.

AC Preamplifier

Model P511-J AC-preamplifier with high impedance
probe (model HIP-5), Grass Medical Instruments
Co., Quincy, Mass. U.S.A.

Acetone

Reagent UV Spectrophotometric Grade, Anachemia
Chemicals Ltd., Toronto, Ont. Canada.

Air

Ultra Pure Air (UPC) - Linde, Ottawa, Ont. Canada.

Analog to Digital Converter

Tekmar analog to digital converter - see "Labmaster".

Carborundum Stone

Trade name for an abrasive of silicon carbide.

Chart Recorder

LinearCorder Mark VII, Watanabe Instrument Corp.,
Tokyo, Japan.

Computer

Compaq Deskpro (with Intel 8086 microprocessor,
640 kB RAM, 30 MB H.D.), Compaq Computer Corp.,
Houston, Texas, 77070 U.S.A.

DDT

p,p'-DDT and p,p'-DDE: Aldrich Chemical Co.
Inc., Milwaukee, Wisconsin.

Dissecting Microscope

DRC Stereomicroscope, Carl Zeiss, D-7082 Oberkochen,
West Germany.

Force Transducer

Model UC-2, Gould Inc., Measurement Systems Division,
Oxnard, California, U.S.A.

Force Transducer Amplifier

Model MA-1, BLB Electronics, Ottawa, Ontario, CANADA.

Gelman Micropore Filter

Gelman Metrical Membrane Filter (0.45 μ m pore size),
Gelman Sciences Inc., Ann Arbor, Michigan 48106 U.S.A.

Glass Tubing - No Filament

Corning Glass Works, Corning, New York, U.S.A.

Glass Tubing - With Filament

Borosilicate Glass Capillaries with Internal Filament for Intracellular Microelectrodes, Model 1B100F-4, Lot #10736-03G, World Precision Instruments, New Haven, CT 06513 U.S.A.

Hot Wire Anemometer

Hot Wire Anemometer, Model W141-4. Weather Measure Corporation, Sacramento, California.

Intracellular Recording Amplifier

Model 701, WP Instruments, New Haven, Conn., U.S.A.

Intramedic P.E. Tubing

Clay Adams, A Division of Becton Dickinson and Co., Parsippany, New Jersey, U.S.A.

Labmaster

Axon Instruments, Inc., Burlingame, California 94010 (TL-1 interface) and Scientific Solutions Incorporated, Solon, Ohio 44139-3377 (A/D converter and daughterboard) U.S.A.

Lucifer Yellow (CH)

Lucifer Yellow CH, Sigma Chemical Co., St. Louis, Missouri, U.S.A.

Microelectrode Puller

Model M1, Industrial Science Assoc., Inc., Ridgewood, New Jersey, U.S.A..

Micropore Filter - Disposable

Minisart NML (0.2 μ m), Sartorius, Federal Republic of Germany.

Minuten Pins

Carolina Biological Supply Co., Burlington, North Carolina, 27215 U.S.A.

MOPS Buffer

Sigma Chemical Co., St. Louis, Missouri, U.S.A..

Oscilloscopes

Tektronix Instrument Corp., Portland, Oregon, U.S.A..

Oxygen

Ultra Pure Oxygen (UPC) - Air Products, Ottawa, Ontario, CANADA.

Pal Pen

Model H-352, Rolf C. Hagen Inc., Montreal, P.Q.,
CANADA.

"PCLAMP" Version 4.0

Axon Instruments, Inc., Burlingame, California 94010
U.S.A.

"PECAN" Analysis Program

Basic language computer program written by Dr. B. L.
Brezden (Dept. of Biology, Carleton University) to sort
PCLAMP data for instantaneous burst frequency and burst
duration.

Racal Store-4 Tape Recorder

Lockheed Electronics Co., Inc., Plainfield, New Jersey,
U.S.A.

"SIGMAPLOT" Graphics Program

SIGMAPLOT Graphics Program (Version 3.1), Jandel
Scientific, Sausalito, California 94965 U.S.A.

Silver Paint (Conductive)

Conductive Silver Paint, JBS #711, J.B.EM Services
Inc., St. Laurent, P.Q., CANADA.

Skinner Solenoid Valve

Honeywell Ltd., Toronto, Ont. CANADA.

Spike Analyzer

Model Sta-1, Medical Systems Corp., New York, U.S.A.

Stimulator

Model SD48 Stimulator, Grass Medical Instruments Co.,
Quincy, Mass., 02169, U.S.A.

Stimulus Isolation Unit

Model SIU-5, Stimulus Isolation Unit, Grass Medical
Instruments Co., Quincy, Mass.

"SYMPHONY" Spreadsheet Computer Program

SYMPHONY Spreadsheet Program, Lotus Development
Corporation, U.S.A.

ABBREVIATIONS

- A6:** the sixth (terminal) abdominal ganglion.
- A5-:** the fifth abdominal ganglion and associated connectives.
- ABX:** anode break excitation.
- A/D:** analog to digital.
- AHP:** after-hyperpolarization (of action potential).
- AP(s):** action potential(s).
- AVNC:** abdominal ventral nerve cord.
- CAP:** compound action potential.
- CDM:** coxal depressor muscles.
- CI:** (widespread) common inhibitor.
- CNS:** central nervous system.
- CTF (joint):** coxal-trochanter-femur joint.
- D₁₋₃ or I₁₋₃:** inhibitory axons in N5r1.
- D_f:** fast depressor motor neuron of the metathoracic ganglion - branch contained in N5r1 to CDM.
- D_s:** slow depressor motor neuron of the metathoracic ganglion - branch contained in N5r1 to CDM.
- DUM:** dorsal unpaired median - refers to neurons that are not paired and reside in a dorso-medial position within a ganglion.
- DUM5r1:** dorsal unpaired median neuron of the metathoracic ganglion that has an axon branch in N5r1 (to coxal depressor muscles).
- DUMETi:** dorsal unpaired median neuron that sends an axon to the extensor tibiae muscle of the hindlimb of some orthopterans (eg., locusts, "weta", but not cockroach). The extensor tibiae muscle is the sole target of this DUM neuron.
- ETi:** extensor tibiae muscle.
- GA(s):** giant axon(s). If "v" or "d" preceeds GA, then it refers specifically to a "ventral" or

"dorsal" GA (eg., vGA).

IPS: inches per second.

L.E.D.: light emitting diode.

LY: Lucifer Yellow carbohydrazide. A highly fluorescent naphthalimide dye that was used to identify DUM neurons.
(dilithium 6-amino-2-[(hydrazinocarbonyl)amino]-2,3-dihydro-1,3-dioxo-1H-benz[de]isoquinoline-5,8-disulfonate; see Stewart, 1978.)

MN: motor neuron.

ms: milliseconds.

N5r1(R & L): refers to the nerve which innervates coxal depressor (extensor) muscles of the hindlimb (right and left) - the fifth nerve root, ramus 1.

N6Br4(R & L): refers to the nerve which innervates coxal levator (flexor) muscles of the hindlimb (right and left) - the sixth nerve root, branch B, ramus 4.

non-DUM5r1: dorsal unpaired median neuron of the metathoracic ganglion which does not have an axon branch in N5r1.

PSI: pounds per square inch.

RP: resting potential.

SD: standard deviation.

SNS: stomatogastric nervous system.

$t_{0.5}$: half-time.

T3: the metathoracic ganglion.

$\dot{V}_{max}$: rate of rise of an action potential.

VNC: ventral nerve cord.

WCI: widespread common inhibitor.

WP(s): wind puff(s).

INTRODUCTORY COMMENTS

1. Paragraphs inset from the normal left margin and in smaller type are of interest but not directly pertinent to the text.
2. Names of manufacturers of apparatus used and various suppliers are listed in the "Glossary of Suppliers", and are generally not included in the "Materials and Methods" sections.
3. There are two characteristic size classes (defined by soma diameter as large or small) of dorsal unpaired median (DUM) neurons in the metathoracic ganglion of the cockroach. In this thesis, I have dealt exclusively with DUM neurons of the large size class.
4. DUM5r1 refers to a metathoracic DUM neuron of the large size class which sends an axon into nerve root 5, ramus 1 (N5r1) of the metathoracic ganglion. Other DUM neurons studied, without an axon in N5r1, are referred to as "non-DUM5r1".

INTRODUCTION

Cockroaches injected with a sublethal dose of DDT often recover behaviourally (between 24 and 48 h post-injection) from signs of DDT poisoning. However, electrophysiological examination of excised ventral nerve cords (VNCs) from these "behaviourally normal" cockroaches still showed the abnormal symptoms of repetitive firing (RF) typical of poisoning by DDT (Elia, 1982; Elia & Gardner, 1984).

These earlier experiments left two questions unanswered.

(1) Did the *in vitro* recorded repetitive firing also occur *in vivo* (at various times after DDT injection)?

(2) Does a neural mechanism exist to account for the apparent dissociation of behavioural and neurophysiological signs of poisoning?

Part I of this thesis was designed to address the first question using a semi-intact preparation that allowed recording from several points from the *in situ* nervous system simultaneously at various times after DDT treatment. Part II investigates the possibility that a dorsal unpaired median (DUM) neuron in the metathoracic ganglion (T3) regulates or modulates motor outputs to the cockroach coxal muscles. The effects of DDT on this neural circuit were also examined.

1. General

The predominant characteristics of the cockroach central nervous system (CNS) are its segmented nature and decentralization of control processes (Pipa & Delcomyn, 1981). Major centres responsible for processing sensory input, motor output and visceral function occur in the brain, thoracic and abdominal ganglia and the stomatogastric ganglia (Fig. 1) (Chapman, 1971; Pipa & Delcomyn, 1981).

The stomatogastric nervous system (SNS) is distinct from the thoracic and abdominal ganglia that comprise the ventral nerve cord (Fig. 1a). The SNS is primarily concerned with digestion and neurosecretion (Mordue & Goldsworthy, 1967; Bignell, 1981). Most of its neurons originate in the frontal ganglion and protocerebrum and innervate the retrocerebral neuroendocrine complex (RNC - corpus cardiacum and corpus allatum), foregut, posterior crop region, salivary glands, and presumably the midgut (Guthrie & Tindall, 1968; Brown & Nagai, 1969; Whitehead, 1971; Bignell, 1981). Brown & Nagai (1969) showed that innervation of the hindgut arises from the terminal abdominal ganglion (A6).

Although the SNS is not considered further in this thesis, it is mentioned here because of its probable involvement in poisoning by neuroactive pesticides. There is strong evidence to suggest that pesticides like DDT and

Figure 1: (a) Diagram showing the major connections of the stomatogastric nervous system (SNS) of the cockroach. Note that for clarity the nerve (ncal) connecting the corpus cardiacum (CC) and corpus allatum (CA) is extended and the CA displaced down (ventrally). If included in this diagram, the VNC would begin at the SOG and extend to the right. (A more detailed outline of the SNS is given by Penzlin (1985).) (b) A dorsal view of the brain (SAG), VNC and cerci of the cockroach. Some details of peripheral nerve roots are included for the metathoracic ganglion (T3 - see also Appendix 1). Extracellular recordings were made from N5r1 and N6Br4 (T3) and the AVNC (A1-A6). Intracellular recordings were made from DUM neurons located in T3. ((a) after Bignell (1981).)

Key: CA, corpus allatum; CC, corpus cardiacum; FG, frontal ganglion; IG, ingluvial ganglion; PG, proventricular ganglia (two); SAG, position of supraoesophageal ganglia (proto-, deuto-, and tritocerebrum); SOG, suboesophageal ganglion; I-III, nervi corporis cardiaci; an, aortic nerve; fc, originally pars frontalis (Nesbitt, 1941), renamed frontal connective (paired); ln, labral nerve (paired); nc, nervus connectivus; nca, nervus corporis allati; oen, oesophageal nerve; oesn, oesophageal salivary nerve; rn, recurrent nerve; sdn, salivary duct nerve; sog n, sub-oesophageal to corpus allatum connective; T1-T3, thoracic ganglia; A1-A6, abdominal ganglia; numbers around T3 in (b) refer to nerve roots.

pyrethroids, which tend to have a general effect on axonal excitability, involve the SNS in the overall expression of the poisoning syndrome (Samaranayka, 1974, 1978; Orchard & Osborne, 1979; Orchard, 1980; Singh & Orchard, 1982).

2. Central Nervous System (CNS)

The brain, thoracic and abdominal ganglia are joined together by "paired interganglionic connectives" to form the ventral nerve cord (VNC) (Fig. 1b). The VNC and its branches to the periphery are surrounded by the neural lamella - a connective tissue sheath composed of collagen and various glycosaminoglycans (dermatin, keratin, and chondroitin sulphates) (Ashhurst & Costin, 1971a,b; Pipa & Delcomyn, 1981; Ashhurst, 1984). The neural lamella seems to function primarily as a support structure and does not act as a barrier to ions and water soluble molecules (*eg.*, La^{3+} ; micro- and macro-peroxidases (1.8 and 40 kD, respectively) (Treherne & Pichon, 1972; Lane, 1974; Treherne, 1974).

(a) Blood-brain barrier

The *perineurium* lies immediately beneath the neural lamella. It is a layer of overlapping cells which surrounds the CNS and is believed to be responsible for the blood-brain permeability barrier (Fig. 2). Tight junctions (zonula occludens) associated with this structure were first

Figure 2: (a) Left - Connectives of the cockroach CNS. The outer layers contain the neural lamella and perineurium which surround other glia and the axons. Right - Schematic of the close associations among the various cells in the outer layers of the CNS. The tight junctions (t.j.) are believed to be responsible for the blood-brain barrier. (g.j., gap junctions; s.j., septate junctions - from Treherne & Schofield, 1981.) (b) "Major features of a typical insect ganglion". The cortex contains the neural lamella, perineurium and nerve cell bodies. In the neuropile, synaptic contacts are made and integration of various inputs occurs. Axon tracts and interneurons are not visible in this figure. (from Pitman, 1985.)

described by Lane and Treherne (1972) and are thought to be responsible for the perineurial blood-brain barrier. This was demonstrated both electrophysiologically (Schofield & Treherne, 1984; Schofield *et al.*, 1984a,b; Hendy & Djamgoz; 1987) and in diffusion studies in which La^{3+} and peroxidases were shown not to reach beyond the inner clefts between perineurial cells (Lane & Treherne, 1972a; Treherne *et al.*, 1973; Lane, 1974; 1981).

Without the perineurium, the CNS would be subjected to the fluctuating ionic environment of the haemolymph (Treherne & Pichon, 1972; Burrows, 1979). One of the consequences of having a blood-brain barrier as found in some insects is that the inner reaches of the neuropile may be as much as 1 mm away from the haemolymph. This distance is too great to rely on diffusion from the haemolymph to supply the immediate requirements of the CNS for nutrients and oxygen. Instead, a complex network of tracheoles delivers air to all tissues. In a ganglion, tracheoles ramify to such an extent that no neuron is more than about 9 μm away from a tracheole (Burrows, 1979). The numerous neuroglia that surround most neurons (Treherne, 1985) are believed to assist in the homeostatic control of the ionic environment of the CNS and may also provide nutrients and remove metabolic biproducts from nerve cells (Treherne, 1979; Pipa & Delcomyn, 1981). Recently, it was suggested that glia may play an important role in the development of the moth CNS (Tolbert & Oland, 1989).

The interdigitating nature and the orientation of the lateral membranes of perineurial cells make it difficult to firmly establish the existence of true tight junctions (Lane, 1981). Lane cited several examples of putative tight

junctions from the literature whose authenticity was questionable. However, the "tight junction hypothesis" first proposed by Lane and Treherne (1972) has now been questioned as the source of the permeability barrier (Shaw & Henken, 1984a,b). Contrary to previous reports (see Lane, 1981), Shaw and Henken found that La^{3+} penetrated between and behind perineurial cells to a previously unreported, monolayer of cells ("sheath cells") situated between the neuroglia (those glia that are directly associated with axons) and perineurium (Shaw & Henken, 1984a,b). These sheath cells formed the outer edge of the barrier to La^{3+} . Furthermore, no evidence was found to support the existence of tight junctions in any of these or between perineurial cells. They asserted that the severe constrictions and lengthening of the extracellular space between cells was sufficient to restrict the access of ions and water-soluble molecules to the inner neuroglia and axons (Shaw & Henken, 1984a,b).

Thus, the exact location and structural identity of the elements responsible for the blood-brain barrier are still in question.

The blood-brain barrier remains intact in a carefully isolated cockroach CNS until it is treated with hypertonic urea (3 M, Schofield *et al.*, 1984b) or the neural lamella is surgically removed (see Schofield & Treherne, 1984).

(b) *'Macroscopic' anatomy of the CNS*

The ganglia of the cockroach (and insecta in general) are made up of a central neuropile and a peripheral cortical region (Fig. 2) (Lane, 1974).

i) Cortex

The outer, cortical region of the ganglion contains the cells responsible for the blood-brain permeability barrier (see above) and the cell bodies of neurons which originate in that ganglion. These cell bodies may belong to interneurons, modulatory neurons or motor neurons. They supply axons that remain within the ganglion of origin (local interneuron), or have an axon that travels between the ganglia of the CNS or that terminates at a peripheral muscle cell. (The paired, interganglionic connectives contain only axons and no neuronal cell bodies.) Most somata are positioned ventrally or ventrolaterally in the cortex (Pipa & Delcomyn, 1981). However, dorsal unpaired median (DUM) neurons and the slow depressor motor neuron (D_S) are positioned dorsally (Fourtner & Pearson, 1977). Consequently, the ventral cortex is considerably thicker than the dorsal cortex (Lane, 1974).

The cell bodies appear to serve a trophic rather than communicative or integrative role, as there are no direct somal synaptic contacts made in the cortex (Burrows, 1980; Treherne, 1980; Pitman, 1985). However, some somata are known to produce substances which are used in

neurosecretion, in which case they could chemically 'communicate' with other cells (Tobe & Stay, 1981).

ii) Neuropile

The neuropile contains glial cells (neuroglia), axon tracts, bundles of axons and dendrites of neurons. The neuropile is devoid of neuronal cell bodies. This is the region within the ganglion where direct communication between nerve cells and integration of inputs occurs (Siegler, 1984).

The juxtaposition of glial cells to axons within the neuropile is quite variable. Some small axons lie naked, while a group of medium sized axons may share the same glial cells (Lane, 1974). Some larger axons (such as the giant axons) are individually wrapped by at least several layers of glial cells (Lane, 1974; Pipa & Delcomyn, 1981). The extracellular spaces that remain between the neuroglia and axons or adjacent neuroglia tend to be extremely narrow (16 μm or 10-20 μm , respectively), except for regions that expand into "lacunae" (Treherne, 1974; Treherne & Schofield, 1981; Treherne, 1985). It has been suggested that the lacunae act as extracellular cation reservoirs and thus help to regulate the microenvironment of the CNS. This is based on evidence that these areas contain hyaluronic acid which forms an anion matrix in the lacunae (Ashhurst & Costin, 1971b; Treherne & Schofield, 1981; Ashhurst, 1984)).

An interesting calculation made by Treherne and Schofield (1981) showed that the amount of Na^+ immediately available to a giant axon could only support about 30 action potentials (APs). If the supply was expanded to include the Na^+ available in the long, single mesaxon cleft (see Fig. 2) then the maximum frequency of firing would still be severely limited by the long half-time required to equilibrate the two sources of Na^+ ($t_{0.5} = 1.2 \text{ s}$ - Treherne & Schofield, 1981). Also, from evidence based upon Na^+ pumping by squid axons, they suggested that axonal Na/K exchange alone could not maintain sufficient Na^+ in the extracellular space to support the characteristic high frequency of APs recorded in giant axons. Thus, it seems more likely that there are a number of active and passive processes (axonal and glial Na/K exchange, buffering by the anion reservoir) that to allow the axons to function under the restrictions imposed by a confined environment.

3. Identified Metathoracic Neurons

Cohen and Jacklet (1967) produced an elegant, three-dimensional representation of the positions that "motor" neuron somata occupy in the cockroach metathoracic ganglion. They mostly employed the procedure of Wallerian degeneration (Fourtner & Pearson, 1977; *i.e.*, somatic RNA ring formation) to identify the motor neurons of a particular nerve root. Their work indicated that many of the neurons (about 100 somata with diameter $> 20 \mu\text{m}$) are arranged in bilaterally symmetrical pairs and that the position of these somata in different animals was very similar (Cohen & Jacklet, 1967).

The introduction and perfection of injection techniques with cobalt (Pitman *et al.*, 1972; 1973 by iontophoresis into

soma and backfilling; Gorcs *et al.*, 1979 by backfilling) or Lucifer Yellow (Stewart, 1978; Bishop, 1980) allowed particular somata within the metathoracic ganglion (T3) to be associated with functionally identifiable motor neurons. (In cockroach, most functionally identified motor neurons are involved in walking (Fourtner & Pearson, 1977) or flight (Ritzmann *et al.*, 1983).)

Ritzmann & Camhi (1978) examined neurons in T3 that were activated during the escape or startle response. Specifically, this included neurons which contribute axons to nerve 5r1 and nerve 6Br4 (nerve notation after Pipa & Cook, 1959). Nerve 5r1 and 6Br4 innervate antagonistic groups of muscles in the coxa, which when activated, cause extension (depression) and flexion (levation or protraction) of the femur, respectively (Pearson & Iles, 1971; Ritzmann & Camhi, 1978). Both N5r1 and N6Br4 are active during the initial and continued response to a startle stimulus (for example, air currents directed at the cerci or electrical stimulation of the abdominal VNC (AVNC)). Thus, recording from these nerves is a sensitive monitor of behaviour.

(a) *Motor neurons and muscles associated with N5r1*

i) Motor neurons

Nerve 5r1 of the metathoracic ganglion (T3) contains an axon of the fast depressor motor neuron (D_f , diameter 25 μm), the slow depressor motor neuron (D_s , diameter

15 μm), three inhibitory motor neurons (D_{1-3} or I_{1-3} , diameter 5 μm), and at least one DUM neuron ($\leq 5 \mu\text{m}$ - Pearson & Iles, 1971; Iles, 1972; Chesler & Fourtner, 1981; Denburg, 1982). (Several fine axons ($< 1 \mu\text{m}$ diameter) remain to be identified (Chesler & Fourtner, 1981).)

The somata of D_f and the inhibitory fibres are located ventrally within T3 whereas the somata of D_s and the DUM neurons are located dorsally (Pitman *et al.*, 1972 (D_f); Pearson and Fourtner, 1973 (D_s , D_{1-3}); Plotnikova, 1969; Crossman *et al.*, 1971, 1972 (DUM neurons)).

ii) Innervation of coxal depressor muscle

It has been demonstrated that there is very little variation not only in the somatic position of the neurons of N5r1 but also in the innervation pattern at the level of the coxal depressor muscle (Pearson & Fourtner, 1973; 1975; Denburg, 1982). Coxal depressor muscles (CDM) 177d, 177e, 177d', 177e', 178 and 179 are all innervated by axons contained in N5r1 (Fig. 3 and Appendices 1a & b) (muscle notation after: Carbonell, 1947; Pearson & Iles, 1971). CDM 177d and 177e are classified as slow muscles and each receives an axon from the slow depressor motor neuron (D_s), common and widespread inhibitory fibres (D_{1-3}) and at least one DUM neuron. The other coxal depressor muscles (177d', e'; 178; 179) do not appear to receive axons from the inhibitors (Pearson & Iles, 1971). Muscles 177d' and e' receive mixed slow (D_s) and fast (D_f) innervation. The only

Figure 3: Diagram showing the ventral surface of the cockroach in the area of the metathorax. The right leg has been raised to show the dorsal surface of the coxa. Nerves 5r1 and 6Br4 and some of the associated muscles (177e, 178 depressors; 182a,b levators) are also shown. See Appendix 1(a,b) for a more detailed view of the innervation patterns and associated muscles of N5r1 and N6Br4. (ANT, anterior; Tro, trochanter) (After Pearson & Iles, 1971.)

identified source of input to muscles 178 and 179 is from D_f (Chesler & Fourtner, 1981; Ritzmann & Pollack, 1981; Denburg, 1982). However, Denburg and Barker (1982) suggest that the intermediate and fast CDM may also receive some innervation from a DUM neuron. (A similar pattern of DUM neuron innervation to mixed and fast fibre-type muscles has been described in the extensor tibiae muscle of the locust hindlimb (Evans, 1985).)

Octopamine has been suggested as the transmitter substance of cockroach (and locust) metathoracic DUM neurons (Evans, 1978a,b; O'Shea & Evans, 1979; Evans, 1981; Denburg & Barker, 1982; Evans & Siegler, 1982; Morton & Evans, 1984). Both DUM neuron somata and axon terminals have been shown to synthesize octopamine (Hoyle & Barker, 1975). Using production of [3H]-octopamine in coxal depressor muscles (CDM) as a measure of the intensity of DUM axon terminal innervation, Denburg and Barker (1982) found that CDM 177d and 177e (which receive only slow motor, inhibitory and DUM neurons) contained the highest level of [3H]-octopamine. The amount of [3H]-octopamine found in the muscle with mixed fast and slow innervation (177d',e') was about eight times less. The fast CDM (178, 179) contained about half of the amount found in intermediate fibres (Denburg & Barker, 1982). After axotomy and regeneration of N5r1, the same unequal distribution of octopamine terminals was restored. Thus, the majority of DUM neuron axon

terminals appear to be located in muscles 177d and 177e (slow (tonic) muscle).

A similar differential distribution of DUM neuron terminals appears to exist in the extensor tibiae muscle of locust hindlimb. Regions of primarily slow and/or intermediate fibre types gave the strongest responses (increased amplitude of twitch tension, increased rate of relaxation, increased c-AMP) to perfusion with octopamine (Evans, 1985). However, although twitch tension of the fast muscle fibres was apparently not affected by octopamine, the relaxation rate was increased in all regions of the muscle innervated by the fast extensor motor neuron. An increase in amplitude and rate of twitch relaxation has also been recorded from the dorsal longitudinal flight muscle of locust in response to perfusion with octopamine or stimulation of DUMDL¹ (Whim and Evans, 1988; Malamud et al., 1988).

Most studies concerned with the effects of octopamine or DUM neuron excitation on the mechanical properties of muscle contraction have used locust extensor tibiae or dorsal longitudinal flight muscles. However, Chesler and Fourtner (1981) speculated that a DUM neuron might have evoked an increase in residual tension in their cockroach coxal depressor muscle (177d) preparation.

Electrical stimulation of nerve root 5 at an intensity far greater than that required to recruit excitatory and inhibitory fibres, consistently increased residual tension in CDM 177d. (This increase was not observed when motor fibres alone were

¹ DUMDL is thought to be the sole DUM neuron that innervates the dorsal longitudinal flight muscles of the locust (Hoyle, 1978; Watson, 1984; Whim & Evans, 1988).

excited.) Because of the very small diameter of DUM axons and the high intensity of stimulation required, it was suggested that it might have been the DUM neuron activating the residual tension (Chesler & Fourtner, 1981).

Some support for this increase in residual (or basal) tension was published in a series of abstracts (Tanaka & Washio, 1984; 1985; Washio & Tanaka, 1986). (However, this work has yet to be published as a full paper.) But, it has been shown that proctolin may be co-released with the putative neurotransmitter L-glutamate from the slow depressor motor neuron (Ds) in CDM of cockroach (Adams & O'Shea, 1986). The effect of proctolin (Adams & O'Shea, 1983; Adams & O'Shea, 1986) on the CDM was to cause a prolonged increase in the basal tension, similar to that seen by Chesler and Fourtner (1981). A similar response to proctolin has also been observed in locust extensor tibiae muscle (May *et al.*, 1979; Evans, 1984) and in the lobster opener muscle preparation (Kravitz *et al.*, 1984). Therefore, without further investigations, the role played by octopamine and/or proctolin in the contractile properties of cockroach skeletal muscle remains unclear.

It appears that some similarities exist in the patterns of DUM neuron innervation in cockroach coxal depressor (Denburg & Barker, 1982) and locust extensor tibiae (Evans, 1985a) muscles. The effects of octopamine on cockroach

muscle have not been well enough defined to make a valid comparison with locust. However, given the homologies that do exist (CNS, peripheral innervation patterns, octopamine receptors) it seems likely that some of the effects of octopamine (perhaps released from DUM neurons) on cockroach muscle might be similar to those of locust.

(b) *Motor neurons and muscles associated with N6Br4*

i) Muscles innervated

As with N5r1, the identified somata associated with axons of N6Br4 remain in about the same position in different individuals (Pearson & Iles, 1970; Fourtner & Pearson, 1977). The muscles innervated by N6Br4 are responsible for causing flexion movements of the coxa-trochanter-femur (CTF) joint during leg protraction (swing phase or levation) (Hughes, 1952). The flexor muscles of the metathoracic coxa associated with this movement are the anterior and posterior main coxal levators (181B,C & 181A/182A-D, respectively) (Pipa & Cook, 1959; Appendices 1a & b). Anterior coxal levator 180 (innervated by N3b) does not appear to actively contribute to the flexion of the CTF joint (Pearson & Iles, 1970).

ii) Motor neurons

Unlike innervation of the coxal depressor muscles by N5r1, innervation of the coxal levators is quite complex.

There are at least 12 known motor neurons (excitatory and inhibitory) that send axons in N6Br4 to muscles 181 and 182 (Fourtner & Pearson, 1977). This complexity of innervation is the antithesis of the relatively 'simple' dendritic branch patterns of the flexor motor neurons observed within the metathoracic ganglion (Fourtner & Pearson, 1977; Siegler, 1984). These characteristics (i.e., simple dendritic tree and many different motor neurons to the same group of muscles) suggest that different motor neurons are active during different behaviours. Thus, the muscles are able to produce many refined femoral flexion movements (Fourtner & Pearson, 1977; Siegler, 1984). (The opposite is true for the coxal depressor muscles.)

Not all of the 12 motor neurons (MN) that innervate muscles 181 and 182 are spontaneously active or recruited during walking (Pearson, 1972; Ritzmann & Pollack, 1981). Of the 12 motor neurons (numbered by axon diameter: the smallest is labelled 1, the largest is labelled 12), motor neurons 3, 4, 5, 6 and 7 are most commonly activated (Pearson *et al.*, 1970). Motor neuron 3 is a branch of the widespread common inhibitor (which also has a branch in depressor muscles). It appears to exert its greatest influence over a motor neuron that is involved with the tonic control of femur position (MN 4) (Pearson & Iles, 1970). (Motor neuron 7 is also involved in tonic control of the femur.)

Two slow flexor motor neurons (5 & 6 - to muscles 182C, D) are active during walking or other sudden movements and are much less affected by the inhibitor (Pearson & Iles, 1970; Pearson, 1972). An unidentified fast flexor motor neuron is also active during walking (Fourtner & Pearson, 1977).

Although a DUM neuron with an axon in N6Br4 has not been identified, there is evidence to suggest that this might be the case. First, morphological data from several independent sources has shown that at least two DUM neurons send an axon into nerve root 6 (Crossman *et al.*, 1971; Arikawa *et al.*, 1984). Also, Evans (1985) has indicated that the flexor tibiae muscles of locust respond to perfusion of octopamine in a manner similar to the extensor tibiae muscle (its antagonist). However, further experiments are required before the question of DUM neuron innervation of flexor musculature of the cockroach can be answered.

4. The Escape Response of the Cockroach

(a) The role played by the cerci

The ability of a cockroach to detect and suddenly respond to an approaching hand ('predator') has been attributed to the cerci and giant fibre system (Roeder, 1948; 1967; Westin *et al.*, 1977; Camhi *et al.*, 1978; Camhi &

Tom, 1978; Camhi, 1980). The cerci are a pair of horn-like, wind-sensitive structures located on the end of the abdomen (Fig. 4a). The ventral surface of each cercus contains about 200 filiform (thread) hairs which are organized into 14 columns (Nicklaus, 1965). Nine of these columns can be consistently identified in different animals according to the response properties of the associated receptor cell and axon. Each hair is in a socket which allows it to bend most readily in one plane that is column specific (Fig. 4b). All the hairs of the same column have the same "preferred" plane of deflection (Nicklaus, 1965; Ritzmann, 1984). Movement of the hair in one direction along its plane induces a depolarization in the receptor cell and a burst of action potentials (APs) in the sensory neuron. Movement in the opposite direction inhibits any ongoing spontaneous APs (Nicklaus, 1965). The preferred plane of deflection and directionality of the response is different in each column. Thus, the filiform hair-receptor complexes are responsible for not only detecting the movement of air but also for 'coding', in the form of action potentials, the direction from which the air current originated. Processing of this information by the CNS results in an 'appropriate' behavioural response (Westi. *et al.*, 1977; Camhi *et al.*, 1978). This functional, columnar specificity is preserved from animal to animal.

Figure 4: (a) Portrait of the cockroach *Periplaneta americana* (L.). Note that the cerci extend well beyond the end of the abdomen. (b) The ventral side of a segment from the left and right cercus. The small circles represent the wind sensitive filiform hairs (or "thread hairs" - labelled a, d, f-i, k-l). The directional sensitivities of the filiform hairs are indicated by the small arrows and lines in rectangular boxes. The direction of wind for optimal excitation is shown for each hair. Note that the pattern of sensitivity between the left and right cercus is a mirror image. (After Camhi (1984) and Ritzmann (1984).)

The behavioural latency² of the escape response of *Periplaneta americana* is one of the shortest of any animal (Camhi, 1978). In experiments using high speed cinematography to record the attempted escape from an attacking toad, the cockroach was observed to begin its turn away from the toad in about 40 ms (time from beginning of tongue extension to cockroach movement - Camhi *et al.*, 1978; Plummer & Camhi, 1981). Using controlled wind puffs, the behavioural latency could be reduced to 11 ms (Camhi & Nolen, 1981). Other experiments revealed that a minimum wind puff velocity of 12 mm.s^{-1} (with an acceleration of $600 \text{ mm.(s}^{-2})$) can evoke an escape response, while even lower velocities (3 mm.s^{-1}) caused slowly walking cockroaches to stop moving (Camhi & Nolen, 1981; Plummer & Camhi, 1981). The mechanism by which a cockroach is able to distinguish background 'noise' (errant air currents) from the lunge of a predator involves the acceleration of the air front advancing toward the cockroach (Plummer & Camhi, 1981). Wind stimuli accelerating at $600 \text{ mm.(s}^{-2})$ or greater are required to evoke escape behaviour.

(b) *Involvement of giant axons (GAs) in escape*

The receptor cell of each filiform hair is responsible for transducing the mechanical deflection of the hair into a neural event (resulting in a burst or inhibition of action potentials in the receptor cell's axon) (Westin, 1979). The APs travel to the terminal abdominal ganglion (A6) via cercal nerve XI, where they synapse with giant axons of the VNC (Pichon & Callec, 1970; Callec *et al.*, 1971; Callec, 1974; Blagburn *et al.*, 1984). The giant axons are believed to be responsible for the transmission and subsequent

² Behavioural latency is the time between the arrival of a stimulus at a mechanoreceptor and the initiation of a behavioural response.

excitation of motor neurons commanding escape behaviour (Westin *et al.*, 1977; Ritzmann, 1984; Ritzmann & Pollack, 1986).

Below is a brief review of some of the connections made in the cercal-GI-motor neuron system that result in an 'appropriate' behavioural response to a wind stimulus.

Both extracellular (Dagan & Camhi, 1979) and intracellular (Nicklaus, 1965; Westin, 1979) recordings from cercal nerves indicated that all cercal filiform hairs of a column responded optimally (with a high frequency burst of APs) to a wind puff from a specific direction. Wind puffs from other directions produced less or no response and each column has its own characteristic receptive field. To investigate whether this specificity was maintained in the GAs, intracellular recordings from identifiable GAs were made while stimulating the cerci with wind puffs (Westin *et al.*, 1977). (Buno *et al.* (1981) performed a similar series of experiments, except that they used direct, mechanical stimulation of the filiform hairs and recorded extracellularly from GAs.)

Giant axons (also referred to as giant interneurons) are typically separated into a ventral group containing four axons and a dorsal group containing three axons (vGAs and dGAs, respectively - Fig. 5a). Bilateral symmetry is preserved so that there are seven GAs on each side of the VNC. The soma of each GA is located in A6 on the side of the ganglion contralateral to its axon (Fig. 5b). (In A6, the primary axon of a GA leaves the soma and

Figure 5: (a) Cross section of the 5th abdominal ganglion showing the approximate positions and numbering scheme for the giant interneurons (GI). (b) and (c): Dorsal view of the terminal abdominal ganglion (A6) showing the left GI 3 in (b) and GI 7 in (c). Drawings in (b) and (c) are from camera lucida tracings of cobalt filled preparations. Stubs of the cercal nerves can be seen at the bottom (posterior) of each ganglion. (A, anterior; D, dorsal; P, posterior; V, ventral - From Daley et al., 1981.)

crosses the neuropile before turning in an anterior direction to exit from the ganglion (Daley *et al.*, 1981).) The position of the soma and dendritic branch pattern of a GA in A6 are characteristic of that neuron and can be used to identify a GA after intracellular recording and injection of a marker substance (Daley *et al.*, 1981). A GA can also be identified in one of the other abdominal or thoracic ganglia by the location of its axon relative to other giant axons (Spira *et al.*, 1969a; Camhi, 1976; Westin *et al.*, 1977).

The ventral and dorsal GAs appear to travel as a continuous unit in the VNC as far as the suboesophageal ganglion (Farley & Milburn, 1969; Parnas *et al.*, 1969; Spira *et al.*, 1969a,b). The dGAs taper abruptly in T3 and continue on as very fine processes. The vGAs taper slowly but in each thoracic ganglion they narrow to about 25% of their pre-ganglionic diameter, widening as they exit the ganglion (Parnas *et al.*, 1969; Spira *et al.*, 1969a,b).

By exposing the cerci to wind from different angles around the longitudinal axis of the body the specific angles from which each GA would respond to wind were mapped (Westin *et al.*, 1977). The intensity of the response (AP burst frequency and duration) of each GA (except GA 2 & 4) varied with the direction of the wind stimulus. Each GA had an optimal receptive field over which it produced a high frequency burst of APs. This directional sensitivity was consistent from animal to animal (Westin *et al.*, 1977). For example, GA 3, 5, 6, and 7 are all direction sensitive. They are insensitive to wind from at least one entire quadrant (the area around a cockroach was divided into four, 90° quadrants). GA 3 & 6 responded to wind from anywhere in front of the cockroach while GA 5 & 7 responded maximally to

winds ipsilateral (rear and front, respectively) to the GAs position. GA 1 responded maximally to wind directed at the cerci from the ipsilateral rear quadrant. However, it also responded weakly to wind from all other angles (Westin *et al.*, 1977).

The mean spike frequency in a single GA ranged from 207 ± 61 Hz to 354 ± 79 Hz. The duration of these bursts was 10-20 ms for vGI 2 & 3 and longer (25-40 ms) for vGI 1 & 4 and all dGIs (Westin *et al.*, 1977).

Although 8 to 12 GAs (including both sides of the connectives) are concurrently excited by a wind puff from any direction, Ritzmann (1984) and Camhi (1984) have shown that wind direction could in fact be accurately interpreted from activity in just three GAs. However, it is likely that the 'extra' activity normally available to the cockroach is used by the CNS in a way that we do not yet entirely understand.

Thus, the directional sensitivity to air movement, first encoded by the filiform hair-receptor complexes of the cerci, is transmitted 'intact' to specific axons of the AVNC. This information must then be relayed to the motor neurons involved in the escape response.

(C) *Relation of GA excitation to activation of motor neurons*

The strong correlation that typically exists between giant fibre systems and escape behaviour has been well

documented (Bullock, 1984). Bullock also noted that many animals (both vertebrate and invertebrate) have quite rapid escape responses and yet do not rely on a giant axon system to mediate this response. Thus, although it seems likely that the giant axon system of the cockroach is involved in escape behaviour (Roeder, 1947; 1967; Westin *et al.*, 1977), evidence directly supporting this hypothesis has been difficult to gather and has only recently been published (Ritzmann & Pollack, 1986).

Some experiments which directly support the hypothesis that the giant axons mediate escape behaviour have involved intracellular stimulation of individual (Ritzmann & Camhi, 1978; Tobias & Ritzmann, 1984a,b) and paired GAs (Ritzmann, 1981; Ritzmann & Pollack, 1981) while recording motor responses from N5r1 and N6Br4; selective GA lesioning followed by behavioural testing and recording from motor nerves (Westin & Ritzmann, 1982); and intracellular recording/stimulation from identifiable GAs and metathoracic local interneurons believed to be part of the pathway between GAs and motor neurons (Ritzmann & Pollack, 1986).

i) Intracellular stimulation of GAs

Results indicated that intracellular stimulation of a single GA could consistently evoke activity in the slow depressor motor neuron (D_s - if GA 5 was stimulated) or in a levator motor neuron (if GA 6 was stimulated) (Ritzmann & Camhi, 1978). Although a single AP in a GA was usually not

efficacious, brief trains of APs (which more closely resembled 'natural' excitation of GAs by the cerci) consistently evoked motor neuron activity.

Earlier work by Dagan and Parnas (1970) cast doubt on the likelihood that the GAs mediated escape behaviour. They indicated that stimulation of a GA (extracellularly), with single current pulses or trains of stimuli up to 200 Hz, would not recruit motor neurons. Only later was it reported that the lowest burst frequency of any single GA during wind puff stimulation (which evokes escape behaviour) was about 207 Hz, while values around 300 Hz were more common (Westin *et al.*, 1977). Furthermore, the frequency recorded over the first few spikes of a burst often reached 900 Hz.

Intracellular stimulation of a single GA with high-frequency stimulus trains could evoke a motor response (Ritzmann & Camhi, 1978). However, the delay from stimulation to motor AP (about 38 ms) was much greater than would be expected from a monosynaptic GA-motor pathway. But, input evoked by more natural stimulation (a wind puff directed at the cerci) is considerably more complex than a train of APs in a single GA. Since a wind puff will normally excite 8 to 12 GAs, there is probably summation and integration of inputs occurring at strategic interneurons, which determine the final motor output (Ritzmann, 1984; Siegler, 1984). Support for this notion of co-operativity and enhancement between GA outputs was shown in experiments where two GAs were impaled and co-excited while recording from motor nerves. Experiments indicated that paired

stimulation of GAs decreased the delay to onset of motor activity and also increased motor output (Ritzmann, 1981; Ritzmann & Pollack, 1981).

ii) Selective GA lesions

Intracellular stimulation of GA 5 consistently evoked activity in the slow depressor motor neuron (D_S) and sometimes evoked activity in D_S and, subsequently, levator motor neurons (Ritzmann & Camhi, 1978; Ritzmann & Pollack, 1981).

It is also known that a wind puff originating in the rear right quadrant strongly excites GA 5 (Westin *et al.*, 1977). Right GA 1, 2 & 4 would also be excited by this stimulus, but none of these are as directionally specific (to the ipsilateral rear quadrant) as GA 5. The behavioural response to this wind puff stimulus is an extension of the right hindlimb, and a turn to the left with forward movement (*i.e.*, turn away from the source of the wind and run).

After conduction in right GA 5 was blocked by the intracellular injection of CoCl_2 , wind puffs from the ipsilateral rear quadrant failed to excite ipsilateral D_S (Westin & Ritzmann, 1982). In another preparation, blocking GA 1 (which was shown to have at least some excitatory input to D_S) did not affect depressor motor neuron output. It was suggested that because the motor response to excitation of GA 1 is normally quite labile, GA 1 would not respond well in any dissected preparation which had to be stimulated at

least several times during experimental set-up (Westin & Ritzmann, 1982; Ritzmann, 1984). Camhi (1984) noted a similar lack of response to lesioning of GA 1.

In summary, the evidence outlined above supports the hypothesis that the GAs can excite motor neurons of the metathoracic ganglion. However, if only one or two GAs were stimulated in a dissected animal, escape behaviour could not be evoked to the extent seen in non-dissected animals. Also, minimum behavioural latency (11-16 ms) was not obtainable. Part of this discrepancy might be explained by the submaximal stimulus protocols used (at most only 2 GAs were excited intracellularly at any one time), whereas a wind puff normally excites from 8 to 12 GAs (Westin & Ritzmann, 1982).

Also, restraint of an animal has been shown to be responsible for inhibition of the escape system in crayfish (Krasne & Wine, 1975) and goldfish (Eaton & Hackett, 1984). In cockroaches, both Camhi and Ritzmann (Ritzmann, 1984) have noted a similar suppression of the escape response in secured animals.

5. DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane]

The brief review presented below concerning DDT is intended to act as an introduction to aspects of DDT that are pertinent to this thesis. Many areas that are important in understanding the mode of action of DDT (such as negative temperature coefficient of toxicity, structure-activity relationships, metabolism and resistance), have not been reviewed here. Each of these topics has been presented in other reviews (Narahashi, 1971; 1978; Elia, 1982; Matsumura, 1985).

(a) *General introduction*

DDT was one of the most widely used insecticides in the world. At the time of its introduction (1943-1944, Italy; 1945, North America) (Dunlap, 1981) it appeared to have some very desirable characteristics: its synthesis was relatively easy and inexpensive; it has low mammalian toxicity while being extremely toxic to insects; and its low volatility and rate of bio-degradation translated into persistence after application (on crops and, not intentionally, in 'run-off' ecosystems). With time, some of these 'qualities' became the nemesis of DDT.

Although its use has been banned in most parts of the world, (since 1972 in North America), its effects are still being felt in the global ecosystem. It has been one of the most thoroughly studied insecticides (Matsumura, 1985). Its

extreme hydrophobicity (2 ppb in water) (Matsumura, 1985) has resulted in physiological effects on a large assortment of biological systems.

For example, although it now seems unlikely that DDT affects the *in vivo* functioning of the Na/K or Mg-ATPases (Cheng & Cutkomp, 1975; Cutkomp *et al.*, 1976; Dary & Cutkomp, 1984; Elia & Gardner, 1987), DDT's effects on a Ca^{2+} or (Ca+Mg)-ATPase are still of interest (Trams & Lauter, 1977; Ghiasuddin & Matsumura; Ghiasuddin *et al.*, 1981; Luo & Bodnaryk, 1988). DDT's action on cultured, whole cell preparations (Scheffczyk & Buff, 1984; Buff & Brundl, 1984; Yoshida *et al.*, 1985); on fractioned components of nerve membranes (Camejo *et al.*, 1969; Barnola *et al.*, 1971; Rashatwar & Matsumura, 1986); and on lipid bilayers (Jackson & Gardner, 1978; Chefurka *et al.*, 1987) have also been studied. DDT's effects on other systems within the insect (such as in the release of factors from the corpus cardiacum) have also been investigated (Orchard, 1979; Singh & Orchard, 1982). But, most numerous are studies which investigated DDT's effects on conduction of action potentials in axons and nerves (Narahashi, 1971; 1979; Matsumura, 1985).

In summarizing these studies, it may be concluded that there is not likely to be a single factor responsible for the ultimate lethality of DDT (Samaranayaka, 1978; Gardner & Vincent, 1978). Instead, DDT's toxicity is probably a result of the combined effects on at least several physiological processes that together conspire to kill the animal.

(b) *Effect of DDT on nerve membrane excitability*

Probably the best characterized symptom of DDT poisoning is its effect on the nervous system - a characteristic repetitive, high frequency discharge of action potentials (APs) (Welsh & Gordon, 1947; Roeder & Weiant, 1948; Narahashi, 1971; 1976; 1978; Elia & Gardner, 1984). Studies using intracellular microelectrodes revealed that an initial, evoked action potential was usually followed by a plateau phase of depolarization (depolarized after-potential) (Narahashi & Yamasaki, 1960).

Figure 6: An example of DDT-induced repetitive firing in the ventral nerve cord of the cockroach recorded extracellularly. Activity was evoked by a single electrical stimulus of just greater than threshold intensity delivered to the AVNC. Initially, at least two axons are discharging repetitively. (arrow, position of stimulus artifact; 1 mV, 50 ms.)

Often superimposed on this plateau was a high frequency train of APs (repetitive firing or after-discharge). The trains normally come to an abrupt end (Fig. 6). From these

experiments, it was evident that the rising phase of the AP ($\dot{V}_{\max}$) was not affected by DDT (Narahashi & Yamasaki, 1960). However, it appeared that alterations in the Na^+ -inactivation mechanism and/or the potassium conductance could have been responsible for changes seen in the AP. This hypothesis was supported when Narahashi & Haas (1967; 1968) published evidence from voltage clamp experiments on lobster giant axon which indicated that the peak transient current (an inward current, normally carried by Na^+) was slow to turn off (i.e., altered inactivation kinetics) and that the steady state current (an outward current, normally carried by K^+) was delayed (1.4-1.6 fold) and reduced to about 37% of that found in untreated axons (Narahashi & Haas, 1968). Consequently there was a prolonged, residual steady state inward current, apparently carried by Na^+ .

An alternate hypothesis (van den Bercken & Vijverberg, 1980a,b) proposed that the "m-gates" of the Hodgkin and Huxley (1952) model failed to close on repolarization (their 'normal' state at resting membrane potential). As "h-gates" reopened after repolarization the 'stuck' m-gates would allow an abnormal increase in Na^+ conductance to occur, leading to another (or series) of APs.

After further refinement of techniques and voltage clamp electronics, Lund & Narahashi (1981, 1983) showed that the steady state inward current ("tail current") was a symptom of altered inactivation kinetics. It was also a

principle factor in the depolarized after-potential and repetitive discharge.

It is interesting to note that many of the effects of DDT and certain pyrethroids (particularly type I, eg., tetramethrin) on nerves or axons are very similar (Wu *et al.*, 1980; Lund & Narahashi, 1981). In both cases, repetitive discharges can be evoked by single electrical stimuli. Also, the tail currents produced in both DDT and pyrethroid treated preparations are qualitatively very similar (Narahashi, 1988), though tail currents provoked by DDT were shorter than those provoked by any of the pyrethroids tested (Lund & Narahashi, 1983).

Patch clamping of tetramethrin-treated neuroblastoma cells (Yamamoto *et al.*, 1983) indicated that single Na^+ -channels behave in a way consistent with the behaviour of whole axons treated with the pyrethroid (tetramethrin). They found that the conductance of single Na^+ -channels in tetramethrin treated neuroblastoma cells did not change. However, instead of finding only a single population of channels with an average open time of 1.8 ms (controls) there was also a second population with an average open time of 17 ms. This second population of Na^+ -channels opened in a less predictable fashion than did 'normal' Na^+ -channels and also tended to remain open long after the return of the holding potential to 'resting' levels. It was suggested that this prolonged open state could account for the tail currents observed in whole axon, voltage-clamp preparations (Narahashi, 1988).

In summary, DDT can induce hyperexcitability in the nervous system. The primary cause of this hyperexcitability appears to be a result of DDT's effect on the Na^+ -inactivation mechanism, causing a prolonged, inwardly directed current. This, in combination with a delayed and decreased potassium conductance, results in a burst of high frequency APs (repetitive firing) following a single stimulus. Single channel studies support the notion that Na^+ -channels remain open longer than normal after DDT or pyrethroid treatment.

6. Dorsal Unpaired Median (DUM) Neurons

(a) General

Since their original description in *Locusta migratoria* by Plotnikova (1969), dorsal unpaired median (DUM) neurons have been identified in all ganglia of the locust CNS, except the suboesophageal ganglion (Braunig, 1988). In fact, they may be a ubiquitous feature of the insect nervous system, appearing in widely divergent species (Evans, 1980; Watson, 1984; Evans, 1985b). ('Typical' DUM neurons have also been reported in cockroaches (Crossman *et al.*, 1971), the Jerusalem cricket (Hoyle, 1985), a primitive orthopteran (cricket-like, the New Zealand weta; Hoyle, 1985), hawkmoth

(Evans, 1985b), fireflies (Christensen & Carlson, 1981; 1982) and lepidopteran larvae (Brookes, 1988; Brookes & Weevers, 1988).

DUM neurons are most readily distinguished by their characteristic morphology and physiology. They are distinctive when compared to the giant axons and motor neurons described above because DUM neurons are not bilaterally paired. During embryogenesis, DUM neurons arise from a single, unpaired median neuroblast (Goodman & Spitzer, 1979). In the mature animal, they appear in a group as individual neurons, each with a single primary neurite emanating from its soma (Watson, 1984). The primary neurite descends into the neuropile and then turns in an anterior direction after which it bifurcates to form a pair of "bilaterally symmetrical" lateral neurites which immediately bend toward the lateral margins of the ganglion (Fig. 7). The lateral neurites branch extensively within the ganglion before making an exit into one or more of the peripheral nerve roots. (The primary neurite also may send out several secondary neurites.)

(b) *Metathoracic DUM neurons of cockroach and locust*

Initial studies of DUM neuron physiology and morphology were primarily concerned with metathoracic (T3) DUM neurons in cockroach (Crossman *et al.*, 1971a,b; 1972) and locust (Crossman *et al.*, 1971a,b; 1972; Hoyle, 1974; Hoyle *et al.*, 1974). Later, came studies on DUM neurons associated with the firefly light organ (Christensen & Carlson, 1981; 1982).

Figure 7: Diagram of a typical dorsal unpaired median (DUM) neuron in the metathoracic ganglion. The soma (S) sends out a single neurite which descends into the neuropile before branching. The branches running to the anterior (AC) and posterior connectives (PC) are designated by a dotted line because of their inconsistent occurrence. (LB, lateral branch; N2-N6, peripheral nerve roots - after Crossman *et al.*, 1971b.)

Hoyle (1974) showed that the myogenic rhythm in locust proximal extensor tibiae muscle could be inhibited by a DUM neuron. Hoyle *et al.* (1974) examined the mode of action of this 'identifiable' DUM neuron (DUMETi³). More recent studies have examined the distribution of biogenic amines (e.g., octopamine) in DUM neurons and mushroom bodies of the CNS (locust & cockroach, Evans, 1978a; Dymond & Evans, 1979); specific reinnervation of coxal depressor muscles by T3 DUM neuron(s) (cockroach, Denburg & Barker, 1982); and a re-examination of branch patterns of metathoracic DUM neurons (cockroach, Arikawa *et al.*, 1984).

Cockroach and locust T3 DUM neurons have many physiological and morphological properties in common. The somata of T3 DUM neurons in both animals produce large, overshooting action potentials (60-110 mV) usually of 2-5 ms duration followed by a long duration after-hyperpolarization (30-50 ms; Crossman *et al.*, 1971; Goodman & Heitler, 1979). The somata may be spontaneously active (0.2-0.5 Hz - higher in locust DUMETi than cockroach DUM neurons). Alternatively, an AP can usually be evoked by direct stimulation of the soma or by stimulating a peripheral nerve root that contains an axon of the impaled DUM neuron. (The latter method of stimulation results in an antidromic AP which conducts back to the soma and also down the contralateral DUM axon (Crossman *et al.*, 1972; Evans & O'Shea, 1978).

³ DUMETi is a DUM neuron in the metathoracic ganglion of the locust. Its sole peripheral target is the extensor tibiae muscle of the hindlimb.

Morphologically, an equivalent of locust DUMETi has not been found in cockroach. However, the usual symmetrical nature of lateral neurites of DUM neurons in locusts are also typically found in cockroach (Evans, 1980; Arikawa *et al.*, 1984; Watson, 1984). But, in both cockroach and locust the positions that individual somata occupy in T3 changes from animal to animal (in contrast to the 'stable' positioning of motor neurons), thus making it difficult to locate the same DUM soma in different animals.

DUMETi is uniquely identifiable because it sends a peripheral axon only to nerve 5b1 right and left, which innervate extensor tibiae (ETi) muscles. Also, it is thought to be the only DUM neuron with an axon in this nerve (Hoyle, 1974; Evans, 1980). Similarly, DUMDL (the DUM neuron that projects to the dorsal longitudinal flight muscles of the locust) is also 'uniquely' identifiable (Hoyle *et al.*, 1980; Watson, 1984; Malamud *et al.*, 1988; Whim & Evans, 1988). (Evidence from an earlier reference (Altman & Tyrer, 1977) indicated that there may be two DUM neurons which send an axon into nerve root 1. However, this evidence seems to have been superseded by these more recent findings.)

Early attempts to define the peripheral root innervation patterns of individual DUM neurons using Procion Yellow met with little success (Crossman *et al.*, 1971). A later paper described a single, peripheral branch pattern (revealed by electrophysiological and dye techniques). This was thought to be common to all DUM neurons of the large, excitable dorsal group (Crossman *et al.*, 1972). This single, common pattern of root innervation has been

questioned (Arikawa *et al.*, 1984). Arikawa *et al.* thought that there were three types of T3 DUM neurons in the cockroach, each identifiable by its peripheral branch pattern. These consisted of DUM neurons that send an axon into: (a) nerve roots 2 and 3 (DUM2,3); (b) nerve roots 3 and 6 (DUM3,6); and (c) nerve roots 3, 4, 5, and 6 (DUM3,4,5,6). More recently, the branch patterns proposed by Arikawa have been supplanted by ones presented in abstract form by other members of his group (Tanaka & Washio, 1984). Tanaka and Washio (1984; Tanaka & Washio, 1985; Tanaka & Washio, 1986) suggested that DUM neurons can be identified according to the following peripheral branch patterns: DUM2,3; DUM2,3,4; DUM5; DUM3,5; and DUM3,5,6. (DUM3,5,6 is also believed to have axons in the anterior and posterior connectives.) However, these abstracts have not yet been confirmed in the literature.

One of the complicating factors in distinguishing between DUM neurons is that not always the same number of DUM somata are visible. Although most workers report eight large dorsal somata (Crossman *et al.*, 1971; Arikawa *et al.*, 1984; Washio & Tanaka, 1986); Tanaka and Washio (1984) have revealed that there can be 9 to 10 somata in this group. Clearly, a detailed morphological investigation, similar to that performed by Watson in locust (1984), is required before any firm understanding of cockroach DUM neurons can be attained. In locust, the number of large, metathoracic DUM somata has also been reported as variable (Watson, 1984; Evans, 1985).

(C) *Possible functions of DUM neurons in cockroach*

Initially, it was believed that two types of DUM neurons existed in T3 of the cockroach. Excitation of one type caused a decrease in tetanic tension of coxal depressor muscle, while excitation of the second type caused an increase in basal tension (Tanaka & Washio, 1984). This view was later changed to one in which a single DUM neuron (specifically DUM3,5,6) released two modulatory substances simultaneously (one (octopamine) which decreased the amplitude of muscle contraction, and the other (a peptide) caused an increase in basal tension - Tanaka & Washio, 1985; Washio & Tanaka, 1986). Although basal tension increased linearly with an increase in frequency of APs in DUM3,5,6, a single AP was apparently capable of evoking both responses. However, this hypothesis can be questioned because of the possibility that endogenous proctolin might have been released during their experiments. It has been demonstrated that the pentapeptide proctolin is co-released (with L-glutamate) from the slow depressor motor neuron (Adams & O'Shea, 1983; 1986). The effect of proctolin on the muscle was to produce tonic tension in the depressor muscles (the same muscle used by Tanaka & Washio) and to indirectly influence relaxation properties of phasic responses (Adams & O'Shea, 1986).

Therefore, until more evidence becomes available, the mechanisms of action and function of DUM neurons in the metathoracic ganglion of the cockroach remain unresolved.

(d) *Locust DUMETi - Effects on muscle contraction*

A clearer picture exists for the DUM neuron innervating the extensor tibiae muscle (DUMETi) of locust hindlimbs. DUMETi has been described morphologically (Hoyle *et al.*, 1974), physiologically (Hoyle & Dagan, 1978; O'Shea & Evans, 1979; Evans, 1985a), biochemically (Evans & O'Shea, 1977; 1978; Morton & Evans, 1984), and pharmacologically (Evans, 1981; 1982; 1985b; Harrow *et al.*, 1982). Its modulatory influence over the extensor tibiae muscle has also been elucidated (Hoyle & O'Shea, 1974; Evans & O'Shea, 1977; 1978; Buchan & Evans, 1980; Evans & Siegler, 1982).

The function first attributed to DUMETi was the inhibition of the myogenic proximal bundle of the hindlimb extensor tibiae (ETi) muscle (Hoyle, 1974; Hoyle & O'Shea, 1974). Evoking action potentials (APs) in DUMETi or perfusion of the ETi muscle with octopamine inhibited the myogenic rhythm (Hoyle, 1974). However, the extensive pattern of innervation by DUMETi over the entire ETi muscle implied that inhibition of the proximal muscle was not DUMETi's only role (O'Shea & Evans, 1979). Further experiments revealed that activation of DUMETi on its own had no effect on the ETi muscles. However, in combination with low frequency (1 Hz) stimulation of SETi⁴ or FETi⁴, DUMETi's modulatory influences became apparent. When SETi

⁴

SETi and FETi refer to the slow and fast motor neurons that innervate the extensor tibiae muscle of locust hindlimb.

and DUMETi were stimulated concurrently, or D,L-octopamine (10^{-6} M) was briefly perfused over the muscle during stimulation of SETi, three differences in twitch tensions from control preparations were noted: (1) amplitude of twitch tension increased, (2) the rate of contraction increased, and (3) the rate of relaxation of twitch tension increased. Under these experimental conditions, the greatest effect was seen in the rate of relaxation of the muscle from twitch tension (maximum increase of 150% - O'Shea & Evans, 1979; Buchan & Evans, 1980). All three effects lasted much longer than the period of stimulation or perfusion. (From their records (Buchan & Evans, 1980), there appears to be a very slight decline in basal tension (tonus) of the muscle. The possible significance of this is outlined below.)

When FETi and DUMETi were concurrently excited (or ETi was perfused with octopamine while stimulating FETi), the most pronounced effect was again the increase in rate of relaxation from twitch tension. However, significant changes in amplitude and rate of contraction were not observed (O'Shea & Evans, 1979; Buchan & Evans, 1980).

One of the main criticisms of this type of experiment might be that SETi and FETi rarely fire (*in vivo*) at a constant rate of 1 Hz. This criticism was addressed by stimulating SETi at more physiological frequencies and then determining the effect of concurrent DUMETi activation or

perfusion with octopamine (Evans & Siegler, 1982). Their results revealed that the effects of DUMETi or octopamine depended upon the frequency of APs in SETi. If SETi was stimulated at a frequency greater than 1 Hz, the primary effect of octopamine appeared to be a decrease in maintained tension (Evans & Siegler, 1982). This effect was maximal when stimulating SETi at about 5 Hz and declined at higher frequencies.

Normally, a brief, high frequency burst of APs in SETi will induce a "catch-like" contraction. This catch-like contraction was prevented by perfusion with octopamine (10^{-6} M). Further, when a stimulus protocol was used to excite SETi which mimicked the *in vivo* walking frequency, they found that tension in the ETi muscle between steps did not decrease to baseline levels unless octopamine was perfused (Evans & Siegler, 1982). (Activation of the common inhibitor did not alter tension between steps.)

Evans (1985b) has hypothesized that the physiological role of DUMETi activation is to prepare and maintain the muscles in a state of 'rapid responsiveness'. Because DUMETi fires prior to activation of motor output (Hoyle & Dagan, 1978; Sombati & Hoyle, 1984a), octopamine would be present to modulate the first contraction of the muscle for a jump or stepping movement. Thus, DUMETi's initial and prolonged effects on the muscle would ensure a continued state of

"arousal and increased behavioural responsiveness" (Evans & Siegler, 1982; Evans, 1985b).

However, Hoyle (1982) indicated that the weta (a primitive orthopteran) displayed physiological responses to octopamine more similar to the walking leg of lobster than locust. Instead of decreasing maintained tension (as in locust), in weta, octopamine facilitated tetanic tension and induced a "catch-like" contraction in hindlimb ETi muscle after a burst of tetanic stimulation in SETi. This 'catch' may have been induced and maintained by the depolarized state the muscle cells exhibited after perfusion with octopamine. Thus, it is not truly a "catch" since it may have been actively maintained by steady depolarization of the muscle membrane potential. In lobster dactyl opener muscle, there is no alteration of resting potential.

(e) Possible central effects of DUM neurons

Watson (1984) described in detail the morphology and axon paths of individual DUM neurons within the metathoracic ganglion (T3) of the locust. His studies were primarily concerned with DUM neurons which send axons into the peripheral nerve roots. While these neurons appeared to contain numerous zones of synaptic input on secondary branches, putative output synapses within T3 were very rare (Watson, 1984). However, there was a frequent proliferation of "fine beaded processes" within T3 which were suggested to have been associated with axonal regeneration or remnants left over from original development of the nervous system.

(That they did not degenerate in mature animals was not addressed.) Similar beaded processes have been reported to occur in autonomic nerves that innervate vertebrate smooth muscle (Marshall, 1962 - cited in Eckert, Randall and Augustine, 1988). These varicosities are also a prominent figure in monoamine-containing neurons in the central nervous system of vertebrates and are usually associated with high concentrations of monoamines (Cooper et al., 1986). It is thought that the varicosities represent transmitter release sites.

In the locust metathoracic ganglion, a previously habituated synapse(s) on a motor neuron (LaFFLTi) could be dishabituated and responses potentiated by iontophoretic application of octopamine or by intracellular stimulation of a DUM neuron (Sombati & Hoyle, 1984a). The dishabituation was evoked whether the habituation was induced by mechanical means (touch or sound) or by electrical stimulation. A similar dishabituation occurred when LaFFLTi was excited by electrical stimulation of one of its collaterals (a more "precise" method of evoking synaptic events on FETi - Sombati & Hoyle, 1984a). These results are supported by evidence that indicates octopamine injected into the mesothoracic ganglion of *Manduca* greatly enhances motor responses (Claassen & Kammer, 1986).

A possible relation between octopamine release (from DUM neurons?) in T3 and the recruitment of specific

behaviour patterns (*viz.*, rhythmic stepping or flight motor activity) has also been suggested (Sombati & Hoyle, 1984b; Claassen & Kammer, 1986). However, the suggestion was questioned (Whim & Evans, 1988) because in one case, octopamine appears to be potentiating sensory input to the flight motor neurons (thus producing a general, rather than specific, increase in arousal in the CNS - Claassen & Kammer, 1986), and in the other case it was suggested that octopamine was acting directly on a motor pattern generator (Sombati & Hoyle, 1984b).

Other evidence supports the suggestion that biogenic amines (noradrenalin, dopamine, serotonin) can initiate walking motor patterns (in spinal cats and spinal rabbits) and locomotion (*Aplysia*, *Limax*); and also feeding behaviour in *Limax* and *Helisoma*. Octopamine and serotonin also affect motor patterns in lobster and crayfish (see Claassen & Kammer, 1986).

A hypothesis was devised in an effort to try and tie together all the various characteristics of DUM neurons (Hoyle, 1985). This hypothesis (*Orchestration Hypothesis*) suggests that specific DUM neurons act together in clusters. Each cluster is responsible for determining and initiating specific behaviours. Thus, the DUM neuron clusters must be active before recruitment of motor neurons. This hypothesis is supported by circumstantial evidence (in locust) (Hoyle, 1985), but has not been directly tested.

In summary, from the studies described above, it appears that DUM neurons can perform an important modulatory role in both the CNS and at the periphery.

DUM neuron modulation of muscle contraction has been thoroughly described for the locust extensor tibiae muscle. The case for modulation in the CNS is not as clear. Morphological evidence supports the hypothesis that DUM neurons of the cockroach might modulate CNS activity. In the metathoracic ganglion of the locust, a DUM neuron(s) appears to dishabituate and potentiate input to motor neurons. Iontophoretic injection of octopamine into narrowly defined areas of this same preparation was shown to evoke specific motor patterns. This is supported by similar work in *Manduca*. Therefore, one might speculate that DUM neuron activity and octopamine will be found to have similar effects on cockroach CNS.

MATERIALS AND METHODS

General - (Parts I & II)

1. The Experimental Animal

Male *Periplaneta americana* (L.) were used in all experiments. Cockroach colonies, as pesticide-free as possible, have been maintained in the Department of Biology (Carleton University, Ottawa) since 1979. The colonies consisted of three 150 litre tanks (91 cm x 31 cm x 41 cm) and two incubators where many individual housing units were set up to accommodate (in isolation) the progeny of single oothecae. An ootheca destined for an individual housing unit was hatched in a large Petri dish (150 mm X 25 mm - lab-tek "pure virgin polystyrene", round disposable) and kept there until 10 to 15 mm in length, after which the animals were transferred to a plastic container (pal pen - 24 cm x 13 cm x 13 cm). The animals raised in this way were of known age and lineage (see Methods Part II, Section (d)). All animals used in experiments of Part I were taken from one of the three 150 litre glass tanks that comprised the main colony. Animals used in Part II were taken from one of the tanks (captured just after the imaginal moult and held in isolation until required) or from one of the two incubators. Experiments were performed at room temperature (range: 22 to 27°C).

Rolled oats (small flake "quick" oats) were the staple nutrient source, supplemented every second week with either wheat germ or "Mixed Cereal" pabulum (Heinz or Mead-Johnson). Water was available to the cockroaches from test tubes plugged with glass wool. Temperature and humidity in the glass tanks were not controlled and ranged from 22 to 26°C and 60 to 75% relative humidity (R.H.). The temperature and humidity in the two incubators were maintained at 29°C and about 75% R.H., respectively. Lighting for the tanks was variable but approximated a normal 12/12 (light/dark) cycle. The incubators were kept on a rigid 12/12 (light/dark) cycle. Light for the tanks was provided by indirect sunlight and overhead fluorescent tubes. Fluorescent tubes (cool white) provided the sole source of lighting in the incubators.

Animals were carefully observed during capture by hand. Any untreated animal that did not attempt to escape during capture was not used in an experiment. (There are several reasons why an animal may not attempt to escape during capture, one of which is a "malfunctioning" cercal sensory system.)

2. Compounds Tested

(a) *Injection procedure (in vivo)*

Initial experiments of Part I and all experiments of Part II involved injecting animals between the third and fourth abdominal sternite with one of the following compounds: $1.0 \pm 0.05 \mu\text{l}$ of physiological saline (see "Salines" below); $1.0 \pm 0.05 \mu\text{l}$ of acetone (Reagent UV Spectrophotometric Grade); $1.0 \pm 0.05 \mu\text{l}$ of acetone containing $0.017 \mu\text{mol}$ of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (p,p'-DDT); and $1.0 \pm 0.05 \mu\text{l}$ of acetone containing $0.1 \mu\text{mol}$ of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE). Both the p,p'-DDT and p,p'-DDE were Aldrich analytical grade (>99% pure). The dose of p,p'-DDT used was that determined to cause about 50% mortality in 96 h when injected as described above (LD50 96 h - Elia, 1982). p,p'-DDE has been shown to be "non-toxic" to cockroaches up to a dose of about $1.6 \mu\text{mol}/\text{cockroach}$ (dissolved in acetone and injected as described above - Elia, 1982). Other aspects of the injection procedure were as previously outlined (Elia, 1982).

At no time was the animal anesthetized with CO_2 or other anesthetic.

(b) *Localized perfusion of the ventral nerve cord
during in situ recording*

The second group of experiments of Part I involved the sequential, localized perfusion of A5 and associated connectives (-A5-) with physiological saline, 0.2 or 0.4% acetone in saline, and 2.0×10^{-5} M or 4.0×10^{-5} M p,p'-DDT in saline. (These animals were not previously exposed to DDT.) The volume of acetone or DDT in acetone required to achieve the "concentration" desired in the bathing medium was vigorously injected into a suspended disposable syringe containing 50 ml of physiological saline just prior to use. (The disposable 60 cc syringe and Intramedic P.E. 60 tubing used to deliver the solution containing DDT were discarded after each experiment.) Gravity feed from the syringe provided a constant flow rate of $2.5 \text{ ml} \cdot \text{min}^{-1}$ for 20 min. A period of 5 to 15 min was then allowed before reinstituting perfusion with normal saline. It should be noted that since stock solutions of DDT were prepared in acetone, both acetone and DDT were present in the DDT-saline perfusate. Also, concentrations of acetone in saline were never allowed to exceed 0.4% because of the acetone side effects noted at concentrations that exceeded 0.6% acetone in saline.

3. Normal Salines

A constant flow of physiological saline (2.5 to 3. ml.min⁻¹) was passed over exposed tissues during dissection procedures. Following dissection, perfusion of the preparation became more localized as all or part of the AVNC was enclosed in a miniature electrode chamber (see below, Methods - Part I and II).

The composition of the saline used in all experiments of Part I was: 210 mM NaCl, 2.9 mM KCl, 1.8 mM CaCl₂, 0.2 mM NaH₂PO₄, and 1.8 mM Na₂HPO₄ (pH 7.2 - Elia & Gardner, 1984).

As an isolated preparation was employed in experiments of Part II, extra effort was made to maintain the integrity of the cells of the nervous system by oxygenation of the saline. To accomplish this without unwanted change in saline pH, the saline described by Ritzmann & Pollack (1986) (derived from Callec & Sattelle (1973) and Carr & Fourtner (1980)) was used. It contained 214 mM NaCl, 3.1 mM KCl, 9.0 mM CaCl₂, 3.0 mM MOPS ((3-[N-morpholino]propane sulfonic acid) - free acid), and 3.9 mM MOPS-Na ((3-[N-morpholino]propanesulfonic acid) - sodium salt). (The higher calcium concentration is also supported by evidence from Treherne (1966) which suggested the intercellular calcium concentration (cockroach CNS) could be as high as 18 mM.) Carr & Fourtner (1980) showed that the buffer MOPS permitted a higher concentration of calcium to be present.

(Vigorous bubbling of ultra pure oxygen for 3 to 4 h in the perfusion reservoir did not cause the pH to change from 7.2.)

All salines (Part I and II) were filtered through a Gelman micropore filter (0.45 μ m pore size) and subsequently stored at 4°C in tightly sealed glass storage bottles.

4. Stimulation Techniques

Both the *in situ* semi-intact preparations of Part I and the isolated preparations of Part II allowed extracellular electrical stimulation of the AVNC and stimulation of the cerci with WPs. For all experiments, the stimulus protocol was based upon a repeating sequence of five wind puffs directed at the cerci or five electrical stimuli delivered to the AVNC (single pulses or trains of pulses) at two second intervals (Fig. 8). (Thus, five stimuli would be delivered in 10 s.) Not less than 60 s, and usually 2 or 3 min, was allowed before a stimulus sequence was repeated.

Wind puffs represent a natural, although complex form of stimulation. The delivery and "recognition" of the stimulus (WP) by the animal (via the wind sensitive cerci at the end of the abdomen) was more variable and difficult to control than direct electrical stimulation of a nerve (AVNC). However, it is useful because it allows examination of nerve activity that putatively occurs *in vivo*. Direct

electrical stimulation, on the other hand, allows a more precise control of induced excitation in a nerve.

Figure 8: Stimulus protocols - (a) Single electrical stimuli, (b) trains of electrical stimuli and (c) wind puffs. Shown in each case is a single stimulus sequence (five stimuli in 10 s). Note that the figures are not drawn to scale.

A drawback of extracellular electrical stimulation of the AVNC was that recruitment of fibres for a given stimulus is determined by: (a) the proximity of an axon to the stimulating electrodes and (b) the diameter of individual axons (larger axons being recruited at lower intensities of stimulation). Thus, at any particular intensity of electrical stimulation, the axons recruited may not exactly

correspond to those recruited during the escape response. Thus, when possible for comparison, experiments employed both WPs directed at the cerci and direct electrical stimulation of the AVNC. (When the cercal nerves or A6 were damaged during dissection, only data derived using direct electrical stimulation of the AVNC was analyzed.)

Controlled, small WPs were created using ultra pure air under high pressure. A regulator with low pressure gauge was connected to a "normally closed" Skinner solenoid valve with 0.95 cm diameter Tygon tubing. A Grass SD5 stimulator was used to precisely control the opening of the solenoid valve. The 15 cm of tube just prior to the point of wind puff expulsion behind the cercus was 0.95 cm plexiglas rather than Tygon. This allowed three fine mesh screens to be fixed inside the tube to break up the laminar flow that may have developed in the tubing *en route* to expulsion. Thus, the cerci were presented with a uniform bolus of air. The end of the WP tube was positioned 2 cm back and just right of centre behind the cerci (Fig. 9). (A free-ranging animal subjected to this type of wind puff would usually move forward and to the left (Westin *et al.*, 1977; Ritzmann & Camhi, 1978).) Wind puff pressure ranged from 2.5 to 5.5 psi. A Gould-Statham virtual isometric force transducer with a small sensor was placed above and between the cerci to "sense" the wind to which the cerci were being subjected (Fig. 9). The electrical response of the force transducer was amplified and filtered (ca. 1000X, 100 Hz), and then

Figure 9: Positions of wind puff delivery tube (WPT), detector (DT), tension transducer (UC2) and cerci. Only the terminal abdominal segments of the semi-intact cockroach are shown (TS). (WS, one of 3 wire screens inside the wind puff tube.)

recorded on magnetic tape using a Racal Store 4 tape recorder with FM electronics. Using a hot wire anemometer (model W141-4) the system was calibrated so that the approximate velocity of 10, 60 and 100 ms WPs was known. (Calibration graphs for 60 and 100 ms WPs are presented in Appendix 2.)

Electrical stimulation of both the AVNC (using tungsten wire electrodes) and motor nerve 5r1 Right or Left (R or L - suction electrode) was accomplished with a Grass SD48 stimulator and SIU-5 stimulus isolation unit. The stimulus intensity used to evoke activity in the AVNC ranged from threshold voltage to a supramaximal stimulus at 3 to 5 times

threshold (duration 0.2 ms). Both single pulses and trains of stimuli were employed (see below, Methods, Part I & II).

The protocol for stimulation of motor nerve 5r1 R or L using a suction electrode typically called for single pulses of 1 ms duration and 0.5 Hz stimulus frequency. The stimulus intensity used was that required to evoke an AP in the DUM soma and axon, usually between 2.5 and 4.0 volts. (The actual voltage delivered to the preparation (AVNC and N5r1) was attenuated by the stimulus isolation unit by a factor of 10.)

5. Recording Equipment

Tektronix 502A and 564B oscilloscopes were used to monitor activity during the experiments. A Racal Store 4 reel-to-reel 4 channel tape recorder with FM boards was used to record data in all experiments. Records were transcribed (hardcopy produced) using a Watanabe four channel LinearCorder thermal pen chart recorder.

(a) Extracellular Recording

All extracellular recordings were made using Grass P511-J AC preamplifiers. Miniature electrode chambers designed to record from and stimulate the AVNC contained five or eight 0.08 mm diameter tungsten wire electrodes. Leads from a chamber were circuited through a switching box

which allowed rerouting of recording and/or stimulating leads on the AVNC without physically altering their positions.

The suction electrodes employed to record from or stimulate motor nerves (all experiments) were based on designs described by Theophilidis & Burns (1982) and Miller (1984). Some of the advantages of using the suction electrode described below include: (a) it can be used to record from very fine nerves, (b) the form of the recorded AP is very sharp and of short duration, thus allowing a more precise calculation of high frequency events, and (c) the gold coating and attached silver wire act as the reference for the electrode, eliminating the need for an isolated lead in the common bath.

Glass tubing for suction electrodes (1.2 mm diameter without filament) was pulled on an Industrial Science Associates micropipette puller (Model M1). (Because the tips were eventually trimmed to approximate the diameter of the nerve, the microelectrode resistance was not a critical factor.) A gentle curve or angular bend of the stem was then made over a low intensity flame. After preparing 6 to 10 pieces of glass tubing, they were mounted with tips up on a plexiglas base and coated with gold in a Jeol vacuum evaporator. A 0.3 mm diameter silver wire was cleaned with emery paper and then wrapped around the gold coated shank with enough wire remaining to reach past the end of the stem to a crocodile clip mounted on the shaft of the holder

(Fig. 10). Conductive silver paint was then applied to the silver wire and shank of the micropipette. This physically and electrically secured the silver reference wire to the gold coating. A plexiglas holder with a hollowed and threaded end to fit a cap with O-ring seal for the micropipette (Fig. 10) effectively isolated the reference wire (outside the micropipette) from the inside of the micropipette and plexiglas chamber.

Figure 10: Gold coated suction electrode (right) and assembly (left) used for recording from or stimulating motor nerves. (AG, silver wire (reference electrode); AGP, area coated with conductive silver paint; AU, gold coating; CC, crocodile clip for AG; CL, chlorided silver wire; EC, end cap; EH, hole to insert glass electrode; LS, locking screw; PI, glass pipette; PE, polyethylene tubing; PH, plexiglas rod with hollowed end; RG, rubber gromet; SW, shielded wire; SY, syringe used for suction.

The electrode holder housed both the active lead (connected to an AC preamp) and the suction source (5 ml disposable syringe and PE 60 tubing with a cap screw to lock the plunger) used to draw a nerve into the tip of the micropipette. Finally, the tip of the micropipette was carefully broken with fine forceps (#5 Dumonts) to a size that approximated the diameter of the cut nerve end. A jagged break was smoothed or the tip opening slightly broadened with very fine grit emery paper. The best recordings were made when the nerve formed a tight seal in the end of the micropipette.

(b) Intracellular Recording

All intracellular recordings were made using a WPI Model 701 Dual Microprobe System. This allowed current to be passed through the microelectrode using a "bridge" circuit. The bridge was balanced in the bath, prior to entering a cell, by passing a short duration current through the microelectrode from the stimulator and adjusting the bridge balance control on the WPI amplifier. Minimum pulse offset and transients were sought. The balance was checked and corrected in a similar fashion, if necessary, after impaling a cell. The microelectrode holder contained a silver/silver chloride lead which connected the inside of the microelectrode to the high impedance input stage (WPI). The bath (reference) electrode consisted of a Pasteur pipette (with appropriately curved tip and shortened stem) filled with either 3% agar in 0.5 M

LiCl or 3% agar in 2 M KCl. This modified Pasteur pipette reference electrode was secured in a plexiglas holder which was filled with either 0.5 M LiCl or 2 M KCl. A chlorided silver wire inside the holder was attached to a cable which completed the circuit from the bath to the amplifier. (Six silver wire reference electrodes were chlorided together in a chamber containing 0.1 M KCl. Six volts and about 50 μ A per electrode were used to deposit the chloride on the reference electrodes. Electrodes were considered to be chlorided when a uniform black coat covered the silver wire. Uneven or 'spottily' coated electrodes were not used.)

Thick wall borosilicate glass capillary tubing with internal filament (1.0 mm O.D., WPI Lot # 10736-03G) was pulled on a modified Industrial Science Associates micropipette puller (model M1) (Jacobson & Mealing, 1980). A schematic of the modifications made to the puller is presented in Appendix 3. Briefly, the changes involved adding a voltage controller, finer heat controls and a large capacitor to drive a second more powerful pull during the heating of the capillary tube. The timing of the second pull was adjusted using a micrometer with vernier control and L.E.D. sensor to detect movement of the shaft sleeve. As the glass begins to soften, the initial pull begins to draw-out the glass. At a point set by the micrometer, the shaft sleeve interrupts the L.E.D. beam which closes a microswitch and thence activates the second more powerful pull. This arrangement provided better control

over microelectrode resistance and geometry (i.e., short pulled length and medium-high resistances).

Heater coils were made from 0.03 mm stainless steel (full hard) and spot welded such that they formed a closed loop that would completely surround the glass capillary (equidistantly) (S.L. Jacobson, personal communication). The part of the capillary which remained stationary during a pull (and thus within the coil) was discarded.

The first piece of glass pulled on the day of an experiment was discarded (in case there were any inconsistencies in the coil because of dust deposited since last used). For each other piece of glass pulled the solenoid voltage, coil current, pulled length (shoulder to tip) and microelectrode resistance were recorded. Typical values used were 1.82 V, 285-287 mA, 5.5 mm and 20-25 Mohms when filled with 2 M KCl. If filled with 4% Lucifer Yellow Carbohydrazide (LY) in 0.5 M LiCl the microelectrode resistance increased to about twice this value.

Microelectrodes destined to be filled with the fluorescent dye Lucifer Yellow (LY) (to mark selected DUM neurons) were kept dry in a covered one litre beaker. The beaker and plastic bag cover were rinsed in distilled water before use. This helped maintain a humid atmosphere within the beaker. (This "wetting procedure" was instituted in an attempt to solve a problem of steadily increasing

microelectrode resistance after the pulled capillaries were filled and placed in the preparation bath. It was thought that an increase in static charge attracted minute particles of dust to the tips of the microelectrodes (where charge density would be greatest) during the time between pulling and the actual filling (ca. 2-3 h) of the capillaries. A humid atmosphere considerably improved the situation.)

A stock volume of 4% Lucifer Yellow in 0.5 M LiCl (about 100 μ l total volume) was kept refrigerated until use in a tightly sealed Beam capsule (the type used for embedding in electron microscopy). Microelectrodes were filled to their shoulder region by placing the end of the stem in the solution of LY, which had been warmed to room temperature. The stem was then backfilled with 0.5 M LiCl using a 31 gauge needle attached to a 10 ml disposable syringe. Syringes used to backfill capillaries or electrode holders were attached to a Sartorius disposable micropore filter device (0.24 μ m pore size).

Capillaries to be filled with 2 M KCl were placed in a covered one litre beaker containing sufficient 2 M KCl to completely cover the capillaries. When required for recording, microelectrodes were backfilled with 2 M KCl using a 31 gauge needle and syringe as described above for LY microelectrodes. The filled electrodes were rinsed with distilled water before use.

Only 3 or 4 glass capillaries were pulled at any one time. They were discarded if not used on that day.

6. Data Analysis

Hardcopy was made of each experiment using a Watanabe thermal pen four channel chart recorder. Tape speed was reduced from 15 IPS (recording speed) to $1 \frac{7}{8}$ IPS (playback speed) with a chart paper speed of 25 or 50 mm/s. These settings allowed spikes to be well separated which facilitated "by hand" analysis. To develop an impression of longer term ongoing spontaneous activity or activity before, during, and after a sequence, hardcopy was also made using a tape playback speed of $3 \frac{3}{4}$ IPS and chart paper speed of 10 mm/s.

Experiments were analyzed using the PCLAMP data acquisition computer program (Axon Instruments). Options within the PCLAMP program were manipulated in a way that allowed the analysis of AVMC records for spike frequency (Appendix 4). Output from PCLAMP was analyzed for instantaneous frequency and bursts of APs (burst duration and frequency of APs within a burst) using a program developed in our laboratory (PECAN). The PECAN criteria used to analyze all 'burst' data from experiments presented here were: (a) the minimum number of spike that constituted a burst was four, and (b) the minimum frequency

to initially accept and then continue a burst was 200 Hz. (A burst was considered to be terminated when the instantaneous spike frequency within the burst dropped below 200 Hz.)

Output from PECAN provided information concerning instantaneous frequency, burst duration, the number of spikes in a burst and overall frequency of firing within a burst. These data (except instantaneous frequency but including tape footage counter) were reorganized and tabulated using SYMPHONY (a spreadsheet program and macros). A copy of each option file from PCLAMP is included in Appendix 4.

The process of analyzing AVNC data recorded on tape required the conversion of the analog signal (stored on magnetic tape) to a digital signal which could be recognized by the computer and PCLAMP program. The analog to digital conversion required AVNC records to be fed from tape through a window discriminator into the LABMASTER for A/D conversion and interfacing with the computer (Deskpro, Compaq). (The LABMASTER consists of a TL-1 interface with 100 KHz A/D conversion board and daughter board which resides in the computer.) The function of the window discriminator was twofold: (a) it allowed selection of spike amplitude. Low level spontaneous activity or noise could thus be excluded, and (b) spikes entering the window activated a gate within

the discriminator which then caused PCLAMP to begin to collect data points (i.e., A/D conversion of spikes).

METHODS - PART I(a)

The first series of experiments were designed to determine whether repetitive firing activity in the AVNC and motor nerves could be related to behaviour during the initial signs of DDT poisoning. Also, once behavioural signs of poisoning are no longer evident (i.e., the animal appears behaviourally "normal") (Elia & Gardner, 1984), does repetitive firing still exist in the nervous system of an 'intact' animal?

1. The Preparation

To address these questions a preparation and chamber were devised that allowed recording from the nerves of a partially dissected (semi-intact) animal that had been injected with a LD50 96 h dose ($0.017 \mu\text{mol}$) of DDT 1 h, 24 h or 96 h previously (control animals were injected with $1 \mu\text{l}$ of saline or acetone). Under a constant flow of physiological saline, the abdomen was eviscerated from the dorsal surface revealing the AVNC. Collaterals of the AVNC were cut (from A5 to T3) to allow the placement of a miniature recording chamber beneath the AVNC. To expose motor nerves 5r1 and 6Br4 a window was cut in the dorsal surface of the right metathoracic coxa, distal to the coxal rim (Fig. 11). (Some overlying tracheal tubing and muscles

Figure 11: Diagram showing the ventral surface of the cockroach in the area of the metathorax. The right leg has been raised to show the dorsal surface of the coxa. Nerves 5r1 and 6Br4 and some of the associated muscles (177e, 17u depressors; 182a,b levators) are also shown. See Appendix 1(a,b) for a more detailed view of the innervation patterns and associated muscles of N5r1 and N6Br4. (ANT, anterior; Tro, trochanter) (After Pearson & Iles, 1971.)

were removed.) Once N5r1 and N6Br4 were located, they were severed leaving as much of the branch as possible for attachment to a suction electrode.

The abdomen and right metathoracic leg were secured to a binding post and plasticine ramp which supported the animal (Fig. 12). The remaining legs and head were allowed to move 'freely'. The tarsi contacted a lightly greased, polished plexiglas surface so that attempts at walking or running by cockroach were still possible. Once dissection was completed and the AVNC chamber was in place, perfusion and aspiration were restricted to T3 and the right metathoracic (dissected) coxa. The miniature electrode chamber (Fig. 12) containing the AVNC and eight 0.08 mm tungsten wire electrodes was then drained of saline and sealed with a piece of coverslip a. l grease (vaseline:light mineral oil, 4:1). A small strip of Whatman filter paper in the bottom of the chamber helped maintain a humid environment for the AVNC. The tips of the suction electrodes were then moved into the pool of saline bathing N5r1 and N6Br4. The ends of the two motor nerves were sequentially picked up in their respective suction electrode and recording established.

The post to which the cockroach was attached was positioned 1.5 cm below the surface of the surrounding plexiglas enclosure. This helped to shield the cerci from

Figure 12: (a) Dorsal view of a cockroach in position for recording and stimulation. The abdomen, posterior thorax and right metathoracic leg are secured. (The left legs and plasticine ramp have not been shown.) The recording chamber is shown inserted within the abdomen. (b) An overhead view of the recording/perfusion chamber used in Part I(b). Note the small posterior chamber ("wet") for perfusion of -A5-. The remainder of the preparation (not shown) is the same as that in (a). (c) A cross-sectional view of the recording chamber positioned within the ventral abdominal cavity. (The dorsal tergites were removed during dissection.)

Key: AB, abdominal cavity; ASP, aspiration; ASP2, additional source of aspiration (Part Ib); CER, cercus; CN, cercal nerves; CS, glass coverslip, D, dorsal; dry, sealed chamber not perfused with saline; EC, electrode chamber; P511, AC preamplifier; PER, perfusion; PER2, additional source of perfusion (Part Ib); RS, leads to chamber for recording or stimulating AVNC; SB, support beam; SR, plexiglas support rod; TE, tungsten electrode; TS, terminal abdominal segments; wet, section of chamber perfused (Part Ib); VNC, ventral nerve cord; V, ventral; WPT, wind puff tube; WS, one of 3 wire screens in the WPT; 1, stimulator leads.

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sources of wind other than that produced by the WP system. To further reduce errant drafts and electrical noise, the preparation and chamber were located inside a Faraday cage that was covered with plastic sheeting. The front panel of plastic could be raised or lowered to allow access to the preparation. The Faraday cage sat on a partially inflated 15" automobile tire inner tube. The cage was stabilized by attaching it with several large elastic bands to an outer table-mounted frame. Narashige micromanipulators were used to position the electrodes. An American Optical fibre optics light source provided "cold" illumination.

2. Experimental Protocol

Threshold stimulus intensity (V) (0.2 ms duration, 0.5 Hz) for the AVNC was determined. The form of the compound action potential (CAP) and whether repetitive firing was induced at threshold for the AVNC was noted. The response of the AVNC (and motor nerves) to stimuli of gradually increasing intensity up to maximum CAP activation were recorded. Responses to sequences of WPs (5 WPs - 10, 60 and 100 ms duration; 0.5 Hz - see above, Fig. 8) directed at the cerci were recorded. Stimulus sequences (electrical or WPs) were delivered at no closer than 2 min intervals. Trains of electrical stimuli were also delivered to the AVNC to mimic a burst of activity similar to that observed when a

WP was used. (The objective was to determine the characteristics that a train of stimuli must possess in order to evoke an escape response.) A range of intensities (threshold to 3 x threshold) and train frequencies (100, 300, 600 Hz trains; [50 or 100 ms duration/train; 0.2 ms pulse duration; 0.5 trains/s]) were used (see above, Fig. 8, Materials and Methods - General, Section 4).

The arrangement of the electrodes along the AVNC and use of a switching box allowed rerouting of the stimulus to evoke an AP just posterior to T3. In this way, if a block did exist in the AVNC, its location could be identified (see Materials & Methods, General, Section 5a).

RESULTS - PART I(a)

1. Behavioural Observations

(a) *Saline, Acetone and DDE Injected (Controls)*

Animals injected with 1 μ l of saline, acetone or p,p'-DDE did not display any behavioural signs of poisoning. Their survivability, measured over a 3 week period following injection, was not significantly different from uninjected controls (Elia, 1982). Immediately following injection, animals usually wiped the site of needle penetration with a metathoracic leg whether they had been injected with saline, acetone or DDE. However, they appeared not to suffer any prolonged discomfort from the injection procedure and behaviour soon returned to normal.

Control animals tested with blow puffs¹ prior to and after injection responded by moving away in an appropriate direction.

A crude startle stimulus (i.e., a gentle tap of the flask containing the cockroach on the bench top) usually caused the animal to tighten its grip on the supporting surface (stance solidification) or caused the animal to take a few controlled steps forward.

During capture by hand, just prior to experimentation, control animals attempted to escape in a predictable manner.

¹ "Blow puffs" are similar to wind puffs except that they are not as strictly controlled.

(b) *DDT-Treated Animals - General*

Most animals began to display signs of poisoning within 10 to 15 min of being injected. Typically, intermittent, followed by continuous, tremors of the legs were the first signs of poisoning. Tremors persisted as jittery movement of the limbs became pronounced. At this time, animals could usually not retain their grip if the flask they occupied was turned over (in contrast to controls). Movements became less predictable as signs of poisoning became worse. The animals' response to a startle stimulus or "blow puff" was grossly exaggerated and usually consisted of an uncoordinated escape attempt. (An exaggerated response to a startle stimulus normally outlasted spontaneous tremor and jitter responses and could be evoked when other signs of poisoning had disappeared.)

Within about 24 h of being injected with DDT, animals either appeared behaviourally normal or signs of poisoning had persisted and usually resulted in the mortality of those animals. The apparent behavioural normality at 24 h was not always an indicator of 'recovery' as occasionally one of these animals died within about 96 h of injection.

The responses of 1 h DDT-treated cockroaches to blow puffs and their behaviour during capture was mostly unpredictable. Very often they would begin moving in one direction and then suddenly change direction without further external stimulus.

24 h DDT-treated cockroaches which were not showing overt behavioural signs of poisoning did not respond to a blow puff with a typical escape-type manoeuvre or attempt to escape during capture. Response to a startle stimulus was usually a stance solidification type movement (with varying degrees of exaggeration).

Cockroaches that survived 96 h after being treated with DDT appeared behaviourally normal and responded to a blow puff and startle stimulus in a fashion not noticeably different from controls. Also, they usually attempted to escape in a predictable manner during capture.

2. Experimental Protocol

(a) *DDT- and DDE-Treated Animals - Qualitative*

correlation between behaviour and neurophysiological symptoms of poisoning

Cockroaches were injected with a LD50 96 h dose of p,p'-DDT. Recordings were made from the AVNC and motor nerves (metathoracic N5r1R and N6Br4R), *in situ* at 1 h, 24 h and 96 h post-injection. During early stages of poisoning (1 h to 24 h) CNS disruption and behavioural signs appeared to correlate well. However, this correlation was lost 24 to 96 h post-injection, when repetitive firing in the AVNC was still prominent yet the cockroach no longer displayed overt signs of poisoning. At this time, whether behaviour was

obviously still abnormal or appeared normal, neurophysiological symptoms of poisoning in the AVNC remained.

(b) *Stimulation of the AVNC and cerci*

i) Saline and acetone controls

Using single pulses delivered to the AVNC (0.5 Hz, 0.2 ms duration) threshold intensity was found to range from 2.4 V to 3.0 V. (Threshold intensity for AVNC activation was not different in treated preparations.) At threshold intensity, a large short duration compound action potential (CAP) was usually elicited (Fig. 13a). (With careful manipulation of intensity at the initial threshold value, it appeared to be possible to excite only one or two of the giant axons (GAs) - probably ventral.) With an increase in

Figure 13: Diagram of a typical compound AP (CAP) recorded from the AVNC evoked by a single electrical stimulus (a) threshold intensity and (b) at an intensity for maximum CAP amplitude.

intensity, the CAP became larger (0.5 to 5.0 mV amplitude) and more diffuse as smaller, slower conducting axons were recruited (Fig. 13b). At the stimulus intensity for maximum CAP activation the time between the first AP of the group and the last was not greater than 5 to 10 ms. Repetitive firing (as described previously - Elia & Gardner, 1984) following a single pulse delivered to the AVNC was not observed in any controls.

Wind puff stimulation of the cerci induced a short duration ($n = 8$, mean = 57 ms), high frequency (mean = 800 Hz) burst of activity in the AVNC (Fig. 14). When the cerci were subjected to a WP sequence (5 WPs delivered at 0.5 Hz) there was not a significant difference in the frequency of firing and duration of subsequent bursts in the sequence (Student's t-test, $P > 0.05$ - Fig. 15).

The responses recorded in motor nerves 5r1R and 6Br4R (Fig. 16) were less consistent. Sometimes no activity was observed. At other times, the slow depressor motor neuron and the common inhibitors in N5r1R and the fast flexor motor neuron and common inhibitors in N6Br4R would fire (Fig. 14). These animals generally did not show consistent signs of movement during a WP sequence. In 2 of 8 preparations, noticeably more motor nerve activity was observed along with movement of the legs (a startle or stance solidification movement). Frequently, the widespread common inhibitor (WCI) fired in the motor nerves before any other induced

iv) 96 h DDT-Treated Animals

In animals which had been injected 96 h previously with DDT, A6 was observed to be blocked in only one of the five preparations studied. Repetitive firing was readily elicited in the AVNC either by a WP directed at the cerci (Fig. 15) or by direct electrical stimulation. The mean spike frequencies (AVNC) induced by WPs in 96 h DDT-treated animals were not significantly different from control treated animals (Student's t-test, $P > 0.05$). However, the mean durations of wind puff-induced AVNC bursts and the frequency of firing in N5r1 and N6Br4 were significantly greater than for those of controls (Student's t-test, $P < 0.05$; Fig. 15 & 16).

Comparing 1 h and 96 h DDT-treated animals, the WP-induced firing in both AVNC and motor nerves was not significantly different (Student's t-test, $P > 0.05$) except for the last two WPs of the sequence shown in Figure 15a.

v) 24 h DDE-Treated Animals

These animals did not differ behaviourally from saline and acetone controls. However, although there is a statistical difference (Student's t-test, $P > 0.05$) in spike frequency and burst duration for 24 h DDE and control preparations, the responses of the two types of preparations are very similar (Fig. 15 & 16). This contrasts with the

Figure 15: Activity recorded extracellularly from the AVNC of semi-intact preparations in response to wind puffs directed at the cerci. Animals were injected at various times prior to experimentation with saline, acetone, DDT or DDE. (a) Mean spike frequency (Hz) in each wind puff-induced burst of the sequence. (b) The mean duration of each burst of the sequence. Lines represent a regression on each data set. Bars represent "estimated standard error of the mean". (Bars not visible are too small to be seen.) Only "mean spike frequency" for 96 h DDT preparations were not significantly different from controls ($P>0.05$). Some data points are shifted to the right for clarity.

Figure 16: Motor responses recorded extracellularly from N5r1 and N6Br4 of the same semi-intact preparations illustrated in figure 15 in response to wind puffs directed at the cerci. Mean spike frequency (Hz) in N5r1 (a) and N6Br4 (b) for each wind puff-induced burst of a sequence. Lines represent regression on each data set. Bars represent "estimated standard error of the mean". (Bars not visible are too small to be seen.) All DDT-treated are significantly different from controls ($P < 0.05$). Some data points are shifted to the right for clarity.

motor activity occurred. (Activity in WCI prior to motor output during "reflex" motor activation was also observed by Fourtner & Drewes (1977).)

ii) 1 h DDT-Treated Animals

In animals treated 1 h previously with p,p'-DDT (n = 5), an exaggerated response was recorded at all three recording sites following stimulation of the AVNC with single pulses or the cerci with WPs (Fig. 17). In response to a WP directed at the cerci, the duration of the AVNC burst and the frequency of firing in the AVNC, N5r1 and N6Br4 of 1 h DDT-treated animals were all significantly greater than that recorded in controls (Student's t-test, $P < 0.05$, Fig. 15 & 16). Movement of the unsecured legs, usually more than stance solidification, was observed with most WPs, i.e., despite the high level of afferent activity in the AVNC (endogenous and stimulated) 'filtering' within T3 appeared to be minor, if present at all. Furthermore, no block of T3 was observed and the very short delay between stimulation of the cerci and motor response indicated that the GAS were the likely conduction path for the impulses from the terminal abdominal ganglion (A6).

iii) 24 h DDT-Treated Animals

At 24 h post-injection, 5 of 7 preparations showed a 'long-term' block of conduction in A6. WPs directed at the cerci of these five animals did not induce activity in the

Figure 17: 1 h DDT-treated - An example of the extracellularly recorded activity evoked by a single wind puff (10 ms duration) directed at the cerci. Note the very long duration, high frequency burst of APs in the AVNC. The widespread common inhibitor (CI) was firing prior to the wind puff stimulus (see arrows, N5r1 & N6Br4) and continued for ca. 300 ms after the stimulus. The large spikes in N5r1 are from the slow depressor motor neuron (D_s). There is a reciprocal pattern of bursting in N5r1 and N6Br4 over the first 250 ms after the wind puff. (Minimal amplifier (P511-J) filtering of input from N5r1 and N6Br4 resulted in the baseline drift observed.) (Scale bars: 1 mV, 25 ms.)

AVNC. Severing a cercus or a cercal nerve just distal to A6 in these preparations did not evoke activity in the AVNC or a behavioural response (contrary to the response seen in controls or 1 h or 96 h DDT-treated animals). Electrical stimulation of the AVNC revealed that the abdominal ganglia (A1-A5) and connectives were not blocked and responded with a burst of repetitive firing when a single electrical stimulus was applied. Activity in the motor nerves was readily induced by electrical stimulation of the AVNC proximal to A6.

Most 24 h animals displayed little, if any, behavioural response to a blow puff prior to being secured for dissection. In the two preparations that responded to wind puffs with a burst in the AVNC and motor nerves, only one had responded to blow puffs prior to dissection. Both responded to other forms of sensory stimulation. (As the 24 h data was based only on the two preparations which did respond to WPs, they were not included in Figures 15 and 16. However, for the two that did respond, the AVNC mean spike frequency was less than both controls and other DDT-treated animals (mean = 600 Hz); the value for mean burst duration was between controls and 96 h DDT-treated animals (mean = 80 ms). Activity in N5r1 and N6Br4 evoked by wind puffs was similar to that observed in 96 h DDT-treated animals.)

iv) 96 h DDT-Treated Animals

In animals which had been injected 96 h previously with DDT, A6 was observed to be blocked in only one of the five preparations studied. Repetitive firing was readily elicited in the AVNC either by a WP directed at the cerci (Fig. 15) or by direct electrical stimulation. The mean spike frequencies (AVNC) induced by WPs in 96 h DDT-treated animals were not significantly different from control treated animals (Student's t-test, $P > 0.05$). However, the mean durations of wind puff-induced AVNC bursts and the frequency of firing in N5r1 and N6Br4 were significantly greater than for those of controls (Student's t-test, $P < 0.05$; Fig. 15 & 16).

Comparing 1 h and 96 h DDT-treated animals, the WP-induced firing in both AVNC and motor nerves was not significantly different (Student's t-test, $P > 0.05$) except for the last two WPs of the sequence shown in Figure 15a.

v) 24 h DDE-Treated Animals

These animals did not differ behaviourally from saline and acetone controls. However, although there is a statistical difference (Student's t-test, $P > 0.05$) in spike frequency and burst duration for 24 h DDE and control preparations, the responses of the two types of preparations are very similar (Fig. 15 & 16). This contrasts with the

very different response in burst duration between DDE and DDT-treated animals ($P < 0.05$).

3. Miscellaneous: Peculiarities of DDT-Treated Secured Preparations

Prior to securing and dissecting animals previously injected with DDT, the difference in behaviour of 1 h compared with 96 h cockroaches was obvious (poisoned at 1 h, apparently normal at 96 h). However, the erratic behaviour of 1 h DDT-treated animals was lessened after the animal was secured. It may be that the relatively immobilized state reduced afferent input and spontaneous activity. This observation is in agreement with the general decreased responsiveness observed in secured preparations (Ritzmann, 1984). However, compared to controls, 1 h DDT-treated animals still displayed exaggerated responses (both neural and behavioural) to stimuli despite the 'calming' effect of restraint.

Paradoxically, an almost reverse effect was observed with 96 h animals. Once secured, their behavioural response to a wind puff was stronger than that observed prior to restraint. The reasons for these observations remain unclear.

4. Preliminary Conclusions From These Experiments

Repetitive firing was observed in the 'intact' nervous systems of DDT-poisoned cockroaches and thus was unlikely to be an artifact in previously reported *in vitro* experiments (Elia & Gardner, 1984).

Behavioural signs of poisoning appear to correlate well with neurophysiological changes in the nervous system of DDT-poisoned animals until about 24 h post-injection. After this, only subtle behavioural clues reflected the poisoned nature of the cockroach.

Results indicated that a block in the terminal abdominal ganglion (A6) may have been responsible for alleviating some of the behavioural signs of poisoning at about 24 h post-injection (*i.e.*, those signs that would be induced by input from the cerci). However, it seems unlikely that the block in A6 could account for any long-term behavioural recovery as only one of five animals at 96 h post-injection showed a block in A6. (Prior to dissection, all 96 h cockroaches appeared 'normal', though the animal with a block in A6 responded only slightly to a blow puff.)

Because of the apparent normal behaviour of 96 h DDT-treated animals, it was hypothesized that motor nerve activity in these animals would not be significantly different from controls. But, the experiments described above indicate that there was a significant difference.

Perhaps the act of securing and dissecting the cockroach impaired or altered the ability of the nervous system to screen-out DDT-induced extraneous activity. Also, if motor nerves were still hyperexcitable due to direct action of DDT, then any modulation of AVNC activity by T3 might be obscured. The experiments of Part Ib address these issues.

PART I(b)

In the first series of experiments (Part I(a)) the p,p'-DDT (0.017 μ mol/cockroach, LD50 96 h) was injected intra-abdominally. Recordings were made from the nervous system at various times post-injection (1 h, 24 h, 96 h). However, because the DDT was injected *in vivo*, all nerves in the animal were likely to be exposed to the DDT. If hyperexcitability was also directly induced in motor nerves by DDT, it would be difficult to ascertain the degree to which afferent AVNC repetitive firing activity might have been modulated (down) within T3. Therefore, to try to discern whether or not T3 had the ability to discriminate against DDT-induced repetitive firing from the AVNC, a preparation chamber was constructed which allowed recording from or stimulation of the rostral AVNC (A4 to A1) while at the same time permitting localized perfusion of A5 and adjacent connectives (-A5-) with various test solutions. Thus, repetitive firing (*i.e.*, extraneous, afferent input to T3) could be evoked in the AVNC without subjecting the whole animal to DDT. Recordings from motor nerves 5r1R and 6Br4R (*i.e.*, T3 efferent activity) were the same as described for Part I(a).

METHODS - PART I(b)

1. The Preparation

The preparation and apparatus were similar to that used in Part I(a) except animals were not pretreated with DDT and a separate perfusion and aspiration system was installed (Fig. 12). This allowed the selective treatment of -A5- with the solutions outlined below. The recording/stimulation part of the AVNC chamber contained 5 tungsten wire electrodes. Recordings from motor nerves of the right metathoracic coxa were made using suction electrodes. Motor nerves and T3 were always perfused with normal saline alone.

2. Experimental Protocol

Initially, -A5- was perfused with saline from a 50 ml disposable syringe (this reservoir was replaced after each experiment). Baseline levels of spontaneous activity were established. Responses to sequences of 5 WPs (0.5 Hz; 10, 60 or 100 ms duration; 2.5 - 5.5 psi) directed at the cerci and electrical stimulation (5 single pulses; 0.5 Hz; 0.2 ms duration; threshold intensity to maximum CAP activation) of the AVNC were recorded. Once baseline levels of activity were established, 0.2% or 0.4% acetone in saline (50 ml total volume) was loaded into the reservoir for perfusion of -A5-. (The quantity of acetone required in 50 ml to make a

0.2% or 0.4% solution was vigorously injected into the saline contained in the reservoir just prior to perfusion.) The same protocol for recording and stimulation was used for saline alone. After perfusing with the acetone/saline solution, the same reservoir was refilled with 50 ml of saline containing DDT in acetone. The volume of DDT stock solution vigorously injected into the saline was such that the final concentration was either 2.0×10^{-5} M or 4.0×10^{-5} M with an acetone concentration of 0.2% or 0.4%.

RESULTS - PART I(b)

1. Behavioural Observations

(a) *Perfusion of -A5- with saline and acetone*

Once dissected and set-up for recording, animals usually remained calm and did not struggle in an attempt to escape. Occasionally, animals were seen to groom a foreleg (prothoracic) or an antenna with their mandibles, or spontaneously attempt to walk (which resulted in 'stationary walking' on the lightly greased, polished plexiglas surface).

When the concentration of acetone in the -A5- perfusate was greater than 0.6%, notable behavioural (and neuro-physiological) effects were observed. These included brief episodes of sudden intense struggling followed by periods of hyperexcitability. This behaviour was not observed when -A5- was perfused with 0.2% or 0.4% acetone as long as mixing had been sufficient. However, when perfusing with 0.4% acetone in saline, animals occasionally responded with a stronger than usual stance solidification movement to the initial WP of a sequence, but not to the later WPs of that sequence.

(b) *Perfusion of -A5- with p,p'-DDT*

Perfusion of -A5- with 2.0×10^{-5} M p,p'-DDT did not induce behaviour different from that observed in acetone or saline controls.

Perfusion of -A5- with 4.0×10^{-5} M p,p'-DDT produced behaviour similar to that seen in acetone controls. Generally, animals did not respond with movement to WPs or low intensity stimulation of the AVNC. However, occasionally an increased level of arousal in the CNS seemed to exist (similar to that observed in 0.4% acetone controls). If at this time the cerci were stimulated with a WP, the animal usually responded with a stance solidification movement or attempted to run away.

2. Stimulation of the cerci with wind puffs (WPs)

(a) *Perfusion of -A5- with saline alone and 0.4% acetone in saline*

When -A5- was perfused with saline alone, WPs evoked bursts in the AVNC with a spike frequency and duration of about 900 Hz and 22 ms, respectively (Fig. 18a,b). Perfusion of -A5- with 0.4% acetone in saline produced similar results except that the duration of the WP-induced AVNC burst was significantly longer than when -A5- was perfused with saline alone (Student's t-test, $P < 0.05$ - Fig. 18b). Spiking in N5r1 and N6Br4 of acetone controls

Figure 1B: In situ perfusion experiments – Activity recorded extracellularly from the AVNC of semi-intact preparations in response to wind puffs directed at the cerci. Mean spike frequency (a) and mean burst duration (b) are plotted against position in the wind puff sequence. Connective A5–A6 and ganglion A5 of each preparation was perfused with saline, 0.4% acetone in saline and the indicated DDT solution. Lines represent a regression on each data set. Bars not visible are too small to be seen. Data for 2.0×10^{-5} M DDT are offset to the right for clarity.

was also greater than that recorded in saline controls (Student's t-test, $P < 0.05$). Figure 19 illustrates this tendency.

(b) *Perfusion of -A5- with p,p'-DDT*

When -A5- was perfused with 2.0×10^{-5} M p,p'-DDT, responses to WPs directed at the cerci recorded in the AVNC and motor nerves were not significantly different from acetone controls (Student's t-test, $P > 0.05$ - Fig. 18 & 19).

When 4.0×10^{-5} M p,p'-DDT was applied to -A5- the durations of WP-induced AVNC bursts were significantly longer than both saline and acetone controls ($P < 0.05$). However, the frequency of firing in these bursts was significantly less than both controls (Student's t-test, $P < 0.05$ - see above, Fig. 18a). The motor responses recorded in N5r1 and N6Br4 were mostly not significantly different from saline (or acetone) controls (Student's t-test, $P > 0.05$ - Fig. 19a,b). Those that were significantly different ($P < 0.05$) are marked with an asterisk.

These data indicate that perfusion of -A5- with 4.0×10^{-5} M DDT results in an AVNC response (to a WP) significantly greater than controls. However, in contrast to *in vivo* treated preparations, the exaggerated AVNC response in locally perfused preparations did not result in an exaggerated motor response.

Figure 19: In situ perfusion experiments – Activity recorded extracellularly from (a) N5r1 and (b) N6Br4 of the same semi-intact preparations illustrated in figure xx in response to wind puffs directed at the cerci. The mean wind puff-induced motor response (spike frequency) was plotted against its position in the wind puff sequence. Lines represent a regression of each data set. Bars represent "estimated standard error of the mean". (Bars not visible are too small to be seen.) Data points significantly different from acetone controls are marked with an asterisk (* – $P < 0.05$).

DISCUSSION PART I(a & b)

1. Overview:

Previous experiments (Elia, 1982; Elia & Gardner, 1984) showed that DDT injected into cockroaches had long-lasting effects on the nervous system (VNC). The neurophysiological symptoms of poisoning seemed to far outlive the behavioural consequences which were only apparent up to 24-48 h after injection (in those animals that eventually did recover). This apparent dissociation of neurophysiological symptoms of poisoning and behavioural signs had previously not been described in the literature. (Gammon (1978) using cockroaches and a topically applied, lethal dose of DDT observed repetitive firing shortly before behavioural signs of poisoning appeared. However, his observations did not include any part of the recovery phase that is associated with a sublethally poisoned animal. His experiments showed that the nervous system and behaviour of animals severely poisoned with DDT both remain unstable (and apparently parallel each other) until the animals' final demise (Gammon, 1978).)

2. Part Ia

Responses recorded in the AVNC of minimally dissected, semi-intact preparations described in Part I after wind puff or electrical stimulation were very similar to responses recorded in isolated AVNCs (Elia & Gardner, 1984). The only notable difference was the higher level of ongoing spontaneous activity in the semi-intact preparations. These results indicate that repetitive firing previously observed in isolated preparations (from animals not displaying behavioural signs of poisoning) (Elia & Gardner, 1984) was not an artifact of the isolation procedure.

3. Cerci-GA-Motor Nerve Circuit in Controls

In controls, the use of a 'natural' stimulus (wind puffs (WPs)), evoked responses in the AVNC similar to those predicted from the work of Westin *et al.* (1977) (see also, Camhi & Nolen, 1981; Ritzmann & Pollack, 1981) *i.e.*, a short duration, high frequency burst of activity in the AVNC which induced activity in motor nerves (N5r1 & N6Br4) with a very short delay (11 to 20 ms). This short delay between action and reaction is indicative of the escape response (Camhi, 1980) and is believed to be mediated by the giant axons (GAs) of the AVNC (Roeder, 1948; Camhi *et al.*, 1978; Ritzmann, 1984; Ritzmann & Pollack, 1986). (Controlled wind puffs produced a behavioural latency as little as 11 ms

(Camhi & Nolen, 1981; Camhi, 1984), which is one of the shortest of any animal.)

The behavioural response to a wind puff, i.e., an evasive manoeuvre, is very predictable. The most uniform and consistent response (behaviour and excitation of specific GAS) occurs when a wind puff stimulus is delivered from directly behind the animal (Westin *et al.*, 1977) (used in all experiments presented here).

In experiments described in this thesis, not all control animals responded with a vigorous motor response. This was probably due to the semi-secured nature of the preparation, despite the relative free-range of motion available to the head, anterior thorax and legs (except the right metathoracic) over or on the greased, polished-plexiglas surface. The reduced responsiveness in secured preparations noted here has also been observed by others in cockroaches (Camhi & Nolen, 1981; Ritzmann, 1984) and in crayfish (Krasne & Wine, 1977; 1984). Ritzmann (1984) reported that immobilizing a single leg was sufficient to suppress vigorous escape behaviour. It should be noted that visual cues are also used by an escaping cockroach (Meyer *et al.*, 1981). Thus, the lack of changing visual stimuli may contribute to the suppression of escape behaviour in restrained preparations.

The actual mechanism of suppression induced by restraining the animal remains obscure. However, there are

natural circumstances that exist during which the escape response would be behaviourally inappropriate and must be suppressed. For example, in a restricted space where other cockroaches are moving about, during the mating 'frenzy', or during running and flight.

Recently, a peripheral mechanism (cercal displacement-induced nerve pressure-block) has been implicated in the suppression of activity from the cerci that would otherwise invade the AVNC during flight or running (Libersat *et al.*, 1987; Goldstein & Camhi, 1988; Libersat & Camhi, 1988). This physical, non-synaptic mechanism appears to be independent of the inhibition that can occur in giant axons of A6 by cercal chordotonal organs (Hue & Callec, 1983; Bernard *et al.*, 1983; Goldstein & Camhi, 1988).

4. Wind Puff-Induced Activity in the Nervous System of DDT-Treated Animals

Spike frequency in the AVNC and motor nerves of DDT-injected animals was very high compared to controls. The duration of the AVNC burst in response to a wind puff (WP) appears to be one of the factors that might have been responsible for the initial exaggerated behavioural responses observed in animals injected with DDT. But, in these animals, it is not clear whether extraneous afferent input to motor neurons (via T3) or hyperexcitability of motor nerves *per se* was responsible for the large motor response recorded. Cockroaches treated with DDT 96 h prior

to experimentation appeared normal and did not respond to a blow puff in a hyperexcited fashion before dissection. Repetitive firing in the AVNC did exist in these animals but had to have been suppressed (at some level) in the nervous/muscular system for behavioural signs not to have been observed. A block in A6 could contribute to the lack of behavioural response to wind puffs at 24 h post-injection. However, because of the experimental stimulus protocol employed, this block in A6 (at 24 h) prevented any observation of suppression by another part of the CNS. At 96 h, a block in A6 was not responsible for suppression of extraneous input to the CNS. Neither did the other abdominal ganglia alter afferent activity (except in terminal stages of poisoning, when parts of the nervous system begin to 'shut-down'). Therefore, remaining possible sites of suppression and/or modulation of extraneous activity include: (a) the thoracic ganglia (T3, T2, T1); (b) the muscle cells innervated by thoracic motor neurons; and (c) a combination of (a) and/or (b) with modulatory influence supported by the brain. The possible modulation of activity in T3 is the subject of the remainder of this discussion.

5. Cerci-GA-Motor Nerve Circuit in DDT-Treated Animals

The giant axons (GAs) of the cockroach VNC extend uninterrupted from A6 to the suboesophageal ganglion (Parnas *et al.*, 1969; Spira *et al.*, 1969a,b). The diameter of dorsal giants decreases abruptly in T3. Ventral GAs suddenly taper after entering each thoracic ganglion, widening again as they exit the ganglion. This change in diameter and synaptic input to the ventral GAs within the thoracic ganglia creates regions of "low safety factor", *i.e.*, regions that cannot support a long duration, high frequency burst of action potentials (Castel *et al.*, 1976; Parnas *et al.*, 1976; Spira *et al.*, 1976). Based upon this evidence, it was hypothesized that DDT-induced repetitive firing might be suppressed in T3 by the region of low safety factor in the GAs. This suppression might serve to reduce behavioural hyperexcitability by blocking input to motor neurons from the GAs, especially during a wind-puff induced escape response.

This assumes that the GAs are responsible for mediating escape behaviour evoked by wind puff stimulation of the cerci. There is strong evidence to support this suggestion (see Introduction). However, the possibility that a parallel, non-giant axon pathway might mediate escape behaviour should not be disregarded (Parnas & Dagan, 1971). Such a parallel, non-giant axon pathway appears to exist in the crayfish (Krasne & Wine, 1984).

Results of experiments in Part I(a) indicated that the circuit involved in the escape response (from cerci to motor output in the metathoracic leg) was intact and functional in control and DDT-injected animals. Three pieces of evidence supported this: 1/ recordings made from motor nerves N5r1 and N6Br4 of the metathoracic coxa, and movement of other legs in response to wind puff stimuli; 2/ usually, there was only a short latency between wind puff stimulation of the cerci and recording of activity in motor nerves (11 to 20 ms), (which made the use of a non-giant axon pathway less likely, see Appendix 6 for calculations); and 3/ the behavioural response (escape) made to a blow puff prior to dissection. Thus, it seems unlikely that a long-term block in the GAS in any of the thoracic ganglia prevented the expression of DDT-induced repetitive activity. Therefore, under the conditions of experiments described here, the regions of low safety factor in giant axons do not appear to block input to motor neurons of T3.

In an injected animal, all nerves are likely to be exposed to DDT. Therefore, even if modulation (down) of afferent AVNC activity was occurring in T3 it may not have been recognizable because of hyperexcitability directly induced in the motor axons (and consequent repetitive firing). Another concern was that the process of securing the cockroach and inserting a miniature recording chamber into its abdomen might have somehow altered the ability of

the CNS to process (filter-out) the DDT-induced extraneous input. Some of these concerns were addressed by experiments of Part I(b) which were designed to better assess whether extraneous afferent input (induced by DDT) could be 'filtered-out' by T3. The preparation used was the same as that of Part I(a) except that a provision in the AVNC chamber was made which allowed the localized perfusion of the fifth abdominal ganglion (A5) and associated connectives (-A5-).

6. Part I(b)

When -A5- was perfused with a solution of saline containing 4×10^{-5} M DDT the response recorded in the AVNC (both frequency and duration of burst) was significantly different from controls (both saline and acetone). However, motor responses were not different from acetone controls and only marginally different from saline controls. These data indicate that DDT-induced repetitive firing in the AVNC was not being expressed as repetitive firing in the motor nerves. Since the abdominal ganglia did not suppress this repetitive firing, it is plausible that elements within T3 might be responsible for filtering-out (or modulating down) the extraneous input. Closer examination of this "black box" might reveal a mechanism by which extraneous activity

could be suppressed before reaching the motor neurons (see Part II, Discussion).

PART II

Experiments of Part I indicated that filtering of extraneous afferent input induced by DDT could have occurred in T3. DUM neurons in T3 of locust were suggested to have modulatory actions on a motor neuron within T3 (Sombati & Hoyle, 1984) and directly on muscles which the DUM axons innervate (for example, Hoyle & O'Shea, 1974; Evans & O'Shea, 1977; 1978). To investigate this possibility in cockroaches, experiments were designed to determine: (1) the involvement of the T3 dorsal unpaired median (DUM) neurons in the cercal/AVNC escape response and (2) how DUM neurons are affected during early stages of poisoning by DDT.

Also, a method of breeding cockroaches was established to see if different individuals with the same genetic heritage showed consistency in position of particular DUM neurons (especially DUM5r1).

METHODS - PART II

The nervous system (T1 to A6) and nerve roots of T3 were excised with the three terminal abdominal segments and cerci attached (Fig. 20). (The intact cerci functioned as an additional means of evoking activity in the AVNC.)

Figure 20: The isolated preparation and chamber used in experiments of Part II. The wet and dry sides of the chamber are connected by a narrow isthmus which is sealed with grease after the VNC is in place. (ASP, aspiration; DUM, microelectrode to DUM neuron of T3; GS, grease seal; N5r1R & L, suction electrodes to N5r1R & L; O2, oxygen to chamber; P511, amplifier leads; PER, perfusion; REF, reference electrode for the microelectrode; RS, record/stimulate leads to chamber; TS, terminal abdominal segments with cerci attached; WPT, wind puff tube; $\pm$, stimulator leads.)

1. The Preparation

Animals were injected intra-abdominally with a LD50 96 h dose ($0.017 \mu\text{mol}/\text{cockroach}$) of p,p'-DDT 1 h or 96 h prior to the experiment. Control animals were injected with $1 \mu\text{l}$ of saline.

Ganglia A6 to A3 were enclosed (dorsal surface up) in the posterior end of a Sylgard chamber which contained five 0.08 mm ($0.003''$) tungsten wire electrodes for recording or stimulating. This chamber was sealed with a cover slip and isolated from the anterior chamber by a grease bridge. The terminal abdominal segments were secured in place above and behind the posterior chamber using vaseline. A2 to T1 were pinned to the floor of the anterior part of the Sylgard chamber (dorsal surface up) and received a continuous supply of oxygenated saline (Fig. 20). (A small needle also delivered oxygen directly to the bath.) The total volume of the anterior chamber was $\leq 0.5 \text{ ml}$. The flow rate of saline ranged from 2.5 to $3.5 \text{ ml}\cdot\text{min}^{-1}$. The metathoracic ganglion was partially secured to the Sylgard by impaling distal branches of nerve roots 2 and 3 with 0.25 mm diameter MINUTEN pins. The entire surface of T3 was then desheathed⁵ using fine forceps (#5 Dumonts) which had been sharpened to

⁵ The desheathing process removed the neural lamella and may or may not have disrupted, but did not remove, the underlying perineurial cell layer (see Discussion).

very fine points on a Carborundum stone. Once the sheath was removed, T3 was further secured with MINUTEN pins.

Following dissection and isolation of the preparation in the recording chamber, the entire dorsal surface of T3 was "desheathed". This procedure removed the neural lamella (the outer collagenous (acellular) sheath) but for the most part left behind the underlying perineurium. If animals were used within three to five days of the imaginal moult, the neural lamella could be removed without any apparent disruption of the contiguity of the cellular perineurium. After about two to three weeks from the imaginal moult the neural lamella could be removed with some disruption of the perineurium. In these preparations, the loss of perineurial contiguity (and hence the permeability barrier (Lane & Treherne, 1970; 1972; Schofield & Treherne, 1984)) was subtle (if it occurred) and cells below the perineurium were generally not damaged. (If it was suspected that cells beneath the perineurium were damaged, the preparation was discarded. Because the DUM neurons lie immediately beneath the perineurium, their integrity was, in part, used as a measure of damage incurred.)

Suction electrodes (the same as described previously in Part I) were used for recording and stimulating N5r1R and L. After extracellular electrodes to the AVNC and motor nerves were set in place, an intracellular microelectrode (filled with 4% Lucifer Yellow in 0.5 M LiCl; or 2 M KCl) was prepared and moved into position above the group of T3 DUM neurons. Microelectrode resistance and D.C. balance were checked and adjusted as necessary. A Carl Zeiss dissecting stereomicroscope (model DRC) with 16X eyepieces and [2x-4x-8x] changer was used during the impaling procedure.

The tip of the microelectrode was positioned just above the target cell and slowly advanced. A shift of -2 to -6 mV in the digital display of the amplifier indicated that the surface of the preparation had been contacted. At this point, a gentle tap on the cage baseplate or a slight further advance was sufficient to cause the microelectrode to enter the DUM soma. Once inside, the resting potential was allowed to stabilize (5 to 10 min) after which the microelectrode balance was rechecked and adjusted (see Materials and Methods - General 6.(b)).

2. Physiological and Morphological Identification of DUM5r1

After impalement, a putative DUM soma was tested to determine if: (1) the cell impaled was excitable (and thus likely to be a DUM soma) and (2) the impaled cell had an axon branch in N5r1R and L. DUM somata which were spontaneously active were recognizable by their large, usually overshooting, APs followed by an after-hyperpolarization of 12 to 22 mV. If the spontaneous APs in such a DUM soma could be correlated with coincident APs in N5r1R or L then this DUM neuron was classified as 'DUM5r1'. To further verify that the impaled cell was DUM5r1, N5r1L (or R) was

stimulated (1 ms duration, 0.5 Hz, intensity usually 2.5 to 4.0 V) in an attempt to excite the DUM soma and contralateral DUM axon by antidromic conduction in the DUM axon.

When the DUM neuron was not spontaneously active, soma APs were induced by anode break excitation (usually from -2 to -5 nA) or depolarization (D.C. or pulses). One of these procedures usually evoked a somal AP which was then compared with activity in N5r1R and L to determine if the DUM soma in question was DUM5r1. The antidromic stimulation procedure was employed for verification. At the completion of most experiments, the DUM soma was filled with Lucifer Yellow (LY) by applying a current through the microelectrode of -2 to -5 nA for 30 to 90 min with a brief check of resting potential every few minutes. After the microelectrode was withdrawn from the cell, the injected LY was allowed to diffuse down the axons for 1 to 5 h. T2 to A1 (inclusive) were then excised and mounted under a coverslip on a depression slide (in saline). The preparation was viewed under a Zeiss Fluorescent Photomicroscope II with 50 watt mercury burner and a high pass barrier filter with a cut-off wavelength of 500 nm.

3. Position of DUM5r1 in the DUM Neuron Group of T3

One of the factors which makes the study of identified DUM neurons more difficult than identified motor neurons is that their somata do not always occupy the same position within the DUM neuron group. For this reason the following studies were undertaken.

(a) Animals from the same or different oothecae (same lineage)

To investigate the possibility that a sub-population of cockroaches derived from the ootheca of a mated pair (same parents) would have DUM5r1 in a consistent position within the DUM group, a single male was mated with four virgin females. A female was temporarily isolated when found to be in the process of expelling an ootheca. The ootheca was collected, tagged, hatched and cared for under conditions previously described (see Materials and Methods - General, Section (a)). The offspring from each single ootheca were raised to maturity in individual housing units. Photographs and hand drawings of the DUM soma group and axon morphology were kept for each animal after at least one DUM neuron had been filled with Lucifer Yellow. Drawings and photographs were later compared for the similarity of: (1) general superficial positioning of DUM somata within the DUM neuron group, and (2) the specific position of DUM5r1 in each group.

(b) *Offspring from the same ootheca (unknown lineage)*

Oothecae were also collected from females inhabiting the general colony. The offspring of these oothecae (of unknown lineage) were raised in the same way as oothecae of "known lineage".

4. Preliminary Experiments with 'Zero'-Calcium Saline

Following the above experimental protocol, but before iontophoresis of Lucifer Yellow, some preparations were bathed in a saline of "zero-calcium"⁶ concentration (214 mM NaCl, 3.1 mM KCl, 10 mM MgCl₂, 3.0 mM MOPS and 3.9 mM MOPS-Na). Once the membrane potential had 'restabilized' in zero-calcium saline, the amplitude of evoked APs was measured. (Soma APs were evoked by stimulating N5r1L or R, by anode break excitation of the soma, or by depolarization of the soma. Spontaneous APs while in zero-Ca²⁺ saline were rare.)

5. Activation of T3 DUM neurons

The stimulus protocols employed to evoke the escape response were similar to those used in experiments of

⁶ Without the use of a suitable Ca²⁺ chelator (eg., EDTA or EGTA), the Ca²⁺ concentration in the saline should be regarded as being very low but not truly "zero".

Part I. Responses to sequences of 5 WPs (0.5 Hz; 60 or 100 ms duration; 2.5 to 5.5 psi) directed at the cerci and electrical stimulation (5 single pulses; 0.5 Hz; 0.2 ms duration; threshold intensity to approximately three times threshold intensity) of the AVNC were recorded. Trains of electrical stimuli were also delivered to the AVNC to mimic a burst of activity produced by a wind puff. A range of train frequencies (50, 100, 300, 600 Hz trains; [50 ms duration/train; 0.2 ms pulse duration; 0.5 trains/s]) were used (see Fig. 8, p 58).

Not all stimulus regimens were tested on each preparation. The objective was to establish a regimen of AVNC or cercal stimulation that was effective in evoking activity in DUM5r1. Thus, there were more responses recorded for 100 and 300 Hz trains (especially in controls). Stimulation of the cerci with wind puffs was also effective and presented for controls. Unfortunately, due to technical problems, cercal-evoked responses in DDT-treated animals were only recorded in one of eight preparations. Although the neural pathway linking the cerci to motor nerves was patent prior to dissection, something happened during the dissection or transfer of the preparation which rendered the circuit inoperable.

RESULTS - PART II

1. General

Animals used in Part II were injected with physiological saline or a LD50 96 h dose of p,p'-DDT at 1 h or 96 h prior to dissection. Their behaviour between the time they were injected and used in an experiment was not different from that described in Part I for similarly treated animals.

The responses recorded in the nervous systems (AVNC and N5r1) of 1 h and 96 h DDT-treated animals were not significantly different. Therefore, these results were pooled.

In most experiments, the physiologically identified DUM neuron (DUM5r1 - see below Section 2) was injected with Lucifer Yellow after completion of the experimental protocol. This helped confirm that the DUM neuron under investigation sent an axon into N5r1. (The location of the DUM5r1 soma in relation to other DUM somata was also noted (see below, Section 2).)

After filling a DUM soma with Lucifer Yellow, a fuzzy outline of the "primary neurite" was seen to lead from the soma into the cortex of T3, and then, at the point of bifurcation, to form the "lateral neurites" (Fig. 21a, see also Fig. 7) (notation of Watson, 1984). The lateral

neurites of the two injected DUM neurons travel together close to the dorsal surface of the ganglion. The neurites cannot be distinguished separately until they reach the lateral margins of the ganglion (Fig. 21a). At the lateral margins of T3 the lateral neurites gave off branches into particular nerve roots (Fig. 21c; see also Fig. 26). In most cases the distribution of DUM axons into peripheral nerve roots was symmetrical (left and right). However, in a few cases a unilateral fill of a nerve root occurred. On the side that failed to fill with Lucifer Yellow, the axon appeared to stop short of the nerve root and/or a build-up of the dye was seen at the point of blockage. This was assumed to indicate DUM axon damage.

In addition to the major branches which enter the nerve roots from the lateral neurites, a sometimes profuse supply of small "secondary neurites" was observed to emanate from the primary and lateral neurites. Some of the secondary neurites of cockroach DUM neurons (labelled "a", "c", & "d" in Fig. 21a & b) corresponded to the generalized scheme shown to exist in locust DUM neurons (Watson, 1984).

Figure 21(a,b): Sketch shown above was traced from a photograph of a portion of a cockroach VNC (T3 and A1). It shows the approximate positions of the group of DUM somata (DUM) and the Lucifer Yellow filled somata and axons (not to scale) in relation to the whole ganglion (T3). Dotted lines represent the lateral neurites (LN) and some of the secondary neurites which are visible in the photograph (at right). The boxed area delineates the area of interest in Figure 21(b)(iv) (see next page). Figure 21(c) shows two different preparations with Lucifer Yellow filled DUM axons in nerve roots of T3. (Scale bars in all figures represent 150 μ m.)

Figure at right: (a) - montage (fluorescent micrographs) showing two DUM neurons of the metathoracic ganglion that were injected with Lucifer Yellow. (Anterior is at the top.) The two Lucifer Yellow filled somata in the DUM group appear white. (Each DUM soma projects a single primary neurite (not visible) which bifurcates into a left and right lateral neurite.) The two lateral neurites (LN) in each hemiganglion travel together in an axon tract, but can be seen to separate near the lateral margins of the ganglion. A right "c" secondary neurite and right and left "d" secondary neurites can be seen to arise from the lateral neurites. The secondary neurites are more visible in Figure 21(b) (see next page). One of the filled DUM somata sent axon branches (Di) into the T3-A1 connectives (lower photo). These axons were traced as far as the first abdominal ganglion (A1). Tracheal tubes (TT) can also be seen within the ganglion. The preparation was desheathed prior to impaling the DUM neurons. (Figure 21(b) on next page.) (Nomenclature of Watson, 1984.)

(a)

Figure 21(b): Photographs of areas from the same preparation shown in Fig. 21(a). (i) and (ii) show the left and right lateral neurites (LN) near the peripheral margin of the neuropile. Secondary neurites labelled "a", "c" and "d" can also be seen. In (i), a d-neurite begins in an anterior direction and then bifurcates (B). One process travels posteriorly, under the lateral neurite (LN - out of focus). The other visible branch proceeds in an anterior direction. Although the d-neurite in (ii) does not make an obvious anterior projection from the LN, it follows a posterior path similar to the d-neurite seen in (i). The photographs from (ii) to (iv) were taken sequentially, at increasing magnifications and depths of focus. They follow the right d-neurite in a posterior-medial direction in the ganglion. (iii) The d-neurite and its varicose-like swellings (v) are shown (small arrows). The position of the slow depressor motor neuron soma (not visible) is at the bottom of the photograph. (iv) The d-neurite from (iii) (out of focus) enters the photograph from the right. A point of reference relating the two photographs is indicated by an asterisk (*). Several very fine branches from the "a" secondary neurites (which emanate from the primary segment (not visible)) can be seen to also contain varicose-like swellings along their lengths (small arrows). (Nomenclature of Watson, 1984.) (Scale bars, 150 μ m.)

Figure 21(c): Two photographs of T3 nerve roots (dorsal view) from different animals, each with a single DUM neuron (DUM5r1) injected with Lucifer Yellow.

In (i), the right side of T3 is shown. This DUM neuron had axon branches (DA) in nerve roots 3 (N3) and 5 (N5). In (ii), left nerve roots 4 (N4) and 5 (N5) are shown (taken from a different animal than in (i)). The left margin of T3 is visible on the right side of the photograph. This DUM neuron had axon branches (DA) in nerve roots 3, 4, 5 and 6 (only N4 & N5 are visible in this photo). The DUM axon in N4 bifurcate. to send an axon into each of the main branches of N4. (Scale bar, 150 μ m.)

2. Identification and Mapping of DUM5r1

(a) *Physiological Methods*

Three pieces of physiological data were used to identify DUM5r1 as the soma sending an axon to motor nerve 5r1 right and left (N5r1R & L). The first involved the recording of "spontaneous" action potentials (APs) in the DUM soma simultaneous with spikes in N5r1R and L. Very often several APs would be evoked during impalement of the soma and/or the cell would be spontaneously active at a low frequency (0.2 to 0.5 Hz). If the impaled soma was DUM5r1 then a correlation could be made between a somal AP and a low amplitude spike in N5r1 (Fig. 22). (In N5r1, the slow depressor motor neuron (D_s) usually provided a background firing rate (variable, 1-15 Hz) with a spike amplitude that was distinctly larger than the DUM axon spike. Low frequency spontaneous firing in any of the common inhibitors (CI) occasionally occurred in N5r1. CI activity was distinguished from a DUM axon spike by the CI's repetitive occurrence, the different shape of CI spikes and the lack of correlation with the DUM soma AP. The fast depressor motor neuron (D_f) fired only very rarely (except in DDT-treated preparations) and its large spike amplitude precluded it from being mistaken for the DUM axon spike.)

Figure 22: Action potentials recorded in the AVNC, N5r1 and DUM5r1 during impalement of DUM5r1 with a microelectrode. The sudden drop in the DUM trace was the point of entry of the microelectrode (curved arrow). Note the occurrence of related DUM5r1 APs and DUM axon spikes in N5r1 (arrowheads). The other spikes in N5r1 were probably from one of the common inhibitors. Low amplitude spontaneous spikes can also be seen in the AVNC. (Scale bars: 50 mV (DUM5r1), 0.15 mV (AVNC, N5r1), 50 ms (all traces).)

The second method used to identify DUM5r1 was to induce an AP in the DUM soma and correlate this with the response in N5r1R and L (Fig. 23). (The soma was directly excited by a brief electrical pulse applied through the microelectrode.)

Figure 23: Action potentials (APs) evoked in DUM5r1 (DUM) after a brief hyperpolarization of membrane potential to about -75 mV (curved arrow, "anode break excitation"). Note the related spikes in DUM5r1 and N5r1 (arrowheads). (Scale bars: 20 mV (DUM5r1), 0.15 mV (N5r1), 50 ms (all traces).)

The third method used to identify DUM5r1 was to electrically stimulate N5r1 while recording from a putative DUM5r1 soma and contralateral N5r1. The subsequent

antidromic DUM axon spike travelled back to the initial segment of the DUM neuron in T3 where it initiated an AP in the DUM5r1 soma. It also continued on as a spike in the contralateral N5r1. A successful correlation appeared as a small stimulus artifact followed by a DUM soma AP (50 to 110 mV amplitude with 12 to 22 mV AHP - with appropriate delay for antidromic conduction from the point of stimulation); following the DUM5r1 soma AP would be a contralateral N5r1 low amplitude spike (Fig. 24).

Figure 24: Antidromic electrical stimulation of N5r1L (2.0 V, 1 ms duration) while recording from a DUM soma (DUM5r1) and N5r1R. Note the sudden onset of the soma AP (i.e., no pre-depolarization) and the matching (delayed) spike recorded in N5r1R (arrow). (SA, stimulus artifact; scale bars: 20 mV (DUM5r1), 0.05 mV (N5r1), 5 ms (both).)

(b) *Morphological*i) DUM5r1 and axon branches - Randomly selected individuals

It has been reported in the literature that unlike motor neurons of the central nervous system (CNS), the exact position of individual somata within the DUM group varies from preparation to preparation. Results presented here indicate that in randomly selected individuals, the probability of finding a DUM5r1 (soma) in the anterior-midline or anterior-right position in the DUM group was about 70% (total n = 35 - Fig. 25). DUM5r1 was also

Figure 25: Representation of the approximate geometric positions within the DUM soma group where DUM5r1 was found to reside in randomly selected individuals. (a) anterior-midline or anterior-right position (70% probability); (b) dead centre, anterior-left or mid-left position (29% probability); and (c) posterior to group midline (1%).

occasionally found in the centre, anterior-left or mid-left position of the group ($n = 10$). There was but one case of DUM5r1 positioned posterior to the group midline.

Lucifer Yellow iontophoresed into DUM5r1 somata "successfully" migrated into the nerve roots of 19 preparations. Two preparations showed the lateral branches splitting and sending a single axon into both N3 and N5 (Fig. 26c). One preparation displayed branches in only N5 and N6. The remainder (16) were divided equally between two root innervation patterns: (a) to N3, 5, and 6; or (b) to N3, 4, 5, and 6 (Fig. 26a,b).

Figure 26: Dorsal view of nerve root branch patterns of DUM5r1 in T3 (small branches within T3 are not shown). Axons of DUM5r1 were identified using Lucifer Yellow and fluorescence microscopy. The single preparation with a DUM5r1 axon to N5 and 6 is not shown. (a) N3, 5 and 6 ($n = 8$ of 19); (b) N3, 4, 5 and 6 ($n = 8$ of 19); and (c) N3 and 5 ($n = 2$ of 19). (Drawings are not to scale. A, anterior; P, posterior; numbers refer to peripheral nerve roots.)

ii) Animals from the same or different oothecae
(same lineage)

Nine experiments used cockroaches derived from the same female/male parents. The position of DUM5r1 in eight of the nine animals was anterior-right of centre (as in Fig. 25a, above). The non-conforming DUM5r1 was positioned anterior-left of centre. Unfortunately, in only three of the nine animals could a positive identification be made of which nerve roots (other than N5) were innervated by the DUM5r1. In two of the three preparations that were successfully filled, DUM axons were seen in N3, 4, 5 and 6. The third indicated innervation of N3 and 5.

iii) Offspring from the same ootheca
(unknown lineage)

In a single example of three males from the same ootheca, DUM5r1 was positioned anterior-right of centre in two of the three (Fig. 25a). The third had DUM5r1 positioned just posterior to dead centre (Fig. 25c).

3. Resting Membrane Potentials of DUM Neurons

DUM neurons studied were grouped into two categories:
(a) those that send an axon to motor nerve 5r1 (DUM5r1) and
(b) those that were identified as not having an axon in N5r1 (non-DUM5r1). As the resting potential of individual DUM

cells tended to vary with time (see Fig. 27), the mean of the resting potential range of each cell was used to determine the overall mean resting potential for DUM5r1 and non-DUM5r1 somata (see Fig. 27 & 28).

The mean resting potential of control DUM5r1 somata was -45.1 mV ($n = 25$, standard deviation (SD) = 5.7 mV). A mean resting potential for non-DUM5r1 somata was -48.0 mV ($n = 35$, SD = 5.6 mV). Although the number of DDT-treated preparations was not as large as for controls, there appeared to be a tendency for the resting potentials of DDT-treated DUM5r1 (-49.1 mV, $n = 10$, SD = 4.4) and non-DUM5r1 (-53.4 mV, $n = 6$, SD = 4.9) somata to be more negative than for controls. However, these differences are not significant ($P > 0.05$).

A feature of both control and DDT-treated preparations was the tendency of the resting potential to vary during a recording session. The symbols connected by lines in Figures 27 & 28 illustrate the movements of the resting potential of individual cells with time. These shifts in resting membrane potential did not appear to be periodic and were not directly associated with the slow depolarization and spontaneous spiking that is often observed in these cells (Fig. 29). Also, they were distinctly different from changes that occasionally occurred due to a sudden increase in microelectrode resistance. (When an increase in microelectrode resistance occurred, the membrane potential

Figure 27: Scatter plots of resting potentials of T3 DUM5r1 somata for controls (a) and DDT-treated (b) animals. Representative examples are presented. Connected points are from a single cell, each from a different individual. The dotted line is the overall mean DUM5r1 resting potential.

Figure 28: Scatter plots of resting potentials of T3 DUM somata (other than DUM5r1) for controls (a) and DDT-treated (b) animals. Representative examples are presented. Connected points are measurements from a single cell, each from a different individual. The dashed line is the overall mean non-DUM5r1 membrane potential.

increased in a hyperpolarizing direction and the noise-level of the baseline also increased substantially.)

Figure 29: Low frequency spontaneous action potential in DUM5r1 and N5r1R. (Scale bars: 20 mV (DUM5r1), 0.05 mV (N5r1), 5 ms (both).)

4. Dependence of Resting and Action Potentials on Extracellular Calcium Ions

An experimental variable that probably influenced the change of resting and action potentials with zero- Ca^{2+} saline was the degree to which the perineurial cells, other glial cells and the DUM group surrounded and 'shielded' the impaled DUM soma. Thus, the results with zero- Ca^{2+} saline have value on a qualitative level and are preliminary in nature.

(a) *Resting potential in zero- Ca^{2+} saline*

When a DUM soma was perfused with "zero"- Ca^{2+} saline there was a rapid depolarization of the resting membrane potential (RP) (Fig. 30(a,b)). The membrane potential decline stopped and plateaued within eight minutes of the start of zero- Ca^{2+} saline in all but one preparation. (Three of the five preparations ended the decline of RP within four minutes.) The mean decline was 25% of the initial resting potential value (SD = 9.68%) with a minimum and maximum decline of 15% and 44%.

The recovery of RP after reintroducing normal saline was less rapid than the decline. Upon return of normal saline, four of the seven trials displayed a rapid initial rate of RP recovery followed by a less rapid phase until RP was restored (Fig. 30a,b). Two cells recovered only 50% of their pre-zero- Ca^{2+} membrane potential within 32 min of reinstituting normal saline. Parenthetically, the trial in which the RP returned at a steady slow rate to normal levels (Fig. 30a) was the second of a duplicate run on a single cell (open and closed circles). The return to normal RP was rapid during the first run on this cell.

It should be noted that in only one of the seven trials did the rapid decrease in RP induced by zero- Ca^{2+} saline cause a cell to enter into a brief discharge of APs. Cells that were spontaneously active at the time of initiation of

Figure 30: Perfusion of T3 and nerve roots with "zero"- Ca^{2+} saline. The entire dorsal surface of T3 was desheathed. Data are plotted on two graphs (a,b) for clarity. Open and closed symbols of the same shape are duplicate trials from the same experiment.

zero- Ca^{2+} saline usually stopped firing during the decline to a new, more depolarized RP.

(b) *Action potential and after-hyperpolarization
in zero- Ca^{2+} saline*

Perfusion with zero- Ca^{2+} saline resulted in a decrease of action potential (AP) amplitude and little or no change (with two exceptions) of the after-hyperpolarization (AHP) (Table 1). It is evident that the decrease in AP amplitude was quite variable, with a mean decrease of 26.7% (SD = 17.8%).

Table 1: The effects of zero- Ca^{2+} saline on the resting and action potentials of DUM5r1. Superscripts refer to the methods used to excite action potentials in DUM5r1 (listed at the bottom of the table). (n. = no response.)

<u>Normal Saline</u>		<u>Zero-Ca^{2+} Saline</u>		<u>Normal Saline</u>	
RP	AP + AHP	RP	AP + AHP	RP	AP + AHP
(mV)	(mV)	(mV)	(mV)	(mV)	(mV)
-56	88 + 12 ²	-36	35 + 14 ¹	-54	90 + 14 ¹
-54	90 + 14 ¹	-43	73 + 13 ¹	-49	n.
-47	64 + 24 ¹	-40	48 + 15 ²	-47	66 + 15 ³
-50	66 + 15 ³	-41	56 + 14 ¹	-51	n.
-56	93 + 17 ¹	-43	76 + 18 ¹	-42	55 + 26 ¹
-48	68 + 21 ⁴	-45	40 + 20 ¹	-47	n.
-51	80 + 12 ²	-43	73 + 16 ²	-46	78 + 12 ²

¹ APs in DUM5r1 evoked by stimulating DUM axon in N5r1L

² Spontaneous action potentials

³ APs induced by anode break excitation (-5nA)

⁴ APs induced by train (600 Hz) delivered to AVNC

When normal saline was reintroduced, recovery of AP amplitude roughly paralleled the recovery of resting potential. Cells which regained pre-zero- Ca^{2+} RP levels also regained pre-zero- Ca^{2+} AP amplitude (Table 1).

The role played by inactivation in reducing the size of the AP in zero- Ca^{2+} was not fully investigated. However, anode break excitation (ABX) using brief (5 to 10 s) hyperpolarizing currents did not reactivate a full AP. On several occasions the cell remained refractory to ABX. (This refractoriness was not unusual as it was also occasionally observed in preparations perfused with normal saline.)

5. Recruitment of DUM5r1 During the Escape Response

(a) Wind puffs (WPs) - Controls and DDT-treated animals

i) Responses recorded in the AVNC and motor nerves

In both controls and DDT-treated animals, the responses recorded from the AVNC to stimulation of the cerci with 60 and 100 ms WPs were similar to those observed in Part I (see Results Part I(a) - Section 2(b)). However, in both cases the mean frequency of firing within a WP-induced AVNC burst was notably higher in the isolated preparations of Part II (for controls, 1100 Hz, Fig. 31) compared to the semi-intact control preparations of Part I (800 Hz, see Fig. 15). In controls, the mean duration of these AVNC bursts were also

Figure 31: Activity recorded extracellularly from the AVNC of isolated preparations in response to wind puffs directed at the cerci. Mean spike frequency (a) and mean burst duration (b) for each wind puff were plotted against position in the wind puff sequence. A burst in the AVNC was defined as being terminated when the instantaneous spike frequency within that burst fell below 200 Hz. Lines represent a regression on each data set. Bars represent "estimated standard error of the mean". (Bars not visible are too small to be seen.) "n" represents the number of preparations. Each data point represents the mean of 24 samples (controls) or 6 samples (p,p'-DDT injected).

consistently longer in the isolated preparations compared to semi-intact preparations (for controls, 77 ms and 57 ms, respectively). In DDT-treated animals, the mean frequency of firing within an AVNC burst was higher in isolated preparations (1050 Hz vs. 900 Hz) but the characteristic long duration (ca. 175 to 200 ms) AVNC burst was the same in both semi-intact and isolated preparations.

Unfortunately, in only one of eight DDT-treated preparations were WPs effective in evoking activity in the AVNC and motor nerves. In this preparation, DUM5r1 was not recruited during the response to the wind puff stimulus. To recruit this DUM5r1 required electrical stimulation of the AVNC with 100 Hz trains at three times threshold intensity (even though single stimuli at threshold intensity were sufficient to evoke AVNC and motor activity). Clearly, this was an unusual preparation.

Motor responsiveness (measured as the frequency of firing evoked in N5r1 by a WP stimulus) in isolated, control preparations of Part II was approximately six times greater than the average control response recorded in semi-intact preparations of Part I (compare control curve in Fig. 16a with that of Fig. 32a). This level of firing in Part II controls is similar to that seen in DDT-treated preparations of Part Ia (see Fig. 16).

The mean time required for APs to travel from the A5-A6 connective to the site of recording in N5r1 was 8.1 ms (SD = 3.69, n = 90) for controls and 4.6 ms (SD = 2.13,

Figure 32: Activity recorded extracellularly from N5r1 of isolated preparations in response to wind puffs directed at the cerci. Mean spike frequency (a) and mean burst duration (b) for each wind puff are plotted against position in the wind puff sequence. Lines represent a regression on each data set. Bars represent "estimated standard error of the mean".

n = 34) for DDT-treated. It takes about 3 ms for a signal to travel from the cerci to the A5-A6 connective. Therefore, conduction time from cerci to metathoracic coxal leg muscles was about 11 ms and 8 ms (control & DDT, respectively), which is close to the behavioural latency previously described (see Introduction).

ii) Responses recorded in DUM Neurons (WP stimuli)

The efficacy of a particular stimulus protocol (WP to the cerci or electrical stimulation of the AVNC) in evoking activity in DUM5r1 was judged by two characteristics of the DUM5r1 response: (a) whether a change in membrane potential (EPSP, IPSP, APs) was induced by the stimulus and (b) the delay between initiation of the stimulus and the beginning of a response in DUM5r1 ("delay to onset").

Minimum activation in DUM5r1 was typically a hyperpolarization or depolarization of one to several mV amplitude (without a subsequent AP). DUM5r1 was also seen to respond with an EPSP-like depolarization followed by an AP. A maximal response (under current experimental conditions) was two, or, on rare occasions three, APs following in quick succession (2 or 3 within 300 ms). After DDT treatment, despite the evidence of hyperexcitability throughout the nervous system, DUM5r1 would not fire repetitively beyond that described above (3 APs in 300 ms). The notable exceptions to this were the

occasions when ABX of a DDT-treated DUM5r1 would evoke a burst of APs at about 40 Hz (see below).

Wind puffs usually evoked a depolarization and/or AP in DUM5r1 in three of four control preparations that responded to WPs. A typical example of a WP-induced AP in DUM5r1 and associated activity in the AVNC and N5r1 of a control is presented in figure 33.

In controls, the mean time between the WP-stimulus and beginning of depolarization in DUM5r1 was about 48 ms (SD = 10.59, n = 35). This time is too slow for the giant axons (GAs) to have been responsible for 'direct' excitation of DUM5r1. Therefore, given the short delay for a motor response and the relatively long delay for a depolarization in DUM5r1 (Table 2), excitation of DUM5r1 was clearly secondary to recruitment of motor neurons.

In one of the four control preparations in which WPs were used, DUM5r1 responded to WPs with a low amplitude (mean = -1.6 mV, SD = 0.49), long duration (mean = 203 ms, SD = 89.3) hyperpolarization (Table 2). The mean delay associated with this response (20 ms, SD = 4.91) was considerably shorter than for the depolarizing responses induced by WPs. Similar, and some longer delay, hyperpolarizing responses were evoked by electrical stimulation of the AVNC in two other control preparations.

Figure 33: Control - Activity evoked by two consecutive wind puffs (60 ms duration) of a sequence. The initiation of the wind puff (time = 0) is marked with a dotted line. Note that the activity in the AVNC and N5r1 precedes activity in DUM (DUM5r1). The arrow marks a DUM axon spike in N5r1. (Scale bars: 100 mV (DUM5r1 only), 100 ms.)

Figure 33 Supplement: The first part of the trace in figure 33 (previous page) was enlarged for clarity. The beginning of the wind puff is marked by an arrow. The DUM5r1 axon spike is marked by an arrowhead. (Scale bars: 70 mV (DUM5r1); 0.15 mV (AVNC & N5r1); 50 ms.)

Table 2: Response characteristics of DUM5r1 to selected stimulus protocols (see Appendix 6 for an entire listing). Electrical stimuli were delivered to the distal AVNC. Wind puff stimuli were delivered to the cerci (control data only). Of six controls, three responded with a depolarization to any stimulus and three responded with a hyperpolarization. A minimum of 20 samples were taken for each value listed.

Notes: AP Amplitude, overshoot + afterhyperpolarization (mV); depolarizing responses listed under "+", hyperpolarizing responses listed under "-" as (); "Threshold", minimum stimulus intensity required to evoke activity in the AVNC; "*" indicates no response; about 4 ms should be added to "Delay to Onset" values evoked by electrical stimulation of the AVNC to make them comparable to wind puff induced values.

Stim Intensity & Treatment	Delay to Onset (ms)		<u>EFSP + and IPSP (-)</u> Ampl (mV) Dur (ms)		Delay to AP (ms)	AP Ampl (ovsh + AHP, mV)
	+	-	+	-	+	+
.....						
Controls (WPs)	48 (20)		3.1 (1.6)		156 (203)	81 70

Single Electrical Stimuli

Threshold

DDT	25 (48)		3.0 (2.9)		106 (324)	122 79
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2xThreshold

Controls	80 (8.2)		1.0 (4.1)		100 (271)	* *
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DDT	14 (13.3)		2.0 (3.1)		37 (305)	44 79
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>2xThreshold

Controls	15 (*)		* (*)		* (*)	47 62
----------	--------	--	-------	--	-------	------------

DDT	15 (23.4)		4.9 (2.8)		145 (290)	35 78
-----	-----------	--	-----------	--	-----------	------------

300 Hz Trains

Threshold

Controls	47 (<10)		* (7.6)		* (447)	71 70
----------	----------	--	---------	--	---------	------------

DDT	30 (*)		2.8 (*)		302 (*)	37 79
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2xThreshold

Controls	* (19)		* (6.1)		* (524)	* *
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DDT	64 (*)		3.1 (*)		365 (*)	138 78
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It should be noted that in each control preparation, DUM5r1 responded with either a depolarizing or hyperpolarizing response, but not both. The polarity of the response remained constant over the course of the experiment, despite changes of stimulus protocol.

Thus, it appears that other variables in the nervous system may be controlling DUM5r1 excitability and polarity of response.

(b) *Electrical stimulation of AVNC*

Representative examples of the characteristics of DUM5r1 activation by various stimulus protocols delivered to the AVNC are listed in Table 2. A complete listing of all protocols tested and responses recorded can be found in Appendix 5. The variability in response of DUM5r1 (in both controls and DDT-treated preparations) to various stimulus protocols makes it difficult to determine any statistically significant trends in the data. However, several recurring tendencies were apparent. These are outlined below.

i) Controls

The response of the AVNC to single electrical stimuli was not different from that described in Part I (see Results Part I, 2(b)). In controls, single electrical stimuli evoked typical compound action potentials (CAPs) whose

"shape" (reflecting axon recruitment) mostly depended upon the intensity of the stimulus (see Fig. 13).

In controls, single electrical stimuli and 50 Hz trains of threshold intensity delivered to the AVNC were not effective in evoking activity in DUM5r1 or N5r1. However, single stimuli of two and three times threshold intensity induced either hyperpolarizing ($n = 3$) or depolarizing ($n = 3$) responses in DUM5r1, depending on the preparation under test (Table 2). The polarity of these responses did not change for other, more intense stimulus protocols. Also, the magnitude of the concurrent motor response did not appear to depend upon the polarity of the DUM5r1 response.

Higher train frequencies (100 (see Appendix 5) and 300 Hz at threshold intensity) could evoke activity in DUM5r1 with a delay to onset similar to that evoked by WP stimulation (Table 2). Also notable is the very short delay to onset of response in DUM5r1 in the preparations which responded with hyperpolarizations (of soma RP).

ii) DDT-treated animals

In DDT-treated preparations an increase in single pulse stimulus intensity shortened the delay to onset of DUM5r1 activation and induced a more consistent, stronger depolarization in DUM5r1 (Fig. 34; Table 2). Also, there was a shorter delay to the evoked AP in DUM5r1 (Table 2).

Figure 34: DDT-treated - Activity evoked by a single electrical stimulus at just greater than threshold intensity delivered to the distal AVNC. The two examples shown are consecutive stimuli from a stimulus sequence (the top set of traces continue below). The time of stimulation of the AVNC is marked by a dotted line. Note the much greater AVNC and motor responses seen here compared to figure 33 (but similar DUM5r1 responses). DUM5r1 APs always occurred after initiation of activity in the AVNC and motor nerve 5r1. The arrow indicates a DUM5r1 axon spike in N5r1. (Scale bars: 40 mV (DUM5r1 only), 100 ms.)

However, at higher frequencies of AVNC stimulation, the time between stimulus and response in DUM5r1 was sometimes greater than that observed for single stimuli. This may have been due to excitation of an inhibitory pathway to DUM5r1, which if co-activated would delay, but not prevent, excitation of DUM5r1.

It should be noted that of eight DDT-treated preparations, in only one did DUM5r1 respond with just a hyperpolarization of its membrane potential. In one other DDT-treated preparation, DUM5r1 responded with both hyperpolarizations and depolarizations. The remainder ($n = 6$) responded with purely depolarizing responses. This contrasts with the 50:50 split between depolarizing and hyperpolarizing responses in controls.

6. Miscellaneous Observations

(a) *Anode break excitation (ABX) of DUM5r1*

This method of excitation was used mainly to help determine whether: (a) the impaled cell was excitable and (b) the impaled cell was DUM5r1 (see Results Part II, Section 2(a)). DUM somata of control preparations usually responded to ABX with a brief (<1 s) burst of APs. The frequency of APs in the burst was quite variable and ranged from 10 to 30 Hz. No DUM neuron of a control preparation was observed to fire APs at a frequency of greater than

30 Hz. However, DUM neurons in DDT-treated preparations commonly fired APs at 40-45 Hz (one preparation managed 60 Hz) (Fig. 35). Durations of the burst were also generally greater than in controls. (The absolute value of burst frequency in DDT-treated DUM neurons is much less than that in axons of the CNS (which typically fire at >200 Hz).)

(b) "Shutdown" of T3

In three DDT-treated animals, a complete silencing of spontaneous and evoked output to N5r1 occurred. This was accompanied by a decrease in resting potential (in two cases it decreased from -49 mV to -37 mV and -46 mV to -40 mV), cessation of ongoing spontaneous activity and a period of inexcitability in DUM5r1. Shutdowns were usually preceded by a very high frequency continuous train of activity in the AVNC and/or in N5r1. In two of the preparations the shutdown persisted for ten minutes before activity slowly returned.

Figure 35: Action potentials in DUM5r1 and N5r1 induced by the sudden release (B) of anodal polarization ("anode break excitation" (ABX)) of DUM (DUM5r1) in a DDT-treated preparation. This particular burst of APs lasted about 2 seconds. The frequency of firing in DUM5r1 over the first 200 ms is 60 Hz. Activity recorded in the AVNC was spontaneous. (Scale bars: 40 mV (DUM5r1 only), 200 ms.)

Summary of Results - Part II

Results of Part II revealed several interesting characteristics about DUM neuron morphology. First, DUM5r1 was consistently located (90% occurrence) in an anterior-right position (Fig. 25) within the DUM neuron group in animals originating from the same parental stock. In offspring of females in which mating was not strictly controlled, DUM5r1 was found on a less consistent basis (70% occurrence) in the anterior-right or anterior-midline position within the DUM neuron group.

The mean resting membrane potential of DUM5r1 and non-DUM5r1 neurons in DDT-treated animals were not significantly different from controls.

Activity in DUM5r1 could be evoked by wind puffs directed at the cerci. However, DUM5r1 always fired well after the motor burst had begun. DDT appeared to facilitate synaptic inputs to DUM5r1. This was deduced from its 'willingness' to fire and generally shorter delay to activation. Also, the delay between stimulation of the cerci or AVNC and the response in DUM5r1 was shortened in DDT-treated preparations.

DUM5r1 does not appear to directly modulate activity of a motor neuron within T3 that sends an axon into N5r1, although it does have many branches within T3 and axons that go to other nerve roots.

Neurophysiological symptoms typical of DDT-poisoning (repetitive firing, depolarized afterpotentials and general hyperexcitability) were not readily evident in the DUM neurons.

DISCUSSION - PART II

1. Desheathed T3 and the Identification of DUM5r1

Two purposes were served by desheathing the dorsal surface of the metathoracic ganglion (T3): (1) it greatly facilitated the impalement of DUM neurons, and (2) it reduced the barrier between externally perfused solutions and the DUM somata.

After desheathing T3, outlines of DUM somata were usually visible beneath the layer of perineurial cells. The extent of perineurial thickness varied among animals used and occasionally obscured the DUM somata. In these cases, penetrations were made 'blind' or superficial perineurial cells were carefully removed. It was also rare that the process of desheathing caused physical evidence of perineurial cell damage. However, other evidence indicates that the act of desheathing removes the neural lamella, and breaks down the blood-brain barrier (Treherne & Schofield, 1984; also see Introduction).

Damage to cells underlying the neural lamella was unlikely as action potentials could be successfully recorded in DUM somata and their axon branches in peripheral nerve roots. Also, iontophoretic injection of Lucifer Yellow with subsequent fluorescence microscopy provided additional supportive evidence that major physical damage did not occur. Usually, the fluorescence was of such an intensity

that other non-injected cells (both DUM somata and perineurial cells) were laterally illuminated and showed no visible signs of damage (see Fig. 21).

2. Morphology of DUM5r1

(a) *Peripheral branch patterns*

It was previously reported for cockroach (Arikawa et al., 1984) that any DUM neuron with an axon in nerve root 5 also had axons in nerve roots 3, 4, and 6 (i.e., DUM3,4,5,6). Evidence presented in this thesis supports the existence of at least two distinct branch patterns for DUM neurons having an axon in nerve root 5r1 (DUM3,5,6 and DUM3,4,5,6). These were the patterns observed in 16 of 19 preparations. Two other patterns represented were DUM3,5 and DUM5,6. It is possible that differences in patterns of innervation might arise because an axon(s) of a damaged nerve root failed to fill. However, in cases presented here, this seems unlikely because it would have required the same nerve root on both sides of T3 to be damaged. Also, in none of these cases was there evidence to suggest that the lateral neurite (within T3) of the DUM neuron bifurcated at a root level other than those identified (i.e., not truncated branch or stub-ending). When a nerve root or branch in T3 was crushed or cut the DUM axon filled with Lucifer Yellow only to the point of trauma or section.

Two of the DUM patterns mentioned above (DUM3,5; DUM3,5,6) are supported by the literature. Tanaka and Washio (1984) identified a DUM5, DUM3,5 and DUM3,5,6 (but not DUM3,4,5,6) in T3 of cockroach. (For comparison, Watson's detailed study of DUM neuron morphology in locust (1984) showed that there were three types of DUM neurons that send axons into nerve root 5: DUM5 (DUMETi), DUM3,4,5 (deep tract) and DUM3,4,5 (superficial tract).)

The cockroach data suggest that the patterns of peripheral innervation by DUM neurons may be variable and/or dependent upon some as yet undetermined aspect of development.

Injecting Lucifer Yellow into two DUM neurons in the same preparation showed that more than one DUM neuron sent an axon into nerve root 5. But, in each of the three trials performed only one of the two axons in N5 was observed to bifurcate and send a branch into N5r1. Also, in five individuals 6-8 DUM somata were impaled without finding more than one DUM neuron with a branch in N5r1. These data suggest that DUM5r1 is unique (i.e., it is the only DUM neuron that sends an axon into N5r1). This possibility was further supported by the finding that only a single DUM neuron (DUM3,5,6) had physiological effects on muscle 177d (Tanaka & Washio, 1985; Washio & Tanaka, 1986). The sole innervation to muscle 177d is attributed to N5r1 (Pipa & Cook, 1959).

Therefore, it appears that DUM5r1 may be a specific, identifiable DUM neuron. Although its overall peripheral branch pattern appears to vary among different individuals, it is the only DUM neuron to send an axon into N5r1 of the metathoracic ganglion.

(b) *DUM5r1 soma position*

It was reported that a DUM neuron with an axon in nerve root 5 (DUM3,5,6) was usually found somewhere in the anterior half of the cluster of DUM somata in T3 (Tanaka & Washio, 1985). Results reported here for animals with the same parental genetic inheritance showed that DUM5r1 was consistently located in the anterior-right position within the DUM neuron cluster (90% occurrence). This helps to rapidly identify and impale the same DUM neuron in different preparations and greatly reduces the risk of incurring cell damage during the search for the 'correct' neuron.

The consistent location of DUM5r1 in 'related' animals also implies that the positions of DUM somata within the cluster may not be entirely random. Further experiments are necessary before firm conclusions can be made on this matter.

In summary, it appears that the DUM neuron identified using the procedures outline above is a 'unique', identifiable DUM neuron.

3. Resting and Action Potentials

Resting potentials recorded in control DUM5r1 and non-DUM5r1 somata (mean -46 and -48 mV) were not significantly different. Those recorded in DDT-treated preparations tended to be more negative than controls, although these differences were not significant. The values for resting potentials are between values previously reported in the literature (-30 to -40 mV, Arikawa *et al.*, 1984; -50 to -60 mV, Crossman *et al.*, 1971; -40 to -60 mV for locust DUMETi, Goodman & Heitler, 1979). Arikawa *et al.* attributed their low potential measurements to damage incurred while desheathing the dorsal surface of T3. (All DUM neuron preparations presented in this thesis were desheathed. However, it was unusual for the membrane potential to be less negative than -40 mV. Preparations with such low potentials were not used.) Crossman *et al.* (1971, 1972) also desheathed the dorsal surface of T3.

DUM cell action potential and after-hyperpolarization amplitudes and durations reported in this thesis closely correspond with those previously reported for cockroach (Crossman *et al.*, 1971) and locust (Hoyle & Dagan, 1978; Goodman & Heitler, 1979).

4. Possible Modulation of DUM Neurons

Spontaneous slow depolarizations of membrane potential (with a frequency of 0.1-0.5 Hz) usually produced APs. The occurrence and frequency of spontaneous APs in DUM neurons has been previously described (Crossman *et al.*, 1971; 1972). What has not been described are the shifts in membrane potential that occur which apparently are not associated with the spontaneous depolarizations that lead to APs (refer to Fig. 27 & 28). (Though, the occurrence of spontaneous APs did seem to be affected by the shifts in membrane potential.)

Also, there were periods during recording when the DUM soma became relatively inexcitable (spontaneous APs ceased, external stimuli were ineffective at evoking a somatic AP). These periods of inexcitability were most obvious after an 'unprovoked' shift (hyperpolarization or depolarization) in membrane potential, but also occurred in the absence of large changes in potential (*i.e.*, the simultaneous occurrence of inexcitability and shift in potential may have been fortuitous.) Hoyle (1985) also noted periods of inexcitability in locust, during which he could not evoke the jump response. He suggested this was not due to habituation, but instead may have been caused by the presence of only a subthreshold amount of the appropriate neuromodulator at the time of testing (Hoyle, 1985).

The possibility that the cause of the inexcitability and shift in membrane potential was a 'fault' in the microelectrode was unlikely because: (1) withdrawal of the microelectrode from the cell revealed a normal microelectrode resistance; (2) microelectrodes that increase in resistance while inside the cell always caused the membrane potential to appear much more negative, usually well beyond the physiological range, (3) random baseline noise did not change, and (4) microelectrode balance was rechecked inside (and later outside) the cell.

Also, the cessation of activity could not solely be accounted for by a change in membrane potential because applying a current through the "bridge" circuit of the microelectrode amplifier (to simulate the changes observed in membrane potential) did not block the induction of spontaneous APs in the soma.

Therefore, it was hypothesized that these somata were made inexcitable by an endogenous substance released from within the VNC. Modulation of neuronal excitability by other neurons within the nervous system is not uncommon in both vertebrates and invertebrates (Kaczmarek & Levitan, 1987). Although direct synaptic contacts are not found on DUM somata, it has been shown that DUM somata contain receptor sites for acetylcholine (ACh) and octopamine (Harrow *et al.*, 1982; D. B. Sattelle, personal communication). Because a complete pharmacological profile has not yet been published for cockroach DUM somata, the

existence of other 'modulatory' receptor sites cannot be discounted.

There are many examples of modulation of activity in central neurons by the biogenic amines octopamine, dopamine and serotonin (eg., in locust (Sombati & Hoyle, 1984a,b); and in the hawkmoth (Classen & Kammer, 1986); see Introduction.) However, one of the most thoroughly studied of identified neurons is cell R15 in the abdominal ganglion of *Aplysia*. This cell's activity can be modulated by serotonin, dopamine and synaptic inputs (Benson & Adams, 1987). Dopamine appears to inhibit burst activity by direct hyperpolarization of the membrane whereas perfusion with serotonin has a longer lasting, inhibitory effect which is dependent upon a second messenger system (Benson & Adams, 1987). Stimulation of the branchial nerve innervating cell R15 can also induce long-lasting inhibition (Parnas & Strumwasser, 1974). However, although the alterations of excitability due to synaptic input and perfusion with serotonin are very similar, evidence does not support serotonin as the synaptic transmitter substance in *Aplysia* (Benson & Adams, 1987). This leads one to suspect that serotonin may be acting as a 'true' neuromodulator/neurohormone, rather than as a classic transmitter substance.

The similarities between modulation of cell R15 in *Aplysia* and known modulatory events in insect (some of which are c-AMP dependent) (Evans, 1985b; O'Donnell & Singh, 1988)

make it tempting to look for parallels between the R15 system of modulation and the possible modulation of DUM neurons of cockroach.

A modulatory system that has been less studied but pertains to insects (and thus may be more comparable) has been described recently. O'Donnell and Singh (1989) have shown that the membrane of *Rhodnius* oocytes contain receptors for octopamine. Low concentrations of octopamine caused a decrease in excitability of the oocytes (which normally support Ca^{2+} -dependent APs - O'Donnell, 1985) without a change in membrane potential. Higher concentrations of octopamine also caused a slight hyperpolarization of the cell. Use of various pharmacological agents indicated that the receptors are probably octopamine-type 2 (Evans, 1985; O'Donnell & Singh, 1989) and that they activated the c-AMP second messenger system (O'Donnell & Singh, 1988). The effects on membrane potential and decrease in excitability they described are very similar to those observed here in DUM5r1.

Further investigations are needed to clarify whether DUM neurons of cockroach are possible target sites for specific neuromodulatory substances.

5. Perfusion of DUM Neurons with Zero- Ca^{2+} Saline

(a) *Membrane potential*

Perfusion of the dorsally desheathed, metathoracic ganglion with zero- Ca^{2+} saline caused a rapid depolarization of the DUM5r1 membrane potential. This suggests that perfusion solutions had ready access to the DUM somata. However, the DUM neurons were perfused *in situ* and thus most of their associated glial cells were present. The variability of change in membrane potential (magnitude and rate) between different DUM neurons during perfusion may reflect the penetration rate of a solution through the occluded intercellular spaces around these cells. It has also been suggested (Goodman and Heitler, 1979) that an intact metathoracic ganglion (of locust) regulates calcium ions. This, if it occurs, may have further influenced results obtained during zero- Ca^{2+} perfusion experiments.

Narahashi (1966) reported little or no change in resting potential of cockroach giant axons when they were perfused with low- Ca^{2+} saline (0.18 mM). Similarly, Frankenhaeuser (1957) found perfusion of frog myelinated nerve fibre with solutions containing from 0.04 to 27 mM CaCl_2 caused little, if any, change in resting potential. However, when a zero- Ca^{2+} solution was used the membrane potential depolarized. Also, long-term exposure to zero- Ca^{2+} salines has been reported to cause an irreversible "progressive depolarization" of membrane potential in

cockroach (Narahashi, 1966) and squid (Frankenhaeuser & Hodgkin, 1957) giant axons. Frankenhaeuser and Hodgkin (1957) suggested that this treatment causes the membrane to lose its selective permeability and become a "poor insulator". But, a progressive depolarization was not observed in results reported in this thesis. Rather, a depolarization to a new stable membrane potential occurred. This suggests a zero- Ca^{2+} concentration was not attained. However, this was not unexpected because: (1) the saline did not contain a calcium chelating agent, (2) glial cells surrounded the DUM somata, and (3) magnesium replaced calcium in the zero- Ca^{2+} saline used here (Mg^{2+} is reported to have a stabilizing effect on the membrane similar to Ca^{2+} (Frankenhaeuser & Hodgkin, 1957)).

Therefore, it is possible that micromolar amounts⁸ of Ca^{2+} (or equivalent cation) were available in sufficient quantity to prevent loss of membrane integrity, but not sufficient to support the membrane permeability at its normal resting level.

A better experimental procedure for study of membrane potential ionic requirements may be to use DUM neurons isolated from their native environment. Under these circumstances, better control of the ionic environment and of membrane potential (during current and voltage clamp) should be possible (Thomas, 1984; Pitman, 1988).

⁸ Contaminants in analytical grade salts used in the saline would produce a minimum of 2.2 μM of calcium.

(b) *Action potentials in zero- Ca^{2+}*

Action potentials in locust DUMETi are dependent on both Na^+ and Ca^{2+} (Goodman & Heitler, 1979). A similar dependence of the AP in unidentified DUM neurons of cockroach has also been suggested (M. E. Adams, personal communication). Experiments presented in this thesis indicate that AP amplitude is decreased (mean 27%) in zero- Ca^{2+} saline, although not eliminated. This raises the question: which ions and channels were responsible for the AP observed in zero- Ca^{2+} saline?

Narahashi (1966) has shown in giant axons of cockroach that at the normal resting potential (-76 mV) there is only about 30% residual Na-inactivation. When the giant axons were bathed in low- Ca^{2+} saline (0.18 mM Ca^{2+}) the resting potential remained about the same, but Na-inactivation increased to greater than 50%. At a holding potential of -45 to -50 mV (similar to the RPs of DUM neurons) greater than 95% Na-inactivation existed whether the giant axons were bathed in normal saline or low- Ca^{2+} saline (Narahashi, 1966; Pichon, 1974; Pichon & Ashcroft, 1985).

If the Na-channels of DUM somata are similar to those found in giant axon plasma membrane, then the Na-channels of DUM somata would be 90-95% inactivated at normal resting potential. They would be more than 95% inactivated at the more positive RP produced when perfused with zero- Ca^{2+} saline. Therefore, if these conditions prevail in DUM somata, it would be unlikely that the Na-channels could

carry the current necessary for generation of the action potential.

It could be argued that the observed resting potentials for DUM neurons were much lower than would be recorded *in vivo*. This explanation is not likely because: (a) other authors have recorded similar RPs in DUM neurons of intact ganglia from both cockroach and locust (Crossman *et al.*, 1971; Goodman & Heitler, 1979); (b) 'stable' intracellular microelectrode impalements of DUM somata often lasted for over 90 min; and (c) it was not unusual to record overshooting APs in DUM somata with a RP of -45 to -55 mV.

For the DUM neuron, it would seem energetically wasteful to produce Na-channels in the somal membrane without a specific function. It thus seems possible that somal Na-channels could have different activation/inactivation kinetics to those found in the giant axon membrane Na-channels. However, inactivation/voltage curves for DUM somata of the cockroach have yet to be compiled.

If a sodium current does not account for the reduced amplitude APs in zero-Ca²⁺ saline then two other possible mechanisms could be that: (a) some calcium ions remain after perfusion with zero-Ca²⁺ saline. However, Goodman and Heitler (1979) showed that if the Na-current in DUMETi was blocked, the remaining Ca-current alone was insufficient to generate an AP; and (b) it is possible that an ion species, other than Ca²⁺ (eg., Na⁺), was creating sufficient inward

current to generate the observed APs by using the Ca-channel. There is evidence which suggests that Na^+ can pass through a Ca-channel under conditions of very low external Ca^{2+} (Tsien *et al.*, 1987).

In summary, even though it was difficult to control the ionic conditions in the environment immediately surrounding the *in situ* DUM somata, it was apparent that a reduction in external calcium concentration had a strong effect on both resting and action potentials. However, it remains unclear whether sodium and calcium ions are required to drive the AP current in cockroach DUM neurons.

6. Activation of the Escape (Startle) Response

(a) AVNC and motor responses to wind puffs

The magnitude (burst frequency and duration) of responses to wind puffs recorded in the AVNC of semi-intact preparations (Part I) were only about 3/4 of those recorded in isolated preparations (Part II). However, motor responses to these wind puffs were about six times greater in isolated preparations than for *in situ* preparations. These results indicate that the mechanism responsible for the general lack of vigorous response noted in secured semi-intact preparations (Ritzmann, 1984; Hoyle, 1985) was not exerting as much influence over the isolated preparations.

Because the two types of preparations are structurally very similar posterior to T3, it seems plausible that input from ganglia rostral to T3 and/or thoracic and head sensory input are important in controlling the overall level of arousal (excitability) in the nervous system.

The hypothesis that the GAs mediated activity responsible for escape behaviour (Westin *et al.*, 1977; Camhi, 1980; Camhi & Molen, 1981; Ritzmann, 1984) is supported by the very short latencies (time from cercal stimulation to motor output) recorded in isolated preparations (11 ms & 8 ms, respectively for controls & DDT). Such short latencies are only possible if the cercal input was conveyed in the AVNC by the giant axons to the interneurons of T3 responsible for integration and excitation of motor neurons. A small axon pathway in the AVNC would have required a calculated minimum of 14 ms to produce a motor output (see Appendix 6 for calculation.; and, Ritzmann & Pollack (1986)).

The hypothesis is also supported by the data which indicates both DUM5r1 and motor activity could be induced using threshold intensity trains of stimuli delivered to the AVNC (single stimuli were sufficient in DDT-treated animals). (Threshold intensity stimulation primarily recruits only the giant axons.)

3

OF/DE

3

MicroD

(b) *Activation of DUM5r1 - General*

Results showed that DUM5r1 could be activated by wind puffs directed at the cerci or by an appropriate electrical stimulus delivered to the distal ventral nerve cord. Previous workers have shown that stimulation of the AVNC near the metathoracic ganglion (T3) could evoke activity in unidentified DUM neurons (Crossman *et al.*, 1971; 1972; Hoyle & Dagan, 1978). However, some DUM neurons have axon branches which enter anterior and posterior connectives of T3 (Tanaka & Washio, 1985) and have been traced as far as the A1-A2 connective (see Results Part II). Thus, stimulating abdominal or thoracic connectives near T3 could have directly excited DUM axons, which would then propagate antidromic spikes back to the soma. However, by stimulating the distal part of the AVNC, as in the experiments in Results Part II, it is unlikely that DUM axons of T3 would be directly activated to spike. In this case, the activation of a DUM neuron in T3 would arise from afferent AVNC input, similar to that evoked by a wind puff stimulus directed at the cerci.

In controls, wind puff stimuli typically evoked one or two APs in DUM5r1 (see Fig. 33). Also, there were occasions when an AP was followed by a small depolarizing potential that did not reach threshold for a full soma AP. The simplest explanation for the failure of the second AP might be that excitatory input to the DUM neuron was insufficient

to evoke more than one AP. Alternatively, the failure may have been caused by a high level of residual inactivation from the previous spike. To help distinguish between these two possibilities, DUM5r1 was stimulated at increasing frequencies from 0.5-5 Hz using a method of excitation that was known to be supra-threshold for a soma AP and does not depend upon synaptic input (N5r1 antidromic stimulation). Under normal circumstances, the maximum firing rate that the soma could maintain over a short duration (ca. 5 s) without failure was about 2 Hz. At stimulus frequencies of 3-5 Hz, some APs failed to invade the soma. Thus, even if excitatory inputs were greater, DUM5r1 appears to be unable to support a high frequency of APs (under normal conditions). This inherent inability to support long duration, 'high' frequency trains of APs has also been shown to be a characteristic of DUMETi (Hoyle & Dagan, 1978), although it seems DUMETi can support maximum rates of firing which are generally higher (10 Hz for ca. 1 s; maximum of 26 Hz) than for DUM5r1.

Using a method of stimulation which reduced residual Na-inactivation and thus enhanced DUM neuron responses (ABX), DUM5r1 could be made to fire APs for short time periods at 30-45 Hz. The high frequency response was especially evident in DDT-treated preparations. These data suggest that the low resting potential may contribute to the apparent inability of DUM5r1 to fire repetitively at high frequency. However, the answer is not complete because holding the resting potential at -55 to -60 mV did not promote high frequency bursts of APs in DUM5r1. (After-hyperpolarizations usually persisted for 30-50 ms, which

theoretically would have allowed frequencies up to 33-20 Hz, respectively.)

Long duration, high frequency activity in DUMETi (locust) does not seem to be functionally necessary as the primary effect of DUMETi is to activate a c-AMP second messenger system (in the extensor tibiae muscle - see Evans, 1985). (Second messenger systems can both amplify and produce prolonged effects in the target cell.) The prolonged effect of octopaminergic DUM excitation has also been described in the extensor tibiae muscle of the Jerusalem cricket and New Zealand weta (Hoyle & Field, 1983; Hoyle, 1984). In these animals, a short burst of activity in DUMETi is sufficient to produce a long-lasting, catch-like tension in the extensor tibiae muscle.

In the only reported example of "DUM" neuron modulation of contraction in skeletal muscle of cockroach (Tanaka & Washio, 1985), a single spike or short burst of activity in the DUM neuron was apparently sufficient to evoke an effect in the muscle.

Therefore, although DUM5r1 seems incapable of sustaining a high rate of AP discharge, a large number of APs are probably not required for it to exert its effects over muscle contraction kinetics (or perhaps central modulation). Experiments using a natural form of stimulation indicated that a typical DUM5r1 discharge

consists of one to several APs, which is similar to reports in the literature for locust DUM neurons.

(c) DUM neuron activity in DDT-poisoned animals

Given the important role DUM neurons play in modulation of both central and peripheral activities (Evans & Siegler, 1982; Evans, 1985b; Sombati & Hoyle, 1984), it was of interest to determine whether DDT could change the normal, low frequency firing pattern in DUM5r1 to one consisting of trains of repetitive APs. Then, if they did 'burst' in a fashion similar to other neurons, would this result in a 'supermodulated' state. Thus, could this account for some of the uncoordinated behaviour observed in DDT-poisoning? Alternatively, if DUM neurons were themselves 'modulated down' (i.e., activity suppressed), would this result in a decreased sensitization and a reduction of behavioural signs of poisoning?

Results of experiments presented in this thesis indicate that in animals treated with DDT, DUM5r1 was much more readily recruited by afferent input from the AVNC. However, individual APs recorded in DUM5r1 were not significantly different from those observed in controls. As with controls, maximum response in DUM5r1 to a wind puff or electrical stimulation of the AVNC consisted of 1-3 APs over 300-500 ms. Under 'normal' conditions of excitation, DUM5r1 did not repetitively fire as typically observed in other

excitable cells of the CNS. However, whether the threshold for activation of DUM5r1 was decreased by DDT and/or inputs to DUM5r1 were much facilitated by DDT remains to be resolved. Since the general state of arousal in the CNS tends to be an important factor in determining final neural (behavioural) output (Hoyle, 1985), this may have contributed to the ease with which DUM5r1 was recruited. Indeed, in controls excitatory vs. inhibitory responses recorded in DUM5r1 were evenly divided. In DDT-treated animals, the bias was heavily in favour of excitation of DUM5r1 (7 of 8 preparations).

Also, in 2 of 8 DDT-treated preparations, a strong hyperpolarizing current (ca. 5 s) followed by its sudden release (anode break excitation) caused a long duration (ca. 2 s), high frequency (45 Hz) burst of APs in DUM5r1. This intensity of firing in DUM5r1 was not seen in any controls. This result suggests that under the influence of DDT, DUM5r1 is more capable of 'high' frequency bursts, but this characteristic is usually not expressed.

Therefore, it appears that DUM neurons of T3 do not directly contribute to the DDT-poisoning syndrome by firing long trains of action potentials, although activity in DUM5r1 is not suppressed. However, what is yet to be determined is the level of activity in DUM5r1 that is necessary to adversely affect muscle contraction or central modulation.

(d) *Disagreement with the "Orchestration Hypothesis"*

The *Orchestration Hypothesis* proposes that each behaviour, or part of a behavioural sequence, is determined by a different specific cluster of DUM neurons (Hoyle, 1985; p. 620).

It has been shown that DUM neurons in the locust (specifically DUMETi and DUMDL) always fire prior to any "spontaneous or reflexly generated" motor neuron activity (Hoyle & Dagan, 1978; Hoyle, 1985; Whim & Evans, 1988). This, and other evidence involving apparent mediation by octopamine of behaviour and potentiation of input in the CNS, led to the creation of a hypothesis to account for: (1) the timing of activity in DUM neurons, (2) their octopaminergic nature, (3) apparent modulation of behaviour, (4) abundant presence in T3, and (5) extensive branch patterns (Hoyle, 1985).

Results of experiments with DUM neurons of the cockroach, do not support the orchestration hypothesis. First, a naturally evoked behaviour (wind puff-induced escape response) activated DUM5r1, but not until well after motor neurons were firing action potentials. (It might be argued that this is only a single DUM neuron and may not have been one in the 'correct' cluster. However, Lucifer Yellow staining indicated that DUM5r1 was likely to be the sole source of DUM innervation to coxal depressor (extensor) muscle, which is the equivalent of DUMETi and the extensor

tibiae muscle in locust.) Second, the delay to the recruitment of non-DUM5r1 neurons was similar to DUM5r1. And finally, in DDT-treated preparations there were many indications that inputs to both DUM and motor neurons were greatly potentiated. Even under these circumstances of extreme potentiation, DUM5r1 was never observed to fire prior to activation of motor neurons.

Therefore, it appears that evidence from DUM5r1 does not support the orchestration hypothesis, at least in cockroach. Instead, it is suggested that DUM5r1 acts to modulate the characteristics of muscle mechanics, perhaps in a manner that produces optimum background tension, whether at rest or during movement (walking, running). The discharge of the common inhibitor prior to excitatory motor input probably 'readies' the muscle for future contractions (Fourtner & Drewes, 1977). Changes in basal tonus during sudden or rapid movements would be produced by a short burst (1-3 APs) in the appropriate DUM neuron(s). A long discharge of APs in the DUM neuron would not be necessary because of the prolonged (and amplified) effects of octopamine (and/or other peptide?) on skeletal muscle.

GENERAL SUMMARY

Experiments of Part Ia showed that in animals injected with DDT, repetitive firing occurred in the intact nervous system of cockroaches after they had recovered behavioural 'normality'. For normal behaviour to be expressed in these otherwise poisoned animals, it was hypothesized that the DDT-induced repetitive firing is 'filtered-out' at some point in the nervous system. Results indicated that the abdominal ganglia (A1-A5) were not the site of suppression of DDT-induced extraneous activity. However, this DDT-injected semi-intact preparation was not suitable for determining whether the thoracic ganglia (T3 in particular) were responsible for the suppression of extraneous activity which allowed normal behaviour.

When only a portion of the AVNC was made hyperexcitable by a localized treatment with DDT (Part Ib), it then became evident that 'filtering' of DDT-induced extraneous activity could occur in the metathoracic ganglion (T3) (as this extraneous activity did not appear in motor nerves 5r1 and 6Br4).

The dorsal unpaired median neurons of T3 (in locust) are known to modulate both motor neuron activity and kinetics of muscle contraction (Evans & Siegler, 1982; Sombati & Hoyle, 1984). It has been suggested that these DUM neurons might play a similar role in cockroach. Thus,

the possible involvement of T3 DUM neurons in the DDT poisoning syndrome was investigated (Part II).

Activation of T3 DUM neurons in the cockroach always followed activation of motor neurons. This does not support the Orchestration Hypothesis formulated for locust DUM neurons (Hoyle, 1985).

Results indicated that although activity in an identified DUM neuron (DUM5r1) was not suppressed in DDT-poisoned animals, DUM5r1 did not fire repetitively (as is characteristic of axons of the CNS). Furthermore, when DUM5r1 was excited by input from the AVNC, evoked action potentials were not different from controls. However, there was a bias favouring excitation of DUM5r1 in DDT-poisoned animals compared to controls. DUM5r1 appeared not to influence motor neuron activity within T3. Further experiments with a semi-intact preparation are necessary to determine the level of activity required in DUM5r1 to affect another putative target site (coxal depressor muscles).

CONCLUSIONS

Repetitive firing recorded in isolated CNS preparations (from behaviourally normal, but DDT-treated animals) was not an artifact of isolation as it also occurred in semi-intact preparations.

In an animal poisoned by DDT, suppression of behavioural signs of poisoning appears to be accomplished by at least two different neural mechanisms. The degree of expression or ability to observe these filter mechanisms seems to depend upon the time since treatment with DDT.

There appears to be a direct correspondence between early behavioural signs and neurological symptoms of DDT-poisoning. At about 24 h after injecting DDT, a block in the terminal abdominal ganglion (A6) prevents input from the cerci from reaching the CNS. At about 96 h after injecting DDT, filtering of extraneous activity (which alleviates behavioural signs of poisoning) probably occurs in the thoracic ganglia, but, does not occur in any of the abdominal ganglia.

The giant axons do not suffer from long-term block during sublethal DDT-poisoning. Therefore, the "zone of low safety factor" hypothesis is not supported.

An identifiable DUM neuron was shown to exist in the metathoracic ganglion. Its position was consistently located in the anterior-right position of the DUM group in

genetically related individuals. It appeared to be the only DUM neuron with an axon branch in N5r1.

A preliminary account of the Ca^{2+} -dependence of resting and action potentials of DUM neurons was described. Both resting and action potentials of *in situ* DUM neurons were reduced in zero- Ca^{2+} saline.

Some evidence was provided to support the thesis that the excitability of DUM neurons is modulated. The source of modulation, although not experimentally identified, may be of at least one of two origins: (a) direct synaptic input, and (b) neurosecretory.

DUM neurons can be excited by input originating in the cerci or by stimulation of distal AVNC connectives. The maximum response (frequency of APs) in an identified DUM neuron (which has an axon branch to coxal muscles) is relatively low compared to the output of motor neurons.

The maximum responses recorded in DUM neurons of DDT-poisoned animals were not different from controls. However, the tendency of a DUM neuron to be excited, rather than inhibited, was much greater in DDT-treated preparations.

During an escape response, the long delay prior to DUM neuron activation indicated that its firing is not critical to the initiation of a motor response. This evidence does not support the *Orchestration Hypothesis*, as proposed for DUM neurons of *Locustidae*.

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APPENDIX 1(a)

Appendix 1(a): Muscles of the right metathoracic coxa of the cockroach (dorsal view). Layers of muscles were sequentially dissected (i..iv) to reveal the coxal levator muscles (181, 182) and the coxal depressor muscles (177d,e, 178, 179). Muscle 180 is not used in flexion of the CTF joint (see text). (After Carbonell, 1947.)

APPENDIX 1(b)

Appendix 1(b): Innervation of coxal depressor and levator musculature by N5 and N6 of the metathoracic ganglion. Top: The number designations of muscles illustrated in Appendix 1(a) are presented in (i, above). Bottom: The branches of nerves 5 and 6 innervating coxal muscles (designated above) are labelled (ii, below). Recordings in this thesis were made from N5r1 and N6Br4. (After Pipa & Cook, 1959.)

Appendix 2: Calibration of 60 and 100 ms wind puffs (WPs).

Wind puff velocity (m.s^{-1}) is plotted against (a) WP pressure (PSI) and (b) WP amplitude (V). Each point is the mean of 10 sample WPs. Dotted lines enclose the range employed during an experiment. Error bars are too small to be seen.

APPENDIX 4

PCLAMP option files for "Fetchex" and "Fetchan".

Fetchex

#	Value	Explanation	Parameter file xxx
12	512	Number of Samples / Episode (8, 16, 24,... 2048)	
13	390	Number of Episodes / Trial (1..1024)	
14	50	Clock Interval (μ s) (≥ 18 ; even number)	
15	0	Mode: 0-Event-triggered, 2-throughput	
18	0.95	Fraction of episode to take after accept/trigger	
24	60	Time to wait for event before abandoning (s)	
34	0	Tape windup time (s) (>0 for tape recorder control)	
19	5	Delay, accept/trigger-to-stimulus (μ s) (≥ 4)	
20	5000	Duration, stimulus (Timer 3) (μ s) (< 64000)	
25	0	Analog output, Channel 0: Holding Level (mV)	
33	125	Gain from DAC to cell (mV @ cell / mV @ DAC)	
49	5	Analog In ch0: Gain from cell to ADC (mV @ ADC / pA @ cell)	
63	0	Post-acquisition data display: 0-none, 1-display	
70	1	Data display: 0-display as points, 1-display as lines	
61	1	Amplification factor (not 0)	
62	0	Vertical offset (fraction of screen height)	
44	1	Polarity of stimulus out (0-active low, 1-active high)	
45	1	Polarity of disable out (0-active low, 1-active high)	
46	1	Polarity of accept/trigger in (0-falling edge, 1-rising edge)	
35	0	Environmental Information--for reference only	
36	0	Cell identifier	
37	24	Temperature ($^{\circ}$ C)	
38	0.1	Criterion Time (ms) (use -1 for zero)	
39	0	Threshold Current (pA)	
40	0	Gain multiplier	
41	6000	Lowpass Filter Cutoff Frequency (Hz)	
42	0	Autosample Gain and Filter frequency: 0-disable, 1-enable	
51	0	Labmaster: ADC option (0-none or PRK, 1-PGL, 2-PGH)	
53	5	ADC range, $\pm$ V (PGL: 0.02, 0.1, 1, 10)(PGH: 1.25, 2.5, 5, 10)	
54	5	DAC range, $\pm$ V	
55	12	ADC resolution, bits (8..14)	
56	12	DAC resolution, bits (8..14)	

continued Appendix 4

Fetchan

#	Value	Explanation
1	4	0-Browse, 1-Sums, 3-Edit, 4-Idealized record
4	0	Interaction level: 0-Auto, 1-Accept/Reject (Sums,Subtract,Ideal rec)
5	100	Ignore gaps briefer than this, μ s (Ideal rec)
6	1	1 erases after each episode
7	1	Start hunt at: 0-discriminator trigger, 1-episode start (Ideal rec)
8	3	Base & Delta: 0-Auto, 1-Adapt Both, 2-Base only, 3-Hold (Ideal rec)
9	0	Begin at this episode number
10	0	0-Rezero analysis output now, 1-continue (Ideal rec)
12	1	Display Amplification factor (Y multiplier; not 0)
14	0	Digital Gaussian filter cutoff frequency (Hz) (0=no filter)
16	0	Factor to multiply passives before Subtr (Sums (file select) or rec)
17	10	Number of points to use for baseline averaging (Subtr rec)
18	0	0-Omit hunting marker, 1-Show marker & Wrap counter (Ideal rec)
19	1	Plot style: 0-points, 1-lines
20	0	Vertical offset for display (fraction of screen height)
21	0	Additional offset for each part (512 samples)

Appendix 5

Response characteristics of DUM5r1 to stimulus protocols (Part II). Electrical stimuli were delivered to the distal AVNC (between A5-A6). Wind puff stimuli were delivered to the cerci (control data only). Of six controls, three responded with a depolarization to any stimulus and three responded with a hyperpolarization. A minimum of 20 samples were taken for each value listed.

Notes: AP Amplitude, overshoot + afterhyperpolarization (mV); depolarizing responses listed under "+", hyperpolarizing responses listed under "-" as (); "Threshold", minimum stimulus intensity required to evoke activity in the AVNC; "*" indicates no response; 3 to 5 ms should be added to "Delay to Onset" values evoked by electrical stimulation of the AVNC to make them comparable to wind puff induced values (see Appendix 6).

Stim Intensity & Treatment	Delay to Onset (ms)		EPSP + and IPSP (-) Ampl (mV) Dur (ms)		Delay to AP (ms)	AP Ampl (ovsh + AHP, mV)
	+	-	+	-		
.....						
Controls (WPs)	48 (20)		3.1 (1.6)	156 (203)	81	70

Single Electrical Stimuli

<u>Threshold</u>						
DDT	25 (48)		3.0 (2.9)	106 (324)	122	79
<u>2xThreshold</u>						
Controls	80 (8.2)		1.0 (4.1)	100 (271)	*	*
DDT	14 (13.3)		2.0 (3.1)	37 (305)	44	79
<u>>2xThreshold</u>						
Controls	15 (*)		* (*)	* (*)	47	62
DDT	15 (23.4)		4.9 (2.8)	145 (290)	35	78

50 Hz Trains

<u>Threshold</u>						
Controls	* (*)		* (*)	* (*)	*	*
DDT	28 (*)		3.0 (*)	41 (*)	58	76

<u>2xThreshold</u> Controls	* (59)	2.2 (4.1)	251 (491)	*	*
DDT	19 (25)	2.7 (2.7)	29 (197)	87	78
<u>>2xThreshold</u> Controls	* (*)	* (*)	* (*)	*	*
DDT	8 (*)	6.1 (*)	322 (*)	*	*

100 Hz Trains

<u>Threshold</u> Controls	40 (*)	* (*)	* (*)	146	43
DDT	27 (*)	5.0 (*)	4 (*)	52	74
<u>2xThreshold</u> Controls	15 (*)	7.5 (*)	178 (*)	75	57
DDT	25 (33)	3.7 (1)	33 (212)	70	77
<u>>2xThreshold</u> Controls	19 (*)	* (*)	* (*)	51	53
DDT	15 (*)	14 (*)	460 (*)	40	94

300 Hz Trains

<u>Threshold</u> Controls	47 (<10)	* (7.6)	* (447)	71	70
DDT	30 (*)	2.8 (*)	302 (*)	37	79
<u>2xThreshold</u> Controls	* (19)	* (6.1)	* (524)	*	*
DDT	64 (*)	3.1 (*)	365 (*)	138	78
<u>>2xThreshold</u> Controls	* (7)	* (6.5)	* (419)	*	*
DDT	21 (*)	* (*)	* (*)	30	92

600 Hz TrainsThreshold
Controls

8 (54)	* (2.1)	* (353)	*	*
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DDT

20 (*)	3.9 (*)	242 (*)	*	*
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2xThreshold
Controls

10 (19)	* (3.5)	* (307)	74	80
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DDT

33 (*)	1.9 (*)	343 (*)	110	82
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>2xThreshold
Controls

* (*)	* (*)	* (*)	*	*
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DDT

10 (*)	* (*)	* (*)	12	81
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APPENDIX 6

Calculation of action potential conduction times from the cerci to N5r1 or N6Br4 of the metathoracic coxa. Both giant and non-giant axon pathways are considered. Distances are the mean of 10 representative animals $\pm$ the standard deviation.

Time Calculations:

1. Mid-cercus to the terminal abdominal ganglion (A6).
2. Terminal abdominal ganglion (A6) to the metathoracic ganglion (T3).
3. Motor neuron (mid-T3) to the site of recording from N5r1 or N6Br4 in the metathoracic coxa.
4. Estimated synaptic delay in the terminal abdominal ganglion (A6).
5. Estimated synaptic delay in the metathoracic ganglion (T3).
6. Estimated delay associated with activation of muscle fibres.
7. Total estimated time for an action potential to travel from the cerci to motor nerves in the metathoracic coxa (N5r1): (a) using the giant axon pathway in the AVNC, and (b) using a 'hypothetical' non-giant axon pathway in the AVNC.

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1. Average distance from mid-cercus to A6: 6.5 ± 0.47 mm

Approximate conduction velocity: 1 m.s^{-1}

Time taken to travel from mid-cercus to A6: 6.5 ms

Based upon conduction velocities for axons of specific diameter (Pearson *et al.*, 1970). (This is a liberal estimate since receptors are also located near the base of the cerci.)

Camhi & Nolen (1981) estimate the minimum time from onset of wind at the cerci to recording a response in the A5-A6 connective as 2.5 ms. This time seems optimistic and is probably better represented by a value of 3-5 ms.

2. Average distance from A6 to mid-T3: 14.8 ± 0.95 mm

Conduction velocity in ventral GAs: $6-7 \text{ m.s}^{-1}$

Conduction velocity in dorsal GAs: $4-5 \text{ m.s}^{-1}$

(Parnas *et al.*, 1969)

Conduction velocity in non-giant axons: 2 m.s^{-1}
($1.5-3.5 \text{ m.s}^{-1}$)

(Dagan & Parnas, 1970)

Time taken to travel from A6 to mid-T3 using

ventral giant axons: 2.3 ms

dorsal giant axons: 3.3 ms

non-giant axon path: 7.4 ms

(Difference between vGAs and non-giant axon path: 5.1 ms)

3. Average distance from mid-T3 to
site of recording from N5r1: 3.6 ± 0.34 mm

Estimated conduction velocity: 2.7 m.s^{-1} (D_s)
(Pearson & Iles, 1971) $3-5 \text{ m.s}^{-1}$ (D_f)

Time taken to travel from
mid-T3 to site of recording: 1.3 ms or 0.9 ms

Time estimated by Camhi & Nolen (1981), T3 to
presynaptic neuromuscular terminal: 1.5 ms

(Mean conduction time from DUM5r1 to
the recording site from N5r1 was
about 3 ms.)

4. There is evidence that the cercal to GA synapse in A6 is monosynaptic (Callec *et al.*, 1972; Callec, 1974). Thus, a synaptic delay of 0.4-1.0 ms is likely, depending upon the immediate history of the synapse. This delay represents a small percentage of the total time and applies to both axon pathways. Therefore, it is not critical. An average delay might be 0.7 ms.

5. The delay that occurs within T3, between the giant axons and motor neurons, is more difficult to estimate because it is a polysynaptic pathway. Also, it is subject to facilitation and habituation, depending upon the general state of arousal of the animal. The delay within T3 may also be different for a non-giant axon pathway. Evidence from an equivalent part of the escape system of the crayfish indicates that this segment of the non-giant axon pathway is slower than for the giants. Rittenman & Pollack (1986) have shown it to be a polysynaptic pathway. Camhi & Nolen (1981) suggest an absolute minimum delay to activation of motor neurons of 1 ms.

6. From Krasne & Wine (1976), the delay associated with initiation of muscle contraction (in crayfish) after the arrival of an action potential was 10-15 ms. This time is shorter in cockroach, although there seems to be some discrepancy as to how much shorter. Delcomyn (1984) indicates it is only slightly less whereas Camhi & Nolen (1981) suggest it can be as short as 1.5 ms.

7. Behavioural Latency (time between onset of a wind puff at the cerci and initial movement of a leg),

Giant Axon Path: 10 ms

Non-Giant Axon Path: 15.1 ms

This assumes that equal amounts of time are required for both axon paths in A6 and T3.

The main difference is the conduction time along the AVNC, the non-giant axon path requiring about 5 ms more than the vGA path.

Therefore, the calculations support the suggestion that the GAs were excited and responsible for the activation of motor neurons with axons in N5r1. However, it must be noted that the values presented above usually represent minimums and generally do not account for times that may be required for summation of inputs or delay circuits. Optimal stimulus is also assumed.

END

18/02/91

FIN