THE FUNCTIONAL ANATOMY AND PHYSIOLOGY OF THE NEURONES OF THE SNAIL *HELIX ASPERSA*.

by

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CONTENTS.

Page

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ABBREVIATIONS

The following abbreviations have been used in this thesis:

Ach	Acetylcholine
ChE	Choline esterase
ChA	Choline acetylase
DMAE	Dimethylaminoethanol
5-HT	5-Hydroxytryptamine
5-HTP	5-Hydroxytryptophan
LSD	Lysergic acid diethylamide
GABA	Gamma-aminobutyric acid
Dopamine	3-Hydroxytyramine
R.P.	Resting potential
A.P.	Action potential
e.p.s.p.	Excitatory postsynaptic potential
i.p.s.p.	Inhibitory postsynaptic potential
conc.	Concentration

INTRODUCTION

For many years, the process of synaptic transmission was considered by some authorities to be an electrical process analagous to that which propagates the nerve impulse. There is now considerable evidence and support for the idea that synaptic transmission is a chemical process. When the impulse reaches the end of an afferent axon it initiates the release of a chemical from vescicles attached to the presynaptic membrane. The chemical passes through the presynaptic membrane and diffuses across the synapse, a matter of 200 Å or less. Once across the gap, the chemical combines with a receptor protein molecule and in some way alters the pore size of the membrane. This in turn alters the permeability of the postsynaptic membrane and allows the passage of ions to occur. The ionic change will alter the resting potential of the neurone, and may lead to a flow of current over the soma membrane in the form of an excitatory or an inhibitory potential wave. In this process there is a close correlation between chemical and electrical events.

The nature of the chemical transmitter in the vertebrate autonomic nervous system has received a great deal of attention. The classical experiments of Elliott, Dale, and Loewi were concerned with this system from the point of view of transmission at the neuromuscular junction. A considerable success was achieved in the analysis of the chemical transmitter at the cat cervical ganglion, (Feldberg and Gaddum, 1934). More recently attention has been turned to the nature of the chemical transmitters in the central nervous system. Due to the complexity of the vertebrate central nervous system this has proved a difficult problem. One solution to this problem might be to study a more simple nervous central nervous system than the vertebrate one, one with fewer neurones.

In many ways the pulmonate gastropods (snails) provide an excellent opportunity for this neurophysiological investigation. There are about 10^{10} neurones in the human brain compared with about 10^4 in the snail brain. The larger cells in the pulmonate brain are 700 μ (Aplysia) and 200 μ (Helix). Though these very large cells are not numerous, there are many cells between 80- 110 μ and these are peripherally arranged in the ganglia within easy reach of an electrode, or a drug. Many of the cells in the Helix brain are spontaneously active and this is often useful when

investigating the effect of a drug, since an active cell will often be more sensitive than an inactive cell.

The peripheral nerves leading to the brain of Helix are easily available and can be electrically stimulated. In this way one can set up orthodromic and antidromic stimulation of a cell. Observing the type of potential induced by stimulating an afferent axon will indicate the type of response to look for when a chemical is applied to the neurone artificially. It is important to know if stimulating by a certain afferent axon will induce full action potentials and/or excitatory potentials. On stimulating a particular nerve the shape of the induced action potential may be characteristic. It may subsequently be possible to produce a similar potential shape after the addition of a drug. If this is possible then a subthreshold concentration of the drug together with a subthreshold stimulation may facilitate one another to produce a normal response. The addition of a potentiator or antagonist may not only affect the application of the drug but also the stimulation of the afferent axon to the experimental neurone. It is possible to carry out all these types of experiments on the snail brain. It was for these reasons that the present investigation into the nervous system of the garden snail Helix aspersa was carried out. It was hoped to find out the way in which the nerve cells reacted

to electrical and chemical stimulation and in particular to determine the probable nature of the natural chemical transmitters.

PLAN OF THE INVESTIGATION

The initial experiments were conducted using isolated Helix brains, and recording the spontaneous activity from the dorsal surface of the suboesophageal ganglia by means of fine extracellular tungsten electrodes. This method of recording was replaced by one using glass intracellular electrodes. A variety of drugs which had been associated with transmission in the vertebrate central nervous system were tested on this preparation. At this time no attempt was made to try and identify individual neurones. The results showed that it was important to know more about the individual cells in the brain. The connexion between the nerve trunks associated with the suboesophageal ganglia and the neurones in the ganglia were investigated, and the experimental cells were marked by the ionophoretic injection of ferrocyanide ions into the cells. The approximate position of large dorsal neurones could easily be identified using a binocular microscope. The possible chemical transmitters could then be applied to neurones which could be identified both anatomically and electrophysiologically.

REVIEW OF THE LITERATURE

(i) ELECTRO-PHYSIOLOGICAL PROPERTIES OF SELECTED INVERTEBRATE NEURONE MEMBRANES.

The electrical properties of postsynaptic membranes have been widely investigated using glass microelectrodes for intracellular recording. This review will be confined to the study of the properties of a few selected invertebrate neurone membranes. The situation in the vertebrates, with particular reference to the spinal motoneurone, has been recently reviewed by Eccles (1961), in a Ferrier lecture. The main interest here will centre around neurones from the central nervous system of molluscs and on the properties of the sensory stretch receptor of the crustaceans. It is hoped that these studies on the electrical activity of the invertebrate neurones will indicate some of the parameters that a chemical transmitter will have to satisfy.

MOLLUSCAN NEURONE

The membrane responses to stimulation have been mainly confined to three molluscan genera; Helix, Aplysia, and Onchidium. The neurone can be stimulated orthodromically

or antidromically via an axon leading to the experimental neurone or a second microelectrode can be introduced into the cell. A current can then be passed directly into the neurone by means of this second electrode. The advantage of this method is that one is able to alter the bioelectric potentials directly of the experimental cell without much chance of interference from interneurones. It is possible that the operation of changing the biolectric potentials of one neurone might well influence interneurones which in their turn could influence the experimental cell.

Tauc, (1954) first studied the response of neurones in the abdominal ganglion of Aplysia depilans to direct intracellular stimulation. When he applied a stimulation of short duration, 20msec, he obtained a full action potential. The latent period between the application of the stimulus and the action potential varied from a few milliseconds to 150msec, depending on the voltage applied. When the cell had a resting potential of -40- 60mV, the action potential had a height of 80- 120mV. A stimulus of longer duration, 100msec, was followed by a multiple response resulting in several action potentials being produced.

Tauc, (1955) next investigated the response of both orthodromically and intracellularly stimulated neurones from the suboesophagael ganglionic mass of Helix pomatia. He was

able to obtain excitatory postsynaptic potentials, full action potentials, and potentials whose height was greater than an excitatory postsynaptic potential but much less than the full action potential. He called this last type a pseudopotential. The response obtained for a given cell depended on the intensity of the applied stimulation. Tauc found that the pseudopotential was a non-propagated, all-or-none response. He suggested that the membrane was divided into zones and at times there was a different threshold from one zone to another. If enough or all the zones were excited simultaneously, then a full propagated response would result. He thought that the presence of pseudopotentials demonstrated the heterogeneity and complex functioning of the membrane. The response to simulation depended on the electrical state of each part of the membrane and the interactions between the different zones of the membrane.

SUMMATION AND FACILITATION

In Aplysia, Tauc, (1955), demonstrated that stimulating orthodromially at a constant rate and intensity initially induced excitatory postsynaptic potentials; these later summated to give a full propagated action potential.

The neurones of the snail Helix pomatia also exhibited summation and facilitation, (Tauc, 1957). When the neurone was stimulated orthodromically at a constant rate and intensity so as to produce excitatory postsynaptic potentials, these summated to form a full action potential. After the action potential, the membrane did not return to the previous level but remained slightly negative, often forming an excitatory postsynaptic potential. The preparation could also show facilitation, though this was not found to occur with intracellular stimulation.

INHIBITORY POSTSYNAPTIC POTENTIALS

Inhibitory postsynaptic potentials were described in Aplysia by Tauc, (1956). Sometimes on stimulating the cell orthodromically the stimulus artifact was followed by a hyperpolarization of the cell membrane which lasted for 200 to 300msec. On stopping this stimulation, there was a period of increased excitation. Tauc concluded that these potentials were due to stimulating an inhibitory axon which connected onto the experimental cell.

INTERACTION BETWEEN EXCITATORY POSTSYNAPTIC POTENTIALS

When two afferent fibres synapse onto the same neurone in Helix, interaction can occur between them, (Tauc, 1960). If a postsynaptic potential precedes one which originates from another axon, then the second postsynaptic potential will be reduced in size. By giving a stimulatory burst to the first afferent axon, the stimulated response from the second can be reduced for many seconds. If both afferent axons are stimulated together, then neither will be diminished. Though facilitation occurs on repeated stimulation via the same presynaptic fibres, there was no facilitation between the two afferent pathways.

LONG LASTING INHIBITION

Long lasting inhibition can also be produced in some cases by the stimulation of excitatory pathways, (Tauc, 1959, 1960). A single stimulus to the afferent fibres can be followed in the cell by a brief depolarization followed by an inhibitory phase lasting 20- 30sec or more. This is accompanied by a hyperpolarizing potential. If the membrane potential is hyperpolarized, this hyperpolarizing wave reversed its polarity at a certain membrane potential. The

reversal potential for normal inhibitory postsynaptic potentials was 20mV lower. Therefore in this case the inhibitory postsynaptic potential was not responsible for the long inhibitory period. On stopping the stimulation the membrane potential continued to hyperpolarize for up to one second before starting to depolarize. The long lasting hyperpolarization was therefore not a summation following each excitatory postsynaptic potential. In one experiment this type of response was observed on stimulation of all the afferent excitatory impulses. While one cell responded in this way another could be depolarized and excited. The response was associated with membrane of the experimental cell rather than the pre-synaptic ending. This type of inhibition cannot be observed in either Aplysia or Helix at certain times of year.

Inhibitory potentials have been recorded by Holmgren & Frank, (1961), in the snail, Helix. They recorded from cells in the right parietal ganglion. Initially on stimulating the ipsilateral nerve at a frequency of 1 - 5/sec, each stimulus produced a full action potential but after a while bothethese driven action potentials and the spontaneous ones were replaced by an oscillating hyperpolarizing activity. Often each afferent stimulus was followed by a hyperpolarizing potential.

When the stimulation was stopped, the hyperpolarizing oscillations and inhibitory effects subsided and after 20- 100 sec the spontaneous activity returned. Renewed stimulation resulted in easier inhibition and finally a single stimulus could induce long inhibition. They concluded that this phenomenon was one of active inhibition by the membrane rather than one of fatigue. They associated this observation with the phenomenon of habituation.

RESTING POTENTIAL AND ACTION POTENTIAL SHAPE

The shape of the action potential obtained on stimulating the cell is dependent on the resting potential of the cell, (Tauc, 1957). If the cell had a resting potential of -52mV, then a normal action potential was obtained with a duration of 25msec. As the resting potential was depolarized, so each action potential elongated. When the resting potential was -15mV, the duration of the action potential was 60mV.

The type of postsynaptic potential in certain cases depended on the resting potential of the cell and on the intensity of the stimulation, (Tauc, 1957). He varied the resting potential of a neurone of Aplysia between -46 and -80mV, and then orthodromically stimulated it. As the

resting potential was raised the stimulated inhibitory postsynaptic potential fell and at around -60mV was replaced by an excitatory postsynaptic potential. In the second type of experiment the resting potential was unaltered but the intensity of the stimulus was slowly raised. As the intensity of the stimulus was raised so the inhibitory postsynaptic potential was replaced by an excitatory postsynaptic potential.

ANTIDROMIC STIMULATION

Most of Tauc's experiments have been concer ned with orthodromic or intracellular stimulation but he has also investigated antidromic stimulation of neurones of Aplysia. (1957c). He found that the rise time of an antidromic excitatory potential was faster than an orthodromic excitatory postsynaptic potential. The type of response obtained on antidromic stimulation depended on the resting potential of the neurone. As the resting potential was raised so the antidromically stimulated full action potential was replaced by a pseudopotential.

NON-SYNAPTIC INTERACTION BETWEEN NEURONES

Non-synaptic interaction has been shown to occur between the two large cells termed A and B, in the abdominal ganglion

of Aplysia, (Tauc, 1959). B was hyperpolarized to block any independent activity of the cell. When a spontaneous action potential occurred in A, a depolarising wave resembling an excitatory postsynaptic potential appeared in B. This potential occurred simultaneously with the full action potential recorded in A. Unlike the normal excitatory postsynaptic potential this potential did not show any tendency to facilitate. Tauc considers this passive potential to be due to electronic spread from the membrane of cell A to the membrane of cell B. This phenomenon was not common.

INTERNEURONES

Interneurones have been analysed in the abdominal ganglion of Aplysia, (Tauc, 1959). Tauc hyperpolarized an active cell to remove any spontaneous activity, leaving only postsynaptic activity. With a second electrode he penetrated or approached adjacent neurones until on approaching one, the activity of the first cell changed rate. This was taken as an indication that this was the interneurone responsible for the postsynaptic activity of the first cell.

SITE OF ORIGIN OF THE ACTION POTENTIALS

Both the site of origin of the propagated action potential and for the synapsing of afferent fibres appears to be on the axon, some 350- 500 μ from the soma (Tauc, 1960a, 1960b). After the removal or inactivation of the soma of the neurone of Aplysia it was found possible to record both orthodromically and antidromically stimulated potentials. The recording electrode was placed in the axon, about 350 μ or 2mm from the soma. When recording electrodes were placed in both the soma and axon and the cell stimulated orthodromically two full action potentials were recorded. In both cases the potential recorded from the axon preceded the potential recorded from the soma. When the axon electrode was placed 2mm from the soma, extra excitatory potentials were recorded which were absent from the soma recording.

TRACING NEURONAL CONNEXIONS

The connexions of two large cell, one in the abdominal ganglion and one in the pleural ganglion of Aplysia, have been investigated by Hughes and Tauc, (1961). They found that the axon of the large neurone in the right part of the abdominal ganglion sent branches to several nerves in the right half of

the body. It also connected with the left pleural ganglion via the cerebral ganglia. Their technique was to stimulate the cell intracellularly and record externally on the nerves leading into the ganglia. A large cell in the left pleural ganglion similarly branches into the nerves in the left part of the body.

THE EFFECT OF LIGHT, OXYGEN, CARBON DIOXIDE AND pH ON CELL ACTIVITY

Excitatory and inhibitory processes initiated by light and infra-red radiations in neurones of Aplysia have been investigated by Arvanitaki & Chalazonitis, (1961). It was found possible to cytologically distinguish three types of cell, the A cell, the Gen cell, and a smaller intermediate cell type. The large A type of cell in the dark may be spontaneously active or inactive. These cells respond to the onset of light showing depolarization and full action potentials. Some of these also responded to 'off'. The 'off' response corresponded to an inhibition process. These A cells contain two pigments, heme-protein and carotene-protein, the relative concentrations varying from cell to cell. The activation to white light has been compared to the activation by monochromatic light of 579mμ, for the heme-protein and 490mμ for the carotene-protein. A beam of 490mμ light usually

elicits an 'off' response when on an A cell; this inhibits spontaneous activity. The activity is re-established when the beam is removed. This light also hyperpolarized the membrane, but there were no inhibitory potentials. The excitatory effects induced by 579mμ are related to a graded generator depolarization of the cell membrane. When the rate of the generator depolarization reaches a given threshold then action potentials are induced. If the light is stopped prior to the generator potential reaching its maximum level, it will continue to depolarize but at a reduced rate. The response depended on the intensity and duration of the light beam. Thus a short flash of high intensity will elicit a response. The subliminal generator potential frequently had tiny depolarizations of about 0.1mV amplitude and 10msec duration. These may be excitatory postsynaptic potentials. The generator depolarization may be made up of a large number excitatory postsynaptic potentials.

In contrast other neurones of the Gen type or B type were always hyperpolarized 10- 20mV when illuminated. Inhibitory potentials were imposed on this hyperpolarization and their frequency increased as the intensity of the light was increased. When the light was stopped, the membrane depolarized to the initial level, the inhibitory potentials subsided and the spontaneous action potentials returned. In an attempt to

find an explanation for this inhibitory response the activity of adjacent cells was simultaneously recorded. Four types of responses were observed. On rare occasions there was no obvious effect on the activity of the surrounding cells. Sometimes there was a decrease in the activity of surrounding cells but no detectable inhibitory potentials. In others there were numerous inhibitory potentials. Lastly in some cases when the light was switched on, hyperpolarization and inhibitory potentials occurred in the Gen type cell while in the surrounding cells there was depolarization and an increase in the action potential rate.

The changes in the electrical activity of nerve cells elecited by a variation in the concentration of carbon dioxide, oxygen, H^+, or OH^- have been investigated and reviewed by Chalazonitis, (1961). The changes under these conditions are termed chemopotentials. Five large neurones and five medium sized neurones are easily recogniseable in the visceral ganglion of Aplysia. The large neurones were classified into three types depending on their spontaneous or induced activity. The A cell type has constant action potentials, with a low frequency of 0.5- I/sec. The Br type has trains of action potentials or slow waves. The duration of the train or burst of activity is several seconds. The mean spike frequency is 4/sec. The B or Gen type possesses spontaneous arhythmic

activity, with intervening hyperpolarizing potentials. These cell types display a different behaviour under the effect of metabolites. A 2 minute anoxia under nitrogen abolishes the 'trains' of potentials of the Br cell. The activity becomes a steady discharge of action potentials. The inhibitory hyperpolarizing wave is abolished. The Br cell then resembles an A cell. After 4 minutes of anoxia the activity of the Br cell degenerates into damped oscillations and the cell stops firing. Under anoxia, the A cell membrane first depolarizes. This increases the rate of the activity and then after a total depolarization of 10- 15mV the cell stops firing. The B or Gen type of cell is less sensitive to anoxia.

Carbon dioxide was found to depolarize all the types of cell examined. The observed carbon dioxide effects occur after 40sec. At this time there is a lowering in the pH of the connective tissue surrounding the A cell. The observed effect under carbon dioxide can be mimicked with ammonia. In this case the effect would appear to be due to OH^-, rather than to H^+.

ONCHIDINIUM VERRUCULATUM

The electrical properties of the large neurones of a marine pulmonate, Onchidinium verruculatum, have been studied by Hagiwara & Saito, (1959). The shape and size of the action

and resting potential was similar to those found in Aplysia. They were able to drive certain neurones by antidromic stimulation of the pedal nerves. They found that the action potential was characterized by a bump on its rising phase. This prepotential became more clearly visible when the membrane resting potential was hyperpolarized. Hagiwara & Saito investigated the voltage-current relations of the soma membrane with the cell held at a set voltage. An inward current was diminished or disappeared in the absence of sodium from the external solution while the outward current was unaffected. From the experiments of Oomura, Ozaki, & Maeno, (1961), it would appear that the neurones of Onchidinium are capable of forming normal action potentials in the absence of sodium ions in the external solution provided the external calcium ion concentration is maintained. A high concentration of external potassium abruptly depolarizes the resting potential to zero.

The neuronal membrane of Onchidinium shows both spatial and temporal summation of excitatory postsynaptic potentials in the formation of the necessary degree of depolarization to induce a full propagated action potential, (Kusano & Hagiwara, 1961). The stimulation of a certain nerve produced similar postsynaptic potentials in several cells and so it was suggested that a presynaptic fibre branches and innervates many neurones. It is possible that different afferent fibres were being

simultaneously stimulated and each was synapsing onto a different neurone.

There was not a one to one relationship between the stimulus and the action potential. Excess calcium in the external solution increased the amplitude of the postsynaptic potential. Excess magnesium decreased the amplitude of the postsynaptic potential. However the maximum amplitude of synaptic potentials reached by repetitive stimulation was almost unchanged by altering the calcium and magnesium concentrations. Thus in the calcium rich solution the conductance of the synaptic membrane was close to the maximum value for a single presynaptic impulse. Kusano & Hagiwara concluded that the increase of amplitude of the synaptic potential produced by repetitive presynaptic stimulation was not due to a simple temporal summation but to the increase of the active area of the synaptic membrane innervated by each presynaptic fibre.

THE CRUSTACEAN CARDIAC GANGLION

This preparation is much simpler than that of the molluscan brain since it contains only nine neurones.

Intracellular recordings were first made from the nine cells of the cardiac ganglion of Panulirus interruptus by Hagiwara & Bullock, (1955).

The ganglion is capable of initiating at regular intervals complex bursts consisting of several to many impulses in each cell. There is a distinct pattern in the whole comlex of nine neurones as well as in the bursts of impulses in each unit. There appears to be a division of labour among these neurones. Certain cells initiate the burst which starts a heart beat while others follow them. During a burst up to 19 potentials occur, (Bullock & Terzuolo, 1957). It was found that two separate spike-like events occurred in the same soma. These had different sizes and frequencies and probabilities of occurrence. They concluded that the diverse forms of activity observed can be reduced to two types. The activity may be characterized by rapid deflexions, which they termed synaptic potentials, and those initiated by a slow depolarization.

At least two separate impulses can originate at different places in the same neurone and occur simultaneously or at any phase relation to each other. These impulses cannot invade the soma except as small 5- 20mV spikes. In one type of cell soma, invasion can produce full potentials. These separate impulses in the soma are able to sum and it is suggested they form the large spike potentials.

Potentials may arise at various phases after a preceding potential. Thus a second potential may occur during the falling phase of the first. It is suggested that the two potentials converge upon the soma from different axonal processes.

The small slow deflections were shown to be possibly presynaptic in origin since when a simultaneous surface recording was made, each small slow deflection in the intracellular record was preceded by an impulse of another neurone.

The response to stimulating the soma with an intracellular electrode has been studied by Hagiwara, Watanabe & Saito, 1959), and they showed that the propagated action potential did not invade the soma membrane of the cell.

Electrical interaction can occur between the large neurones. When two large cells were recorded from and a polarizing potential applied to one, an effect was also recorded in the other one.

These results from the cardiac ganglion indicate the complexity that occurs when several neurones are interconnected. Certain of the phenomena observed are also found in the snail brain and are due to the interaction between many nerve cells.

CRUSTACEAN STRETCH RECEPTOR

The simplest known situation is that shown by the crustacean stretch receptor where there are two neurones which interact, a sensory neurone, and an inhibitory neurone, (Eyzaguirre & Kuffler, 1956). Stretching the muscle fibre depolarizes the nerve and acts as a generator potential. A series of action potentials is set up in the axon of the cell, the potentials probably being set up some 0.5mm away from the soma, (Edwards & Ottoson, 1958). Stimulation of the inhibitory nerve brings the membrane back to the potassium equilibrium level.

CONCLUSIONS

From the examples so far sited it would seem that certain generalizations can be made. The axons appear to be excitable by electrical stimulation and/or field effects. It is possible that axon-axon connection could have transmission in an electrical manner.

Dendrite-soma transmission on the other hand, appear to be of a chemical nature. At least two different chemical systems are required (a) to increase the sodium conductance

and so bring about an action potential and (b) to increase the potassium or perhaps chloride conductance and so bring about a stabilization of the resting potential.

The site of origin of the impulse would appear to be in the axon of all three preparations, (the Molluscan brain, the crustacean heart ganglion, and the crustacean stretch receptor neurone), and probably the afferent fibres synapsing onto these neurones do so onto this region of the axon. This is in contrast to the vertebrate system where the afferent fibres synapse onto the soma of the neurone.

ACETYLCHOLINE

INTRODUCTION

Though it had been thought for some time that a chemical transmitter occurred between the nerve-nerve junction, and also the nerve-muscle junction, it was not clear as to what this transmitter was. Dixon, (1906), tested extracts of frogs hearts, whose vagus nerve had been stimulated, on normally beating hearts and found that they were inhibited. This effect could be abolished by atropine. About the same time it was shown that the suprarenal gland contained compounds which produced a hypotensive effect in excess of the known choline content. Cholinesters were synthesised and it was found that acetylcholine had effects 1,000 to 10,000 times more potent than choline.

Dale, (1914), demonstrated that acetylcholine had two effects. The first was a depressor cardioinhibitor effect, which was similar to the effect of muscarine. The second was a pressor effect, similar to the effect of nicotine. This response only appeared when the first type was abolished by atropine.

Direct evidence for chemical transmission came in 1921,

with the investigations of Loewi. He found that stimulation of the vago-sympathetic trunks of a perfused isolated heart released two antagonistic substances. These when transmitted to a second heart had an effect similar to that of stimulation of the two nerve trunks.

Clear evidence for a role of acetylcholine in the transmission of impulses between neurones came with the investigations of Feldberg & Gaddum, (1934). They perfused the superior cervical ganglion of the cat with Locke's solution containing eserine, and examined the emerging venous flow. Stimulation of the cervical sympathetic nerve caused the liberation from the ganglion of a compound which was pharmacologically identified as acetylcholine. It was further shown that impulses passing along the vagus nerve and antidromic impulses released no acetylcholine, (Feldberg & Vartiainen, 1935). They concluded that the liberation of acetylcholine occurs at the synapse. They estimated that around 10^{-15}g of acetylcholine was released per synapse in response to a single maximum preganglionic volley. A low concentration of eserine potentiated the acetylcholine response whereas high concentrations depressed or blocked it.

Dale, Feldberg & Vogt, (1936), stimulated the motor nerve

fibre of perfused voluntary muscle. They found that this caused the appearance of acetylcholine in the venous fluid. When the muscle was completely denervated, no acetylcholine was released. When the muscle response was blocked with curarine, acetylcholine still appeared in the perfusate. When conduction from the motor nerve fibre to the muscle failed due to exhaustion, then no acetylcholine was released.

It was later shown that the nerve impulse in the motor fibre induces an end-plate potential in the postsynaptic muscle fibre membrane. This end-plate potential in turn gives rise to the muscle action potential, (Eccles, Fatz & Kuffler, 1941). It would appear that the end-plate potentials were induced by liberated acetylcholine from the presynaptic membrane of the motor nerve fibre. With this as a background investigations as to the possible role of acetylcholine in the central nervous system were undertaken. These will now be reviewed in more detail.

VERTEBRATES

THE LOCALIZATION OF ACETYLCHOLINE

The distribution of acetylcholine in the vertebrate brain was investigated by MacIntosh, (1941). He found that in the dog, acetylcholine occurs in the gray matter, and in those parts of the white matter which contain efferent axons, but not in white matter containing only afferent axons. In the cat the distribution of acetylcholine did not run parallel to that of cell bodies or synapses. The cerebral cortex and some nuclei and tracts are relatively poor.

Crossland, Pappius, & Elliott, (1955), concluded that the most likely level for the unfrozen hemispheres in the rat was 2.4 μg/g, and 1.9 μg/g for unfrozen whole adult brain. The content of acetylcholine in whole adult frozen brain was 3.1 μ g/g.

In addition to acetylcholine, two other homologues have been found in vertebrate tissue. Banister, Whittaker & Wijesundera, (1953), found two such compounds in the ox spleen and one of them they were able to identify as propionylcholine.

Kewitz, (1959), isolated γ-aminobutyrylcholine from the brain of the pig.

Another approach is to investigate the powers of the different regions of the brain to synthesise acetylcholine.

Feldberg & Mann, (1946), studied the synthesising abilities of the brain. They found that the synthesising ability of the cerebrum was greater than in the cerebellum. It was also high in the retina. They found a correlation between the brain acetylcholine content and the synthesising enzyme. The greater the acetylcholine content, so the more acetylcholine could be formed by the enzyme from brain extract of that region. The central nervous system had a lower content of acetylcholine, but it had higher powers of acetylcholine synthesis than the autonomic nerves.

Acetylcholine synthesis in 40 regions of the central nervous system was later investigated by Feldberg & Vogt, (1948). In the thalamus, 220- 520 µg acetylcholine /g dried tissue was formed per hour. The anterior roots formed 236%, compared with the thalamus as 100%. The most active regions in the central nervous system were from where the spinal and cranial motor nerves originated, that is the anterior horns and the hypoglossal and vagal (motor) nuclei. These regions have 70% of the anterior root activity. The activity was lowest in sensory nerves and tracts, the pyramidal tracts and the cerebellar cortex. The cerebellar peduncles have moderate activity. The white matter and the corpus callosum have slightly higher activity than the pyramidal tracts.

The caudate nucleus, the region of the supraoptic nuclei and cornu ammonis have an activity slightly higher than the thalamus. The activity of the cerebral cortex is lower than in the thalamus, but it is comparatively uniform in the functionally different areas, for example, motor, somaesthetic, visual and olfactory. It is highest in the olfactory area. Feldberg & Vogt suggest that in voluntary motor pathways, the finding of high enzyme concentration in the anterior horns and motor nuclei of the cranial nerves agrees with the cholinergic concept of the lower motor neurone. The lower values in the pyramidal tract indicate that the upper motor neurone is not cholinergic.

The retina contains large amounts of the enzyme which synthesises acetylcholine but the optic tract is free, that is, acetylcholine is the transmitter at one or more of the synaptic junctions in the retina. The optic fibres run into the lateral geniculate body, and the high activity here suggests a cholinergic nature of the third neurone.

The sensory pathway which starts in the posterior roots is non-cholinergic, but the high activity values for the nuclei into which these fibres pass suggest evidence for the cholinergic nature of the second neurone in the sensory pathway. Fibres from the nuclei gracilis and cuneatus terminate in the thalamus. The third neurone originates

from here and leads to the cortex. This it is suggested is non-cholinergic since there are low activity values in the internal capsule.

The activity in the cerebellar cortex is low. This suggests few or no cholinergic fibres.

Low activity values in the posterior pituitary indicate non-cholinergic fibres. The high activity found in the supraoptic nucleus suggests that cholinergic fibres end in this region. It has been shown by Pickford, (1947), that acetylcholine stimulates the supraoptic neurones. This sensitivity to acetylcholine is evidence for the presence of acetylcholine-sensitive cells from which non-cholinergic fibres emerge.

In contrast, the hypoglossal nuclei provide an instance of central neurones, which are sensitive to acetylcholine, Miller, (1943), and also the origin of cholinergic fibres. This can be compared with the situation in the peripheral nervous system where the sympathetic and parasympathetic neurones are sensitive to acetylcholine, the former are the origin of adrenergic fibres and the latter are the origin of cholinergic fibres.

Feldberg & Vogt conclude that cholinergic neurones only form a fraction of the neurones in the central nervous system; They have a pathway of alternating cholinergic and non-cholinergic

fibres. The same cell may receive both cholinergic and non-cholinergic fibres synapsing onto it.

CHOLINE ESTERASE

Burgen & Chipman, (1951), investigated the relative amounts of choline acetylase, acetylcholine, and cholinesterase in different regions of the brain of the dog. They found a good correlation between the occurrence in a given area of the three compounds except in the cerebral hemispheres where there was a disproportionally high cholinesterase content and in the anterior spinal roots where there was a low cholinesterase content. However Hebb, (1957), points out that it is difficult to account for the function of true cholinesterases solely in terms of acetylcholine function. The cholinesterase in the cerebellar cortex largely occurs in the glial cells and there is no evidence that these cells are capable of synthesising acetylcholine. Motoneurones were found to contain large amounts of true cholinesterase, (Koelle, 1954). The enzyme was located in only certain of the neurones of the brain. This would agree with the idea of alternatively cholinergic and non-cholinergic pathways.

PSEUDO-CHOLINESTERASES

Pseudocholinesterases occur in the central nervous system as well as true cholinesterase. The relative concentrations of each have been investigated by Burgen & Chipman, (1951). They found that the distribution of true cholinesterase was very uneven, the ratio of the highest, the caudate nucleus, to the lowest, the sub-cortical white matter, was 400:I. The similar ratio of the pseudocholinesterases was 18: I. In 80% of the brain areas examined, pseudocholinesterase contributed to over 10% of the hydrolysed acetycholine, and in 30% of the brain areas to over 40%. The activity in the optic nerve due to pseudocholinesterase was 93%, in the corpus callosum 54%, in the hypothalamus 43%, cerebellar hemisphere 2%, in the caudate nucleus 6%, and in the lentiform nucleus 8%. Cavanagh, Thompson, & Webster, (1954), found that the white matter of the human brain was rich in pseudo-cholinesterase. The high activity of the glioma tissue led them to suggest that it was located in the glial cells. Pseudo-cholinesterases were not found in conductive elements of peripheral nerves. They suggest it is confined to the cells of Schwann.

Desmedt & Grutta, (1955), injected inhibitors selective for either aceto- or pseudo-cholinesterases into the external

carotid artery of unanaesthetized cats while recording electrical activity of the cerebral cortex. They found that the activation of brain potentials depended on inhibition of pseudo-cholinesterase.

DIMETHYLAMINOETHANOL. :DMAE

This compound was first suggested as a precursor for acetylcholine by du Vignaud, Chandler, Simmonds, Moyer & Cohn, (1946). DMAE has been reported to occur in the brain, Honegger & Honegger, (1959). The electroencephalogram recording of the rabbit was found to be greatly enhanced following the injection of DMAE, (Goldstein, 1960). However, the observed behaviour of the animal was associated more with overexcitation than with depression. This action of DMAE was antagonized by choline and atropine. It was interesting that the brain electroencephalogram responded as for a depressant yet the animal was overexcited.

Pepeu, Freedman & Giarman, (1960) tried to find out if DMAE was a precursor for acetylcholine in the brain, and to see in the concentrations used, if DMAE could elecit effects referable to an action on the central nervous system. They found little difference in the acetylcholine content of the brain with or without the prior administration of DMAE. From

a study of the biosynthesis of acetylcholine in vitro from DMAE in the presence of various anticholinesterases, they could find little evidence for the importance of DMAE in acetylcholine production. They tested the effect of DMAE on the effect of barbiturate and ethanol which are both central depressants. They found that DMAE greatly prolonged the duration of the depression caused by these two compounds. The effect of DMAE on behaviour was examined. Rats were trained to climb a rope in order to obtain food. The administration of DMAE impaired this ability for 2hr.

They conclude that it is premature to ascribe DMAE actions on the central nervous system to its conversion to acetylcholine. It may have actions of its own or be a precursor for some other compound. They suggest the introduction of labeled DMAE into the animal and the testing for the presence of labeled acetylcholine would be a useful experiment. They conclude that DMAE induces an indifference to food, that is, a temporary decrease in the thresholds for food motivation behaviour.

The possible role of DMAE as a central nervous system stimulant has been reviewed by Murphree, Jenney & Pfeiffer, (1959). It may be concluded that the role of DMAE in the nervous system is far from clear.

APPLICATION OF ACETYLCHOLINE TO THE SPINAL CORD

The action of acetylcholine in the central nervous system will now be considered.

1 μg of acetylcholine was injected into the circulation through the spinal cord of the dog, (Bulbring & Burn, 1941). It was found to produce a discharge of motor impulses which were recorded as a series of contractions of the tibialis anterior. They concluded that the response was due to the direct stimulation of the motoneurones. Comparable effects were produced by eserine. Adrenaline was found to facilitate the action of acetylcholine, while the acetylcholine effect was blocked by atropine.

Eccles, Fatt & Koketsu, (1954) investigated the transmission of impulses from the collaterals of the spinal motor axons to the interneurones or Renshaw cells. They worked on the hypothesis suggested by Dale, (1934), that a cell will release the same transmitter chemical from all its endings. It was known that the motoneurone at the neuromuscular junction released acetylcholine. So they studied the effect of this compound on the system. Impulses in the motor axons set up a prolonged repetative discharge in these Renshaw cells. The transmission to the Renshaw cells can be inhibited by dihydro-β-erythroidine and to a lesser extent by atropine.

The administration of acetylcholine via the arterial blood supply of the spinal cord caused repetitive firing in the Renshaw cells. However there is a wide range in the response of the Renshaw cells to acetylcholine. Anticholinesterases prolong the discharges of the Renshaw cells. The nature of the inhibitory chemical liberated by the Renshaw cell presynaptic membrane onto the motoneurone membrane is not known. The i.p.s.p. so produced can be blocked by strychnine.

Kiraly & Phillis, (1961), tested drugs which potentiated or antagonized transmission at cholinergic synapses, on slow depolarising dorsal root potentials evoked by stimulation of adjacent dorsal and ventral root systems of the isolated toad spinal cord. Some drugs acted specifically on the dorsal root potential evoked by ventral root stimulation. Acetylcholine depressed this potential whilst anticholinesterases in low concentrations potentiated and in high concentrations depressed it. They suggest this offers some evidence for a cholinergic link in the pathway responsible for the generation of depolarising potentials in the dorsal root subsequent to ventral root stimulation.

Motor neurones of isolated cords of the frog, bathed in recirculated nutrient Ringer's solution, were activated antidromically polysynaptically via the dorsal root fibres, and via simpler connexions from descending fibres in the

lateral column, (Crepax & Brookhart, 1960). The eserinized perfusate was assayed for 5-HT activity on the heart of Venus mercenaria. A compound resembling 5-HT appeared in the perfusate after excitation and at rest. Acetylcholine appeared in the perfusate in greater concentration during lateral column stimulation than during dorsal root or antidromic stimulation. They concluded that the role of acetylcholine as a transmitter can only be inferred if there is a quantitative link between acetylcholine produced and motor neurone excitation.

APPLICATION OF ACETYLCHOLINE TO THE VERTEBRATE BRAIN.

The response to acetylcholine has been tested on different regions of the vertebrate brain. These effects up to 1950 have been reviewed by Feldberg, (1950). Action potentials were recorded from the cerebral cortex of the rabbit by Sjostrand, 1937. He added a drop acetylcholine to the cortex surface and recorded a burst of activity. Repeated applications of the same dose or a single application of a stronger concentration inhibited the activity completely.

Crossland & Mitchell, (1956), during an investigation into the nature of the Cerebellar Factor, tested the effect of

acetylcholine on the cerebellar activity. Acetylcholine potentiates the activity of the cerebellum. The Cerebellar Factor however was not acetylcholine as the activity remained on destroying the acetylcholine in the extract.

Acetylcholine was found to stimulate a motor nucleus, the hypoglossal nucleus, (Miller, 1943). He observed the same effects on the application of acetylcholine as when he applied a current to the nucleus. The acetylcholine at a concentration of 1:50 million, rapidly penetrated to the nucleus. The response lasted for only 4- 15 seconds. It was potentiated by eserine and antagonized by atropine. The response caused the tongue to contract and deglutition.

Trephine holes were made in the skull of cats under deep ether and then allowed to recover (Dikshit, 1935). A week later he introduced 0.1- 0.5 μg of acetylcholine into the lateral ventricle of the brain, or deeper into the hypothalamus. This induced sleep. The effect came in after 10- 30 minutes and lasted for 2-3 hours. The injection of saline had no effect.

Reserpine is normally associated with the release of 5-HT and possible other amines, but it has been shown by Malhotra & Das, (1962), that after the injection of reserpine the mean content of acetylcholine in the hypothalamus rose by 25%.

Bradley & Mollica, (1958), recorded single unit activity in the reticular formation of the decerebrate cat. Acetylcholine was injected via the intracarotid, at concentrations of 0.2- 1.0 μg. All the units tested showed a response. The response occurred in two parts. The first response occurred after 0.5- 8 seconds, and the second one after 20-40 seconds. There was no blood pressure effect. Generally both effects were the same, though in some cases there was a primary increase in activity followed by inhibition. Acceleration of the activity was more common than inhibition. Some units responded to both acetylcholine and adrenaline, and here the effects could be the same or different. Some units responded only to acetylcholine.

Krnjevic & Phillips, (1961), used 5 barrel micro-pipettes to investigate the sensitivity of the cortical neurones of the cat to acetylcholine. By this means they were able to record the extracellular electrical activity of single units. The other 4 barrels contained strong solutions of acetylcholine or other compounds which could be released in the immediate vicinity of the neurones by ionophoretic injection. The tips of the micro-pipettes were 5-10 μ, and could easily be inserted into the cortex after the removal of the pial layer. They examined 1,367 cells in various cortical area in 13 cats

anaesthetised with Dial or Chloralose. All the cells were excited by l-glutamate; this action and any spontaneous activity was effectively blocked by GABA. Of the cells investigated, 200 were sensitive to acetylcholine, either by an increase in the frequency of firing of the spontaneous activity or the firing of quiescent cells. To obtain an effect, a current of 0.05-0.I μA was passed through the acetylcholine barrel for a minimum of 2-20 seconds. When the acetylcholine current ceased, the heightened activity persisted for periods of 5-60 seconds. By passing a similar or much stronger outward current through another barrel containing for example sodium sulphate, it was easy to show that the observed excitation could not be ascribed to the electrotonic effects of the flow of current.

Acetylcholine sensitive neurones were also excited, though to a lesser extent, by carbamylcholine, propionylcholine, and succinylcholine, but not by butyrylcholine. They were affected by neostigmine and edrophonium applied in a similar manner. Both these compounds potentiated the action of acetylcholine, or even excited the cells. On the other hand tubocurarine could not be shown to have any blocking action. The only response observed in some cases was a delayed but pronounced excitation. Nicotine had a similar excitatory action, which bore no relation to the acetylcholine sensitivity. Atropine

and hyoscine had a non-specific depressant effect on nearly all the cortical neurones to which they were applied. Gallamine was the only compound which selectively blocked the action of acetylcholine. Acetylcholine sensitive neurones tended to occur in clusters, and they often showed spontaneous activity related to slow waves of the electrocorticogram. Although a few sensitive neurones were found in most area, they were distributed mainly in the primary somatosensory, visual, and auditory receiving areas. The greatest concentration of acetylcholine sensitive cells were found in the primary visual area of some of the cats.

The pharmacological properties of urocanylcholine, murexine, have been investigated by Keyl & Whittaker, (1958). This choline ester has both ganglion stimulating and neuromuscular blocking actions. The neuromuscular blockage was due to the depolarization of the end plate region and, as with decamethonium, cats were most sensitive and rats least sensitive. The action of urocanylcholine was short-lasting in all the species studied, but this they suggest was not due to hydrolysis by plasma esterases.

The pharmacological properties of senecioylcholine, β , β - dimethylacrylylcholine, have been studied by Sekul & Holland, (1961). Preliminary evidence suggests that it is a ganglion stimulating agent. It was found to produce a pressor response

in the absence of atropine. This effect was partially blocked by adrenalectomy and almost completely abolished by ganglionic blockage. The pressor response to senecioylcholine was reversed by adrenergic blockage. This ester produced contraction of the nictitating membrane. This action was antagonized by ganglionic blockage. It produced the contraction of isolated frog rectus abdominis muscle. Acetylcholine owes its muscarinic and nicotinic activities to the presence of three chemically active groups: the keto oxygen, the ether oxygen, and the quaternary nature of the nitrogen atom. In acetylcholine these are arranged to bring about optimal activity. These groups have a similar spatial arrangement in senecioylcholine.

INVERTEBRATES

LOCALIZATION OF ACETYLCHOLINE IN CRUSTACEA AND INSECTA

Acetylcholine has been shown to occur in the nervous tissue of several crustaceans. Smith, (1939), found 12.6 μg/g in the nerves and 45.8 μg/g in the ganglia of Cambarus bartoni. The blood of Cambarus limosus contained 0.7- I.I μg/g.

Schallek, (1945), reported 3.1 μg/g in the ventral ganglion of Cancer; 9.8 μg/g in the nerve cord of Cambarus; 13.2 μg/g in the circumoesophagael ganglia of Limulus; and 0.3 μg/g in the Limulus cardiac ganglion. When he perfused the ventral nerve cord of the lobster, 10% of the acetylcholine passed to the perfusate after 1 hour. Schallek found both bound and free acetylcholine in the lobster nerve cord.

Florey & Biederman, (1960), found acetylcholine only in the sensory axons of the crustacean peripheral nerve, at concentrations ranging from 1.7 - 6.7 μg/g nerve tissue.

The estimation of acetylcholine in insect heads was done by Lewis & Smallman, (1956). They found 32.7 μg/g in Calliphora; 28.3 μg/g in Lucilia; 26.1 μg/g in Musca; 7.8 μg/g in Tenebrio; and 9.8 μg/g in Periplaneta americana. This last insect had 36.7 μg/g in its isolated nerve cord. Lewis & Fowler, (1958), extracted acetylcholine from frozen insect heads. They found 38.7 μg/g in the head of Calliphora. There was no significant difference in the acetylcholine yield depending on whether the head was severed before or after freezing.

Colhoun, (1958), estimated the acetylcholine content of the nervous tissue of Periplaneta americana. He found 135.2 μg/g in the brain and 95.4 μg/g in the thoracic ganglia.

The venom of certain Hymenoptera, for example the hornet, Vespa crabro, has been found to contain acetylcholine, 5-HT, histamine, and a new kinin, (Bhoola, Calle & Schachter, 1960, 1961). The acetylcholine content varied from 18-50mg/g dry venom sac.

Recently it has been shown that the garden tiger moth, Arctia caja contained a high concentration of at least two active cholinesters. The freeze-dried prothoracic gland contained 1-2 mg/g acetylcholine equivalent activity, possibly due to β -dimethylacrylylcholine, (Bissett, Frazer, Rothschild & Schachter, 1960). The abdomen has also been shown to contain an acetylcholine-like substance, (Gill, Parsons & Paton, 1961). This compound is different from the one found in the thorax by (Bissett, Frazer, Rothschild & Schachter, 1960).

Acetylcholine was found in the hypertrophied silk glands of the final instar larva of Arctia caja, (Morley & Schachter, 1961). They found approximately 4 mg/g freeze-dried gland using bioassay and chromatographic techniques. The silk of the cocoon had about 300 μ g/g. This was the only pharmacologically active compound detected in the larva.

It has now been shown that acetylcholine occurs in high concentrations in non-nervous tissues of Lepidoptera, (Morley & Schachter, 1962). Acetylcholine was detected in the adult reproductive systems of both sexes. The highest concentration was found in the ejaculatory duct of the male A. caja at 2.5-

5.5 mg/g. In the female the bursa copulatrix contained up to 8.2 mg/g. Acetylcholine has also been found in several allied species. Acetylcholine-like activity has been found in the Sphingidi, Noctuids, and in a Plutellid.

OTHER CHOLINE ESTERS IN INVERTEBRATES

Several choline esters occur in the invertebrates. Keyl, Michaelson & Whittaker, (1957), found acetylcholine, urocanylcholine, (murexine), and homarine in Murex fulvescens, Thais lapillus, and Urosalpinx cinereus whole tissue or hyprobranchial gland. Another species studied, Thais floridana, was found to contain a new ester, β,β-dimethyl-acrylylcholine (Senecioylcholine). The only physiologically active choline ester found in lobster nervous tissue was acetylcholine. A high acetylcholine equivalence was found in Buccinum undatum. This was not identified. Murexine was first found by Erspamer & Dordoni, (1947), in the hypobranchial gland of Murex trunculus.

In addition to urocanylcholine, and senecioylcholie, Whittaker, (1960), found acrylcholine in the hypobranchial gland of certain marine gastropods.

Acetylcholine has been reported in the ganglia of the octopus and in Aplysia, Bacq, (1935).

CHOLINE ESTERASES

Cholinesterases have been examined histochemically in the nervous system of the lobster, Homarus americanus, (Maynard & Maynard, 1960). Unfixed, frozen sections of lobster brain, eye-stalk, sub-oesophageal, thoracic, stomatogastric, and cardiac ganglia were investigated. The cholinesterases hydrolysed acetylthiocholine more intensely than butyrylthiocholine, but both substrates were hydrolysed at the same sites. In the brain, suboesophagael, thoracic, and stomatogastric ganglia, most neurones have only a small amount of cholinesterase in the cytoplasm of the soma and processes. There was a large amount of cholinesterase in the sheath cells around the soma of the neurones. The cardiac ganglion contained only a very little enzyme.

Maynard & Maynard, (1960b), also found cholinesterases in both the sensory neurones and in the motor and 'accessory' nerve fibres of the muscle receptor organs.

It was found by Nachmansohn & Meyerhof, (1941), that practically all the choline esterase present in the giant fibre of the squid, Loligo paealii, was localized in the sheath. The enzyme activity of the axoplasm was negligible. They consider this as evidence that the enzyme is always concentrated at or near the surface of the nerve cell. They

found a very high concentration of choline esterase in the head ganglion of the squid.

Augustinsson, (1948), found that the dart sac of Helix pomatia was able to hydrolyse esters of choline. Using acetylcholine as substrate he found 906 µl of CO_2 were evolved during 30min from 100mg of dart sac tissue. On a comparable basis 70 µl of CO_2 were formed using acetyl-β-methylcholine; 37 µl of CO_2 were formed using benzoylcholine; 336 µl of CO_2 were formed using acetylsalicylcholine; 107 µl of CO_2 were formed using tributyrin; smaller amounts were formed using acetylsalicylic acid, acetylaneurine and ethyl acetate; and little or none was formed using N-acetyl-p-aminobenzoylcholine, carbaminoylcholine or salicylcholine.

CHOLINE ACETYLASE

The distribution of the enzymes choline acetylase and cholinesterase in relation to acetylcholine have been investigated in the invertebrates as well as in the vertebrates.

Mehrotra, (1960), has studied the development of the cholinergic system in eggs of Musca and Oncopeltus. He found that choline acetylase appeared first, followed much

later by cholinesterase, and finally acetylcholine. The amounts of substrate and enzymes increased progressively till hatching time.

Casida, (1955a,b), surveyed 14 different species of insect and mite in various development stages and 14 organ systems from Periplaneta americana for their esterase activity with reference to acetylcholine, acetyl-β-methylcholine, benzoylcholine, triacetin, and o-nitrophenylacetate. He concluded that insect acetylesterases were a group of related enzymes with widely divergent properties.

FUNCTION OF ACETYLCHOLINE IN THE INVERTEBRATES

ANNELIDA

It has been suggested that acetylcholine transmits impulses across the neuromuscular junction of longitudinal muscles of the leech, Hirudo medicinalis, (Bacq & Coppee, 1937). Bacq, (1937), concluded that the motor nerves of worms were cholinergic. Stimulation of the nerve leading to the leech muscle led to the appearance of a chemical in the surrounding fluid which had the properties of an unstable choline ester. This substance was quickly destroyed in the absence of eserine. He considered the substance to

be acetylcholine. Similar experiments using molluscan motor nerves and muscle did not yield acetylcholine in the surrounding medium. Although there was acetylcholine present in the crustacean nervous system, in, for example, the stellate ganglion, stimulation failed to mobilize it.

MOLLUSCA

Acetylcholine has been suggested as a possible transmitter of inhibition in Aplysia, (Tauc & Gerschenfeld, 1960). Intracellular recordings were made from neurones in the abdominal ganglion. It was found that acetylcholine at a concentration of 10^{-12} g/ml hyperpolarized the membrane and mimicked the inhibitory postsynaptic potentials in cells which had an inhibitory input. Neurones which only had an excitatory input were depolarized by acetylcholine. The inhibitory response of acetylcholine was reversed when the cell membrane was artificially raised. The reversal-level for acetylcholine potentials and inhibitory postsynaptic potentials have equal values. They suggest this indicates a similar selective permeability variations of the membrane. In the cells with excitatory inputs, the equilibrium level is close to the zero membrane potential. This indicates

a different and non-selective permeability change. D-Tubocurarine and atropine in external solutions reduced the inhibitory postsynaptic potentials as well as the action of acetylcholine. Eserine at first lengthened the inhibitory effect and then reduced the size of the inhibitory postsynaptic potentials by inactivating the postsynaptic membrane. Eserine did not affect excitatory postsynaptic potentials. From these observations they concluded that the inhibitory system in Aplysia central nervous system is cholinergic.

Acetylcholine has been applied to the neurones in the central nervous system of the snail Helix pomatia, (Tauc & Gerschenfeld, 1960). They found that for a given cell the acetylcholine effect depended on the resting potential of the cell. If the resting potential is below 50mV, then acetylcholine hyperpolarizes the membrane, inhibiting the activity. If the membrane resting potential is raised above -50mV, then acetylcholine depolarizes the membrane and excites the activity of the cell. The acetylcholine effect depends on the resting potential value, and the critical value varies from cell to cell. The concentration of acetylcholine which gives this response is 10^{-8} g/ml. At a concentration of 10^{-5} acetylcholine, the activity is inhibited and the resting potential may fall. Both effects

are potentiated by eserine.

It has been suggested that acetylcholine transmits the impulses across the synapses between the cardio-acceleratory fibres and the heart muscle of Venus mercenaria, (Prosser, 1940, Welsh & Slocombe, 1952). Prosser, (1940), found that the heart was sensitive to dilutions of 10^{-12} acetylcholine in the spring. Stimulation of the visceral nerve caused inhibition of the heart similar to that caused by the application of acetylcholine. In non-toxic concentrations atropine had no effect on the effect of acetylcholine. Fluid from a heart inhibited by visceral nerve stimulation was often found to depress the beat of an eserinized test heart. Eserine was found to prolong the response to applied acetylcholine and to nerve stimulation.

CRUSTACEA

It was suggested that the heart of decapod crustacea was accelerated by cholinergic acceleratory fibres. Welsh, (1939), found that acetylcholine 10^{-9} accelerated the heart of Panulirus argus. This effect was potentiated by eserine. Atropine inhibited or blocked the heart beat. It also blocked the response to acetylcholine. Wiersma & Novitski, (1942),

stimulated separately the acceleratory and inhibitory nerves to the heart of the crayfish. The response to stimulating the acceleratory nerve or after the addition of acetylcholine were identical. Eserine did not affect the normal heart beat, but it did potentiate the acetylcholine effect. But Florey, (1961), has found that acetylcholine is not present in the cardioaccelerator fibres and the cardiac ganglion of the lobster Homarus americanus. Neither atropine nor eserine had any effect on the stimulation of the acceleratory nerve. Acetylcholine does not appear to be the natural excitatory regulator of the crustacean heart.

The effects of anticholinesterases on synaptic transmission in the crayfish have been examined by Schallek & Wiersma, (1949). High concentrations of both eserine and diisoflurophosphate were needed to block the transmission. They suggest that since high concentrations are required that the anticholinesterases themselves may produce a direct block. This is a giant nerve fibre system and chemical transmission is unlikely at their synapses, (Furshpan & Potter, 1959).

INSECTA

Acetylcholine at concentrations of 10^{-2} M, after 1-5

minutes caused a burst of asynchronous action potentials from the desheathed abdominal nerve cord of the cockroach, followed by block, (Twarog & Roeder, 1956).

During diapause the loss of endocrine activity is parallelled by the disappearance of spontaneous electrical activity and of cholinesterase from the brain of Cecropia, (van der Kloot, 1955). He suggested that low temperatures promoted the accumulation of a cholinergic substance in the brain. This compound might eventually trigger the synthesis of cholinesterase. The resumption of hormone release is accompanied by the return of cholinesterase and electrical activity to the brain.

ARACHNIDA

Acetylcholine has been applied to the ommatidia of the horseshoe crab, Limulus tridentatus, during physiological stimulation, (Kikuchi, Naito & Minagawa, 1960). It appeared to have two actions. Acetylcholine had a summative action with physiological stimulation which could be explained as the sensitization of the chemical produced or acceleration of its liberation during illumination. Acetylcholine also had a depolarizing action on the sensory cell membrane.

CONCLUSION

Before a chemical can be classified as a transmitter of impulses it has to satisfy certain requirements. These are outlined in the discussion on page 206. The evidence for a cholinergic mechanism in the autonomic nervous system and at the neuro-muscular junction is conclusive in the vertebrates. The evidence also supports the suggestion that acetylcholine acts upon the membrane of the Renshaw cells in the vertebrate spinal cord, though the chemical released by these cells onto the postsynaptic membrane of the motoneurone is unknown. It is not acetylcholine. A good correlation has been constructed in the vertebrate central nervous system between the occurrence of choline acetylase, acetylcholine, and cholinesterase. Certain neurones in the brain are also sensitive to physiological doses of acetylcholine. It is therefore likely that there are cholinergic pathways in the vertebrate brain.

The situation in the invertebrate is far from clear. It would appear that in the annelids and the molluscs there is some evidence for a cholinergic mechanism. This is possibly true for the neuromuscular junction of the leech, and between certain synapses in the abdominal ganglion of Aplysia. We have yet to discover the transmitter substance for the synapses in other phyla, but of all the chemicals so far tested, Acetylcholine seems the most probable.

CATECHOL AND RELATED AMINES

INTRODUCTION

The physiological effects of extracts of the suprarenal capsules was first investigated by Oliver & Schafer, (1896). They found that the extracts had a striking effect on muscular tissue generally and especially upon heart and arteries. They suggested that the suprarenal medulla produced a substance which maintained the right amount of tonic contraction which was essential for physiological activity of the tissue. Langley, (1901), extended this work and concluded that the suprarenal extract had a specific stimulating action on sympathetic nerve endings, with little or no effect on cranial and sacral autonomic nerve endings. The degree of the stimulatory action varied with the nerve endings in different tissues. Elliott, (1905), found that stimulation of sympathetic nerves to, for example, the bladder, could be mimicked by the addition of adrenaline.

The actual release of an adrenaline-like substance was first shown by Calabro, (1933). The nature of the active substance was further elucidated by the experiments of Greer, Pinkston, Baxter & Brannon, (1938). They showed the similarity

between the actions of the released substance and noradrenaline. The identity of the specific neurohormone of adrenergic nerves with laevo-noradrenaline was later shown by biological and chemical methods applied to extracts of such nerves, (Euler, 1946, 1948).

The possible role of the catechol amines in nervous transmissions in the central nervous system will now be reviewed.

LOCALIZATION OF CATECHOL AMINES IN THE VERTEBRATE BRAIN

The distribution of adrenaline and noradrenaline has been reviewed by Vogt, (1957). The highest quantities of noradrenaline, 0.4 - I μg/g fresh tissue in the dog, were found in the hypothalamus and gray matter around the aqueduct, and in the area postrema. There are regions which contain hardly any noradrenaline, for example the visual and acoustic cortex, and the cerebellar cortex. The percentage of adrenaline in the hypothalamic sympathin varies from 6-25% in the dog, and from 2.5-II% in the cat.

Carlsson, (1959), has investigated the occurrence and

distribution of catechol amines in the nervous system. Noradrenaline and dopamine, 3-hydroxytyramine, are present in approximately the same amounts, 0.25 and 0.30 µg/g respectively, in the brain of the sheep. Adrenaline occurs in smaller amounts, less than 10% of the noradrenaline level. In the amphibian brain adrenaline appears to be predominant, 1.4 µg/g compared with 0.26 µg/g noradrenaline. The distribution of noradrenaline and dopamine are different in the mammalian brain. The former Dopamine is found to predominate in the corpus striatum, while the highest concentrations of noradrenaline occur in the brainstem, especially the hypothalamus. This agrees with the findings of Vogt, (1957, 1954).

Bertler, (1961), found that the anterior and middle portions of the hypothalamus of man contained large amounts of noradrenaline. Lower levels of noradrenaline were found in the mesencephalon and the fourth ventricle. Dopamine was confined to structures involved in the extrapyramidal system. He also found high concentrations of 5-HT in the hypthalamus, medulla oblongata, corpus striatum and the thalamus.

FORMATION OF ADRENALINE

A knowledge of the amines involved in the synthesis of

of adrenaline is helpful, since any one of these itself may have a role as a chemical transmitter at certain sites in the nervous system. The possible pathway for the synthesis of adrenaline will be briefly reviewed.

The intermediate stages in the formation of adrenaline are as follows:- the oxidation of l-tyrosine to l-dopa; this in turn is decarboxylated to dopamine; dopamine is oxidized to noradrenaline; and this is methylated to adrenaline, (Blaschko, 1957, 1959).

The evidence will be reviewed briefly. The enzyme, l-dopa decarboxylase was first shown to be present in mammalian kidney by Holtz, Heise & Ludtke, (1938). This enzyme was shown to decarboxylate l-dopa to dopamine; it had no effect on N-methyl dopa, (Blaschko, 1942). This enzyme was shown to occur in adrenergic neurones by Holtz, Westermann, (1956). They have found a high concentration of this enzyme in the hypothalamus. The next step in the series was shown when DL-dopa-$2C^{14}$ was incubated with bovine adrenal homogenates. It was found that 50% of the radioactivity was present as dopamine, (Demis, Blaschko & Welch, 1956). There was also a little radioactive noradrenaline.

Hagen, (1956), isolated the radioactive dopamine and re-incubated it with homogenate from the suprarenal gland

of the fowl. In this way he obtained radioactive noradrenaline. The formation of adrenaline from noradrenaline was shown by Bulbring, (1949). It was found that the adrenaline content of the incubated suprarenal gland increased at the expense of the noradrenaline content when ATP was added.

Experiments have also been done on the intact animal. Gurin & Delluva, (1947), administered radioactive phenylalanine to the rat and found that radioactive adrenaline appeared in the suprarenal glands. Intraperitoneal administration of radioactive tyrosine or phenylalanine into rats or rabbits also yielded radioactive adrenaline, (Udenfriend, Cooper, Clark & Baer, 1953).

APPLICATION OF ADRENALINE AND NORADRENALINE TO THE BRAIN

The effects of catecholamines on the central nervous system has been reviewed by Rothballer, (1959). Mainly work dealing with the response of individual neurones to catecholamines will be discussed.

Bradley & Mollica, (1958), recorded from single units in both the mesencephalon and medulla of 46 cats. They

injected 5-25 µg intravenously or 1-5 µg via the common carotid of adrenaline HCl. They tested 36 units in the mesencephalon. In 13 cases there was no effect, in 11 cases there was an increase in the activity, and in the remaining 12 cases there was either a decrease in the activity or complete inhibition. Adrenaline HCl was tested on 31 units in the bulbar reticular formation. In 15 cases there was no effect on the activity, in 14 cases there was acceleration, and in only 2 cases was there inhibition. When adrenaline was injected intravenously, there was a rise in blood pressure, and a delay of 20-30 seconds before a result was observed. With the carotid injection, there was rise in the blood pressure and an effect was observed in 2-5 seconds. In 7 cases which showed an effect, 10-50 µg noradrenaline was then administered intravenously. In 3 cases there was the same effect as with adrenaline, and in the remaining cases no effect was observed.

It has been shown by Quadbeck, (1957), and Weil-Malherbe, Axelrod, & Tomchick, (1959), that intravenously injected adrenaline penetrates the blood-brain barrier of the hypothalamus much more readily than elsewhere in the brain.

Adrenergic blocking agents, including dibenamine, yohimbine, ergotamine, and dihydroergotamine; inhibitors of

amine oxidase, isonizid and iproniazid, inhibitors of catechol O-methyl transferase, sodium p-chloromercuribenzoate, have been applied ionophoretically to neurones of the spinal cord and no action has been observed, (Curtis, 1961).

EFFECT ON BRAIN CATECHOL LEVELS OF THE INJECTION OF RESERPINE OR OTHER CATECHOLS

The administration of reserpine causes the almost complete disappearance of both dopamine and noradrenaline from the brain of the rabbit, (Carlsson, Lindqvist, Manusson & Waldeck, 1958). The injection of dopa, 3,4-dihydroxy phenylalanine, to normal and reserpinized rabbits caused a marked rise in the level of dopamine in the brain, (Carlsson, 1959). The noradrenaline level is not much affected by the administration of dopa. Dopa is thus able to penetrate through the blood-brain barrier. Carlsson suggests a physiological role for dopamine. The corpus striatum forms part of the extrapyramidal system and lesions in this system induce the Parkinsonian syndrome. Administration of reserpine, which depletes the dopamine in the corpus striatum, may produce a syndrome similar to the Parkinsonian syndrome.

Carlsson suggests that dopamine is involved in the control of motor functions.

The effect of reserpine on the metabolism of dopamine and noradrenaline in the brain has also been studied by Bertler, (1961). He found that dopamine was depleted from the brain at a faster rate than noradrenaline. The dopamine content fell to 50% of the normal level in 15 minutes, while the corresponding time interval for noradrenaline was 45 minutes. The dopamine in peripheral tissues was only slightly reduced after 13 hours while the brain dopamine was reduced to insignificant amounts. 5-HT is affected in a similar manner. It is suggested that reserpine may act by blocking the active transport of the catecholamines into the granules. Reserpine does not affect the formation of catecholamines from dopa.

DISTRIBUTION OF CATECHOLS IN THE INVERTEBRATES

Ostlund, (1954), investigated the distribution of catecholamines in several invertebrate phyla, using bio-assay techniques. He found very little adrenaline-like compounds in the protozoa, coelenterates, ehinoderms, crustacea, molluscs, and tunicates. He found 0.13 μgm/gm

adrenaline and 0.48 μgm/gm noradrenaline in the ventral ganglionic chain of Lumbricus terrestris. They also occurred in Arenicola marina. In Noctiluca, sea anemone, and Mytilus edulis he found an unidentified amine which he called catechol 4. This compound was not adrenaline, noradrenaline, dopamine, dopa, tyrosine, or 5-HT. In insects he found dopamine widely distributed in the concentration range of 5-15 μg/g. The percentage of dopamine varied during the life cycle, being highest in the larva. The ratio of dopamine: noradrenaline: adrenaline was 100:10:1.

von Euler, (1961), has reinvestigated the occurrence of catecholamines using a fluorimetric technique. He found 0.015 μg/g noradrenaline and 0.003 μg/g adrenaline in Lumbricus terrestris, and 0.33 μg/g noradrenaline in the larvae of Pieris brassicae. The concentrations of adrenaline and noradrenaline in the aschelminthes, molluscs, other annelids, crustacea, echinoderms and tunicates were less than 0.005 μg/g. Amphioxus contained 0.16 μg/g noradrenaline but there was no evidence for adrenaline. von Euler used whole animal extracts except in the case of the echinoderm where only the visceral organs were used.

Erspamer & Boretti, (1951), have shown the presence of p-hydroxyphenylethanolamine, octopamine, in the posterior salivery glands of Octopus vulgaris. von Euler, (1953),

has also found noradrenaline in these glands. In addition to these, Hartman, Clark, Cyr, Jordan & Leibhold, (1960), have shown the presence of 5-HT, histamine, dopamine, and the amino acids tyrosine, and histidine, in Octopus apollyon and Octopus bimaculatus salivary glands.

Blaschko, (1959), suggests that octopamine formation occurs via the decarboxylation of tyrosine to tyramine and on to octopamine. This compound has also been found in the urine of rabbits after treatment with monoamine oxidase inhibitors, Kakimoto, & Armstrong, (1960).

Recently Erspamer & Glasser, (1960), found leptodactyline, the first naturally occuring m-hydroxyphenylalkylamine, in the skin of certain species of the genus Leptodactylus. This compound has a free OH group in the meta position of the benzene ring, this is important for the intensity of the pharmacological effects. The physiological significance of this compound in the skin is obscure.

Catecholamines have been little tested on invertebrate neurones. However Gerschenfeld & Tauc, (1961), tested the response of adrenaline and noradrenaline on neurones in the visceral ganglion of Aplysia depilans. They tested their response on two types of cells. They tested them on cells which they could accelerate, D-cells. Noradrenaline at

concentrations up to 10^{-6} was found to hyperpolarize and inhibit the D-neurones and activate the H-neurones. Adrenaline has the same effect but is about five times less active.

Twarog & Roeder, (1957), studied the effect of adrenaline and noradrenaline on the activity of the desheathed last abdominal nerve cord of Periplaneta americana. Adrenaline, at concentrations of 1×10^{-4} M, increased the activity and induced it in bursts. At 1×10^{-3} M, adrenaline blocked the stimulation of the cervical nerve. Between 1×10^{-3} M and 10^{-2} M, noradrenaline blocked the response.

Harlow, (1958), used two preparations to test the response of drugs on the nervous system of Locusta migratoria. The isolated leg was made to kick by stimulation of the crural nerve, and a reflex retraction of the tibia of the hind-leg was initiated by stimulating the tarsus by heat. Compounds were applied directly onto the ganglion. Adrenaline had no effect on either preparation.

McGeer, McGeer, & McLennan, (1961), tested several compounds on the crayfish stretch receptor neurone. They found that 3-hydroxytyramine was the most active compound tested, having approximately 100 times the activity of GABA. The activity of noradrenaline was comparable to GABA. All the compounds

which were appreciably inhibitory had an acidic and basic function and could be orientated in such a way that these groups were approximately 4 Å apart. The blocking powers of picrotoxin, dibenzylene and chlorpromazine were examined. Picrotoxin only weakly blocked 3-hydroxytyramine, and 60% of the GABA activity. The other two compounds were ineffective against GABA but blocked 3-hydroxytyramine almost completely. All three blocked the activity of Factor I.

CONCLUSION

The role of the catechols in transmission in the sympathetic nerve-muscle junction of vertebrates is established. The function of the catechols in the brain is unknown. Adrenaline and noradrenaline are found in the same areas of the brain. The relative proportions appear to vary from species to species. In certain regions of the brain catechols are absent. Brain adrenaline may be associated with the function of acetylcholine since it is found to potentiate the action of the latter. Catechols are found in the brain-stem nuclei of the reticular formation. Adrenaline stimulates neurones in the bulbar reticular formation. Certain of the enzymes envolved in the synthesis of the catechols occur in

the brain. It would appear that there is as yet, no clear example of an adrenergic system in the spinal cord.

Although the annelids and possibly the molluscs contain catechol amines their function is not known. Both adrenaline and noradrenaline have been found to be active on the neurones of Aplysia, and dopamine was found to be very active on the crustacean stretch receptor neurone. It is possible that the catecholamines may play an important role in other invertebrate synapses.

HISTAMINE

INTRODUCTION

Histamine was first synthesized in 1907 by Windaus and Vogt, who prepared it from imidazolepropionic acid. The natural occurrence of histamine was first demonstrated by Barger & Dale, (1910), who isolated it from ergot.

Histamine can be isolated from extracts of probably all animal and plant tissues, (Sollmann, 1957). It is located chiefly in the mitochondria, (Copenhaver Nagler & Goth, 1953). The mast cells of the blood and the skin have a relatively high content of histamine which is liberated by their destruction. Histamine is also a constant constituent of nervous tissue and for this reason it is considered here as a possible chemical transmitter. Histamine is formed by the decarboxylation of histidine and is destroyed by histaminase.

LOCALIZATION OF HISTAMINE IN VERTEBRATE NERVOUS TISSUE

Histamine was first determined quantatively in nervous tissue by Kwiatkowski (1943), in a number of mammals. In the cat and dog he found less than 0.I μg/g in the spinal cord and medulla oblongata, 0.16 μg/g or less in the cortex, 0.28 μg/g in the mid brain, and 1.5-2.0 μg/g in the cerebellum. The vagus was rich in histamine, up to 4.5 μg/g, and the lumbar sympathetic contained 1.0-1.4 μg/g. During degeneration, the histamine content of the sciatic nerve was found to rise.

Harris, Jacobsohn and Kahlson, (1952), found values up to 43.0 μg/g and 76.0 μg/g respectively in the anterior and posterior lobe of the pituitary. They found 0.7-8.3 μg/g in the hypothalamus, 3.6-25.0 μg/g in the median eminence, and 4.0-15.8 μg/g in the sympathetic ganglion, but none in the cerebellum. Lastly Adam, (1961), determined quantatively the histamine content of the central nervous system and hypophysis of the dog. He found a mean value of 14.3 μg/g in the hypophysial stalk, 11.4 μg/g in the posterior lobe, and 7.7 μg/g in the anterior lobe of the pituitary. These regions are rich in mast cells, the greatest number being in the hypophysial stalk. The following values were found in

the hypothalamus, 0.9 μg/g in the ventral, 0.46 μg/g in the dorsal, 0.74 μg/g in the corpora mamillaria, and 0.34 μg/g in the supraoptic region. It is considered that these regions do not contain mast cells. The area postrema of the medulla oblongata contained 0.92 μg/g.

White, (1959), investigated the formation and catabolism of histamine in brain tissue of the cat, pig and dog. Minced brain tissue was incubated with ^{14}C-histidine and the ^{14}C-histamine formed was determined by an isotope dilution technique. The formation of histamine was found to be greatest in the hypothalamus, as much as 1.1 μg/g being formed in 3 hours from 40 μg ^{14}C-histidine in the dog. The cerebellum yielded 0.002 μg/g. The reaction was inhibited by semicarbazide and hydrazine. When the cat brain was incubated with ^{14}C-histamine, the principal metabolite formed was ^{14}C-methylhistamine. White found that, while the brain region richest in histamine was also highly active in forming it, the cortex metabolized histamine at a faster rate, indicating more histaminase than in the hypothalamus.

The findings of White, (1959), and Adam, (1961) agree for the hypothalamus but not in the case of the area postrema. Adam found much more histamine present than White suggested from his experiments. Adam suggests that the area postrema

makes only a small amount of its histamine, the remainder being acquired from the blood flowing through it or from the cerebral cerebrospinal fluid that bathes it. However, he found that the latter contained less than 0.001 μg/ml histamine.

The distribution of histamine closely parallels that of 5-HT and noradrenaline already mentioned. The highest concentrations of the three are found in the hypothalamus and the area postrema, intermediate concentrations in the mid-brain, and the least in the cerebral cortex and white matter, Adam, (1961).

However, Kahlson (1960) points out that caution must be exercised in postulating physiological functions only on the distribution of a compound and its enzymes in a system. He found that starvation, the removal of the pituitary gland or adrenals, the complete inhibition of the histamine destroying enzyme histaminase, or the inhibition of histidine decarboxylase (which converts histidine to histamine) by semicarbazide, had little effect on histamine tissue values.

EFFECT OF RESERPINE AND HARMALINE ON HISTAMINE CONTENT OF TISSUES

Reserpine does not appear to have any effect on histamine content of the brain, (Waalkes, Coburn & Terry, 1959). This

is not the case with the histamine in the optic ganglia of the squid, Eledone moschata, (Bertaccini, 1961). The histamine content of the optic ganglia varies from 1.70-4.00 µg/g, depending on the time of year. He also investigated the 5-HT and catecholamine content, the former ranged from 1.95-4.30 µg/g, and the latter from 0.90-1.20 µg/g. 24 hours after the administration of reserpine, the histamine level had fallen from 4.00 to 0.45 µg/g. The 5-HT and catecholamine levels were also depleted. Half an hour after the addition of harmaline, a monoamine oxidase inhibitor, the level of histamine had risen from 4.00-10.00 µg/g, and the level of 5-HT had risen from 4.20-11.80 µg/g.

From this rate of increase Bertaccini calculated that the turnover of histamine in ganglionic tissue must be very high, the half life being approximately 15 minutes. The half life of 5-HT in this tissue was about 10 minutes. Bertaccini concludes that histamine, 5-HT, and the catecholamines may be associated with chemical transmission in the central nervous system of the Octopoda.

APPLICATION OF HISTAMINE

The evidence for a physiological role for histamine in the central nervous system will now be considered.

Fuche & Kahlson, (1957), showed that in rabbits, on distruction of the adrenal medulla, the intravenous infusion

of 50 μg/Kg histamine caused a significant lymphopenic response which was parallel to the response after injection of corticotropin. The histamine response did not occur after hypophysectomy. They suggested that histamine was a possible chemical mediator of the cerebral control of the anterior pituitary gland.

In his investigation into the nature of the Cerebellar Factor, Crossland (1960), tested the effect of histamine on the cerebellar activity. He found that 70 seconds after the injection of 0.05 μg of histamine stimulated the activity of the cerebellum. There may be a correlation between histamine response and certain regions of the cerebellum. Antihistamine compounds or removal of the nodulus and uvala of the cerebellum remove the motion sickness response. This latter point was shown by Wang & Chinn, (1956), in the dog. Crossland, (1960) has also found an enzyme which destroys histamine in the cerebellum. He suggests this enzyme might protect the cerebellum from exogenously formed histamine which is carried to the cerebellum in the blood.

Trendelenburg, (1954), has shown that histamine stimulated the hypothalamus, and also to potentiate the response of the superior cervical ganglion to preganglionic stimulation, (1955).

Feldberg and Sherwood, (1954), developed a technique in which a cannula was permanently implanted into the lateral ventricle of the cat brain. Drugs were injected through this cannula into the unanaesthetised cat. 150-200 µg of histamine injected through this cannula induced licking with or without swallowing, profuse salivation, retching, defaecation and tachypnoea with panting. The effect of the injection of 5-HT, adrenaline and acetylcholine was also tested. 75-500 µg of 5-HT caused muscular weakness, tachypnoea and bursts of profuse salivation. Adrenaline and noradrenaline at a concentration of 20-80 µg, produced a condition resembling light sodium pentobarbitone anaesthesia. This occurred after 10 to 20 minutes. During the first few minutes after the injection, licking movements and swallowing occurred, followed by retching, vomiting and on one occasion, defaecation. Acetylcholine at 10-20 µg after a few seconds elicited a peculiar high-pitched cry. During the next few hours the animal appeared subdued, detached and stuporous.

Feldberg & Greengard, (1956), found that on arterial injection of 10-20 µg of 48/80, histamine appeared in the venous effluent. About 30% of the histamine content of the sciatic nerve was released. There was no increase in the amount of histamine released when the amount of 48/80

injected was increased.

LOCALIZATION OF HISTAMINE IN INVERTEBRATES

Histamine has already been mentioned to occur in the brain of the Octopoda. The distribution of histamine in the invertebrates has been investigated by Ungar, Ungar & Parrot, (1937). They found histamine-like compounds in coelenterates, sponges, echinoderms, annelida, crustacea, and molluscs. The nervous tissue of Octopus vulgaris was found to contain 200 µg.

Histamine has been found in certain moths, (Bisset, Frazer, Rothschild & Schachter, 1960). They found 750 µg/g freeze-dried abdominal tissue in the white ermine, Spilosoms lubricipeda and in the cinnabar, Hypocrita jacobaeae. They found lesser amounts in several other moths.

Histamine has been found in the venom of the hornet, Vespa crabro, (Bhoola, Calle & Schachter, 1960, 1961). They found 14-30mg/g of dry venom sac. They also found acetylcholine and 5-HT, in concentrations of the same order.

Recently histamine has been shown to occur in very high concentrations in the heart of the crab, Carcinus maenas, (Kerkut & Price, 1961). The heart was found to contain

1212 μg/g histamine. The pericardial organs and the ventral pericardium also contained large amounts. The stellate ganglion had a concentration of 21 μg/g.

Histamine may be an important constituent of the coelenterate nematocyst. The histamine content of the tentacles of the anemone Actinia equina ranged from 50-150 μg/g freeze-dried tissue (Mathias, Ross & Schachter, 1960). Other species contained little or no histamine. It may be noted that besides histamine two other compounds were found in greatest concentration in the tentacles. These were tetramethylammonium and a toxic protein-like substance. Homarine in high concentrations also occurs widely.

The effect of histamine has been little studied on the invertebrate nervous system. The effect of certain drugs was tested on the synaptic transmission in the ventral nerve cord of Cambarus clarkii, (Schallek & Wiersma, 1948). They found that histamine at a concentration of 1×10^{-5} g/ml had no effect. On the same preparation, acetylcholine and adrenaline at 5×10^{-3} g/ml had no effect either. Only from the investigations of Bertaccini, (1961), has histamine been suggested as a possible chemical transmitter in the transmission of nerve impulees.

The action of histamine on the molluscan heart seem to vary depending on the species (Krijgsman & Divaris, 1955).

CONCLUSION

The hypothalamus is the most likely site for the role of histamine as a central transmitter. The presence of histamine in the octopus optic ganglion suggests a possible transmitter role for histamine in parts of the molluscan nervous system, but much more is required than the mere presence of a compound before a transmitter role can be suggested. Histamine in invertebrate tissue appears to be associated with attack or defence. Its presence in certain stages of the moth life cycle and absence from others is suggestive of a metabolic role.

5-HYDROXYTRYPTAMINE AND 5-HYDROXYTRYPTOPHAN

The investigation into the distribution and role of 5-HT has been very intense and extensive in the last few years. The first major step was taken by Erspamer and Asero, (1952), when they identified enteramine, a substance that is present in the enterochromaffin cells and which affects gut motility, as 5-HT. Enterochromaffin cells have been found in the gastroint-stinal mucosa of all the vertebrates so far examined and also in Amphioxus and the ascidians.

5-HT is present in the blood platelets and the organs associated with them, for example, the spleen and lung tissue. It is also present in the mast cells. It has been found to occur in the central nervous system and for this reason will be revieved here as a possible transmitter substance between neurones. TWo other indolealkylamines are known to occur in nervous tissue. Trytamine has been shown to be present in the brain, (esa, Redfield & Udenfriend, 1959); whilst melatonin(5-methoxy-N-acetyltryptamine), is present in the pineal gland and in mammalian peripheral nerve, (Lerner, Case & Takahashii, 1960).

LOCALIZATION OF 5-HT IN THE VERTEBRATE BRAIN

It was first reported in the vertebrate brain by Twarog & Page, (1953), and Amin, Crawford & Gad um, (1954). Using

one-way paper chromatography and the Venus heart and isolated byssus retractor muscle of Mytilis for bioassay, Twarog and Page demonstrated the presence of 5-HT in acetone extracts of dog, rat and rabbit brain, in conc. of 0.1-0.36 μg/g tissue.

Amin, Crawford & Gaddum, (1954) found that the distribution of 5-HT closely resembled that of noradrenaline. They found 5-HT only in the gray matter. They found 0.28 μg/g in the hypothalamus, 0.215 μg/g in the area postrema, 0.205 μg/g in the mid-brain and 0.17 μg/g in the nuclei gracilis and cuneatus of the dog. For their bioassay they used the oestrus rat uterus. They concluded that the 5-HT in the thalamic areas was concerned with autonomic activities and that the regions associated with transmission of sensory impulses to the cerebrum contained none. The main site of 5-HT production and storage in mammals appears to be in the intestine. If this organ is removed in the rat, then 5-HT metabolism falls, (Bertaccini, 1960).

5-HTP DECARBOXYLASE AND AMINE OXIDASE

There seems to be some correlation between the distribution of 5-HT in the brain and the enzyme 5-HTP decarboxylase, (Gaddum & Giarman, 1956). This enzyme decarboxylases 5-HTP, the precursor of 5-HT, to form 5-HT. Monoamine oxidase,

which breaks down 5-HT, is widely distributed in the brain but is found in greatest conc. in the hypothalamus, (Bogdanski & Udenfriend, 1956). The overall scheme of formation and metabolism of 5-HT has been formulated by Udenfriend, Titus, Weissbach & Peterson, 1956). Trytophan is hydroxylated by a hydroxylase enzyme to 5-hydroxytrytophan. This is decarboxylated to 5-HT by 5-HTP decarboxylase. Under the influence of amine oxidase, 5-HT is metabolized to 5-hydroxyindole acetic acid. From their experiments on the biosynthesis of 5-HT, Price & West, (1960), conclude that the brain must be considered to be one of the chief sources of the enzyme 5-HTP decarboxylase.

It is not yet clear if the brain can hydroxylate tryptophan to 5-hydroxytryptophan.

APPLICATION OF 5-HT TO THE VERTEBRATE BRAIN

The effect of 5-HT ejected close to the neurones in the lateral geniculate nucleus of the cat was studied by Curtis & Davis, (1961). In the presence of 5-HT, the orthodromic response of neurones which responded monosynaptically and occasionally repetitively to an optic nerve volley, was suppressed. When the neurones were excited by l-glutamate, 5-HT had no effect. The antidromic spike response was

unaltered by 5-HT. Curtis & Davis conclude that since 5-HT does not affect antidromic nor l-glutamic stimulation, 5-HT does not appear to significantly modify the membrane conductance of the neurones. They suggest it may act by combining with excitatory synapses.

It appears that 5-HT is not capable of penetrating the blood-brain barrier. This can be overcome by the injection of 5-HTP which can penetrate this barrier. This leads to an increase in the brain 5-HT content, (Udenfriend, Weissbach, & Bogdanski, 1957).

There is however some discrepancy in the interpretation of the response to increased conc. of brain 5-HT. In some cases there is a marked central stimulation, (Bogdanski, Weissbach & Udenfriend, 1958). Others have reported a central depression, (Lewis, 1958), (Glasser & Mantegazzini, 1960).

The effect of electrostimulation on the brain 5-HT content has been investigated by Breitner, Picchioni, Chin & Burton, (1961). After generalized convulsive electrical stimulation, the rabbit, rat and cat 5-HT content increased. Localized brain stem stimulation had no effect on the 5-HT content. They conclude that the benefits of localized stimulation in mental disease would appear not to be due to a change in the 5-HT conc.

EFFECT OF RESERPINE, LSD AND IPRONIAZID

To assist in determining the function of 5-HT certain chemicals have been employed. Reserpine has been found to release 5-HT. Lysergic acid diethylamide, LSD, antagonizes the action of 5-HT. Iproniazid is used as a monoamine inhibitor.

The level of 5-HT in the brain is greatly reduced after the administration of reserpine, (Passonen & Vogt, 1956). The tranquilization caused by reserpine injections takes place after brain 5-HT conc. are dramatically decreased, (Shore, Pletscher, Tomich, Carlsson, Kuntzman & Brodie, 1957). They suggest that reserpine acts by rapidly entering the brain and, by an unknown means, irreversibly affects the 5-HT binding sites and then disappears. 5-HT is then released, and this unstable free form is metabolized by the action of monoamine oxidase. 5-HT continues to be synthesized in the body, and the low value in the brain represents the balance between rate of synthesis and metabolic transformation. The effect of reserpine persists until the binding sites regain their binding capacity or until new sites are formed.

It has however been shown by Kirpekar, Goodlad & Lewis, (1958), that reserpine also releases catecholamines, histamine and ATP.

When iproniazid is administered, it causes a 2-3 fold increase in endogenous brain 5-HT levels, (Weissbach, Bogdanski, Redfield & Udenfriend, 1957). When cats are treated with the monoamine oxidase inhibitory, Phenylisopropyl-hydrazine, the effects of 5-HT injected into the lateral cerebral ventricle are greatly intensified and prolonged. The affects of adrenaline and tryptamine are not intensified and are prolonged only to a slight degree, (Schain, 1961).

With the aid of amine oxidase inhibitors it has been shown that the half life of cerebral 5-HT is not longer than 10-30 minutes, (Udenfriend, 1958).

Amineoxidase inhibitors block the oxidative deamination of a number of biogenic amines which are substrates for monoamine oxidase. These drugs may act on other enzyme systems, (Erspamer, 1961).

The depressant action of 5-HT on the brain of the cat is antagonized by LSD. From the experiments of Rothlin, (1957), it is shown that while brom-LSD is as active as LSD in antagonizing 5-HT, it produces none of the mental disturbances. This suggests that the observed mental disturbances may not be due to the antagonism of 5-HT.

5-HT AND THE AUTONOMIC SYSTEM

In addition to its central effect, 5-HT has also been

shown to occur in the perfusate of the superior cervical ganglion of the cat, (Gertner, Paasonen & Giarman, 1957). They added iproniazid to the perfusate and after 2-3 hours they found 10-20 μg/min. 5-HT. When both iproniazid and 5-HT were present in the perfusate, 5-HT appeared after 15 minutes.

DISTRIBUTION OF 5-HT IN THE INVERTEBRATES

5-HT was first associated with the molluscan heart when Erspamer & Ghiretti, (1951), found that it stimulated the heart of the species studied. They doubted if it was the cardio-regulator since it was absent from the posterior salivary gland of certain species whose hearts were very sensitive to 5-HT. Welsh, (1953), extended this study to the heart of Venus mercenaria. He first showed it to be present in the nervous system of V. mercenaria, (1957), at a conc. of 15 μg/g wet ganglion tissue.

These studies were greatly extended with the advent of the spectrofluorometer. It was found by Udenfriend, Weissbach & Clark, (1955), that 5-HT could be measured spectrofluormetrically. As has already been mentioned, Welsh & Moorhead, (1960), determined by this method the quantitative distribution of 5-HT in several invertebrate phyla, with special reference to their nervous system content.

They found that the nervous tissue of the bivalve molluscs contained more 5-HT than was found in any other nervous tissue examined. They found 40 μg/g of Venus ganglia. The cephalopod nervous system had the least 5-HT of the molluscan nervous tissues examined. Crustacean nervous system contained very little 5-HT. The pericardial organs however contained 2.5 - 4 μg/g. 5-HT was found in the heads of insects, 0.05 - 0.16 μg/g.

The identification and distribution of 5-HT in the sea anemone, Calliactis parasitica, has been studied by Mathias, Rose & Schachter,(1957, 1960). They found 500-600 μg/g freeze-dried tissue in the coelenteric tissue, and 6-7 μg/g in the tentacles. Other species contained little or no 5-HT.

A similar situation occurs in the Octopoda, where the salivary gland of Octopus vulgaris contains approximately 500 μg/g, whereas that of Octopus macropus contains none, (Erspamer, 1954). This type of distribution is perplexing if a function of fundamental importance for the substance is to be considered.

5-HT has also been identified in the venom of the wasp, (Jaques & Schachter, 1954).

Meng, (1960), investigated the mechanism of the control of the heart beat of Helix pomatia. By paper chromatographic techniques and testing the elutions on the heart he found

evidence for the presence of 5-HT and acetylcholine. However Jullien, Cardot & Ripplinger, (1961), Cardot & Ripplinger, (1961), using chemical chromatographic techniques failed to find any 5-HT. Among the compounds they identified were phenylalanine, histidine, tyrosine, alanine and glutamine.

Recently 5-HT, at a concentration of about 10^{-7} has been found in the snail brain (Kerkut & Cottrell, 1962).

THE PRESENCE OF ENZYMES ASSOCIATED WITH 5-HT

It has been shown that the formation of 5-HT from 5-HTP by an homogenate of Venus ganglia takes place at a high rate, (Welsh & Moorhead, 1959). The incubation of homogenates of the fluke, Fasciola, with 5-HTP and pyridoxal phosphate for 1 hour resulted in a 100-300 fold increase in the amount of 5-HT in the reaction mixture, (Mansour, Lago & Hawkins, 1957). 5-HT, but neither acetylcholine nor catechols, stimulated the endogenous respiration of excised gill of Modiolus demissus and Mytilus edulis, (Moore, Milton & Gosselin, 1961). The respiration was found to be stimulated by the addition of 5-HTP. 2-bromoLSD inhibited the effect of 5-HT, while LSD mimicked it.

If the branchial nerve is cut in situ just distal to its origin from the visceral ganglion then the ciliary

activity ceases, (Aiello, 1957). He found that gill tissue extract and 5-HT activate the quiescent cilia.

Blaschko & Milton, (1960), showed that homogenates of gill plates of Mytilus edulis used oxygen when 5-HT was added. The oxidation of 5-HT was not due to the presence of amine oxidase but to that of an enzyme which catalysed the oxidation of other 5-hydroxyindoles, e.g. 5-HTP and bufotenine. The oxidation was cyanide-sensitive but was not inhibited by iproniazid.

FUNCTION OF 5-HT

MOLLUSCA

The effect of 5-HT was tested on two types of cell from the abdominal ganglion of Aplysia depilans, (Gerschenfeld & Tauc, 1961). They found that 5-HT excited both the cells with excitatory inputs and those with inhibitory inputs. But the response was ten times greater on the cells with excitatory inputs. However interneurones may mask the true effect on the recorded cell. It was found that on occasion 5-HT excited an inhibitory interneurones which inhibited the 5-HT response from a cell with inhibitory inputs. This could be avoided by local injection of the drug.

PLATYHELMINTHE

The rhythmical activity of Fasciola was stimulated by 5-HT and by LSD, (Mansour, 1957). This effect was peripheral and not mediated through the central ganglion. This action was antagonized by bromolysergic acid and diethylamide.

INSECTA

Concentrations of 10^{-2}M 5-HT and a number of tryptamine analogues blocked the development of an action potential and of tension in the fibres of the metathoracx extensor tibialis and flexor tibialis muscle of the locust, Schistocerca gregaria, (Hill & Usherwood, 1961). High concentrations of these compounds failed to block the action potential in fibres of the crural nerve which supplied 'fast' motor axons to the extensor and flexor tibialis. They concluded that these compounds blocked neuromuscular transmission by preventing the release of transmitter compounds from nerve terminals or blocking the receptor sites at the junctional regions of the muscle cells.

CONCLUSIONS

West, (1958), estimated the 5-HT content and the 5-HTP decarboxylase activity of several tissues of seven species

of mammals. From his results he suggested the following functions for 5-HT in the body:- (1) the control of the central nervous system activity; (2) to stimulate peristalsis in the gut; (3) to control capillary permeability in the tissues; (4) it was important as a haemostatic agent. Only the first conclusion concerns us. There are three pieces of evidence which favour a possible role of 5-HT as a chemical transmitter. 5-HT is found in the brain, but in varying concentrations from region to region. The rate of turnover of 5-HT is fast. Lastly 5-HTP, which can penetrate the blood-brain barrier, is decarboxylated by brain tissue to form 5-HT. This in turn can be oxidized by the brain. Lastly it may be mentioned that Brodie & Shore, (1957), propose that 5-HT and noradrenaline are chemical mediators of mutually antagonistic centres in the brain.

As in the vertebrates, the role of 5-HT in the invertebrates appears to be diverse. There seems to be a link between 5-HT and defence or attack, since it is found in both coelenterate tentacles, possibly nematocysts, and in the venom of wasps. Welsh considers that either 5-HT or a chemical analogue is produced by the cardioregulator nerves but it has not yet shown that 5-HT is released by them. The role of 5-HT in either neuromuscular transmission or nerve-nerve transmission is even less clear. The presence

of 5-HT in molluscan nervous tissue and the presence of a decarboxylating enzyme might be indicative of a transmitter function. 5-HT would seem a possible candidate for the role of nerve-nerve and nerve-muscle transmission in the gastropods.

GABA AND RELATED COMPOUNDS

Gamma amino butyric acid is of great interest partly due to the reactions that occur when it is added to the nervous system, and partly due to the light it throws on the pharmacology of the nervous system. Most of the work has been carried out on the vertebrate nervous system, and this vertebrate work will be briefly discussed first of all to provide a general picture before the invertebrate examples are considered.

VERTEBRATE STUDIES

Three groups of workers first reported the occurrence of GABA in the mammalian brain in 1950. Prior to this, Gale in 1940 had shown that certain bacteria could convert glutamic acid to GABA by a bacteria decarboxylase, and Steward, Thompson & Dent, (1949), had found GABA to occur in potato extracts.

Awapara, Landua, Fuerst & Seale (1950), analysed chromatographically the quantitative distribution of GABA in the human brain. They found that the highest conc., 100 μg/g fresh tissue, occurred in the caudate nucleus; the

lowest conc., 30 μg/g fresh tissue, was found in the white matter. Levels in other homoiotherms ranged from 110 μg/g fresh tissue, in the pigeon, to 40 μg/g fresh tissue in the rabbit. Thus there is a regional distribution of the compound in the brain.

Using chromatographic techniques, Roberts & Frankel (1950), showed that GABA and the brain extract had the same Rf. They also incubated C^{14} labeled glutamic acid with fresh mouse brain acetone powder, and demonstrated the presence of radioactive GABA. They suggested that GABA was formed via the α-decarboxylation of glutamic acid.

Lastly Udenfriend (1950), using the isotope derivative method, identified GABA in mouse brain extracts.

In the study of tissues and body fluids in various vertebrates including fish, amphibian, reptilian, avian and mammalian species, GABA has been detected unequivocally only in the brain and spinal cord, (Roberts & Eidelberg, 1960).

GABA, FACTOR I AND THE CRUSTACEAN STRETCH RECEPTOR

It was in 1954 that Florey observed that a vertebrate brain extract would inhibit the crustacean stretch-receptor response. He called this extract Factor I.

Florey & McLennan (1955), placed an irrigation chamber in contact with cat brains and collected the exudate. This was assayed for activity on the crayfish stretch receptor. The inhibitory action reached a maximum in 20 minutes. Transmission could be blocked with Factor I in the inferior mesenteric ganglion and the stellate ganglion using a conc. of I:10^5. No effect on transmission could be obtained in the superior cervical ganglion. Neuromuscular transmission was also unaffected. Factor I was next applied topically in conc. of 0.05 BE to the exposed spinal cord, (Florey & McLennan 1955b). The monosynaptic stretch reflex evoked by mechanical stimulation of the patellar tendon was inhibited in 5 sec. The recovery time depended on the conc. applied, and this might suggest enzymic breakdown. When Factor I was applied during the elicitation of a polysynaptic flexor reflex, there was no inhibition of the reflex. In some cases the response was enhanced. However with additional applications this enhancement was not observed. Factor I also caused an increase in the hypoglossal nucleus activity when topically applied.

The next advance came when Bazemore, Elliott & Florey (1956), identified GABA in the purified extract of Factor I.

An investigation was carried out by McLennan (1957) to see if all the physiological actions of Factor I could be duplicated by GABA. He also compared the actions of

α-amino-β-hydroxybutyric acid and carnitine. The squid rectum and the cat knee-jerk reflex were affected differently by Factor I and GABA. α-amino-β-hydroxybutyric acid reacted similarly to GABA. He concluded that Factor I contained inhibitory compounds in addition to GABA. McLennan also found that after the application of Factor I or GABA, stimulation of the motor cortex failed to cause movement of the contralateral hind leg. The preparation recovered after 10 minutes.

The Factor I story was rather confused by the finding of McLennan that Factor I did not contain GABA (1958). He suggested that GABA had been broken down from a larger molecule during the preparation of the Factor I extract of Bazemore, Elliott & Florey, (1956).

Further evidence against Factor I containing GABA was presented by Honour & McLennan, (1960), when they showed that monosynaptic stretch reflexes and transmission through the inferior mesenteric ganglion were blocked by Factor I but not by GABA, β-guanidinopropionic acid, and γ-guanidinobutyric acid. γ-aminobutyrylcholine, a substance shown by Asano, Noro & Kuriaki, (1960), to be present in mammalian brain, was inactive on all the test preparations. None of these compounds can explain the actions of Factor I.

APPLICATION OF GABA TO THE BRAIN

The rest of this account will be devoted to the effect of GABA and related compounds on the brain and spinal cord.

Purpura, Girado & Grundfest (1957), tested the effect of GABA painted on the cerebral cortex and cerebellar cortex. They found that the surface negative postsynaptic potentials of the apical dendrites produced by various stimulations were reversed by GABA. The resulting positive potential was almost a mirror image. GABA eliminates the surface negative dendritic postsynaptic potential but does not induce the positive potential when applied to the cerebellar cortex. The difference it is suggested is due to the fewer inhibitory inhibitory synapses in the cerebellar cortex. They suggest that GABA hyperpolarizes the synaptic membrane of the apical dendrites, and selectively blocks the depolarization associated with excitatory synapses. β-alanine they found gave the same effect as GABA but more weakly. They conclude that the action of GABA is specific.

The action of GABA on the cortical electrical activity of the cat has been studied by Iwama & Jasper, (1957). They topically applied 0.02- I.0% of GABA in unanaesthetized cats with partial destruction of the brain stem. After the application of GABA the amplitude of the spontaneous

electrical activity was often doubled. Sensory evoked potentials recorded 0.5-I.Omm beneath the surface of the cortex were unaffected by surface or deep GABA injection. They concluded that GABA had a selective depressant action upon the structures in the most superficial layers of the cortex.

Another approach to the problem of deciding the role of GABA is to add compounds to the brain which lead to a rise or fall in the conc. of GABA, and also to observe the effect on the intact animal.

The addition of certain hydrazides which depress the glutamic acid decarboxylase activity cause convulsions. The level of GABA in brains treated in this way is lowered, (Killam, 1957). Similarly the addition of hydroxylamine which inhibits the GABA-ketoglutaric acid transaminase, causes an increase in the GABA conc. in the brain. This rise in conc. is accompanied by an increase in the seizure threshold, (Baxter & Roberts, 1960).

It should be noted that the level of glutamic acid changes inversely with that of GABA and the observed responses could be due to this.

APPLICATION OF GABA TO SPINAL CORD NEURONES

The next step was to test these amino acids (glutamic

on cells acid, GABA, etc.), the resting potential of which could be recorded. This was done by Curtis & Phillis, (1958); Curtis, Phillis & Watkins, (1959), and Curtis, Phillis & Watkins (1960). They recorded from neurones in the spinal cord of the cat which they could drive anti- or orthodromically. The compound to be tested could be applied ionophoretically through one of the barrels of a multibarrel electrode. The centre barrel which was longer than the rest recorded the potential. The other barrels each contained a substance which could be ejected on to the experimental cell.

Their first experiments were conducted using GABA and β-alanine, (Curtis, Phillis, Watkins, 1959). It was found that neither compound affected the resting potential of the motoneurone, but the membrane was less excitable when stimulated either anti- or orthodromically; i.e. a voltage which would normally result in the formation of an action potential failed to do so. Both e.p.s.p.'s and i.p.s.p.'s were blocked. The effects of both GABA and β-alanine were not blocked by strychnine. This compound acts by selectively blocking the inhibitory synapses. Thus GABA and β-alanine cannot wholely act by blocking the inhibitory synapses.

GABA and β-alanine blocked the responses from not only motoneurones but also from dorsal horn cells, small motoneurones, and Renshaw cells. Both compounds also blocked the excitatory

response of dorsal horn cells to glutamic acid and of Renshaw cells to acetylcholine.

Thus these compounds act on the postsynaptic neurone membrane, and do not inhibit the release of these chemicals transmitters. Both the chemically and electrically excitable areas of the membrane are blocked.

In motoneurones the inhibitory and excitatory transmitters alter the permeability of the membrane to produce i.p.s.p.'s and e.p.s.p's, (Coombs, Eccles & Fatt, 1955a & b).

If the cell was surrounded by inhibitory transmitter, one would expect a hyperpolarization of the resting potential to the i.p.s.p. level. The excitatory transmitter would still produce e.p.s.p.'s but these would be smaller.

A specific inhibitory compound applied to motoneurones would be expected to hyperpolarize the membrane to the equilibrium potential abolish the orthodromically produced i.p.s.p's and reduce the size of the e.p.s.p's. But with GABA and β-alanine there was no effect on the resting potential and both the e.p.s.p's and i.p.s.p's were depressed or abolished. The fact that the intravenous injection of strychnine did not prevent their action also was evidence against them as natural inhibitory transmitters.

Curtis, Phillis & Watkins, (1959), therefore suggested that

both in the spinal cord and the brain that GABA and β-alanine were non-specific depressants.

GABA and related amino acids have also been tested on the neurones in the brain stem of the cat, (Curtis & Kiozumi, 1961). These neurones responded in a similar fashion to those in the spinal cord.

APPLICATION OF GLUTAMIC ACID TO SPINAL CORD NEURONES

Glutamic acid has also been shown to occur in the vertebrate brain, (Williams, Schurr & Elvehjem, 1950). This amino acid occurs in the rat brain in greater conc. than any other amino acid, (McIlwain, 1955). Glutamic acid in the presence of glutamic acid decarboxylase, forms GABA and is the precursor of GABA in nervous tissue, (Roberts & Frankel, 1950). This enzyme is found only in the nervous tissue, (Albers & Brady, 1959). Curtis, Phillis, & Watkins, (1960), investigated the action of glutamic acid and related acidic amino acids on the motoneurones of the cat spinal cord. The technique of ionophoretic injection was the same as in the case of GABA. They found that the injection of glutamate, asparate, and cysteate extracellularly, close to the experimental motoneurone, caused excitation. These ions excited motoneurones, interneurones, and Renshaw cells by

depolarizing their membrane. If this depolarization was sufficient it initiated spike potentials.

How do these compounds act? Do they act on the outside or inside of the neurone membrane? Curtis, Phillis & Watkins, (1960), concluded that it was not likely to be due to their entry into the cell. This conclusion was based on three pieces of information. The neuronal membrane is considered to be impermeable to glutamate ions, (Shanes, 1958). When glutamate ions are injected intracellularly into the neurones there is no effect on the resting potential, (Coombs, Eccles & Fatt, 1955). Activation by these compounds is not due to their power of chelating calcium since more powerful chelating agents fail to excite the neurone, (Curtis, Perrin & Watkins, 1960).

The normal excitatory transmitter would be expected to act at specific points, receptor sites, on the membrane; in a similar fashion to Ach. on the Renshaw cell membrane. This acetylcholine action can be blocked by dihyrdo-β-erythroidine which forms a complex at these sites, so blocking the acetylcholine effect. But in the presence of dihydro-β-erythroidine, these amino acids still excite the membrane. Therefore these ions do not combine with the specific receptor sites to exert their action. Thus their action appears to be a non-specific and unrelated to excitatory

synaptic transmitter action.

Curtis & Watkins, (1960), also doubt the presence of enzymes to break these amino acids since they, and also the dextro and laevo forms of glutamic acid, all exert their action for a similar period. It is unlikely that each enzyme would function at the same rate.

Glutamic acid was found to excite every neurone tested in the brain stem of the cat, (Curtis & Koizumi, 1961). These authors found that acetylcholine, 5-HT, noradrenaline and tubocurarine failed to influence neurones in the medullary and mesencephalic reticular formation. Curtis & Watkins, (1960), have compared the structures of these depressants and excitants to see if there is a correlation between their structure and function. They found that when the α-carboxyl group of the excitant acidic amino acid was removed, it became a depressant. A powerful excitant when decarboxylated became an equally powerful depressant, e.g. glutamic acid to GABA; aspartic acid to β-alanine, and cysteic acid to taurine. They found the following correlations between structure and function; (1) at least one amino and one carboxyl group was required for activity; (2) the optimum activity is obtained when the amino group is β or γ with respect to an acidic group; (3) the excitant depolarization is solely due to the extra carboxyl group which for maximum

activity should be α with respect to the amino group; (4) D & L forms exert the same effect; (5) and the addition of substituent groups into the carbon chain of the amino group abolished the activity.

The properties of these compounds which have to be remembered in formulating a method of action are as follows; (1) slight structural modifications have a large effect on the action; (2) the excitatory action is a direct link with depolarization; (3) the depressant action does not affect the membrane potential and diminishes both i.p.s.p's and e.p.s.p's; (4) all these amino acids affect both cholinergic and non-cholinergic neurones; (5) the excitatory action is antagonized by the depressant action when the latter is added and vice versa.

With these properties in mind Curtis & Watkins suggested a possible mode of action of these compounds based on receptor sites on the membrane involving either two or three charged centres. These charged centres would react with the ionized amino and carboxyl groups.

Recently Curtis & Watkins, (1961), have found analogues of glutamic acid and GABA which are more potent than these two amino acids. These responses were first shown in neurones in the toad spinal cord, (Curtis, Phillis & Watkins, 1961). The investigation was extended to two mammalian

preparations, the interneurones of the cat spinal cord and cells from the exposed parietal cortex. N-methyl-D-aspartic acid was found to be approximately ten times as potent an excitant, and D-homo-cysteic acid three to six times. Among the depressants tested, 3-amino-I-propane-sulphonic acid was two to five times as potent a depressant as GABA.

INVERTEBRATE STUDIES ON GABA

OCCURRENCE OF GABA

Attempts have been made to find GABA in invertebrate nervous tissue. Kuffler & Edwards, (1958), claim to have found GABA in the crustacean nervous system but present no direct evidence. Florey & Chapman, (1961), chromatographed extracts of peripheral and central nervous system of crabs. They showed the presence of a single ninhydrin-positive substance which was not GABA, and could not find GABA. Glutamic acid decarboxylase was also absent.

Homogenates of bee brain are able to convert glutamic acid to GABA, (Frontali, 1961). This demonstrates the presence in the bee brain of the enzyme glutamic acid decarboxylase. The same author states that GABA has been found in the free state in extracts of bee nervous tissue.

OCCURRENCE OF GLUTAMIC ACID

Glutamic acid, at conc. of 24± 8mg/100ml, was detected in the haemolymph of the cockroach, Periplaneta americana, by Stevens, (1961). Glutamic acid was also found in the sera of Limulus, Cancer and Homarus, at conc. of 0.30, 0.26-0.33, 0.59-1.44, and 0.16 mg/100ml respectively, (Stevens, Howard, & Schlesinger, 1961).

In the snail, Helix aspersa, glutamic acid has been found to be the most abundant amino acid in the brain, (Kerkut & Cottrell, 1962). It occurred at a concentration of 0.20 μM/g brain. Hydrolysed brain extracts yielded a trace of GABA.

APPLICATION OF GABA TO INVERTEBRATE NEURONES

The crustacean stretch receptor was found to be very sensitive to GABA and is used for the bioassay of GABA, (Bazemore, Elliott, & Florey, 1956). The membrane in the crayfish stretch receptor under the action of GABA were investigated by Hagiwara, Kusano, & Saito, (1960). They found that GABA and related compounds imitated the membrane changes produced by the inhibitory presynaptic impulses. The conductance increase of the postsynaptic membrane was in part dependent on the concentration of chloride across the membrane.

Hirchar, (1960), tested the effect of GABA on the activity of the desheathed isolated fifth abdominal ganglion of the crayfish, Oronectes virilis. He found that it had no effect. β -alanine caused either excitation or inhibition, depending on the concentration applied; 10^{-4}M was the threshold for excitation and 10^{-2}M for inhibition.

Slow post-synaptic potentials have been recorded from the giant motor fibres of the crayfish, by Furshpan & Potter, (1959). These potentials are associated with an inhibitory effect. It was found that GABA at conc. of 3-5 x 10^{-5}g/ml mimicked some of the effects of the slow potentials.

The effect of GABA was tested on two types of neurones from the abdominal ganglion of Aplysia depilans, Gerschenfeld & Tauc, (1961). Certain cells which have inhibitory inputs and are inhibited by acetylcholine were depolarized and accelerated by GABA. Other cells which have excitatory inputs and are depolarized by acetylcholine were always hyperpolarized and inhibited by GABA. However in certain cases the cells with inhibitory inputs were inhibited by GABA.

GABA and glutamic acid also play important roles in metabolism. This subject has recently been considered by Roberts & Eidelberg, (1960), and Roberts, Baxter & Eidelberg, (1960).

CONCLUSION

Though there has been some confusion about the presence or absence of GABA in the nervous system, it is present in the vertebrate, and also in the mollusc nervous system. It is not present in the crustacean nervous system. There are two inhibitory substances present in the nervous system; they are called Factor I for the vertebrate preparation, and Substance I for the crustacean preparation. These are not GABA.

The action of GABA and glutamic acid on neurones would appear to be non-specific. This would remove their possible function as chemical transmitters in the nervous system. Their most likely role would be in metabolism via the tricarboxylic cycle. GABA might be a possible precursor for other compounds. GABA has a marked affect on the crustacean stretch receptor but there is no evidence for a natural function in this system. The same may be suggested for its role in the crustacean heart ganglia. Since GABA is present in the molluscan brain, it would seem necessary to test its action on the snail neurones to determine the physiological and pharmacological actions.

THIAMINE AND COCARBOXYLASE

It was shown by Minz, (1938), that 4-8 times more thiamine diffuses into the solution surrounding excised nerves when they are excited than when they are quiescent. It was found that on analysing stimulated and non-stimulated nerves which on excitation had been put into liquid air, more thiamine was present in the excited than in the quiescent extract, (von Muralt, 1942). Von Muralt thought that the excitation had shifted the "bound" thiamine into "free" thiamine. In nerves poisoned with iodoacetate, about 5% of the "free" thiamine disappeared during stimulation into an unknown compound, compound X, (Wyss & Wyss, 1945).

Thiamine is active in organisms in the form of thiamine pyrophosphate or cocarboxylase. It was thought possible that this coenzyme might play a part in the synthesis of acetylcholine from choline, (Minz, 1946). High doses may cause a contracture of the leech dorsal muscle. But this reaction is not potentiated by eserine, (Minz, 1932). If cocarboxylase is capable of transforming choline to acetylcholine, then the mixture should exert an effect on the leech muscle which is potentiated by eserine. Cocarboxylase alone had no effect on the leech muscle in the presence or absence of eserine. Cocarboxylase enhances the contracture produced

by choline on the leech muscle. This increase was even greater in the presence of eserine. Thus the muscle may aerobically and in the presence of cocarboxylase transform choline into an ester the action of which is potentiated by eserine. It is known that cocarboxylase catalyzes the decarboxylation of pyruvic acid, forming acetic acid. Banga, Ochoa, & Peters, (1939), Ochoa, (1941), have shown that decarboxylation is associated with phosphorylation of adenylic acid to yield adenosine triphosphate. Adenosine triphosphate intensifies the activity of choline acetylase which synthesises acetylcholine. Thus via the action of cocarboxylase the cell has acetic acid and adenosine triphosphate, both concerned with the synthesis of acetylcholine.

The effect of adding antimetabolites to the solution bathing stimulated single myelinated nerve fibres was investigated by von Muralt, (1958). He used four antimetabolites; oxy-thiamine, a competitor for cocarboxylase; neopyrithiamine, a competitor for thiamine; purified thiaminase, from carp intestines; and extracts from the fern, Pteris aquilina. The latter two compounds destroy the thiamine molecule. The addition of fern extract raised the node threshold from 50mV to 80mV after one minute. After 7 minutes, the threshold was 110mV, and the action

potential height had fallen. It recovered when it was returned to normal Ringer. The action of neopyrithiamine was next examined. After 1 minute in contact with this compound the threshold had risen from 50-80mV, the height of the potential had fallen and the repolarization time had greatly increased. von Muralt associates this change in shape with either an increase in the external sodium concentration or inactivation of the sodium transport system. Oxythiamine had little effect on the preparation. Neopyrithiamine did not affect the resting potential, indicating that it had no effect on the potassium permeability. von Muralt concluded that thiamine had little effect on the resting membrane but was important during activity of the fibre. Cocarboxylase in addition might be important for metabolism.

von Muralt injected labeled thiamine-S^{35} into animals with severe avitaminosis. When the animals were normal they were killed and the nerves plunged into liquid iso-propane. It was found that the chemical composition of the excited and quiescent nerves was different. At rest a compound X appeared with thiamine in autoradiograms, while in the excited nerve it disappeared and thiamine-phosphate appeared. It would appear that on stimulation, the form of thiamine in the nerve changes. Petropulus, (1960), using the same

preparation found that fern extract reduced the size of the action potential and hyperpolarized the membrane. The effect of the fern extract could be completely blocked by the addition of thiamine.

There is an enzyme in Helix dart sac and blood which will hydrolyse acetylthiamine, (Augustinsson, 1946, 1948).

CONCLUSION TO THE REVIEW OF THE LITERATURE

This account of the literature concerned (a) the electrical responses of the molluscan brain, crustacean heart ganglion and the crustacean stretch receptor; and (b) the effects of various drugs on the nervous systems of vertebrates and invertebrates. Though the two sections may appear to be quite distinct, it would seem that if the transmission between nerve cells is due to the liberation of chemicals, then these chemicals should be capable of producing the described electrical effects. In some respects it is as if the chemical and electrical analysis of the nervous system were fragments of a story in two different languages; the reader has to try and construct the complete story as well as a dictionary of the two languages. A knowledge of the changes in the membrane that take place during excitation and inhibition

indicate the type of responses that the 'natural' transmitter substance(s) will have to mimic.

In reviewing the types of compounds that have been considered as possible chemical transmitters, it is more than likely that many more are yet to be discovered. In this respect it should be remembered that many of the chemicals that can be found in the brain will have a metabolic or a structural role and not a transmitter function. The neurones must have enzymes present that will allow synthesis and breakdown of the transmitter. The transmitter must have a similar effect to natural stimulation and it must be released in concentrations comparable to those that will allow experimental verification.

The present investigation is concerned with the nature of the functional connexions between the nerve cells in the snail brain. A certain amount of work has been done on this subject, mainly electrical studies by Tauc and his colleagues on the abdominal ganglion of Aplysia. The neurones show most of the properties that one associates with nerve cells throughout the animal kingdom. The review of the chemical nature of the transmitters has indicated the probable chemicals that can be tested for transmitter action. It is now intended to describe the electrical

actions of snail brain neurones in some detail, and then to study the way in which similar electrical effects can be brought about by chemical stimulation. The types of response have been divided into two, depending on the concentration of the drug. From the literature it would appear that transmitters occur in concentrations in the order of I x 10^{-6}g/g tissue. Concentrations in excess of this have been labeled as pharmacological and they often have a different type of response on the activity of the cell. The responses to be described in Section One of the drug experiments are largely in the way of preliminary screening experiments to see which compounds are active at physiological concentrations and as to whether these are acceleratory or inhibitory in nature. Sections Two and Three are concerned with the responses of marked cells. Where ever possible comparisons will be drawn between electrically and chemically stimulated responses.

METHODS

Apparatus

The microelectrodes used ranged in resistance from 5 to 30 megohm. They were connected to a cathode follower by means of silver-silver chloride wire. A 6B 57 valve with a grid current of approximately 10^{-10} amps was used in the cathode follower. This led to a Tektronix 502 oscilloscope. The potentials were monitored on a twin channel tape recorder. A 35mm Shackman camera was attached to the oscilloscope. In this way a continuous record of the experiment could be taped and selected potentials filmed. The tape records were replayed either onto an Ediswan Pen recorder or the camera.

A square wave stimulator with a Radio frequency probe delivered a pulse of variable duration, voltage and frequency. A frequency of one impulse per second with a width of 2.5msec was found to be a convenient form in which to explore possible connexions. During the stimulation of each nerve the voltage was gradually raised. There was a nine-way switch and it was possible to select each of these channels in turn and stimulate the appropriate nerve.

THE ANIMAL

The garden snail, Helix aspersa, was used in all the experiments described in this study. The gastropod molluscs were chosen because of their relatively simple central nervous system and because of the presence of large brain cells. The presence of large nerve cells in this animal was recorded by Nabias in 1894. This animal was chosen in preference to any other gastropod because of its comparatively large size, the ease with which it can be identified, and because it can be collected locally in large numbers.

In preliminary experiments it was found that aestivating and hibernating snails had low resting potentials and the cells were not spontaneously active. For this reason cells were always activated prior to use. This was done in the following way. A quater of an inch of water was placed in a glass jar and a bright light was shone onto it. Several pieces of filter paper were also added together with the snails to the glass jar. In this way a warm humid environment, together with a ready food supply was created. The snails soon emerged from their shells and began to crawl around. After five or six hours in this environment they were ready for experimentation.

Snails entering their third year were generally used

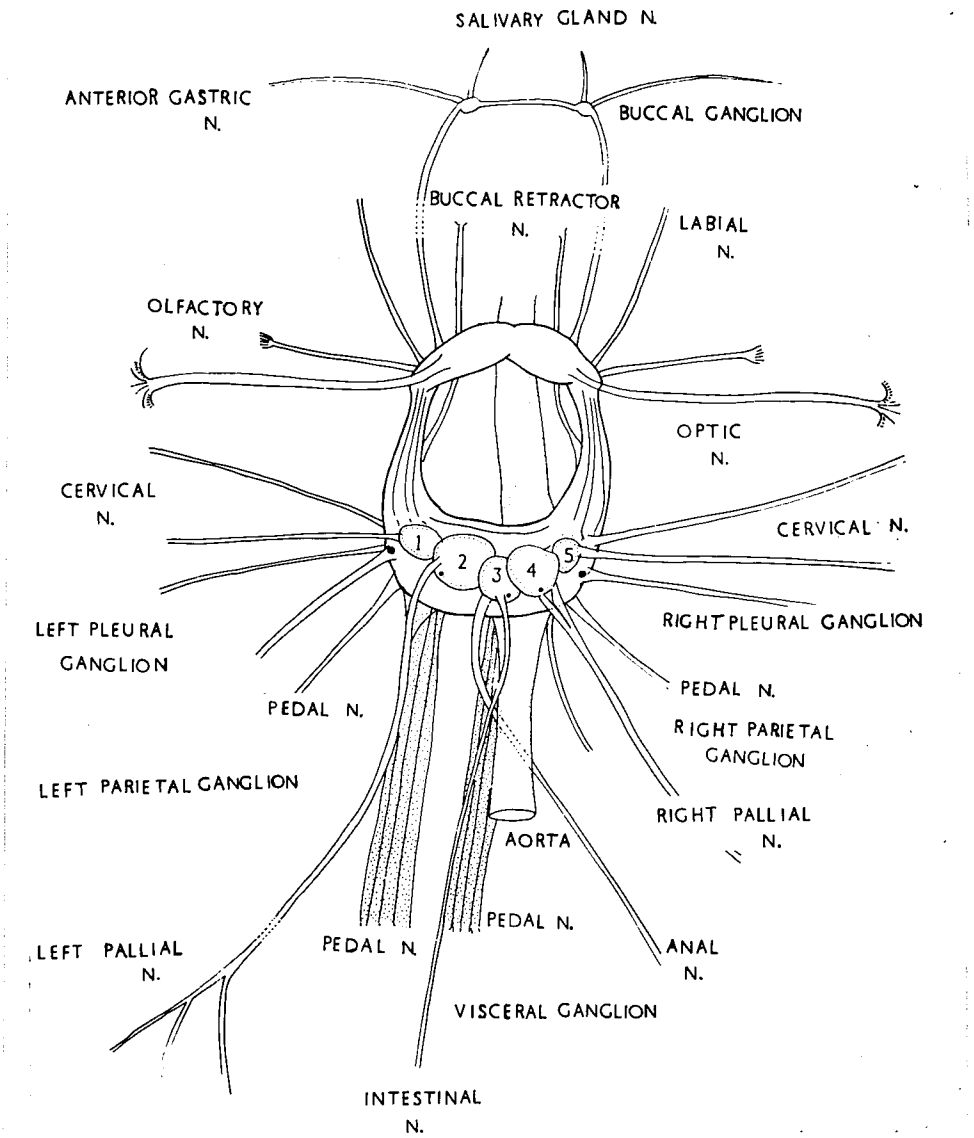


Figure 1 shows the dissection of the nervous system of a young Helix aspersa to show the major ganglia and the nerve trunks leading into them. The dissection is shown from the dorsal surface.

in this study. The nerves of younger snails were usually not long enough. Older snails developed pigmentation and calcium deposits within the ganglion tissue and generally the resting potentials of many of their neurones were low or difficult to record.

PREPARATION

In the initial experiments an isolated brain of Helix aspersa was used. At first tungsten external electrodes were used. When glass microelectrodes were used the connective tissue was removed to expose the neurones.

In the in vivo experiments the snail was removed from its shell and extended and pinned onto wax in a bath. The brain was exposed and the oesophagus was cut and removed to one side, to expose the sub-oesophagael ganglion. The nerves were exposed and hooked onto the external silver stimulating electrodes. Nine of these electrodes were used in each experiment. The preparation was viewed under a binocular microscope during the experiment, and a magnification of 25x and 50x was used.

Fig. 1 shows a snail brain with the major nerves used in the stimulating experiments. This was a young specimen in

which the pleural ganglia, (1 and 5), had not yet fused with the parietal ganglia, (2 and 4). Between the two parietal ganglia is the visceral ganglion, (3). Beneath this complex of ganglia are the two pedal ganglia. These seven ganglia are connected with the supra-oesophageal ganglia by two pairs of connectives.

Leaving each parietal ganglion is a single large nerve trunk, the pallial nerves. These pass mainly to the lateral body wall musculature. There are two large nerve trunks leaving the visceral ganglion. The more dorsal of them is the intestinal nerve, and the more ventral of them the anal nerve. These send branches to the body viscera, the heart, etc. Associated with the pleural ganglia are three pairs of cervical nerves. There are ten pairs of pedal nerves radiating from the pedal ganglia. These innervate the muscles of the foot.

Passing between the five dorsal and the two ventral ganglia of the sub-oesophageal ganglionic mass is the aorta.

From the supra-oesophageal ganglia nerves run to the eyes, the olfactory tentacles, the buccal mass and ganglia, the reproductive system, and the collumellar muscle.

RINGER SOLUTION

Throughout the experiments described in this study a 70% frog Ringer solution was used, (Cardot, 1921).

All the drugs used in this study were made up in this solution, the pH of which was 7.2. Carbon dioxide was blown into the solution and the pH remeasured, it was 7.19. Several of the drug solutions were tested for their pH. The results are summarised in the following table:

DRUG	pH	CONCENTRATION OF DRUG
Acetylcholine	7.4	5×10^{-5} g/ml
Histamine hydrochloride	6.7	1×10^{-4}
	7.1	1×10^{-5}
	7.2	1×10^{-6}
Dopamine	7.1	1×10^{-4}
Senecioylcholine	7.2	1×10^{-4}
Urocanylcholine	7.2	1×10^{-5}
Butyrylcholine	7.15	1×10^{-4}
Crotonylcholine	7.0	1×10^{-4}

At physiological concentrations the pH does not appear to be affected by the addition of the drug.

APPLICATION OF DRUGS

In all experiments known amounts of drugs were released over the preparation via a pipette. Initially Iml of solution was applied either close to or over the experimental cell. In later experiments smaller amounts of liquid, 0.1 to 0.3ml, were applied using the same method. The smaller volume it was hoped would reduce any mechanical response resulting from the addition of larger amounts. The lowest concentration of the drug was applied first and the concentration gradually increased.

All the drug concentrations are expressed in terms of grams per millilitre. Thus a solution of 10^{-6} contains one part of the drug in a million parts of water. The only liquid used, DMAE, was made up v/v instead of w/v.

Dilutions of 10^{-6} and more dilute are considered to be physiological, while those more concentrated are considered to be pharmacological. The experimental bath contained 10ml of solution, so in determining the final concentration of a drug this had to be taken into consideration.

Concentrated test solutions were made up and stored in the refridgerator. They were diluted during the course of the experiment and applied, the most dilute first.

RECORDING ELECTRODE

For the initial experiments a fine tungsten glass external electrode was used. This recorded from within the surface of the whole suboesophageal ganglionic mass. The connective tissue was not removed from over the ganglia. This meant that applied drugs had to first penetrate the connective tissue over the neurones. It was found that on removing the connective tissue the nerve activity disappeared, though the reason for this was not clear. The results from these experiments are not described in this account.

It was thought necessary to record from individual neurones, and for this purpose glass microelectrodes were made. They were manufactured from Pyrex glass tubing with an external diameter bore of 4mm and an internal diameter bore of 2.0mm. These were pulled on a locally constructed vertical machine, initially under gravity and then under the influence of a solenoid.

The microelectrodes were first filled with absolute alchohol under reduced pressure at approximately 60°C. When they were filled with the alcohol it was displaced by distilled water and then in turn this was displaced by 3M KCl.

MARKING OF THE CELL

The electrodes used in the marking experiments were pulled in the normal way but they were filled with 2.5M KCl and 0.5M $Na_4Fe(CN)_6$. IOH_2O, sodium ferrocyanide. Activity could be recorded from a penetrated neurone for several hours without deteriation of the shape of the action potential. At the end of the experiment a current was passed through the electrode, the inside of the electrode acting as the cathode, and the bath as the anode. A D.C. voltage of up to 30V could be applied across the electrode tip. The sudden application of this voltage to the cell in the intact brain of an in vivo preparation often resulted in a movement of the animal and the electrode then came out of the cell. To overcome this, 0.35M $MgCl_2$ was added prior to the application of the voltage. The preparation was left in this solution for ten minutes.

It was often found that the electrode tip quickly polarized and the current then fell to zero. When this happened the polarity of the electrode and the bath was reversed several times. This generally removed the polarization and permitted the passage of a current. However, on occasions this had no effect. Then a voltage of 170V D.C. was substituted for the first supply and this

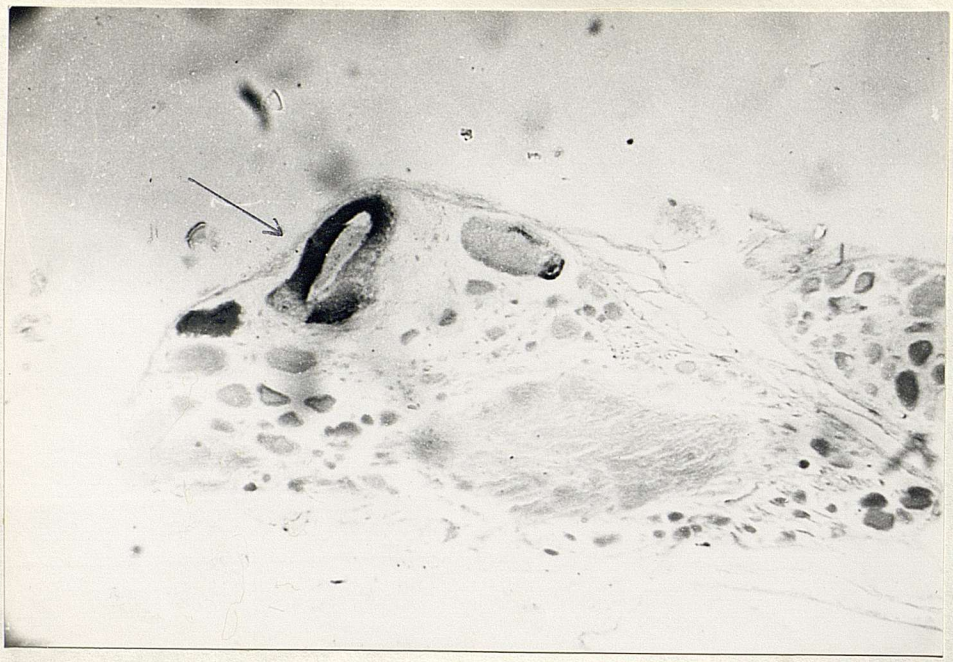
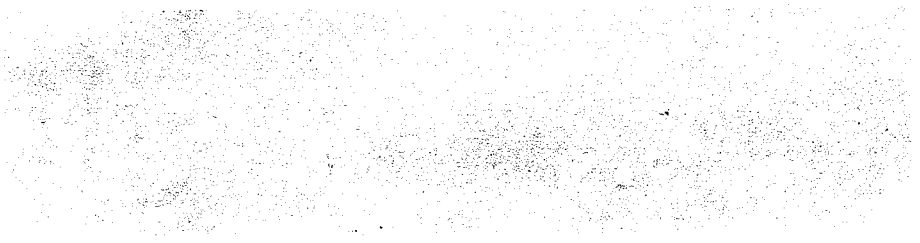


Figure 2 shows a section through the sub-oesophageal ganglionic mass of Helix to show a marked cell. The marked cell is blue against a pale red eosin background. Only the cytoplasm has stained.

passed through the electrode for a few seconds to depolarize it. The 30V D.C. supply was then replaced and the current passed. With a current of 5 to 8 μamps, the current was allowed to flow for 10 minutes. The electrode was then removed and a few drops of I.IM $FeCl_3$ solution applied over the cell. If the marking was successful, then a Prussian blue colour filled the cell. This was due to the ejected ferrocyanide ions combining with the ferric chloride.

The brain was then removed from the animal and fixed in 90% alcohol. The brain was then cleared, embedded and sections were cut at 10 μ and lightly stained in eosin. A photograph of a marked cell can be seen in Fig. 2.

The stain was restricted to one cell. It is interesting to note that in many cases the nucleus did not take up the stain.



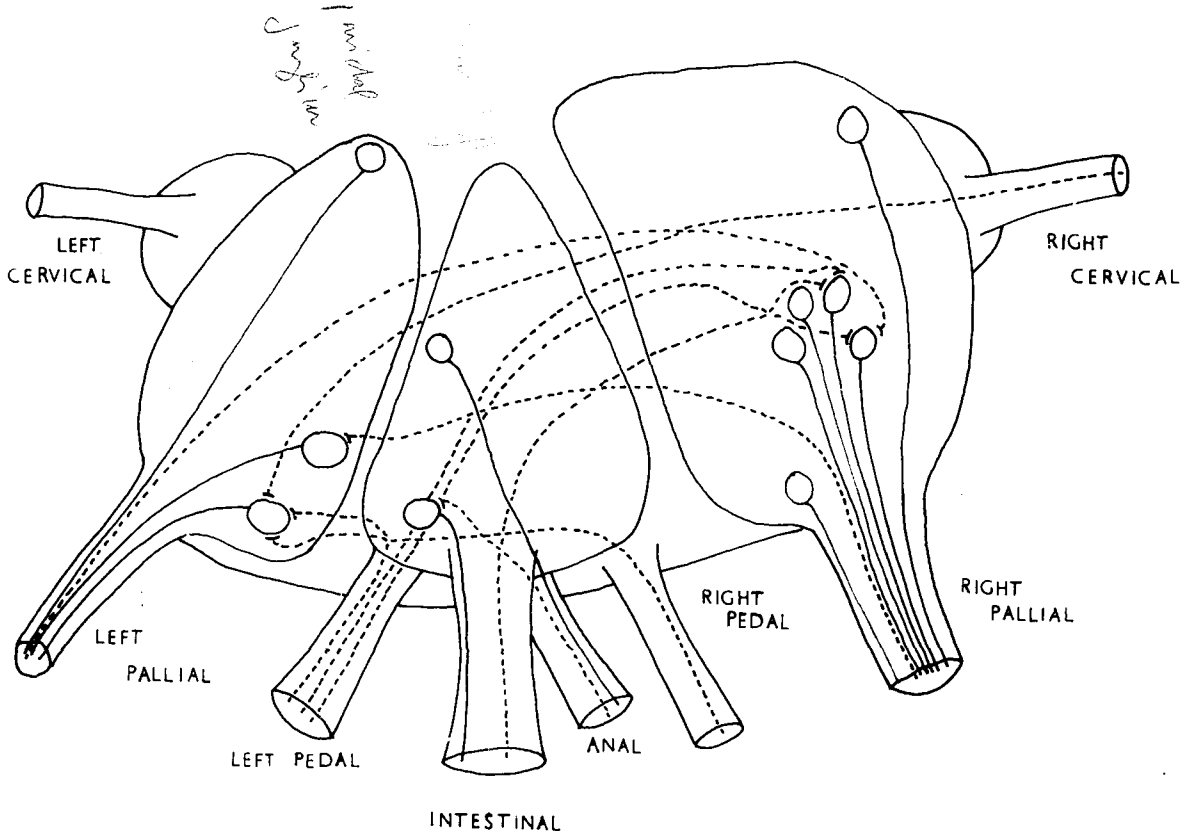


Figure 3 shows the positions of neurones capable of being driven antidromically and also in some cases orthodromically. The neurone axon is always in the nerve trunk which leads directly into the ganglion in which the neurone is situated. In the right parietal ganglion can be seen a small group of neurones with similar efferent axons.

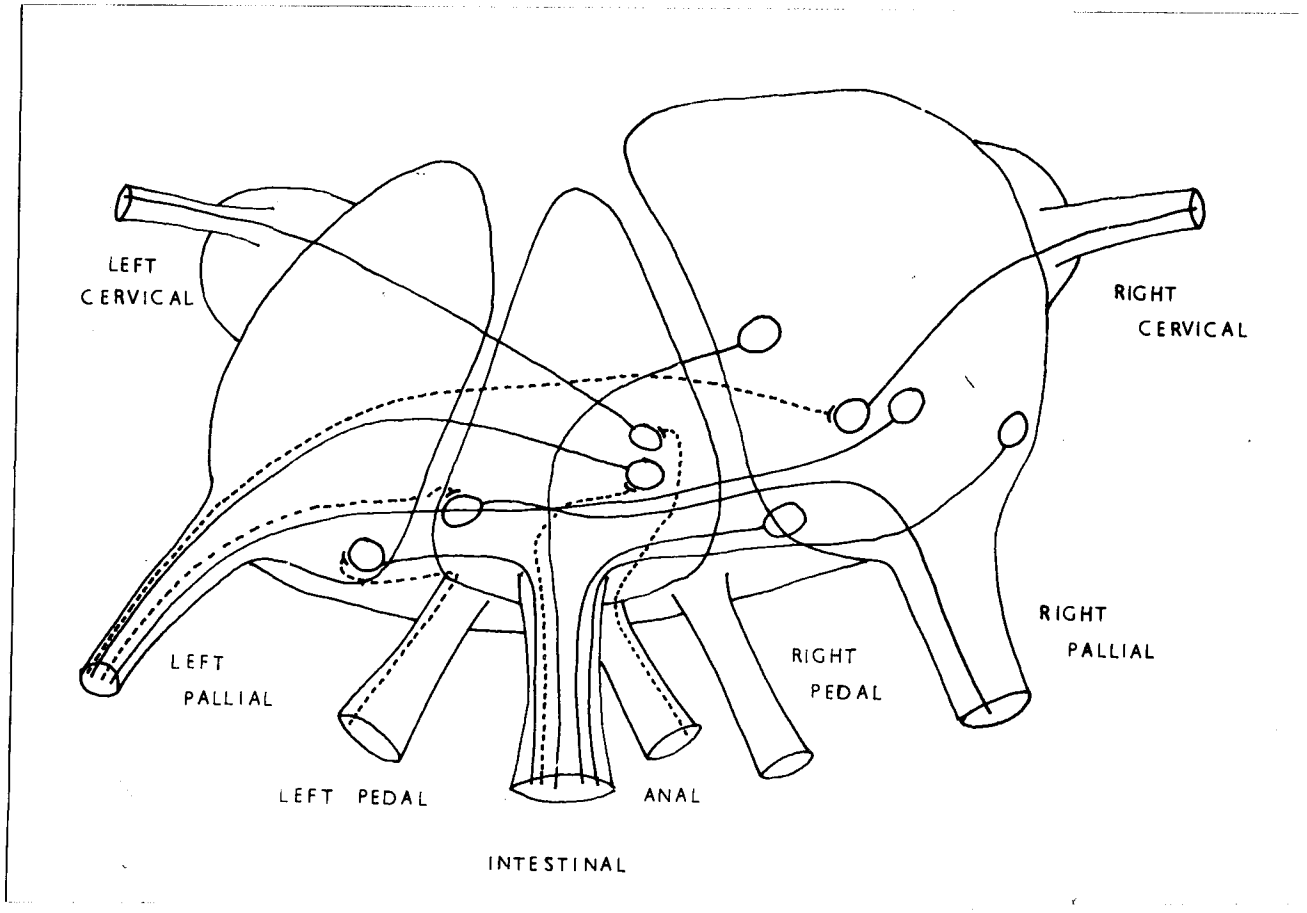


Figure 4 shows the positions of neurones capable of being driven antidromically and also in some cases orthodromically. The neurone axon is always in a nerve trunk which leads into a ganglion other than the one in which the neurone is situated.

RESULTS

PHYSIOLOGICAL NEURONOGRAPHY

In an attempt to trace some of the anatomical connections of the neurones in the snail brain, 236 experiments were carried out using the physiological neuronography technique. The results are summarised in figures 3 to 8.

From figures 3 and 4 it is not clear as to whether the efferent axons of the neurones emerge into the nerve trunk leading from the ganglion in which they are situated or whether they emerge from an adjacent ganglion nerve trunk. Both situations obviously occur, but is is suggested that the pathways of figure 3 will prove to be the usual route. Small groups of cells are present in both parietal ganglia which lead out directly via the emerging pallial nerves. From figure 4 it can be seen that there is no tendency for the efferent axons of neurones in, for example, the right parietal ganglion, to emerge via the left pallial nerve. There is only one occurrence of such pathway in figure 4. The lack of this crossing over of axons may be contrasted with that found in both vertebrate and annelid systems. It would appear that the intestinal nerve has efferent axons from neurones of both parietal ganglia. So far the maximum number of synapses onto a neurone is three, that is, action potentials have been driven orthodromically following the

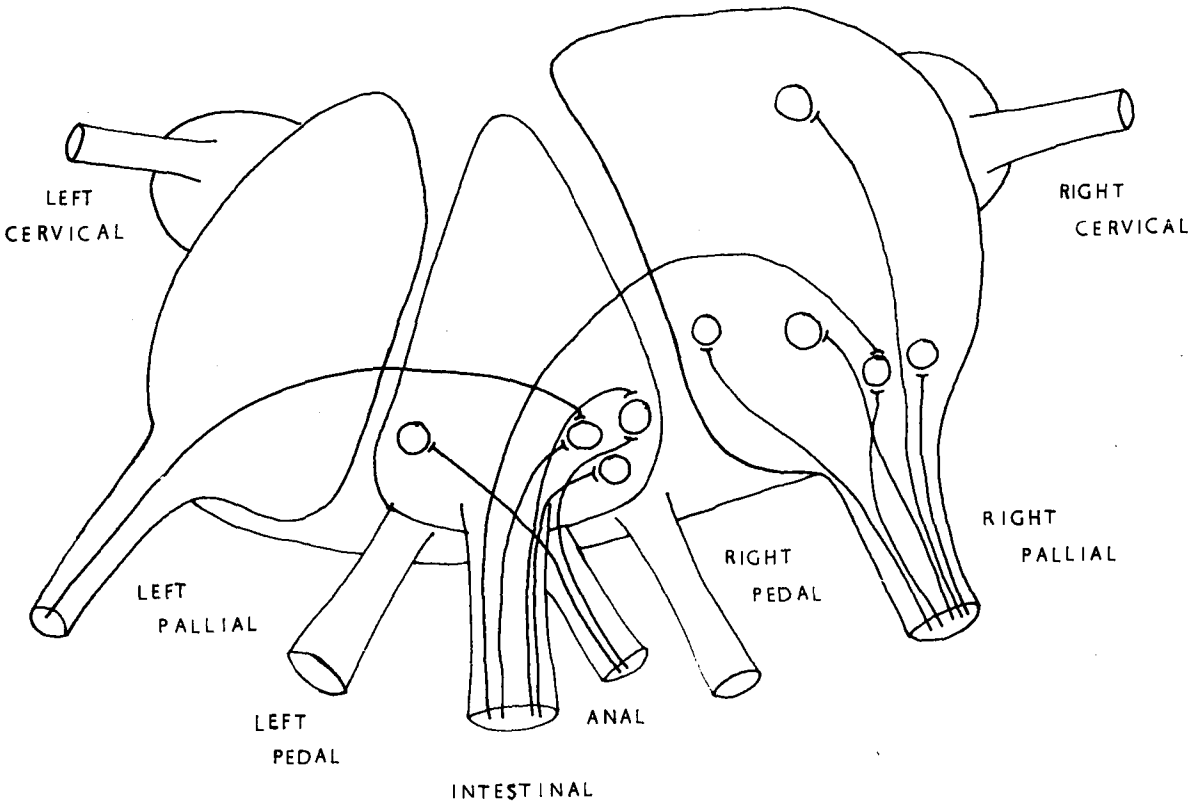


Figure 5 shows the positions of neurones capable of being driven only orthodromically. The afferent axon is in the nerve trunk which leads directly into the ganglion in which the neurone is situated. In three cases described in the figure a second afferent axon also synapses onto the neurone, in this case from a nerve trunk which leads into an adjacent ganglion.

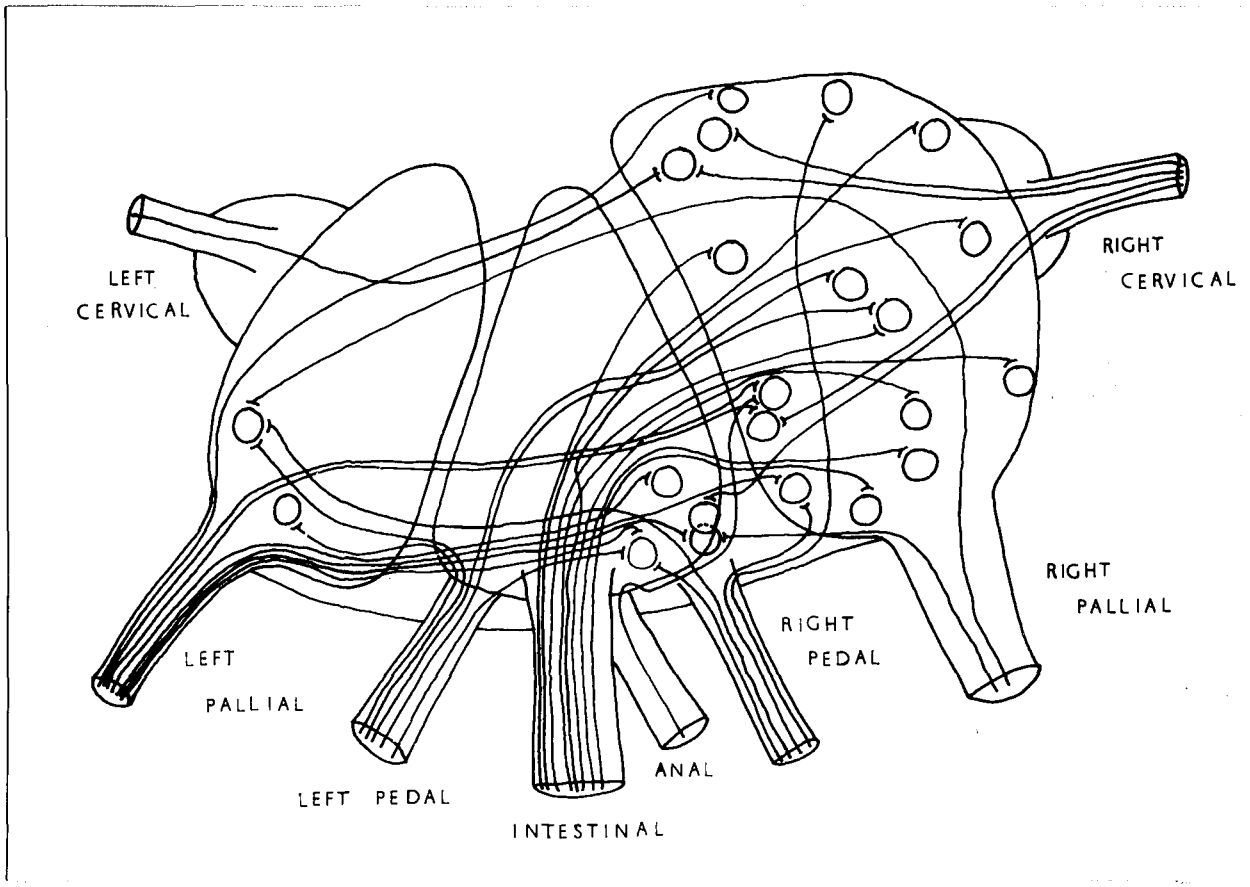


Figure 6 shows the positions of neurones capable of being driven only orthodromically. The afferent axon(s) is in a nerve trunk which leads into a ganglion other than the one in which the neurone is situated. As many as three axons synapse onto the same neurone. There are many more examples of this type of synapsing than of the type found in figure 5.

stimulation of three different nerve trunks.

The pathways outlined in figures 3 to 8 are all in response to driving the action potentials of the neurone. In many additional cases, the activity of the neurone has been altered without directly driving the cell activity. Some of these experiments will be described later. It is highly probable that many of the orthodromic synapses shown in the figures synapse via interneurones. It should be remembered that there is no real evidence concerning the morphological nature of the synaptic connections. There is no direct evidence for soma-axon connections and it has been suggested that most of the synapses will be between axons in the neuropil, (Nisbet, 1961).

In figures 5 and 6, cells which could only be stimulated orthodromically are shown. The vast majority of such neurones receive synapsing axons which enter the brain via nerve trunks which do not enter the ganglion in which the neurone is situated. As can be seen from figure 6, most of the experimental neurones are located in the right parietal ganglion. These cells receive a large number of synapsing axons from the left pallial nerve and the intestinal nerve. It is suggested that the neurones in the left parietal ganglion will likewise receive afferent synapsing axons from

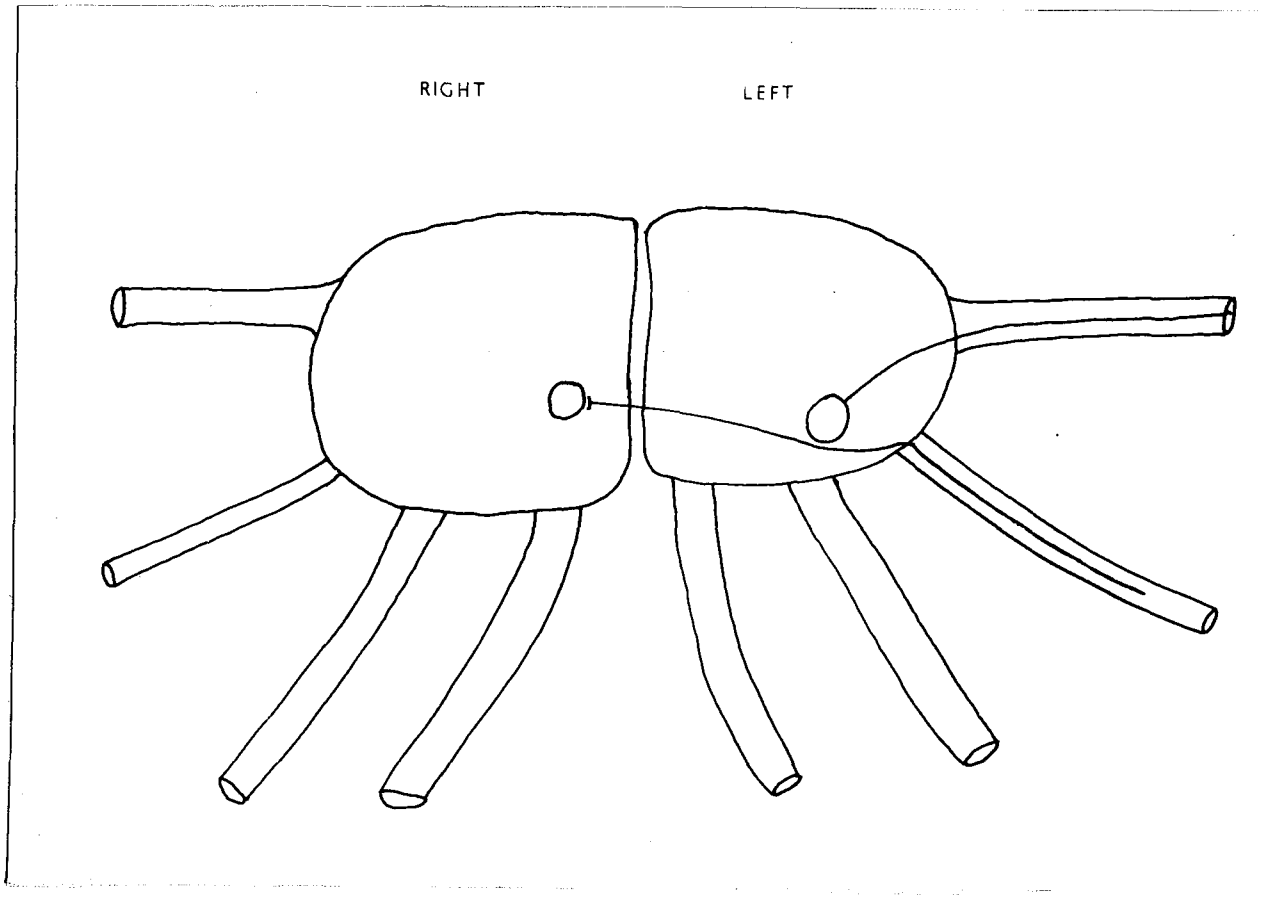


Figure 7 shows two neurones so far traced in the pedal ganglion. One was driven antidromically and the other orthodromically. These studies are to be extended. The nerves shown in the figure are three of the ten pairs of pedal nerves.

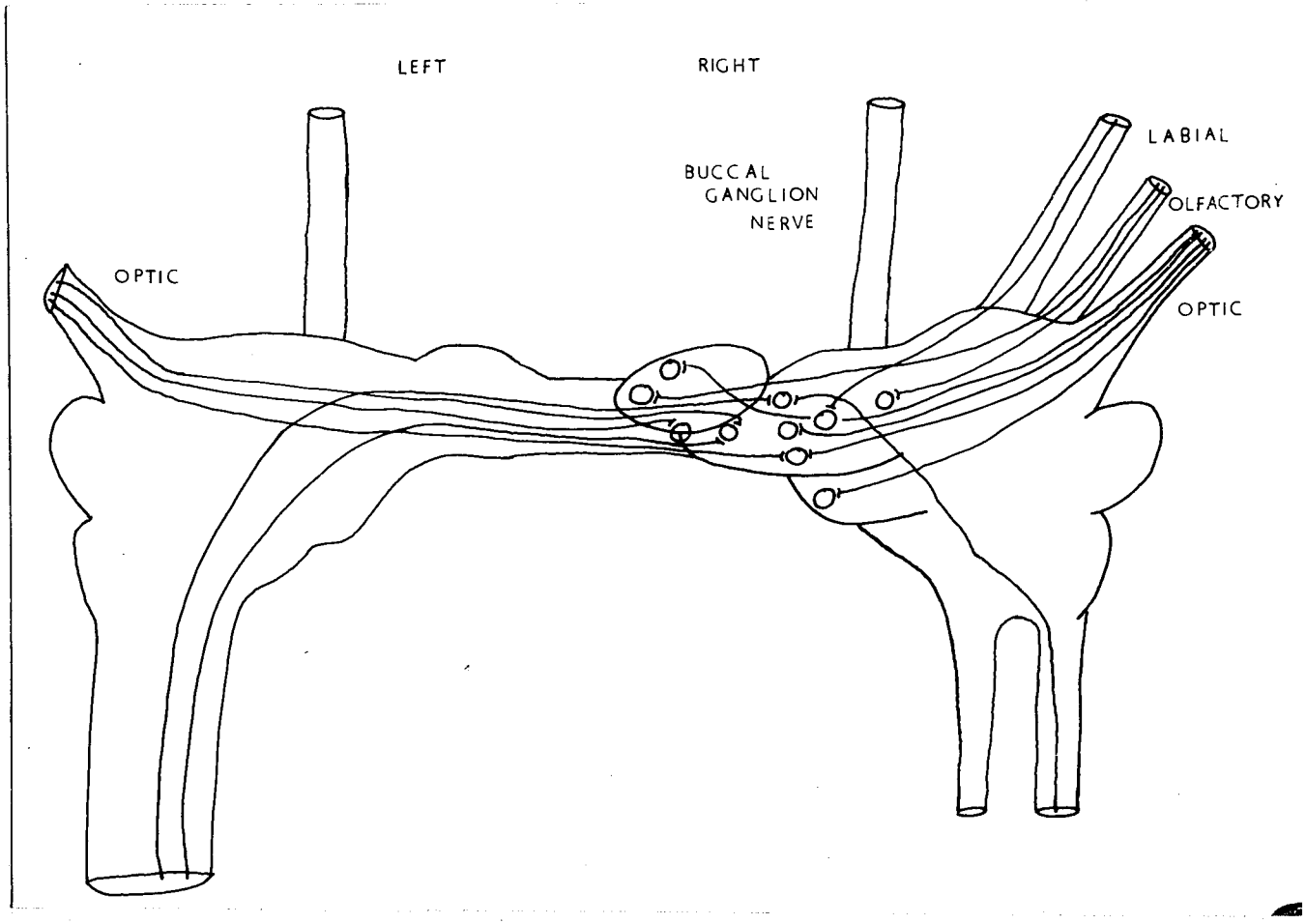


Figure 8 shows the positions of neurones so far mapped in the right supra-oesophageal ganglionic mass. Only orthodromically stimulated links have so far been observed in this region. These studies are to be extended.

the right pallial nerve.

The nine ganglia of the snail brain did not prove all equally accessible to microelectrode examination. This was partly due to the availability of large cells, and also the availability of nerves via which the cells could be stimulated. In head region many of the nerves are short, less than 1mm in length and proved difficult to stimulate. The cells in the supraoesophageal ganglia are rather small, less than 50 μ in diameter.

Figures 7 and 8 are the results of preliminary studies of mapping the neuronal connections in the pedal and supra-oesophageal ganglia. It is hoped at a future date, to greatly extend these studies.

A series of the stimulation experiments will now be described to indicate the types of responses obtained when the nerve cells are stimulated through the axons.

AN ANALYSIS OF THE RESPONSES OF THE NEURONE MEMBRANE TO STIMULATION.

When a nerve leading to the brain of a snail is stimulated, one of several things may be observed in the experimental cell. There may be no effect. Sometimes the spontaneous activity of the cell is accelerated or

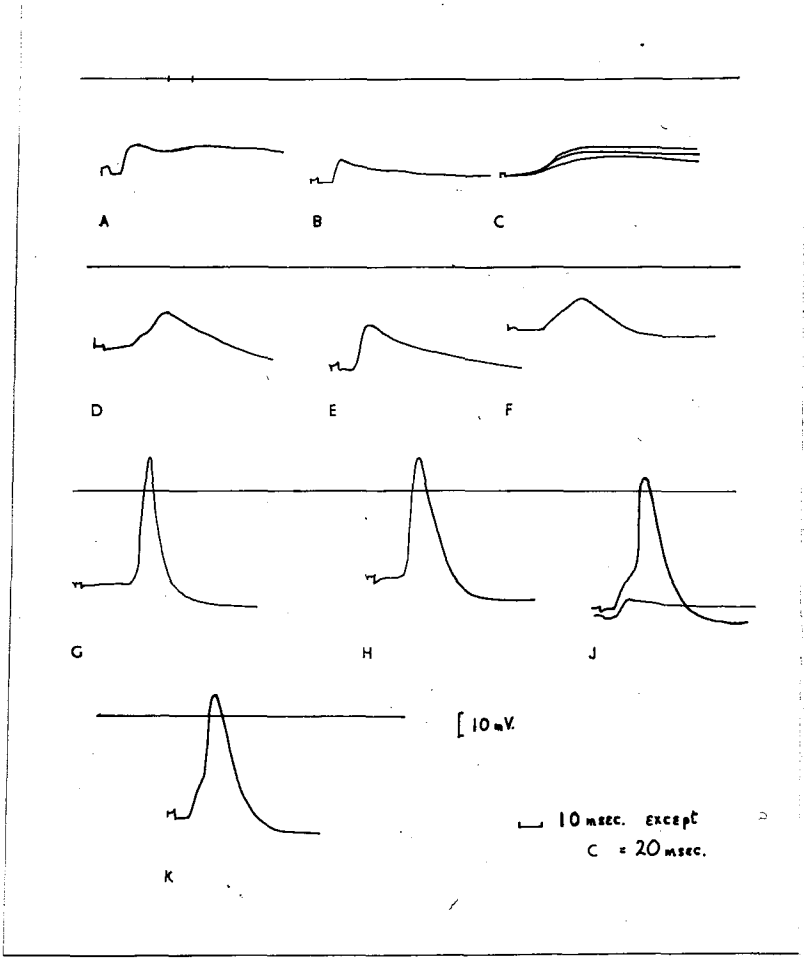


Figure 9 compares the shapes of orthodromically and antidromically driven e.p.s.p.'s, pseudopotentials, and full action potentials. A). and B). are antidromic and C). is an orthodromic e.p.s.p. D). and F). are orthodromic and E). is an antidromic pseudopotential. G). and H). are orthodromic and J). and K). are antidromic action potentials. The delay time in K). is 6.5msec and in G). it is 20msec.

inhibited. The cell may adapt to this stimulus or it may alter its reaction. Stimulation may drive the cell, producing full action potentials, pseudopotentials, excitatory or inhibitory potentials or a combination of these bioelectric phenomena. The cell may show adaptation, summation or facilitation. The cell may be capable of being driven anti- or orthodromically. There may be ipsilateral and contralateral antagonism or synergism. Many times only one of a pair of nerves elicits a response. Examples of these different types of responses will now be given.

In describing these experiments the stimulus voltage is normally given. The frequency of stimulation unless specifically stated is one per second and the width of the stimulus is 2.5msec.

THE PROBLEM OF ORTHO- AND ANTIDROMIC STIMULATION

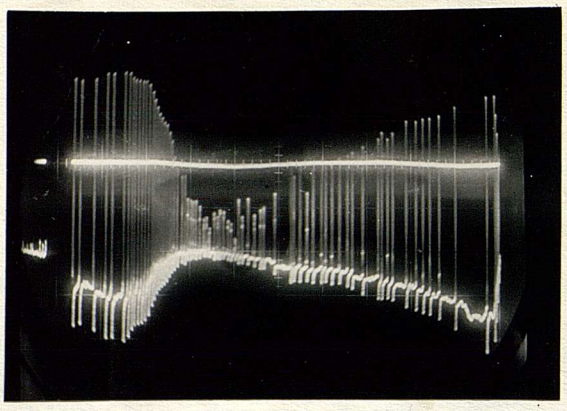
One of the problems in tracing the connexions in the central nervous system is to distinguish between orthodromic and antidromic stimulation. In this study three main criteria have been used.

A short delay time between the stimulus artifact and the potential has been taken as an indication of antidromic stimulation, figure 9. In figure 9G there is a much larger

delay time between the artifact and the action potential. This is an orthodromic stimulation. In this case the rate of conduction along the nerve has to be taken into consideration. It would appear that the rate of conduction along the pallial nerves does not exceed 100cm per second, that is one millimetre per milli-second. The rate of conduction along the intestinal and anal nerves is less, that is 50cm per second or half a millimetre per milli-second.

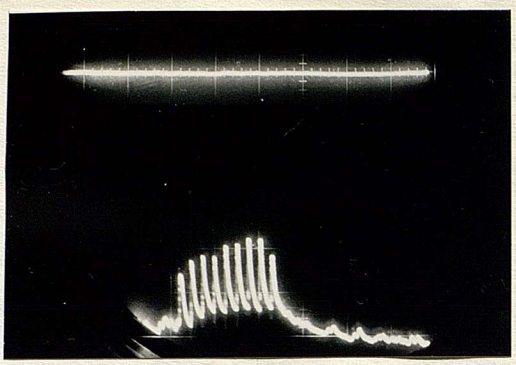
The second criterion depends on the fact that an electrically induced excitatory potential has a relatively fast rise time compared with a chemically induced excitatory postsynaptic potential. This is shown in figure 9. Figures 9A and B are e.p.s.p.'s induced by antidromic stimulation while figure 9C is one induced chemically by orthodromic stimulation. Figures 9D, E and F show three pseudopotentials, one resulting from antidromic stimulation (E), the others, D and F from orthodromic stimulation. The orthodromic pseudo potentials, figures 9D and F have a longer rise time than the antidromic potentials figure 9E.

The third criterion is the presence of a step on the rising phase of the full action potential, figures 9J and K. The step of the antidromic spike occurs at the firing level of the cell soma. It represents the transition from the electronic spread of the spike in the axon to the spike



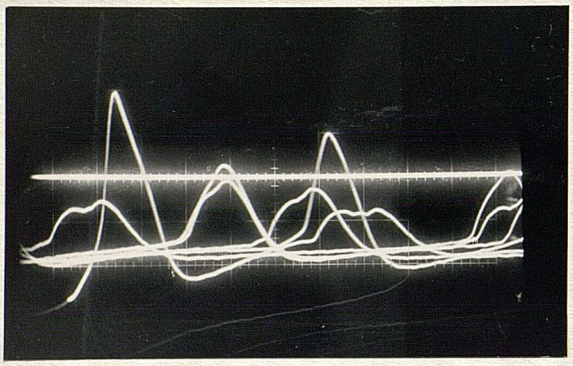
A.

5 sec.



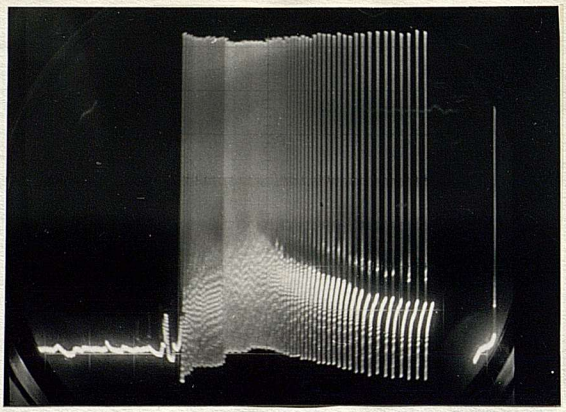
B.

5 sec



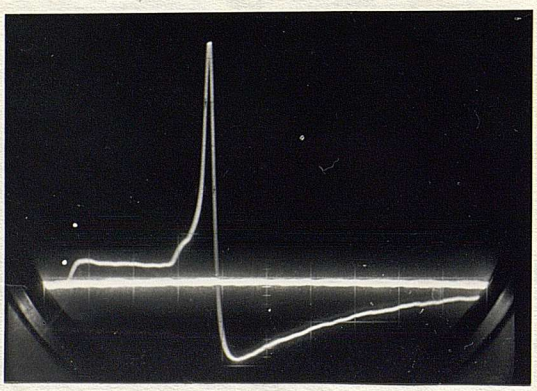
C.

10 msec.



D.

5 sec.



10 mV.

E.

60 msec.

Figure IO .

Figure IO shows spontaneous and induced generator-type potentials.

A). Spontaneous activity in the form of a long depolarization, accompanied by an increased rate and a fall in the height of the action potentials.

B). A driven generator potential with superimposed pseudopotentials.

C). The shape of the action potentials shown in A). The action potential had a duration of 20msec.

D). Stimulation of the left pallial nerve of cell 229 produced a burst of activity.

E). An action potential with a large positive afterpotential. The afterpotential was about I8mV in height.

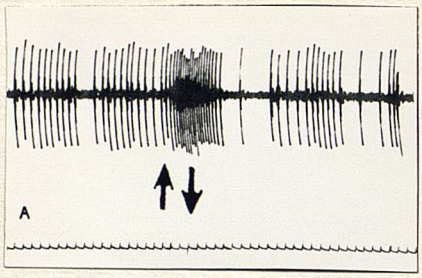
All the figures, except B)., are from the same neurone.

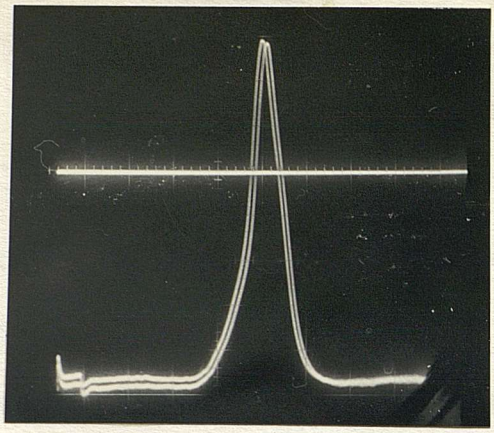
potential of the soma, and so indicates an antidromic propagation. Figures 9G and H represent orthodromic stimulation along different nerves. All four records, G to K are from the same neurone.

GENERATOR-TYPE POTENTIALS

Figure 10A shows the type of spontaneous activity recorded from a cell. As the rate of activity increased so the resting potential depolarized and the height of the action potentials fell till the resting potential level was -23mV. The resting potential then slowly repolarized to its previous level of 43/32mV at each action potential. The shape of the action potentials can be seen in figure 10C. This was a generator-type potential which lasted for about 40 seconds. This cell could not be driven and while raising the voltage of the stimulus to the right pallial nerve the resting potential fell to zero and did not recover.

Cell 236 was a spontaneously active cell which could be driven orthodromically to show both full action potentials and e.p.s.p.'s. Figure 10 B shows the response to stimulating the cell via the left pallial nerve. The cell membrane responded by a depolarization of the resting potential from -65 to 55mV which was maintained during the period of





10msec. [10mV

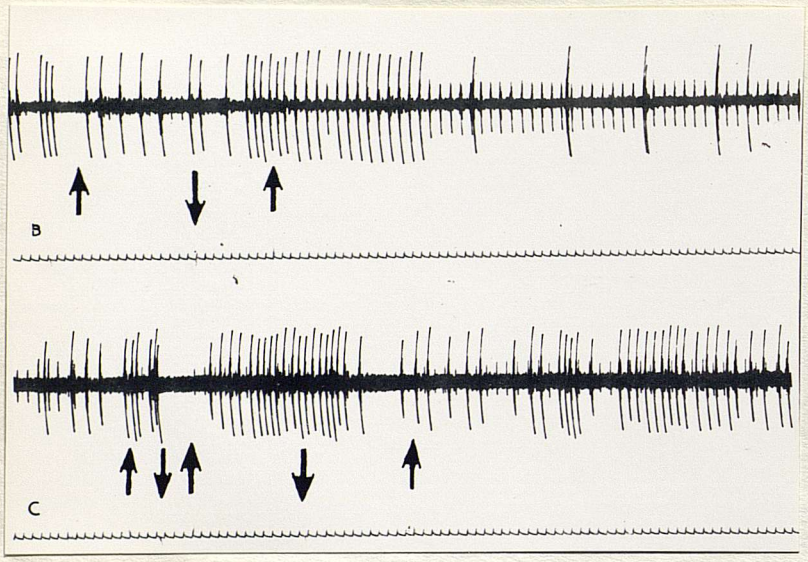


Figure II shows both acceleratory and inhibitory responses to stimulation, in the absence of driving the cell; and the replacement of spontaneous activity by driven action potentials.

A). Acceleration due to the stimulation of the right pedal nerve.

B). The inhibition of spontaneous activity and the driving of the cell via the left pallial nerve which adapts.

C). Acceleration via the right buccal nerve.

D). Driven action potentials on stimulation via the left pallial nerve. The duration of the action potential is 27msec.

stimulation. This resembled a generator potential. On stopping the stimulation, the resting potential slowly repolarized to its previous level.

ACCELERATION OF SPONTANEOUS ACTIVITY

Cell No. 144 was spontaneously active. Stimulation could accelerate the spontaneous activity of this cell, figure 11A. In this case the right pedal nerve was stimulated with a frequency of I/sec and a voltage of 3.3V. A burst of activity could be obtained by stimulating the right buccal nerve, figure 11C. The spontaneous activity of the cell could be inhibited by stimulating the left pallial nerve with the same voltage. Stimulating via this nerve produced e.p.s.p.'s. These adapted after 18sec. The voltage of the stimulation was then raised and the cell was redriven, figure 11B. Even at this higher voltage the cell soon adapted. The presence of the e.p.s.p., and the shape of the potential indicated that the cell was being driven orthodromically, figure 11D. It is possible that the cell was being driven naturally via this pathway, the left pallial nerve, and stimulation inhibited the natural rhythm.

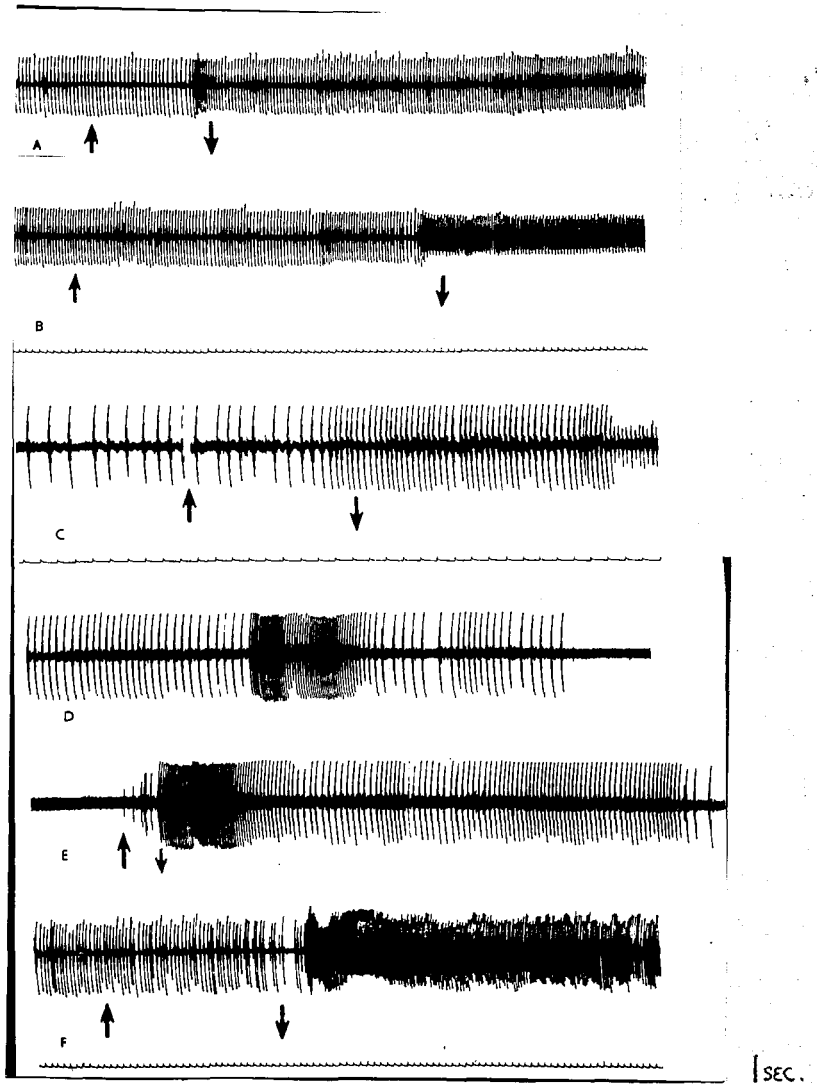


Figure I2.

Figure I2 shows more non-driven responses which lead to both physiological and pharmacological effects. A). and B).; D). and E).; are from the same neurones; C). and F). are from different neurones.

A). Stimulation via the left pallial nerve caused physiological acceleration.

B). Stimulation via the right cervical nerve caused pharmacological acceleration.

C). Stimulation via the right cervical nerve was initially physiological but later became pharmacological.

D). This was a spontaneous burst of activity.

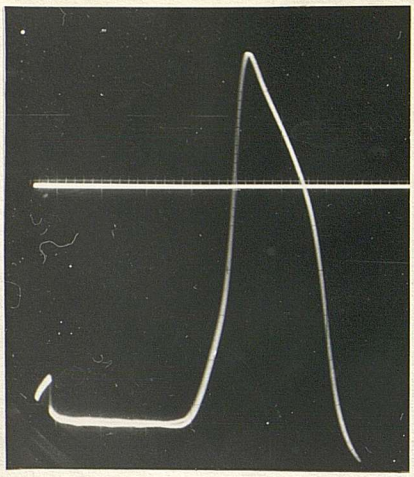
E). This was a burst of activity induced by stimulating the left pallial nerve.

F). This shows an inhibitory pause followed by pharmacological acceleration after stopping stimulation of the right pallial nerve.

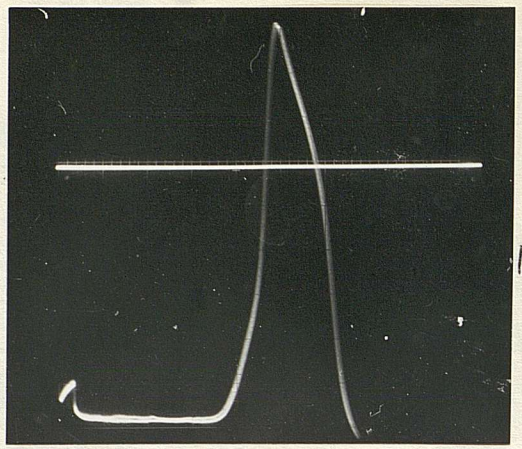
The same cell can exhibit both a physiological and pharmacological acceleration effect depending on the nerve stimulated. Cell No. 235 was spontaneously active, but could not be driven. Stimulation of the left pallial nerve with a voltage of 4V caused slight acceleration, figure 12A. This was accompanied by an increase in the duration of the action potential. The action potential sobn returned to its normal shape. Stimulation of the right cervical nerve at the same voltage also accelerated the activity, but this lead to a more drastic pharmacological response, figure 12B. The cell did not recover from this stimulation. This stimulation probably released a pharmacological concentration of chemical transmitter. In another cell (No. 229), stimulation of the right cervical nerve produced an acceleratory effect which gradually became pharmacological, figure 12C. This cell had action potentials of 2 heights, figure 10C. As has already been mentioned, the activity of some cells occurred in bursts, figure 12D. This burst could be induced by stimulating the left pallial nerve, figures 10D, 6 and 12E. The action potential in this case had a very large positive afterpotential, figure 10E.

CESSATION OF STIMULATION

Stopping the stimulation to a cell may have more effect on the spontaneous activity than the actual stimulation itself. An example will be described. No. 161 was an active cell in the visceral ganglion with a rate of 2/sec. action potentials. Stimulating the anal nerve with a voltage of 5.6V, drove full action potentials with a delay of 36msec. The driven action potentials did not affect the rate of the spontaneous activity. Thus, the spontaneous activity, if it was due to synaptic action, was being stimulated via another nerve. Stimulating the right pallial nerve with a voltage of 4.5V partially inhibited the activity. When this stimulation was stopped, there was a short inhibitory period and then the rate of the activity greatly increased, figure 12F. The rate of activity slowly fell, but did not return to its previous level. The height of both the action and resting potentials fell, and the duration of the action potential increased. These did not return to normal. Later on restimulating the right pallial nerve, the rate again increased, but the action potentials were replaced by small depolarizations. Gradually both bioelectric potentials of the cell decreased in size and finally the cell depolarized to zero. This was an extreme response which is only



A.



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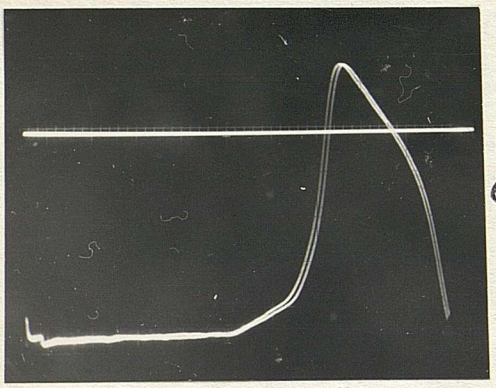
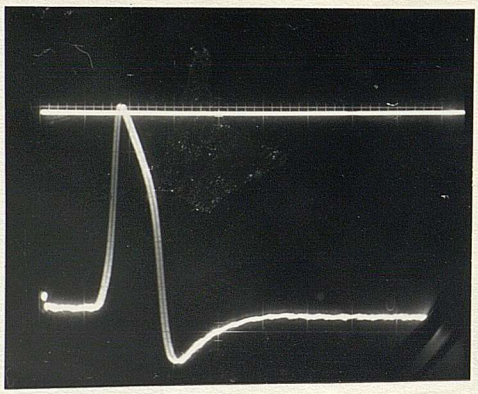


C.



D.

Figure I3.

A, B, C, ⊢ 10 msec. [10 mV

D ⊢ 20 msec.

Figure I3 shows that the action potential shape can change firstly during the stimulation of a given nerve trunk and secondly from one nerve trunk to another. In the latter case the resting potential was also reduced and this was a pharmacological response from which the cell failed to recover.

A). Shows the shape of the potential on stimulating the right pallial nerve. The duration of the potential was 26msec.

B). Later on stimulating the same nerve as in A). with the same voltage, the duration of the potential had increased to 32msec.

C). Shows the shape of the potential on stimulating the intestinal nerve with the same voltage as in A). and B). The duration of the potential, including the prepotential, was 47msec. The normal action potential of the cell failed to recover.

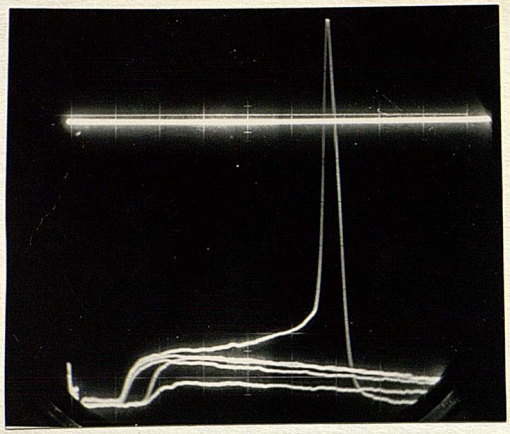
D). Shows the shape of the potential on restimulating via the right pallial nerve. The duration of the potential was approximately 57msec.

mimicked by acetylcholine 10^{-3} g/ml.

CHANGE IN SHAPE OF DRIVEN POTENTIAL WITH TIME

Driven action potentials can change their shape with time. Generally they increase in duration and often fall in height. This is termed a pharmacological type of potential. Two experiments will be cited.

The first example (No. 202) occurred in the right parietal ganglion and was spontaneously active with a rate of 1.5/sec. Stimulating the right pallial nerve with a voltage of 4.5V inhibited the spontaneous activity and drove the cell orthodromically. The delay was 38msec, figure 13A. The response was all or none; there were no e.p.s.p.'s and no adaptation. During the course of driving the cell, the action potential changed in shape, figure 13B. Stimulating the intestinal nerve with a voltage of 9.0V drove the cell orthodromically with a delay of 45msec, figure 13C. In this case there was no inhibition of the spontaneous activity, no adaptation, and no e.p.s.p.'s. The response was all or none. There was a difference in shape between the two driven action potentials. The potential driven via the intestinal nerve had a longer duration and a reduced overshoot. The resting potential was also reduced. The cell did not



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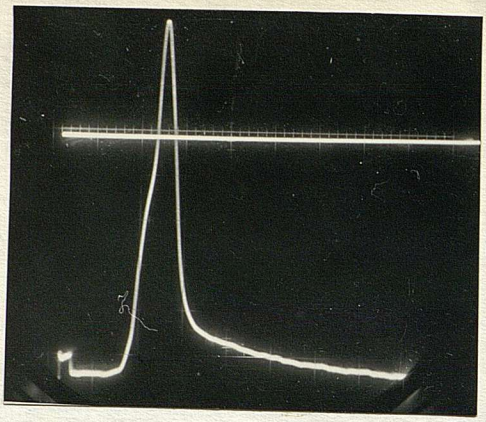

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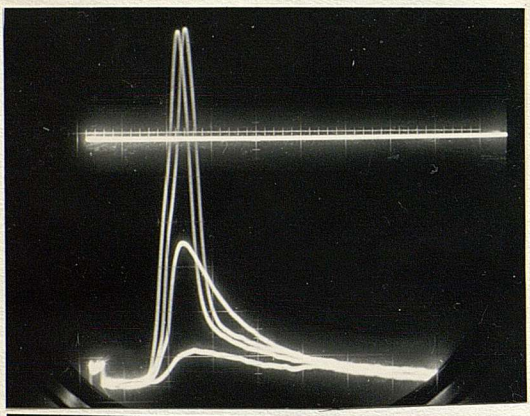

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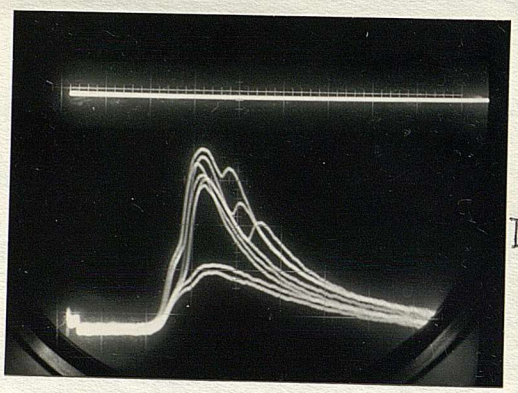

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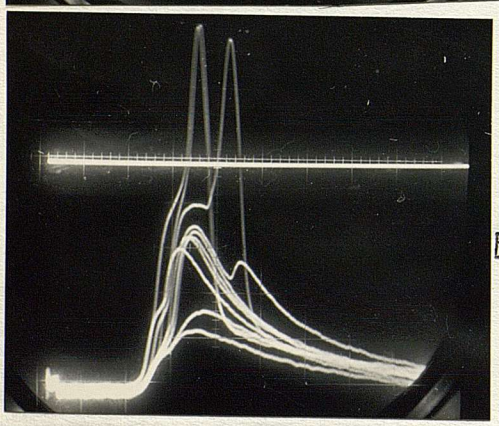

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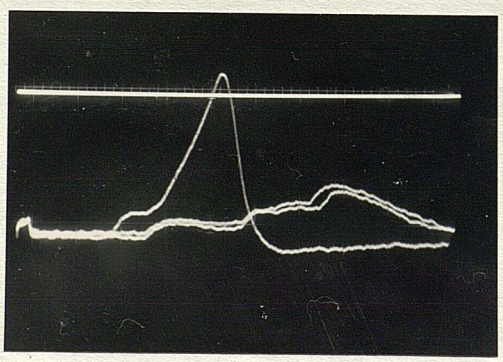
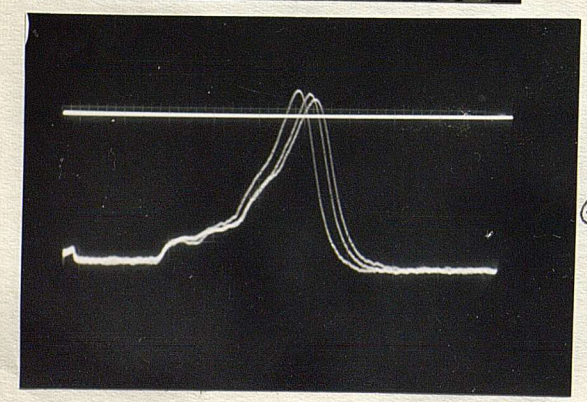
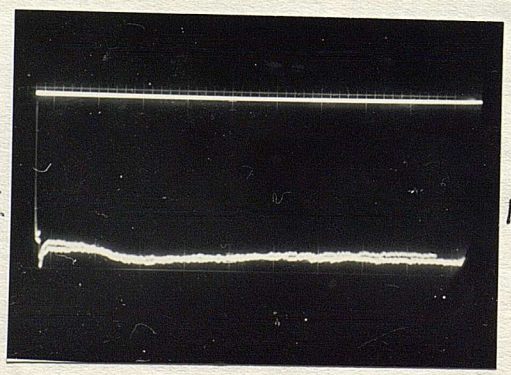


F.



G.



H.

Figure 14.

20 msec.

[10 mV

Figure 14 shows examples of both facilitation and summation of e.p.s.p.'s to form full action potentials.

A). Shows facilitation of e.p.s.p.'s to form full action potentials on stimulating the anal nerve. The delay time was 20msec and the duration of the action potential was about 20msec.

B). Another cell shows full action potentials without any summation or facilitation.

C)., D). and E). are further examples of a cell showing facilitation and also summation.

F). and G). are examples of summation to form pseudopotentials and full action potentials. The duration of the complete potential in G). is about 80msec. Stimulation is via the intestinal nerve.

H). Shows the shape of i.p.s.p.'s stimulated via the right pallial nerve. The height of the potential was 2mV.

A). ; B)., C)., D). and E). ; F)., G). and H). are from the same neurones.

recover from the intestinal nerve stimulation, and on restimulating via the right pallial nerve the driven potential was greatly altered, figure 13D. The action potential had a large positive afterpotential.

THE SUMMATION OF e.p.s.p.'s TO FORM PSEUDOPOTENTIALS AND ACTION POTENTIALS

Figure 14A shows the response to stimulating the anal nerve with a pulse voltage of 12V. This cell, No. 228, was a resting cell with a resting potential of -71mV. The driven e.p.s.p.'s facilitated to give full action potentials This was temporal facilitation. These action potentials quickly adapted and diminished to e.p.s.p.'s. On raising the voltage to 14V the e.p.s.p.'s again facilitated to produce full action potentials. The delay time was independent of the voltage applied. This was an antidromic stimulation, the nerve cell showing a very long prepotential. It was an acetylcholine sensitive cell. It was also possible to stimulate this cell orthodromically via the left pallial nerve with a voltage of 12V.

The next cell to be considered was also silent with a resting potential of -58mV. The cell was stimulated

orthodromically with a voltage of 5.0V. At first full action potentials could be driven with a delay of 22msec, figure 14B. The cell then began to adapt and e.p.s.p.'s were initially driven which facilitated to first pseudopotentials and then full action potentials, figure 14C. Later in the experiment both facilitation and summation occurred during the formation of a full potential, figures 14D and E. It would appear that facilitation preceded summation in this cell. The full action potentials had a negative afterpotential.

In cell No. 163, the intestinal nerve was driven with a voltage of 6.0V full action potentials and pseudopotentials could be driven, figure 14F. As can be seen in the figure, e.p.s.p.'s summated to produce a pseudopotential. This was a silent cell with a resting potential of -35mV. In figure 14G can be seen the summation of at least three e.p.s.p.'s to form a full action potential. It would appear that often two e.p.s.p.'s would sum to form a pseudopotential and a third one would trigger the full potential. This response depended on the state of the membrane since it is seen in the first figure that similar summation may only form a pseudopotential. This cell did not show obvious facilitation. The cell did not adapt. Stimulating the righ pallial nerve with a voltage of 9.0V drove small i.p.s.p.'s, figure 14H. These quickly adapted.

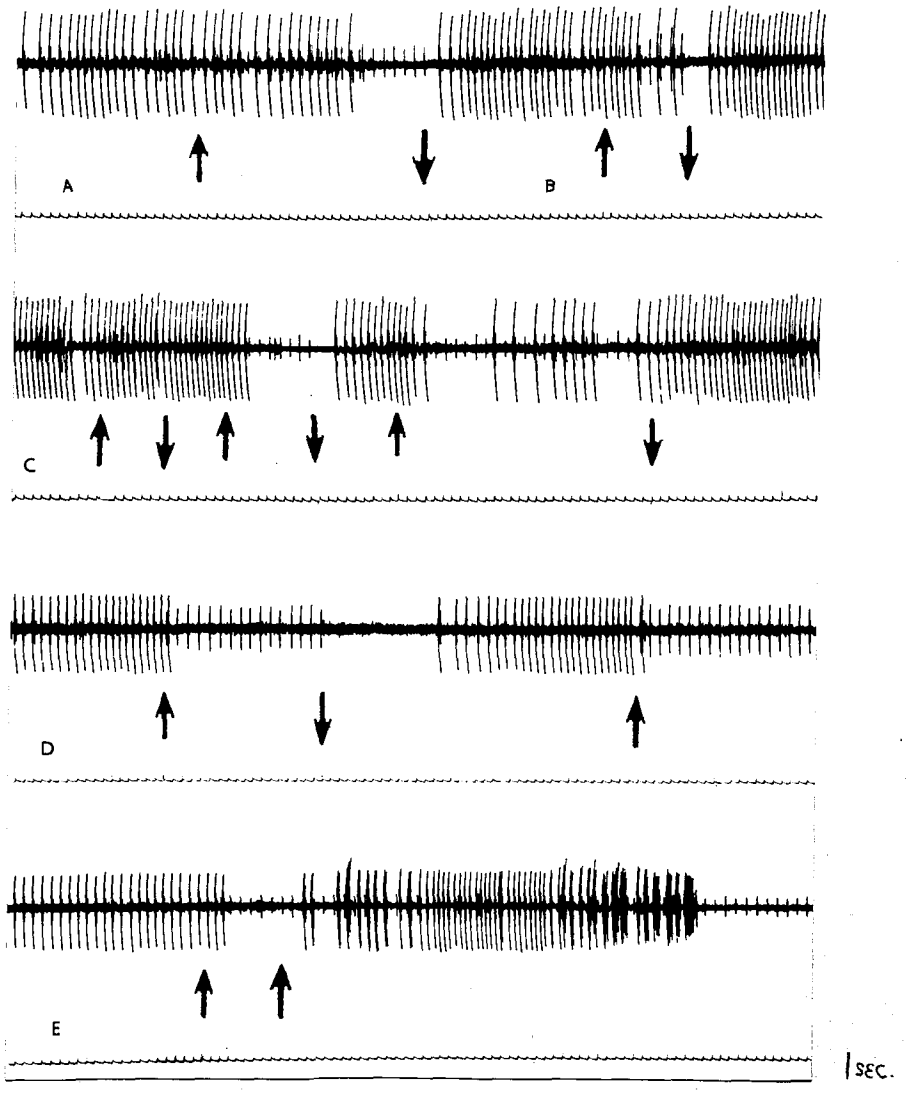


Figure I5.

Figure I5 shows inhibitory responses on stimulation in the absence of i.p.s.p.'s. In the trace the height of the stimulus artifact is never more than half the height of an action potential.

A). Shows inhibition on stimulating the right pallial nerve.

B). Shows inhibition on stimulating the left pallial nerve.

C). Shows inhibition on stimulating the left pedal nerve.

D). Shows inhibition on stimulating the left pedal nerve.

E). Shows inhibition on stimulating the left pedal nerve. This inhibition was blocked by the addition of nicotine, which later had a pharmacological effect on the cell activity.

A), B)., and C).; D). and E). are from the same cell.

TYPES OF INHIBITION

Inhibition can occur in three forms:-

(1) cessation of activity without a noticeable change in resting potential;

(2) a marked hyperpolarization of the resting membrane;

(3) driven i.p.s.p.'s.

Figures 15A, B and C show the result of an experiment of cell No. 207-2 in which stimulation of the right pallial nerve, the left pallial nerve, and the left pedal nerve inhibited spontaneous activity. There were no i.p.s.p.'s and no change in the resting potential of the cell. The inhibitory effect fell off with time, adaptation, and was not blocked by nicotine.

This type of inhibition was found to occur several times. In the case of cell No. 201, where there was inhibition but no i.p.s.p., inhibition lasted for up to 13sec after the cessation of stimulation, figure 15D. There was, however, some hyperpolarization of the cell, the resting potential rose from 40 to 48mV. Nicotine blocked the inhibition, figure 15E.

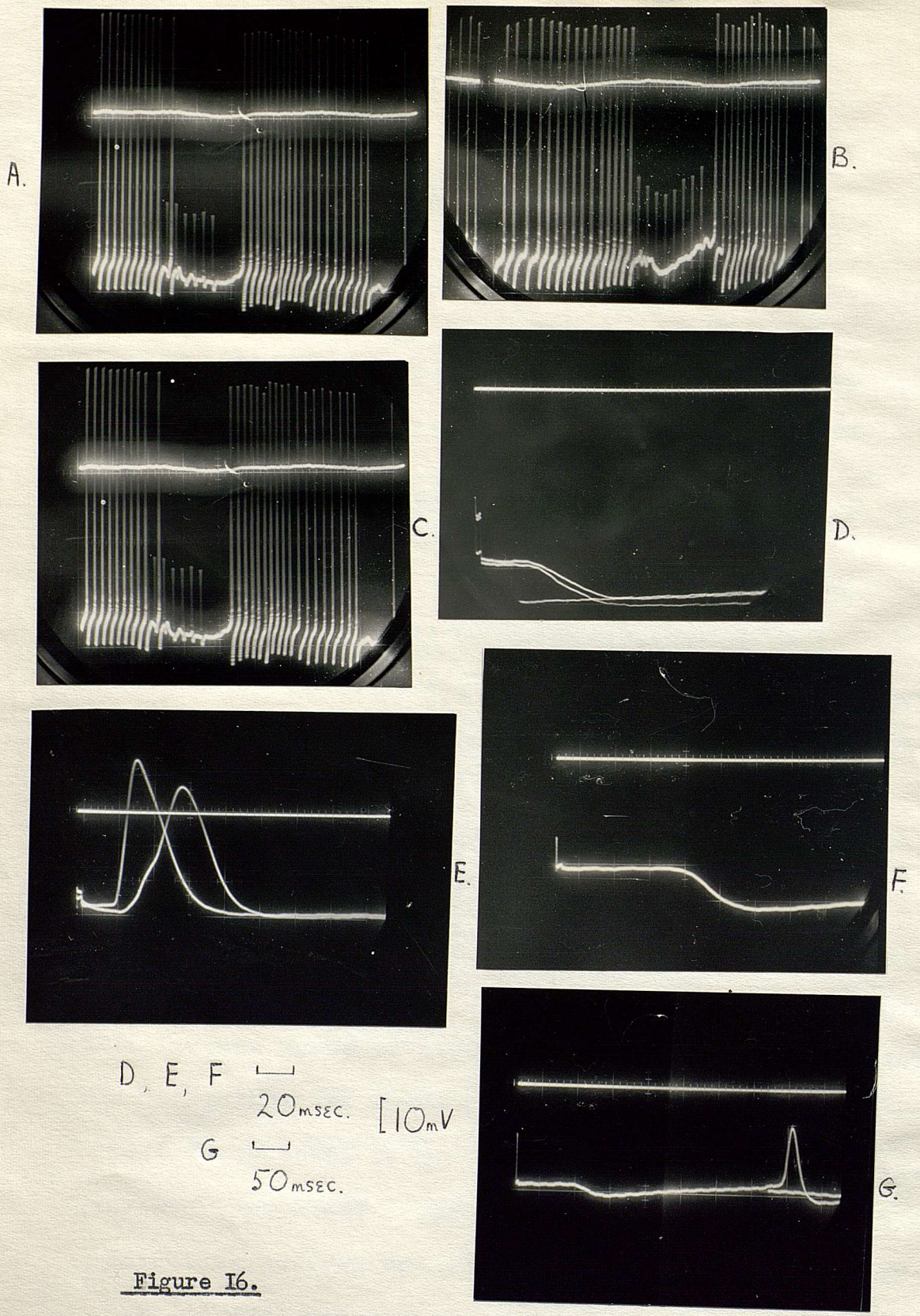


Figure I6.

A)., B). and C). ; D). ; E)., F). and G). are from the same neurones.

Figure 16 shows induced inhibition accompanied by i.p.s.p.'s.

A). The first group of stimulations via the left pallial nerve, each stimulation produces an i.p.s.p. The inhibition lasted for about 4 sec. after the stopping of the stimulation.

B). The second burst of stimulation via the same nerve. The induced i.p.s.p.'s were quickly replaced by a sudden hyperpolarization of 3mV which then gradually adapted.

C). The third burst of stimulation via the same nerve, from which the cell adapted and the activity returned during the stimulatory period. In this case there were neither i.p.s.p.'s nor a marked hyperpolarization.

D). Shows the shape of a driven i.p.s.p. on stimulating the right pallial nerve. The i.p.s.p. had a hyperpolarization of 10mV.

E). Shows the shapes of driven and spontaneous action potentials. The driven action potential had a duration of 50msec and the spontaneous potential a duration of 74msec. The height of the former was 40mV and of the latter 35mV.

F). Shows the shape of an i.p.s.p. driven by stimulating the same nerve as in E). but later in the experiment. The height was 10mV.

G). Shows a driven i.p.s.p. followed approximately 150msec later by a small action potential. The cell was beginning to run down (the resting potential was beginning to deteriorate).

I.P.S.P.

Cell No. 220 was a spontaneously active cell. Stimulating the left pallial nerve at a voltage of 6.0V, completely inhibited the spontaneously activity, figure 16A, which gradually adapted. The method of inhibition was of interest. Figure 16A shows inhibition involving at each stimulus an i.p.s.p. together with a gruadual hyperpolarization. When the stimulation was stopped, the RP slowly depolarized and the activity continued. When the same voltage was reapplied, there was a much more marked hyperpolarization which gradually adapted, figure 16B. On applying the same voltage for the third time, there was neither a marked hyperpolarization nor i.p.s.p.'s, and the activity returned while the nerve was still being stimulated, figure 16C. The final inhibition lasted for 8-10sec.

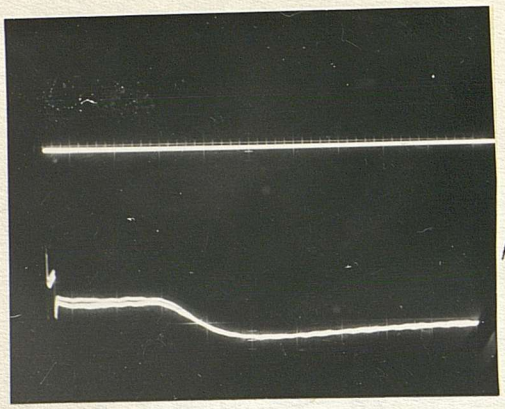
When stimulating the right pallial nerve was stopped, there was an increase in the rate of activity. This cell was insensitive to acetylcholine.

Cell No. 197 was a silent cell with a resting potential of -41mV. Stimulating the intestinal nerve with a voltage of 5.6V, drove pseudopotentials with a delay of 40msec. There was no adaptation. Stimulating the right pallial nerve with a voltage of 5.0V it was possible to drive i.p.s.p.'s.

These were accompanied by a hyperpolarization to -53mV, figure 16D. These gradually adapted and the resting potential depolarized towards its previous level.

In stimulating the intestinal nerve with a voltage of 5.6V drove full action potentials in cell No. 148, with a delay of 20msec. There were no e.p.s.p.'s and no adaptation. Both driven action potentials and spontaneous ones occurred, figure 16E. The height of the spontaneous action potential was less than the driven potential. Later stimulating on the same nerve with the same voltage inhibited the activity and drove i.p.s.p.'s, figure 16F. The delay for the i.p.s.p.'s was 40msec. These adapted to driven action potentials again with a delay of 30msec. With the same voltage, width and frequency, stimulating the same nerve could produce a variety of responses. On raising the voltage to 9.0V, i.p.s.p.'s and inhibition again occurred. The value of the resting potential prior to a driven action potential or i.p.s.p. was the same. At the higher voltage the cell again adapted and the i.p.s.p.'s were replaced by action potentials. It was possible to find a driven i.p.s.p. followed by an action potential, figure 16G. Toward the end of the experiment the cell began to run down.

In another cell (No. 202) the driven i.p.s.p.'s did not interfere with the spontaneous activity. On stimulating



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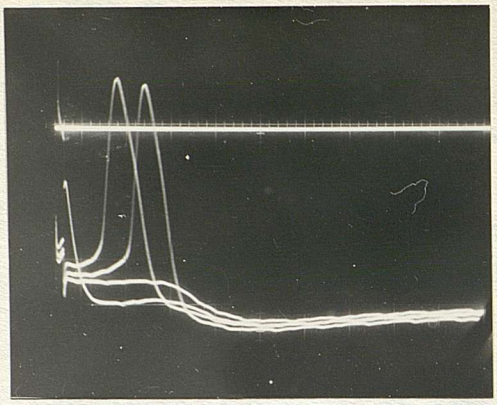

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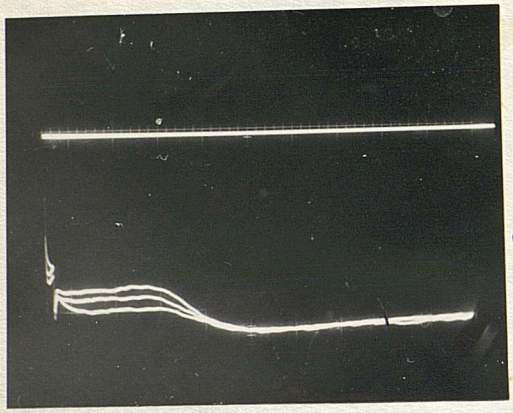

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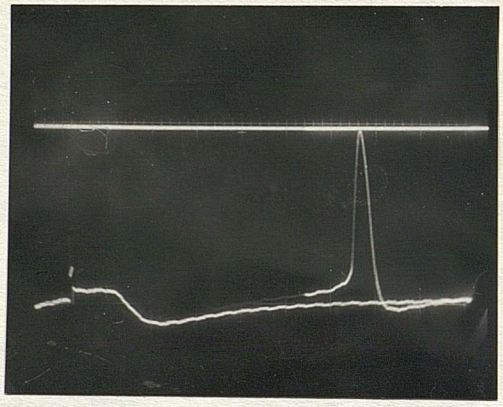

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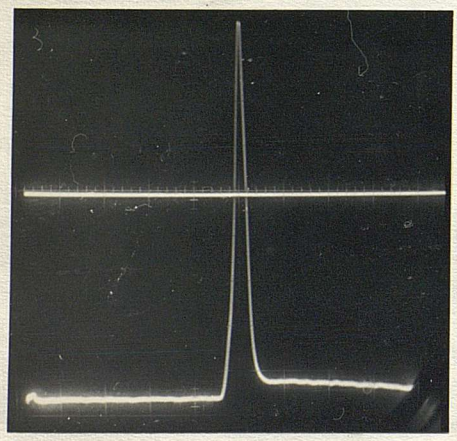

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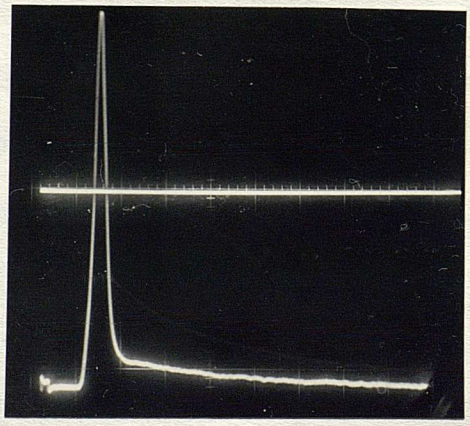


F.

Figure 17.

[10m V.

A, B, C. ⌊ 20 msec. D. ⌊ 50 msec. E, F. ⌊ 10 msec.

Figure I7 shows more inhibitory phenomena associated with i.p.s.p.'s. Both i.p.s.p.'s and action potentials occurred together. E). and F). are records from the same neurone stimulated via the same nerve trunk at two points. The difference in delay time can give an indication of the rate of conduction along the nerve.

A). Shows the shape of the i.p.s.p. induced by stimulating the right pallial nerve. The time delay was 48msec and the height was 8mV. The duration of the potential was over 200msec.

B). Shows the presence of both i.p.s.p.'s and full action potentials.

C). Shows three superimposed i.p.s.p.'s each smaller as the resting potential hyperpolarized.

D). Shows both driven i.p.s.p.'s and spontaneous action potentials. The duration of the i.p.s.p. was 230msec.

E). and F). Shows the delay time in response to stimulating at two different points along the intestinal nerve.

A)., B)., C). and D). ; and E). and F). are from the same neurones.

the right pallial nerve at a voltage of 4.0V, i.p.s.p.'s were driven, figure 17A. Figure 17B shows that while i.p.s.p.'s were being driven the spontaneous activity still continued. The size of the i.p.s.p. would appear to be associated with the resting potential level, figure 17C. From the figure it can be seen that as the resting potential was hyperpolarized, so the size of the i.p.s.p. was reduced. Figure 17D also shows a driven i.p.s.p. and a spontaneous action potential. The cell did not adapt to the stimulation.

RATE OF CONDUCTION ALONG PERIPHERAL NERVE

In some experiments it was possible to place two sets of stimulating electrodes on the one nerve trunk. From the difference in the delay time between the stimulus artifact and the action potential, and knowing the distance between the two sets of electrodes, it was possible to calculate the rate of conduction along the nerve. An example is shown in figures 17 E and F, where the electrodes were 16mm apart and the difference in the delay time was 76msec. This gave a rate of conduction of 21cm/sec.

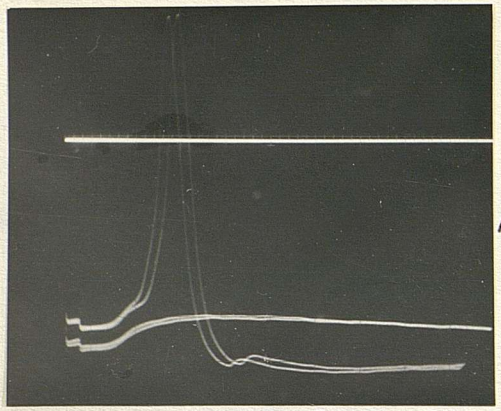
Though there was some scatter, there appeared in general to be two peaks of conduction, one fast and one slow. These are shown in the following table:-

NERVE	FAST RATE ANTI-	SLOW RATE ORTHO- DROMIC CONDUCTION
Intestinal n.	70cm/sec.	30cm/sec.
Anal n.	70cm/sec.	30cm/sec.
Left Pallial n.	100cm/sec.	30cm/sec.
Right Pallial n.	100cm/sec.	30cm/sec.
Pedal nerves	-	30cm/sec.

The fast might be associated with antidromic conduction whilst the slow rate could be due to orthodromic conduction and the passage of the action potential over a synapse. In many of the slow cases, the stimulation gave rise to an e.p.s.p. which summated to form a full action potential. The pallial nerves conduct at a faster rate than the intestinal and anal nerves.

RECORDING FROM DIFFERENT AREAS OF THE SOMA

The following experiment would support the idea that the shape of the action potential is partially dependent on the point of recording in the soma. Cell No. 139 was active with rate of action potentials of 4/sec. The cell



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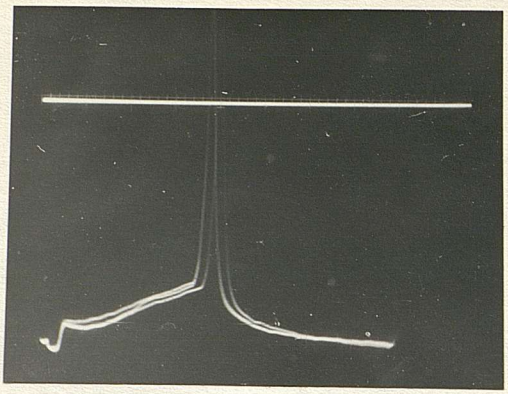

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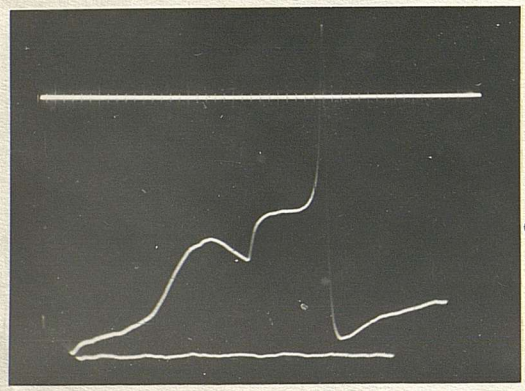

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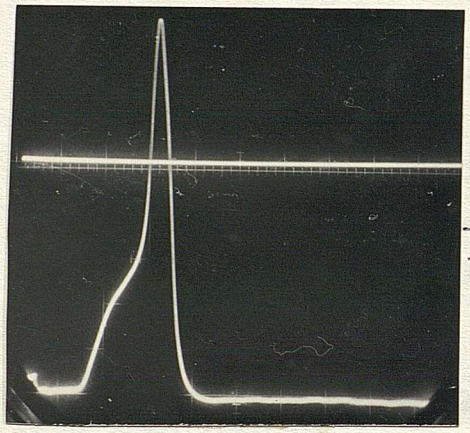

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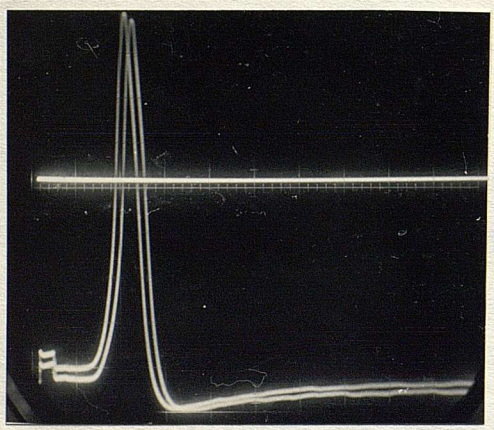

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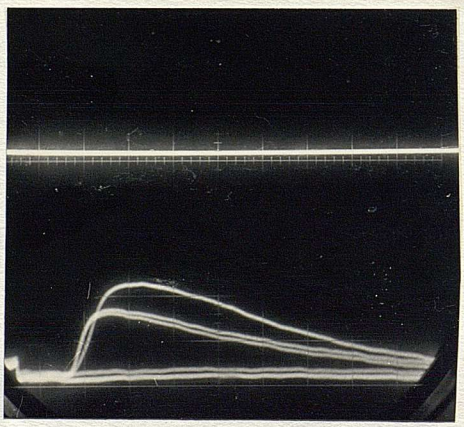


F.

Figure I8.

A, D, E, F ⌊ 10msec B, C. ⌊ 20msec. [10mV.

Figure I8 shows that the shape of the action potential and afterpotential varies depending on the point of recording in the soma. E). and F). are from another cell which adapted from full action potentials to e.p.s.p.'s.

A). Shows the shape of the action potential and afterpotential in response to orthodromic stimulation via the left pallial nerve. The e.p.s.p. has a slow rise time. The delay time is 7msec. There is a small depolarizing potential during the afterpotential. This was absent when recording from another area of the soma.

B). Shows the shape of the action potential in response to antidromic stimulation via the right pallial nerve. Here there is a sharp rise time and a delay time of 5msec.

C). Shows the shape of a full action potential induced during the recovery time of a pseudopotential. The action potential probably synapsed via a synapse different from the one which induced the pseudopotential.

D). Shows the shape of an antidromic action potential driven on stimulating the intestinal nerve. The delay time is IOmsec.

E). Shows the same action potential as in D). but 6 sec. later.

F). Shows that the action potentials of D). have adapted to pseudopotentials. F). was filmed I2 sec. after D).

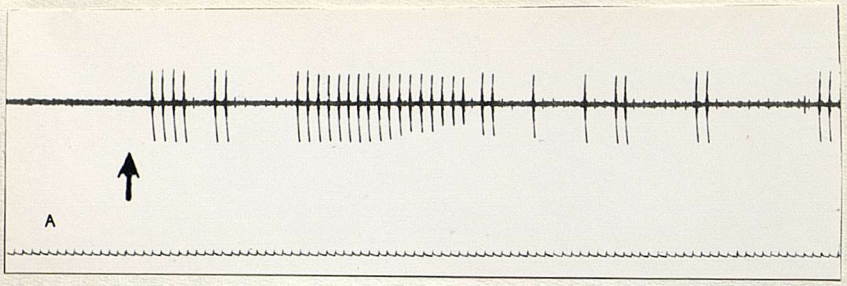
A) and B). ; C). ; and D)., E). and F). are the same neurones.

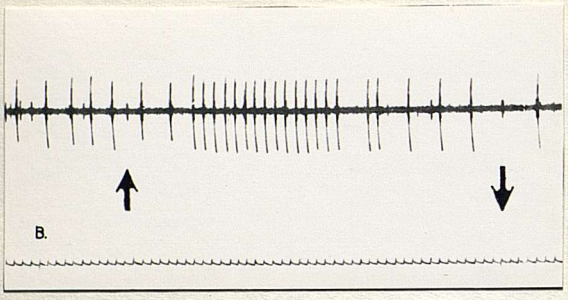
was located in the visceral ganglion. It could be driven orthodromically via the right pallial nerve with a stimulus voltage of 4.5V, and a delay of 8msec. The cell gave both e.p.s.p.'s and full action potentials, figure 18A. The latter had an interesting depolarization following the positive afterpotential. The cell did not adapt. Later the microelectrode came out of the cell. The cell was repenetrated and could be driven antidromically via the left pallial nerve with a stimulus voltage of 3.3V. The action potential was of normal shape, figure 18B. The delay was 6msec, and there were no e.p.s.p.'s. The response was all or none. An adjacent cell showed a good example of summation, figure 18C.

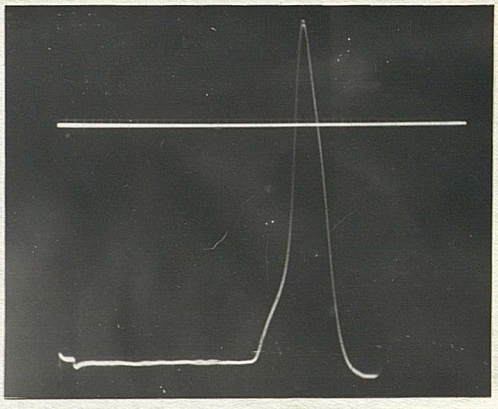
DRIVEN CELLS WHICH ADAPTED WITH TIME

Cells which could be driven antidromically or orthodromically adapted with time. The adaptation of the full action potentials could either be to e.p.s.p.'s or a complete adaptation where the stimulus had no effect on the cell. In either case these cells were usually silent.

Antidromic stimulation of the intestinal nerve with a voltage of 4.0V drove full action potentials in a silent

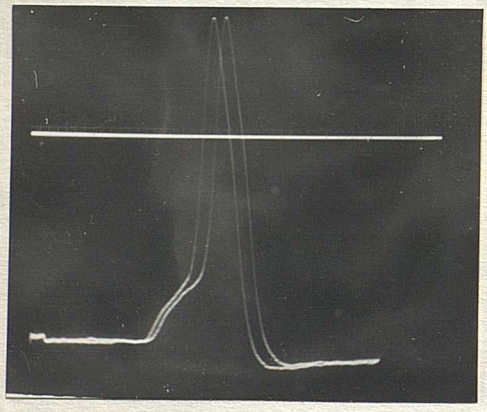


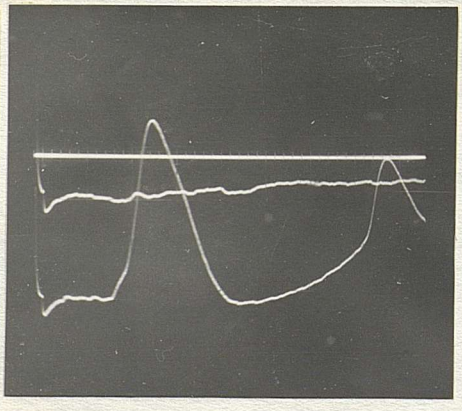




C.

D.





E.

Figure I9.

C, D ⌐¬ 10 msec. E ⌐¬ 20 msec. [10 mV.

Figure I9 shows another cell which quickly adapted when driven; above a certain voltage input via the stimulated nerve the cell ceased to adapt.

A). Shows that the stimulation of the intestinal nerve drove full action potentials which adapted.

B). Stimulation of the left branch of the left pallial nerve drove full action potentials which adapted.

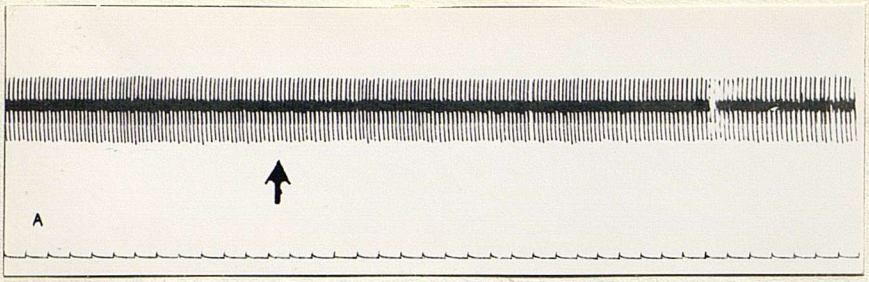
C). Shows the shape of the action potential driven orthodromically in A). The time delay was 38msec.

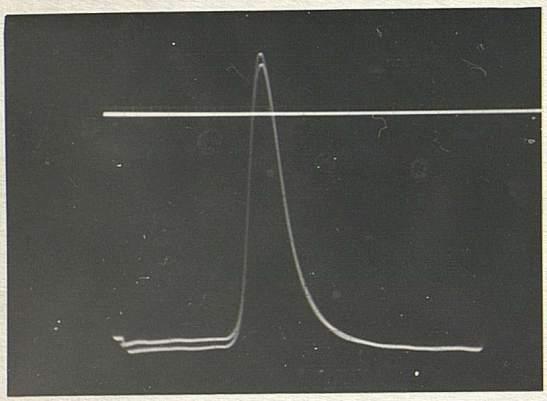
D). Shows the shape of the action potential driven in B). The delay time was 22msec. This delay time was rather long for antidromic stimulation.

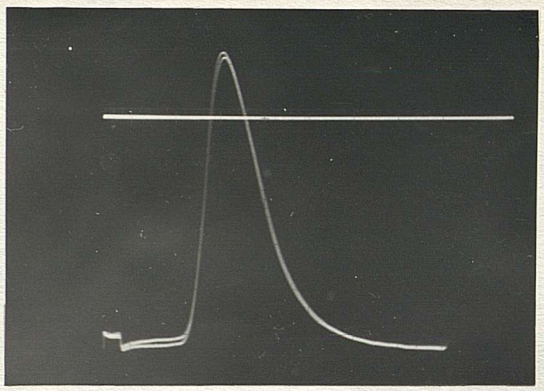
E). Shows the effect of nicotine on the driven action potential and on the resting potential of the cell.

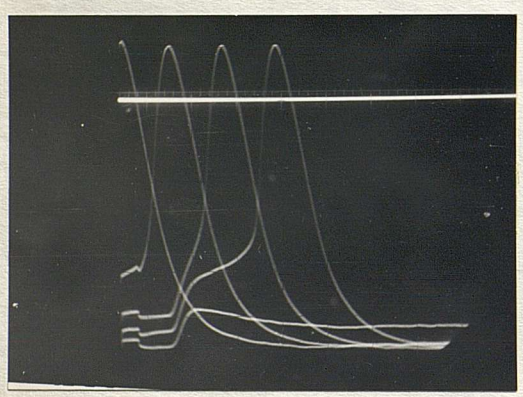
cell, No. 152, with a resting potential of -56mV, figure 18D. Initially the action potential was preceded by a soma potential but this disappeared, figure 18E. After 7sec the full action potential was replaced by e.p.s.p.'s which facilitated to form small pseudopotentials, figure 18F. On raising the voltage the full potentials could again be driven for a few seconds.

It is possible by varying the voltage to change an adapting cell into a non-adapting cell. The presence or absence of e.p.s.p.'s can depend on the nerve stimulated. The type of response obtained on stimulation would appear to depend on the properties of the synaptic membrane stimulated. It is possible that certain synaptic membranes are capable of graded responses while others are not. Cell No. 206 in the visceral ganglion had a resting potential of -66mV. Orthodromic stimulation of the intestinal nerve with a voltage of 3.3V drove full action potentials, figure 19A and C. The response was all or none, there were no e.p.s.p.'s, and the cell quickly adapted. Raising the voltage to 3.9V redrove the cell for 21sec before it adapted. At a voltage of 4.7V there was no adaptation. Stimulating the left fork of the left pallial nerve with a voltage of 4.5V, the cell was also driven orthodromically, figures 19B and D. Here it may be noted that the full action potential threshold









B, C, D. 10 msec. [10 mV.

Figure 20

Figure 20 shows the response from a cell which could be driven on three nerves and which did not adapt on any of them. In this preparation it took 7msec to cross from the right to the left parietal ganglion.

A). Stimulation of the anal nerve drove full action potentials which did not adapt, summate or facilitate.

B). Shows the shape of the action potential driven orthodromically via the anal nerve in A). The delay time was 22msec.

C). Shows the shape of the action potential driven orthodromically via the right pallial nerve. The delay time was I3msec.

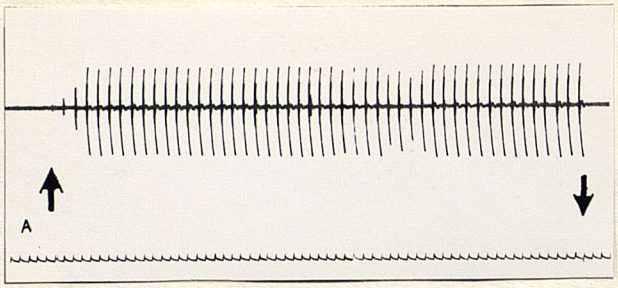
D). Shows the shape of the action potential driven antidromically via the left pallial nerve. Here there are e.p.s.p.'s and axon-soma prepotentials of the full action potentials. The delay time was 7msec.

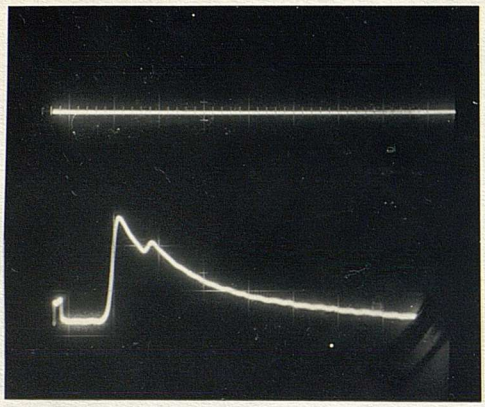
would appear to be less than when the intestinal nerve was stimulated. The resting potential is lower in this case. There were no e.p.s.p.'s and the cell adapted slowly. Later in the experiment stimulating via the same nerve it was possible to drive e.p.s.p.'s as well as full action potentials, these e.p.s.p.'s summed to form full action potentials. On the addition of nicotine there was no effect for 75sec and then the resting potential and driven action potential fell in height, figure 19E. There was no blockage of the driven action potential.

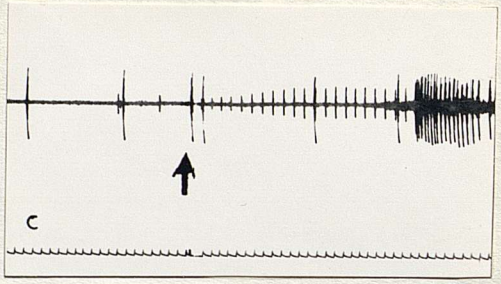
DRIVEN CELLS WHICH FAILED TO ADAPT WITH TIME

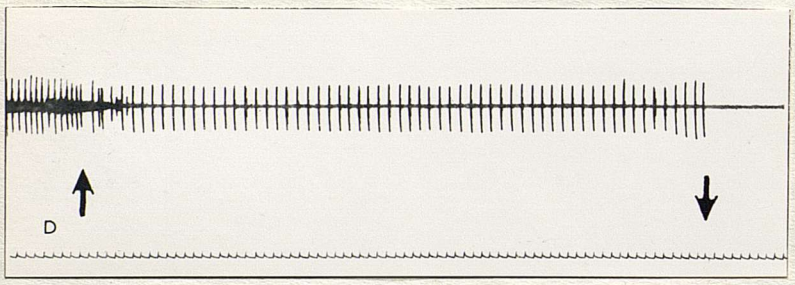
The majority of the cells which did not adapt to being driven were spontaneously active. Some cells could be driven both orthodromically and antidromically without showing adaptation.

The cell to be described (No. 210) could be driven by three nerves. This was a cell in the left parietal ganglion with a rate of activity of 6/sec. Orthodromic stimulation of the anal nerve with a voltage of 3.9V drove the cell, figure 20A and B. Each stimulus drove one full action potential, there were no e.p.s.p.'s and no adaptation. The









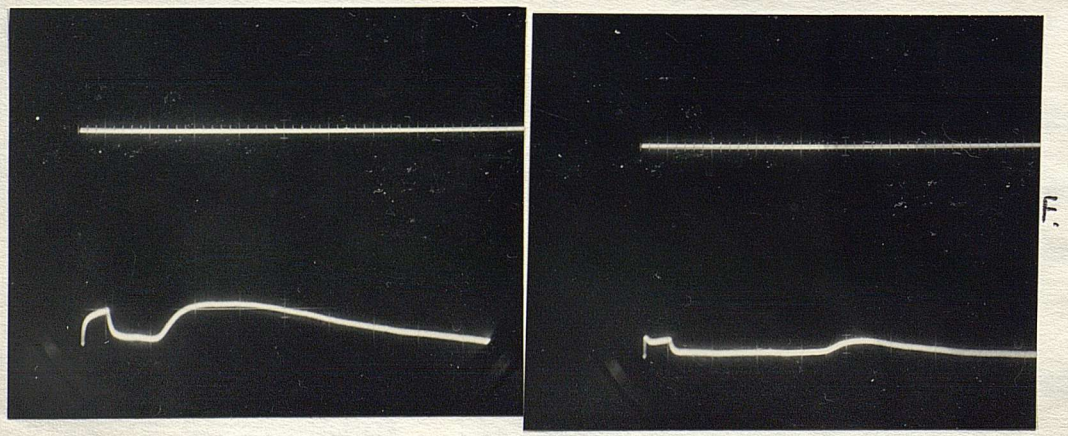


Figure 21.

B. ⌐ 20 msec. E, F. ⌐ 5 msec. [10 mV.

Figure 2I shows that driven pseudopotentials and e.p.s.p.'s may also fail to adapt. In these cells it was impossible to induce full action potentials.

A). Stimulation of the intestinal nerve drove a pseudopotential which did not adapt.

B). Shows the shape of the pseudopotential of A).

C). Stimulation of the intestinal nerve initially inhibited and then accelerated the spontaneous activity.

D). Stimulation of the left pallial nerve drove an e.p.s.p. which did not adapt.

E). Shows the shape of the antidromically driven e.p.s.p. of D). The time delay was 5msec.

F). Shows the shape of the orthodromically driven e.p.s.p. via the left pedal nerve. The time delay was I8msec.

A). and B).; and C)., D)., E). and F). were the same neurones.

delay was 26msec. With the same voltage it was also possible to orthodromically drive full action potentials via the right pallial nerve, figure 20C. The delay was 15msec. Later it was possible to antidromically drive the cell, via the left pallial nerve with a voltage of 3.3V here there were occasional e.p.s.p.'s, figure 20D. There was a delay of 8msec. There was no adaptation. Allowing for the rate of conduction along the right and left pallial nerves to be the same, then it took 7msec for the orthodromic impulse to pass through the ganglionic mass from the right to the left parietal ganglion. The axon-soma prepotential of the antidromic potential can be seen in the figure.

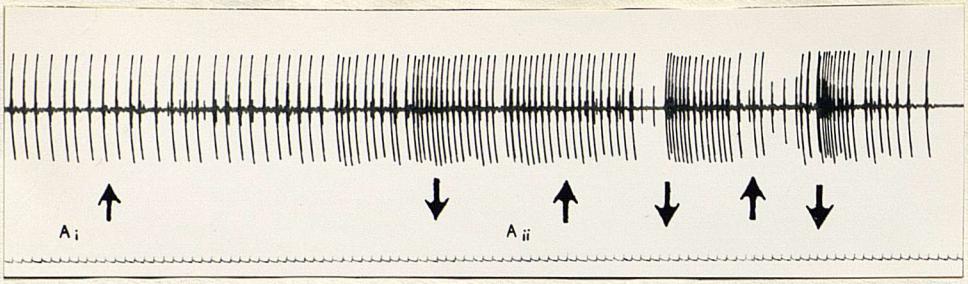
Pseudopotentials can also fail to adapt. Cell No. 151 was in the right parietal ganglion with a resting potential of -62mV. Stimulating the intestinal nerve with a voltage of 5.0V drove pseudopotentials with a delay of 20msec, figure 21A. There was no adaptation, and neither e.p.s.p.'s nor full action potentials could be driven. The pseudopotential had an interesting second peak superimposed on the recovery period, figure 21B. This might be due to a postsynaptic potential stimulated via another pathway. The sharp rise time of the pseudopotential indicates antidromic stimulation but the delay time was rather long.

E.p.s.p.'s can also fail to adapt. Cell No. 139 was

active in the left parietal ganglion. Stimulating the intestinal nerve with a voltage of 4.0V initially inhibited the activity and then accelerated it, figure 21C. Antidromically stimulating the left pallial nerve with a voltage of 3.3V drove e.p.s.p.'s with a delay of 6msec, figure 21D and E. Increasing the voltage failed to induce either pseudopotentials or full action potentials. The cell did not adapt. Orthodromically stimulating the left pedal nerve with a voltage of 3.3V slowly rising to 6.0V, also drove e.p.s.p.'s with a delay of 19msec, figure 21F. No action potentials could be induced and there was no adaptation.

SYNERGISTIC RESPONSES BETWEEN IPSI- AND CONTRALATERAL NERVES

In many animals there is a synergistic-antagonistic relationship between ipsi- and contralateral nerves. The functional significance of this may be found in the necessity to have antagonistic muscle movements when an animal, for example, turns to the left or to the right. The precise functional situation in the parietal ganglia is not clear, but it was thought worth while investigating the reactions of the cells in the ganglia to stimulation of the ipsilateral and contralateral nerves.



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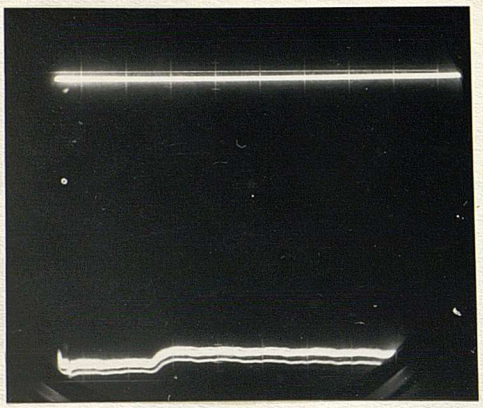

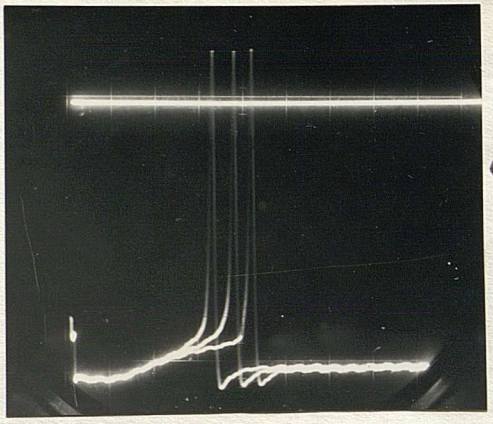

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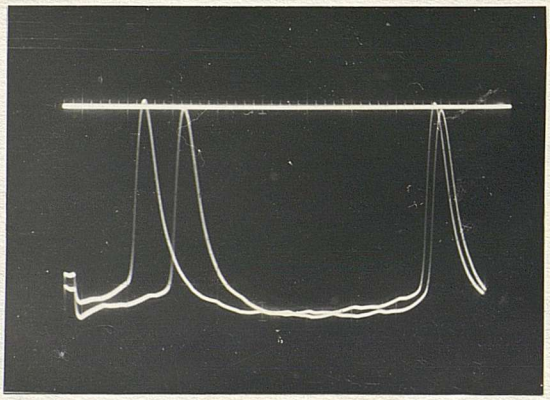

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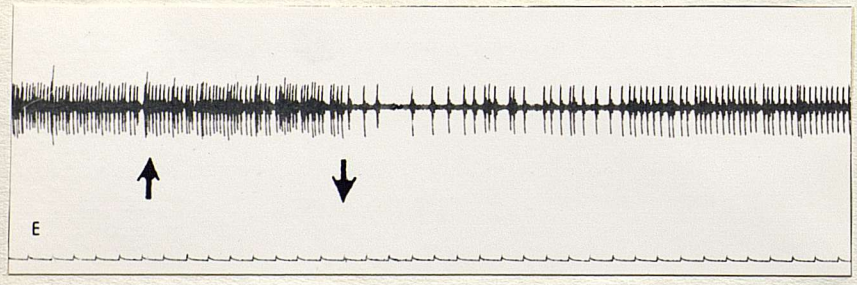


1 sec

Figure 22.

B, D ⌊ 20 msec. C. ⌊ 50 msec. [10 mV.

Figure 22 shows that both nerves of paired nerve trunks may influence the activity of the same cell. The response may be synergistic or antagonistic.

A). (i). Stimulation of the left pallial nerve accelerates the activity while

(ii). stimulation of the right pallial nerve inhibits the activity.

B). Shows the shape of the e.p.s.p. in response to stimulating the right pallial nerve. The delay time is 40msec.

C). Shows the shape of the action potential in response to stimulating the left pallial nerve. The delay time is about 50msec.

D). Shows the shape of the action potential in response to stimulating the left pallial nerve. The delay time is about 10msec.

E). The response on stopping the stimulation to the right pedal ganglion.

A). and B). and C). and D). are from the same neurones.

On stimulating the left pallial nerve with a voltage of 6V, the spontaneous activity of a cell in the right parietal ganglion was accelerated, figure 22A(i). When the right pallial nerve was stimulated at the same frequency, width and voltage, the spontaneous activity was inhibited, figure 22A(ii). This inhibition gradually adapted. There were no i.p.s.p.'s. When stimulation via the right pallial nerve ceased, there was a burst of activity. The cell then became silent. Stimulation of the intestinal nerve at a voltage of 4V induced a burst of activity. These were e.p.s.p.'s.

In another experiment stimulating the right pallial nerve with a voltage of 3.9V drove e.p.s.p.'s of 4mV height and 105msec duration, with a delay of 40msec, figure 22B. Stimulating the left pallial nerve at a voltage of 3.5V drove full action potentials with a delay of 50msec, figure 22C. The response gradually adapted. These were both orthodromic stimulations. The cell responded to acetylcholine by an increase in the rate of the spontaneous activity and the formation of e.p.s.p.'s.

It was also possible to affect the spontaneous activity of the same cells via both pedal and both pallial nerves. Cell No. 181 was in the right parietal ganglion and it had a spontaneous frequency of 6/sec. Stimulation of the left

pallial nerve with a voltage of 4V drove full action potentials with a delay of 40msec, figure 22D. There were no e.p.s.p.'s and the cell did not adapt. The rate of the spontaneous activity was no affected. Stimulating the right pallial nerve with a voltage of 4V also drove full action potentials with a delay of 24msec. The cell did not adapt. On stopping the stimulation of the right pedal nerve with a voltage of 4.5V, the spontaneous activity was inhibited, figure 22E. It gradually recovered. Stimulating the left pedal nerve with a voltage of 3.3V accelerated the activity. This acceleration was inhibited on stopping the stimulation. There was no change in the action potential shape. This acceleration included the appearance of e.p.s.p. type depolarizations. The addition of 5-HTP accelerated this cell.

CONCLUSIONS

These experiments demonstrate certain properties of the neurone membrane of the snail.

(1) It is possible to drive a cell either anti- or orthodromically.

(2) It is possible to distinguish between anti- and orthodromic stimulation.

(3) The driving may or may not affect the spontaneous activity. This spontaneous activity may occur in a variety of forms.

(4) The cell may or may not adapt to stimulation. It is more likely to adapt if the cell is silent. The cell adapts more often to orthodromic than to antidromic stimulation. This may be associated with the fatigue of the synapse.

(5) The driven cell may form e.p.s.p.'s, i.p.s.p.'s, pseudopotentials, or full action potentials. On stimulation it may produce one of these bioelectric phenomena, two or more of them, or none of them.

(6) The cell may exhibit temporal or spatial summation or facilitation.

(7) The size of the i.p.spp.'s is associated with the resting potential size.

(8) There are several types of inhibitory response, that is, inhibition may occur without a noticeable change in the resting potential, it may occur via a hyperpolarization of the resting potential, or it may be associated with i.p.s.p.'s.

(9) There may be synergism between ipsi- and contralateral pairs of nerves or between nerves on the same side of the body.

(10) The shape of the driven action potential depends on the site of recording along the soma.

(11) A cell may produce more than one height of action potential.

(12) The action potential shape may differ depending on the nerve stimulated. This may be associated with the release of different chemicals at the different presynaptic membranes.

(13) The rates of conduction differ in the different nerve trunks.

(14) During the course of an experiment the membrane properties of a cell may vary.

These experiments allow the following inferences:-

1. It is possible to drive a cell so that stimulation via one nerve will bring about normal action potentials, and these can be inhibited by stimulating another nerve.

2. It is possible to drive the cell by stimulating both nerves, the action potentials being of the same shape and nature.

3. It is possible to drive the cell by stimulating both nerves, the action potentials having a different nature and being distinctive for the given nerve.

4. It is possible to obtain full action potentials when one nerve is driven, and e.p.s.p.,pseudopotentials from another nerve.

These results give some indication of the complexity of synaptic connections that must be considered to occur in the snail brain.

THE EFFECT OF DRUGS ON THE BIOELECTRIC POTENTIALS OF HELIX NEURONES

INTRODUCTION

The results to be described are divided into four sections. In the first section the overall effects of the possible transmitter substances examined are described. The positions of most of the cells in this section were not recorded. All the cells occurred in the parietal, pleural, or the visceral ganglia.

In the second section the interactions of drugs have been tested by applying a series of drugs to marked neurones. In the third section the effect of a drug on the same cell in different brains has been examined. Preliminary results are given in this section and further experimentation is hoped to be carried out in the near future.

In section four the effect of four choline esters has been examined.

SECTION ONE

Over 300 neurones have been examined for their response to different possible chemical transmitters in the nervous system.

In the initial experiments no attempt was made to identify the experimental neurones. Possible transmitter substances were merely tested and their effects on the bioelectric potentials of the cell recorded. In the earlier experiments the effect on the rate of the activity was taken as the main criterion for drug effect. In later experiments photographs were taken to show the more detailed effect of the drug on the resting potential and the shape of the action potential.

The overall response to each drug will be described together with appropriate illustrations of the drug effect.

The overall response can be seen in Table I.

TABLE I. A Summary of the Overall Drug Responses on the Activity of Helix aspersa Neurones.

DRUG	RESPONSE AND THRESHOLD CONCENTRATION ACCEL.		INHIBIT.		NONE	NO. OF EXPERIMENTS
Acetylcholine	22	: 10^{-8}	5	: 10^{-7}	41	68
Dimethyl-amino-ethanol	-	:	5	: 10^{-9}	7	12
Adrenaline	3	: 10^{-6}	2	: 10^{-8}	17	22
Nor-adrenaline	4	: 10^{-9}	1	: 10^{-6}	14	19
Dopamine	6	: 10^{-10}	10	: 10^{-11}	11	25 (2 did both)
Phenylalanine	-	:	5	: 10^{-9}	4	9
5-HT	9	: 10^{-9}	-	:	15	24
5-HTP	12	: 10^{-9}	2	: 10^{-6}	11	25
Histamine Base	3	: 10^{-6}	-	:	1	4
HCl	7	: 10^{-9}	1	: 10^{-7}	8	16
GABA	4	: 10^{-10}	2	: 10^{-10}	10	16
Glutamic acid	2	: 10^{-7}	2	: 10^{-7}	18	22
β -alanine	1	: 10^{-6}	-	:	5	6
Thiamine HCl (Aneurine HCl)	2	: 10^{-8}	1	: 10^{-8}	5	7 (I did both)
Cocarboxylase	3	: 10^{-9}	2	: 10^{-8}	8	12 (I did both)

(i).

(ii).

(iii).

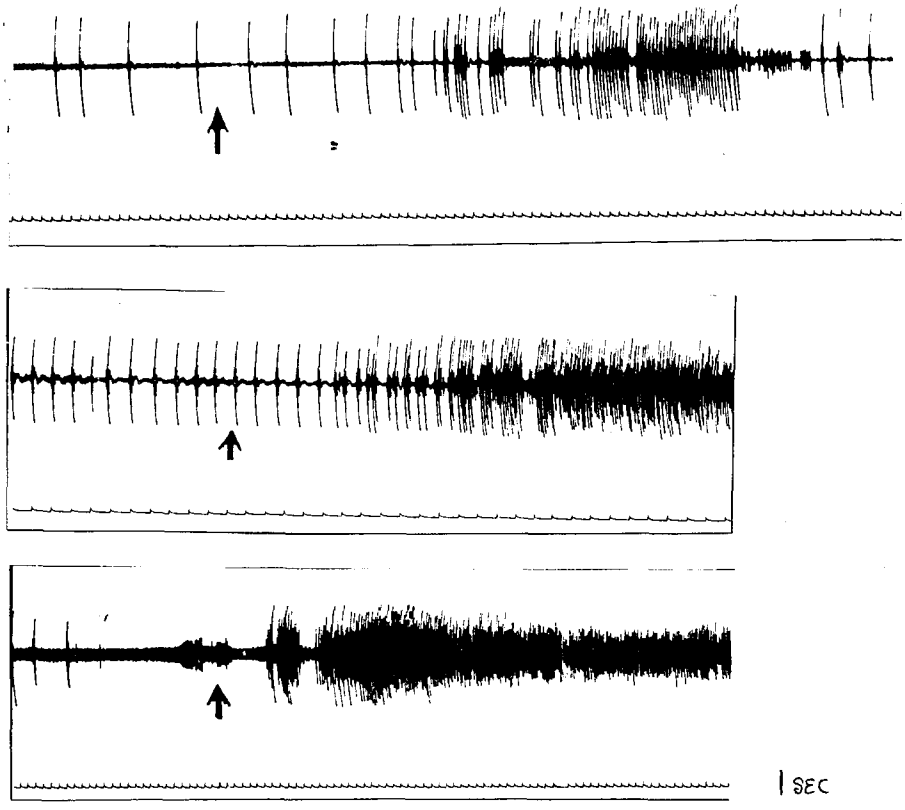


Figure 23 shows the three types of response to acetylcholine at a concentration of about I x 10^{-6}. The first record is a physiological response which occurred after 25sec. The second response is semi-pharmacological and occurred after 6sec, the cell recovered from this dose. The third response is pharmacological and occurred after 6sec, the cell did not recover from this dose.

ACETYLCHOLINE (Ach.)

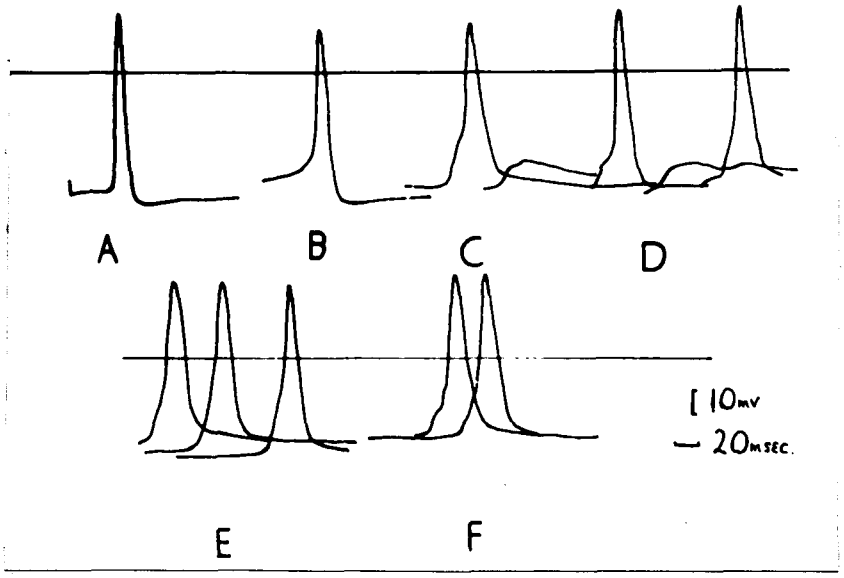
The effect to Ach has been tested in 68 experiments. 22 neurones were either activated or their activity accelerated by acetylcholine at physiological concentrations. The activity of 5 neurones was inhibited by acetylcholine and there was no physiological effect on the remainder. The threshold concentration for acceleration was 10^{-8} and for inhibition it was 10^{-7}. Ach was tested on both isolated brain preparations and on brains in situ. The results differ slightly as can be seen in the following table:-

Ach. response

	Acceleration	Inhibition	No response
isolated	10	4	25
in situ	12	1	16

At a concentration of 10^{-6}, Ach. can have one of three effects. In some cases there is an increase in the rate of A.P.'s without any change in their shape. The rate of the activity after such a response always returns to normal. Such an acceleratory response can be seen in fig. 23(i). The response starts after 24 seconds.

(i).



(ii).

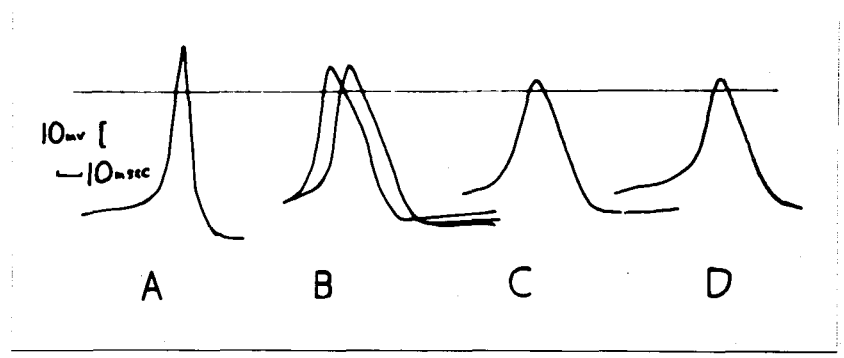


Figure 24 shows the effect of acetylcholine on the shape of the action potential to (i). a semipharmacological and (ii). a pharmacological response.

(i). A). is before, B). 20sec after, C). 24sec after, D). 29sec after, E). 34sec after, F). 4Isec after the application of acetylcholine.

(ii). A). is before, B). I8sec after, C). 45sec after, D). 60sec after the application of acetylcholine. In this case the duration of the action potential increased from 20msec to 40msec.

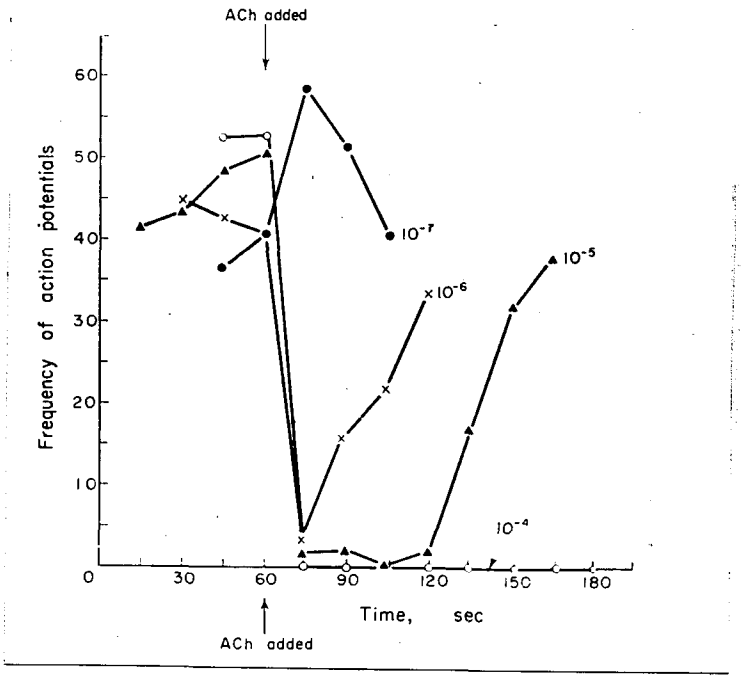


Figure 25 is a graph to show the effect of adding various concentrations of acetylcholine to a single preparation. IO^{-7} g/ml caused an acceleration; IO^{-6} g/ml caused a slight inhibition. The inhibition was more marked at the concentrations IO^{-5}, IO^{-4}.

The second type of response can be regarded as semi-pharmacological in that there are slight changes in the shape of the A.P. and in the R.P. height, accompanied by an increase in rate of activity. Such a response can be seen in fig. 23(ii). The response starts after 6 seconds. The increase in rate of A.P.'s is accompanied by the appearance of e.p.s.p.'s, Fig. 24(i). The A.P. is slightly increased in duration and the positive afterpotential is replaced by a negative afterpotential, fig. 24(i)C. The cell recovers from this effect.

The third type of response has a much more drastic effect on the bioelectric potentials of the cell. This response occurs about 7 seconds after the addition of the Ach., fig. 23(iii). There is a vast increase in the rate of the A.P.'s and e.p.s.p.'s and the R.P. and A.P. fall in height. The A.P. duration greatly increases, fig. 24 (ii) C and D.

The response to Ach. on a single neurone can vary depending on the concentration applied. In an experiment 10^{-7} caused a slight increase in the activity; 10^{-6} caused a decrease in the rate of activity for 1 minute, after which the activity increased. As can be seen from fig. 25, Ach. caused an increase in the duration of inhibition as the added concentration was increased. This experiment shows that Ach. has a dual effect on the spontaneous activity

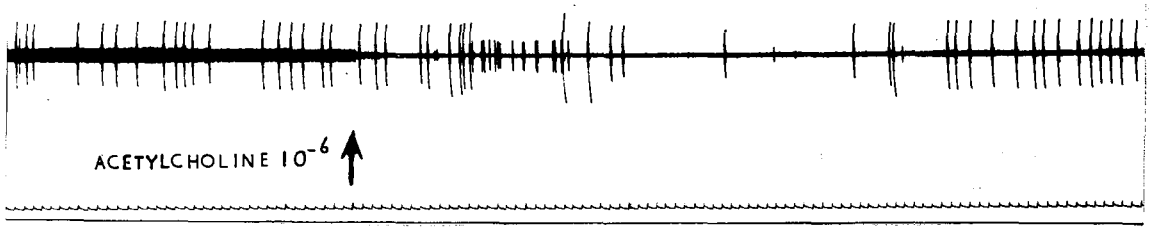


Figure 26 shows an inhibitory response to acetylcholine I x 10^{-6}. I4sec after the addition of the acetylcholine, the full action potentials were replaced by e.p.s.p.'s and then the cell was silent. The resting potential was hyperpolarized. The normal activity returned after 55sec.

depending on the concentration. Other cells examined show only one type of response to Ach. either that of inhibition or acceleration. The threshold concentration to bring about the acceleration of the activity was found to be 10^{-8}, while that for inhibition was 10^{-7}.

Although the general effect at physiological concentrations is one of acceleration in a few experiments these concentrations resulted in the inhibition of the activity. As can be seen in the fig. 26, the addition of Ach. 10^{-6} induced a few e.p.s.p.'s, followed by a period of inhibition. The activity returned to the normal rate after about 55 seconds.

PROTECTION AGAINST THE EFFECT OF ACETYLCHOLINE

A concentration of Ach. of the strength of 10^{-4} caused a brief increase in the activity and then a marked inhibition. The cell usually did not recover from such an effect. Certain chemicals tended to protect the membrane from the action of 10^{-4} Ach. These chemicals either prevented the complete depolarization of the membrane or reduced the period of complete inhibition. The effect was most marked after the application of β -phenylalanine > glutamic acid > cocarboxylase. The chemicals are arranged in order of

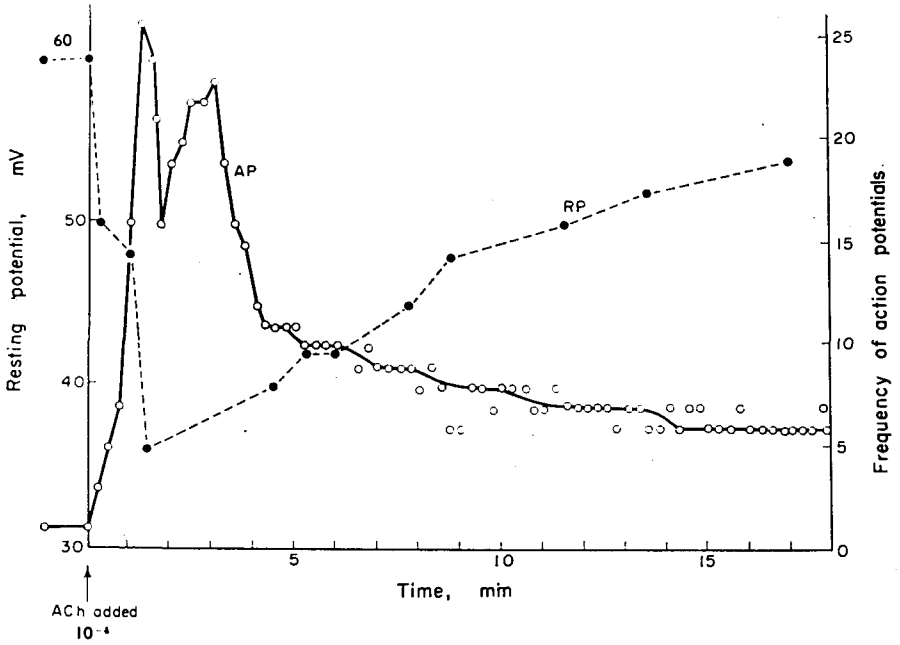


Figure 27 shows the action of 10^{-4} acetylcholine. The resting potential (●) and the frequency of the action potentials (o) are shown in the figure. Acetylcholine caused a sudden decrease in the resting potential and an increase in the frequency of the action potentials. As the resting potential returned towards the initial level, the frequency of the action potentials decreased.

decreasing protection against Ach. GABA and noradrenaline did not appear to provide any such protection to the membrane.

Fig. 27 shows an experiment where the membrane was first treated with β -phenylalanine and then Ach 10^{-4} was added. The R.P. fell from -60 to -36mV and the activity rose from I per 15 secs to 30 per 15 secs. As the R.P. slowly recovered the activity slowly decreased in rate. If Ach. 10^{-4} had been added to the preparation prior to the phenylalanine, then the R.P. of the cell would most likely have been depolarized to zero.

The possibility that the response to Ach. depended on either the R.P. of the cell or upon the difference between the maximum/minimum R.P. was investigated. Table 3 shows the results from fifteen active preparations in which the maximum/minimum R.P. prior to an action potential was recorded. There is a tendency for those experimental neurones which did not respond to Ach. to have a very small difference between their maximum/minimum R.P. But this does not seem to be the only criterion, since a neurone in which the maximum/minimum R.P. was 25mV did not respond to Ach. The value of the R.P. of the cell prior to the addition of the Ach. appears to be equally unimportant. Silent cells with R.P.s between -30 and -70mV were accelerated by 10^{-6} Ach, table 2.

CONCLUSION

The acceleratory response of Ach. does not appear to depend on the difference between the maximum and minimum R.P. The type of response of the cell to acetylcholine appears to be independent of the R.P. of the cell.

Table 2. R.P. and the effect of Ach. on silent neurones.

Neurone	Acceleration		Inhibition	No response
	Physiological	Pharmacological		
1	-70mV.			
2	43mV.			
3	56			
4	30			
5			-72mV	
6				-45mV
7				62
8				52
9				50
10				62
11				62

Table 3. Table to compare the maximum/minimum R.P. of active neurones with Ach. response.

Neurone	Acceleration		Inhibition	No response
	Physiological	Pharmacological		
1	10mV			
2	16mV			
3	8			
4	16			
5	17			
6		14mV		
7		7		
8			10mV ----->	
9				10mV
10				25
11				10
12				8
13				6
14				4
15				18

EFFECT OF ATROPINE AND/OR ESERINE

The effect of these compounds varied according to the nature of the cell under investigation. In some cases the action of acetylcholine was facilitated by the addition of eserine, (10^{-5}) on other occasions eserine could produce an acceleration by itself. Sometimes atropine, (10^{-4}), could produce an acceleration of the spontaneous activity, the response lasting for up to 55 seconds after the atropine had been added. In general atropine tended to reduce the effect of acetylcholine. After prolonged washing the addition of acetylcholine alone could still bring about an acceleration, thus indicating that the effect had been inhibited by atropine.

DIMETHYLAMINOETHANOL (DMAE)

This drug was tested on 12 neurones. It inhibited or reduced the activity of 5 of them. In no experiment was there a clear case of acceleration. The threshold concentration for inhibition was 10^{-9}.

In one experiment 10^{-10} to 10^{-7} had no effect. 10^{-6} hyperpolarized the R.P. to -55mV from -40mV and 10^{-5} depolarized the R.P. to zero.

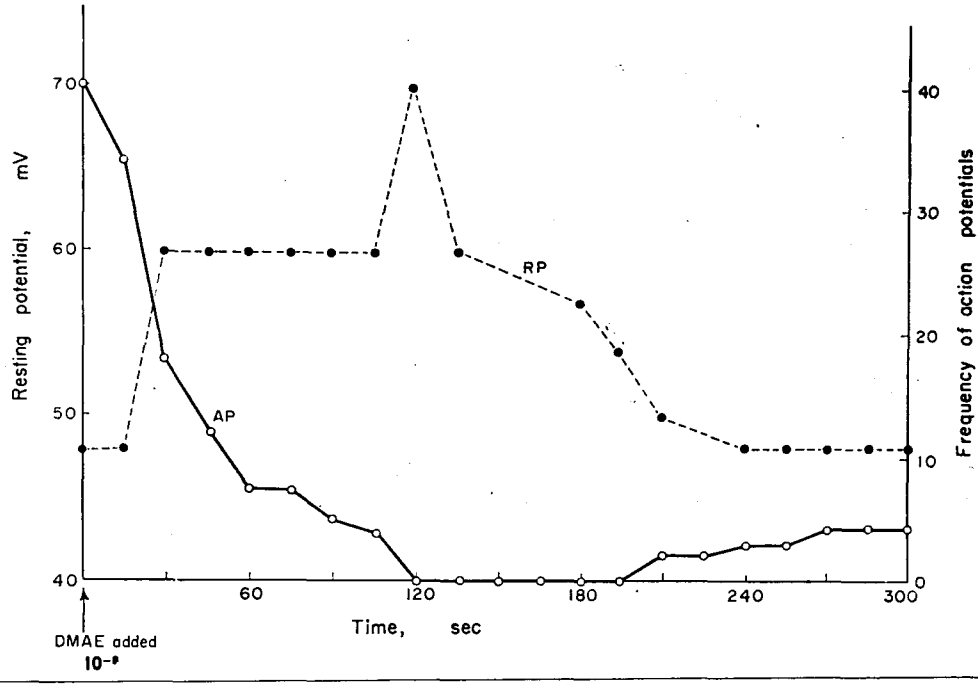


Figure 28 shows the action of dimethyl-amino-ethanol, 10^{-8}, (DMAE). DMAE caused an increase in the resting potential (●) and a decrease in the frequency of the action potentials (o).

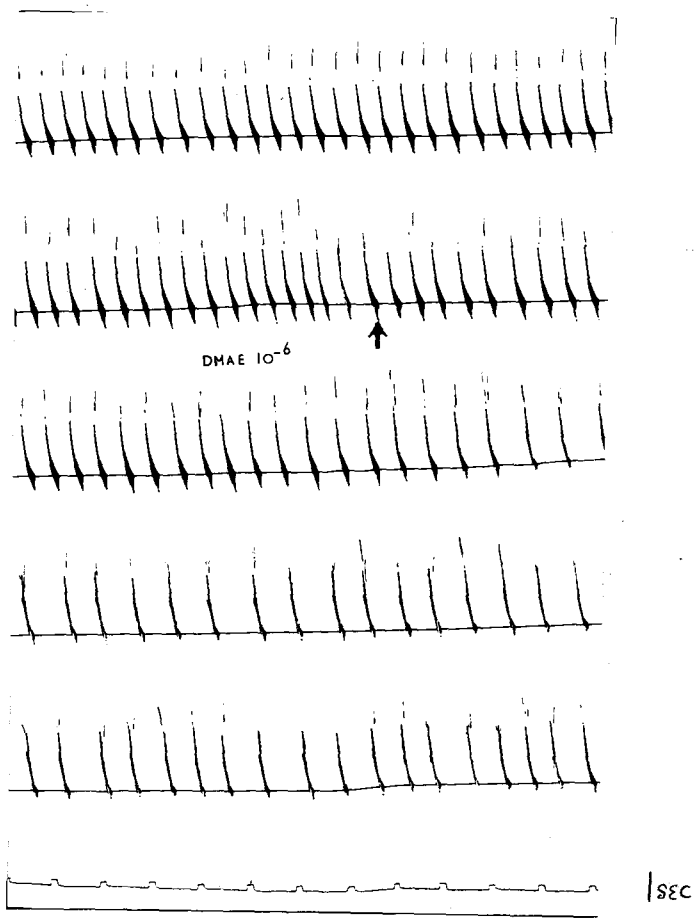


Figure 29 shows the response to 10^{-6} DMAE. The activity of the cell was partially inhibited, but it slowly recovered.

In another case there was little response till 10^{-3} was added, this inhibited the activity for 45 seconds. 10^{-2} after initial acceleration depolarized the membrane to zero.

Fig. 28 shows the effect of adding 10^{-8} DMAE to a spontaneously active neurone. The frequency of the activity fell from 40 per 15 sec. to zero. At the same time, the R.P. showed a hyperpolarization from 45 to 70mV. As the R.P. slowly depolarized to its initial level, so the frequency of the A.P.'s increased.

Higher concentrations of DMAE, 10^{-4}, 10^{-3}, 10^{-2}, tended to depolarize the R.P. to zero. This was the only drug apart from Ach. and adrenaline which did this.

Fig, 29 shows the response to 10^{-6} DMAE, the R.P. was hyperpolarized by 3mV.

5-HYDROXYTRYPTOPHAN, (5-HTP)

This drug was tested on 25 neurones. In 12 cases it accelerated the spontaneous activity or induced activity in a silent cell. Only in 2 cases did it inhibit the activity. The remaining neurones were unaffected by 5-HTP at physiological concentrations. The threshold concentration for excitation was 10^{-9} and for inhibition was 10^{-6}. 5-HTP induced both full action potentials and small excitatory

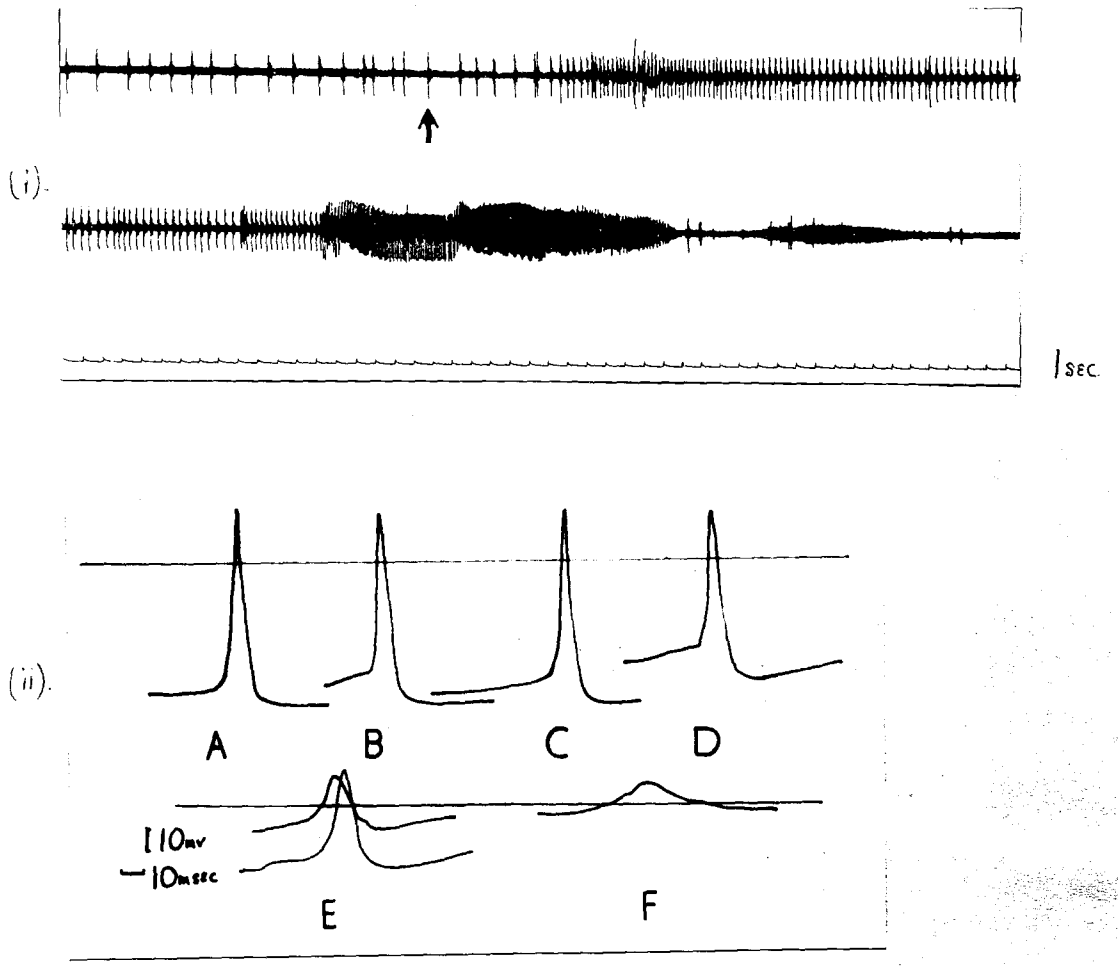


Figure 30.

Figure 30 shows the response of a cell to IO^{-9} 5-HTP. A physiological acceleration was replaced 45sec after the application of 5-HTP by a pharmacological response. The change in the shape of the action potential can be seen in (ii). As can be seen by comparing A and B, there is little change in shape with a physiological acceleratory response. The duration of A). was I7msec, and of B)., was I2msec. 5-HTP can induce e.p.s.p.'s of a similar shape to driven e.p.s.p.'s, A). and B)., (iii)

(i). The response of neurone I8I to the application of 5-HTP IO^{-9}.

(ii). The change in shape of the action potential on the addition of IO^{-9} 5-HTP.

A). before, B). I8sec after, C). 24sec after, D). 3Isec after, E). 48sec after, F). 54sec after the application of 5-HTP.

(iii). The shape of an e.p.s.p. A). driven and B). induced by 5-HTP.

potentials.

As in the case of Ach., examples of the types of response to this drug will be given in section two and three. In an experiment 10^{-9} 5-HTP caused acceleration after 5.5 secs, fig. 30(i) and (ii). This response was physiological for 38 secs and then it became pharmacological. The A.P. lost height and its duration increased and the R.P. fell in height. The cell did not recover.

Often during an acceleratory response A.P.'s and e.p.s.p.'s are formed as in the case of Ach. In a recording from a neurone in the left parietal ganglion, only e.p.s.p.'s could be obtained either by driving the cell or by the application of 5-HTP. The height of the driven e.p.s.p.'s was 8mV and those induced by 5-HTP were around 6mV. The duration of the former was 140msecs and of the latter 115msecs, fig. 30 (iii). The height of the driven e.p.s.p.'s was not constant and some of them coincided with the size of the 5-HTP induced e.p.s.p.'s.

As in the case of Ach. the possibility that the response of the neurone to 5-HTP might depend upon the level of the R.P. was investigated. The value of the R.P. was noted prior to the addition of 5-HTP in fifteen experiments. R.P. values between -33 and -80mV were unaffected by 5-HTP, while R.P. values between -62 and 50mV were accelerated by

5-HTP, table 4. There does not appear to be any clear correlation between the size of the difference between the maximum and minimum R.P. and the response of the cell to 5-HTP, table 4.

Table 4. To compare the difference in maximum/minimum R.P. before and after the addition of 5-HTP.

Neurone	Response to 5-HTP		No response	Resting Potential
	Acceleration:	Inhibition:		
1			6 : 7	63
2			15 : 15	65
3			14 : 10	80
4	7 : 4			64
5			17 : 17	57
6			-40	-40
7	-50 : 23			-50
8			-64	-64
9			-68	-68
10	18 : 18			53
11		15 : 15		42
12			7 : 7	33
13	23 : 30			52
14	-62 : e.p.s.p's			-62
15	7 : 13			58

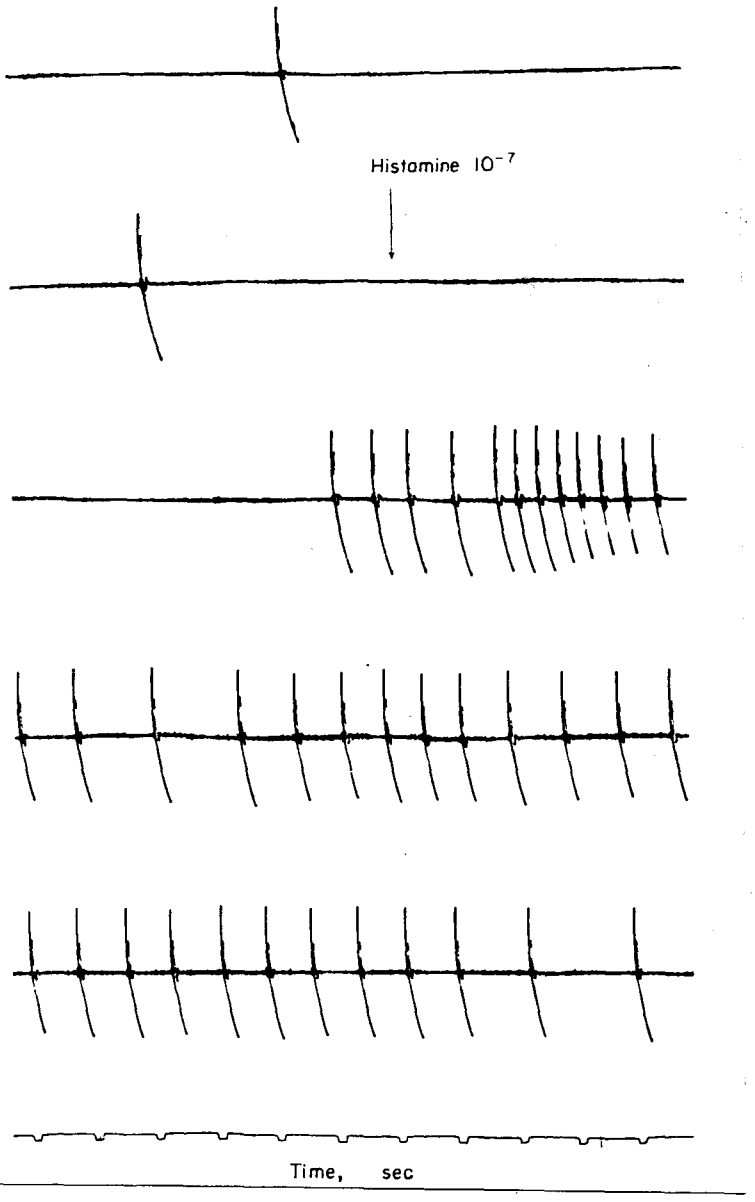


Figure 3I shows the response of a cell to 10^{-7} histamine HCl. The response occurred 10sec after the application of the histamine. The cell slowly recovered to its original frequency.

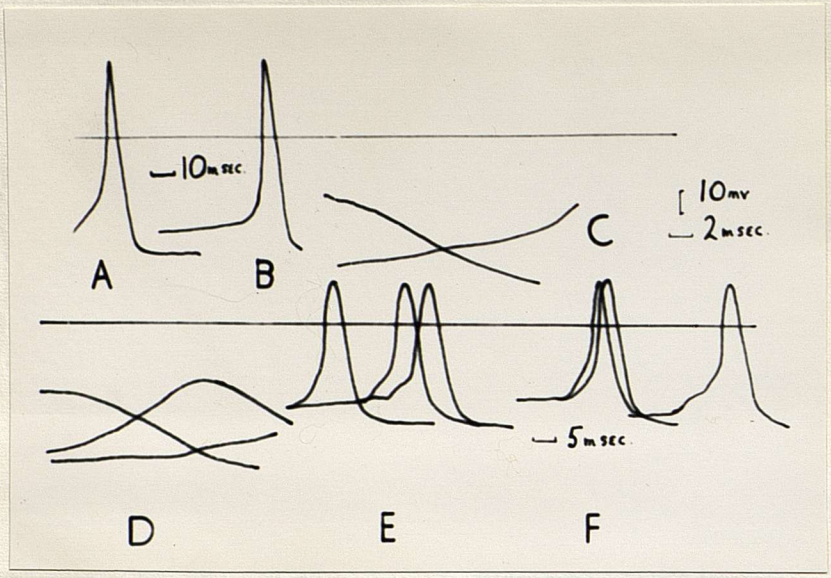
5-HYDROXYTRYPTAMINE. 5-HT

24 cells were tested with this drug. Nine of these were accelerated by either 10^{-6} or more dilute solutions. There were no clear examples of inhibition. The threshold concentration for acceleration was 10^{-9}. In most cases the 5-HT response occurs only once either at 10^{-9} or at 10^{-6}. Very rarely is there a graded response, proportional to the concentration applied. Fig. 45 (i), page 177, shows the effect of 10^{-6} 5-HT on the spontaneous activity of a neurone also activated in a similar manner by reserpine. The response to reserpine is shown in 44 G.

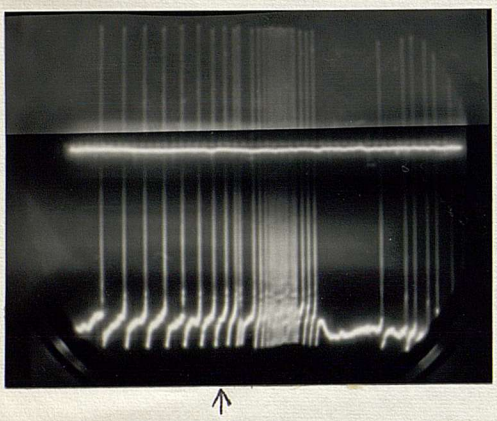
HISTAMINE HYDROCHLORIDE

Histamine hydrochloride, like the free base, was overwhelmingly an acceleratory drug. Histamine hydrochloride was tested on 16 neurones. 7 neurones were accelerated by it and 1 was inhibited. The remaining neurones were unaffected. The threshold concentration for acceleration was 10^{-9} and for inhibition 10^{-7}. The response took place 5-10 secs after the addition of the compound. Fig. 31 shows the effect of adding 10^{-7} histamine HCl to a cell accelerated

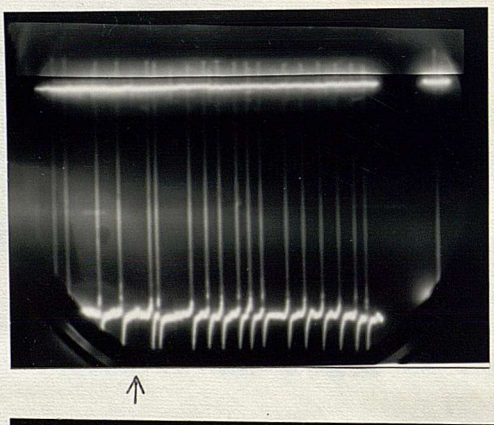
(i)



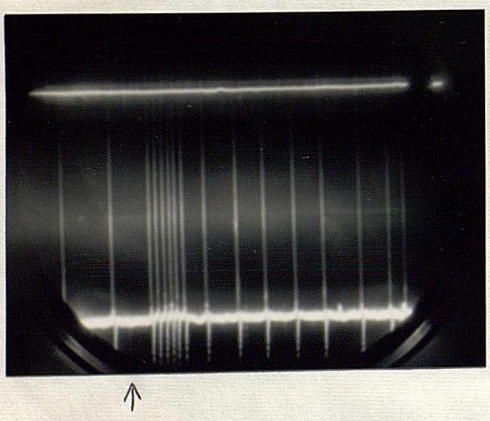
(ii).



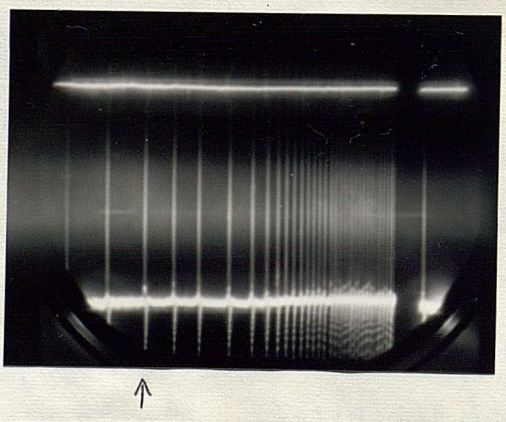
A.



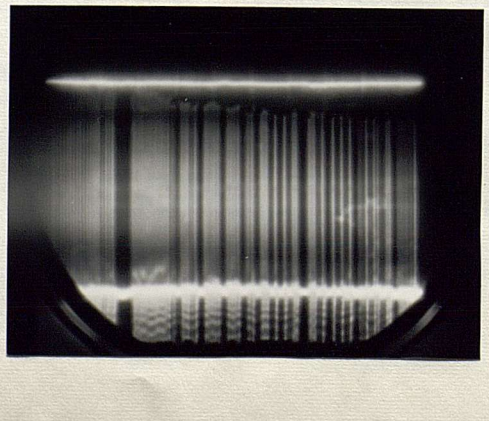
B.



C.



D.



E.

5 sec. [10m V.

Figure 32.

Figure 32 shows the filmed response of a cell to histamine and the antihistamine compound, mepyramine maleate.

(i). The effect on the shape of the action potentials of two neurones from the same brain, to histamine base $I \times IO^{-6}$.

A). and B). before, C). I68sec after, D). I78sec after, the application to the first neurone.

E). before, F). after the application to the second neurone.

(ii). Filmed response to histamine base of the first neurone.

A). the response to histamine base $I \times IO^{-6}$.

B). the response to mepyramine maleate $I \times IO^{-6}$.

C). the response to histamine + mepyramine maleate, $Ix\ IO^{-6}$.

D). the response to histamine base $I \times IO^{-6}$.

E). the continuation of film D).

C)., D). and E). were taken on A.C. The action potential had a large positive afterpotential.

by histamine. This cell was also accelerated by 10^{-6} and 10^{-5} histamine. In one experiment at 10^{-5} it was possible to inhibit the preparation for 4.5 minutes after the first application. But after the second application the activity was inhibited for 2.75 minutes and only for 15 seconds after the third application of 10^{-5}.

HISTAMINE BASE

In addition to the experiments on histamine hydrochloride, a few experiments have been undertaken using histamine free base. It had been suggested that the acceleratory response obtained from the histamine hydrochloride might be a pH effect, but the tested solutions had a pH of 7.4. Histamine base has so far been tested only on the large cells in the right parietal ganglion. On three occasions it caused the acceleration of the activity. The results are discussed in section two, page 176 .

The effects of histamine and the antihistamine compound, mepyramine maleate, were investigated, fig. 32(ii). Histamine 10^{-6} alone, accelerated the A.P.s without any radical change in their shape, there were no e.p.s.p.'s, fig. 32(ii)A. After the burst of activity there was a compensatory pause similar to ones observed after natural or stimulated bursts

of activity. The R.P. slowly depolarized and the activity returned to its previous level. The addition of antihistamine on its own at 10^{-5} had no effect on the spontaneous activity, fig. 32(ii) B. Histamine and the antihistamine added together greatly reduced the response to histamine alone, fig. 32(ii) C. Later the addition of histamine 10^{-6} alone caused another burst of activity much longer than the first response to histamine, fig. 32(ii) D. The addition of more antihistamine 10^{-5} resulted in the fall in height of the A.P.s. The preparation did not fully recover. The shapes of the A.P.s in response to the last application of histamine can be seen in fig. 32(i) A to D. Fig. 32(i) E and F shows the response of another neurone to histamine.

In table 5 are compared the effect on the maximum/minimum R.P. difference before and after the addition of histamine free base in the first four cases and of histamine hydrochloride in the last. Only in one case is there a radical change after the addition of the drug. This result is compared with the effect of Ach. 10^{-6} on the same neurones. In two of the three cases tested Ach had an effect. In both of these the histamine response gave A.P.s and e.p.s.p.'s. The acceleratory effect of histamine would not appear to be associated with a change in the maximum/minimum R.P.

Table 5. To show the effect of histamine hydrochloride and free base on the maximum/minimum R.P.

Neurone	Before application:	After application:	Ach. effect
1	82/75 = 7mV	70/50 = 20mV	no effect
2	67/63 = 4	68/63 = 5	A.P.'s and e.p.s.p.'s
3	55/37 = 18	58/42 = 16	not tested
	77/57 = 20	78/56 = 22	not tested
4	72/64 = 8	75/66 = 9	A.P.'s and e.p.s.p.'s

The first three neurones were tested with histamine free base and the fourth with histamine hydrochloride. In all the cases the histamine had a physiological acceleration effect.

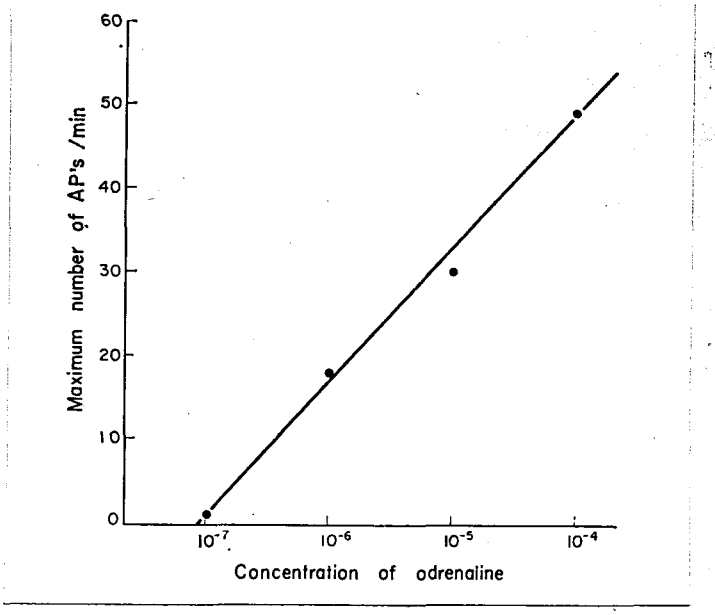


Figure 33 shows the response of a cell to adrenaline at different concentrations. In this graph the maximum number of action potentials per minute was plotted against the added concentration of adrenaline. All the data came from the same cell. The higher concentrations of adrenaline caused a higher frequency of action potentials.

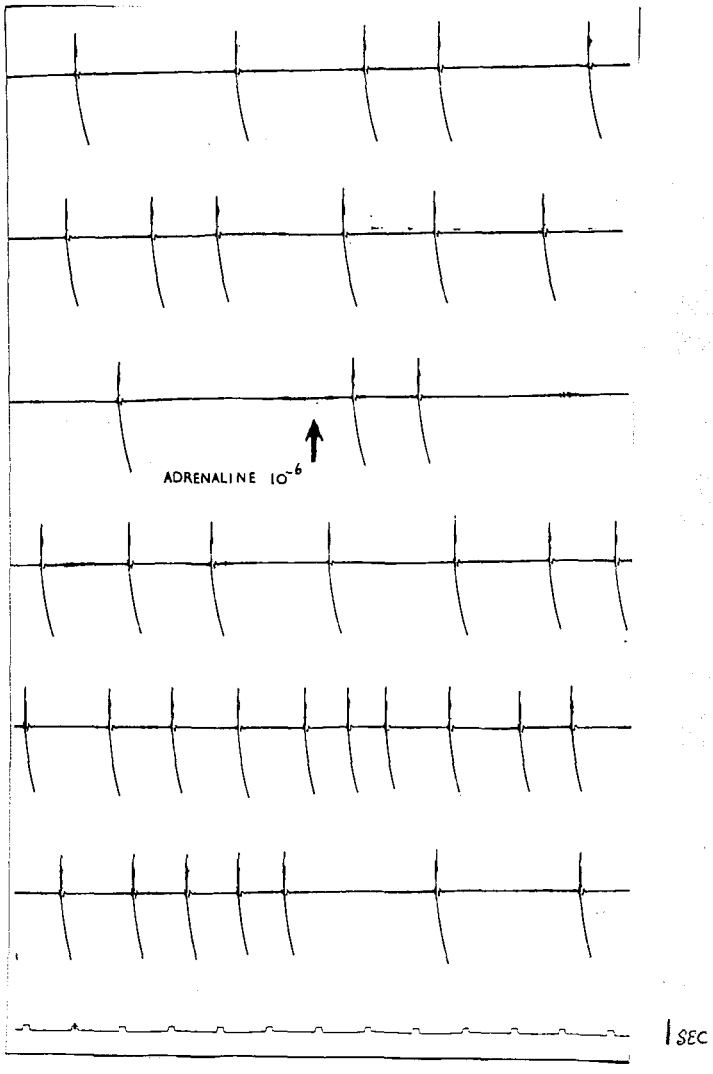


Figure 34 shows the response of a cell to the addition of 10^{-6} adrenaline. The cell activity soon returned to its normal rate.

ADRENALINE

Only 5 of the 22 preparations on which adrenaline was tested gave a good response, and of these 3 were acceleratory. The threshold for acceleration was 10^{-6}, and for inhibition 10^{-8}. In many cases 10^{-4} caused acceleration of a physiological nature but the responses are only recorded if they are 10^{-6} or more dilute. Generally 10^{-4} does not depolarize the membrane to zero. This behaviour differs from Ach., the latter at 10^{-4} generally results in the complete depolarization of the R.P. Even after 2 hours in a solution of 10^{-4} adrenaline the spontaneous activity was unaffected in several experiments. In some cases however, the R.P. was depolarized to zero by 10^{-4}. The responses to adrenaline both at physiological and pharmacological concentrations were extremely varied. Fig. 33 shows the effect of adding increased concentrations of adrenaline, and for each concentration plotting the maximum number of A.P.'s obtained per minute after the addition of the concentration.

Fig. 34 shows the response to the addition of 10^{-6} adrenaline to an active cell. The acceleratory effect occurs after approximately 18 seconds and lasts for about 12 seconds.

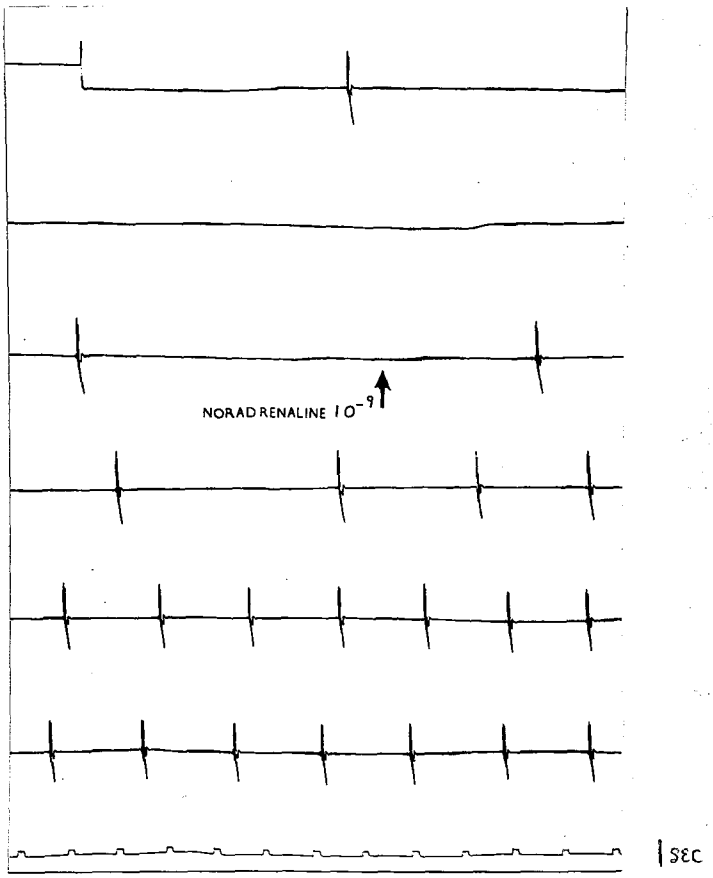


Figure 35 shows the response of a sparsely active cell to the addition of 10^{-9} noradrenaline.

NOR-ADRENALINE

Only 5 of the 19 neurones tested with this drug showed a conclusive response, and of these, 4 were acceleratory. The threshold for inhibition was 10^{-6} and for acceleration was 10^{-9}. At the more dilute concentrations the response to nor-adrenaline was physiological. It tended to affect the height of the A.P. more than the R.P. 10^{-5} was the greatest concentration tested and this did not in any experiment depolarize the R.P. to zero. Ach 10^{-4} added after a series of nor-adrenaline concentrations depolarized the R.P. to zero.

Fig. 35 shows the effect of noradrenaline on a sparsely active cell. At a concentration of 10^{-9}, noradrenaline excited the cell activity.

3-HYDROXYTYRAMINE. (DOPAMINE).

Dopamine was tested on 25 neurones. This drug inhibited the activity of 10 neurones and accelerated the activity of 6 neurones. In the remaining 11 experiments, dopamine had no effect. The figures for the overall effect of this drug are misleading in so far as the inhibitory effects are

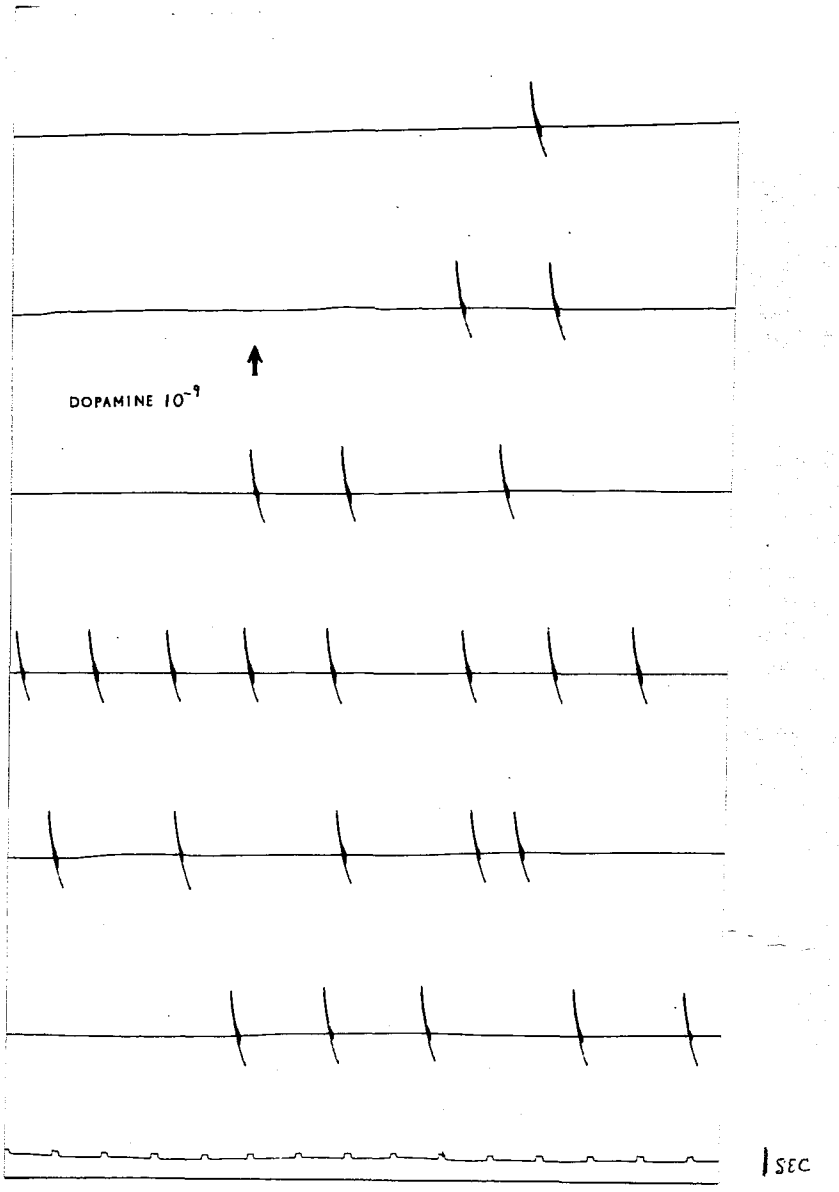


Figure 36 shows the response of a cell to 10^{-9} dopamine. The response started after 15sec and the main burst lasted for 25sec.

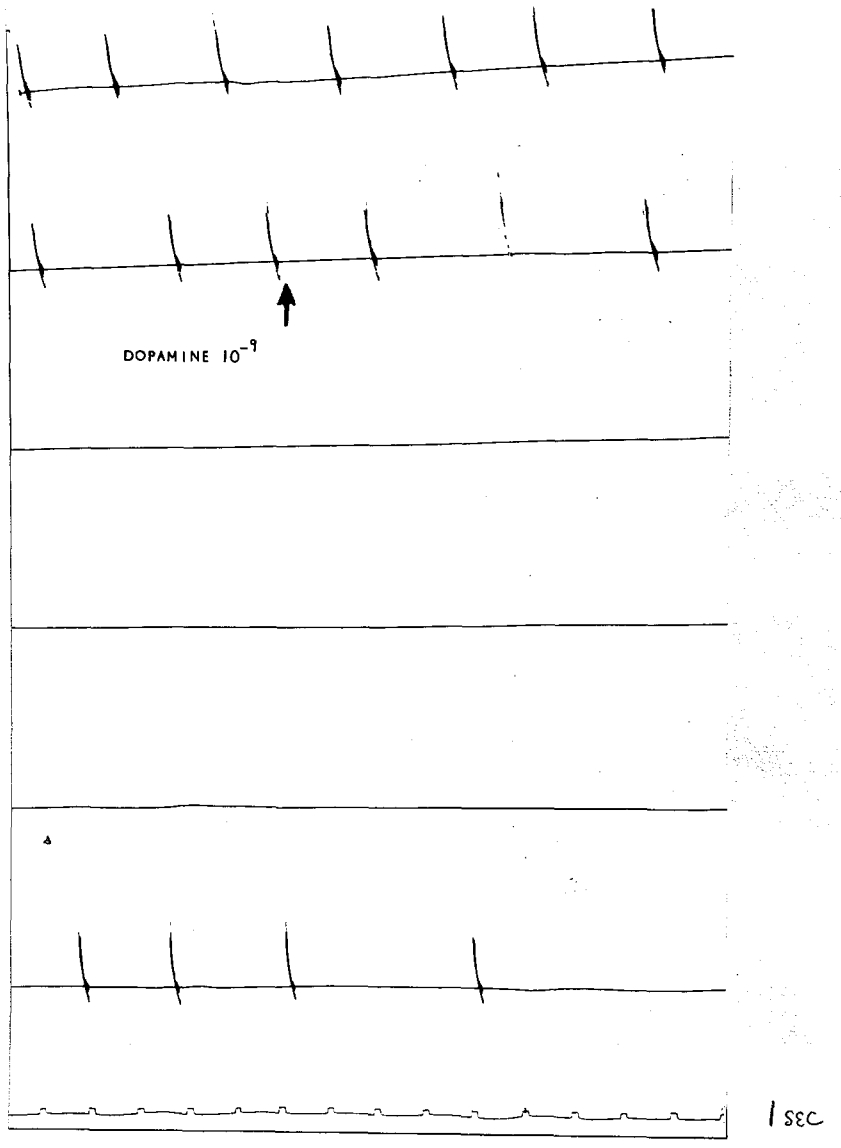


Figure 37 shows an inhibitory response of a cell to the application of 10^{-9} dopamine. The effect occurred after 8sec and lasted for about 45sec. During this period the resting potential was hyperpolarized.

much more startling than the acceleratory ones in the majority of experiments. Dopamine was the best hyperpolarizing agent at physiological concentrations so far examined. The threshold concentration for acceleration was 10^{-10} and for inhibition was 10^{-11}.

ACCELERATION

Fig. 36 shows the response of a cell with occasional activity to the addition of 10^{-9} dopamine. During the addition of the drug there were two action potentials and then after 7 seconds the activity appeared. The cell gradually recovered from this acceleratory burst of activity.

INHIBITION

10^{-10} dopamine concentration reduced the rate of the spontaneous activity of a cell. A concentration of 10^{-9} inhibited the activity completely for 45 seconds, fig. 37, whereas a concentration of 10^{-8} inhibited the activity for only 25 seconds. 10^{-7} inhibited the activity for 15 seconds, and more concentrated solutions, 10^{-6} & -5 reduced the rate for shorter periods.

In several experiments with this drug, the response was found to increase as the concentration applied was raised.

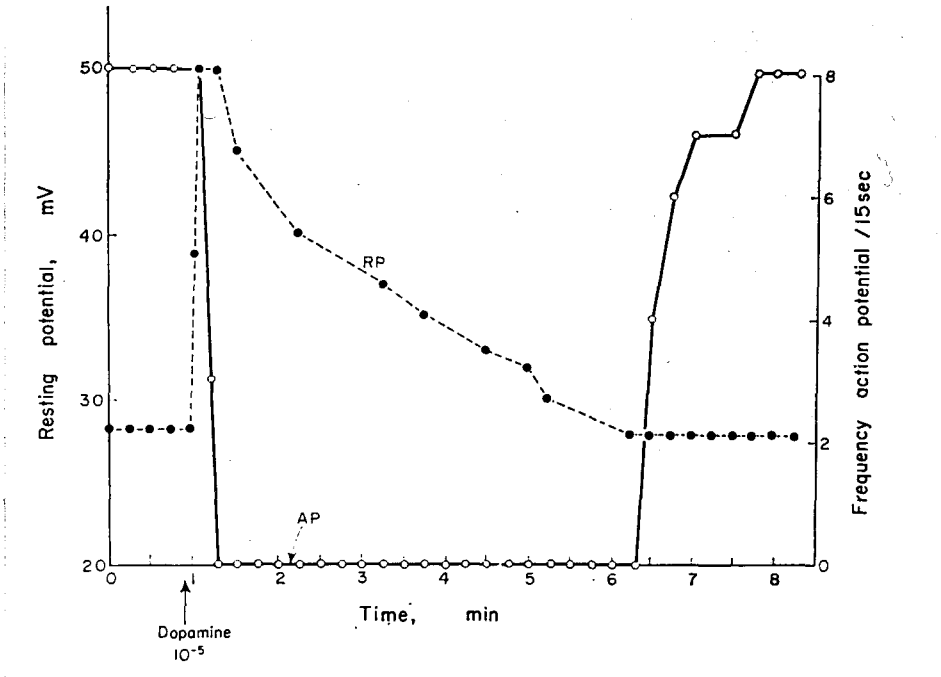


Figure 38 shows the action of 10^{-5} dopamine on a cell. Dopamine caused a sudden increase in the resting potential (●) and a decrease in the frequency of the action potentials, (o). As the resting potential returned to the normal level, so the frequency of the action potentials returned to normal.

Another neurone, number 129, exhibited a mixed response to dopamine. 30 seconds after the addition of 10^{-8} there was a slight increase in the rate of the activity. 10^{-7} inhibited the activity for 83 seconds, though it took 60 seconds before it had an effect. 12 seconds after the addition of 10^{-6}, the activity was completely inhibited for 65 seconds. The first addition of 10^{-5} completely inhibited the activity after 4 seconds by hyperpolarizing the membrane for 312 seconds. This response can be seen in fig. 38. This concentration caused a decrease in the rate of activity from 50 per 15 seconds to zero. At the same time it hyperpolarized the membrane from 28 to 50mV. As the resting potential slowly decreased to its original value, so the action potentials increased in frequency back to their original level. Since the activity was directly associated with the value of the resting potential it is suggested that this is an autoactive neurone. It is possible however that an interneurone was also affected by the dopamine and had an influence on the observed response. As with all concentrations of dopamine applied to this cell the activity completely recovered. Addition of more dopamine 10^{-5} again hyperpolarized the membrane for 329 seconds. The ability to both accelerate at one concentration and to inhibit at another concentration was most striking.

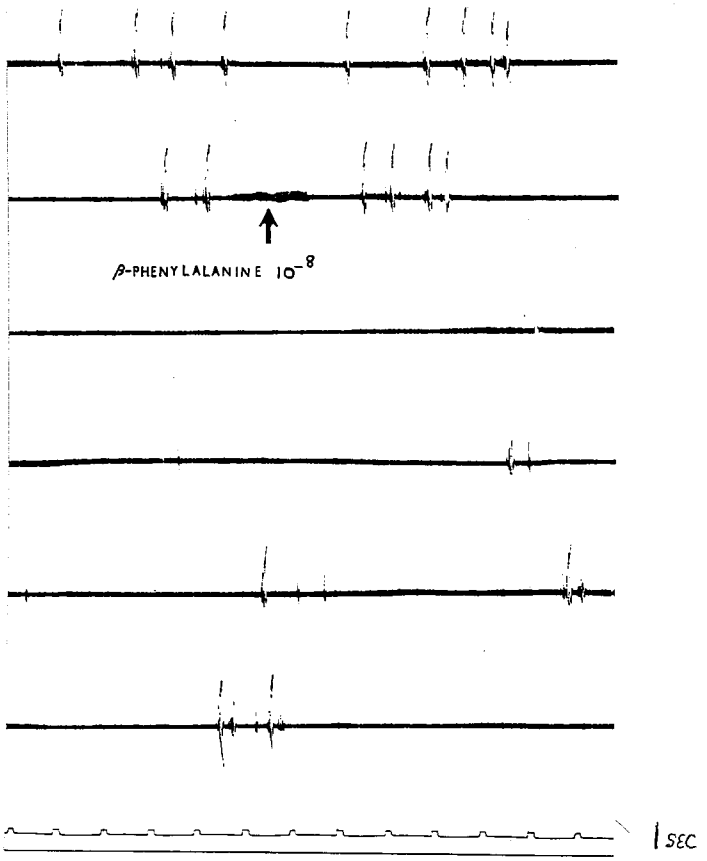


Figure 39 shows the response of a cell to 10^{-8} β-phenylalanine. The inhibition occurred after about 6sec and lasted for about 35sec. During this period there were a few e.p.s.p.'s.

PHENYLALANINE

Only 9 tests were made with this drug. Of these 4 had no effect. In 4 of the remaining 5 experiments phenylalanine hyperpolarized the membrane and inhibited the activity. In the other experiment the addition of 10^{-11} phenylalanine caused an initial burst of activity and then depolarized the membrane to zero. The threshold for inhibition was 10^{-9}. As in the case of glutamic acid, phenylalanine protected the membrane from complete depolarization after the addition of 10^{-4} Ach. The response to this drug occurred 5-10 seconds after its addition.

Fig. 39 shows the effect of phenylalanine on the spontaneous activity of an active neurone. A concentration of 10^{-8} inhibited the activity for approximately 27 seconds and then the activity slowly recovered. Excitatory depolarizations occurred first and these were followed by full action potentials. The response to phenylalanine occurred after 3 seconds.

γ -AMINOBUTYRIC ACID. (GABA)

GABA was tested on 16 neurones; 4 of these neurones showed acceleration and 2 inhibition. The thresholds for

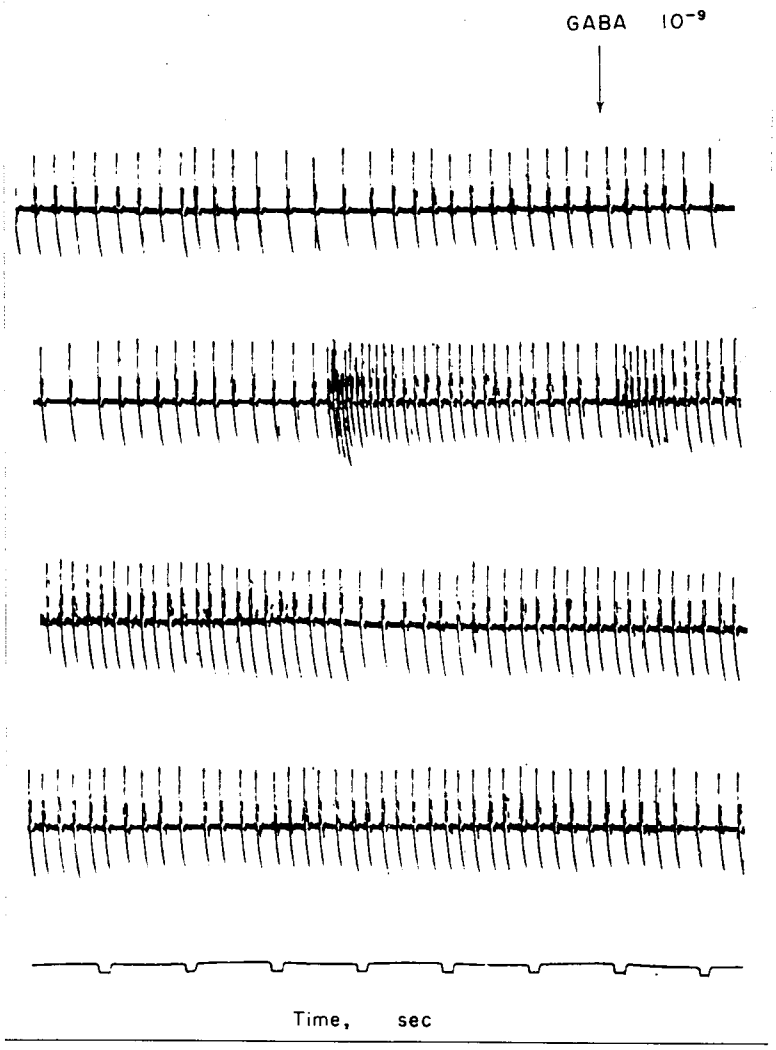


Figure 40 shows the acceleratory response of a cell to 10^{-9} GABA, γ-aminobutyric acid. The response occurred after 6sec and lasted for about 20sec.

both were 10^{-10}. The usual concentration for the acceleratory response was 10^{-8}. Fig. 40 shows the response after adding 10^{-9} GABA to a spontaneous neurone. Five of the experiments were carried out on in vivo preparations, none of these were affected by the drug.

GLUTAMIC ACID

Glutamic acid was tested on 22 neurones. In 2 cases it caused acceleration and in two cases it caused inhibition. The acceleratory responses were delayed, occuring after a minute, while the inhibitory responses occurred in 5 to 10 seconds. Glutamic acid usually did not depolarize the membrane. In certain experiments 10^{-5} glutamic acid were followed by a range of concentrations of Ach. In these experiments it was noted that the R.P. was often not depolarized to zero after 10^{-4} Ach. In the absence of glutamic acid the membrane is usually depolarized by this concentration of Ach. Glutamic acid had little effect on the shape of the A.P.

β -ALANINE

This drug was only tested on 6 in vivo preparations. In 5 it had no effect. In the sixth case, fig. 48(i), it

greatly accelerated the spontaneous activity and reduced the height of the A.P., fig. 48(ii). The A.P. gradually recovered.

COCARBOXYLASE

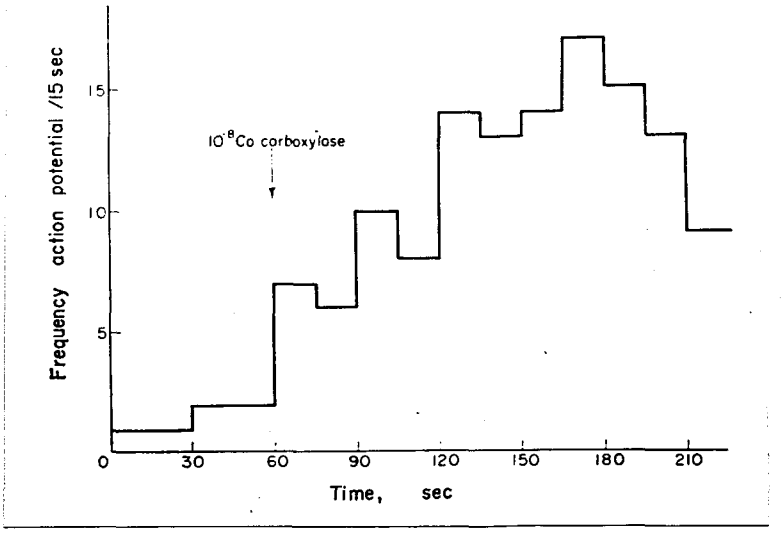
12 neurones were tested with this drug. 8 of these cells were not affected by cocarboxylase. In 2 preparations the activity was accelerated, in one it was inhibited, and in one there was both acceleration and inhibition depending on the concentration applied. The threshold for acceleration was 10^{-9} and for inhibition it was 10^{-8}.

In one experiment 10^{-8} cocarboxylase inhibited the activity for 13.5 minutes, while 10^{-7} inhibited the cell for 4.5 minutes. 10^{-6} had no effect. 10^{-5} inhibited the activity for 20 minutes, and then it returned at its previous rate.

In another experiment 10^{-7} caused acceleration after $1\frac{3}{4}$ minutes. This acceleration lasted 1.5 minutes. None of the other concentration had any effect.

Cocarboxylase can cause both acceleration and inhibition depending on the concentration applied. Thus 10^{-9} caused an initial burst of activity, while 10^{-5} and 10^{-6} inhibited

(i).



(ii).

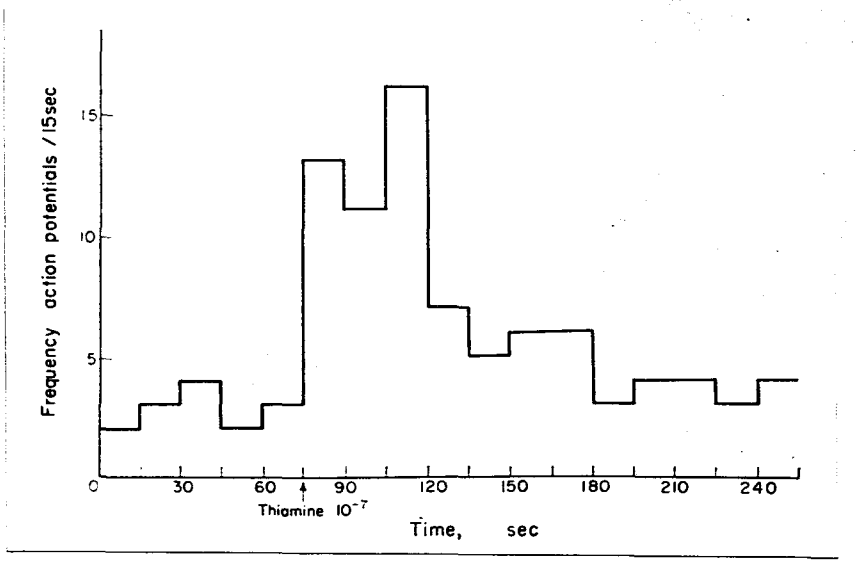


Figure 4I shows the response of two cells, one to 10^{-8} cocarboxylase (i); the other to 10^{-7} thiamine. The addition of both compounds caused an increase in the frequency of the action potentials. The thiamine response lasted for about 100sec.

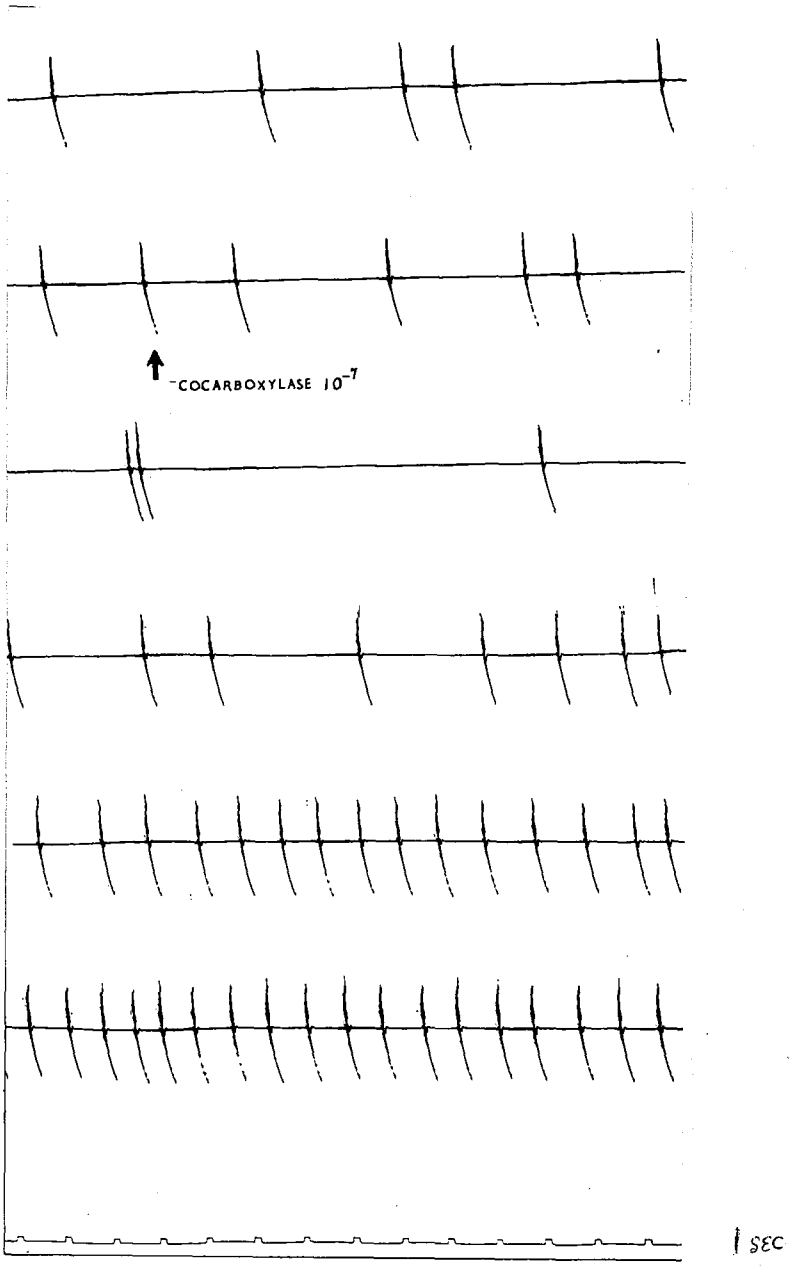


Figure 42 shows the response of a cell to 10^{-7} cocarboxylase. There was initial inhibition followed by prolonged acceleration. There is a break in the record of 85sec between the third and fourth trace of the figure.

the activity. 10^{-7} and 10^{-8} had no effect.

In fig. 41(i) can be seen the response to the addition of 10^{-8} cocarboxylase to a preparation. 10^{-7} gave a much reduced response while more concentrated solutions had no effect.

Fig. 42 shows the response of an active cell to the addition of 10^{-7} cocarboxylase. There was an initial decrease in the rate of action potentials but after approximately 100 seconds there was a great increase in the rate of activity. After about 2 minutes the activity returned to its normal rate. The application of both more dilute and more concentrated solutions of cocarboxylase had no effect on the activity rate of the cell.

THIAMINE HCl

Thiamine HCl was tested on only 7 neurones. 5 of these were not affected by it. One neurone was accelerated and one was both accelerated and inhibited depending on the concentration added. The threshold concentration for acceleration was 10^{-8} and for inhibition was 10^{-8}. Thiamine HCl did not depolarize the membrane to zero in any of the experiments. In one experiment thiamine HCl hyperpolarized

the R.P. by 6mV for 1¼ minutes. 10^{-7} accelerated this neurone, fig.41(ii). 10^{-4} to 10^{-6} had no effect. In another experiment 10^{-8} accelerated the activity. This acceleration was maintained and none of the other concentrations applied had any effect.

CONCLUSIONS

From the foregoing account, certain conclusions may be made;

1. All the drugs tested have an effect on the bioelectric potentials of the snail brain neurones.

2. In most cases, a given drug accelerates some cells, inhibits others, and has no effect on others.

3. The response of a cell may vary depending on the concentration of the drug applied.

4. There is usually an overall tendency to inhibit the activity, for example β-phenylalanine, dopamine, or dimethylaminoethanol; or to accelerate the activity, for example, 5-HTP, 5-HT, or histamine; while a few drugs do both equally, for example, glutamic acid, cocarboxylase or thiamine.

5. When a drug has an acceleratory effect, it can be either physiological or pharmacological; the response usually depends on the concentration applied.

6. A physiological concentration alters only the rate of the activity, and has little effect on the shape of the action potential or the height. A pharmacological acceleratory effect increases the duration of the action potential and often reduces the height of both the action and the resting potential. In some cases the cell may fail to recover.

7. Pharmacological responses generally occur much sooner than physiological responses after the application of the drug.

8. There was no clear link between the maximum/minimum resting potential and the type of drug response. There was a tendency for an increased response as the height of the resting potential was reduced.

9. Certain drugs, for example, acetylcholine, 5-HT, 5-HTP, and histamine were able to induce e.p.s.p.'s as well as full action potentials.

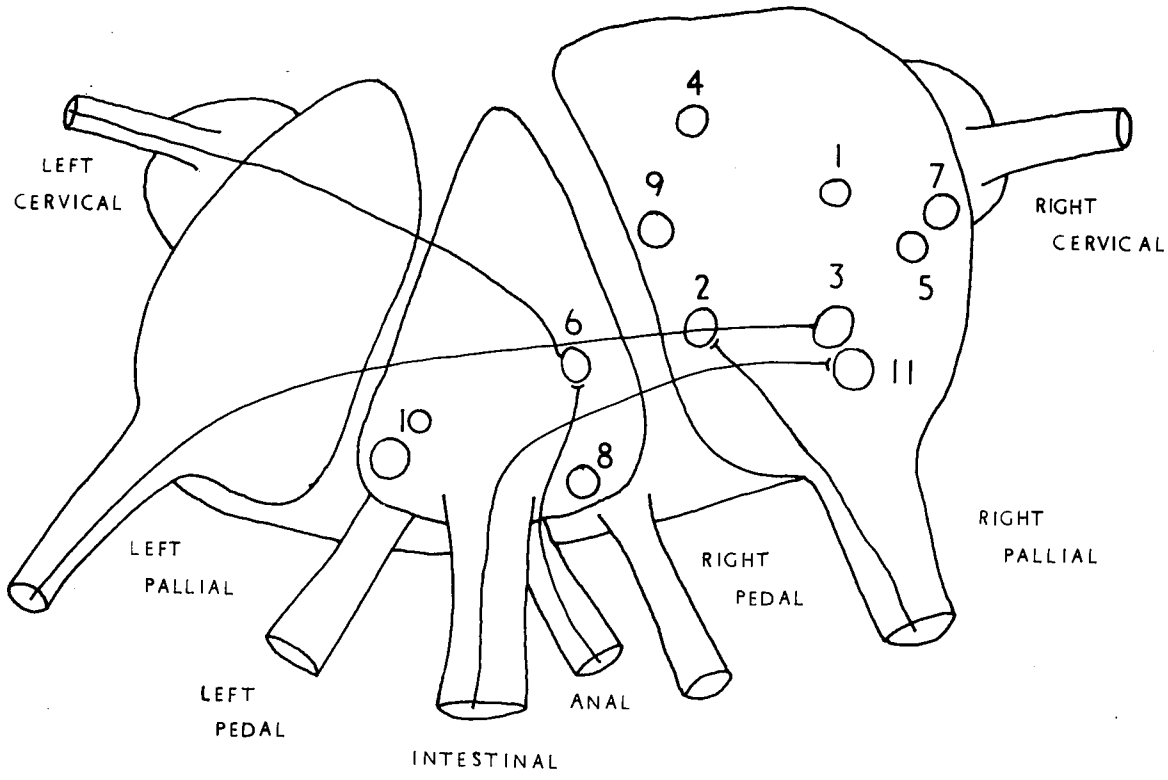


Figure 43 is a diagram of the suboesophageal ganglionic mass to show the positions of the eleven neurones used in section two.

SECTION TWO

THE EFFECT OF SEVERAL DRUGS ON ONE NEURONE IN A GIVEN BRAIN

INTRODUCTION

In the previous section, the various drugs were applied to exposed neurones in the snail brain. The response to these drugs were variable. It was decided that one way of simplifying the situation was to study the effect of the drugs in sequence on specified cells in the brain. The results of these experiments will now be described.

The eleven neurones to be described in this section are shown in position in fig. 43.

Neurone I lies in the right parietal ganglion. It is anterior to the very large cell and slightly smaller, about 100-120 μ in diameter. The spontaneous activity was irregular, occuring in bursts, followed by a period of reduced activity. This form of activity can be seen in (G) of fig. 44. The drugs were added in the order seen in the figure.

The following results were obtained:-

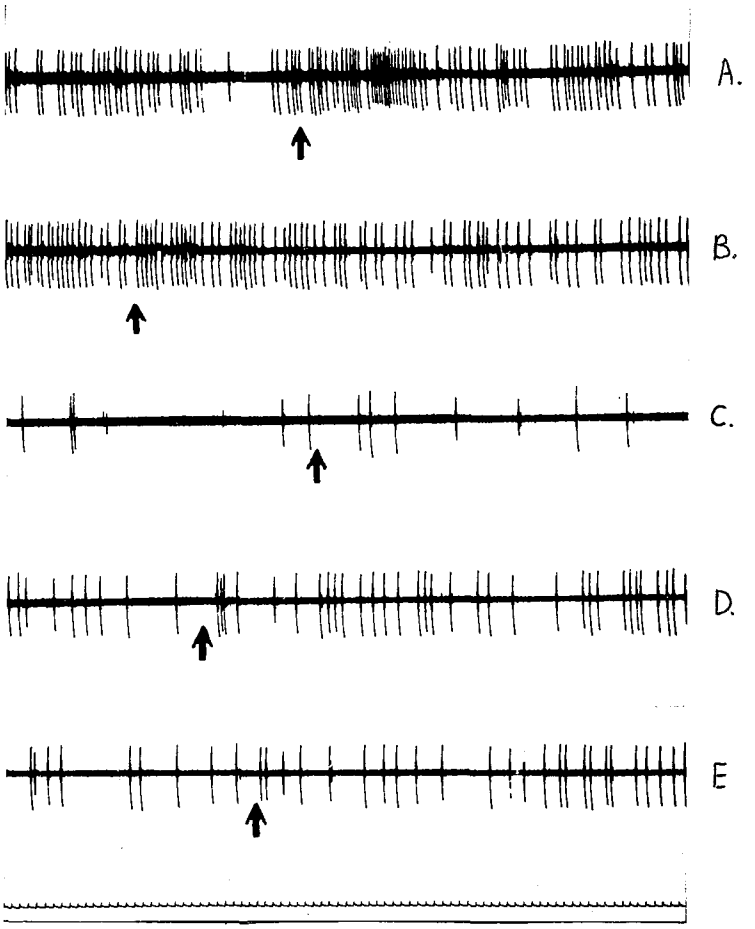


Figure 44, a pen recording, shows the response of neurone I to the application of ten drugs, in the following order:- A). Glutamic acid IO^{-6}; B). β -alanine IO^{-6}; C). Histamine base IO^{-6}; D). Acetylcholine IO^{-6}; E). Mepyramine maleate IO^{-6}.

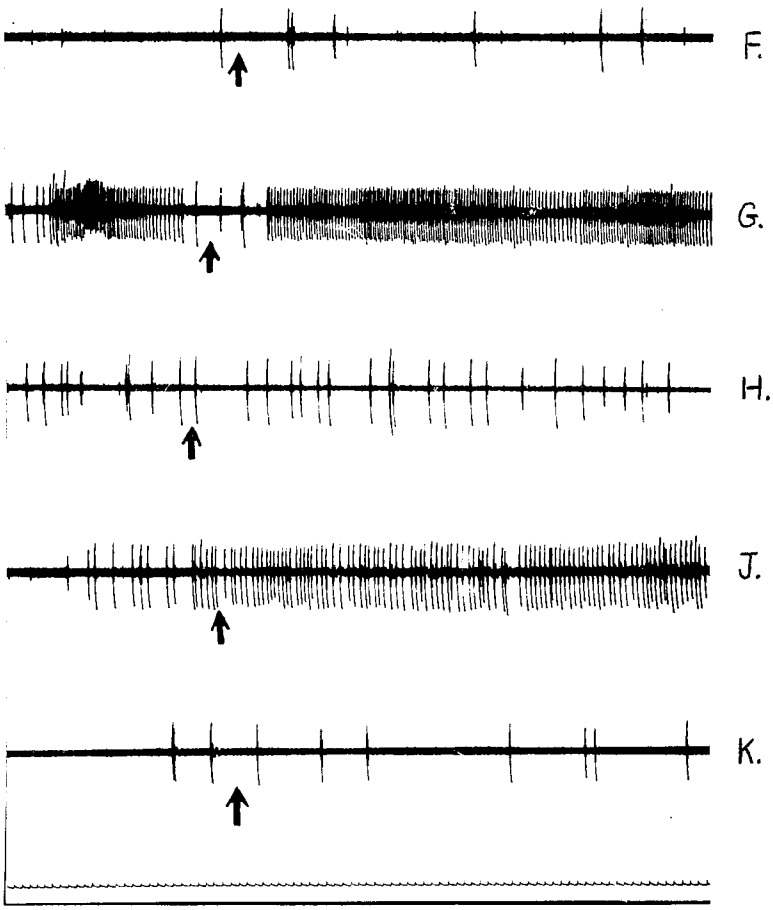


Figure 44 continued.

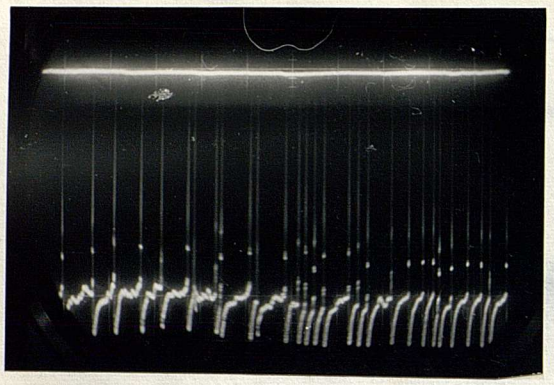
F). 5- HTP IO^{-6}; G). Reserpine approx. IO^{-5};

H). Eserine IO^{-5}; J). GABA IO^{-6};

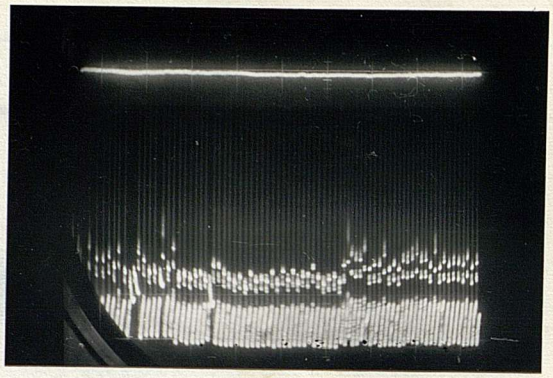
K). Dopamine IO^{-6}.

The spontaneous activity of the cell sometimes occurred in bursts as can be seen in G). prior to the addition of reserpine.

(i).



A.



B. — 5sec [10m V.

(ii).

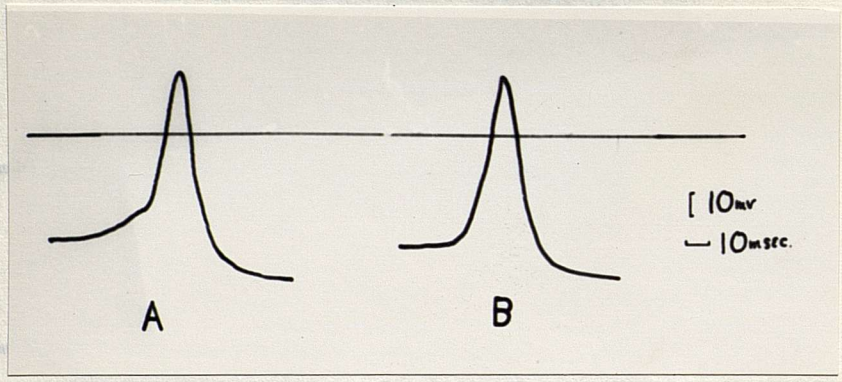


Figure 45 shows the filmed response to adding 5-HT 10^{-6} to Neurone I of figure 44, and the shape of the action potentials.

(i).A). Shows the rate of the action potentials before and during the addition of 5-HT.

B). Shows the rate after the addition of 5-HT.

(ii). Shows the shape of the action potentials A). before and B). 90sec after the addition of 5-HT. This was a physiological response.

Glutamic acid	-	no effect
β -alanine	-	no effect
Histamine Base	-	no effect
Ach.	-	no effect
Mepyramine maleate	-	no effect
5HTP	-	no effect
Reserpine	-	prolonged acceleration
Eserine	-	no effect
GABA	-	no effect
Dopamine	-	no effect
5HT	-	prolonged acceleration (See fig. 45(i)).

All the concentrations applied were 1 x 10^{-6}g/ml

The responses to both reserpine and 5HT were physiological, there being no change in either the shape of the A.P. or the size of the R.P., fig. 45(ii). The addition of a second dose of reserpine following the addition of eserine had no effect. It is possible that the amine store liberated by the first application of reserpine was exhausted. Neither after the addition of reserpine nor 5HT were there any e.p.s.p.'s.

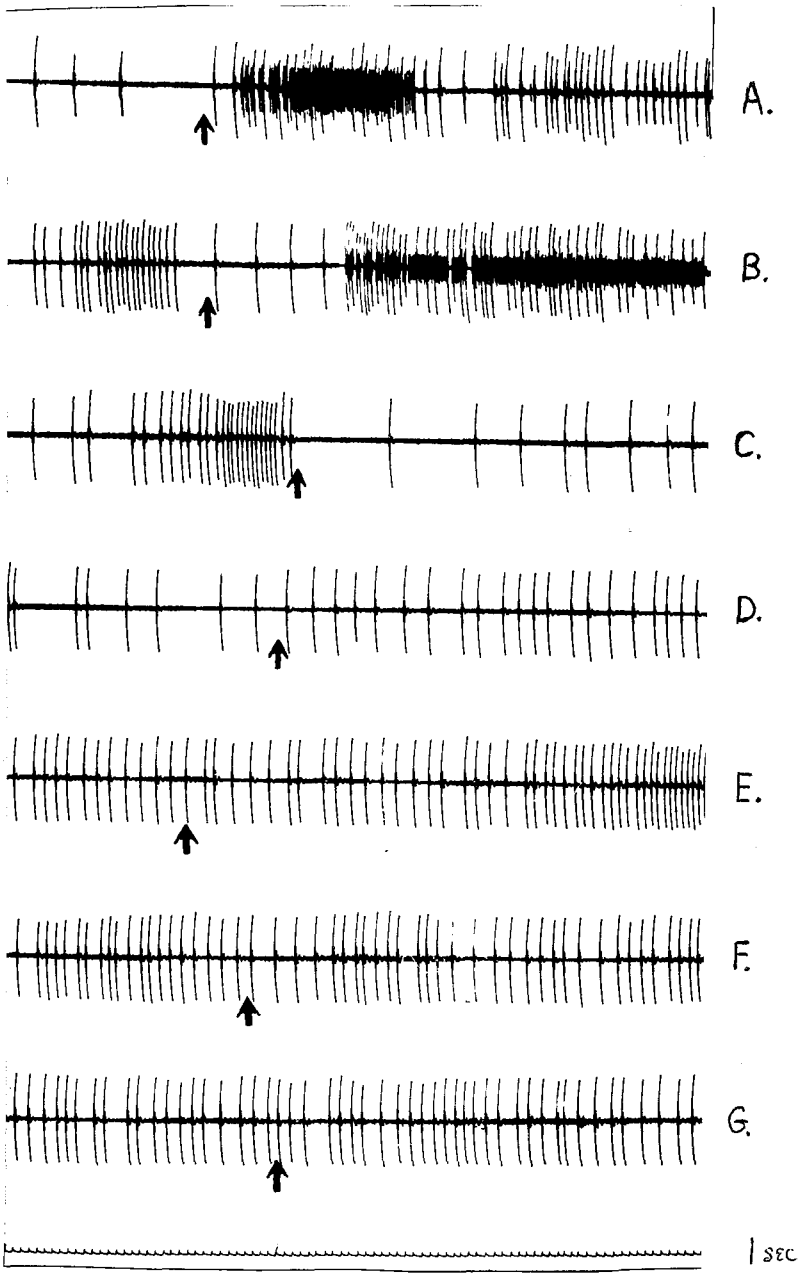


Figure 46 shows the response of neurone 2 to the application of seven drugs, in the following order:- A). Histamine base 10^{-6}; B). Acetylcholine 10^{-6}; C). 5-HTP 10^{-6}; D). Acetylcholine and Atropine 10^{-6}; E). β-alanine 10^{-6}; F). Glutamic acid 10^{-6}; G). GABA 10^{-6}. Histamine and Acetylcholine accelerated the activity. During part of the experiment, the activity occurred in bursts.

It is concluded that this cell was sensitive to 5-HT. The addition of reserpine appeared to liberate an amine which greatly accelerated the activity of the cell. It is tentatively suggested that the amine may have been 5-HT. It is strange that 5-HTP had no effect on the cell activity.

Neurone 2 was a large cell in the posterior region of the right parietal ganglion. The responses to the drugs tested can be seen in fig. 46. The drugs were tested in the following sequence:-

Histamine base	-	acceleration (fig. 47)
Ach.	-	acceleration (fig. 47)
5HTP	-	no effect
Ach. + Atropine	-	no effect
β -alanine	-	no effect
Glutamic acid	-	no effect
GABA	-	no effect
Ach.	-	acceleration
Eserine	-	acceleration (fig. 47(ii) C)

With the exception of atropine and eserine which were 10^{-5}, the drug concentrations were 10^{-6}.

The addition of histamine had little effect on the shape of the A.P., fig. 47(iii) γ . It can be seen by comparing

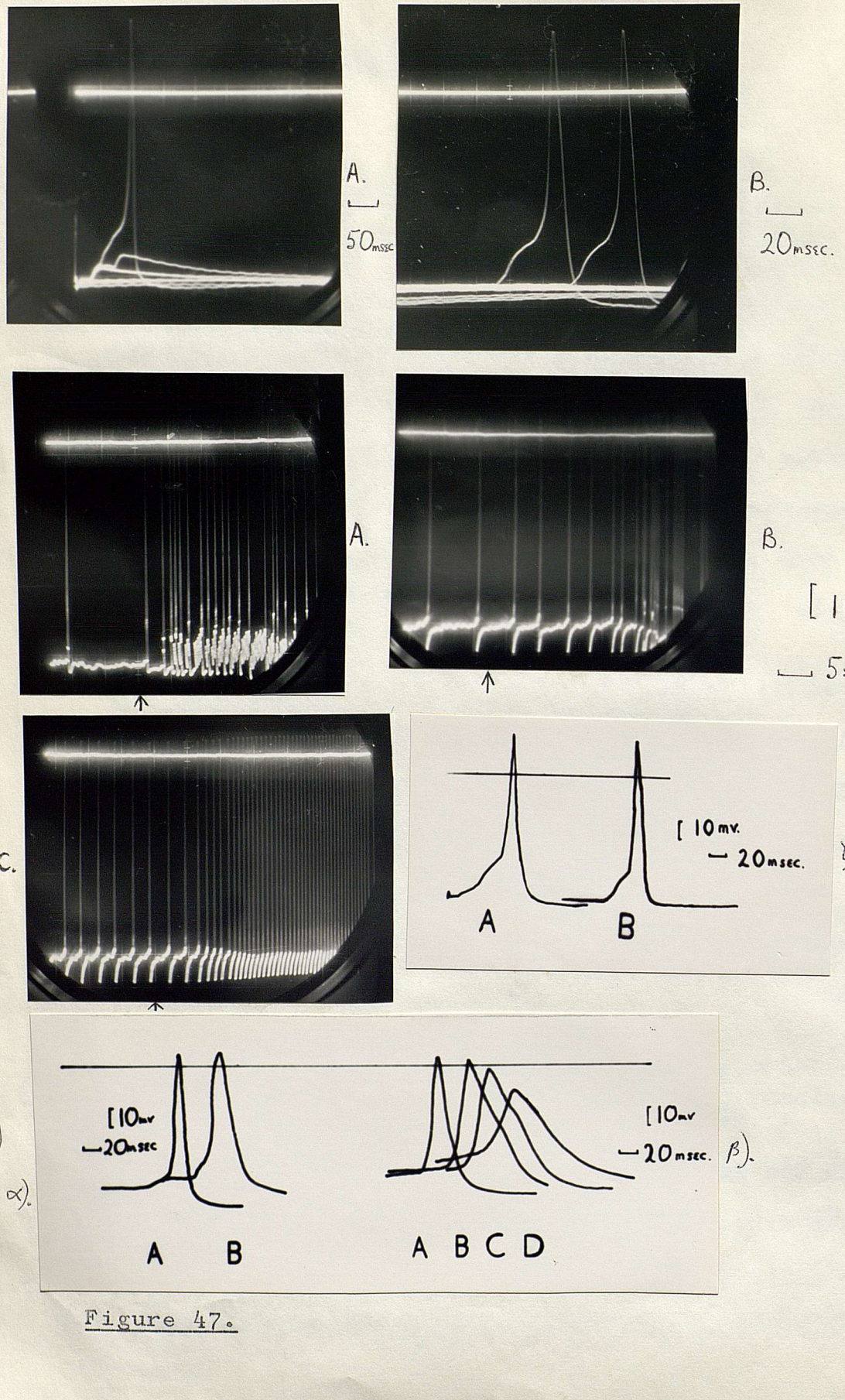

(i).
A.
50msec
B.
20msec.
(ii)
A.
B.
10mV.
5sec.
C.
10mv.
20msec.
A
B
γ).
(iii)
10mv
20msec
A B
10mv
20msec.
A B C D
α).
β).

Figure 47.

Figure 47 shows the shape of some of the action potentials and some filmed response to drugs of neurone 2, figure 46.

(i). A). before and B). after the application of histamine base. A). is a driven action potential with a duration of 20msec and B). is a spontaneous action potential of 20msec duration.

(ii). A)filmed response to histamine base 10^{-6}. This is a film of the pen recording of figure 46A.

B).filmed response to acetylcholine 10^{-6}. This is not shown in figure 46.

C). filmed response to eserine 10^{-5}. This is not shown in figure 46.

(iii). The effect of acetylcholine, eserine, and histamine base on the shape of the action potentials.

α). A). before and B). 53sec after the addition of acetylcholine.

β). A). 50sec after, B). 73sec after, C). 81sec after, D). 156sec after the addition of eserine.

γ). A). before and B). after the addition of histamine base 10^{-6}.

fig. 47(ii)A with fig. 47(ii)B and C that after the addition of histamine base, a positive afterpotential develops. The responses to both histamine and Ach. includes an increase in rate of A.P.s and the induction of e.p.s.p.'s. The acceleration due to the histamine begins 5 seconds after the addition and lasts for 23 seconds. After this period the cell settles down to a steady action potential rate which is considerably faster than before the addition of histamine, from approximately 1 A.P. every 8 seconds to 1 every second. The response to Ach. begins after 18 seconds and lasts 73 seconds, the activity then tending to come in bursts followed by periods of reduced activity. The responses to both histamine and Ach. have little permanent effect on the A.P. shape, fig. 47(iii)α, γ. Both responses can be considered to be physiological. The cell membrane is able to respond physiologically to at least two drugs under experimental conditions. Atropine added with Ach. blocked the response to Ach. Later additions of 10^{-6} Ach. had no effect, though 10^{-5} did elicit a much reduced acceleratory response. Eserine added alone caused an increase in A.P. rate, though no e.p.s.p.'s, after 8 seconds, see fig. 47(ii)C. The effect to eserine was pharmacological, envolving a change in shape of the A.P., fig. 47 (iii)β. The eserine effect would appear to be different in nature to that

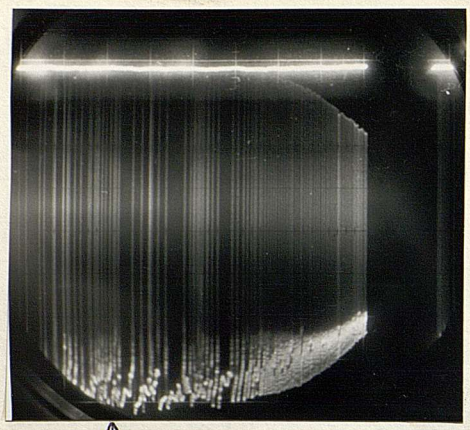
after Ach. in this experiment. The activity largely recovered but the addition of Ach. 10^{-5} and eserine caused a pharmacological effect from which the A.P.s failed to recover. Ach. 10^{-5} on its own accelerated the A.P.s and induced e.p.s.p.'s.

CONCLUSIONS

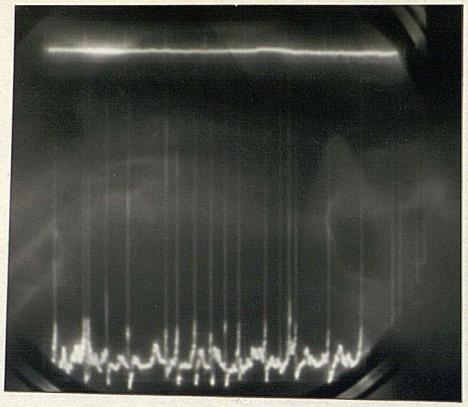
This cell was apparently equally sensitive to histamine base and acetylcholine. Atropine blocked the response of acetylcholine while eserine on its own had an acceleratory effect. Atropine however, did not inhibit the spontaneous activity. This would suggest that the spontaneous activity was not due to the release of acetylcholine. The acetylcholine response occurred after 23 seconds, while the eserine response occurred after only 8 seconds. This is further evidence for eserine effect independent of a potentiation of the acetylcholine response. The response to histamine occurred after 5 seconds.

Neurone 3 is a large cell in the posterior region of the right parietal ganglion. The drugs were added in the following order:-

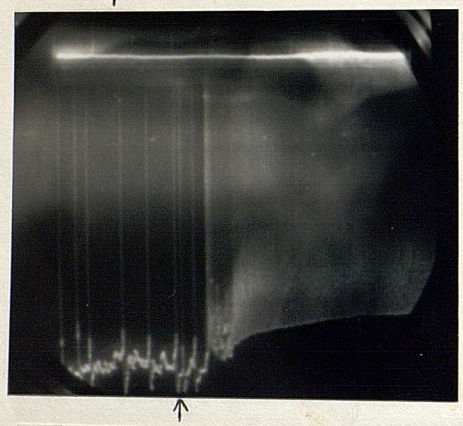
(i).



A.



B.

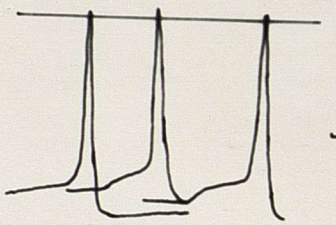


C.

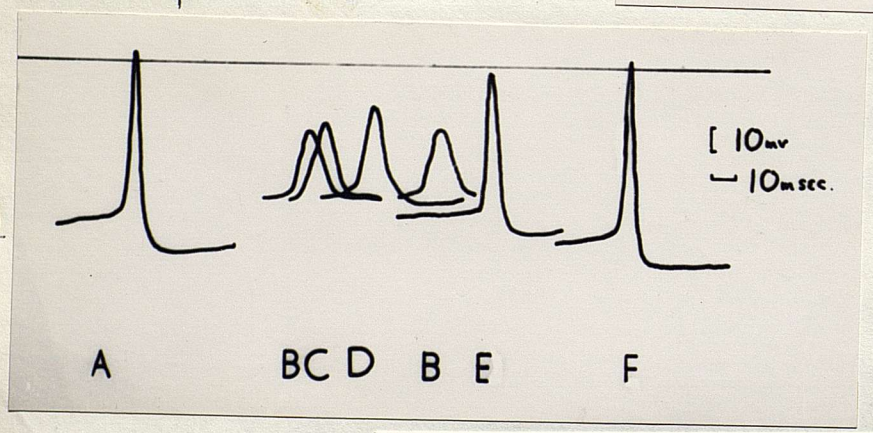
[10mV.

5sec

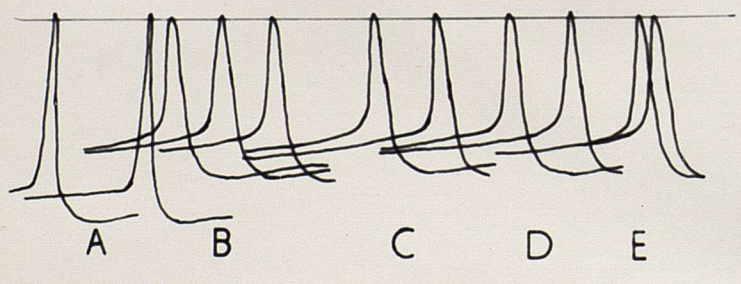
(iii)



(ii).



(iv).



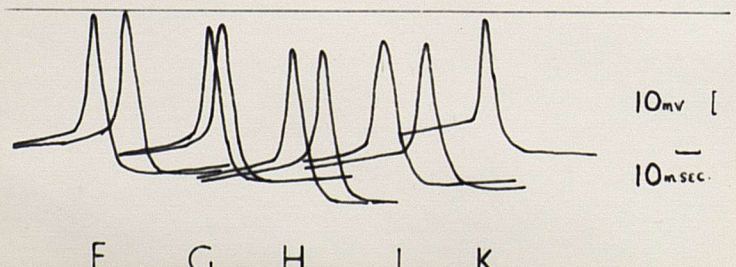


Figure 48.

Figure 48 shows the filmed response to β-alanine and histamine and the shape of the action potentials after the addition of these drugs together with the response to 5-HTP. The responses were from the same cell.

(i).A). Shows the filmed response to β-alanine IO^{-6}.

B). Shows the filmed response before and C). after the application of histamine base IO^{-6}.

(ii). Shows the shape of the action potential after the application of β-alanine IO^{-6} filmed in (i). A).

A). before and B). 47sec after, C). 52sec after,

D). 58sec after, E). 80sec after and F). I75sec after the application of β-alanine.

(iii). Shows the shape of the action potential after the addition of 5-HTP IO^{-6}. A). before and B). 55sec after and C). 65sec after the application of 5-HTP.

(iv).Shows the shape of the action potential after the application of histamine base IO^{-6}. A). before and

B). 48sec after, C). 53sec after, D). 58sec after,

E). 64sec after, F). 7)sec after, G). 76sec after,

H). 82sec after, J). 90sec after and K). I35sec after the application of histamine base.

Ach.	-	no effect
β -alanine	-	acceleration
5HTP	-	no effect
Glutamic acid	-	no effect
GABA	-	no effect
Histamine Base	-	prolonged acceleration
Ach.	-	prolonged acceleration
Histamine base	-	acceleration

In each case the concentration added was 10^{-6}.

The β -alanine effect was pharmacological, fig. 48(i)A, but the A.P. shape and R.P. fully recovered, fig. 48(ii)A to F. The R.P. was less affected by β -alanine than was the A.P. height. The absolute height of the A.P. fell from 73 to 30mV, fig. 48(ii)B. The positive afterpotential disappeared. The acceleration occurred after 18 seconds and lasted for 50 seconds. There were no e.p.s.p.'s.

Fig. 48(iii) shows that there was no change in shape after the addition of 5HTP.

Histamine base caused acceleration after 4 seconds. There was little change in the duration of the A.P., fig. 48(iv), but the height was reduced and the R.P. also fell. The positive afterpotential was maintained. The increased activity lasted several minutes. There were no e.p.s.p.'s.

The first addition of Ach. at the beginning of the experiment had no effect. The second addition after the histamine base caused prolonged acceleration with both A.P.'s and e.p.s.p.'s.

The second addition of histamine base was pharmacological and the cell did not recover.

CONCLUSION

The addition of acetylcholine after β -alanine, 5-HT, glutamic acid, GABA, and histamine base accelerated the activity, while the addition of acetylcholine prior to the application of the compounds had no effect. This would suggest a possible potentiating response by one of these compounds. This could be verified by penetrating this neurone in a number of brains and doing a sandwich test of the response of acetylcholine, the compound, acetylcholine. The acetylcholine response occurred after approximately 40 seconds, while the histamine response occurred after 3 seconds.

Neurone 4 is situated in the right hand corner of the visceral ganglion. The drugs were added in the following

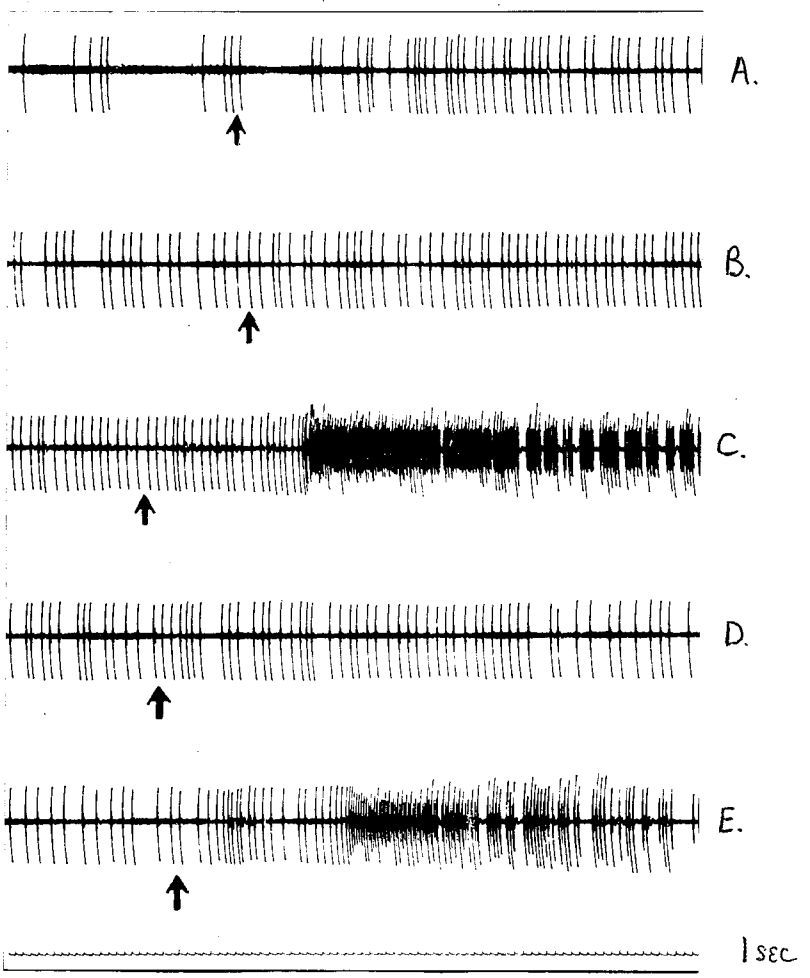


Figure 49 is a pen recording which shows the response of neurone 4 to the application of ten drugs in the following sequence:-

A). 5-HTP IO^{-6}; B). Glutamic acid IO^{-6};

C). Acetylcholine IO^{-6}; D). Atropine IO^{-5};

E). Acetylcholine and Atropine IO^{-6};

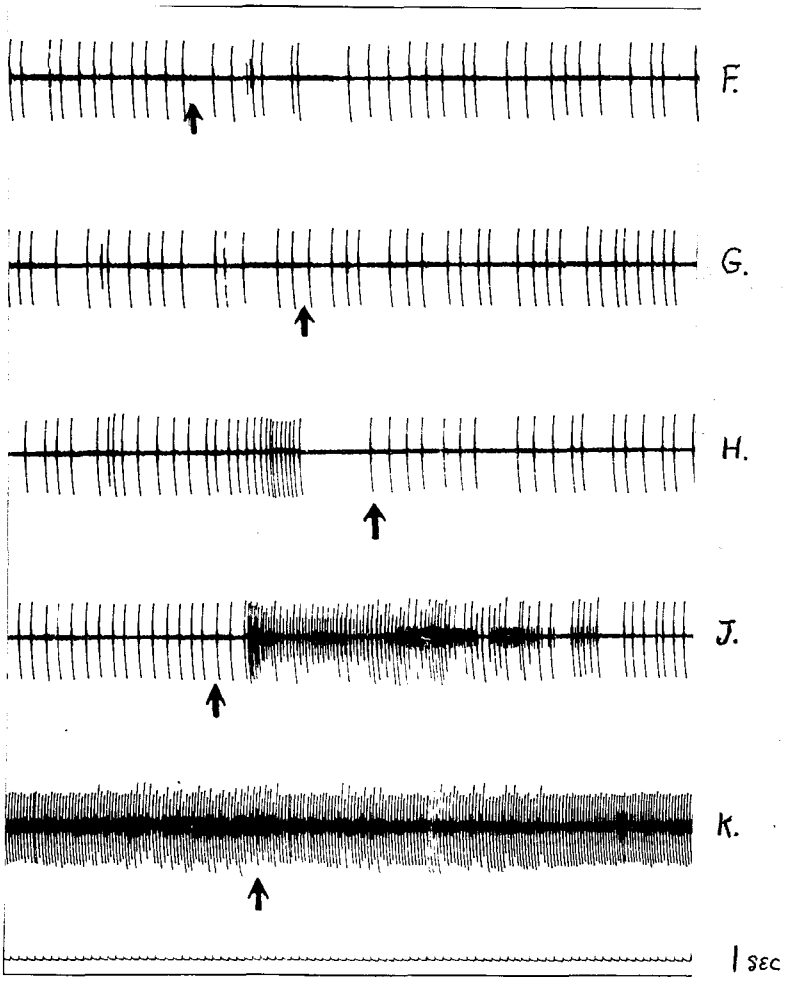


Figure 49 continued:

F). β-alanine 10^{-6}; G). GABA 10^{-6};

H). 5-HT 10^{-6}; J). Histamine base 10^{-6};

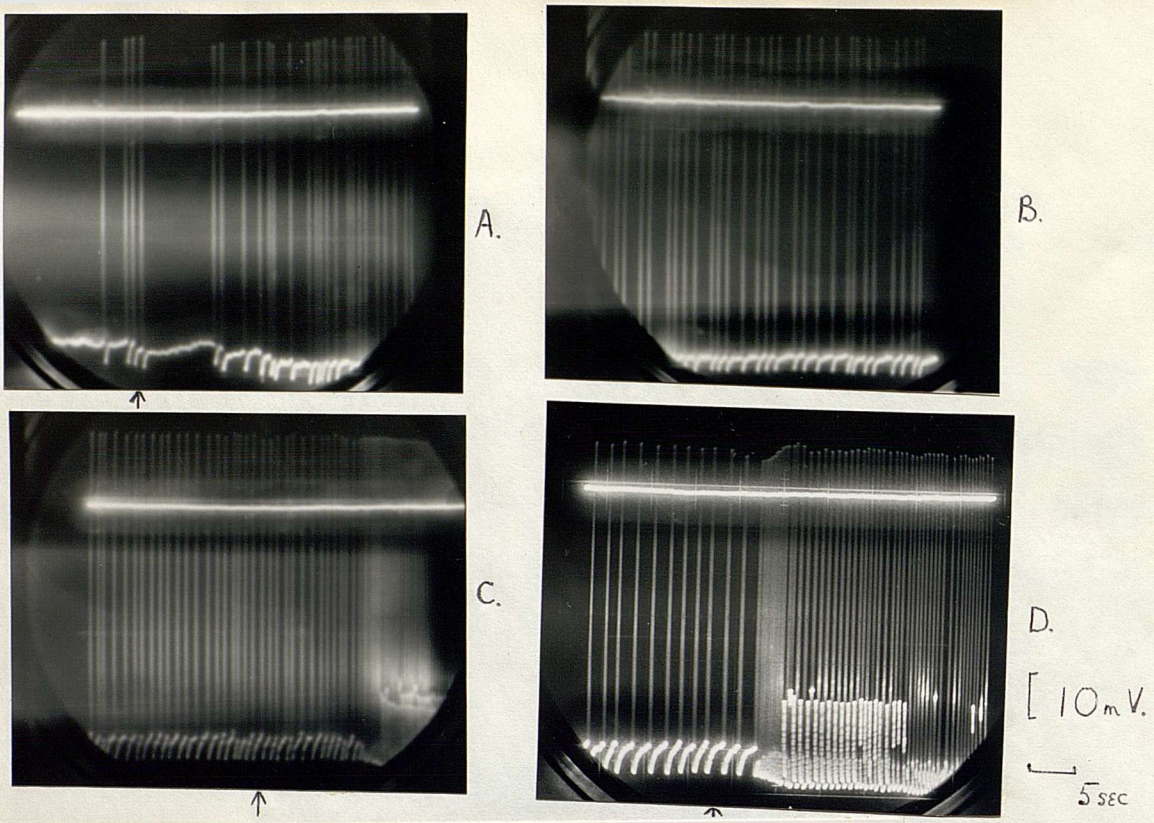
K). Dopamine 10^{-6} .

order at a concentration of 10^{-6}, fig. 49:-

5HTP	-	acceleration
Glutamic acid	-	no effect
Ach.	-	acceleration
Atropine	-	no effect
Ach. + Atropine	-	reduced acceleration
β -alanine	-	no effect
GABA	-	no effect
5HT	-	no effect
Histamine base	-	acceleration
Dopamine	-	no effect

5HTP caused slight maintained acceleration 10 seconds after the addition. The response was physiological and there was no change in the shape of either the A.P. or the R.P. height, fig. 50(i)A and B and (ii) α .

Ach. also had an acceleratory effect on the neurone. This response was physiological, fig. 50(i)C and (ii)β. The A.P. rate was increased and e.p.s.p.'s were induced. The A.P. lost the positive afterpotential, fig. 50(ii)β,C-F, and developed a negative one. This also occurred when the cell was driven, fig. 50(ii)β G. The Ach. response occurred after 20 seconds and lasted for 217 seconds. The addition


(i)
A.
B.
C.
D.
10m V.
5 sec

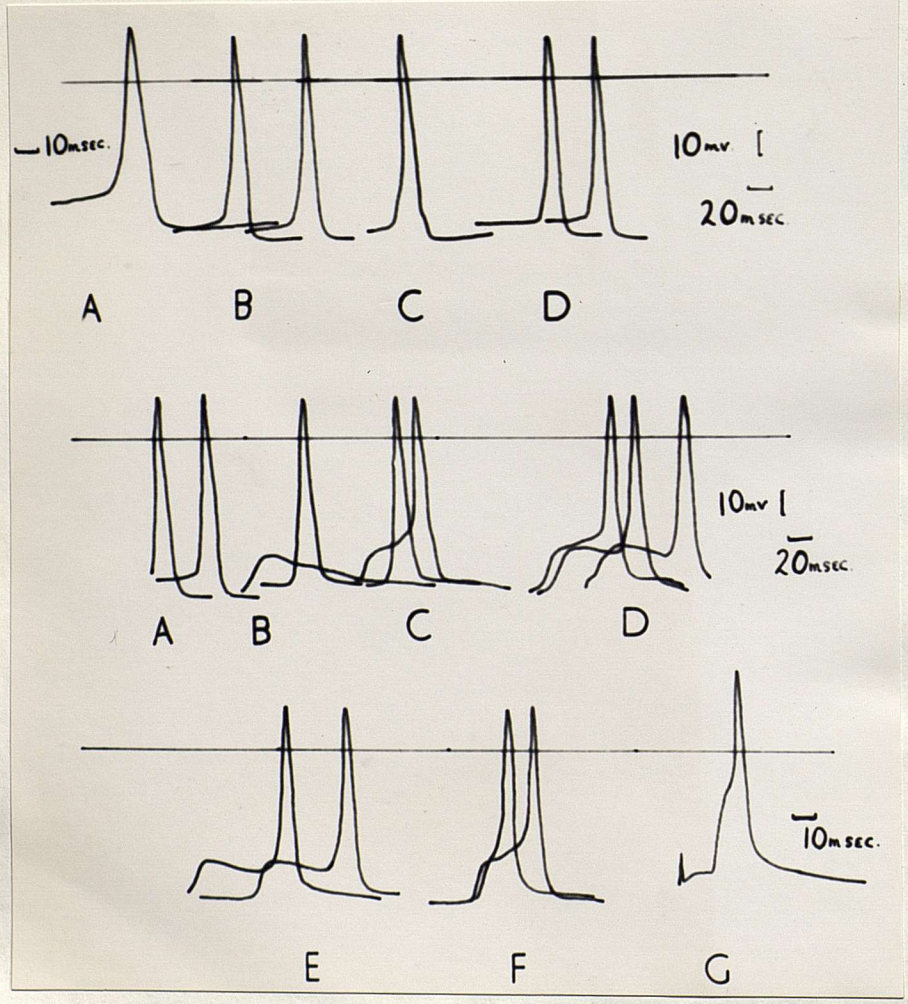

10msec.
10mv
20msec
α).
A
B
C
D
(ii).
10mv
20msec
β).
A
B
C
D
10msec.
E
F
G

Figure 50.

Figure 50 shows the filmed response to 5-HTP, acetylcholine and histamine and the shape of the action potentials after the addition of 5-HTP and acetylcholine. This was neurone 4.

(i).A).Shows the filmed response during and B). after the addition of 5-HTP IO^{-6}.

C). Shows the filmed response to acetylcholine IO^{-6}.

D). Shows the filmed response to histamine IO^{-6}.

(ii).α). Shows the shape of the action potential of neurone 4 to 5-HTP IO^{-6}; A). before and B). 44sec after, C). 50sec after and D). 56sec after the addition of 5-HTP.

β). Shows the shape of the action potential A). before and B). 106sec after, C). 111sec after, D). 118sec after, E). 134sec after, F). 149 sec after the addition of acetylcholine IO^{-6}.

G). is the shape of a driven action potential.

of Atropine had no effect. When Ach. and Atropine were added together, the response, which was reduced, occurred after 22 seconds. The acceleratory effect lasted for 42 seconds.

GABA and 5HT had no effect.

Histamine base at a concentration of 10^{-6} after 4 seconds accelerated the spontaneous activity and induced e.p.s.p.'s. The response lasted for 46 seconds. This response occurs in two parts. Firstly there is a period lasting 3.5 seconds, during which the activity is very fast. The A.P. height at the start of this period is reduced from the normal by 4mV and during the 3.5 seconds gradually recovers to normal. Also during this period the R.P. hyperpolarizes by 2-3mV, fig.50(i)D.

Dopamine had no effect on the activity.

CONCLUSION

This was another cell which was activated by both histamine and acetylcholine. In this case the acetylcholine was effective prior to the application of histamine. The histamine response resembled the reduced response of acetylcholine and atropine. Both drugs induced excitatory potentials as well as an increased rate of full action potentials. The histamine response occurred much sooner after its application than the

acetylcholine response did. The delay after the application of acetylcholine was approximately the same each time.

Neurone 5 is situated anterior to and right of, the large cells in the right parietal ganglion. This also was a silent cell, with a R.P. of -50mV. The following drugs at a concentration of 10^{-6} were tested:-

Glutamic acid	-	no effect
β-alanine	-	no effect
Ach.	-	no effect.

None of the drugs had an effect. At the beginning of the experiment there were slow hyperpolarizations, resembling very slow i.p.s.p.s.

Neurone 6 occurs in the anterior portion of the right parietal ganglion toward the inside edge. On penetrating this cell bursts of A.P.s were obtained but the cell became silent before the addition of thedrugs.

They were added in the following order at concentration of 10^{-6}:-

Ach.	-	no effect
Ach.	-	no effect
Glutamic acid	-	no effect

5HTP	-	no effect

None of the drugs tested had any effect on this cell.

Neurone 7 occurs in the same region as neurone 5. This was a spontaneously active cell. The following drugs were tested:-

5HTP	-	no effect
Glutamic acid	-	no effect
Ach.	-	no effect

None of the drugs tested had an effect.

Neurone 8 occurs in the posterior right region of the visceral ganglion, slightly post. to neurone 4. This was an active preparation. The following drugs at a concentration of 10^{-6} were tested:-

Glutamic acid	-	no effect
5HTP	-	no effect
Ach.	-	no effect
5HT	-	no effect

None of the drugs tested had an effect.

CONCLUSION

Neurones 5 to 8 were insensitive to the drugs applied. This shows that the action of these drugs is selective. A drug which was non-selective would excite or inhibit the bioelectric potentials of every neurone to which it was added. It also shows that acetylcholine applied after 5-HT, 5-HTP and glutamic acid is not necessary potentiated by them.

Neurone 9 is a large cell in the right parietal ganglion. It was spontaneously active during the experiment. The following drugs were added in sequence:

Glutamic acid	-	no effect
5HTP	-	no effect
Ach.	-	acceleration followed by inhibition.

13 seconds after the addition of Ach. there were a few e.p.s.p.'s and the activity ceased for 25 seconds, save for a single A.P. Another dose of 10^{-6} Ach. depolarized the membrane to zero.

CONCLUSION

The response to acetylcholine occurred after only 7 seconds.

This is a much shorter delay time than is normally observed with 1 x 10^{-6}. This type of response is associated with higher concentrations of acetylcholine. This is shown with neurone 10, where a concentration of 5 x 10^{-6} has a comparable effect after 8 seconds. This cell would appear to be more sensitive than the average to acetylcholine.

Neurone 10 occurs in the far left posterior corner of the visceral ganglion. The following drugs were tested:-

5HTP	-	no effect
GABA	-	no effect
Ach.	-	acceleration.

Ach. at a concentration of 5 x 10^{-6} increased the rate of activity after 8 seconds. There were no e.p.s.p.'s. The effect was pharmacological in nature. The height of the A.P. and R.P. fell and the former elongated. This process continued until the membrane was completely depolarized. After about 15 seconds the activity occurred in bursts of from 4 to 5 with a gap of 1.5 seconds. The positive afterpotential was not replaced by a negative one.

CONCLUSION

The response to acetylcholine had a short delay. This

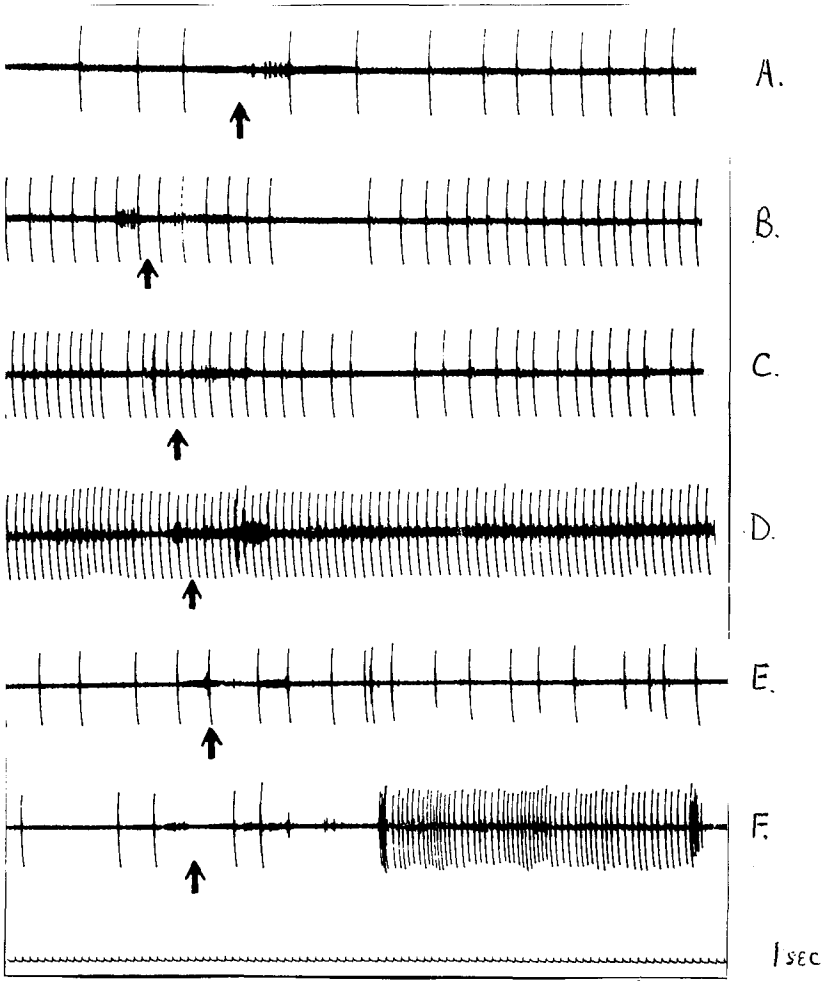


Figure 51 shows the response of neurone 11 to the addition of five drugs and an extract of snail heart added in the following order:-

A). Acetylcholine 10^{-6}; B). 5-HT 10^{-6};

C). Dopamine 10^{-6}; D). GABA 10^{-6};

E). β-alanine 10^{-6}; F). Heart extract.

The heart extract accelerated the activity of the cell.

is indicative of a pharmacological response.

Neurone 11 is a large cell in the right posterior parietal ganglion. The following drugs were tested in concentration of 10^{-6}, fig. 51:

Ach.	-	no effect
5HT	-	slight inhibition
Dopamine	-	slight inhibition
GABA	-	no effect
β -alanine	-	no effect
Snail heart extract	-	acceleration

Since the activity of this neurone was irregular, the slight inhibition after both 5HT and dopamine is probably due to that, fig. 51B & C. 24 seconds after the addition of the heart extract there was a burst of activity which lasted 42 seconds, fig. 51F. This burst was immediately preceded by a much faster burst lasting around a second. After 42 seconds there was a muscular contraction and the electrode came out of the cell. It was possible to repenetrate the cell, the activity being one A.P. per 3 seconds. The addition of 0.3M $MgCl_2$ inhibited the activity and then after 80 seconds, the activity returned at 1 A.P. per second.

CONCLUSION

It is interesting that the heart extract had a delayed effect, similar to the type of response observed with a physiological eoncentration of acetylcholine or 5-HT.

NICOTINE

The effect of nicotine has been studied in a few experiments, with regard to its possible role as a synaptic blocking agent. In experiments using isolated brains, it was found that a concentration of nicotine down to 10^{-3} inhibited the activity of the cell but only slightly affected the resting potential. Concentrations as low as 10^{-6} would completely inhibit the activity.

Nicotine had an effect on both the resting and action potential of a cell in a brain in situ. Nicotine added to a cell whose activity was inhibited by stimulating the left pedal nerve, not only blocked the inhibition but later depolarized the cell to zero. In an experiment in which the cell was driven, nicotine slowly depolarized the cell to zero without blocking the activity.

CONCLUSIONS

Nicotine in the intact brain is able to depolarize the

resting potential of the neurone to zero and stop their activity. In the isolated brain it reduced the resting potential to approximately 40mV and blocked the activity. Due to the fact that it would appear to have a pharmacological effect of its owh, its use was discontinued in these experiments.

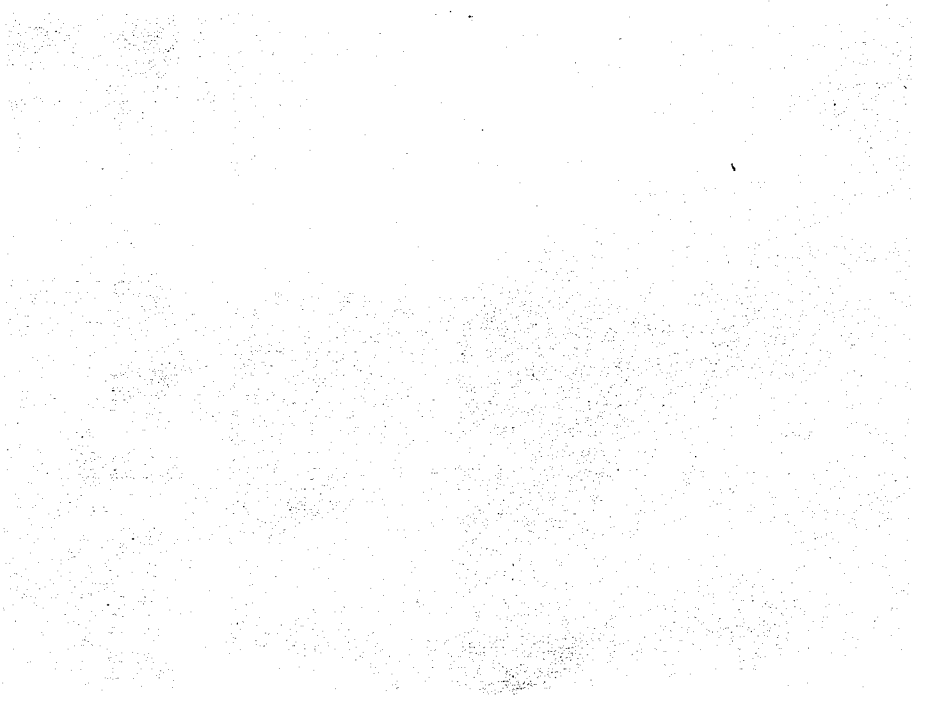
GENERAL CONCLUSION OF SECTION TWO

From the eleven neurones tested with three or more drugs it would appear that no drug affects every cell to which it is applied. Certain compounds such as acetylcholine, histamine, and 5-HTP are more likely to affect the cell than others, for example, glutamic acid and GABA. It is difficult from the experiments to suggest any "sequence" effect.

These experiments indicate the usefulness of compounds such as atropine, eserine and reserpine in assessing the potentialities of a given drug. Certain safeguards must be born in mind when using these compounds. Each may have an effect on its own, independent of a possible potentiation or antagonism of a proposed transmitter. This is true in the case of eserine where it may have an acceleratory effect. This response differs from the acceleration observed after acetylcholine. Though the eserine effect was physiological it occurred after 8 seconds and there were no e.p.s.p.'s.

The acetylcholine effect occurred after 24 seconds and this was accompanied by e.p.s.p.'s.

There would appear to be two types of response. The response to the applied drug may occur either 4 to 6 seconds after its application or 20 to 40 seconds after its application. The former may be associated with a general permeability increase of the whole soma, while the latter may be associated with activation via the synaptic regions of the axon.



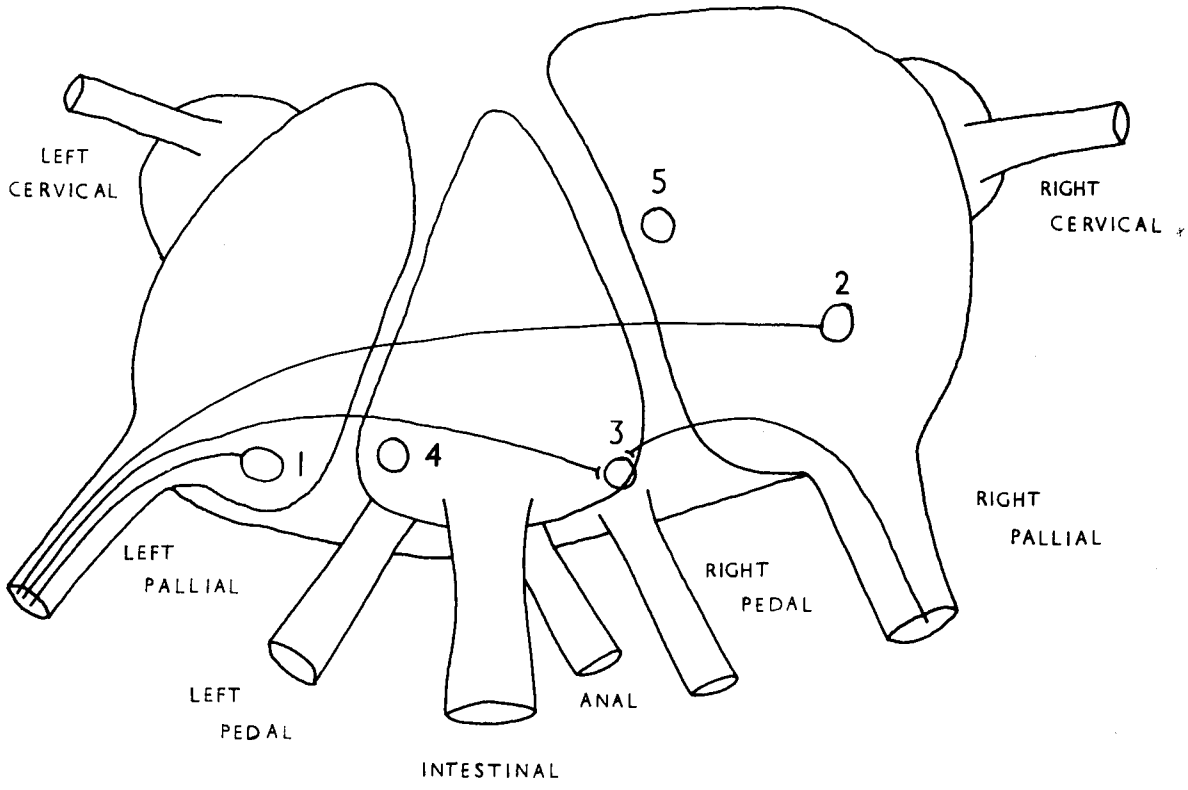
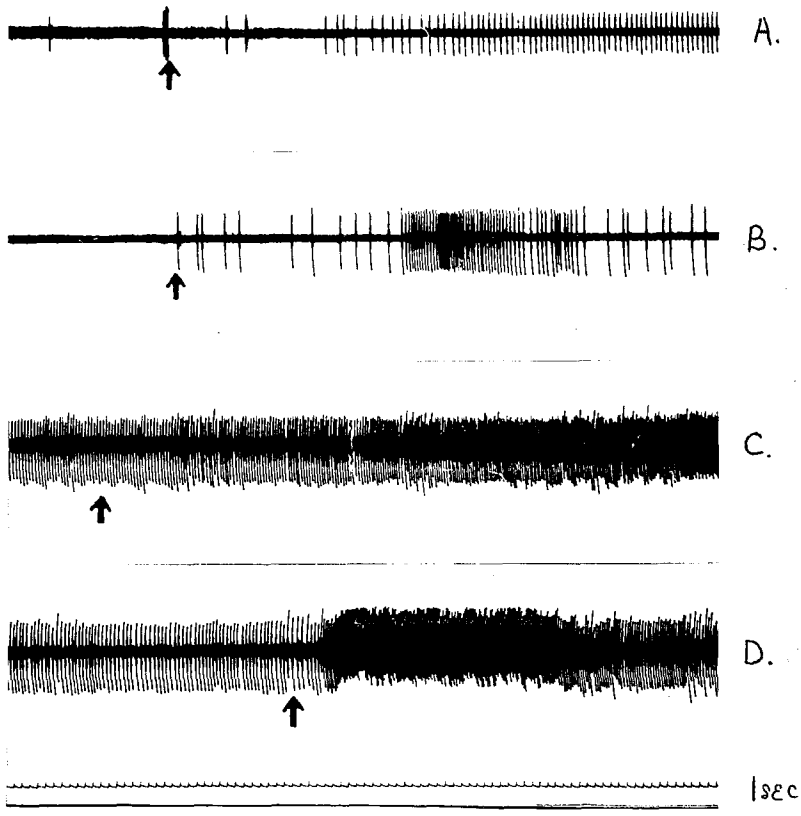
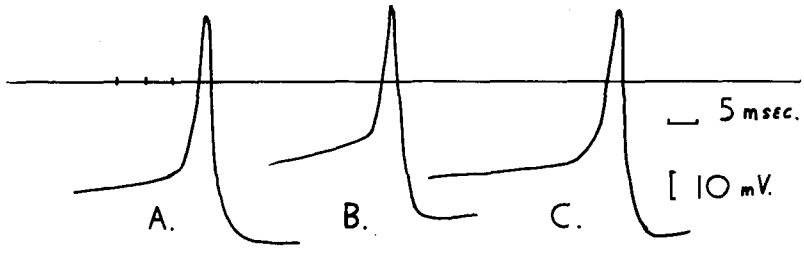


Figure 52 is a diagram of the suboesophageal ganglionic mass to show the positions of the five experimental neurones, each of which is sensitive to a given drug.



(i)



(ii)

Figure 53 shows the response of cell I from four different brains to the application of 5-HTP 10^{-6}.

(ii). Shows the shape of the action potential A). before and B). and C). after the addition of 5-HTP to brain B. The duration of the action potential increased from 5 to 8msec.

SECTION THREE

THE EFFECT OF A DRUG ON THE SAME CELL FROM SEVERAL DIFFERENT BRAINS.

In testing drug response it is important to show that a given drug has the same effect on a known neurone from one brain to another. In the snail brain it is possible to identify certain of the larger neurones. These range in size from 120-200 μ. Fig. 52 indicates the positions of 5 of these neurones located in the left and right parietal and visceral ganglia. The cells can be identified partly on their anatomical position and partly by their ability to be driven by stimulating specific peripheral nerves.

A 5-HTP SENSITIVE CELL

This is neurone 1 in fig. 52. The response from four experiments are shown in fig. 53. In two of these experiments the cell was virtually silent and in the other two the cell was very active. In fig. 53A, the addition of 10^{-5} 5-HTP induced e.p.s.p.'s. The shape of these can be compared with the driven e.p.s.p.'s, fig. 30(iii), (page 160). Fig. 53B shows the response after the addition of 10^{-6} 5-HTP to the same neurone in another brain. The main response occurred

approximately 30 seconds after the addition of the drug. The change in shape of the action potential can be seen in fig. 53(ii). The threshold for the firing of each action potential was reduced from 38 to 23mV. The maximum afterpotential was reduced from -63mV to -55mV. The duration of the action potential remained almost constant.

The response followed a physiological pattern rather than a pharmacological one. The response lasted approximately 24 seconds. There were no e.p.s.p.'s. There was a slight increase in the size of the positive afterpotential. This is contrasted to the situation often found in physiological experiments with acetylcholine, where the positive afterpotential disappears and gives way to a negative afterpotential, fig. 50(ii)β, E, (page 184). In fig. 53C and D the neurone was firing rapidly and on the addition of 10^{-6} 5-HTP, the rate of the activity greatly increased with little change in shape. In C, the response occurred after 40 seconds, while in D the response occurred after 4 seconds.

The action potentials of this neurone were characteristically large, with an overshoot of around 20mV, the duration was short, of the order of 6 to 10msec, and there was a large difference between the maximum/minimum resting potential. Acetylcholine has no effect on this neurone.

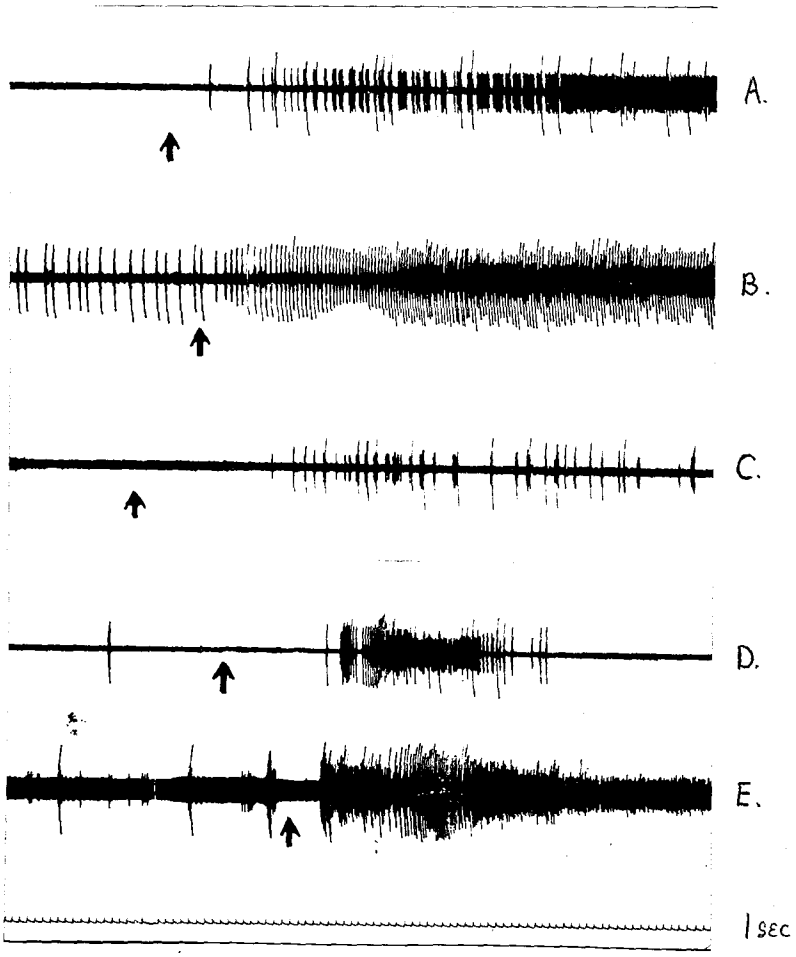


Figure 54 shows the response of cell 3 from four different brains to the application of acetylcholine IO^{-6}. In each case the cell was excited by the drug. A). and B). are from the same brain. The first application of acetylcholine induced mainly e.p.s.p.'s while the second application induced mainly full action potentials.

In these experiments there would appear to be link between the size of the resting potential and the degree of the response to 5-HTP. The resting potentials in the four experiments were, for A) -60mV, B) -54mV, C) -52mV, and D) -45mV. As the resting potential was lowered, so the response increased. The response appeared to increase both in overall duration and intensity and in delay time prior to the main effect. It is hoped to do further experiments to either confirm or refute this suggestion.

AN ACETYLCHOLINE SENSITIVE CELL

This is neurone 3 in fig. 52. Fig. 54A and B show the response of adding 1 x 10^{-6} acetylcholine consecutively with a gap of 170 seconds. Prior to the first application of acetylcholine, the cell had a resting potential of -70mV, and prior to the second application, the resting potential was -67mV. The first application led to a few action potentials but mainly e.p.s.p.'s while the second application led almost entirely to action potentials. The delay in B is 3 seconds, and the delay in A is 10 seconds, C, D and E are separate experiments. The delay in C was 20 seconds, in D 15 seconds, and in E, 4 seconds. The shape of the action potential after

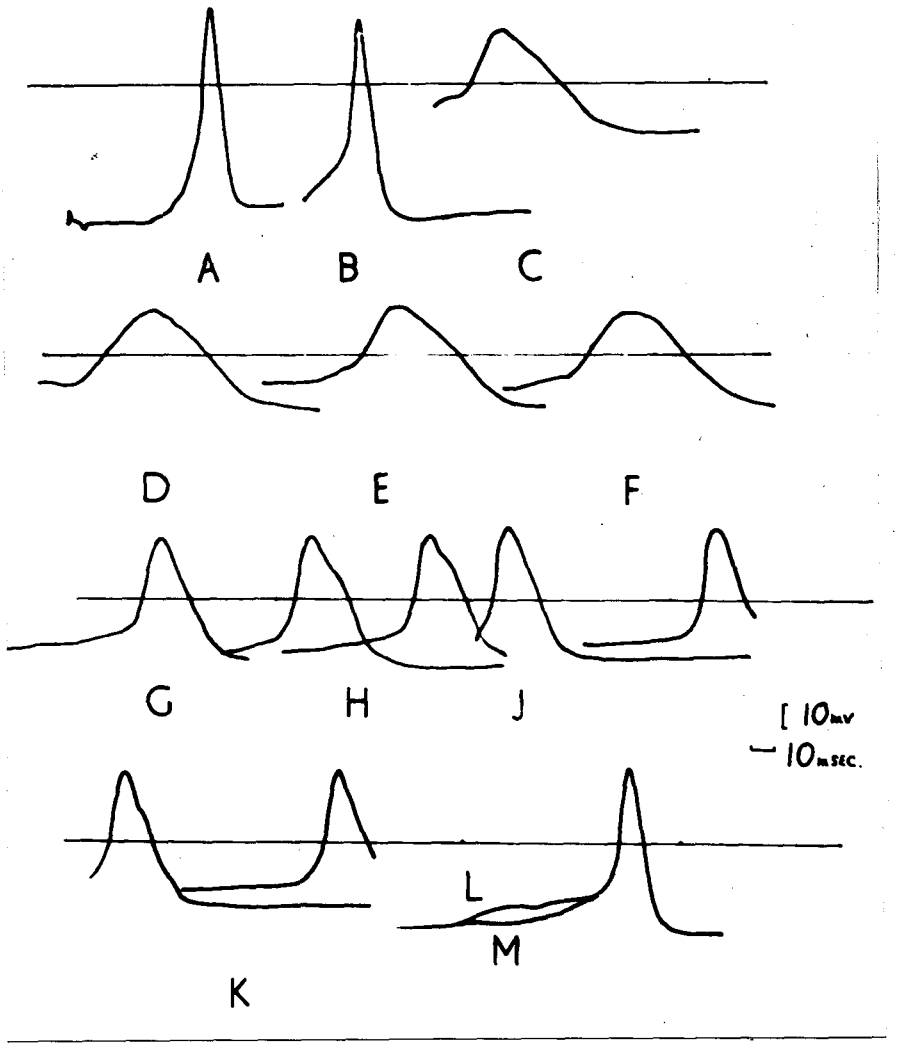


Figure 56 shows the effect of acetylcholine 10^{-6} on the shape of the action potential from neurone 3 of brain E). of figure 54.

A). is a driven action potential.

B). before and C). 30sec after, D). 38sec after, E). 46sec after, F). 53sec after, G). 143sec after, H). 150sec after, J). 155sec after, K). 160sec after, L). 215sec after and M). 223sec after the acetylcholine.

the application of acetylcholine in E is shown in Fig. 55. This was a pharmacological effect from which the cell partially recovered. The resting potential in E was -52mV. In this experiment the driven action potential, fig. 55A has a negative afterpotential. A similar shape can simetimes be seen after a physiological acetylcholine response, fig. 50iiβ.

There would appear to be a correlation between the height of the resting potential and the response to acetylcholine; the lower the resting potential so the greater is the response to acetylcholine. The delay time for a response is much shorter for a pharmacological effect than for a physiological one. This agrees with the experiments in which histamine has a short delay time and acetylcholine a long one. In this case it was suggested that the histamine excites the soma membrane while the acetylcholine excites the synaptic membrane only. A pharmacological concentration of acetylcholine could also affect the soma.

In fig. 52 three other cells are tentatively associated with a possible chemical transmitter. Cells 4 and 5 are suggested to be sensitive to acetylcholine, and cell 2 was found to be more sensitive to histamine.

It is hoped to continue this part of the investigation and see the extent to which it is possible to identify a specific neurone chemically.

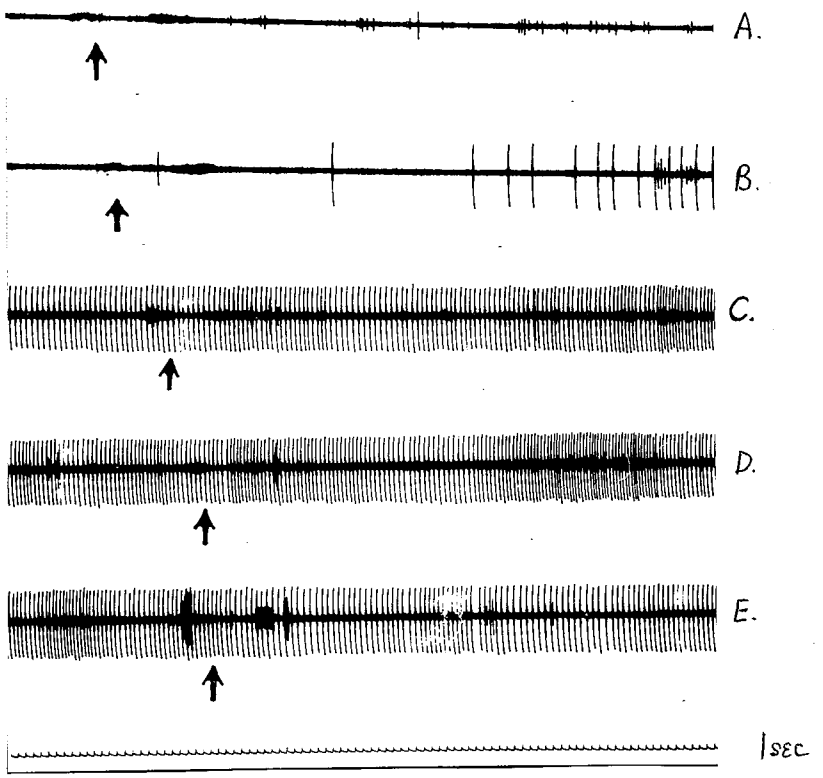


Figure 56 shows the response to drugs of neurone No. 173. The drugs were added in the following order:-

A). Urocanylcholine 5x 10^{-7}; B). Crotonylcholine 7x 10^{-7};

C). Butyrylcholine 5x 10^{-7}; D). Acetylcholine 10^{-6};

E). Senecioylcholine 10^{-6};

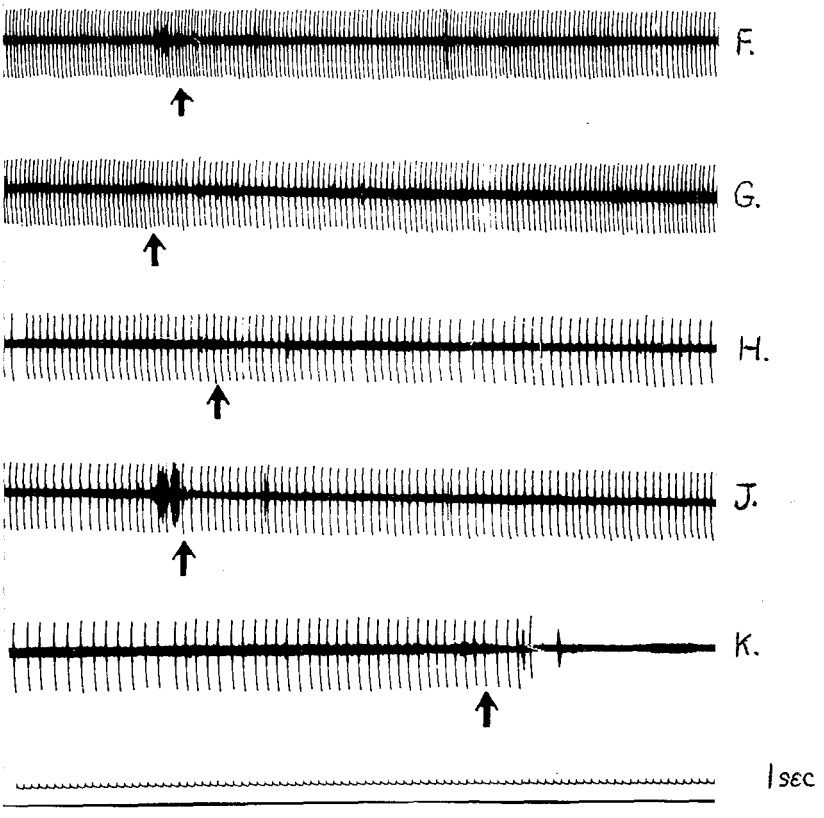


Figure 56 continued:

F). Butyrylcholine 5x 10^{-7}; G). Adenosine diphosphate 10^{-6}; H). 5-HTP 10^{-6}; J). 5-HT 10^{-6}; K). Acetylcholine 10^{-4}.

Urocanyl and crotonylcholine induced activity in a silent cell. The other drugs had little effect, except 10^{-4} acetylcholine which depolarized the cell to zero.

SECTION FOUR

CHOLINE COMPOUNDS OTHER THAN ACETYLCHOLINE

Within recent years, interest has developed in the other choline esters such as urocanylcholine, crotonylcholine, butyrylcholine, senecioylcholine, etc. Thanks to Dr. V.P. Whittaker, of the Agricultural Research Council's Department of Biochemistry, Brabraham, I was given a small amount of each of these choline compounds to carry out preliminary experiments on their action on the snail neurone.

In these experiments a neurone was exposed, and a microelectrode inserted into it. A series of drugs was then applied in a known order and their effects noted for this one cell. Though one cannot state that there was no interaction of effects or tachyphylaxis, the results give some indication as to the relative effects of the compounds.

Details of four experiments will now be given to demonstrate the type of effects that these choline compounds had.

In a typical experiment, No. 173, the 5 choline compounds together with adenosine diphosphate, 5-HTP, and 5-HT were tested in the following order with the results shown in fig. 56. In this experiment the activity was slightly arhythmic as can be seen in trace D and E. These are A.C. pen recordings

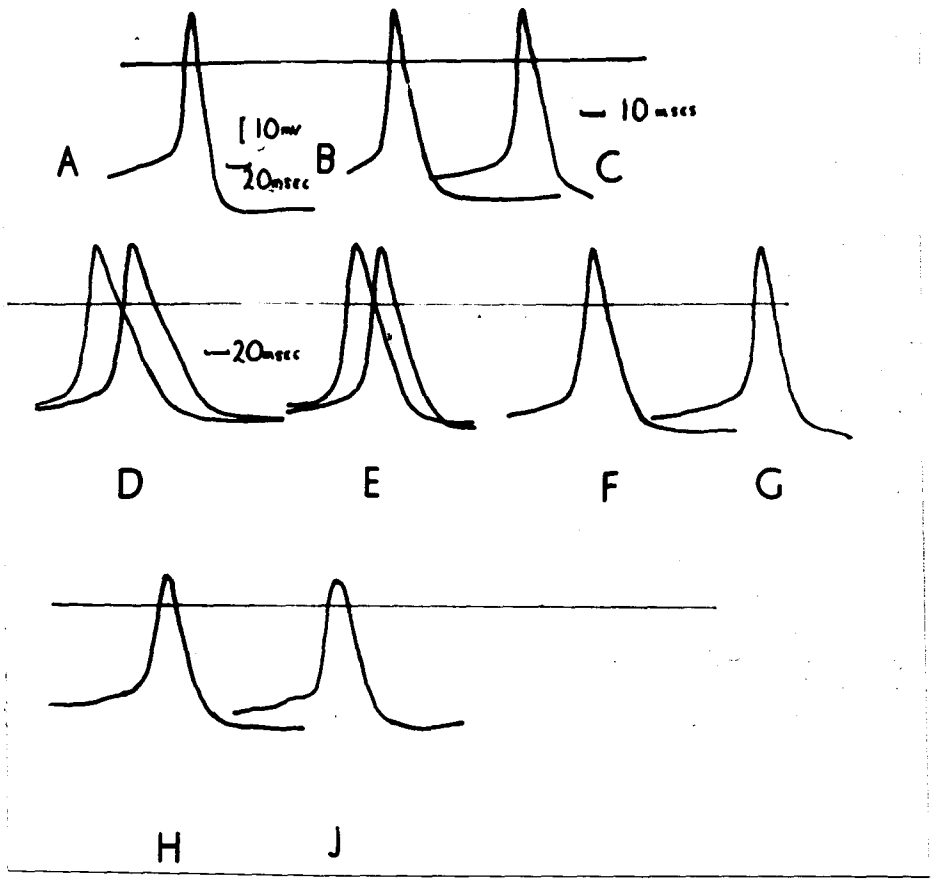


Figure 57 shows tracings taken from photographs of the shape of the action potentials before and after the addition of butyrylcholine, acetylcholine and adenosine diphosphate.

A). before and B). 160sec after, C). 166sec after the addition of butyrylcholine. D). 58sec after, E). 63sec after, F). 68sec after, G). 108sec after acetylcholine. H). II2sec after, J). 120sec after adenosine diphosphate.

but the resting potential values were recorded during the experiment. The shape of the action potentials can be seen in fig. 57, before and after the application of three of the drugs.

Urocanylcholine	5×10^{-7}	e.p.s.p.'s;
Crotonylcholine	7×10^{-7}	induced activity on an inactive cell;
Butyrylcholine	5×10^{-7}	no effect;
Ach.	1×10^{-6}	no effect;
Senecioylcholine	1×10^{-6}	no effect;
Butyrylcholine	5×10^{-7}	no effect;
Adenosine diphosphate	1×10^{-6}	slight inhibition;
5-HTP	1×10^{-6}	no effect;
5-HT	1×10^{-6}	no effect;
Ach.	1×10^{-4}	depolarization of resting potential to zero;

Both urocanylcholine and crotonylcholine could induce activity in a silent cell. Urocanylcholine depolarized the membrane from -50 to -46mV to produce e.p.s.p.'s after 17 seconds, and crotonylcholine produced action potentials after 46 seconds. In neither case was the response very dramatic and in no way resembled the response obtained from an Ach. sensitive cell. The other two choline compounds and Ach. at physiological doses had no effect. Butyrylcholine did

not affect the rate of the activity, nor the shape of the action potential, fig.57 B & C.

Ach at 10^{-6} did not alter the rate of the activity, but increased the duration of the action potential, fig 57D. The shape of the action potential gradually recovered, fig. 57E, F & G.

ADP, besides slightly inhibiting the activity, reduced the size of both the A.P. and R.P. The overshoot of the A.P. was reduced from 27 to 13mV, which did not fully recover, fig. 57H & J. Neither 5HT nor 5HTP had any effect on the activity or shape of the A.P.

Ach. at 10^{-4} after 5 seconds reduced the R.P. to zero and the cell did not recover.

The choline compounds were tested on a large cell in the right parietal ganglion.

Butyrylcholine	5×10^{-7}	-	acceleration and inhibition;
Urocanylcholine	5×10^{-7}	-	no effect;
Crotonylcholine	7×10^{-7}	-	no effect;
Ach.	10^{-6}	-	no effect;
Senecioylcholine	10^{-6}	-	inhibition;

3 seconds after the addition of butyrylcholine there was an increase in the A.P. rate, this was followed by a period of

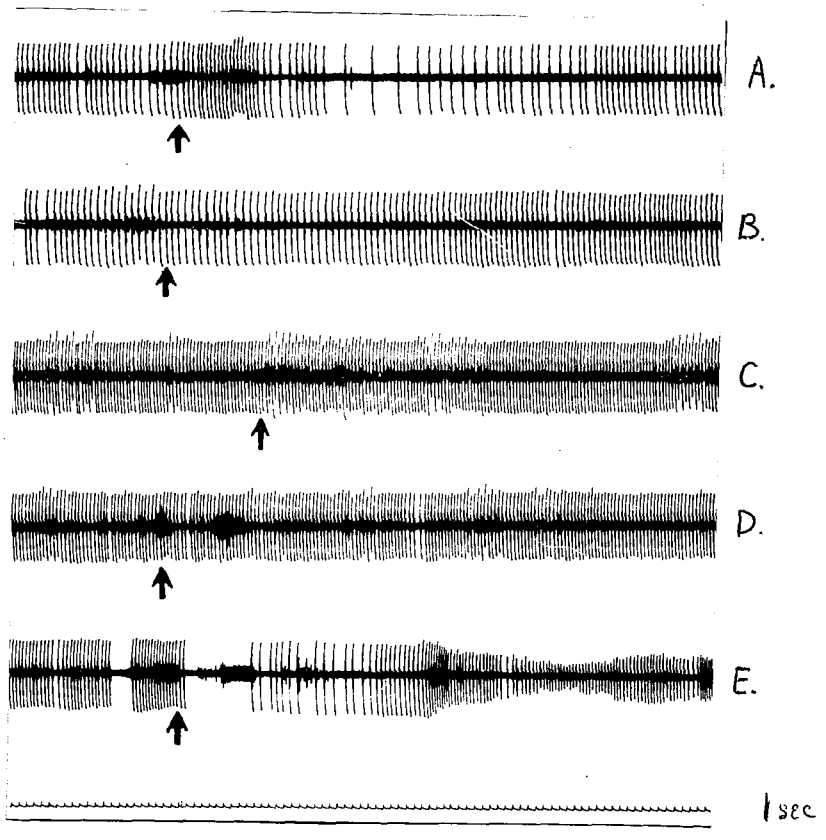


Figure 58 shows the effect of five choline esters on a single neurone.

A). Butyrylcholine $5x\ 10^{-7}$; B). Urocanylcholine $5x\ 10^{-7}$; C). Crotonylcholine $7x\ 10^{-7}$; D). Acetylcholine 10^{-6}; E). Senecioylcholine 10^{-6}.

Butyrylcholine initially accelerated and then inhibited the activity while senecioylcholine inhibited and then had a pharmacological effect on the activity.

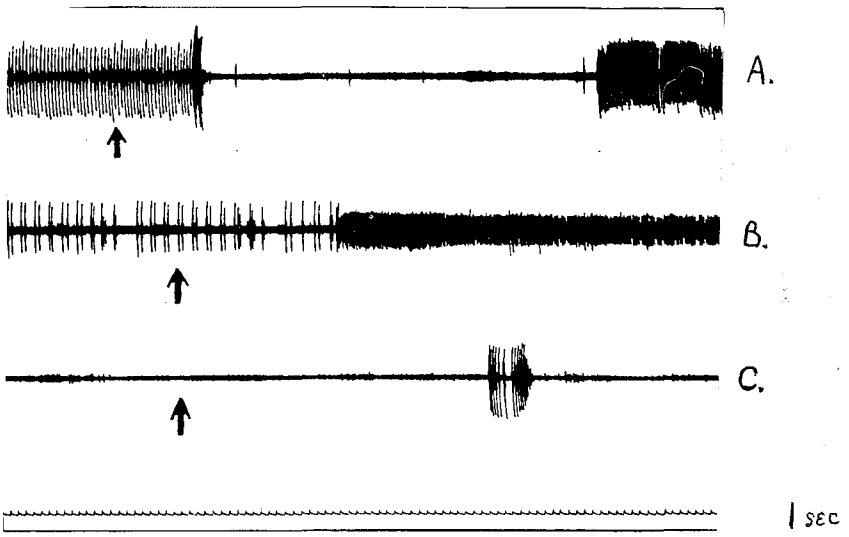


Figure 59 shows the effect of butyrylcholine on three neurones from the visceral ganglion of the same brain.

A). Butyrylcholine applied at 7×10^{-7};

B). Butyrylcholine applied at 5×10^{-9};

C). Butyrylcholine applied at 5×10^{-8}.

The first response was pharmacological in nature, as also was the second response.

inhibition, Fig. 58A. There were no e.p.s.p.'s. Urocanylcholine, Ach., and Crotonylcholine had no effect, Fig. 58B, D & C. 1.5 seconds after the addition of senecioylcholine the activity ceased, the R.P. falling to zero, fig. 58E. After 8 seconds depolarizations occurred, similar to e.p.s.p.'s, but the membrane failed to recover completely. This was a pharmacological response similar to that obtained after the addition of Ach. 10^{-4}.

Two problems should be considered in studying the effect of choline compounds on the cell. If a given concentration of a drug depolarized the cell membrane to zero, would this concentration depolarize (a), all the cells in the brain, (b), all the cells sensitive to that drug, or (c), have no influence on the response of the other neurones. The second problem is whether a given concentration will facilitate the response to a second dose or conversely render the membrane insensitive.

The effect of butyrylcholine at a concentration of 7×10^{-7} was tested on three neurones from the visceral ganglion of the same brain.

The first neurone was spontaneously active with a rate of 2 to 3/sec. 10 seconds after the application of butyrylcholine, there was a burst of 8 action potentials in one second and the R.P. fell to zero, fig. 59A. 32 seconds later the R.P. rose to -10mV and after 19 seconds suddenly rose to -48mV slowly

depolarising to -42mV at each action potential. The action potential had a negative afterpotential. The overshoot of the action potential was reduced from +20mV to +8mV and the duration had increased from 8msec to 30msec. Gradually a pharmacological response appeared, the height of the action and resting potentials falling and the duration of the action potential elongated.

It was decided to test a range of concentrations on the second neurone, starting with 5×10^{-10}. This concentration had no effect. 21 seconds after the addition of 5×10^{-9}, the A.P.s were replaced by a prolonged burst of e.p.s.p.'s, fig. 59B. These were of 10mV height and 80msec duration. There was no effect on the R.P. 82 seconds later the R.P. fell from -35mV to zero. The cell did not recover.

The third neurone was silent with a R.P. of -40mV. 5×10^{-10} and 5×10^{-9} had no effect. In fact the R.P. hyperpolarized to -44mV. 40 seconds after the addition of 5×10^{-8} there was a burst of A.P.s lasting 4.5 seconds and the R.P. depolarized to zero, fig. 59C.

CONCLUSION

The concentration of butyrylcholine which depolarized one neurone does not have a similar long term effect on all the other neurones. The neurones would appear to differ in their

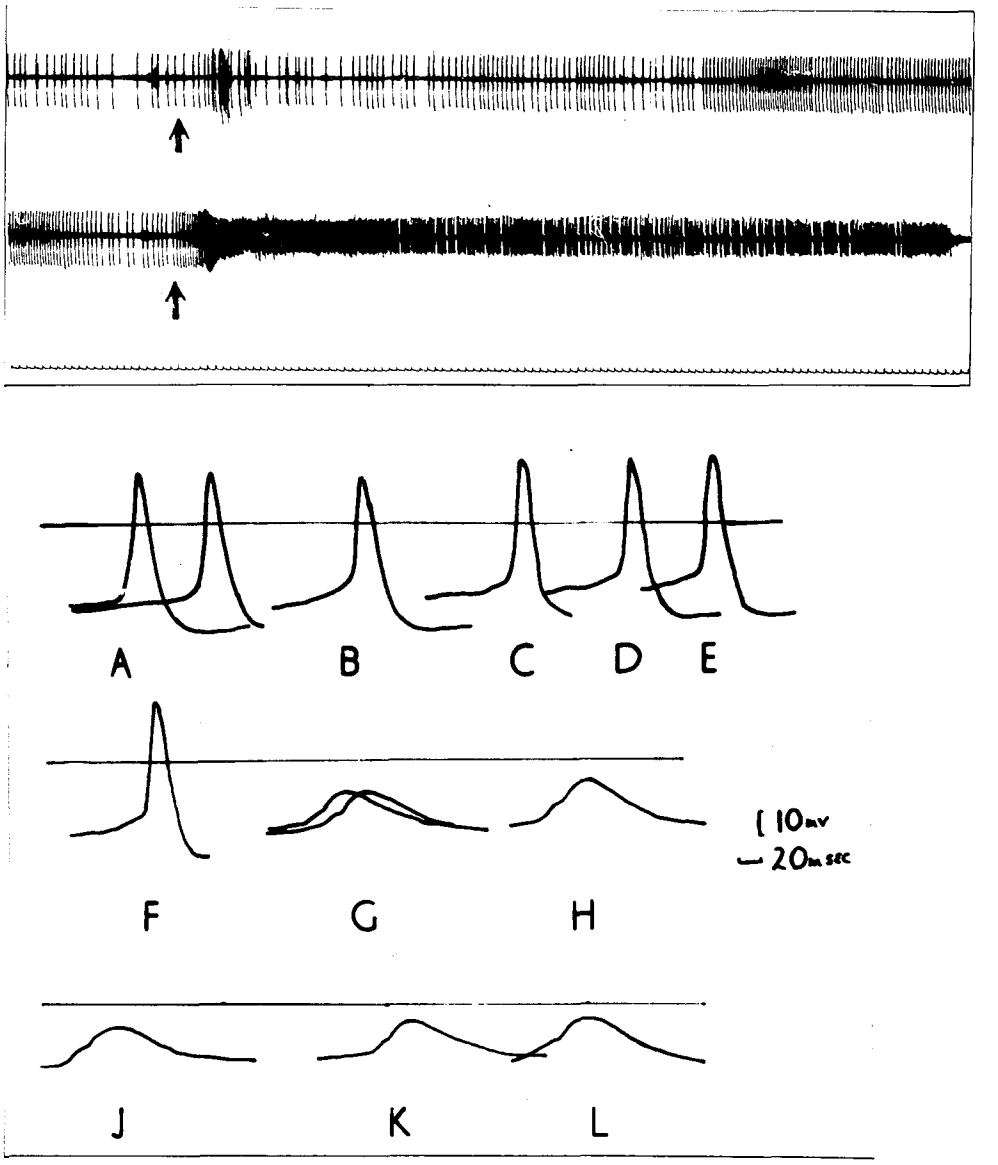


Figure 60(i) shows a pen recording of the effect on a cell of two applications of $5x10^{10}$ butyrylcholine. The two traces are continuous.

(ii) shows the shape of the action potentials of (i).

A). before and B). 33sec after, C). 40sec after, D). 48sec after and E). 57sec after the first application. F). 20sec before and G). 25sec after, H). 37sec after, J). 42sec after, K). 48sec after, L). 72sec after the second application of butyrylcholine.

sensitivity to a given drug. It is interesting in this experiment that all three neurones were responsive to butyrylcholine.

In experiment No. 178, two applications of 5×10^{-10} butyrylcholine were added to the neurone. The first addition caused a gradual increase in the A.P. rate which was maintained, fig. 60(i) and (ii)A. 3 seconds after the addition of the second dose there was a prolonged burst of A.P.s and e.p.s.p.'s, fig. 60(i)B and (ii)G-L. After 91 seconds the R.P. fell to -12mV and did not recover.

CONCLUSION

It would appear that a second dose of a drug is by the first application facilitated.

The overall effect of the choline compounds on the activity of the neurones can be seen in Table 6.

NUMBER OF EXPERIMENTS

EFFECT AND THRESHOLD

COMPOUND	PHARM.	PHYSIOL. ACCEL.	PHYSIOL. INHIBIT	NO EFFECT	TOTAL
Butyryl.	6: 5×10^{-10}	2: 5×10^{-7}	1×10^{-6}	3	11
Senecioyl.	1: 1×10^{-6}	in one experiment the A.P. was replaced by an e.p.s.p. at the same rate	1×10^{-6}	2	5
Urocanyl.	2: 5×10^{-7}	1: 5×10^{-7}	-	1	4
Crotonyl.	-	2: 7×10^{-7}	-	3	5

Butyrylcholine was tested on 11 neurones. In 6 of these experiments the ester had a pharmacological effect, that is, the concentration applied depolarized the cell potential to zero and it did not recover. In 2 experiments the activity was accelerated by butyrylcholine, and in one of these the acceleration was followed by a period of inhibition, fig. 58A. The threshold for acceleration was 5×10^{-7}. Certain neurones would appear to be very sensitive to butyrylcholine, since the threshold for a pharmacological effect was 5×10^{-10}.

It is suggested that in the case of those cells exhibiting a pharmacological effect that at a lower concentration the ester would have a physiological effect. This type of response can be seen in fig. 60(i)A, where a pharmacological response was followed by prolonged acceleration.

Senecioylcholine was tested on only 5 neurones. This was the only ester to exhibit initial inhibition of the activity of one cell at a concentration of 1×10^{-6}, fig. 58E. In one case a concentration of 1×10^{-6} had a pharmacological effect. In another experiment senecioylcholine had no effect on the rate of the activity but the full action potential was replaced by an e.p.s.p. In the remaining 2 experiments the ester had no effect.

Urocanylcholine was tested on only 4 neurones. In 2 cases it had a pharmacological effect at a concentration of 5×10^{-7}. In one case it depolarized the resting potential sufficiently to induce e.p.s.p.'s. In this respect it resembles the action of acetylcholine. It had no effect on the remaining neurone.

Crotonylcholine was tested on 5 neurones. This compound did not exert a pharmacological effect at the concentration applied. In this respect it resembled acetylcholine. On two occasions it either induced activity, fig. 56B, or accelerated the spontaneous activity. In the remaining three experiments it had no effect.

CONCLUSIONS

All four choline esters had an effect on the bioelectric potentials of the snail neurone. The physiological response was mainly one of acceleration but in the case of senecioylcholine there was a clear example of inhibition. In three out of four cases the ester appeared to be more potent in its effect than acetylcholine. This would suggest that the neurone membrane was more sensitive to them or that they were destroyed at a slower rate or not at all. It is hoped that these compounds can be retested on cells known to be acetylcholine sensitive.

DISCUSSION

Before one can postulate that a chemical compound is a likely transmitter, certain criteria have to be fulfilled.

(1) The substance must be shown to be present in, and synthesised by, the relevant neurones.

(2) It must be liberated from these cells when they are stimulated, and it must be capable of stimulating or inhibiting other neurones standing in synaptic relationship.

(3) The amount required to demonstrate this excitatory or inhibitory action must be similar to the amount of the compound released on stimulation.

(4) The nervous system must have enzymes present capable of destroying the transmitter substance quickly.

(5) The substance must have the same type of effect on the postsynaptic membrane as occurs on stimulation of the presynaptic axon.

The snail neurones are not extremely sensitive to drugs. The abdominal ganglion neurones of Aplysia are sensitive to a concentration of 1 x 10^{-12}g/ml acetylcholine, (Tauc &

Gerschenfeld, 1960); the heart of frogs at certain times of the year appear to be sensitive to around 1 x 10^{-15}g/ml acetylcholine; the heart of Venus mercenaria is sensitive to 1 x 10^{-9}g/ml 5-HT, (Twarog & Page, 1953); the heart of Helix is sensitive to 1 x 10^{-15}g/ml, (Kerkut & Laverack, 1960); the leech muscle is sensitive to 1 x 10^{-9}g/ml acetylcholine, (Minz, 1955); and the alveolar muscles of the frog's lung are sensitive to 1 x 10^{-16}g/ml acetylcholine, (Dijkstra & Noyons, 1939). The lowest threshold recorded in this study on the neurones of Helix was 10^{-11}g/ml for the inhibitory effect to dopamine.

The responses shown in the table, (page 151) are to the application of physiological concentrations of the drug, that is, a concentration which is likely to occur in the snail brain. This concentration was arbitrarily set at 1 x 10^{-6}g/ml, since from the literature this would seem to be the maximum concentration occurring naturally in the brain.

The responses from each group of drugs will now be briefly discussed, together with their individual possibilities as likely transmitters in the snail brain.

ACETYLCHOLINE AND DMAE

Acetylcholine has been found both in this and other investigations to affect the activity of molluscan neurones. During the present study an acetylcholine-like substance in

the snail brain has been extracted using the rat jejunum as a bioassay. It was present at an equivalent concentration of 5-10 μg/g. Chromatographic analysis also indicated that a choline compound is present, in this case it most resembled urocanylcholine, though the evidence is not decisive. This choline ester, together with butyryl-, crotonyl-, and senecioylcholine have been tested on the snail brain. These esters tend to be more sensitive than Ach. Helix neurones were most sensitive to butyrylcholine but urocanylcholine resembled Ach. in inducing e.p.s.p.'s on a resting cell. Carbaminoylcholine has been found to be twenty times as active and butyrylcholine 0.3 as active as Ach. on the heart of Helix pomatia, (Megemont, Dastugue and Bastide, 1960).

Choline esterases occur in snail blood, (Augustinsson, 1946), and the response to Ach. can be potentiated by eserine and reduced by atropine. Certain cells in the visceral ganglion are sensitive only to Ach. The evidence is reasonably good for the role of a compound like Ach. as a transmitter in the snail brain.

In addition to the acetyl ester of choline, four other esters were tested on the snail neurones. These were the butyryl, crotonyl, urocanyl and senecioyl esters of choline. Butyryl, urocanyl, and senecioylcholine were found to have pharmacological effects at concentrations which would bring about a

physiological response from acetylcholine. Crotonylcholine most resembled acetylcholine in the form of its response. None of these compounds have yet been shown to be present in the snail, but then there is not decisive evidence that acetylcholine is present.

Sometimes after the addition of Ach. the positive afterpotential of the action potential is replaced by a negative afterpotential. If the positive afterpotential is due to the return of the resting potential towards the potassium potential, then this failure would be associated with an interference of potassium conductance by Ach. Excitation is normally associated with an increased permeability of the postsynaptic membrane to all ions. Thus the Ach. could increase the sodium conductance, and so the inward flow of sodium ions, while it decreases the potassium conductance, and so the outward flow of potassium ions. It would be very useful to have some information about the ionic events during the response of a drug.

DMAE only inhibited the activity, in no case did it excite the activity. There is no evidence for the presence of DMAE in the snail, nor for its conversion to Ach., but this could be tested more rigidly by using C^{14} DMAE

CATECHOLAMINES AND PRECURSORS

There is no evidence for the presence of either adrenaline or nor-adrenaline in the snail, but it is hoped to reinvestigate this question, using a sensitive spectrofluorimetric technique. Neither compound was particularly active in its effect on the snail neurones. With respect to their acceleratory effect, nor-adrenaline was approximately a thousand times more active than adrenaline. Tauc has found that nor-adrenaline was more active than adrenaline on Aplysia neurones.

In over half the experiments in which it was tested phenylalanine inhibited the activity and hyperpolarized the resting potential. This amino acid is present in the brain of the snail. It would be interesting if the presence of adrenaline is established to see if radioactive phenylalanine injected into the snail can be recovered as adrenaline. From the earlier experiments it would appear that phenylalanine applied prior to the addition of 10^{-4} and 10^{-5} acetylcholine blocks the depolarizing effects of this drug at high concentrations.

Dopamine, has been found in the insects but as yet not reported in the Mollusca. Working in the department, Price (unpublished) isolated a compound from the brain of gastropod snails which gave a spot with an Rf similar to that for

dopamine. Ostlund, (1954), did not find dopamine in the Mollusca, but did find an unidentified catecholamine which he termed "catechol 4". If dopamine can be identified in the snail brain then from the present studies, it would rank as a potential inhibitory transmitter. Dopamine was found to inhibit the spontaneous activity and to hyperpolarize the resting potential. It did so regularly at concentrations of 10^{-9}. It is not yet known if this compound acts selectively on inhibitory synapses or merely blocks all synaptic connexions.

THE INDOLEALKYLAMINES

5-HTP has been proposed in vertebrate studies as the precursor for 5-HT. The former compound has not yet been identified in the snail brain, but 5-HT has been shown to be present in a concentration of around 10^{-8}g/g, (Cottrell, unpublished observations). From the work of Cardot and Ripplinger, (1961), it had previously been reported to be absent but these workers employed a method that probably destroyed the 5-HT during the extraction. In this study both compounds have been found to be specifically active on certain marked cells. Like Ach., and in contrast to histamine, the excitatory response occurs after a delay of up to 20 seconds.

Both compounds are almost entirely excitatory in nature and have thresholds around 10^{-9}. The threshold for inhibition is 1000 x more concentrated. Only two experiments have given inhibitory results out of a total of 49, (21 of which were excitatory). As with Ach. and histamine, both 5-HT and 5-HTP not only increased the rate of the activity but also induced e.p.s.p.'s. In this way they resemble electrical stimulation.

HISTAMINE

This compound has not been shown to be present in the snail, but histidine, the amino acid precursor for histamine, is present. In the initial experiments this compound was used as the hydrochloride but it was thought that it might be exerting its action through its pH rather than the histamine molecules on their own. The pH was tested and at 10^{-6} and weaker concentrations the histamine had no affect on the pH. In later experiments on marked cells, the free base was substituted for the hydrochloride. The response was found to be the same. Histamine is excitatory in nature, but its action occurs 4 to 6 seconds after its addition. This delay time contrasts with that for acetylcholine (10^{-6}) which is over 20 seconds. Pharmacological concentrations of acetylcholine (10^{-4}) on the other hand have a short delay

time. It may be suggested that histamine is not a natural transmitter but that it acts on the soma membrane and causes a general depolarization of the resting potential.

There would appear to be a distinct difference between acceleration due to a physiological concentration of acetylcholine and that due to histamine on the same neurone. In the case of the acetylcholine, the acceleratory response occurs after 20 to 30 seconds, while that to the histamine occurs after 4 to 6 seconds. It is possible that the acetylcholine response is local, occurring only at the synapses at the proximal end of the axon. It will take several seconds for an external application of the drug to penetrate to the synapses of the experimental cell. On the other hand the histamine could act all over the soma surface and cause quick general depolarization. The response to acetylcholine at pharmacological levels may also be quick, resembling that of histamine. Here as well it may be acting directly on the soma membrane surface rather than via the postsynaptic membrane. Certain drugs take up to a minute before they induce a result. Here of course one must consider the problem of interneurones. The drug could be acting via an interneurone and the experimental cell soma could itself be inactive to the drug. It might be necessary to use isolated neurones for such tests to make sure that the

experimental soma membrane is receptive. So far it has not been possible to isolate single cells from this preparation and to obtain suitable records from them.

Histamine does not have effect on all neurones, so that there is some selectivity in its action.

THE GABA GROUP

This group contains glutamic acid and β-alanine in addition to GABA. From vertebrate studies it would be expected that GABA and β-alanine would have an inhibitory effect on the activity while glutamic acid would accelerate the activity. The results obtained in this present investigation were rather disappointing. While it has been found that glutamic acid stimulated all the vertebrate neurones on which it was tested, (Krnjevic and Phillis, 1961), in this study glutamic acid excited only 2 out of the 22 neurones on which it was tested. GABA was only tested on 16 neurones and β-alanine on 6, and it is clearly necessary to test these drugs on more cells before any definite conclusions can be made. GABA, alanine, and glutamic acid have been found to be present in the snail. Glutamic acid has a probable metabolic role in vertebrate tissue, (Strecker, 1957), and it could have the same effect in the snail brain.

THE VITAMIN B_I GROUP

The response to both cocarboxylase and thiamine (aneurine) hydrochloride was not conclusive. However the two excitatory experiments with thiamine were more conclusive than the one inhibitory response. This would agree with previous workers who associated thiamine with excited nerves. Cocarboxylase has been associated with metabolism and in the snail it both accelerates and inhibits nerve activity. There is no evidence for the presence of either compound in the snail though Augustinsson, (1946, 1948), has described an enzyme in Helix dart sac and blood which will hydrolyse acetylthiamine, as yet there is no evidence of a transmitter role for either chemical.

THE EFFECT OF THE RESTING POTENTIAL ON DRUG ACTION

In a later series of experiments, the effect on the resting potential was recorded. These experiments were done mainly with acetylcholine and 5-HTP. There would appear to be no correlation between the size of the maximum and minimum resting potential and the response of the drug. The maximum resting potential is the maximum value of the positive afterpotential while the minimum resting potential is the potential at which the action potential begins its sharp rise. It is possible that where there is a large difference, (a large positive

afterpotential), the cell membrane has been damaged during the penetration of the soma and this makes it easier for a drug to affect the cell activity. The link between positive afterpotential size and decay time and damage has been investigated by Moore and Cole, (1960), for squid axons. They found that the rate of recovery of the positive afterpotential increased after the initial cutting of the mantle. They suggest that the more damaged an axon, the faster the decay of the positive afterpotential. In the experiments with Helix neurones it would appear that during the course of an experiment the decay time of the positive afterpotential also increased.

It may be concluded that the maximum/minimum resting potential does not affect the response to a drug. There is no correlation between the acceleration of the activity by a drug and this value. Normally the addition of a given concentration of a drug to a silent cell has a different effect to that when the cell is active.

The response to some extent depends on the value of the resting potential, and it has been suggested by Tauc that the response of a cell to a drug depends entirely on the cell's resting potential. The cell responds so as to restore the resting potential to a certain level, the equilibrium potential. When the cell is hyperpolarized above this level, then a drug

will tend to depolarize the neurone. When the cell is depolarized below this level, then the application of a drug will tend to hyperpolarize the resting potential. In the experiments described here it was not possible artificially to change the resting potential, but the normal resting potential of a given cell varies from one brain to the next. From the experiments so far done, it would appear that with a drug which normally accelerates the activity, the lower the resting potential so the greater is the response, fig 54 A and B.

There was also the possibility from the initial experiments that there was a change in the response of a given drug when it had been preceded by another drug. From the earlier experiments it appeared that certain amino acids when present prevented concentrated solutions of acetylcholine from depolarizing the cell resting potential to zero. Phenylalanine, glutamic acid and cocarboxylase were found to do this. In section two, (page 176) several drugs were tested in a known sequence on a single neurone. There was no clear correlation between the response of the cell to a drug and the sequence in which it was applied.

The investigations described only indicate which chemicals have an effect on the neurone membrane of snail brain cells.

Further studies will have to be necessary to locate the relevant enzyme systems and to show that the chemicals are released on stimulation of the relevant axons.

This study has been undertaken on the assumption that a chemical transmitter diffuses across the synapse from the pre- to the postsynaptic membrane. While this concept is widely held, there are dissenters. According to Nachmansohn, (1959, 1961), the propagating agent along the nerve fibre, as well as across synaptic junctions, is electric current. The action of Ach. occurs within the conducting membrane and is an intracellular process. Ach. exists in the resting condition in a bound and inactive form. When the membrane is excited Ach. is released. The free ester acts upon a specific receptor, a protein, produces the change in ionic permeability, and generates the potential. The free ester is then attacked by acetylcholinesterase. The receptor return to its resting condition and the barrier to the ion movements will be re-established. The fact that Ach. is found at the synapses is, according to Nachmansohn, due to the lack of a barrier against Ach. as a methylated quarternary ammonium salt.

It is generally considered that a chemical transmitter, such as acetylcholine is present in the tissue in an inactive form bound to a protein or lipoprotein. Acetylcholine is released from this complex by a variety of stimuli, especially

ion movements. The free ester diffuses across the synapse and combines with a receptor protein in the postsynaptic membrane. This alters the ionic movements across the membrane and may stimulate the structure concerned. Acetylcholine must then dissociate itself with the receptor protein and combine with cholinesterase which it is suggested is also on the membrane. Zupancic, (1953), does not understand why acetylcholine should combine with the active receptor protein first rather than combining with the active cholinesterase receptor initially. He suggests that both will have similar active groups and will be in close spatial vicinity in the postsynaptic membrane. He suggests that the receptor protein is not similar to but identical with cholinesterase. The following picture then emerges; the released free ester molecule of acetylcholine combines with tissue cholinesterase, that is, the receptor protein; acetylcholine acts upon it, causing stimulation, and is then hydrolysed to inactive compounds. The receptor protein is then ready to act upon the next molecule of acetylcholine.

This view of the action of Ach. is of interest, but it will require considerable experimental support before it can be accepted.

THE PROBLEM OF HETEROGENEITY

In the experiments which make up the bulk of the results of Section I, the effect of the drug in relation to the spontaneous activity of the neurones alone was recorded. As can be seen from the table (page 151) in most cases the drugs both accelerated and/or inhibited the activity. There was usually a clear majority for either an overall inhibitory or acceleratory response. The overall response for acetylcholine, 5-HTP, histamine base and hydrochloride, noradrenaline, and 5-HT was acceleratory. The overall response for phenylalanine, dopamine, and DMAE was inhibitory. The responses from adrenaline, glutamic acid, β-alanine, GABA, cocarboxylase, and aneurine hydrochloride are not sufficiently obvious for a conclusion.

However from these results it is clear that there is a marked heterogeneity in the response of the neurones of the snail brain to drugs. This heterogeneity in response has also been shown to occur in the vertebrate brain, (Bradley & Mollica, 1958). It has also been proposed that there are alternate cholinergic and non-cholinergic pathways in the vertebrate brain, (Feldberg, 1957). It became obvious that the experimental technique would have to be modified. It was for this reason that the experimental neurones were marked

at the end of each experiment.

In a study to see if the drug experiments on the neurones were consistent, a given drug was tested on an identified marked cell in different brains. These experiments are still in progress but certain cells are consistantly responsive to a given drug or in some instances two drugs, and to no other ones that are applied.

It also became apparent that more must be known about the connexions between the axons and the neurones and between the neurones themselves. The stimulating experiments were then carried out on marked cells to try and map areas of the ganglia with a common function. It was hoped that cells with common connexions would be sensitive to the same chemicals. The axonic connections also helped to identify the neurones.

In the mapping experiments it has been assumed that a neurone has only one axon but this does not always appear to be the case. It has been suggested that the axon of the giant neurones of the abdominal ganglion of Aplysia divides and sends branches into several nerve trunks on the ipsilateral side of the body, (Hughes & Tauc, 1961). The axons of the supramedullary neurones of the puffer fish also appear to send branches to several dorsal root nerves, (Bennett, Crain & Grundfest, 1959). In this investigation a few neurones have possessed short delay times on stimulation of two different

nerve trunks but only the shorter delay time has been taken to indicate antidromic stimulation.

The measurement of the rate of conduction along the nerve trunks presents a problem. It has been shown that there are slow and fast conducting fibres in the intestinal nerve, (Schlote, 1955). This mixture of fibres also occurs in Mya, (Horridge, 1958). The fastest rates of conduction in the gastropods would appear to be around 100cm/sec, and the slowest around 20cm/sec. This agrees with the values found in the present investigation. It has also been shown that the intestinal nerve of Helix contains neurones, (Schlote, 1955).

Nisbet, (1961), suggests that the delay time in the abdominal ganglion of Archachatina may be as low as 10 to 15msec. In this preparation, the transmission from the right pallial nerve to a neurone in the left parietal ganglion, close to the entry of the left pallial nerve would appear to be as low as 7msec.

The present study shows that there are groups of cells whose axons pass down the same nerve trunks. It is suggested that these cells may have a similar function. There are also indications that cells in the same area of a ganglion respond to the same drug. Experiments to confirm these suggestions are in progress.

It has already been mentioned that in contrast to most systems so far investigated the neurone axons pass out via ipsilateral rather than contralateral nerve trunks. However, there is some evidence for a link between a pair of nerve trunks and a given neurone. Thus if the efferent axon passes down the right pallial nerve, then there will be an afferent axon from the left pallial nerve which will synapse onto it. This pair of fibres may function antagonistically or synergistically.

INHIBITION

Inhibition occurs in the snail nervous system, as in the vertebrate central nervous system. It parallels the motoneurones of the mammalian spinal cord in that i.p.s.p.'s may be present. However these are not necessary for inhibition, and they can also occur in the presence of full action potentials. Their function is not clear, but at times they do accompany complete inhibition of the activity. These inhibitory synapses may be distinct from the excitatory synapses but there is evidence that when the membrane resting potential is hyperpolarized below its equilibrium potential, then the i.p.s.p. becomes a depolarizing potential, (Coombs, Eccles and Fatt, 1955, Tauc, 1957). The structure of the inhibitory

synapses of the crustacean stretch receptor has been investigated by Peterson and Pepe, (1961), using the electron microscope and they found little difference from the structure described for excitatory synapses.

I.p.s.p.'s are associated with an increase in permeability to potassium ions and possibly chloride ions in the mammalian motoneurone, (Coombs, Eccles and Fatt, 1955a). The ionic changes involved in the snail are not known but it has been suggested by Tauc, (1958), that they are the same as for the cat motoneurone. However it may be recalled that the gastropod neurones do not behave entirely as mammalian neurones with respect to ion influence. Oomure, Ozaki and Maeno, (1961) have shown that normal action potentials can occur in Onchidinium in the absence of sodium. They suggest that calcium is important in excitation. Not all inhibition in the vertebrates is associated with chloride ion movements as well as potassium. Hutter, (1962), states that while inhibition in the vertebrate heart is associated with an increase in potassium ion movement, there is little change in chloride ion movement.

On the other hand it has been suggested by Chalazonitis and Arvanitaki, (1957), that the i.p.s.p.'s are equivalent to the positive afterpotential of the full action potentials.

Inhibition in the snail can also occur in the absence of i.p.s.p.'s. This may or may not be accompanied by a hyperpolarization of the resting potential. When inhibition of the activity occurs in the absence of a resting potential change it may be that an inhibitory chemical blocks the excitatory synaptic sites, so blocking the orthodromic action potentials without affecting the cell resting potential.

I.p.s.p.'s would appear to be a device for the regulation of the resting potential and a means of attempting to restore the resting potential to an equilibrium potential. This potential probably varies from cell to cell, and for a given cell, depending on the state of the membrane.

The height of the action potential can vary with the resting potential. Sometimes when the cell is depolarized the height of the action potential falls. As the resting potential repolarizes, so the action potential height returns to its previous level. A fall in the height of the resting potential is often associated with an increase in the permeability of the membrane to sodium. During an action potential, the membrane conductance to sodium greatly increases and the cell potential approaches the potential for a sodium electrode. When the height of the action potential is reduced, it would appear that the sodium conductance is impaired.

This can be produced both electrically and via chemical application. It also occurs naturally.

In certain cells it is impossible to drive full action potentials. These cells may never transmit full action potentials into their soma, in which case, they are similar to the crustacean heart ganglion neurones and to the supramedullary neurones of the puffer fish, Spheroides maculatus, (Bennett, Crain and Grudfest, 1959).

CONCLUSION

In some ways this thesis resembles a 'pilgrims progress'. On looking back at the tortuous path through which the experiments have led, it is clear that a much more straight and direct route could have been taken, and that many of the present conclusions are self evident. Each new series of experiments required an improvement in the experimental techniques. The first experiments were carried out by means of external electrodes recording the activity in the peripheral nerves. Then tungsten-glass semi-microelectrodes were used, and finally glass microelectrodes. At first drugs were applied to the cells which happened to be penetrated by the electrode. Later it became clear that the cells' responses were not

uniform and that it would be necessary to mark and map the cells. A histological investigation was started into the fine structure of the brain and the extent to which the cell numbers and size are constant from one brain to the next. Simultaneously a marking technique has been developed to mark the cell into which the microelectrode had been inserted. The in situ brain has been examined and the peripheral nerves stimulated in order to determine the connections between axons and cell bodies. Specific cells can now be recognised and the way appears to be clear to determine the nature of their chemical sensitivity. Furthermore by linking together subthreshold stimulation of an axon and direct chemical stimulation of the soma, it may be possible to find out the probable nature of the natural chemical transmitters. It does seem, without being unduly pessimistic, that many deep crevasses lie between the present position and the final solution. One of these would appear to be present day lack of availability of possible chemical transmitters. Though new simple organic substances are becoming available, there is often difficulty in obtaining other feasible transmitters. Some chemicals such as ortho- 5;6 dihydroxytryptamine, are suggested to be unstable - a valuable property for a transmitter substance, but one which makes their synthesis in the laboratory difficult. The careful use of enzyme inhibitors

may help to indicate the possible action of transmitters though care has to be taken that one does believe that the inhibitors are absolutely specific under all conditions; they may be inhibiting aspects of metabolism as well as the transmitter substances.

SUMMARY

(1) The literature on the electrical properties of three invertebrate neurone membranes, (i), the gastropod sub-oesophageal ganglion neurones, (ii), the crustacean heart ganglion neurones, (iii), the crustacean stretch receptor neurone, and the occurrence and function of possible chemical transmitters in the vertebrates and invertebrates has been reviewed. Little is as yet known about chemical transmission between neurones in the invertebrates.

(2) The neurones of the snail, Helix aspersa, are well suited to electrophysiological and neuropharmacological investigation using intracellular microelectrodes since the brain cells are comparatively large, being of the order of 80 to 200 μ in diameter.

(3) The nerve cells in the snail brain may be spontaneously active or silent. The resting potentials are generally in the range of -40 to -65mV, the action potentials have an overshoot of up to 30 to 40mV. The duration of the action potentials vary from 2msec to over 100msec. The size and duration of e. and i.p.s.p.'s vary considerably. The rate of the spontaneous activity can be up to 10/sec. The activity may

be rhythmic, arhythmic, or occur in bursts. The action potentials may be followed by positive or negative afterpotentials.

(4) It is often possible to distinguish between orthodromic stimulation and antidromic stimulation of a cell. The rise time of antidromically stimulated excitatory depolarizations is faster than that of orthodromically stimulated e.p.s.p.'s. The delay time for antidromic stimulation is shorter than for orthodromic stimulation. An antidromic action potential often has an axon-soma prepotential prior to the full potential.

(5) The afferent and efferent pathways of axons of the neurones in the parietal and visceral ganglia were mapped. The cells were driven antidromically and orthodromically. The efferent axon in many cases emerged along a nerve trunk leaving the same ganglion as contained the experimental neurone. Up to three axons were found to synapse on the same neurone. Ipsi- and contralateral nerve trunks of the same nerve, for example, the left and right pallial nerves, often link onto the same neurone.

(6) The rates of conduction varied for the different nerves stimulated. The pallial nerves tended to conduct at 100cm/sec, and the intestinal and anal nerves at 50cm/sec. The minimum

time taken to cross from the right to the left parietal ganglion was 7msec.

(7) It was possible to drive full action potentials, pseudopotentials, excitatory and inhibitory postsynaptic potentials. In some cases these were found to summate and/or facilitate to form full action potentials. The driven potentials may adapt with time.

(8) Inhibition of cellular activity can be achieved in a number of ways. I.p.s.p.'s may be driven and these inhibit the activity. When the cell resting potential is altered or the intensity of stimulation varied the i.p.s.p. may give way to an e.p.s.p. The activity may be inhibited with little change in the cell resting potential. Inhibition may be achieved by a hyperpolarization of the cell membrane without i.p.s.p.'s.

(9) The neurones showed great heterogeneity in their response to the applied drugs. Almost all the drugs accelerated, inhibited, or had no effect on some of the neurones on which they were tested. The drugs could accelerate the activity without changing the shape of the action potential; a physiological response, or they could affect both the shape and height of the action potential and the height of the resting potential, a

pharmacological response. A physiological response was associated with a concentration of 1 x 10^{-6}g/ml or a more dilute solution, and a pharmacological response was associated with more concentrated solutions. The size of the maximum/minimum resting potential did not appear to influence the response to the drug.

(10) The response of the snail neurone bioelectric potentials to fourteen drugs was tested. The overall response of three of them, β-phenylalanine, DMAE, and dopamine was inhibitory. The overall response of acetylcholine, 5-HT, 5-HTP, histamine base and HCl and noradrenaline was acceleratory. No conclusion could be made about the overall response from adrenaline, glutamic acid, GABA, β-alanine, cocarboxylase, and thiamine (aneurine) HCl.

(11) Certain acceleratory drugs caused their effect after a delay of 20 or more seconds, for example, acetylcholine; other acceleratory drugs had their effect after 4 to 8 seconds, for example, histamine. It was suggested that the former drugs affected the excitatory synapses while the latter affected the general surface of the soma.

(12) A series of drugs was tested on a single cell. Drugs were also applied to the same cell in several different brains.

Cells specifically sensitive to acetylcholine and 5-HTP were located. There appeared to be no correlation between the order in which the drugs were applied and the drug response.

(13) These studies indicate the marked heterogeneity in the response of the cells in the nervous system of the snail, Helix aspersa, to drugs. They indicate that it is necessary to work on single known cells and to be able to determine the response of the same cell in a number of brains.

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