STUDYING THE ISOLATED CENTRAL NERVOUS SYSTEM; A REPORT ON 35 YEARS: MORE INQUISITIVE THAN ACQUISITIVE

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Abstract—1. The CNS from invertebrate animals such as slugs, snails, leeches, and cockroaches, can be isolated and kept alive for many hours.
2. The electrical and pharmacological properties of invertebrate CNS neurons have many similarities and it is probable that the basic rules governing the CNS evolved more than 600 million years ago.
3. The nerve cells can show sodium action potentials, calcium action potentials, EPSP, IPSP, biphasic potentials, electrogenic sodium pump potentials, and a variety of potassium, sodium, calcium and chloride currents.
4. Invertebrate CNS ganglia contain identifiable individual nerve cells whose properties and responses to neurotransmitters and drugs are constant and repeatable from preparation to preparation.
5. It was possible to set up an isolated CNS-nerve trunk-muscle preparation and study the transport of radioactive material from the CNS to the muscle and from muscle to CNS. This has provided information about axoplasmic transport in both invertebrate and vertebrate preparations.
6. The methods developed from studies of invertebrate isolated CNS preparations have been applied to vertebrate isolated CNS preparations.
7. In addition to thin slices of the mammalian brain, it is possible to keep 5 cm lengths of the whole mammalian spinal cord and brain stem alive for many hours.
8. The isolated mammalian spinal cord has functional ipsilateral and contralateral reflexes, ascending and descending pathways, extensive sensory integrative local area networks, and inhibitory interneuron circuits. Much of the in vivo circuitry is functional in vitro.
9. The isolated mammalian spinal cord and brain stem can be developed to include functional higher brain circuits that will provide increased understanding of the control and integrative action of the mammalian central nervous system.

INTRODUCTION

I have been lucky. I have had very supportive parents, enthusiastic gifted teachers and co-workers, good health, and a resonable amount of energy.

Most of my interests have developed from coming into contact with enthusiastic teachers. When I was at school, teaching was an honoured profession and many of my teachers were people of outstanding academic ability with strong personalities. The enthusiasm for their subject and their interest in teaching had an immediate effect. They acted as powerful catalysts and developed the talents latent in many of their pupils. One became interested in the subject, read around it, and in the end knew much more than would be required by the yearly examination. Subjects, people and places, all came to life and were as real to me as the everyday events.

In the sixth form my main teacher, “Jasper” Machin did not believe in formal teaching; he preferred our discussing different topics, reading around a subject, thinking and writing essays. If I asked him about anything he would give a brief synoptic (Delphic) reply and suggest that I looked it up in the well-supplied library. His normal comment was, “You have got a brain, use it!” In this way we were trained to work independently and think about what we were doing.

At University I found many of the lectures very interesting and it was also stimulating to go to other courses and hear people such as Bertrand Russell lecture. But the real science teaching was done at tutorials (supervisions) where one or two students went for one hour’s instruction each week in a specific subject with a member of the college or staff.

At that time my college did not have a specialist in biological sciences so I could choose anyone in the University who would teach me and I had varied experiences. There was one rather elderly gentleman who used to supervise in the evenings after he had taken a good dinner. The room was warm and stuffy and my colleague and I used to talk very quietly or say very little, to see how long it would take for our supervisor to fall asleep. Often we could get him to doze off within about five minutes, though he would later wake with a start and go on in the middle of the sentence as if nothing had happened. In a successful evening we could get him to fall asleep about half a dozen times, sometimes with a good snoring accompaniment.

Another supervisor did not like reading essays and insisted that our essays on a given topic should be no longer than three sentences and no sentence should contain more than thirty words.

On the other hand, there were excellent teachers who would explain difficult subjects, set interesting
essays, and make sure that their pupils came up to the required standard. In many cases they would also talk about their research work and this was usually very interesting. Enthusiasm is infectious.

In my second year at University, I asked Dr Philip Whiting if he would supervise me and my friend. The supervisions were held at six o’clock in the evening and were supposed to last for an hour or fifty-five minutes, but Philip Whiting got so interested in what he was describing that the sessions often went on for an hour and a half, or even two or three hours.

He was very keen that we should realize that neurology was not based on books but on histological specimens and would set up lots of microscope slides so that we could gaze at Rohon-Beard cells, beautiful Golgi preparations and methylene blue stained neurons: all the elements of the central nervous system in three dimensions. He introduced me to the published works of Cajal and he matched Cajal’s illustration with microscopic preparations of similar sections.

We were also taught other topics and in each case had to look at the basic evidence and learn to evaluate it. In this way I learnt that many of the answers to important questions were not known; that often the evidence in support of an accepted view was not very strong; that one had to go to the original evidence or the latest experiments to evaluate the situation; that there were fashions in science as in other subjects and that the fashionable explanation was not necessarily the correct explanation.

After graduating in Natural Sciences, I stayed on at Cambridge to carry out research on simple central nervous systems. The simplest animal I could find that had a well-developed central nervous system was the starfish, *Asteria rubens*, and I spent three years studying the physiology of its nervous system and the manner in which it controlled the direction of walking and the stepping of the tube feet. This work was done under the expert supervision of J. E. Smith (Eric Smith), who later became Director of the Marine Biological Association Laboratories at Plymouth.

**CNS PHYSIOLOGY**

If the starfish is placed on its back and the tip of one arm stimulated, the tube feet on that arm and on all the other four arms would point away from the site of stimulation, i.e. there was transmission of specific directional information along all five arms, and this was stopped if the radial nerve was cut. If the cut was half-way along one of the other arms, the direction change of pointing would take place over the whole of the starfish up to the position of the cut, but the tube feet beyond the cut would not point in the new direction. The study of this phenomenon and the stepping activity of the tube feet became the basis for my PhD thesis.

I obtained a postdoctoral research fellowship and decided to study the electrical activity of the molluscan central nervous system. At first, I studied *Aplysia* sent to Cambridge from Plymouth but it was difficult to obtain *Aplysia* throughout the year so I decided to work on a more common molluscan species that would allow experiments to be carried out every day, and found that there were hundreds of slugs in my garden. The slug brain is a very beautiful conglomerate of ganglia with nice large nerve cells and proved to be electrically very active.

The first task was to find a suitable Ringer saline to keep the preparation alive and during this work it became clear that the slug CNS was very sensitive to osmotic changes in the bathing saline and that it could detect changes of 1% of the body fluid concentration over 4 min. Such rates of osmotic change were found in the whole intact slug after it had been out in the rain, or when it had been crawling on dry surfaces. The sensitivity was similar to that of the osmoreceptors in the mammalian brain which could detect a 2% change in the osmotic pressure of the blood. This work was initially carried out with George Hughes, and then later at Southampton with Brian Taylor.

One of the problems of working on slugs is that there are several common species of garden slug and its is often difficult to make a correct species identification. For this reason, I decided that it would be easier to work on snails. There are only two species of *Helix* in England and the common species is *Helix aspersa.* (The larger Roman snail, *Helix pomatia,* is found mainly in the Cotswolds.) An advertisement in the local paper offering to pay two pence for large snails brought in offers of several hundred snails (*Helix aspersa*) from small boys in the neighbourhood (Fig. 1).

I had, by this time, moved to Southampton University and helped in starting the Physiology and Biochemistry Department which later was instrumental in the foundation of the Southampton University Medical School.

Working in Physiology, together with Mike Laverack, I showed that tissue extracts from the snail had a cardio acceleratory activity in addition to that produced by 5 HT. I was also interested in the effect of sudden temperature changes on the nervous systems and, with Brian Taylor, showed that a sudden increase in temperature from 20 to 23°C could cause a temporary decrease in the spontaneous activity in the cockroach leg nerve whilst a sudden decrease in temperature from 30 to 25°C could cause an temporary increase in nerve activity. The steady-state activity after the temperature change was in the expected direction, i.e. higher at the higher temperature and lower at the lower temperature. What interested us were the transient changes in nerve activity in the “wrong” direction.

The explanation for this was provided by Tony Ridge who showed, using internal microelectrodes on various preparations, that a sudden increase in temperature caused a hyperpolarization of the resting potential and hence a slowing of the spontaneous activity. Decreasing the temperature led to a depolarization of the resting potential and so a transient increase in the rate of spontaneous activity.

We also measured the $\Omega_{\infty}$ values for changes in membrane potential in crab, insect and frog muscle and found that they were significantly higher than that predicted from the Nernst equation. This suggested that the resting potential might have a metabolic component (electrogenic metabolic pumps) and might not be just a simple physical diffusion potential.
Isolated central nervous systems

Fig. 1. Some of the animals whose isolated CNS we have investigated. (1) Snail, (2) slug, (3) *Tetrahymena*, (4) leech, (5) *Limulus*, (6) cockroach, (7) frog and (8) hamster.

SNAIL NEURONS

The large neurons in the snail brain made them admirable subjects for intracellular recordings using microelectrodes. Furthermore, it was relatively easy to keep the neurons alive for many hours after the brain had been isolated, and so study the effect of possible neurotransmitters on nerve activity (Fig. 2).

In the 1960's it was generally thought that there were only two neurotransmitters, acetylcholine and noradrenaline, and that nerve cells would respond to these. Working with Robert Walker, it quickly became apparent that the nerve cells in the *Helix* brain would respond to many possible neurotransmitters (acetylcholine, noradrenaline, glutamate, dopamine, 5HT, GABA, histamine) and that the transmitter vocabulary of the CNS was much greater than just two words, acetylcholine and noradrenaline.

Another interesting discovery was that the responses of the different neurons in the *Helix* brain were specific to a given neuron and if we wished to get repeatable results we had to work on identified neurons. In this way, it became clear that some nerve cells were stimulated by ACh whilst other nerve cells were inhibited by ACh. Some nerve cells responded to 5HT or dopamine or glutamate, whilst other neurons did not respond. This started a program of work that lasted for many years mapping the neurons in the *Helix* brain and determining the response of the specific neurons to neurotransmitters and electrical stimulation through nerve trunks. It was also necessary to show that these neurotransmitter chemicals were present in the snail brain, and localize them using histochemical and fluorescent methods.
Glen Cottrell measured the levels of ACh and 5HT in the snail brain; Christine Sedden used the Falck Hillarp method for the identification of Helix neurons containing 5HT and dopamine and also measured the dopamine content in the Helix brain; Janet Loker showed that many snail neurons that contained 5HT also responded to applied 5HT; Charles Marsden extended the fluorescence studies and showed the presence of a large dopamine containing neuron in the brain of the snail Planorbis; Mary French identified the axonal pathway in the twelve identified neurons by filling them with Procion Yellow; and John Lambert, Rob Gayton and Janet Loker helped bring the physiological and pharmacological data together to make a map of the snail brain showing the neurons that responded to glutamate, ACh, 5HT, GABA and dopamine.

Glen Cottrell developed this work in his laboratory at St Andrews and, together with his co-workers, showed that stimulation of a snail neuron containing a known transmitter (5HT or dopamine or histamine) could produce post-synaptic potentials in identified follower cells, these potentials being mimicked by local application of the specific transmitter to the follower cell. This is probably the best evidence for 5HT, dopamine or histamine being neurotransmitters in the central nervous system.

Electrophysiological studies showed that many of the properties of Helix neurons were similar to the properties of other animal nerve cells (Fig. 3).
INHIBITORY POST-SYNAPTIC POTENTIALS

The IPSP in snail neurons, for example, was rapidly reversed if chloride ions were injected into the neuron. If a series of anions of increasing hydrated diameter ranging from potassium bromide to potassium neuron. If a series of anions of increasing hydrated diameter ranging from potassium bromide to potassium ion. This indicated that the IPSP in mammalian motoneurons.

ELECTROGENIC SODIUM PUMP

Roger Thomas showed if sodium ions were injected into snail neurons there was a rapid hyperpolarization of the membrane potential by as much as 30 mV. This hyperpolarization was inhibited if ouabain was present or if the extracellular potassium concentration was reduced. The hyperpolarization was due to the stimulation of an electrogenic sodium pump which, due to the high resistance of the nerve membrane, produced a very rapid and big change in membrane potential.

John Lambert showed that some snail neurons had a high sodium permeability relative to the potassium permeability and that these neurons had a greater electrogenic sodium pump component of the membrane potential.

Barbara York showed that the snail neuron was sensitive to the oxygen concentration around the neuron and that reducing the $P_o$ brought about a depolarization of the membrane potential, whilst increasing the $P_o$, hyperpolarized the neuron, the effect being prevented by ouabain or scillaren. Sodium-injected neurons were more sensitive ($\Delta 22 mV$) than changes in $P_o$, or was potassium injected neurons ($\Delta 8 mV$). This indicated that the electrogenic sodium pump component of the membrane potential was more sensitive to $P_o$ than was the electro-diffusion (Nernst) component.

The electrogenic sodium pump had been considered for many years and thought to contribute only one or two millivolts to the membrane potential. The snail neuron provided the first example of a rapid and major sodium in membrane potential due to the activity of the sodium pump, and indicated a more important role for metabolic pumps in nerve and muscle activity. The role of the electrogenic sodium pump in nerve and muscle tissues (especially mammalian cardiac muscle) is now more fully appreciated.

GLUTAMATE AS A NEUROTRANSMITTER

In the search for new neurotransmitters, the general opinion in the 1960s was that the new transmitters would be complicated unfamiliar chemical compounds. We showed in 1962 that a very familiar chemical, L-glutamate, proved to be very good at stimulating snail neurons and later showed that glutamate made insect and crustacean muscle contract, the tissues often responding to nanomol concentrations. Susan Pigott showed that snail neurons were 100 more times more sensitive to L-glutamate than to D-glutamate. Lucy Leake and Avril Shapiro found

that if the snail, crab or insect nerve-muscle preparation was perfused it was possible to obtain glutamate in the perfusate following stimulation of the nerve. We also tested various possible inhibitors such as glutamate diethyl ester, glutamate methyl esters, alpha aminopimelic acid and alpha aminoadipic acid, but none of them were very powerful inhibitors of glutamate. The lack of potent inhibitors has hindered research on the role of excitatory amino acids in the CNS (except for the NMDA system). Nevertheless, it has generally become accepted that glutamate or aspartate type compounds are probable transmitters in the CNS.

RECEPTOR IONOPHORE COMPLEXES

Once it was realized that a given receptor type could be linked to different ionophores (ACh producing depolarizations in some neurons and hyperpolarization in other neurons) it was of interest to study the relationship in more detail.

John Chad developed a ramped voltage clamp system and showed that there were three different types of neurons in Helix brain that responded to ACh. (a) Those hyperpolarized by ACh, due to an increase in chloride permeability, (b) Those depolarized by ACh, due to an increase in sodium permeability. (c) Those depolarized by ACh due to an increase of both sodium and chloride permeability. The ACh receptors linked to the sodium ionophore were blocked by hexamethonium whilst those linked to the chloride ionophore were blocked by decamethonium.

Some snail nerve cells respond to two transmitters. $F_i$ is depolarized by ACh and by 5HT, both receptors being linked to sodium ionophores (Fig. 4). $E_4$ is hyperpolarized by ACh and by glutamate, both receptors being linked to chloride ionophores. Are the same sodium ionophores linked to both the ACh-R and 5HT-R and are the same chloride ionophores linked to both the ACh-R and the Glu-R?

John Chad showed that even when the neuron gave its maximum current to application of ACh, it could pass more current (have more ionophores open) when 5HT and ACh were added together. The increased membrane conductance response to simultaneous application of ACh and 5HT was equal to the sum of the individual ACh and 5HT responses, similarly for ACh and glutamate. It would seem that each receptor (ACh-R, 5HT-R, Glu-R) is linked to its own population of sodium or chloride ionophores.

AXOPLASMIC TRANSPORT IN ISOLATED BRAIN-NERVE TRUNK-MUSCLE PREPARATIONS

The isolated snail brain can survive more than 24 hr in isolation and it is possible to set up brain-nerve trunk-muscle preparations and study the muscle contraction following brain stimulation.

This gave us the idea of putting the brain in an isolated pool of saline containing radioactively labelled material (glucose or glutamate) and having the muscle in a second isolated pool of saline.
(non radioactive) with the two pools connected by the nerve trunk. Care was taken using lanolin barriers to see the radioactive material did not leak directly from the brain pool to the muscle pool.

It was found that radioactivity went from the brain to the muscle along the nerve trunk. It took about 20 min for the radioactivity to travel the 1 cm length of nerve trunk. This rate of transport was much faster than that suggested by Paul Weiss for amphibian nerve. We developed an isolated frog spinal cord-sciatic nerve-gastrocnemius preparations which survived in isolation for more than 20 hr and found that radioactive material could be transported along the amphibian nerve trunk at fast rates.

It was also possible to put the radioactivity in the muscle pool and find that after 24 hr there was radioactivity in the snail brain or frog spinal cord. This antidromic transport was slower (about 1 cm in 24 hr) than the orthodromic transport (Fig. 5).

At a meeting in the USA in 1966 it appeared that many American workers had found that slow rate of axoplasmic transport described by Weiss and had also some results giving fast rates of orthodromic transport but had been cautious about revealing the fast rates. However, they did not think that antidromic transport occurred, or if it did so, it was an artifact! Since then many laboratories have also found antidromic transport of material from the periphery to the nerve cell bodies and it is no longer considered an artifact.

The use of isolated CNS-nerve trunk-muscle preparations that survive well for 12-24 hr has still not yet been fully exploited and this point with be discussed later on in this article.

**INSECT CNS**

Having obtained results from microelectrode studies on intracellular potentials in snail neurons following applications of drugs, it seemed interesting to look at other isolated CNS preparations. The cockroach CNS gave excellent extracellular recordings but for some reason seemed difficult to study with intracellular electrodes. Bob Pitman, using the techniques we have developed for intracellular studies on snail neurons, found several nerve cells in the cockroach isolated ganglia that gave good resting potentials, EP, IPSP and action potentials. Furthermore, the neurons responded well to application of ACh which excited the cells and GABA which inhibited the neurons.

I heard that Edward Rowe, working at Ann Arbor, Michigan, had obtained microelectrode records from cockroach ganglia nerve cells but had difficulty in getting his results accepted. I wrote to him saying that we had similar results and were getting them ready for publication, and asked if he would like to send me a paper on his own work that could be published before ours? He did so and we followed with publication.

One summer, whilst our work was going on, Graham Hoyle paid us a visit and Bob Pitman showed him the work on the cockroach ganglia and the recordings from the nerve cells. Graham had thought that the recordings might not be from nerve cells, but Alan Crossman had filled the cells we recorded from with Procion Yellow which enabled the cells and axonal connections to be identified. Graham Hoyle was convinced by these marked neurons and their action potentials, and went back to
Isolated central nervous systems

A

lanolin

RINGER

lanolin

nerve
cord

muscle

Frog Gastrocnemius--sciatic--nerve cord

B

cut dorsal roots

isolated nerve cord

muscle

C-14 Ringer

lanolin

Ringer

Fig. 5. Axoplasmic transport along the frog sciatic nerve trunk, between the isolated nerve cord and the peripheral muscle. (A) The frog nerve cord is isolated, together with the sciatic nerve and the gastrocnemius muscle. A double lanolin barrier is placed between the nerve cord and the muscle so that radioactive material can be placed in either the nerve cord compartment, or the muscle compartment, and no direct leakage of labelled material can take place. When the muscle is soaked in Ringer containing labelled glutamate for 24 hr, radioactive material is found in the sciatic nerve trunk and also in the nerve cord. (B) The effect of cutting the dorsal roots. Radioactive material still appears in the nerve cord, even when the dorsal roots have been cut and the cut plugged with lanolin.

Eugene to work on these cells which he called dorsal unpaired median cells (DUM). With his colleagues he showed that they contained octopamine as a transmitter/modulator. I later got Graham to write a chapter on these neurons for Comprehensive Insect Physiology.

ISOLATED INVERTEBRATE CNS PREPARATIONS

The isolated snail brain provided excellent opportunities to study the membrane potentials of specific nerve cells, their IPSP, EPSP, long term inhibition and excitation, electrogenic sodium pumps, reaction to anoxia, and their synaptic pharmacology. With the techniques learnt from Helix neurons, and the help of my colleagues listed in the bibliography at the end of this article, it was possible to apply these techniques to other isolated invertebrate CNS preparations such as those of Hirudo medicinalis (leech), Periplaneta americana (cockroach) Schistocerca gregaria (locust), Limulus polyphemus (King crab) and Tetrahymena, the ciliate protozoan that is almost equivalent to a free-swimming neuron (Fig. 1). We had intended to look at the CNS of Peripatus but Ernst Florey and Graham Hoyle got there before us!

The success with invertebrate CNS preparations indicated that the vertebrate CNS might function quite well in isolation and I decided to have a look at the mammalian system to see what could be done there. Neuroanatomists and histologists have been studying the functional anatomy of the mammalian CNS for the last hundred years and we know more about its structure and connections than for most other central nervous systems. The wiring of the CNS is of supreme importance and the anatomical background, together with modern computer analysis of the electrical activity (information transfer, should allow greater understanding of the functioning of the CNS.
Henry McIlwain and his colleagues had found that stimulating rat brain slices doubled the rate of respiration of the slice and that these brain slices showed good electrical activity. It looked as if isolated pieces of the mammalian CNS might survive quite well.

Together with Jeff Bagust, Kevin Green, Caroline Herron, Ian Forsythe, Nagi Ibrahim and Melanie Kelly, we set to work to see the extent to which we could get large pieces of the mammalian CNS functional. We were able to get good electrical activity from thin and thick slices of the rat cortex but I could get large pieces of the mammalian CNS quite well.

It is possible to remove the whole spinal cord and brain stem from 3–6-week-old hamsters, mice and rats (animals that are fully mobile and active) and get good electrical activity from these preparations. The hamster has long spinal roots and so we tend to use it especially to study reflex activity in the dorsal and ventral roots. Furthermore, the preparation has developed from the initial hemisected spinal cord to our present preparations of the intact whole spinal cord.

These preparations show the following activity:

1. Spontaneous activity in the dorsal and ventral roots.
2. Stimulation of a dorsal root gives ipsilateral monosynaptic and polysynaptic responses in the ventral root. These are reversibly blocked by saline containing zero calcium and 2 mM Mn, i.e. involve synaptic transmission.
3. Stimulation of the dorsal root will give polysynaptic responses in the contralateral ventral root.
4. Preparations of the isolated spinal cord can have identified peripheral nerve trunks going to antagonistic muscles and the activity in these motor nerves studied following stimulation of ipsilateral and contralateral sensory nerves.
5. Stimulation of a dorsal root sends activity up and down the spinal cord. The ascending action potentials can reach the cuneate nucleus, synapse there, and pass onwards to the brain stem.
6. Stimulation of the brain stem sends activity down the spinal cord and, depending on the site of brainstem stimulation, will augment or inhibit the local spinal sensory-motor reflex.
7. Stimulation of dorsal roots sends activity across the spinal cord to the contralateral dorsal horns as well as up and down the cord. There is a sensory network in the spinal cord that could allow modulation of sensory inputs ipsilaterally and contralaterally. This suggests that the spinal cord has important local area network connections in addition to the through tracts going to and from the brain (Figs 6 and 7).
8. The patterns of spontaneous activity in the dorsal roots of the spinal cord indicate that there are segmental pattern generators in the spinal cord that are functionally linked together.

9. The segmental pattern generators that are in adjacent segments of the spinal cord are more closely correlated in their activity than those that are far apart in the spinal cord. Complete transection of the spinal cord removes the correlation between the segmental pattern generators either side of the cut.
10. The neurons in the spinal cord respond to changes in the bathing saline. Changes in ionic composition, the presence of drugs, blocking agents and anaesthetics can all bring about a quick response. There is no blood–brain barrier preventing access to the neurons and synapses.
11. Many of the neurons in the spinal cord respond to drugs applied in the bathing saline. The response is quick, the drug can be easily washed off and the preparation recovers. This is the advantage of not having a blood–brain barrier and should allow more detailed study of the pharmacology of the CNS.
12. There are many interneurons functioning in the isolated spinal cord and stimulation of adjacent ventral or dorsal roots, or lateral pathways can inhibit segmental reflex activity. The inhibition is probably due to Renshaw cells and other glycine systems.

There are thus many neurons and functional circuits active in these isolated mammalian CNS preparations and they remain functional in vitro for up to 24 hr after isolation. Conditions are not ideal because the oxygenation of the deeper parts of the preparation is not sufficient and many nerve cells die from anoxia, but it is possible to improve the oxygenation of the system.
Fig. 7. Cajal's diagram of a transverse section of the mammalian spinal cord showing the commissures connecting the dorsal horns of both sides of the cord (D and E). It is generally thought that sensory information crosses to the ipsilateral and contralateral ventral horns of that segment (for local motor reflexes) and also travels up the spinal cord to the brain. These dorsal commissures indicate that sensory information is also transmitted to the contralateral dorsal (sensory) horn and provides the morphological basis for the physiological local area sensory network shown in Fig. 6 (and also in the paper by Bagust et al., later in this issue of the journal).

FUTURE OF ISOLATED MAMMALIAN CNS PREPARATIONS

One of the problems in understanding how the CNS works is that we tend to think in two-dimensional models, in terms of diagrams on a page. But the CNS works in three dimensions, probably with many central processing units functioning in parallel, with handshakes and cross-talk going on in all dimensions.

The isolated mammalian spinal cord preparation offers many opportunities to study these systems. It is possible dissect out tracts and pathways and determine the extent to which they interact. The pathways, if functional, can be locally anaesthetized (reversibly blocked) and the differences in firing patterns and interactions studied.

Preliminary experiments show that higher pathways such as the pyramidal tract are also functional in the isolated preparation. With improved technique it will be possible to have simple dissected out circuits with the spinal cord, brain stem, cerebellum, cerebral cortex and basal ganglia all functioning.

The brain and spinal cord can then be considered in terms of a computer with its plug-in boards of circuitry. The dissected-out interconnected anatomical units of the isolated preparation will allow recording from many sites in the CNS simultaneously, the boards (cerebellum, corpora quadrigemina, auditory nerve input, optic nerve input, basal ganglia, cerebral cortex etc.) can be plugged in or out (anaesthetised or recover) and so provide an understanding of the parallel and hierarchical organization of the CNS (Fig. 8).

The complex shuffling back and forth of thousands of electrical signals in the CNS each second is beyond simple one- or two-channel analysis and requires new techniques to enable us to understand what is going on. With the development of sophisticated analytical software for microcomputers that can take the electrical data simultaneously from many sites in the isolated CNS, it will be easier for the research worker to understand the more complex and subtle hierarchical, parallel, differential, error-correcting, conditional probability communications taking place within the spinal cord and brain and so obtain a better appreciation of the multifarious systems at work, i.e. the computers will enable us to understand what is going on, and the isolated mammalian CNS preparations will provide ideal data for such computer analysis.

It is always necessary to check the findings from \textit{in vitro} preparations with those in the intact living animal. The two systems of analysis of CNS function (\textit{in vitro} and \textit{in vivo}) go together, like two hands grasping a problem and will allow greater progress in the understanding of how the CNS works.

RETROSPECT

The study of the isolated central nervous system has led to an increased understanding of the rules determining the activity and interactions of nerve cells.

Studies on the electrical activity of peripheral nerve explained the membrane potential and action potential in terms of one potassium current and one sodium current. Now we know of at least five potassium currents, three sodium currents, four calcium currents and three chloride currents in CNS neurons.

Progress has gone from two chemical synaptic transmitters to at least 30 and probably 100 if one includes modulators. Once there was only one neurotransmitter in the presynaptic terminal; now there can be many in the one terminal; and of course the number of different receptors for a given transmitter has been subdivided so that any respectable transmitter will have at least three receptor subtypes.

It was assumed that a nerve cell at rest is isopotential throughout the whole of that cell. Now it is probable that different parts of a cell can be at
different membrane potentials. Furthermore, the ionic composition within a neuron is probably not uniform and there is compartmentalization with some regions (dendrites) having different ionic content and calcium sequestering properties from other regions of the neuron.

Once all CNS neurons were equal; now you have to know which neuron you are working on so as to get repeatable experimental results. It is also important to appreciate the diurnal, monthly and yearly cycles in neuronal activity in the CNS.

Much of this new information has come from studies on the isolated CNS and it is probable that "you ain't seen nothing yet".

We have been fortunate in participating in some of these discoveries, and this has made the scientific work interesting and exciting. I have benefited greatly from working with enthusiastic, gifted colleagues who have helped make my progress more constructive than destructive, more helping than hindering and more inquisitive than acquisitive.

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