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Visceral Afferent Functions of the Nervous System

P. P. Newman

VISCERAL AFFERENT FUNCTIONS OF THE NERVOUS SYSTEM

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PREFACE

THIS monograph is an expression of my pursuits in a field of research which has attracted successive workers at Leeds for nearly half a century. Interest in visceral afferents from the abdominal region began with McSwiney in 1926 when he traced impulses into the central nervous system and later, in collaboration with Bain, showed that afferents in the splanchnic nerves ascended to the oculomotor nucleus. Conduction pathways and their clinical implications were the subject of Goligher's well-known studies on the pelvic viscera. About the same time, ideas on the central control of the thoracic organs were being developed by Hemingway, Schweitzer & Neil and their lead opened up a new era in cardiovascular thinking which has since been actively promoted by Linden and his associates. It was in this background that I was encouraged to follow the course set by McSwiney for, at the time, very little was known about the cerebral destination of the visceral afferents or the part they played in reflex and regulatory mechanisms. Indeed, there was a curious reluctance on the part of many writers to admit that fibres travelling in the autonomic nerves belonged to a separate projection system which was sensory in function. Although the significance of the hypothalamus had long been recognized, there was no clear picture suggesting that the cerebral cortex might also serve in the neuro-endocrine adjustments of the body.

Notable progress was made in the year 1951 when it was shown that evoked potentials could be recorded from the sensory cortex after stimulating the central cut end of a splanchnic nerve and this was followed by the demonstration of cortical responses to over-distension of the gall bladder, stomach and other hollow viscera. In those days we lacked the advantages of modern equipment, but none of the pioneering spirit or enthusiasm that marked each disclosure and approach to the next problem. We constructed our own amplifying and recording systems, which now seem so primitive, and designed much of the hardware from the workshop bench. The introduction of microelectrode techniques was hailed by neurophysiologists everywhere and the literature during the next decade gave ample proof of its success in rediscovering the nervous system at a unitary level. It was certainly reassuring, if not entirely dramatic, when it was first shown that the discharge of a single brain cell could be influenced by impulses arising in abdominal viscera. The way was then open for a detailed analysis of the discharge properties of the units and to demonstrate their representation at every functional level. A new interpretation was given to the functions of the cerebellum and our knowledge of the reticular network in the brain stem was considerably advanced. It was also evident that feedback from corticofugal influences played an essential role in determining the outcome of complex interactions between afferents of somatic and visceral sources.

Another set of problems then emerged. Once it had been established that visceral afferents belonged to an organized projection system reaching to the cerebral cortex and utilizing feedback control, it was necessary to discover how such impulses could be integrated with the mass of information feeding into the hypothalamic and brain stem centres through which the reactions of the body were mobilized. At the present time it is believed that the limbic regions of the brain fulfil this role. Collectively, they exert a control over the entire autonomic nervous system and therefore make possible the widespread visceral reactions observed in conditions of emotion and stress. There are still many problems associated with emotional disturbances which may be relevant to the circuitry of the limbic system and only by the successful examination of the underlying physiological mechanisms can we hope to achieve a clearer understanding of functional changes in health and disease.

P. P. N.

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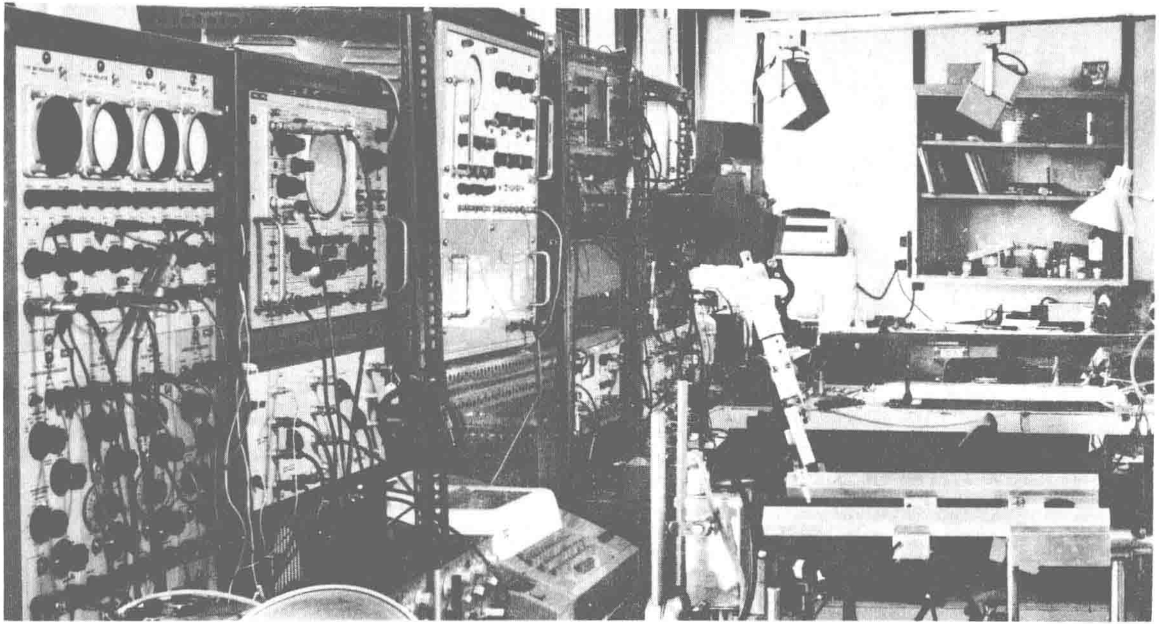
THE subject matter which provides the title of this book was derived in no small measure from discussions with colleagues throughout the world during the International Congresses of Physiology as well as at our own Society Meetings. From them came the inspiration and friendly contacts that are so important to the advance of our research. In particular, I am indebted to V. E. Amassian and his associates at the Albert Einstein College of Medicine, New York, to J. C. Eccles and to the late Warren McCulloch who spent some happy times in my laboratory at Leeds. Most of all I should like to thank J. H. Wolstencroft of Birmingham, and D. H. Paul of Manchester together with various colleagues who have worked with me over the years. I am also grateful to my colleague, Dr A. V. Holden, for reading and criticizing the manuscript and for many helpful suggestions. On the technical side I have been fortunate to have the expertise of Mr Eric French and the assistance of Mr Paul Armitage. Electronic developments have been in the hands of Mr Stanley Stainthorpe and his staff; workshop facilities, initially under the direction of the late Mr Albert Jepson, were cheerfully provided by Mr Stanley Pennington and Mr George Thompson; histological assistance by Mrs Barbara Royle. For the illustrations I am grateful to the late Mr Norman England and his successor, Mr Peter Hargreaves. The entire manuscript was kindly typed by Mrs Marian Stainthorpe. Finally, I should like to express my thanks to the Medical Research Council for many years of financial support.



A neurophysiological laboratory

CONTENTS

1

INTRODUCTION

THE neurophysiologist is concerned with experiments designed to give information on the activities of the nervous system. He will generally begin by testing a hypothesis in a series of pilot experiments and the outcome may be of use to show him the direction for planning his future work. He will continue by observing and measuring the changes produced by a particular agent or stimulus, comparing such changes with those obtained under strictly controlled conditions. The inferences made from the experimental results can often be strengthened by using different methods of approach or using different techniques with appropriate forms of analysis. Eventually, as data accumulate, he may be tempted to express a point of view or to make a report which, although open to criticism, may give encouragement for further investigation. The objects of his research are to record the facts and to make tentative suggestions, leaving others to decide on their value and significance. Sometimes the facts are confirmed by contributions from other sources so that new ideas emerge and principles become established; in this way steady progress is made towards a further understanding of the problem. However, such is the competitive interest of scientific study that results are just as likely to give rise to controversy or doubt and the problem must then be investigated again, perhaps with the help of new techniques or a new approach or else await the attention of another generation of workers.

EXPERIMENTAL METHODS

The era of 'string, pulleys and smoked paper' has been superseded by a multitude of electronic and engineering devices, which have given the modern research worker a distinct advantage. Yet the possession of sophisticated instruments and knowledge of their application do not by themselves produce solutions to problems and in no way detract from the skill and insight of earlier workers. Many outstanding contributions have been made when only the most primitive equipment was available.

General rules of management

There are certain rules in the management of acute experiments which are designed to maintain the animal in good physiological condition. Availability and costs are usually the deciding factors in determining the species used. Cats have proved extremely valuable for investigations on the nervous system and there is a strong case for establishing more breeding farms to ensure standard sizes and weights. The kind of preparation employed will of course depend on the nature of a particular experiment. Decerebrate and spinal preparations have the advantage that they do not require an anaesthetic after completing all the operative procedures. On the other hand, they demand careful haemostasis, constant attention to airway and a watchful eye on the blood pressure. With intact animals, the success of an experiment often depends on the choice of the right anaesthetic and dosage. Commonly preferred anaesthetics are mixtures of Dial and urethane (0·7 ml/kg), chloralose (60–100 mg/kg), sodium pentobarbitone (Nembutal, 30–50 mg/kg) and sodium thiopentone (Pentothal, 45 mg/kg). An initial dose of barbiturate, given by intraperitoneal injection, acts slowly but safely and gives good surgical anaesthesia; thereafter small maintenance doses are given intravenously as required. When ether is used initially, followed by small doses of barbiturates, the skin wounds and pressure points should be infiltrated with a local anaesthetic (1% procaine HCl). In order to maintain the body temperature between 36 and 38°C an external source of heat is required such as an infra red lamp. The blood pressure is monitored at intervals during an experiment and may be prevented from falling below 80 mm Hg by intravenous drips of glucose or dextran.

Methods of stimulation

(i) Mechanical

Rubber balloons are widely used to effect distension of a viscus by expansion. They can be cut to size from a surgical glove and tied over the end of a glass cannula or polyethylene tube. A second tube inserted with the balloon can be used for measuring pressure in the viscus. The balloon is inflated with saline or air delivered from a motor-driven syringe or by hand. A two-way tap on the nozzle allows for rapid deflation. When experiments are performed on abdominal viscera, it is important to cause as little disturbance as possible to the mesentery, which is very sensitive to mechanical stimuli. After testing the action of the balloon, the connecting tubes are fixed to the skin of the abdominal wall, which is then closed in layers.

(ii) Thermal

Heat is an effective physiological stimulus. Many techniques have been described for raising intracranial temperature. In one method, the proximal end of one or both carotid arteries is cannulated and the blood passed through a spiral glass tube in a water-bath before being returned through a cannula inserted into the distal end of the artery. The temperature of the water is raised by an electric heater. The method avoids the danger of spread of heat to the vagi and other structures in the neck, which occurs when heating tubes are placed around the carotid arteries. The temperature of the carotid blood is monitored by a thermocouple inserted through the central end of the lingual artery. A second thermocouple, inserted into the brain, measures the change of temperature which occurs on heating. Irrigation of the cerebral ventricles with warm saline has also been used.

The application of heat to a localized region of the brain has been described in detail by Magoun, Harrison, Brobeck & Ranson (1938). These authors used a low-powered, high-frequency oscillator to pass current between two wire electrodes. A modification of their method uses a silver electrode, insulated except at the tip and mounted in a stereotaxic holder. The electrode is connected to the output of a diathermy or radio frequency unit; the indifferent electrode is a saline pad applied to one limb. The temperature in the region of the electrode tip is recorded by a thermocouple made by brazing together copper and constantan wires. Electrode placements are determined by histological studies in which the track of the electrode can be easily recognized.

The use of thermodes has the advantage of giving any desired temperature so that the effects of cooling can also be observed. Thermodes made from stainless steel tubes, insulated except at the tip, operate by the circulation of hot or cold water between two tanks. Thermal stimulation of surface structures may be effected by a stream of distilled water or saline emitted from the tip of a hypodermic needle. Rapid temperature changes can be accomplished by displacing the stream with fluid from a second needle (Poulos & Benjamin, 1968).

(iii) Chemical

Strychnine has proved a valuable aid in tracing pathways in the central nervous system. The method was introduced by Dusser de Barenne (1916) as an alternative to the method of extirpation for mapping cortical boundaries. He described the action 'setting on fire of the cortex by strychnine'. A small piece of filter paper, soaked in 1% strychnine sulphate and coloured with toluidine blue, is placed on the pial surface in proximity to a pair of recording electrodes. Cortical 'spiking' is observed about one minute after the application. If a second pair of recording electrodes is placed in another part of the brain, responses to strychnine stimulation suggest the existence of a direct functional pathway between the two areas. The method is simple, effective and avoids anatomical lesions.

Chemical substances, acting on the stomach, are introduced by means of an oesophageal catheter or by passing a polythene tube through the mouth. Alternatively, the abdomen is opened in the mid-line and the tube inserted into the pyloric antrum through a small incision in the wall of the duodenum, which is then tied. The resting contents of the stomach are washed out with warm saline solution before introducing the chemical stimulus. The effects of hyperacidity of the gastric mucosa have been studied in this way, using a solution of HCl which is first adjusted to any desired pH.

Substances introduced into the cerebrospinal fluid may be injected through the atlanto-occipital membrane or into one of the cerebral ventricles. It is possible to limit the action of a chemical to any part of the ventricular system by collecting the outflow through an implanted cannula, whilst other parts of the system are perfused separately. A method of multiple cannulation of the cerebral ventricles has been described by Carmichael, Feldberg & Fleischhauer (1964).

The application of a chemical substance to single neurones requires the use of a two-barrelled microelectrode. One barrel is filled with a solution of the substance to be tested and the other is used for recording the electrical response of the neurone. The technique of recording from single neurones will be described below. The chemical substance is released from the tip of the microelectrode by passing a current through the solution either constantly or in pulses. This electrophoretic method controls movement out of the tip by diffusion and obviously can only be used with ionic substances. Comis, Evans & Whitfield (1964) developed a micro-tap, consisting of two concentric pipettes, the outer filled with the chemical solution and the inner serving as the recording electrode. A screw-driven syringe advances the inner electrode towards the tip of the outer pipette and closes the tap. Multi-barrelled pipettes are also used for investigating the action of chemicals on single cells. They are made of fine glass tubes fused together, each containing a different substance. The tubes are arranged radially around a central pipette employed as the electrode recording the responses of the cell (Curtis & Eccles, 1958).

(iv) Electrical

Stimulating electrodes. A pair of silver wires, with tips 3–4 mm apart, is very satisfactory for peripheral nerve stimulation. They can be mounted with a second pair of silver wires for recording the nerve action potential; the part of the nerve between stimulating and recording electrodes is connected to the animal or grounded in order to reduce artifact. For abdominal work, it is useful to fix the multi-electrode assembly to the musculature of the body wall by sutures and also to hold the connecting leads in place before the abdomen is closed. This technique allows continuous stimulation and recording without loss of contact between nerve and electrodes, which may otherwise occur due to respiratory movements.

Bipolar silver wires with ball tips are suitable for stimulating surface areas of the brain. When a unipolar electrode is employed, the shock artifact may be quite large unless the indifferent electrode has a high resistance. Stimulation of structures deep to the surface is effected by a bipolar concentric needle electrode or by means of a fine steel wire, insulated with enamel except at the tip. Both these types of electrode allow a very localized region of stimulation.