EXPERIMENTAL NEUROLOGY

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BY

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CLARENDON PRESS · OXFORD

1961

Oxford University Press, Amen House, London, E.C.4

GLASGOW NEW YORK TORONTO MELBOURNE WELLINGTON
BOMBAY CALCUTTA MADRAS KARACHI LAHORE DACCA
CAPE TOWN SALISBURY NAIROBI IBADAN ACCRA
KUALA LUMPUR HONG KONG

Printed by The Whitefriars Press Ltd. London and Tonbridge

PREFACE

The preparation of the English edition from the German text published in 1957 took about three years. This made it possible to include some important recent advances but also necessitated a shift of emphasis in a few chapters and by this caused a rearrangement of some of the subject-matter. I am grateful to the Thieme Verlag for permitting these adaptations.

During this time the translation was carefully nursed by an English 'foster mother', Miss C. Boulter M.A., whose command of the English language, style, and critical faculties I had learned to appreciate already in my book on Neuroglia. We hope that a concise and clear English account of the German text has been achieved.

Acknowledgements: Sincere thanks are due to Miss A. G. Smith and the other technical staff of the Physiology Dept. and to the staff of the Oxford University Press for their ready and unfailing help.

Oxford, 1961 P. G.

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I

METHODS OF EXPERIMENTAL NEUROLOGY

Introduction

IT is something of a break with tradition to begin a textbook of this kind with a chapter on methods, but this approach is perhaps justified by the fact that the content is based almost exclusively on experimental evidence and thus ultimately on the methods applied. Our knowledge of the nervous system, it will be agreed, is based chiefly on the co-ordination of experimental evidence with the experience gained in clinical neurology; it is, however, important to remember that the results of any experiment have no absolute validity outside that particular experimental setting, and that to draw any general conclusions may be unwise. Therefore the account of the methods most used in neurological research, which this chapter contains, is accompanied by references to the results obtained with them by a number of neurohistologists and neurophysiologists in different types of experiment. It is hoped that this will give an indication of the range of the various methods and some guide to the interpretation of results.

The experimental animal

No problem in neurology can be thoroughly investigated without a sound knowledge of normal neuroanatomy, and when embarking on neurological research it is best to begin with a purely neuroanatomical problem. For this type of experiment the most suitable animal to use is the rabbit, or some similar, small mammal which can be easily kept. An animal high in the evolutionary scale should never be chosen if a lower one suits the purposes of the particular experiment equally well—and the choice is vast: neurological information can be gained by studying even the comparatively simple nerve fibre of the squid. Experiments with higher animals—particularly primates—which have a more developed cortex and can therefore be trained and tested (Fig. 31) must be more elaborate, including the study of neurological and behavioural disturbances caused by lesions, for only thus can results be obtained which can be applied in clinical neurology, which after all is the ultimate purpose of all experimental neurology.

The animal used for the experiment should be in good condition and well looked after both before and after operation, particularly in long-term experiments, much of whose success depends on the proper care of the

animal. (Since the initial preparations for the experiment are so important, students are well advised to seek advice from some laboratory where work of this kind is regularly undertaken.) The animal can be spared much unnecessary suffering if the experiment is carefully planned.

All experiments should be accurately recorded and, if possible, a film made of the animal's condition and test performance before and after the operation. The results of the experiment will be of greater value if the records are very detailed and well kept.[1]

Anatomical methods

The important function of the nervous system—that of transmitting signals—rests on the integrity of cell processes and nerve fibres (the protoplasmic continuations of the nerve cells) which may cover astonishing distances: a descending pyramidal fibre or an ascending sensory fibre of a spinal ganglion cell in man may be as long as one metre. An increase in the number of cells and an increase in the number of cell processes would both contribute to the efficiency and complexity of the nervous system, but it is likely that the human nervous system has reached its high degree of perfection by an increased number of processes rather than of cells. The organization of the nervous system, then, is highly complex, and a variety of problems in experimental neurology can be studied by damaging this system, with the object of discovering by post mortem examination of fibre degeneration or cell changes the exact effects of the damage.

A lesion is made by destroying or removing a certain region of the brain or by severing the afferent or efferent fibres connected with that region. The subsequent investigation will then be directed towards discovering the extent of the anatomical damage, the degree of the elimination of function, or the type of behavioural changes which the lesion has wrought (Figs. 1 and 2). The study of the anatomical or histological damage may of course be an end in itself, but it is also a necessary part of the neurophysiological study of function or of the study of behaviour, for the exact extent and range of a lesion can never be judged accurately without a post mortem examination, and a study of function will be valueless unless related to precise anatomical data. This being so, the reason for starting on a neuro-anatomical problem in experimental neurology is self-evident.

Lesions may be made in various ways: some involve direct injury to the tissue, others damage the tissue by more indirect means. With the more direct surgical or anatomical methods the whole brain may sometimes have to be shrunk in order to achieve greater scope of movement within the narrow cavity of the skull. This is done by injecting a 20 per cent. glucose solution or a 1–2 per cent. urea solution, interperitoneally or intravenously,

[1] Many examples of meticulous operational records will be found in the work of Hess (see particularly Hess, 1951).

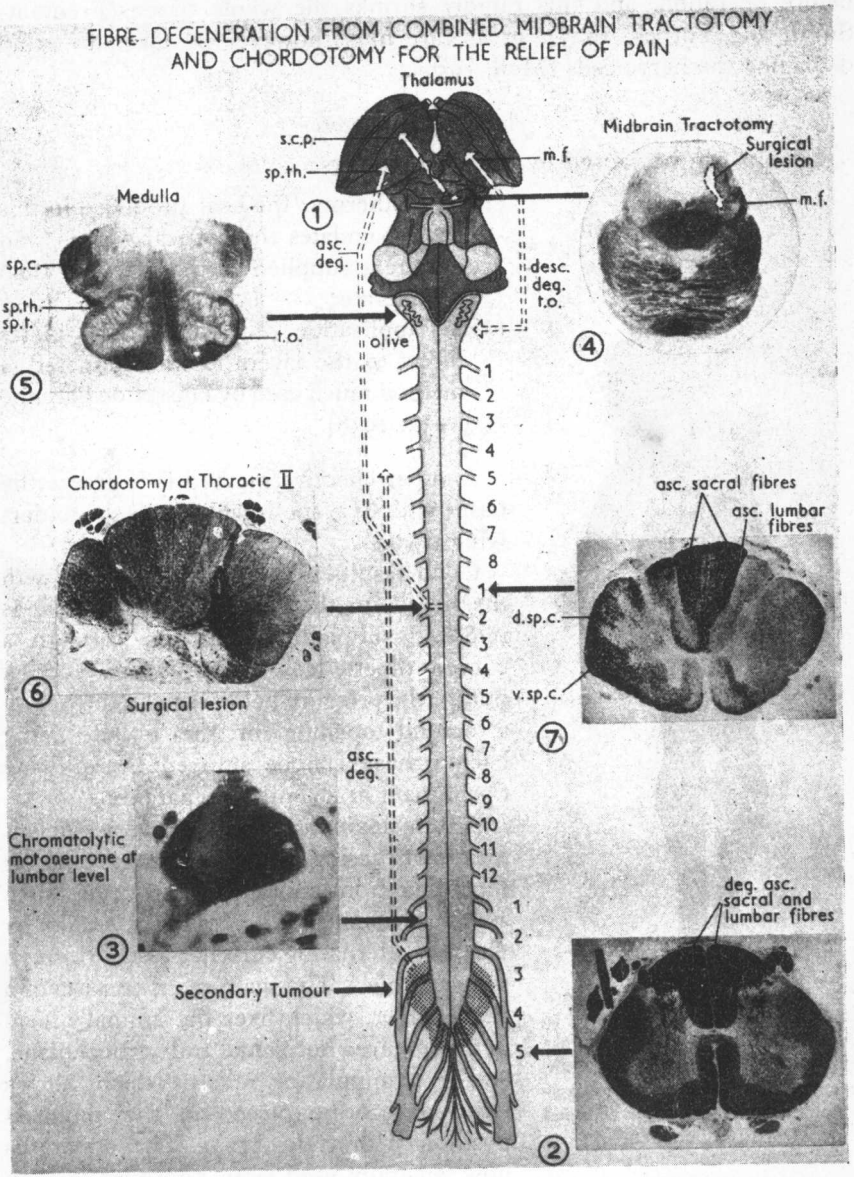

FIG. 1. The histological changes, as shown by a combination of Marchi and Nissl methods, caused by three successive lesions: a secondary tumour and the operations of left midbrain tractotomy and right chordotomy. (The operations were performed to relieve pain.) 1. The level of the lesions and distribution of fibre tracts. 2. The effect of the tumour on ascending fibres of the dorsal column. 3. The effect on motoneurones of ventral root compression caused by the tumour. 4. The level of midbrain tractotomy. 5. The location of descending and ascending degeneration at medullary level. 6. The extent of spinal chordotomy. 7. Ascending fibre degeneration at cervical level.

before operation, and this rapidly shrinks the whole tissue (Fremont-Smith and Forbes, 1927); great care must however be taken to avoid damaging the nerve cells (Moll, 1953).

Anatomical lesions

Lesions can be caused by ***heat*** in two ways:

(1) by diathermy; the heat produced in this case coagulates the cortical vessels, and the areas supplied by these vessels then degenerate;

(2) by application of heated metal plates direct to the layers to be eliminated, a method much used by Dusser de Barenne (1933, 1938).

FIG. 2. The sensory impairment caused by the lesions described in Fig. 1. The area of complete loss of pain sensation is shown black, very reduced sensitivity cross-hatched, and slightly reduced sensitivity shaded.

Another effective method is to freeze the tissue with CO_2 snow, which kills the underlying tissue.

Good results may also be obtained with the suction method: here the brain tissue is removed subpially by suction through a curved pipette connected with a suction pump, the pressure being regulated through a second opening in the pipette. Any haemorrhage can be stopped by applying fibrin foam or a similar preparation.

A stereotaxic instrument is a very efficient means of causing deeper lesions, as also for recording from subcortical structures such as the thalamus. The lesion is made by direct or alternating current passing through the electrodes. The instrument consists of a head-holder, which fixes the animal's head in the required horizontal and vertical plane, and a manipulating stand, which allows electrodes to be placed in the required position within the brain. The apparatus now generally used is an improved version of the original design of Horsley and Clarke (1908), which itself was an improvement on the first instrument with which an accurate lesion could be made, von Trendelenburg's 'Myelotom' (1907).[1] Circumscribed lesions,

[1] Those interested in the history of the development of stereotaxic instruments should read the original paper by Horsley and Clarke, who fully recognized the neurobiological and neurosurgical importance of localized deep lesions.

avoiding the use of electrical current, can also be made with a rotating, sheathed knife fixed to the stereotaxic instrument (Glees *et al.*, 1947).

In order to be able to make comparable lesions (in size and location) in different animals of the same species, the stereotaxic instrument is often used in conjunction with a special topographical brain atlas, based on serial brain sections in standard planes. The planes in which these sections are cut correspond to the planes of the head-holder and therefore to the brain of the particular animal. These brain atlases have been made for stereotaxic work with various animals (for the rat by Krieg, 1946, and de Groot, 1959; for the cat by Hess, 1947, and Desmedt and Franken, 1958; for the dog by Lim *et al.*, 1960, and Adrianov and Merink, 1959; for the thalamus of the monkey by Olszewski, 1952; for the guinea pig by Hoffmann, 1957; and for man by Singer and Yakovlev, 1954, Schaltenbrand and Bailey, 1958, and Jimenez-Castellanos, 1959).

Ultrasonic waves

The destructive effect of high frequency sound waves has been used by brain surgeons either to destroy tumours, to interrupt the frontal lobe connexions, or to eliminate circumscribed cortical areas.

Lindström (1954) gives an account of twenty cases in which the frontal lobe was subjected to the effects of these waves (seventeen cases of malignant disease and three cases of organic brain damage which had produced certain psychiatric symptoms). His work on the human brain was preceded by experiments on rabbits, which proved that the extent of the destruction varied considerably if the skull remained closed, although this was not so if the bone was removed; removal of the dura made no difference. Lindström used a quartz crystal 3 cm in diameter which oscillated 1,000 kHz per second. It was placed between 1·5 and 4 cm from the brain, and pressure waves of 7 watts were transmitted through Ringer solution onto the dura by a special adapter. Fourteen of the brains treated were anatomically examined; hardly any damage could be seen macroscopically, but microscopically glial proliferation appeared in the white substance, particularly near the ventricles and the cerebral vessels.

Similar experiments by G. Peters (1955) on the guinea pig, using radiation lasting from 5 to 12½ minutes at a frequency of 100° kHz, showed damage very similar to that often found in brain trauma caused by road accidents involving a sudden impact.[1]

Lynn and Putnam (1944) found that the effects of ultrasonic waves on the brains of dogs, cats, and monkeys were most marked in nerve cells and, on the whole, glia cells and blood vessels were comparatively little damaged. In the spinal cord of the frog the large nerve cells have been found to be particularly sensitive (Wall *et al.*, 1951) and glia cells and blood vessels pathologically unchanged.

[1] The extensive literature on this subject is discussed by Peters.

American scientists now claim to be able to produce waves of a very much higher frequency than those used hitherto, with much more accurate focusing, but few results have as yet been published.

Chemical lesions

Lesions can be caused by various chemicals, e.g. fat-free substances, D.D.T., mustard gas, anoxia, and hydrocyanide.[1] The effect of chemicals on the brain is discussed in more detail in the following chapter. To what extent lesions can be caused by narcotics is difficult to say, since too little is known of the exact effects of most of them.

Radiation

No attempt has yet been made to produce brain lesions by X-rays for experimental purposes, but the incidental damage caused by this means to nerve cells and neuroglia cells has been studied where radio-therapy has been used for tumours or other neurological disorders (Scholz, 1934; Gerebtzoff and Herve, 1949). Since the development of nuclear physics, radioactive substances and particles of high velocity have become available for biological studies; the high-energy proton beam of a cyclotron has, for instance, been used to cause circumscribed lesions in the spinal cord (Larsson *et al.*, 1959), in the internal capsule (Larsson *et al.*, 1958), and in the cerebral cortex (Malis *et al.*, 1957), and the radioactivity of radon has been used to cause experimental lesions in the spinal cord (Cairns and Fulton, 1930; Glees, Livingston, and Soler, 1951) and cingular gyrus (Glees and Lewin, unpublished).

The study of anatomy

It is the study of the degeneration of nerve fibres and cells which provides the evidence of the precise effect of a lesion. An axonal process of a nerve cell is an integral part of the cell, and if severed from it the peripheral or amputated portion degenerates fairly quickly (Fig. 3). The most characteristic early signs of degeneration are nodular swelling and marked dilatation (Fig. 10); fragmentation of the axone follows this stage (Fig. 5*B*); in peripheral nerve fibres the debris is rapidly removed (Fig. 5*C*), but in central nerve fibres large quantities remain for a considerable time (Glees, 1948).

The success of this part of neurological research depends very much on the quality of the histological techniques used. Fixation has even been termed the Via Dolorosa (*Leidensweg*) of neurohistology by Bargmann (1948), who gives a nice dissertation on the distorting effect fixation can

[1] The toxic effect of cyanide is similar to anoxia: see Stone, 1938, and Olsen and Klein, 1947*a*, *b*.

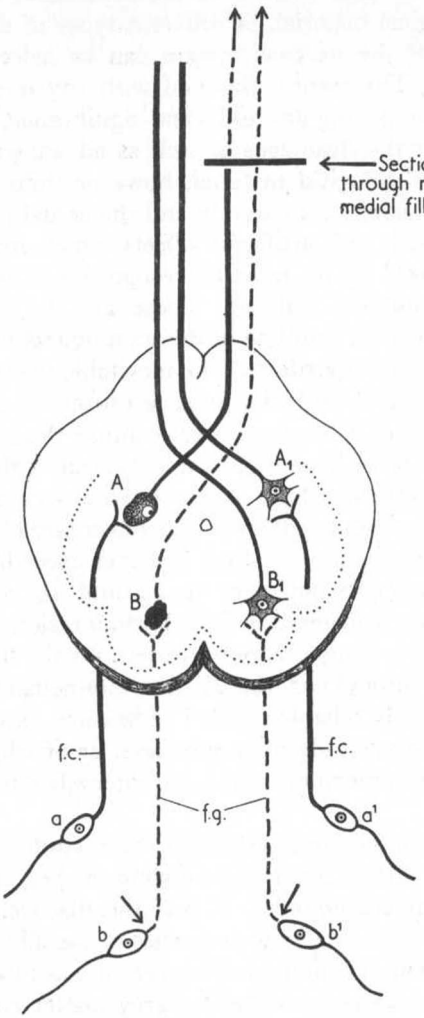

FIG. 3. The combined effect of degeneration of both fasciculi gracilis (see small arrows) and unilateral severence of the postsynaptic axones of the cells of the gracile and cuneate nuclei at the level of the midbrain (see horizontal arrow). The extent of degeneration is shown by the interrupted line. Neurone *B* is affected both by the retrograde Marchi degeneration from the midbrain and by the result of the damage to the spinal ganglion cell, and is totally degenerated. Neurone *A* shows only chromatolysis, which may be only a sign of repair since the proximal part of its axone is undamaged and there is no trace of retrograde Marchi degeneration. *a*, a^1, *b*, b^1=spinal ganglion cells; f.g.=fasciculus gracilis; *f.c.*=fasciculus cuneatus; *A*, A^1=cells of the cuneate nucleus; *B*, B^1=cells of the gracile nucleus. (From Glees *et al.*, *J. Neurol., London* **14**, 281, 1951.)