

OBSERVATIONS ON THE MINUTE STRUCTURE  
OF THE CORTEX OF THE BRAIN AS RE-  
VEALED BY THE METHYLENE BLUE AND  
PEROXIDE OF HYDROGEN METHOD OF  
STAINING THE TISSUE DIRECT ON ITS  
REMOVAL FROM THE BODY.

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I.—CONCERNING THE TECHNIQUE OF THE METHOD.

SINCE the preliminary notice of this process, which appeared in the Autumn number of *BRAIN*, 1900, I have been engaged in making further trials with it, from the results of which it appears as though the reaction is even more capricious and uncertain than I then thought, and that for its success other conditions must be complied with beyond those specifically mentioned in my first account.

A series of trials, closely following those, the results of which I published, on a dozen or more different brains, nearly all resulted in complete failure, no trace of the reaction being obtained except once in the case of a cerebellum, and this was very good.

During this time the weather had been dull, and the bottles containing the tissue to be stained had been placed far from the window of the room they were in.

As nearly all the successes happened during the summer months, it occurred to me that perhaps heat or light had something to do with the matter.

So far although not able altogether to eliminate the effects of heat in aiding the reaction, I think from the trials I have made that at any rate it takes a very subordinate

part; but as regards light this seems to be essential. After many failures, using different proportions of peroxide of hydrogen, different solutions of methylene blue, and keeping the tissue at different temperatures I have obtained a success or modified success in a certain number of pieces which have been kept fully exposed to the light, and perhaps the modified nature of the success is to be ascribed to the season of year, and the extremely dull weather that has been the rule during the time these trials were made.

In November, December and January, during which period these experiments took place, the active properties of light is at its lowest, and very likely as this increases in the summer months the results will be found to get correspondingly better.

The series of trials to show the effect of light were made on pieces of tissue from twenty-four brains; and they were put into a mixture of a 1 per cent. solution of methylene blue—four parts, a 10 per cent. solution of peroxide of hydrogen—one part, and left for nine to eleven days against a window and not interfered with. Of these fourteen turned out more or less successfully, but only five or six were really good, and most of these were pieces of cerebellum.

That very cold weather has a deterrent effect on the process is rendered probable by the fact that during the late cold weather in February and March not a single success was obtained in nearly thirty specimens of different brains, but later on as the weather became warmer, using the same solution and under similar conditions, successes were again the rule.

One other point must be noted—I have never got the reaction in the brains of animals freshly killed or human brains very shortly (a few hours) after death. It seems that a certain stage of decomposition in the tissue is necessary before it takes on the stain in the required way.

As lactic acid is produced during this decomposition I have lately added four to eight drops to a pint of the staining solution, but although some good results have been obtained after this addition, it is yet too early to speak definitely as to the desirability of this addition as a routine practice.

Further trials since the above was written seem to confirm the idea that the addition of lactic acid is desirable. I even add as much as three drops to 40 cc. of the staining mixture (the amount usually employed in individual cases). This addition renders the tissue more brittle—but in cases where pieces of tissue were placed in this strongly acid mixture and others from the same brain in a very faintly acid solution, whilst the former were successful the latter were not, other conditions being the same.

In the previous notice of the method, reference was made to the partial character of the reaction, and it was mentioned that only little patches of the tissue show it, and the first layer and superficial part of the second never stain successfully. To this must be added the fact that in the cerebellum with very few exceptions the reaction occurs at the bottom of a sulcus and usually gets less marked towards the top of the sulcus, and although the granules are deeply stained, yet in this layer there is never any sign of the reaction. No appearance of the processes of the granule, cells, &c.

It is also in the deeper or interior parts of the block of tissue that as a rule the best sections are got, sometimes indeed when the first few outside sections of a block show scarcely any reaction, those more in the centre will show it well.

In passing I may remark, as it may have some bearing on the question as to the nature of the small darkly-stained structures in the cortex, whether they are of nervous or neuroglial origin, that the latter form of cell in the medullary substance of the cortex are often seen, both in the cerebrum and cerebellum, but they stain of different colour to the nerve-cells and processes. Both the cell body and processes are of a somewhat pale reddish tinge.<sup>1</sup>

In the places where the re-action occurs fully the process probably brings out greater detail of structure than any

<sup>1</sup> Occasionally the neuroglial fibres of the first layer in pathological conditions stain clearly as dark, smooth, wavy, or crenated fibres. A similar appearance is sometimes seen in the cortex cerebelli when the radial or Bergmann's fibres stain as above noted, and appear very similar to the basket fibres.

hitherto known method, for it is quite equal to gold, silver or mercury in picking out the fine ramifications of nerve fibres, but further than these it appears to differentiate between different nerve-cells, so that while the pyramidal system and the Purkinje cells are stained faintly others are very darkly stained. The latter, which are further distinguished by size and shape, would appear as though in ordinary methylene blue staining they are not shown at all or probably only their nucleus is stained, for we see in unsuccessful preparations of cortex stained by the peroxide method that there is no trace of them to be found, whilst in slightly successful pieces one of the first indications of the reaction is the picking out more or less clearly of these cells.

While the opaque character of the impregnation with silver or mercury more or less obscures all detail in and immediately around the cell, the present method, even in the case of the darkly-stained cells, allows fully the interior and surrounding detail to be seen.

And finally, another advantage of the method is that it appears to be permanent, for some of the sections I have now before me have been kept for over two years and show no deterioration.

The foregoing remarks regarding the technique of the method must be taken as representing conclusions only provisionally arrived at, and one reason for publishing these observations in what may be regarded as a premature state is that others may be induced to experiment with the complicated conditions necessary for success in this very elusive process, a process, however, which gives promise of being of considerable service to those engaged in the study of the structure of the nervous system in health and possibly also in disease.

Another reason is that as the specimens I have show clearly the appearances on which my deductions are founded, I think it advisable to publish the results without waiting for further developments in the method; for although as mentioned before, so far the sections appear permanent it is yet possible they may deteriorate in process of time, or get

damaged, and with the uncertainties of the method in view, I may not be able to replace them by others as good.

The following summarises the steps in the process as at present adopted :—

(1) An old-standing 1 per cent. solution of methylene blue (Grubler's pat. b. x), kept exposed to light and air till its *pithy* smell has disappeared. Probably the addition of four to eight drops of lactic acid to the pint is an advantage.

(2) A mixture of four parts of this to one of a 10 per cent. solution of peroxide of hydrogen. The pieces of tissue are placed in this mixture in a fairly capacious bottle, and kept exposed to light, and if very cold weather at a temperature of 25° to 30° C. for eight or nine days.

(3) Twenty-four hours or more in 10 per cent. molybdate of ammonia.

(4) Washed in running water about twelve hours (all day).

(5) The following evening and night in several changes of absolute alcohol.

(6) The next morning through several changes of xylol for two hours.

(7) Two hours in paraffin bath.

Following these instructions, in the summer time under favourable conditions of light and temperature, the successes will probably be in the proportion of nine or ten to one failure; whilst during the winter and dull weather the reverse will be the case—only one success to about nine or ten failures.

In the following pages I propose to consider briefly the structures shown by this method in the cortex of the cerebrum and cerebellum, and to suggest what seems to me to be the most probable inferences to be drawn therefrom. In the description given of the molecular layer of the cerebellum I am quite aware that the main features noticed will be familiar to workers in Golgi's method; but even so, they appear worthy to note, inasmuch as this latter method has, I believe, been chiefly limited in its application to the cerebellum of animals or human embryos,

and therefore, at the least, the results I get are a corroboration of those obtained by Golgi's method; especially as it has been contended that the so-called staining of this latter process is due to impregnation about the nervous structures of precipitated metal in a fine state and not to a true staining of these structures.

Of course the limitation of the stain, in my method, to the molecular and Purkinje cell layers is a serious drawback to its utility in tracing the further course of nerve-fibres.

## II.—CEREBRUM.

This method reveals around the pyramidal and giant-cells of the cortex a delicate beaded network (see figs. 1, 2, and 3) loosely enveloping the cell-body and its apex and processes, and extending along the latter as far as it is ever possible to trace them in sections. In some specimens the beaded network can be clearly seen enveloping like an open-work stocking the dendrites of the giant-cells for a distance of 300  $\mu$ . The beads on this network stain, as do the fibres, nearly black, and vary considerably in size and shape, some being round, others of irregular contour, and others ring-like. The large irregular beads sometimes appear as if two or more small ones had blended together. The usual size of these beads is about 1  $\mu$ , but large ones are met with of 2 or 3  $\mu$  in diameter. Where the branching of the network occurs, the fibres always appear to spring from a bead, and not from intermediate portions of the fibril. The distance apart of the beads varies considerably; over some of the giant-cells, where they are generally large in size, they may be clustered very closely together, but a distance of several  $\mu$  usually separates them. Now, this network does not appear as a closed network drawn over the cell; but presents on all sides tags formed by beaded fibres, passing off from it into the surrounding matrix (see figs. 2 and 3), and it is not unfrequently possible to trace some of these fibrils into the stouter fibres, which have all the appearance of being dendrites from neighbouring cells;

also, in like manner, branches can be found which give off fibrils to supply the network of two adjacent pyramidal cells (see fig. 5).

*I wish to emphasise the statement that fibres coming off from different parts of the network can be traced running in different directions in the surrounding matrix, and in some cases can be traced to the thicker branches, evidently proceeding from different cells, because it implies that the cells from the branches of which the network is ultimately formed are in organic continuity one with another by means of this network, and this holds good whether, as some think, the network is an extension of the collaterals of other cells, or whether, as I think, it is an extension of the dendrites of the small dark cells. This beaded network is apparently a very persistent structure, and is still found in brains which are the seat of extensive pathological changes, e.g., in the brains of general paralysis and dementis.*

Two varieties of cells are distinguished by this method—pale and dark. A cursory reference was made in my former communication to this fact, and it was pointed out that whereas the cytoplasm of the majority of the cells (pyramidal and giants) stains very lightly others are picked out which stain very darkly.

The cytoplasm of the pyramidal and giant cells stains uniformly and very lightly, so that the apex and dendrites are not prominent features and can only be discovered near the cell body. The nucleus is plump and consists of darkly stained granules closely packed and contained in a definite and darkly stained membrane. The nucleolus as a rule is paler than the nucleus and indistinctly outlined.

It will be seen that this description applies also to pyramidal cells stained by the intra-vitam method.

Amongst the pyramidal cells we meet with instances of dark staining, more especially among the giants, but the infrequency with which these occur in proportion to the numbers which are lightly stained points to a pathological cause for this difference.

And furthermore, one always meets with more dark-stained pyramidal cells in brains which have undergone

obvious pathological changes than in brains from cases of recent insanity.

Although as just stated in the pale-stained pyramidal cells the dendrites are a very insignificant feature and can rarely be traced far, yet in the brains of certain demented of long standing we find many of the pyramidal and giant cells very deeply stained and showing a great wealth of dendrites which are irregular in contour and can be traced for long distances. Now these processes, both apices and dendrites, appear to give off nearly at right angles numerous very fine fibrils.

Even although we grant that this dark coloration of the cell is pathological, this does not alter the significance of the fact in showing the extensive ramifications of the cell branches, for it will scarcely be admitted that a morbid process will have any marked effect in the production of this extensive ramification, or in causing any decided change in its general configuration. We must admit, I think, that the processes thus shown are normally present although only occasionally stained.

One point must be borne in mind, when the cell and its processes are deeply stained the fine investing network is naturally obscured, and I think that quite possibly the irregularity noticed in the dendrites is due to this investing network and that some at least of the fine fibrils mentioned as passing off from these dendrites are in reality fibrils *coming to* them to join the network.

In deeply-stained giant-cells sometimes the axis cylinder can be seen passing off as a darkly-stained, perfectly smoothly outlined fibre, but which, after passing 50 or 60  $\mu$  from the cell, loses its colour and appears as a smooth, very pale strand; and these stout pale strands representing axis cylinders are generally seen running in the lower part of the cortex.

The dark cells of the cerebrum present considerable diversity in shape and size, but the great majority are between 20 and 24  $\mu$  long and of a more or less spindle shape, although not unfrequently one meets with square or pentagonal forms (see figs. 4, 8, 9).



While the cytoplasm and its extensions stain deep blue, the nucleus appears as a homogenous and black mass. The processes of these cells, which are given off all around the body, spring from it at once as comparatively slender branches which can often be traced for 200  $\mu$  or more. They do not branch much as a rule (I am not referring now to the very delicate threads given off all along nearly at right angles to the main stems), and generally begin as shaggy fibres, passing, as they get finer, into moniliform or beaded fibrils.

It is from the dendrites of these cells that I believe I have been able in several instances to trace directly the beaded fibrils of the network about the pyramidal cells.

Apart from the shaggy processes above referred to, sometimes one sees a perfectly smoothly contoured fibre of small calibre passing off from the cell, but in all cases where I have seen this smooth fibre it could only be traced a short distance before passing out of the plane of the section, or perhaps (as with the axis cylinder of the great cells) losing its colour. As very often this smooth fibre is given off from the upper part of the cell and runs towards the surface of the section, it seems probable that some, at any rate, of these dark cells are Martinotti's cells (see fig. 9).

These dark cells are found scattered throughout the cortex from the second layer inclusive down to the medullary substance.

These dark cells are chiefly if not entirely limited to the region of the granule cells in the occipital cortex so far as my observations show. And they are clustered together in amongst the granules in greater number than I have met with them in either the frontal or ascending frontal cortex.

There is never any sign of a beaded network around them or their processes.

Briefly summarised, the following are the main features shown by this method :—

(1) That there are certain cells, the body and dendrites of which stain very lightly and their nucleus dark, granular, and sharply defined, and that the axis cylinder of these cells, when it can be seen, is smooth and of a very pale blue colour.

(2) That a network of fine beaded fibrils loosely invests these cells and their protoplasmic processes, and that beaded fibrils pass from this network at many different points, and that in some cases these can be traced joining to stouter, somewhat shaggy fibres.

(3) That there are certain darkly stained cells, generally small, often pear-shaped or pentagonal, and which give off fine dark-stained processes, which processes can often be followed for very long distances with very little branching.

The finest branches of these cells are beaded or moniliform, and appear very similar to the beaded fibrils of the network.

(4) These dark cells show no signs of any network around them.

Sometimes fibrils can be directly traced from these cells to the network. (This last observation must be separated from the former because before it can be accepted as a fact it will require further corroboration.)

The above, I submit, are four observations that can be verified by any one inspecting my specimens or preparing others by the same method, and from these I would draw the following inferences: First, as to the origin and significance of the beaded network. There are good reasons against the supposition that it is a supporting network of neuroglial fibres, for in the first place the neuroglial elements do not usually stain by this method, and when they do they stain of a rather pale reddish tint and their fibrils are not so distinct or so dark,<sup>1</sup> and in the second place Golgi's method shows in the ending of certain peripheral sensory nerve fibrils just such a beaded appearance as is seen in the network, and no process that I know of has ever shown anything like a beaded appearance of the neuroglial fibres.

Excluding then the neuroglia I think we may fairly certainly assume that the network is formed by the processes of nerve-cells.

As to their being the extension of the collaterals of axis cylinders we must bear in mind that the axis cylinders, as seen in the lower part of the cortex by this method, appear

<sup>1</sup> See foot-note, page 240, for exception to this statement.

as very pale blue and smoothly contoured fibres, and collaterals are not certainly stained at all. By this I mean I have never seen a dark collateral given off from one of the pale axis cylinders, although of course it is possible that the ultimate terminations of the collaterals may stain differently from the same fibre at its origin from the axis cylinder.

As to its being an extension from the dendrites of the pyramidal cells, these usually are very pale and soon disappear from the plane of the section, and although they sometimes colour deeply and can be traced for long distances, it is not only in these cases, as we should expect if they gave origin to the network that this latter shows, but even when the pyramids and processes do not colour at all.

Now in favour of the assumption that the network is formed by extension of the dark cells we have the fact that the processes of these cells always stain deeply and of the same colour as the network, and in all physical characters, they appear similar to the fibrils of the network.

And finally, I believe that in several cases I have been able to trace a fine beaded fibril coming from a dark cell directly to a network, so that the balance of evidence seems to be in favour of this network being as it were an extension of the ultimate fibrils proceeding from the small dark cells.

If we accept the above interpretation it must lead to considerable alteration in our conception of the arrangement of the nervous elements in the cortex of the brain, *for it implies that there are at least two systems of cells present, the pale or pyramidal variety, and the small darkly-stained variety, and these latter branch in all directions and their dendrites ramify and ultimately form a network about the former, and the whole system of dark cells is in absolute continuity one with another through the medium of the network.*

### III.—CEREBELLUM.

Figs. 9 and 10 give a general idea of the appearance of the cerebellum as shown by this method.

In fig. 9 chiefly the small cortical cells (Shaffer's cells)

are shown, and although numerous delicate fibrils are seen given off from them and interlacing in all directions, yet the photograph gives but a very poor idea of the wealth of fibrils actually shown in the sections, and to see which it is necessary to shift the focus.

In fig. 10, more highly magnified ( $\times 400$ ) the lower half of the cortex only is shown, and the dark cells seen are chiefly basket cells. In the lower border of the figure there is a Purkinge cell enveloped in its sheath of basket fibres.

The Purkinge cells, as a rule, stain lightly, and show no trace of chromophilic material; the nucleus is dark and granular, and with a sharply defined border; the nucleolus varies, but is often pale and indistinct.

Variations in this way of staining have been pretty frequently met with, but as I am dealing with pathological material, it seems probable that these variations are due to morbid conditions, and I have taken as normal the form most frequently met with in the young and in recent cases of insanity, and which is most nearly allied to the staining obtained by the *intra-vitam* method. In the majority of cases the branches of the Purkinge cells either do not stain or else stain very lightly, but occasionally these also are dark, and indeed sometimes darkly coloured branches are seen originating from a cell with pale body. Of course it is only when they are darkly stained that we get a view of the great wealth of branches given off and showing then as a rule, little lateral projections or thorns (figs. 12, 13, 14).

Even if the supposition is correct that a dark colour implies a pathological condition of the cell and processes, this does not necessarily mean that thorns are a pathological feature, but that under normal conditions they do not take on the stain.

The ramifications of the Purkinge cells extend quite up to the surface of the layer, and the main stems do not bear thorns, only the terminal twigs.

These thorns take the shape of a short delicate lateral fibril ending in a knob. I find that they are of common occurrence when the branches from which they spring stain deeply, and when met with they occur all along the processes,

and not one here and there, although Obersteiner says ("Anatomy of Central Nervous Organs" translated by Hill, edition 1900, p. 405), speaking of Golgi's method:—"In perfectly successful preparations of the brains of adults no such excrescences appear, only a granule here and there is seen attached to the processes of an isolated cell."

In the case of a man 68 years old, suffering from melancholia, although there was very manifest atrophy of the branching fibres of the Purkinje cells, yet the lateral projections with knobbed ends were very distinctly shown (fig. 12). The atrophy referred to is not a necessary accompaniment of age, as in another man of 68, the branches were as plump and broad as usual.

In the lower half of the molecular layer in sagittal sections (across the direction of the foliæ) are seen a number of stout (1 to 1.5  $\mu$ ), smoothly contoured, wavy or crinkled fibres, which run for the most part parallel one to another and to the surface of the layer and stain deeply. Not unfrequently one of these fibres bends back abruptly and runs in an opposite direction.

These fibres originate (in part at least) from small (12 to 15  $\mu$ ), oval or pear-shaped cells lying in amongst them and usually with their long diameter in the direction of the fibres. These cells stain deeply, and for this reason it is often difficult to distinguish the nucleus from the cell body. Besides the stout, smooth, parallel fibre, which is said to be the axis cylinder and which passes generally from one end of the cell (and sometimes apparently one from each end), other branches are given off. These generally divide near the cell into very delicate fibrils, which may appear to end shortly, as for example when they run in the direction of the granular layer, or else as when they spring from the upper side, may often be followed for very long distances without further branching. Some of these long, fine branches running upwards can be traced to within a few microns of the surface of the layer and there double back and run down in an opposite direction, traversing in their downward course considerably more than half the width of the layer, so that altogether these delicate processes

can sometimes be distinctly traced for the surprising distance of over 400  $\mu$ .

The parallel fibres give off as they run along, branches at right angles, which, for the most part, run towards the granular layer; sometimes a branch given off from the upper aspect of the fibre (fig. 16) bends down and runs inwards to form the basket work around the Purkinje cell.

Often one is able to directly trace the passage of a parallel fibre or its branch into the basket work and this structure loosely envelopes the cell and the fibres of which it is formed meet together at some little distance below the invested cell.

As a rule the fibres going to form these baskets widen out and appear flat and tape-like in their passage over the cell (fig. 15).

A number of branches coming from different parallel fibres often meet together in a dense mass as they reach the cell (fig. 17).

In the great majority of instances the fibres of the basket work run over the cell in the same direction as its long diameter, but occasionally transverse strands are seen (fig. 16).

Sometimes instead of a right angle branch of parallel fibre running to form the basket work the fibre itself ends by turning down for this purpose (fig. 17).

The flat tape-like looking fibres often attain a width of 2 or 3  $\mu$ , and generally speaking, the whole structure of the basket work is made up of coarse fibres which very loosely envelope the cell. I particularly mention this as Obersteiner (p. 406) speaks of these fibres as "brushes of extremely fine fibres which fix on or closely encircle the aforesaid (Purkinje) cells," although the translator of this work (Dr. Hill) correctly figures the structure in his work on the Golgi method, and it is correctly figured in the latest edition of "Quain's Anatomy."

The basket fibres often envelope the branches of the Purkinje cells some distance from the cell body before passing down to form the basket work proper.

Apart from the above-described system of stout enveloping

fibres, there is, both around the Purkinje cell and its main branches, a network of delicate beaded fibrils, very similar in appearance to that seen about the pyramidal cells; whilst, however, it is only occasionally that these delicate fibrils can be detected over the body of the cell, they are comparatively frequently met with over the branches.

Beaded fibrils can also be seen coursing about in different directions in the surrounding matrix, but generally only in the region of the parallel fibres. I have not been able to determine where these beaded fibrils come from; sometimes they seem to be delicate fibres passing off from a basket fibre. Probably, I think, some at least originate from the small cortical cells higher up in the layer, for these generally present a decided but slightly beaded aspect.

W. Aldren Turner and W. Hunter ("On a Form of Nerve Terminations in the Central Nervous System demonstrated by Methylene Blue," *BRAIN*, vol. xxii., pp. 123-134) failed by the intra-vitam method to detect this delicate lattice work in the cerebellum; and, as by this method, the basket work is also not shown, they argue that this latter is not a nerve arrangement, but neuroglial. I think, however, the fact that it is stained by the present method in such a distinct way and that it can be traced as arising from the darkly stained cells lying in this region will not permit us to accept this view; especially, as the neuroglial elements do not usually stain, and, when they do, they take on a quite distinct colour to that assumed by the nervous elements.

The remaining part of the molecular layer (that is, the outer half), beyond the expansion of the Purkinje cells already alluded to, is occupied by a number of extremely small (9 to 11  $\mu$ ) very dark cells of different shapes (triangular, square, oval, pear-shaped, &c.), which give off numerous delicate branches on all sides, forming an intricate network in this region. These branches are almost always moniliform or beaded, but the beads are generally only very slight swellings on the fibre, and not nearly so clearly defined as those seen in the cerebrum. The cells are most thickly congregated in the middle third of the layer, but they are met with lying quite close to the surface and also down

among the basket fibres near the granular layer, and it is often very difficult or even impossible in this region to distinguish them from the basket cells; the main points by which we can most certainly differentiate them being the rather larger size and more regular disposition of the basket cells, and the fact that it is these cells only that give off stout smooth fibres running in a parallel direction.

Fig. 10 gives some idea of the relative number and variety in shape of these small cells. Their processes can often be followed for long distances, but their ultimate destination cannot with any certainty be made out, although unquestionably they must be brought into very close relationship with the thorny branches of the Purkinje cells amongst which they lie.

Briefly summarised, the results arrived at from the examination of the cerebellum by this method are as follows:—

(1) It has a selective influence in regard to the staining of the different nerve-cells, some (the Purkinje cells) being very lightly stained, and others (small cells) very deeply stained.

(2) The dark cells can be subdivided into those which certainly envelope the body of the Purkinje cells by their branches and those which, although one is not able with certainty to trace their branches to their destination, there are grounds for supposing invest the branches of the Purkinje cells with a fine beaded network.

In conclusion, I will refer briefly to some inferences that I have been tempted to draw from the foregoing observations.

It seems only reasonable to suppose that the difference in colour, size and shape, &c., of the cells we have met with both in the cerebrum and cerebellum is associated with a difference in their function, and as we have considerable evidence in favour of the pyramidal cells being concerned with efferent impulses. I would suggest that the others—the small dark variety—are concerned with afferent impulses, that in a sense they serve as collectors of ingoing currents



which, after passage through them, are distributed to the pale cells with which they are in association.

Although there is considerable difference in the size of the dark cells of the two regions of the brain examined, yet, as they both react similarly to the methylene blue I consider that this, taken along with certain morphological similarities before referred to, will be some justification in associating them together for the purposes of comparison. Now we find that the proportion of dark to pale cells is very different in the cerebrum and cerebellum. In the former they are few in number relatively to the pyramidal cells, in the latter they far outnumber the relatively few Purkinje cells.

Thus we appear to have in the cerebrum a condition the reverse of that found in the cerebellum. In the former there are numerous efferent cells in relation with comparatively few afferent elements, in the latter few efferent in relation with many afferent.

The interpretation that I have given to these facts harmonises with Spencer's theory that the cerebellum is the great organ for co-ordinations in space, while the cerebrum is the organ for co-ordinations in sequence.

#### DESCRIPTION OF FIGURES.

FIG. 1.

Outline traced from a photograph shows the beaded network over a giant-cell. A beaded fibril is seen passing from (? to) the cell from the upper part of the figure, another from the right-hand side. The size and shape of the beads vary; they are generally about  $1\ \mu$  in diameter, some 2 or even  $3\ \mu$ . Their distance apart also varies from  $1.5$  to  $8\ \mu$ . The nucleus of the cell is shown in the lower part of the figure ( $\times 700$ ).

FIG. 2.

Pyramidal cell with dense network of beaded fibrils encompassing it. Fibrils are seen passing off from the network in all directions. Although the nucleus of the cell is shown, yet in the specimen, when it was in focus, the network over the near surface of the cell was thrown completely out of the field of vision ( $\times 600$ ).

FIG. 3.

Outline, traced from a photograph, shows the network extending along the branches of the pyramidal cell. Coarse fibres can be seen at *a* and *b*, from which delicate fibrils proceed to join the network ( $\times 700$ ).

FIG. 4.

Traced from a photograph, shows a small dark cell, with thin, slightly varicose processes given off from it ( $\times 400$ ).

FIG. 5.

Shows a dark, stout fibre, dividing and giving off from each division fine beaded fibrils to two pyramidal cells. A stout, dark fibre runs up to the left side of the upper pyramidal cell, and apparently also helps to form the investing network ( $\times 600$ ).

FIG. 6.

Also shows a stout fibre dividing and giving off one fine branch to the network about the pale cell shown, while the other passes upwards, probably on its way to another network. This fibril also shows beads on it ( $\times 600$ ).

FIG. 7.

A pale cell encompassed by stout fibres, from which fine beaded fibrils are given off to pass over the cell ( $\times 600$ ).

FIG. 8.

A small, dark, pyriform cell, giving off only a few branches from either end; the lower ones appear varicose and can be followed for a long distance without further branching ( $\times 600$ ).

FIG. 9.

A dark cell with ascending axis cylinder (*a-c*) which gradually gets paler in colour as it passes upwards. Note its smooth contour. From a stout process a fine fibril (*x*) passes off, along which are little irregular swellings, closely resembling the beaded fibrils of a network. The figure is traced from the photograph which was reproduced in my article in the autumn number of BRAIN ( $\times 600$ ).

FIG. 10.

Molecular layer of cerebellum with small cortical and basket cells ( $\times 300$ ).

FIG. 11.

Basket cells and fibres with a Purkinje cell invested by the latter ( $\times 400$ ).

FIG. 12.

Darkly-stained Purkinje cell branch with lateral processes showing thorns. Note the atrophy of the branches ( $\times 600$ ). From a man aged 68 years.

FIG. 13.

Darkly stained Purkinje cell branches with thorns, also a small cortical cell whose branches are shown intermingled with the thorny processes. From a man aged 41 years. Note the stout character of the branches compared with those in the previous figure ( $\times 600$ ).

FIG. 14.

From the same as the last, showing thorns ( $\times 600$ ).

FIG. 15.

Basket fibres spreading out over a Purkinje cell into flat, broad, tape-like strands. Some seem to subdivide longitudinally ( $\times 600$ ).

FIG. 16.

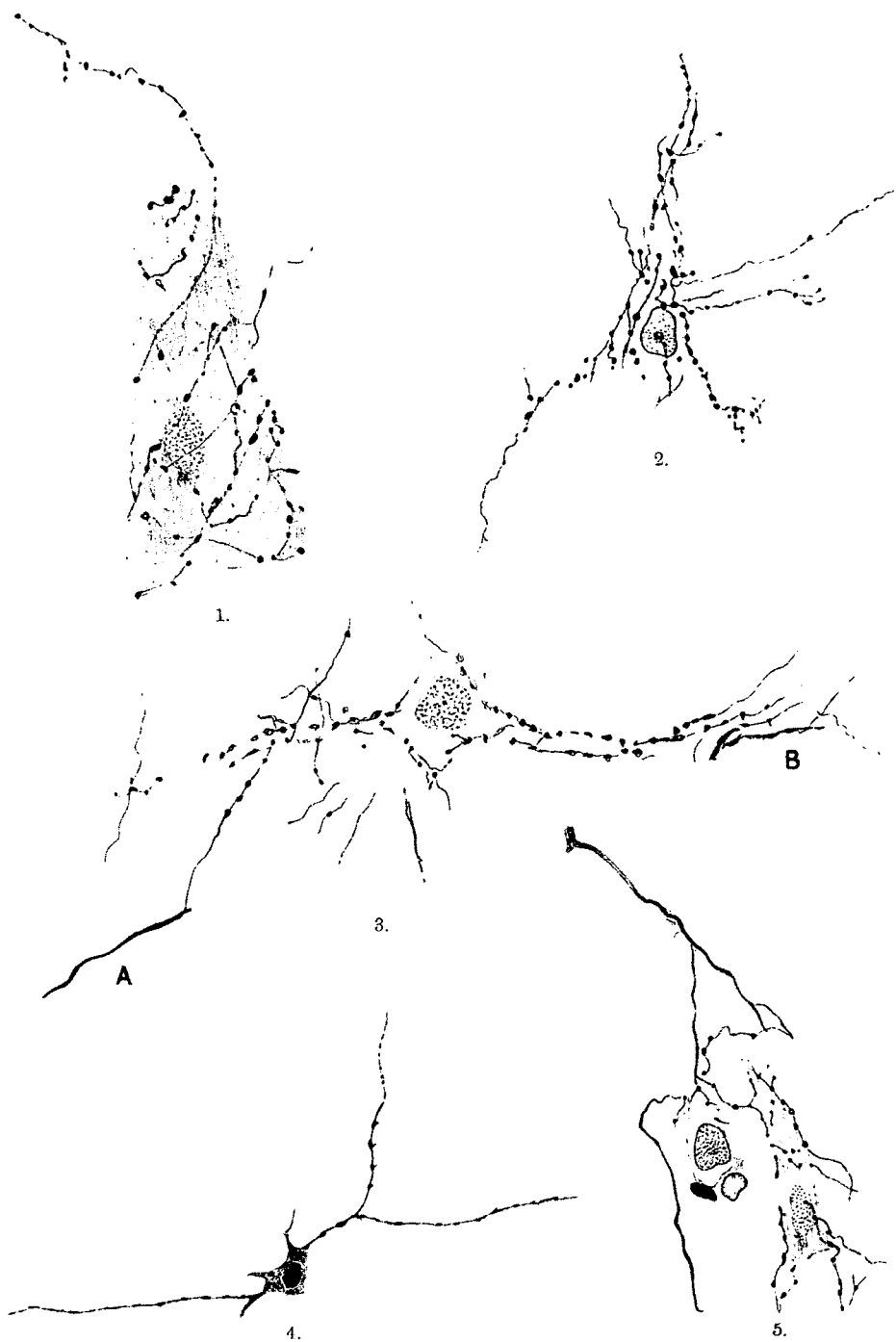
A basket fibre, shown in upper part of the figure, which gives off from its upper side a branch which bends down and goes to help form a basket arrangement around the Purkinje-cell shown in the lower part of the figure. Note the transverse strands and the basket fibres meeting below the Purkinje cell some little distance ( $\times 600$ ).

FIG. 17.

A basket fibre is seen to terminate by bending down to assist in forming the basket work around the Purkinje cell; other fibres are seen coming from other directions and meeting together in a dense cluster to one side of the Purkinje cell, which shows the almost colourless cytoplasm and dark nucleus referred to in the text ( $\times 600$ ).

FIG. 18.

Shows fine beaded fibrils similar to those seen in the cerebrum. Some of these can be seen passing over the Purkinje cell which is just indicated by its slightly darker colouring above the granules ( $\times 600$ ).



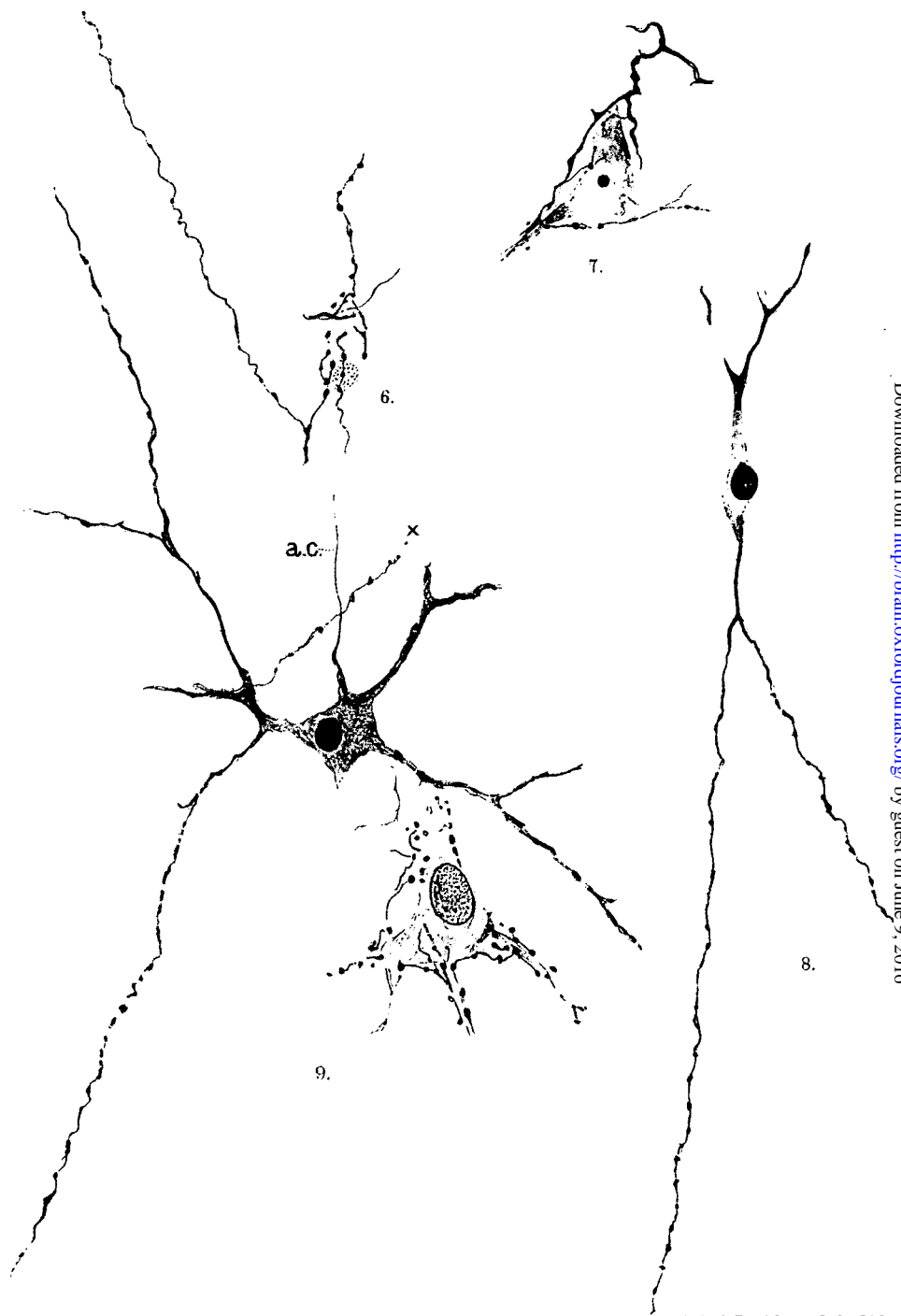




FIG. 10.

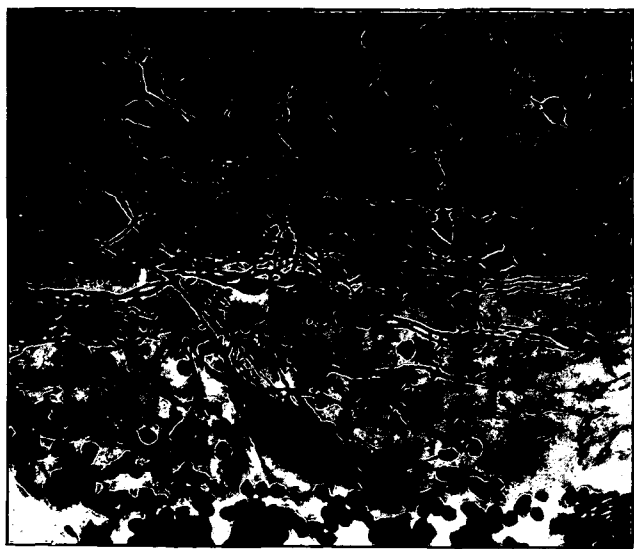


FIG. 11.

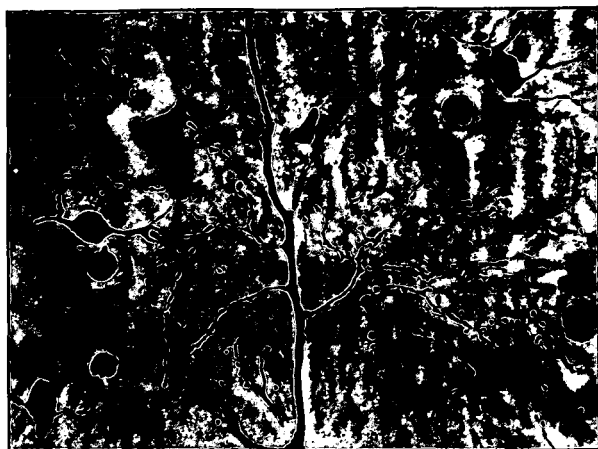


FIG. 12.

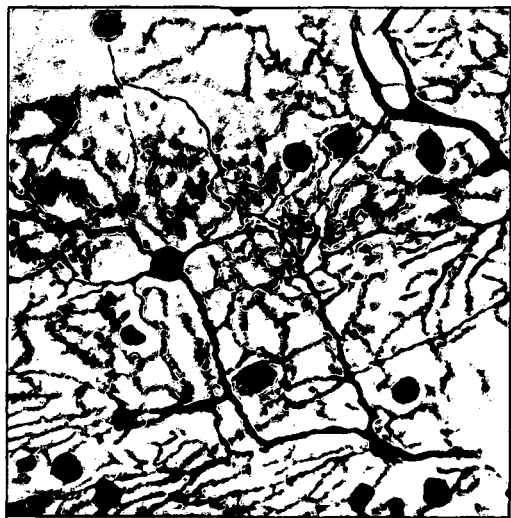


FIG. 13.



FIG. 14.



FIG. 15.

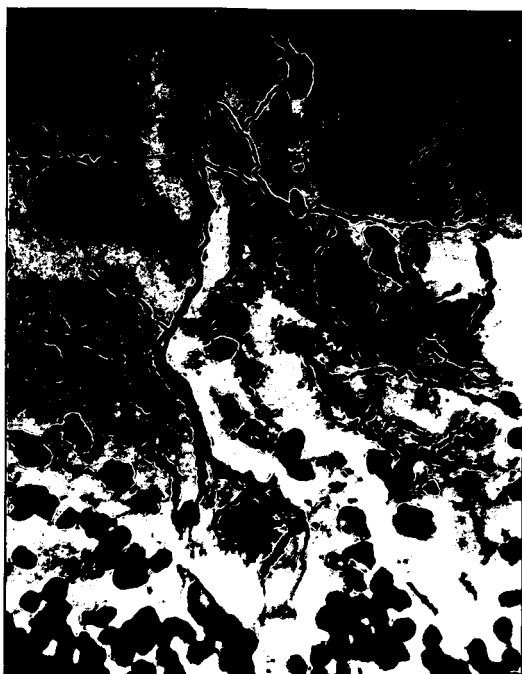


FIG. 16.



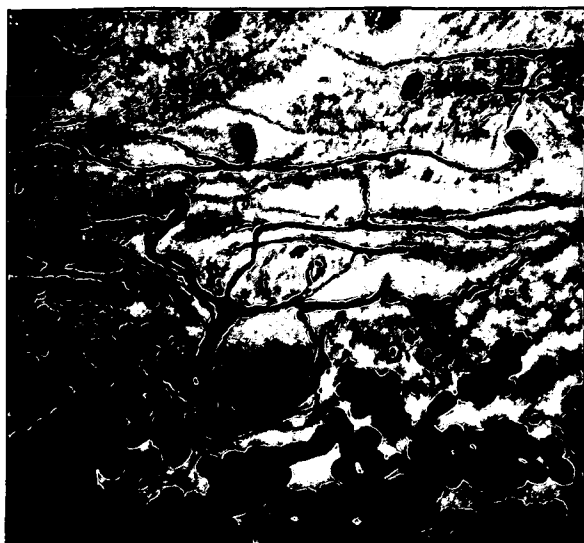


FIG. 17.

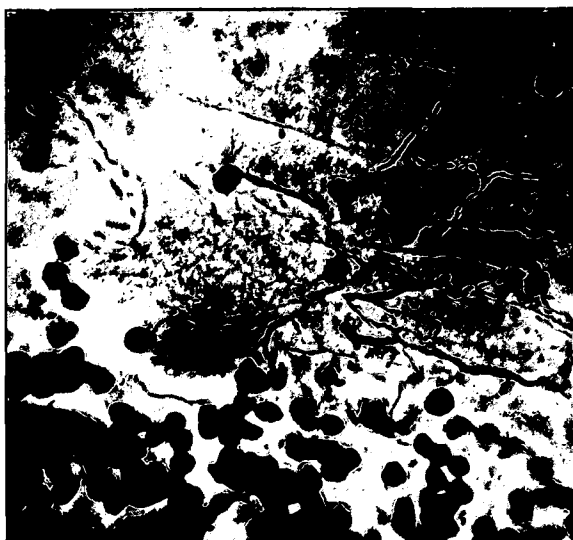


FIG. 18.