

Anatomy. — *Neuropilem and gliareticulum in the cortex of the cerebrum.*

By S. T. BOK. (Communicated by Prof. M. W. WOERDEMAN.)

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The investigation by R. C. RENES¹⁾ concerning the abundance of fibres in the cortex of the cerebrum has brought to light that everywhere between the different cells and vessels of the cortex there is the same total length of nerve fibre per spatial unit (except in those places where the myelin sheaths are numerous and so occupy an appreciable part of the space). In other words the neuropilem is equally dense everywhere in the cortex.

This makes it clear how it is that cortex areas, in which there occur mainly small nerve cells, are much richer in nerve cells than areas with larger nerve cells (by which we mean that in areas with small cells the number of nerve cells per spatial unit — the density of the nerve cells — is greater than in areas with large cells). For generally a larger nerve cell has a larger dendrite complex with a greater total length of dendrites than a smaller cell and because the richness in fibres is the same everywhere, this greater length of dendrites requires more space, so that the space between the nerve cells must be greater as those cells are larger.

Meanwhile RENES' investigation has not yet made it clear through what cause this distribution of fibres is the same everywhere in the cortex.

The constancy found in the length of the nerve fibres per spatial unit suggests that everywhere the fibres have reached their maximal development, that, as it were, the cortex is everywhere filled to the greatest possible extent with nerve fibres. But what is the factor, determining the maximum which brings to a close any further development of ever new branches between those existing already?

This is certainly not the mere fact that the space between the nerve fibres should have become too narrow to allow fibres of sufficient thickness to penetrate: in the BIELSCHOWSKY-preparation everywhere between the thin black-impregnated fibres there is a space which is averagely considerably more than ten times as great as the average thickness of those nerve fibres, so that certainly hundreds of times more fibres of the same size might find a place in the same space in the cortex.

RENES had investigated if, perhaps, the cause may be that there must always be a certain minimal distance between the nerve fibres. If such a minimal distance is indeed prescribed, the cortex space would be "filled up"

¹⁾ R. C. RENES, Over de vezelrijkdom in de hersenschors. Thesis Leyden 1940.

much sooner (then the maximal length of nerve fibre per spatial unit would be much smaller) than if the fibres are allowed to touch. For this purpose he has measured the distance between neighbouring nerve fibres (= the length of their common perpendicular) and in one respect his result seemed to confirm this idea.

He has namely made these measurements in different architectonic fields and in each field separately in layers II—VI (layer I is not sufficiently impregnated in the BIELSCHOWSKY-method). In each field a small area was selected in each of the layers and in each of those areas the fibre distance was measured in a hundred cases. It was seen that in each area examined these distances vary greatly in size, but in all areas their size varies between practically the same minimum (0.4μ) and about the same maximum (1.9μ). So in each field he found a certain minimal fibre distance and this minimal distance appeared to be the same in all the areas examined (for the mutual variations between these minima practically remained below the value of the error of measurement: the average error of the individual measurements was determined at 0.027μ and in the various areas examined the smallest of the distances found varied between 0.40 and 0.44μ , once it was 0.46μ). So it appeared that there is indeed a minimal fibre distance throughout the cortex, which was found everywhere, and within which no two nerve fibres cross each other.

But in two important points the results of his measurements are by no means in agreement with the theory tested.

In the first place the distribution of the various fibre distances was not in agreement with the idea that everywhere in the cortex there is the maximal quantity of nerve fibre which there might be if it was only limited by a minimal distance between the fibres. If this maximum should be reached it might be expected that in many places the fibres would cross at the minimal distance. It is true that greater distances would also occur owing to two fibres crossing at a certain place while they had already been fixed in place through neighbouring crossings, with minimal distance, but the greater distances would become less frequent as they became greater. A frequency curve of the different fibre distances found, would therefore coalesce with the zero line in values less than the minimal distance, at the minimal distance it would rise vertically to the top value after which it would slowly fall to the greater values.

The frequency curve made of the fibre distances found does not fulfil this expectation. After the minimal value of 0.4μ it does, indeed, climb steeply, to fall slowly after reaching the maximum, but the top lies too far past the minimal value (namely at 0.56μ) for this deviation to be attributable to errors in measuring. Only with an average error of measurement of 0.11μ the vertical truncation of the curve in these circumstances would have been flattened to approximately the shape found in the actual measurements. But repeated measuring of the same distance of intersection proved the error to have been no more than 0.027μ , so that

we are justified in attributing a real significance to the gradual climbing of the frequency curve between 0.4 and 0.56 μ . So the distribution of the measurements found is not in keeping with the idea of a maximal filling limited by a minimal distance between the fibres prescribed.

In the second place the value of the minimal distance is contradictory to this idea: if the fibres could not come closer together than the minimal distance of 0.4 μ , the total fibre length per spatial unit could be many times greater than the length found. Neglecting the thickness of the fibre and supposing all the fibres to run parallel (in order to obtain maximal "filling") then a simple calculation shows that per 1000 μ^3 of space there would be 14450 μ of fibre, that is 72 times the length found (200 μ). Owing to the thickness of the fibres this value will probably be a little less, but not much, because the average fibre thickness may be estimated at less than $\frac{1}{4}$ the minimal distance between the fibres, and the total fibre length per spatial unit would be less than 4 % smaller. A greater influence has the fact that the fibres do not run parallel but in many different directions. Owing to this factor the possible length will be a few times smaller than calculated above, but it will certainly be dozens of times greater than the one found in the cortex.

So RENES' calculations show that the "fibre filling" is the same everywhere in the cortex, but they do not throw light on the factors which keep the filling constant. In particular they deny the supposition that there would be here a maximum of fibres, limited only because they must not or cannot approach any nearer than to a certain constant distance. So the constancy of the fibre filling must be owing to some other cause. What cause can that be?

The reconstruction of a small fibre area (measuring $15 \times 15 \times 15 \mu$), made by RENES — a stereoscopic picture of which is given in his thesis — showed that the fibres are not distributed equally about the space, but that comparatively large open spaces are found, between which the fibres are packed as in thin membranes or in rods. These open spaces are of about the same size, the centres of two membranes facing each other are about $5\frac{1}{2} \mu$ apart. So it is probable that the occurrence of these open spaces limits the quantity of nerve fibres and the question arises if the constancy in the fibre filling may be a consequence of a constancy of these open spaces.

About the nature of these open spaces we can state with certainty that they do not consist of cell bodies: they do not contain nuclei. Moreover they are far too numerous for that: from the measurement given it would follow that there ought to be about 9000 in an area of $100 \times 100 \times 100 \mu$, whereas there are only 80 to 120 cells (glia and nerve cells together).

The order in size of these open spaces corresponds to the meshes of the glia reticulum as drawn by SPIELMEYER, by HOLZER, and others. The supposition is obvious that they are vacuoli, between which protoplasm walls or protoplasm rods form the bedding for the nerve fibres.

Thus far it has not proved technically possible to obtain an insight

through microscopic observation into the shapes of these vacuoli and protoplasm walls or rods. A purely theoretical deduction, however, reveals some remarkable circumstances, which suggest a very simple way in which the fibre quantity in the cortex would be limited to the measure found by these very vacuoli.

We may start from the provisional supposition that these vacuoli are non-coherent; then the protoplasm would lie between them in the shape of a froth, and then the above measurement of the froth chambers tells us, according to a calculation which will be given below, that the total edge length in this froth corresponds with the length of nerve fibres found (200μ per $1000 \mu^3$). If the vacuoli should be more or less coherent this agreement would still hold, if only the protoplasm walls were penetrated, as in a sponge, the protoplasm rods being left in the place of the original edges of the froth structure. The fibre filling found would then be determined quantitatively by the simple rule: in one protoplasm rod one nerve fibre.

In the calculation indicated above the open spaces were conceived as chambers in a protoplasm froth and the reconstruction showed that these chambers are about equally large. In a communication about the shape of froth chambers ¹⁾ I could determine that in a froth with about equally large chambers the latter approach the shape of a regular dodecahedron. For the calculation we therefore started from the conception that when we imagine flat planes between the open spaces these planes would be the walls of regular dodecahedrons. This conception only approaches the actual proportions, as has been explained in the communication mentioned, but for our purpose this approximation proves amply sufficient.

In the reconstruction the centres of neighbouring open spaces lie about 15 cm apart. As the enlargement is 27500, they are about $150000 : 27500 = 5.46 \mu$ apart in the preparation, so that the radius of the inscribed globe of the dodecahedrons may be calculated as $\frac{1}{2} \times 5.46 = 2.73 \mu$, from which follows the edge length as 2.46μ ²⁾ and the contents $114 \mu^3$ ³⁾.

Each regular dodecahedron is enclosed by twelve pentagons, meeting in $12 \times 5 : 2 = 30$ edges. In the cumulation 3 dodecahedrons meet in one edge, so that per dodecahedron there are $30 : 3 = 10$ edge lengths or $10 \times 2.46 = 24.6 \mu$ of edge length. Per $1000 \mu^3$ there are $1000 : 114 = 8.75$ dodecahedrons, giving a total of $8.75 \times 24.6 = 215 \mu$ edge length. In the same volume RENES found 200μ of fibre length.

Considering that the conception of a froth as an accumulation of regular dodecahedrons is only an approximation, this agreement is so great as to warrant the conclusion that practically there is as much fibre length in the

¹⁾ See these Proceedings, p. 1180—1190.

²⁾ From the radius $r = 1/20 a \sqrt{250 + 110\sqrt{5}} = 1.11 a$ or
 $a = 0.9 r = 0.9 \times 2.73 = 2.46 \mu$

³⁾ From the contents $c = 1/4 a^3 (15 + 7\sqrt{5}) = 7.663 a^3$
 $= 7.663 \times 2.46^3 = 114 \mu^3$.

neuropilem as the edgelenh would be, if the cortex was a froth with a chamber size agreeing with the data of the reconstruction.

As the distance between the nerve fibres has been measured, it is attractive to use the froth structure with averagely 8.75 chambers per $1000 \mu^3$ as a provisional model, and to ask ourselves what would be the aspect in that model of the crossing of the fibres, of which there is each time one in an edge.

In a froth four edges always meet in one point of junction. A nerve fibre which we imagine in one of those edges will lie in another of those four edges after passing that point: so it occupies two out of the four edges of that one point of junction. Hence in the two remaining edges there will be a second fibre, which passes the first in this point of junction. According to this model there can only pass two fibres through the same junction, otherwise there would be more than one nerve fibre in one rod.

It is clear that the two fibres would intersect each other in this point of junction, if the edges as well as the fibres were mathematical lines without thickness. But in our model both have thickness. The mathematically conceived planes between the adjacent chambers discussed above served only to calculate the edgelenhs, in our model these planes lie in a protoplasm wall of a certain thickness and thus the abstractly conceived mathematical edges also lie in protoplasm rods with a certain thickness, while the junctions where four rods meet are also protoplasm areas of a certain spatial extension. If this extension is large enough as regards the thickness of the fibre the two fibres can cross here and leave a certain distance between them. Then they lie no longer accurately at the site of the mathematical edges, but a little by the side of them, at least in these junctions where they cross one another.

The four edges meeting in one point of junction in a froth, together form averagely equal angles, that is to say, they run from that point like the lines connecting the centre of a regular tetrahedron with its four vertices. If a nerve fibre was situated exactly in the mathematical edge that fibre would make an angle at each point of junction it passes (*viz.* one of averagely just over 109°). If we set out to draw in our model the shortest fibre between two far removed points, which fibre was everywhere to coincide exactly with the mathematical edge, then this would not be a straight line but a broken one, making the angle mentioned at regular intervals, but otherwise approaching the straight line mentioned. It is then seen that this broken line is wound screw-wise round a nearly straight axis (apart from the fact that it consists of a number of straight parts, not being curved, therefore, like a mathematical screw line).

Because the nerve fibres in our model — at least in the points of junction of the edges — should not coincide exactly with the mathematical edges, the possibility arises to truncate and round off the angle which each fibre would otherwise have to make at each point of junction. A fibre thus rounding off every angle, will by this fact be a little shorter than the total

of the mathematical edges it follows and the screw-like line mentioned will approach to a straight line. Maximal shortening would be obtained by such a fibre which would entirely flatten all its angles: such a fibre would be situated in the axis of the screw-wisely broken line of the corresponding mathematical edges. The question may be asked, what would be the distance between two such maximally straightened out fibres.

A purely mathematical treatment of this problem is not possible, as the froth chambers have not the exact shape of a regular dodecahedron. The property discussed, that regular dodecahedrons cannot be piled up exactly fitting, is the cause that the froth chambers must differ a little from regular dodecahedrons, and in the article mentioned on the shape of froth chambers it has been explained that on account of the surface tensions in these shapes, these deviations must a.o. consist in slight curvature of the walls and of the edges, which curvings differ from edge to edge and from chamber to chamber. Meanwhile it is possible in models of regular dodecahedrons to indicate, approximately at least, where these lines must be situated, and it is then seen that the distance of intersection between these axis lines in each point of junction is approximately 0.75 of the edge length. In our model, where the edge length represents 2.46μ , this gives a distance of intersection of 1.85μ and this is the maximal distance which these fibres can have with complete straightening out of their spiral course. This theoretically derived maximal distance of intersection of 1.85μ comes fairly near to the maximal fibre distance of 1.9μ , prevailing in the measurements, so that our model appears readily to account for the maximal fibre distance found by RENES.

Yet another consequence to be derived from the model agrees with a peculiarity of the fibres in the preparations, qualitatively as well as quantitatively.

If the maximal fibre distance was really determined by the above straightening out of a screw-like course to a straight one, then only those few fibres in which the maximal mutual distance of 1.9μ had been found would entirely have given up their screw-like course, while all the other fibres had not quite straightened out to a straight line. So the many fibres should have a screw-like course with a pitch prescribed by our model. When we follow those mathematical rods which wind round a straight line, it is seen that the axis line is again on the same side of those rods if along that axis line a distance has been covered which is a little smaller than the distance between two adjacent dodecahedron centres. According to the model the pitch should therefore be a little less than $2r$ or $2 \times 2.73 \mu$, so a little less than 5.46μ .

As a matter of fact most fibres in a BIELSCHOWSKY preparation do not run straight but with fairly regular undulations. In a fibre which lies about in the direction of the optic axis of the microscope, and which therefore, when the micrometer-screw stands still is seen as a black point (small spot), we see in most cases that this point moves in an approximately circular

direction when the micrometer-screw is turned: the undulation proves to be a consequence of the screw-like course. The pitch of this screw can be easily measured in those fibres which run parallel to the section plane. In the drawings RENES made to determine the length of the fibres, their undulation is plainly visible (see fig. 5 of his thesis) and from it the average pitch of 5μ was measured. This measurement also lies in the same order of size as the measure of a little less than 5.46μ theoretically derived above.

In order to get the nerve fibres in the junctions of the protoplasm rods to cross at the mutual distances found, these junctions must have special dimensions and the question arises what shape the protoplasm froth should have in connection with this requirement. When calculating this we are up against the uncertainty whether the nerve fibres can lie quite peripherally in their protoplasm layer, or if they have always to be surrounded on all sides by a layer of protoplasm of a prescribed thickness. On the ground of the minimal distance of intersection of 0.4μ , RENES already pronounced the supposition that every nerve fibre must be surrounded by a protoplasm sheath 0.2μ thick. Starting from this supposition, a calculation, which will be published in detail elsewhere, shows that in order to obtain the maximal distance towards the neighbouring one of 1.9μ the junction must rather exactly have the shape that would arise if the adjacent froth chambers were globular and just touching. Where, on the other hand, the chambers are more or less flattened by contact, the junctions would be such that the distances of intersection would have to be smaller than the maximal value of 1.9μ . The fibres crossing in these smaller junctions could not have a perfectly straightened out course. The variation of the distances between the fibres might therefore — in part at least — be based on a variation in the thickness of the froth walls (the protoplasm rods respectively). In this connection it is remarkable that already with very slight flattening of the froth walls (a very small penetration respectively) the protoplasm rods between the junctions would become too thin to contain more than one nerve fibre with its sheath 0.2μ thick.

So here the froth structure also acts as a factor determining that per protoplasm rod there is as a rule only one nerve fibre.

The supposition that the protoplasm of the cortex is constructed like a froth (or spongelike as a network of rods, taking the place of the edges in that froth), the chamber size of which corresponds to the dimension of the open spaces in RENES' fibre construction, produces consequences which qualitatively and quantitatively correspond to the following properties, which had come to light through the measurements taken of the nerve fibres:

1. The total length of protoplasm rods (froth edges) is practically equal to the total length of nerve fibre, viz. 215μ and 200μ respectively per cell-free cortex area of $1000 \mu^2$;
2. The distance between two perfectly straightened out fibres, crossing

each other in the junction of the protoplasm rods (1.85μ) practically agrees with the maximal value of the distance between neighbouring nerve fibres in the cortex (1.9μ);

3. According to the supposed froth or reticulum structure of the protoplasm, the fibres which are not entirely straightened out (the great majority) ought to describe screw-like lines with a pitch of not quite 5.46μ , while most of the nerve fibres in the BIELSCHOWSKY preparation do indeed follow screw lines, with a pitch of averagely 5μ ;

4. According to a calculation not included here for lack of space, the complete straightening out of the fibres with its corresponding fibre distance of 1.9μ could occur only in such places where the chambers are not flattened by contact with each other. If the chambers are a little larger (everything calculated with a constant mutual distance between their centres) the flattening (or penetration) of the frothwalls must be attended with screw-like fibres and smaller distances between the fibres. The protoplasm rods between their junctions will then also become so thin that they can contain only one nerve fibre with its layer of protoplasm 0.2μ thick. As we saw above, this is indeed the rule.

It stands to reason that the protoplasm froth or reticulum may be identified with the so-called glia reticulum, the reticulum of protoplasm rods, into which the extreme ends of the differentiated gliacells finally pass and which — according to the drawings occurring in the literature — agrees in order of size with the open spaces in the reconstruction of RENES.

According to the theory discussed in the above, the relation between the unmyelinated nerve fibres and the protoplasm in the cerebral cortex is the same as in the peripheral nerve system.
