SILVER STUDIES OF DEGENERATING THALAMO-CORTICAL CONNECTIONS

A. VAZ FERREIRA, M.D.
[Montevideo, Uruguay]
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During recent years, several investigators have used various silver-impregnation methods for the study of degenerating fibers and terminal endings in the central nervous system. Among them are: Miskolczy ('31, '34); Hoff ('32 a and b); Foerster et al ('33); Snider ('36); Gibson ('37 a and b); Rosiello ('37); Schimert ('38, '39); Szentágothai-Schimert ('41); Szentágothai ('42, '43, '48); Glees ('41, '42, '44, '46); Glees et al ('41, '46); Clark and Meyer ('47); Carrea, Reissig, and Mettler ('47, '48); Meyer ('49); Meyer and Allison ('49); Brodal ('49); Wall ('50); Nauta, and Gygax ('51).

Estable has been using different variations of silver-impregnation methods for tracing degenerating fibers in the central nervous system since 1935 (see Sosa and Andrew '47), paying special attention to the fact that frequently, in order to obtain best results, it is necessary to use specific variations for each problem.

With this in mind, a variation of the Cajal silver-impregnation technic, which would be most accurate for the study of degenerating thalamo-cortical connections, has been sought.

MATERIAL AND METHODS

Small lesions were placed in the various thalamic nuclei of the adult albino rat, using Krieg's stereotaxic machine and coordinates (Krieg, '46). The lesions were made, one in each animal, with the positive pole, using a 29 gauge wire and a current of 2 milliamperes for 5 seconds. The animals were anesthetized with Nembutal injected intraperitoneally. Since even the smallest infection can introduce great difficulties in the interpretation of degenerating fibres, 1 cc. of Di-Sulfalac was given intraperitoneally just before the operation, and another about two hours later.

The animals (ca. 100) were sacrificed after survival times ranging from 1 to 22 days with an overdose of Nembutal. Before the heart stopped beating, both carotids were cut to allow exsanguination.

Our problem has been to demonstrate the various thalamic and hypothalamic nuclei, the cortical areas, the lesion produced by the stereotaxic machine and the fibers in degeneration, both traumatic and Wallerian. Formula 6 appeared best to fulfill these requirements (see Vaz Ferreira, '49). Cajal and his collaborators (Cajal, '10; Cajal and De Castro, '33; Cajal and Tello, '33; Lee, '28) pointed out the following advantages of this variation: it is very constant; the surface of the pieces appears almost without overimpregnation and tissue shrinkage is avoided; the degree of hardening of the tissues is appropriate for obtaining good celloidin sections; and frequently, the silver stains the cell nuclei enough to allow recognition of them. These authors considered this technic accurate for obtaining serial sections of the cerebrum in small mammals. Thin fibers are impregnated especially well.

1 From the Department of Anatomy (Publication #573) Northwestern University Medical School, Chicago, Ill.
2 Rockefeller Fellow, on leave of absence from the Instituto de Investigación de Ciencias Biológicas, Montevideo, Uruguay.
This is notable in the staining of the cortical plexuses. On the other hand, the larger fibers are less intensively stained.

The technique is carried out as follows: The brain is cut by frontal section into slices; these are fixed in 10% solution of chloral hydrate in distilled water for 24 hours (if the solution becomes turbid it is advisable to change it). Then the slices are washed three times in distilled water, for a period of 1 to 2 minutes. They are transferred for 24 hours to 100 cc. of 95% alcohol, to which 10 drops of strong ammonia water are added. The slices are blotted rapidly with filter paper and transferred to a 1.5% of silver nitrate solution in distilled water, and kept in a dark bottle in a 37°C oven. The silver solution is to be changed after 24 hours, and after 5 to 6 days in this bath, they are washed three times (taking on the whole 1 to 2 minutes) in distilled water, and reduced for 20 minutes in the dark in a weak reducing solution (pyrogallic acid 0.5 Gm., formalin 7.5 cc., distilled water 92 cc., acetone 5 cc.). Without being washed, the slices are transferred to a strong reducing solution (pyrogallic acid 1 Gm., formalin 15 cc., distilled water 85 cc.), kept in darkness for 24 hours, washed in distilled water, and embedded in celloidin. It is advisable to check the distilled water for the presence of chlorine.

The washing before reduction is an important step: if too short, the periphery of the tissue will be overimpregnated; if too prolonged, the periphery will be understained. Some trial is advisable. Serial sections are cut at 20 μ, and every section mounted.

Formula 6 has also been found accurate for the study of thalamic degeneration after hemidecortication in the albino rat (Combs, '51).

Other variations of Cajal silver-impregnation, although not as satisfactory as formula 6, can give interesting results. Noteworthy is the formula in which alcohol-ammonia is used as the fixative (Formula 3 in Cajal, '10, and Lee B., '28; paragraph 116 of Cajal, and De Castro, '33). The results are more inconstant and the study of the cortical areas and of the thalamic nuclei more difficult, but, at times, the degenerating fibers appear very well.

RESULTS

The lesion which is produced by the stereotaxic machine varies according to the postoperative survival time. Here, the picture which appears at 6 to 8 days will be described, since it has been found to be the most useful survival time.

Figure 1 exhibits the central cavity (A), which contains some necrotic remnants and a few erythrocytes. Around the cavity is a layer formed by nerve fibers (B) which, although placed in a region which is under the most intensive action of the electric current, are apparently normal on microscopic examination. Because of the heavy fiber-impregnation the layer has a dark appearance. This is even better demonstrated in Figure 5. Cajal ('28, 2nd vol. p. 631 and ff., '11 b, '11 c) described in parts of the central nervous system which had been previously under an intensive traumatic action, the presence of fibers which are apparently normal or almost normal. His interpretation of them is as follows: Wallerian degeneration, with its characteristic beading and later fragmentation, is to a large extent a vital reaction which takes place in the course of several days. However, when fibers are very rapidly killed by an intense trauma, no time is allowed for the appearance of these vital reactions, and so the necrotic fibers retain an appearance not unlike normal fibers. Cajal called them “preserved” fibers. Figures 1 and 5 show the above process occurring when a lesion is produced with the aid of the stereotaxic instrument.

At the periphery of the “preserved” fibers layer, in contact with the layer
marked C (fig. 1), it is frequent to find nerve fibers which, still without formation of beads or only with scant and small beads, are fragmented, thus giving origin to a granular aspect which is shown in the layer marked B (fig. 5). The “preserved” fibers appear very early, even a few hours after the lesion is made, and
persist for many days. Consequently, together with the central cavity, they are useful for the determination of the exact place and extent of the lesion.

Fig. 3. Degenerating fibers in the white matter, 1,000 X.
Fig. 4. Traumatic degeneration in the thalamus, 1,000 X.

At times, the "preserved" fibers maintain their normal arrangement. However, occasionally they acquire a concentric arrangement, having been obviously pushed by the tip of the electrode. Between these two extremes, one finds all transition forms.

Immediately outside the zone of "preserved" fibers appears a layer marked C
(fig. 1), wherein the principal characteristic is the predominance of necrotic tissue. However, “preserved” fibers can be present in small numbers.

The layer marked by letter E (fig. 1) is heavily infiltrated by round cells (phagocytes according to the description of Horsley and Clarke, '08). These cells can be recognized in silver preparations at high magnification, although they are not visible in the present low-power photomicrograph. Occasionally, some nerve fibers are present. On account of the cell infiltration, the layer marked by letter E is the same as the innermost and middle subzones of the edema zone of Horsley and Clarke ('08).

Between layers C and E there appears very frequently in silver-material a fissure, marked D. Its formation is apparently due to the unequal shrinking of layers C and E.
the tissues during the various steps of the technic. This fissure is not exactly in the boundary between the necrotic and the cell-infiltrated layers, but commonly some of these cells are left in contact with the necrotic layer. As pointed out by Horsley and Clarke ('08), the phagocytic cells of the zone of edema do not penetrate into the necrotic layer or into the cavity of the lesion.

Finally, Figure 1 shows a layer, marked F, where, in addition to many normal nerve fibers, one can see fibers which have undergone traumatic degeneration. This is not apparent in the present low-power photomicrograph. Figure 4, at a 1,000 magnification, shows the characteristic large balls of degeneration.

It is important to distinguish traumatic from Wallerian degeneration. The traumatic (Jeavon, '11 a, '28) appears a few hours after the lesion both in the central and peripheral stump of the axons, which have been severed, and extends in general only for 1–2 mm. or even less from the site of injury. This extension varies with many factors. The parts of the axon which are not broken into nerve fibers.
action of the trauma are involved in this type of degeneration. Wallerian degeneration, on the contrary, appears only in the distal portion of the axon, that is, the one which is separated from the trophic center. This extends through all the fiber length and appears later than the traumatic. This distinction is important, since, as pointed out above, the traumatic degeneration appears in both the central and the peripheral stump. Consequently, its presence after a lesion does not allow one to draw conclusions about the direction of a pathway. Since it does not extend more than 2 mm. from the lesion, it can usually be neglected when working in large animals, but in small animals like the albino rat, 1 or 2 mm. are a relatively long extension, and a confusion of the traumatic degeneration with the Wallerian can give origin to a misinterpretation of the connections. With albino rats, killed one or two days after a thalamic lesion, that is, when the Wallerian degeneration has not yet begun, it was possible to determine that the traumatic degeneration never reaches the cortex. However, the same material shows that it can extend from one nucleus of the thalamus to another, thus presenting a difficulty in the interpretation of the direction of an intrathalamic connection.

After longer survival times, the true Wallerian degeneration, which extends to the cortex, appears. In some cases during the 4th day it is possible to detect it but the most consistent and demonstrative results are obtained at 6 to 8 days. At that time, a part of the degenerating fibers shows a marked beading and others are already fragmented.

Figure 7 shows pieces of fragmented fibers through the striatum. Some of these are vacuolar. Frequently, formula 6 gives a pale impregnation to the normal fibers in the thalamic radiations, thus improving the contrast with the well-stained degenerating fibers.

Figure 3 displays a fiber in the white matter in the beading stage, just before fragmentation, while Figure 6 shows a similar structure in the deep layers of the cortex.

For our study of the thalamo-cortical connections, it is sufficient to see the penetration of the degenerating fibers into the deep layers of the cortex. The methods of silver-impregnation that have been used here did not allow the tracing of degeneration to more superficial layers. The question of terminal degeneration in the cortex is too involved for consideration here.

Due to the position of the thalamo-cortical connections, many of them in frontal sections appear transversally cut. Figure 2 shows the cingulum after a lesion has been produced in the anterior nucleus of the thalamus. All of the degenerating fibers are cut transversally, yet the fragments are easily recognized. They are heavily impregnated, irregular in size and shape, at times with vacuoles at their interior.

The disappearance of the degenerating fiber remnants takes place in several days. In two of our rats it was possible to trace the degeneration to the cortex at 11 days. In the specimens which were killed at 17 to 22 days, some remnants were still present, especially around the lesion, but they were not sufficient to allow the tracing of pathways.
CONCLUSIONS

Formula 6 of Cajal is most useful for the study of the thalamo-cortical connections in experimental material. It reveals the normal structures (nuclei of the thalamus and of the hypothalamus, cortical areas etc.), the lesion which is produced by means of the stereotaxic machine, and the fibers in degeneration, both traumatic and Wallerian.

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