

A COMPARISON OF THE MESENCEPHALON AND HIPPOCAMPUS AS SITES OF ELECTION FOR NEGRI BODIES IN RABIES

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DURING recent years various workers have criticized the classical claims of the hippocampus as the site of election for Negri bodies, and a certain amount of evidence has accumulated suggesting that the mesencephalon and in particular the oculo-motor nucleus is a more favourable site.

Thomas & Jackson (1930) in South Africa examined 70 rabbits' brains of which 48 were positive histologically. "In 25 cases (51 per cent) Negri bodies were more frequent in the mid-brain than in the hippocampus. In only 1 (2 per cent) were they more frequent in the latter than in the former. In 11 cases (22 per cent) Negri bodies were absent in the hippocampus but present in the mid-brain, and in 1 case (2 per cent) they were absent in the mid-brain but present in the hippocampus."

In a review quoting this article McKendrick (1931, *loc. cit.*) pointed out that the matter was of considerable importance and invited those who had access to the necessary material to put such statements to the test.

Nicolau & Kopciowska (1932) in their studies on fixed virus in rabbits, using the slow method of Giemsa, found that while Negri bodies were rarely found in the hippocampus they were much more numerous in the "noyau optique basale".

More recently these same authors (1933) working with two strains of fixed virus (Mathis & Jonnesco) have confirmed their previous results. With the Mathis strain Negri bodies were larger and much more numerous in the "noyau optique basale" than in the hippocampus, while with the Jonnesco strain, a virulent Roumanian strain isolated from a wolf, they were so few and so scarce in the hippocampus "que pratiquement ils sont comme inexistants". On the contrary, they were always present in the "noyau optique basale" and in some cases both numerous and large.

This nucleus is described as a formation under the optic chiasma or optic tract and consists of a large number of ganglion cells. It would appear to be identical with the oculo-motor nucleus.

Muratowa (1934), working with mice, found that the first appearance of Negri bodies is in the mesencephalon and, even when the symptoms of rabies have fully developed, Negri bodies may be absent from the hippocampus though present in other parts of the brain.

OUR OBSERVATIONS

Technique

The routine procedure in the Sudan is to send to the Laboratories the suspected brain divided in halves in 5 per cent formol-saline and to remove a thin slice of the hippocampus for section. This has given very satisfactory results on the whole, but as the removal of the brain from suspected animals is often carried out in out-stations by unskilled natives, the brain is sometimes rather torn and damaged in the process, although as a rule sufficient remains of the hippocampus to take a piece for section.

There is also a tendency to cut the brain off short at the upper level of the mid-brain and hence this region is often missing in the brains received. Further, the brain is often received undivided, still surrounded by its membranes, and in these circumstances the denser portions of the brain stem are often poorly fixed, but the formalin usually penetrates sufficiently into the ventricles to fix the hippocampus.

The following series only includes cases in which both mid-brain and hippocampus could be examined, and it extends over the period November 1935 to December 1936.

Method of examination of the mid-brain

Following the suggestion of Thomas & Jackson (1930) a complete transverse section was made whose upper limit was the posterior part of the superior corpus quadrigeminum, and which included a small anterior portion of the inferior corpus quadrigeminum. This portion included the oculo-motor nucleus and on the ventral surface the emerging fibres of the oculo-motor nerve.

In the case of small brains it was generally possible to include the complete transverse section in a single block, but with the brains of larger animals as a rule it was necessary to make two blocks, of which one included the aqueduct.

In the few cases where the medulla was examined, a transverse section was made through the pyramids a little above the decussation.

Staining

After trials extending over years of the many recommended stains, it has been found that Lépine's (1935) is by far the most useful for diagnostic purposes. Admittedly in our hands it has proved a somewhat coarse stain for the finer differentiation of cellular structures, but Negri bodies are clearly stained and stand out from the cell with great distinctness. This stain has proved especially valuable in brains which, owing to imperfect fixation for the reasons previously described, have undergone a certain amount of post-mortem autolysis. In a number of sections Leishman's stain was also used, but in most cases we have found this less efficient than Lépine's.

Sections were cut 3μ in thickness, and in each section all ganglion cells were examined before reporting a negative result. In many cases numerous serial sections of the mid-brain were made to exclude the possibility of a few scattered positive foci. As the results in general were so clear cut, there appeared to be no added advantage in carrying out comparative percentage counts of the cells containing Negri bodies in the hippocampus and mid-brain.

The results, recorded in Table I, are unequivocal and indicate the great superiority of the hippocampus as a site of election for Negri bodies. Of 37 brains examined 10 (27 per cent) were positive in both hippocampus and mid-brain, 18 (48.6 per cent) were positive in hippocampus only and in no case was the mid-brain positive when the hippocampus was negative. Again a number of brains showed numerous and large Negri bodies in the hippocampus while the mid-brain was either negative or the Negri bodies were scanty, small and rather difficult to make out. In a few of the positive mid-brains they were multiple, as many as 12–16 in a cell and embedded in what appeared to be a structureless, acidophil matrix which obscured their contours. This appearance was also noted in cells of the medulla and did not appear to be a staining artefact. As in the same brains the Negri bodies in the hippocampus were always well developed and typical in appearance, it seems possible that the appearances in the mid-brain or medulla might be interpreted as an earlier stage of development. With respect to the oculo-motor nucleus the above findings are also at variance with those of Thomas & Jackson. It is true the large ganglion cells of this nucleus are most distinctive and easily picked out by a $\frac{2}{3}$ in. objective, but in only one case did they contain any Negri bodies. This negative result was so unexpected that a number of serial sections was examined by both of us, some of these sections being stained with Leishman to exclude any possible fallacies due to the stain, but with the above-mentioned exception these cells in all the sections were negative.

The Negri bodies in the mid-brain were usually found in smaller cells whose situation suggested they were cells of the nucleus ruber, but the cells of the superior and inferior corpora quadrigemina were also positive in some cases. The medulla was examined in only 13 cases, 2 of which were negative although positive in the hippocampus. The results suggested that routine examination of this region would be unlikely to be of any particular advantage, and so its examination was discontinued. Our findings thus are at complete variance with those of the above-quoted authors, but it should be noted that the series are not strictly comparable. The present series with one exception (rabbit brain inoculated from human case proved mid-brain – and hippocampus +) were all from brains of naturally infected animals the majority of which were dogs. The other workers were using rabbits or mice experimentally infected with strains of street or fixed virus. It is difficult to see why this difference should affect the results in so marked a manner, but the possibility should at least be borne in mind. Naturally it would be more satisfactory to compare the above results with an exactly similar series, but we have been

Table I. *A comparison of the hippocampus and mid-brain from suspected rabid animals as sites of election for Negri bodies*

No.	Hippocampus	Mid-brain	Medulla	Remarks
4480	+	-	-	
4497	+	+	+	Mid-brain—in large and small cells
4498	+	+	+	Mid-brain—in small cells only
4484	+	-	-	Hippocampus—bodies large and numerous
4517	+	+	+	Hippocampus—fairly numerous Mid-brain—few
4518 (Sheep)	+	-	-	Hippocampus—few bodies
4522 (Donkey)	+	+	-	Mid-brain—few bodies
4527	-	-	-	
4531	-	-	-	Optic nerve—Negri bodies absent
4532	-	-	-	
4533	+	-	One N.B.	Hippocampus—few small bodies Optic nerve—numerous in one part of section
4555	+	-	-	Hippocampus—few small bodies
4553	-	-	-	
4562	-	-	-	
4563	-	-	-	
4564	+	+	+	Hippocampus—numerous and large Mid-brain—small and scanty
4581	-	-	-	
4586	-	-	-	
4588	+	+	-	Hippocampus—numerous and large Mid-brain—small and very few
4592	-	-	-	
4605	-	-	-	
4631	+	-	-	
4640	-	-	-	
4648	-	-	-	
4669	-	-	-	
* Rabbit inoculated from human case	+	-	-	
4954	-	-	-	
4842	+	+	-	Hippocampus—fairly numerous Mid-brain—small and scanty
4869	+	-	-	
4873	-	-	-	
4887	-	-	-	
4888	-	-	-	
4968	-	-	-	
4980	+	+	-	Hippocampus—numerous and large Mid-brain—very scanty, small and indistinct
4987 (Donkey)	+	+	-	Hippocampus—numerous and large Mid-brain—very scanty, small and indistinct
5007 (Donkey)	-	-	-	
5017	+	+	-	Hippocampus—few, but large and typical Mid-brain—very scanty and indistinct

Where not otherwise mentioned the brains are from dogs.

+ = Negri bodies present.
- = Negri bodies absent.
— = not examined.

* The human case was that of a native woman who died in Omdurman hospital, four months after being bitten on the right hand by a rabid dog. Unfortunately it was not possible to obtain a post-mortem, but through a small trephine hole in the skull a small piece of the cortex was removed. Histological examination for Negri bodies was negative, but the rabbit inoculated with an emulsion of the cortex died in 21 days with paralytic symptoms.

unable to find in the literature any records of examination of the hippocampus and mid-brain from naturally infected animals which would be strictly comparable.

In default of fresh evidence on this subject, and bearing in mind that routine histological examination is carried out on the brains of naturally infected animals, we consider the present results demonstrate unequivocally the classical claims of the hippocampus as the site of election for Negri bodies.

SUMMARY AND CONCLUSIONS

A comparative examination of the hippocampus and mid-brain in a series of 37 brains from suspected rabid animals has shown the marked superiority of the hippocampus as the site of election for Negri bodies.

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