AN INTRANUCLEAR FIBRILLAR LATTICE
IN NEURONS

R. L. CHANDLER AND R. WILLIS

Institute for Research on Animal Diseases, Compton, Berkshire, and Department of Anatomy, University College London, W. C. 1

SUMMARY
A new intranuclear structure has been demonstrated in neurons of rat and mouse brain, and studied using conventional electron microscopy and electron stereoscopy techniques. The structure has a crystalline appearance and a variety of profiles was seen in the sections, some showing curvature and one showing ellipticity. The basic component consisted of planes of parallel fibrils; layers of these arrays, usually two in number, crossed each other at an angle, resulting in the appearance of a lattice.

INTRODUCTION
The presence of a lattice structure in the nuclei of rat and mouse neurons has been described by Chandler (1966). The neurons concerned were observed by electron microscopy in ultrathin sections of the hippocampus and thalamus, in the course of a study on experimental scrapie disease (Chandler, 1961; Chandler & Fisher, 1963); the structure was demonstrated in normal rats, from the stocks of two different research institutes, as well as in rats and a mouse concomitantly inoculated with scrapie. The present paper describes the observations more fully, and electron stereoscopy has been used to gain more detailed information.

MATERIALS AND METHODS
Laboratory animals. The majority of the rats were of the white Wistar stock, maintained as a closed colony at the Institute for Research on Animal Diseases for several years. The mice were of the B.S.V.S. stock, maintained similarly. In addition 2 rats were obtained from the Medical Research Council Radiobiological Unit, Harwell, by courtesy of Dr O. A. Trowell. Some of the rats and the mouse in which the intranuclear structure was detected had been inoculated with scrapie agent; the structure was, however, present in normal rats and there was no evidence that its presence in scrapie-affected animals was other than incidental.

Electron microscopy. The animals were anaesthetized with ether, decapitated, and the heads were cleaved sagittally. Small pieces of hippocampus or thalamus were fixed in osmium tetroxide (Caulfield, 1957) or in glutaraldehyde followed by post-osmication (Sabatini, Bensch & Barnnett, 1963). The embedding medium was Araldite (Glauert 1961). Glass knives were used for sectioning. Sections were stained with uranyl acetate...
in alcoholic solution (Stempak & Ward, 1964). The routine specimens were examined in an A.E.I. EM 6 electron microscope at instrumental magnifications of 4000–50000.

Electron stereoscopy was performed on selected specimens using a Siemens Elmiskop 1 electron microscope fitted with a Valdrè cartridge; this permits continuous observation of the chosen structure during tilting through angles of up to \( \pm 22.5^\circ \) about horizontal. Magnifications up to \( \times 40000 \) were used (see Valdrè, 1962.)

**OBSERVATIONS**

The intranuclear structure was only rarely encountered. The general study was, however, extensive; numerous sections from 30 rats and mice were examined. Following its discovery, the search was intensified and the structure was demonstrated in 4 normal rats, including one from the Harwell stock, and in 3 rats and one mouse that had been inoculated with scrapie. Sections of one tissue block, in which the structure had been detected, revealed the structures in two other neurons of that block. The structure was present in both male and female rats.

The structures appear in sections, as a linear, curved or elliptical profile with an overall dimension of from 0.6–1.8 \( \mu \). Such profiles lie in a variety of positions within the nucleus; no particular relationship to a specific region of the nucleus, or to the nucleolus or nuclear envelope, was observed. The chromatin granules sometimes appear less densely packed around the structure.

The structure is seen at low magnification as a small, dense band within the neuron nucleus (Fig. 1, arrow). Fig. 2 shows part of the structure at higher magnification. From this and micrographs described below, the structure is best interpreted as a flat or slightly curved sheet of material consisting essentially of 2 parallel layers of fibrils (but see below), crossing each other at an angle of 60° or more to form a lattice. Fig. 2 shows an oblique section through such a lattice. The average thickness of an individual fibril is about 70 Å; the centre-to-centre spacing is about 110 Å. The layers have a centre-to-centre spacing of about 120 Å. Figs. 4 and 5 are a stereopair of the structure cut at a similar angle to that of Fig. 2. These should be viewed with a stereoscope, and confirm that the lattice is cut obliquely and extends down through the thickness of the section. About 7 fibrils can be counted in the plane near the horizontal; this is to be expected since the sections were approximately 600–800 Å thick (see Peachey, 1958) and the section is in focus throughout its depth. If either Fig. 4 or 5 is considered separately some of the chromatin granules appear as part of the lattice, but when viewed as a stereopair these granules can be seen lying either above and to the right of or below and to the left of, the lattice.

The elliptical profile (ep) shown in Fig. 3 indicates that the lattice may be saucer-shaped. In this example the section has passed through the rim of the structure, and the lattice arrangement is most conspicuous at the poles of the ellipse. Fig. 6 shows the lattice may be curved. This could represent either a section of a curved sheet or of a saucer-shaped structure cut in a plane perpendicular to that of Fig. 3. In region \( x \) of Fig. 6 fibrils of the upper set appear as dots, indicating that they are perpendicular to the electron beam; below can be seen a second set of dots, and below this linear
Intraneuronal lattice in neurons

densities (d). This configuration could be interpreted either as a section of a region of the lattice which is highly curved or bent back upon itself, or that this particular example consists of at least 3 sheets of fibrils.

Other micrographs also suggest that more than 2 layers of fibrils may be involved. Fig. 8 shows an oblique section through the structure. When this section was tilted through 45°, a remarkably different image was obtained, and is shown in Fig. 9. The tilt angle is, in this experiment, too large to enable the pictures to be readily fused in a stereoscope, but nevertheless the value of a tilting mechanism to obtain otherwise inaccessible information is clearly demonstrated. Fig. 9 indicates that 2 lattices may lie very close together, or that a lattice may be bent back upon itself, or that the structure may sometimes consist of 4 sheets of fibrils. Such possibilities are suggested most clearly by the upper region in Fig. 9, where the lattice has been tilted to lie vertically in the electron beam. The sequence from left to right is a row of dots, a dense line, a second row of dots and a second dense line. The dots represent aspects of the vertically oriented fibrils and the dense lines the horizontally oriented fibrils, several of which will lie superimposed and in focus within the thickness of the section. (It is the electron scattering resulting from the sum of their densities that causes the appearance of the dense line, such as d in the lower part of the lattice in Fig. 6.)

Occasionally, profiles such as that shown in Fig. 10 can be observed. The crossed array of fibrils appear curved and give the structure the appearance of a double helix. However, such an appearance is probably due to a marked curvature of the lattice within the thickness of the section, and the orientation of the lattice almost parallel to the electron beam—that is, almost vertical with respect to the section.

Sometimes bundles of fibrils were seen which were not arranged in a lattice. In Fig. 7, for example, fibrils (f) are seen lying close to the lattice (l), and in Fig. 3 fibrils show a less intimate association. Bundles of fibrils identical with those described by Siegesmund, Dutta & Fox (1964) were also observed for the first time in rat and mouse neurons in the course of these studies (Chandler, 1966).

DISCUSSION

The intranuclear fibrillar lattice was initially observed in neurons of the rat and mouse hippocampus and thalamus by Chandler (1966) in osmium-fixed and in glutaraldehyde-fixed, osmium-post-fixed, material. It has since been observed by Dr L. E. Westrum and Dr R. D. Lund (personal communications) in nuclei of neurons of the prepyriform cortex of rats (paraformaldehyde-fixed and osmium post-fixed) and in neuronal nuclei of the superior colliculus of the rat. The animals studied by the latter workers were adult albino rats of unknown strain.

Observations thus suggest that the lattice may be an important structure in neuronal nuclei. The fact that it has not hitherto been recorded is probably in part due to its comparatively small size. If the lattice is considered to be a saucer-shaped sheet of 1.8 μ diameter and 0.1 μ thickness, sections of it should appear on average on about 20 of the 130 600-Å sections required to traverse an 8-μ diameter nucleus. Our rather extensive search has produced only relatively few examples, so the structure is pro-
bably limited to a small proportion of neurons, as is the rodlet described by Siegesmund et al. (1964). Intranuclear fibrous material has also been described in invertebrate neurons by Gray & Guillery (1963) and others.

It is difficult to decide the angle at which the fibrils intersect to form the lattice. Measurements on micrographs suggest an angle of about 60° but this is related to the orientation of the structure within the section and the true angle of intersection could well be larger, even up to 90° (see Figs. 4, 5).

The authors wish to acknowledge the support of Professor J. Z. Young, F.R.S., and the advice and encouragement of Dr. E. G. Gray. We also thank Mr K. Smith, F.I.M.L.T., for skilled technical assistance and Mr S. Waterman for the prints.

REFERENCES


(Received 5 March 1966)

Fig. 1. Low-power view of the lattice (arrowed) within the neuron nucleus of mouse hippocampus. Fixative according to Caulfield (1957), stained with uranyl acetate. × 20 000.

Fig. 2. Higher-power picture of the lattice. Preparation details as in Fig. 1. × 115 000.
Fig. 3. The lattice seen as an elliptical profile (ep) within a neuronal nucleus of rat hippocampus. Note also the presence of non-organized filaments at f. Glutaraldehyde fixation with post-osmication, stained with uranyl acetate. × 65,000.

Figs. 4, 5. Low-angle stereomicrograph pair. When viewed with a suitable viewer the structure should be seen to run diagonally down through the section. Preparation details as in Fig. 1. Tilt angle 4°. × 45,000.
Fig. 6. High-power micrograph of a curved part of the lattice, showing filaments cut end-on (x) and longitudinally (d). Preparation details as in Fig. 1. × 200 000.

Fig. 7. The lattice (l) cut in transverse section is here closely associated with an organized structure of filaments (f). Preparation details as in Fig. 1. × 120 000.
Figs. 8, 9. Higher magnification of Fig. 1. These micrographs show a high tilt-angle pair, demonstrating the marked change in appearance of the structure caused by tilting. Tilt angle 45° about marked axis. × 105,000.

Fig. 10. The intranuclear lattice in the prepyriform cortex of the rat. Note the helical appearance of the filaments at the left-hand side of the micrograph. Formalin-perfused and post-fixed with osmium tetroxide. Double stained with uranyl acetate and lead citrate (Westrum, 1965). × 90,000. (Micrograph by courtesy of Dr L. E. Westrum.)