J. Anat. (1965), **99**, 4, pp. 711–759
With 58 figures
Printed in Great Britain

A histochemical study of cholinergic fibres in the cerebral cortex

BY K. KRNJEVIĆ AND ANN SILVER
A.R.C. Institute of Animal Physiology, Babraham, Cambridge

INTRODUCTION

Several kinds of evidence have led many authors to suggest that cholinergic transmission plays a role in cortical function. Acetylcholine (ACh) and related compounds apparently excite cortical neurones and this effect is abolished by cholinolytic agents which also interfere with normal activity(Bonnet & Bremer, 1937; Miller, Stavraky & Woonton, 1940; Feldberg, 1945; Bornstein, 1946; Bremer & Chatonnet, 1949; Hyde, Beckett & Gellhorn, 1949; Infantellina, 1955; Monnier & Romanowski, 1962; Ling, 1963). The cortex contains a substantial amount of ACh, choline acetylase and cholinesterase (ChE) (MacIntosh, 1941; Feldberg, 1945; Feldberg & Vogt, 1948; Burgen & Chipman, 1951; Pope, 1952; Hebb & Silver, 1956; Stone, 1957; Rosenzweig, Krech & Bennett, 1958; McCaman, 1963; Lewis, Shute & Silver, 1964), and there is a continual release of ACh in the cortex, at a rate bearing some relation to the level of cortical activity (MacIntosh & Oborin, 1953; Mitchell, 1963).

Recent experiments have proved that some nerve cells in the mammalian neocortex can be excited by ACh applied directly from micropipettes (Krnjević & Phillis, 1961, 1963*a*; Spehlmann & Kapp, 1961; Spehlmann, 1963). Further studies (Krnjević & Phillis, 1963*b*) revealed certain characteristic features of these cholinoceptive cells. Although some were found in all regions of the neocortex, often in clusters, they seemed more common in or near the primary afferent areas. Perhaps their most notable feature was an uneven distribution in depth; few were found within 0·8 mm of the cortical surface and they were concentrated particularly within the middle range of depth, between 0·8 and 1·3 mm. Since most of the cells activated by antidromic stimulation of the medullary pyramids could be excited with ACh, cholinoceptive units in general appeared to be deep pyramidal neurones.

Cholinoceptive cells often showed a typically irregular, spontaneous discharge, similar to the projection activity described by Dempsey & Morison (1943). Their ACh receptors had certain well-defined pharmacological properties (Krnjević & Phillis, 1963*c*); they were especially sensitive to the muscarine-like actions of ACh (Dale, 1914) and other muscarinic agents, and were blocked specifically by muscarine-antagonists, such as atropine and hyoscine. These cortical cells thus presented a sharp contrast to spinal cholinoceptive cells with predominantly nicotinic receptors (Eccles, Fatt & Koketsu, 1954).

The most likely explanation for all these findings is that cortical cholinoceptive cells are innervated by cholinergic nerve endings. However, although various cerebral pathways were examined (Krnjević & Phillis, 1963*b*), a distinct cholinergic innervation could not be demonstrated by electrophysiological and pharmacological

means. For example, none of the relatively direct pathways such as the thalamocortical and transcallosal connexions were cholinergic. The most characteristic responses of cholinoceptive cells were delayed and prolonged discharges, such as repetitive responses elicited and stimulating the thalamic relay nuclei or the internal capsule. The slowness of these responses suggested that cholinergic pathways may be multisynaptic or that they consist of slow fibres which travel to the cortex by a relatively indirect route.

An attempt has therefore been made to identify these postulated pathways by a histochemical method. Of the various components of a cholinergic system, only cholinesterase can be readily identified histochemically. With a few exceptions, the distribution of ChE (particularly acetylcholinesterase (AChE)) agrees well with that of known cholinergic neurones in spinal and cranial motor nuclei (Koelle, 1950, 1951, 1954, 1963; Gerebtzoff, 1959; Snell, 1961); it is also closely associated with cholinergic neurones in several autonomic ganglia and some autonomic nerves (Hurley, Shelley & Koelle, 1953; Gerebtzoff, 1959; Okinaka & Yoshikawa, 1962; Sjöqvist, 1963; Koelle, 1963). According to these authors, some ChE appears to be present in all parts of a cholinergic neurone, including the soma and all the ramifications of the axon, although the various parts do not necessarily show this equally clearly.

The cerebral cortex has been examined by several workers using modifications of Koelle's technique (Koelle, 1950, 1954; Ishii, 1957; Gerebtzoff, 1959; Okinaka, Yoshikawa, Uono, Muro, Mozai, Igata, Tanabe, Ueda & Tomonaga, 1961). It is generally agreed that the neocortex contains relatively little ChE, with only moderate staining of a few neurones and scattered fibres producing no meaningful pattern. The present paper describes the results of a systematic re-investigation of the distribution of ChE in the cat's cerebral cortex. Preliminary reports have already appeared (Krnjević & Silver, 1963*a*, *b*).

METHODS

Most of the studies were made on adult cats, but the brains of a number of kittens were also examined. In addition, a more perfunctory survey was made of the brains of three rhesus monkeys (*Macaca mulatta*). In all cases, we used a standard technique based on Lewis's (1961) modification of Koelle's method.

Fixation. The majority of animals had been used in electrophysiological studies of the exposed cerebral hemispheres; they had therefore been anaesthetized for at least 12 h, mostly with a mixture of diallylbarbituric acid and urethane (Dial Compound, Ciba Ltd.), before the head was perfused to fix the brain. The chest was opened within a few minutes of death by an overdose of pentobarbitone sodium (Abbott Ltd.). The descending thoracic aorta was cannulated, the ascending aorta was tied at the base of the heart and an opening made in the auricles to allow the escape of venous fluid. The head and upper limbs were then perfused with the following solutions: about 100 ml/kg body weight of isotonic Na_2SO_4 (16·7 g/l) at 37 °C, over a period of about 10 min; then 100 ml/kg of warm isotonic Na_2SO_4 containing 20 % (v/v) formaldehyde solution (36 %, w/v) over a further period of about 10 min. After another 10 min the skull was opened (or the original opening was extended) so that the whole brain, or various portions, could be dissected out

and placed in a large volume of isotonic Na_2SO_4 containing 10 % (v/v) formaldehyde solution, and then left in the refrigerator at 4 °C for 4 h. The tissue was then rinsed in glass-distilled water at 4 °C and transferred to a solution of 20 % ethanol in distilled water at 4 °C. Tissues could be kept in alcohol at 4 °C for several weeks but almost all brains were sectioned within 5 days of perfusion and mostly within 24 h.

Section cutting. Frozen sections were cut on either a Reichert sledge type, or a Reichert ratchet type, freezing microtome. A few sections of monkey brain were cut in a Slee cryostat. The thickness was in most cases 70 μm.

Histochemistry

Incubation medium. The incubation medium was prepared as follows: 100 mg of acetylthiocholine iodide (Roche Products Ltd. and L. Light & Co. Ltd.) or 110 mg of butyrylthiocholine iodide (Roche Products Ltd.) were dissolved in 4·0 ml glass-distilled water and the iodine precipitated with 7·0 ml M/10 $CuSO_4$. The first additions of $CuSO_4$ were made drop by drop and the solution was well shaken between each addition. It was left to stand for 10 min and then centrifuged at 2000 rev/min for 20 min. 62 mg glycine were dissolved in 10 ml of the supernatant, the pH adjusted to 5·5 with M sodium acetate and the volume made up to 50 ml with distilled water to give a substrate concentration of 6·3 mM. This incubate was usually prepared just before each experiment but occasionally it was kept for up to three days at 4 °C. It was always filtered immediately before use.

Incubation. The tissue sections were incubated at room temperature for $2\frac{1}{4}$ h. Very small sections were put in about 0·5 ml solution in the wells of haemagglutination trays; larger sections of cats' brains needed up to 2·0 ml of medium and those of monkey's brains up to 5·0 ml.

Incubation with inhibitors. In some experiments di-isopropylphosphorofluoridate (DFP: kindly given by Dr B. C. Saunders) was used as an inhibitor; the concentrations were 5×10^{-7} M and 5×10^{-6} M, for cat tissue, and 10^{-6} M and 10^{-5} M for monkey tissue. In these experiments sections were placed for 45 min in the appropriate concentration of DFP in 0·2 M sodium acetate/acetic acid buffer (pH 6·5) before being transferred to the medium, to which inhibitor was also added. Adjacent control sections were put in buffer and incubated without DFP.

Development. At the end of the incubation the solution was sucked off and the sections washed 3 times with distilled water; they were then put in fresh distilled water in clean trays. The stain was developed by immersing the sections for 2 min in a solution of sodium sulphide in 0·2 M acetic acid (1 g/45 ml).

Section mounting. After development the sections were washed in distilled water and left in fresh distilled water until they were mounted on lightly albuminized slides. These were dried at room temperature, and then in an oven at 37 °C for at least 30 min; finally they were taken through absolute alcohol and xylene, and the cover-slips were attached with Gurr's Damar-Xylol.

Other histological techniques. Representative sections, already treated as above, were taken through descending grades of alcohol to water and then counterstained for 1 min in $\frac{1}{2}$ % cresyl violet, $\frac{1}{2}$ % thionin or 1 % carmalum.

Cortical surgery. In a number of experiments, small areas of the cortex of cats were undercut or isolated in a preliminary aseptic operation under sodium pento-

barbitone. The skull was opened and, with a narrow knife blade inserted through the dura, two holes about 5 mm deep were made in a gyrus adjoining the area to be isolated. A right-angled probe was inserted into each hole and directed below the area in question, which was undercut by rotating the probe several times. The blunt tip of the probe could be worked up to the pial surface around the undercut area to complete its isolation on all sides, without damaging the superficial blood supply. To prevent later adhesions to the dura, a piece of polythene sheet was placed over the trephine hole before the muscle and the skin were resutured.

After recovery from the operation, the cats showed no obvious disability, apart from some temporary contralateral stiffness of limbs when lesions were made in the motor area. Several animals tended to purr very readily, like the cats described by Kennard (1955). According to the latter, this is a characteristic result of bilateral anterior cingulectomy, but in our series the lesions were unilateral and they affected mainly the middle suprasylvian or the pericruciate regions.

RESULTS

Observations on the brains of cats

As stated by previous authors, with Koelle's method only a few scattered cells and fibres are stained, without forming any immediately obvious pattern. The overall amount of staining varies considerably from one preparation to another. In some brains hardly any staining is visible unless sections are examined at a very high magnification, when a few clearly stained fibres are nearly always detectable. In other cases, there is so much diffuse staining that the detailed distribution can only be analysed with difficulty. It is not yet clear whether these great differences in staining reflect true differences in the amount of ChE activity in various specimens, or whether they are artefacts caused by unknown factors interfering with the method.

Distribution of staining as seen with low magnification. Figures 1 and 2 show the general distribution of staining. The neocortex has relatively little ChE activity, but in the basal ganglia and the brain stem it is a predominant feature.

Hardly any staining is seen in much of the white matter, such as the centrum semiovale, the internal capsule, the corpus callosum and the anterior commissure. Forebrain areas which show a relatively high degree of staining include the midline supracallosal rudimentary cortex or induseum griseum (Figs. 1, 29) and a thin band below the corpus callosum which is especially clear in Fig. 2; also seen in this figure are the hippocampus and the olfactory bulb, both with distinct zones of ChE activity.

In the pallium the staining follows the curvature of the surface, partly below and partly within the grey matter. The central core of a gyrus is usually only poorly stained, but it is lined on either side by bands of staining which form particularly well-defined loops around the troughs of sulci. In the main part of the gyrus, these bands become wider and more diffuse, except for their outer portion, which stands out as a distinct line or border in the middle of the cortex, at a relatively constant depth of 0·8–1·2 mm from the surface. A variable amount of ChE activity is also present in the plexiform layer; it is poorly developed or absent in the top of the gyri, but it may be dense in the walls of sulci. On the medial aspect of the hemisphere,

the superficial staining often extends continuously over sulci and gyri. Since the staining in the convexity of a gyrus differs from that around a sulcus, the two will be described separately.

Specificity of ChE staining

In the presence of specific inhibitors. To distinguish between true and pseudocholinesterase activity in the cortex, brain sections were treated with acetylthiocholine in the presence of ChE inhibitors, as described in the section on methods.

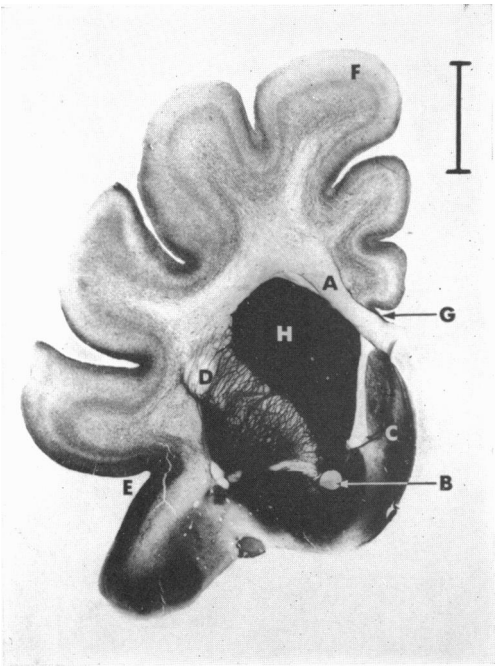


Fig. 1

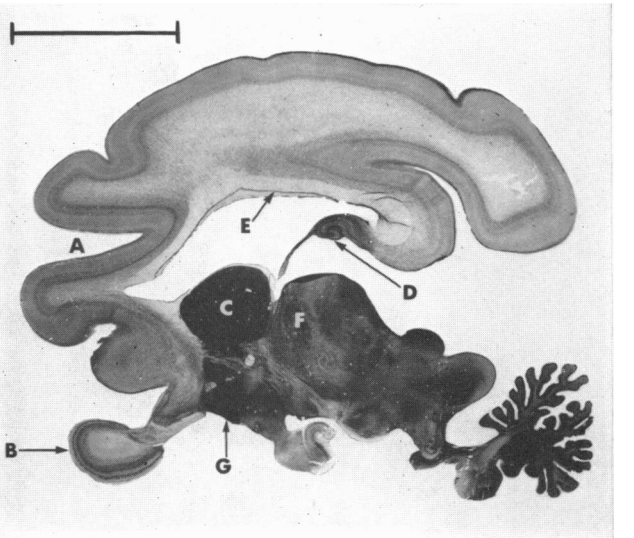


Fig. 2

Fig. 1. Frontal section of cat's forebrain showing general distribution of AChE activity. A, Corpus callosum; B, anterior commissure; C, septum; D, internal capsule; E, rhinal fissure; F, marginal gyrus; G, induseum griseum; H, caudate nucleus. Scale: 5 mm.

Fig. 2. AChE activity in a parasagittal section of kitten's brain. A, Cruciate sulcus; B, olfactory bulb; C, caudate nucleus; D, hippocampus; E, 'subcallosal band'; F, thalamus; G, olfactory tubercle. Scale: 10 mm.

It was found that the fibre staining (Fig. 3) is only little affected by treatment with 5×10^{-7} M-DFP (Fig. 4), but it is largely abolished by the application of 5×10^{-6} M-DFP (Fig. 5). Cellular staining is similarly affected by DFP (Figs. 6–8). Cortical ChE activity is thus due predominantly to true cholinesterase (AChE).

With butyrylthiocholine. When brain sections are treated with butyrylthiocholine instead of acetylthiocholine, even after 8–24 h of incubation there is only minimal staining in the cortex (Figs. 9, 10), with a rather diffuse coloration of the pia and the white matter (Fig. 10). The only substantial cellular staining occurs in glial cells of an astrocytic type that are sometimes visible close to the scar of a chronic lesion.

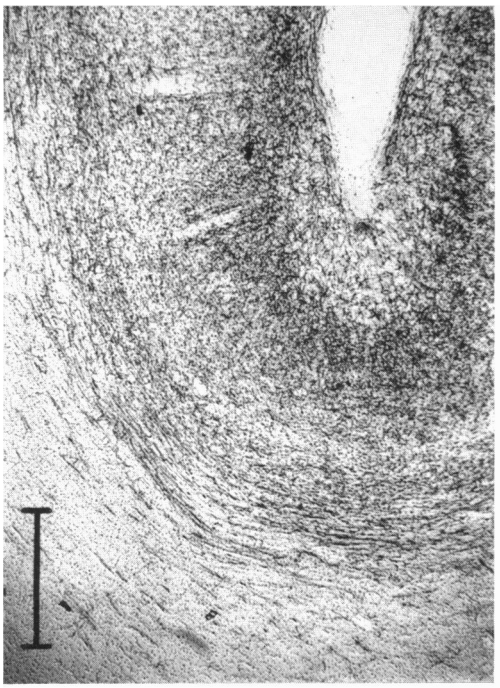


Fig. 3

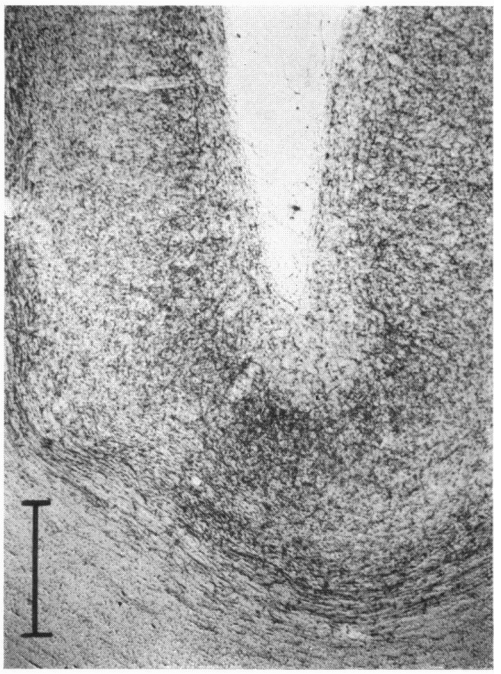


Fig. 4

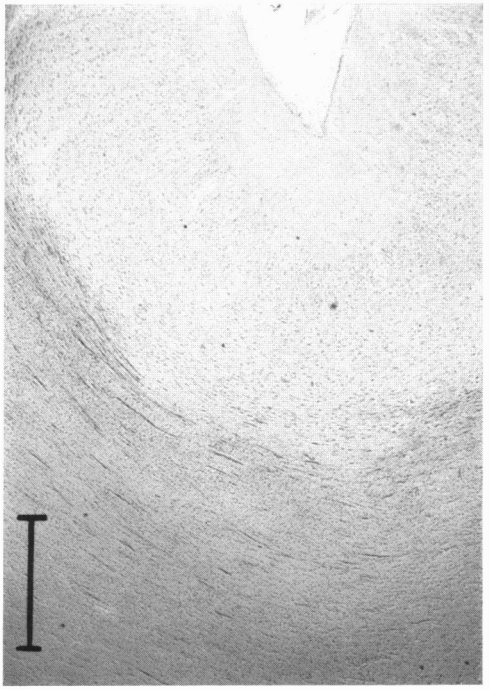


Fig. 5

The striatum also shows some staining after prolonged treatment with butyrylthiocholine (Fig. 10), but this is probably due mainly to AChE, since the activity was not blocked by 5×10^{-7}M-DFP. Marked staining, indicating pseudocholinesterase activity, is only seen in the thalamus and other parts of the brain stem. We have not found any clear evidence of a projection of pseudocholinesterase-containing fibres to the limbic cortex (cf. Okinaka *et al.* 1961; Shute & Lewis, 1963).

AChE activity of the gyral surface

Fibre staining

Most of the observed AChE activity is associated with fibres or a fibrillar network. The characteristic pattern is shown by Figs. 11–16, which give a sequence of views of the central region of a gyrus, ascending along the core of white matter towards the cortical surface.

Subcortical. Whereas the central core remains largely unstained, many clearly stained fibres travel near the boundary between white and grey matter (Fig. 11). They are mostly thin, with a diameter of 1–2 μm. Although many of the fibres pass into the grey matter in the walls of the adjacent sulci, the predominant direction is towards the top of the gyrus. As the fibres approach cellular layers, their orientation becomes less regular and they encroach gradually into the central area (Fig. 12); a continuous network of stained fibres thus spreads below and within the deepest part of the cortex (Figs. 13, 14).

Deep network. The clearest and densest portion of the deep network extends into the cortex for a distance of about 0·5 mm, mainly in layer VI. It consists of distinct fibres, having a general orientation towards the surface though with much twisting and branching; more superficially, many fibres run in a tangential direction and then the staining progressively becomes less well defined (Fig. 15).

A curious feature of the deep network is the presence of large numbers of small but densely stained structures. In Figs. 13 and 14 they are visible as little dots or squiggles apparently scattered at random between the branching fibres. When examined under high power (Fig. 17) they can be seen to have a diameter of about 4–5 μm; careful focusing shows that they are not simply fibres that have been cut across, but their structure is not readily made out. Sometimes they seem to be simply terminal expansions of fibres; in other cases they look more like a fibre coiled on itself, either into a kind of bulb or, when more elongated, into a corkscrew structure. They bear no obvious relation to other cells, as might be expected if they were synaptic terminals.

Zone of more diffuse staining. In the middle of the cortex the overall amount of staining is no less than in the deeper zone, but it is usually not associated with a distinct pattern of fibres (Fig. 15). At high magnification, one sees a profusion of crystals, granular material and fibrils, all interconnected in a poorly defined neuropil. Here and there well-stained fibres can be traced over short distances.

Figs. 3–5. Low-power view of sections of region round posterior cruciate sulcus, treated with different media to show specificity of acetylcholinesterase activity. In Fig. 3 acetylthiocholine was used in the ordinary way; in Fig. 4, the section was pre-treated with 5×10^{-7}M-DFP and, in Fig. 5, with 5×10^{-6}M-DFP—note disappearance of staining in the latter only. This sequence, together with Fig. 9, shows that the cholinesterase activity observed was due to acetylcholinesterase (AChE). Scales: 0·5 mm.

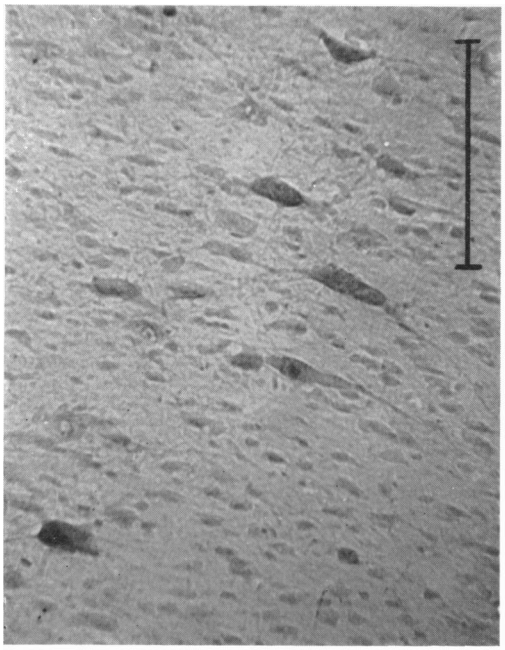


Fig. 6

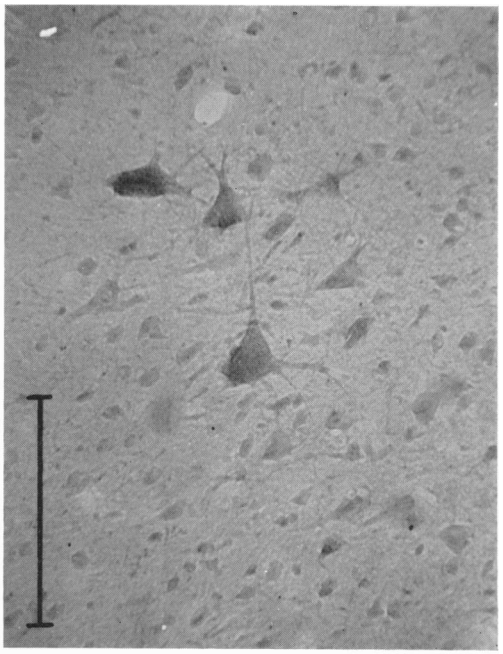


Fig. 7

Fig. 8

Figs. 6–8. Specificity of cellular cholinesterase activity in cortex. All three sections were incubated with acetylthiocholine and they illustrate Betz cells in anterior sigmoid gyrus. Fig. 6 is a control, while the sections in Figs. 7 and 8 were pre-treated with 5×10^{-7} M- and 5×10^{-6} M-DFP respectively. Preservation of staining in Fig. 7 but not in Fig. 8 shows that the cholinesterase activity was principally due to AChE. Scales: 200 μm.

This type of staining is found mainly in layer V but it often overlaps, at least partly, both layer VI and layer IV. Its sharp outer boundary is approximately at the level of the outer line of Baillarger (cf. Fig. 26). It thus extends through the layer of prominent pyramidal cells that is commonly found at a depth of about 1·0 mm (0·8–1·2 mm in different areas) and then either rapidly disappears or becomes much fainter (Figs. 18, 19). In some preparations such diffuse staining or

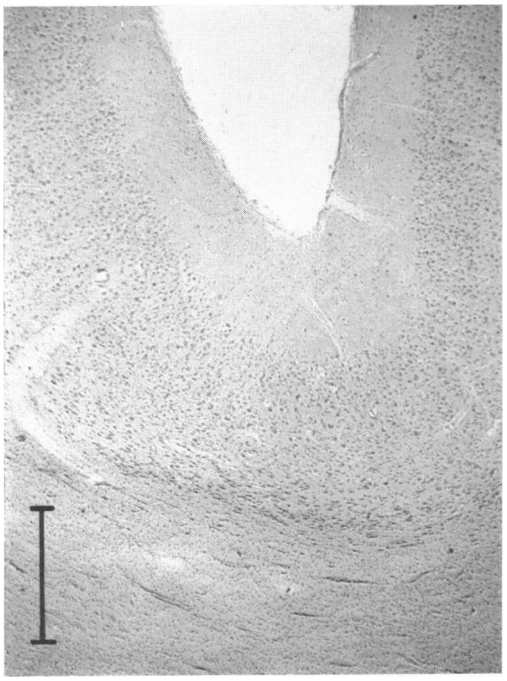


Fig. 9

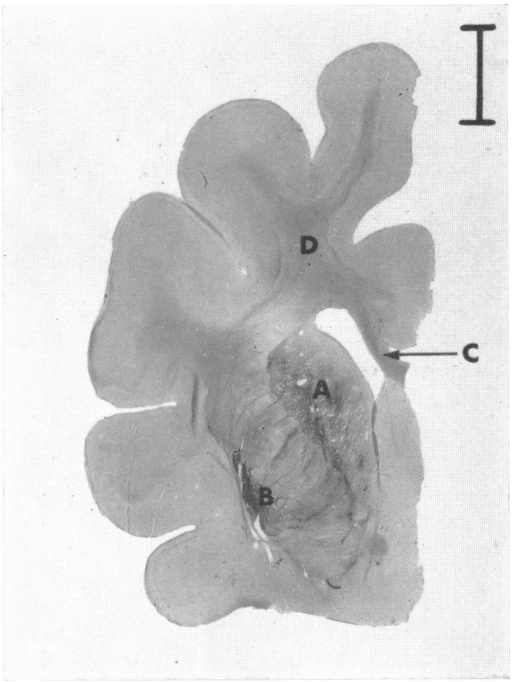


Fig. 10

Fig. 9. The section shown was incubated for 8 h with butyrylthiocholine instead of acetylthiocholine. The virtual absence of staining (cf. Fig. 3) indicates there is little or no pseudocholinesterase in the cortex. Scale: 0·5 mm.

Fig. 10. Very low-power view of frontal section of hemisphere, incubated for 8 h with butyrylthiocholine. Note diffuse staining in white matter, probably due to pseudocholinesterase. Striatal staining, unlike the diffuse staining, was not inhibited by 5×10^{-7} M-DFP, and was thus probably due to AChE. A, Caudate nucleus; B, putamen; C, corpus callosum; D, white matter. Scale: 5 mm.

even a relatively clear network of fibres may persist right up to the plexiform layer (Fig. 16), being then continuous with the denser zone of staining near the surface.

Superficial zone. The layers of cells between about 0·2 and 0·8 mm from the surface (layers II and III) are always the least stained (Figs. 16, 18, 19). It is the sudden transition to this zone which gives to the deeper staining the sharp outer border often visible with the naked eye (Fig. 1).

Staining in the plexiform or marginal layer is particularly variable. In the middle of the gyrus it is usually slight or even absent (Figs. 1, 18, 48). A few tangential

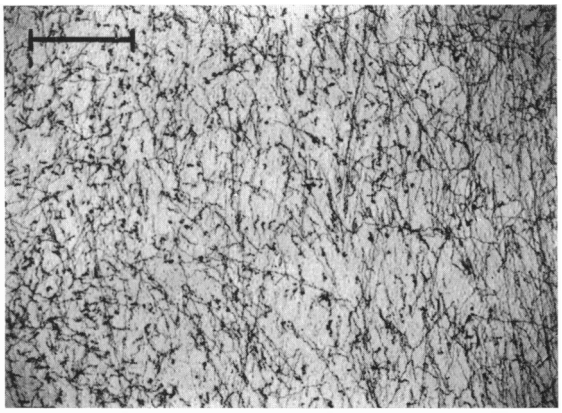


Fig. 13

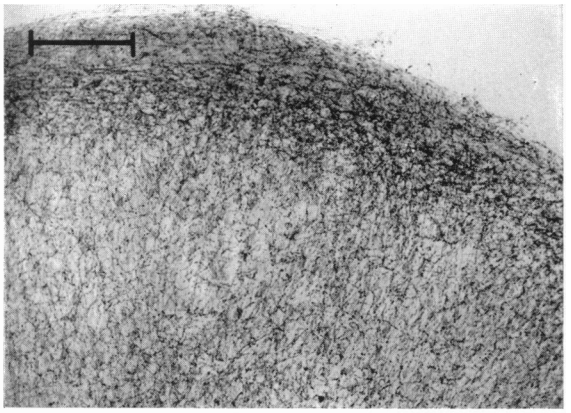


Fig. 16

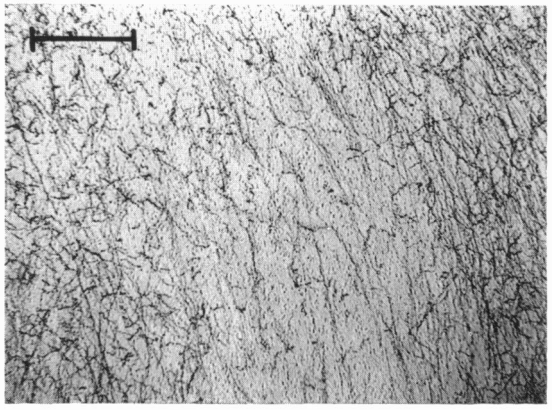


Fig. 12

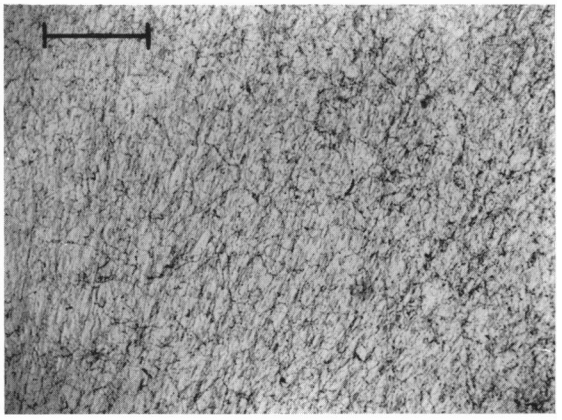


Fig. 15

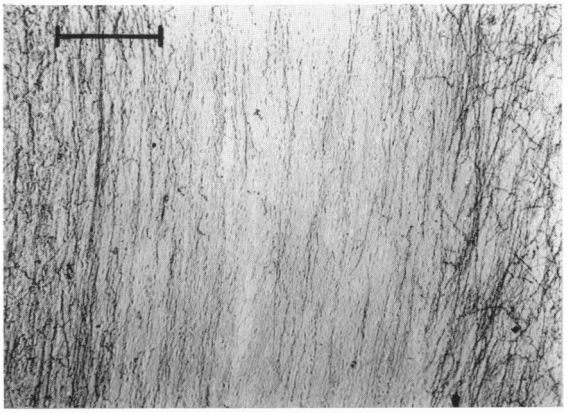


Fig. 11

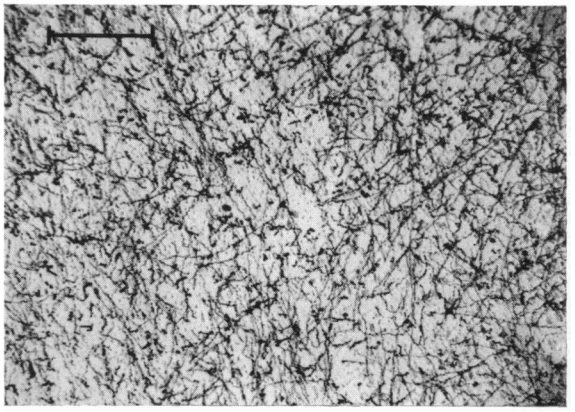


Fig. 14

fibres may be visible (Fig. 20), occasionally extending over quite long distances, even from one gyrus to the next, like U-fibres. In some cases, and especially nearer the fissures, the staining may be more substantial. It then shows tangential fibres, and a poorly organized but relatively dense neuropil, often seen best in the deeper half of the marginal layer, near its junction with the underlying cellular layers. As already mentioned, the superficial fibrillar network may be clearly continuous with the deeper network; even when the two are separated by a zone with only slight or diffuse staining, occasional fibres can be seen ascending directly from the white matter to the plexiform layer, where they divide into horizontal branches which join the superficial plexus. Pial fragments are largely unstained, except for a thin line of granular staining commonly seen over the whole surface of the hemisphere.

Cellular staining

A high degree of AChE activity, comparable with that of known cholinergic neurones in the brain stem and spinal cord or of some cells in subcortical areas (cf. Fig. 24), is never seen in cortical cells. Nevertheless, some cells show very definite activity, in contrast to the majority which are hardly detectable without counterstaining.

Distribution of stained cells

Although a few stained cells are found in all layers they are mostly concentrated in two relatively well-defined groups, corresponding to deep pyramidal cells of layer V and polymorph cells of layer VI.

Pyramidal cells of layer V. Some of the most regular and conspicuous AChE activity is that of the deep pyramidal cells in or near the outer border of the zone of diffuse fibrillar staining (Figs. 18, 19, 21; see also plate 1 in Krnjević & Phillis, 1963*b*). The stained cells tend to be in little clusters and they often make up a distinct layer which is continuous over a limited horizontal distance. Individual cells in a stained cluster show a variable amount of AChE activity, while many surrounding cells are not stained at all, or give a minimal reaction (Fig. 21). Some large neurones are especially well stained. Similarly stained isolated cells are also found at other depths, but they are very rare in superficial layers.

Most of the staining of deep pyramidal neurones is at the soma; it extends for only a short distance along the base of the apical dendrite and not at all into the axon (Figs. 21, 22, 55). In general there appear to be two components of staining: one superficial and one inside the cell. Superficial staining is usually related to the surrounding, more or less diffuse, fibrillar network, which seems to form a system of terminals over the cell body and the proximal part of the dendrite (Figs. 21, 22 and 55). The appearance and concentration of these terminals varies considerably with different cells, but sometimes the surface of the neurone is almost covered with little round bodies or rods.

Figs. 11–13. Continuous ascending sequence, illustrating fibre staining at various levels in a typical gyrus (marginal). Figs. 11 and 12 show the subcortical white matter, and Fig. 13, the inner margin of cortex. Scales: 200 μm.

Figs. 14–16. Continuation of sequence shown in Figs. 11–13. Fig. 14 shows the deep network in layer VI, Fig. 15, the diffuse staining in layer V, and Fig. 16, the superficial zone. Scales: 200 μm.

The deep component of staining is either a diffuse coloration of the cytoplasm or an intracellular accumulation of rod-like particles; it is not always readily distinguishable from superficial staining (Fig. 22). The intracellular staining is usually absent in the region of the nucleus.

Polymorph cells of layer VI. Although many of these cells are usually stained, they form a less conspicuous group than the deep pyramidal cells, partly because

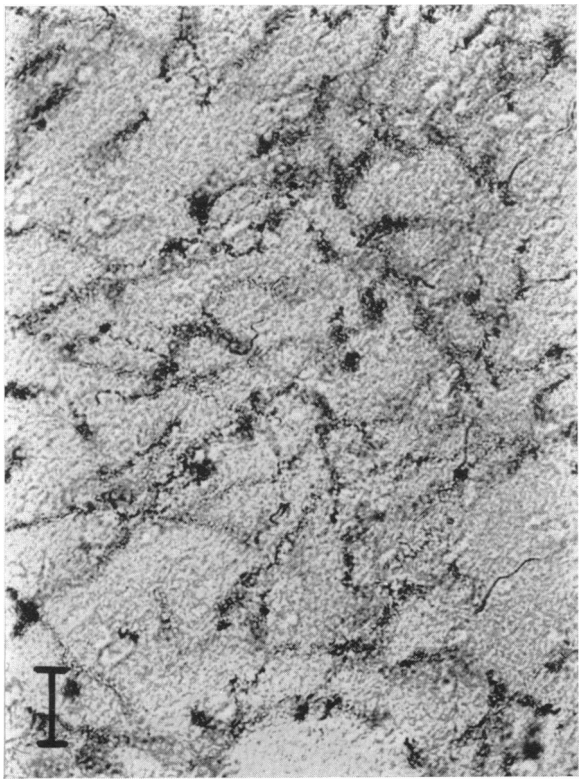


Fig. 17

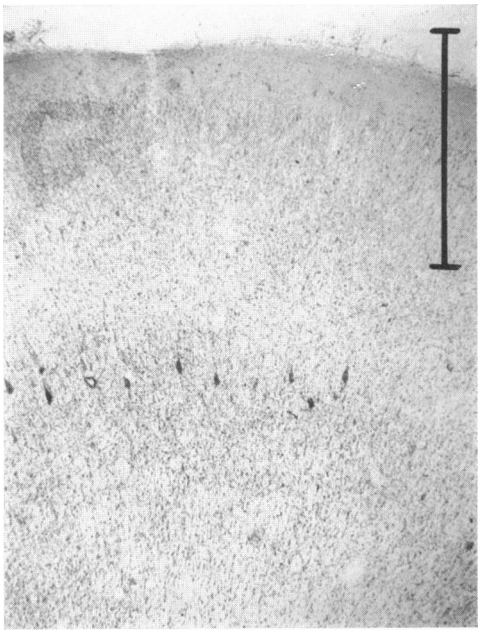


Fig. 18

Fig. 17. High-power view of deep network of fibres in cortex. Scale: 25 μm.

Fig. 18. Deep pyramidal (Betz) cells in outer part of zone of diffuse staining, in anterior sigmoid gyrus. Scale: 1·0 mm.

they are much smaller, and partly because they are not arranged in a distinct layer (Figs. 19, 23). They are associated especially with the deep network and the U-fibres, to which their processes seem to contribute.

Characteristically, the staining of the polymorph cells is relatively dense; it is situated in the cytoplasm, filling the cell body (except for the clear nucleus) and extending into the dendrites and the axon (Fig. 23). The staining of these cells therefore differs qualitatively from that of pyramidal cells, which is mainly superficial; it resembles more closely the staining of some large cells found in the fornix and septum (Fig. 24) or in the brain stem. Stained polymorph cells are found

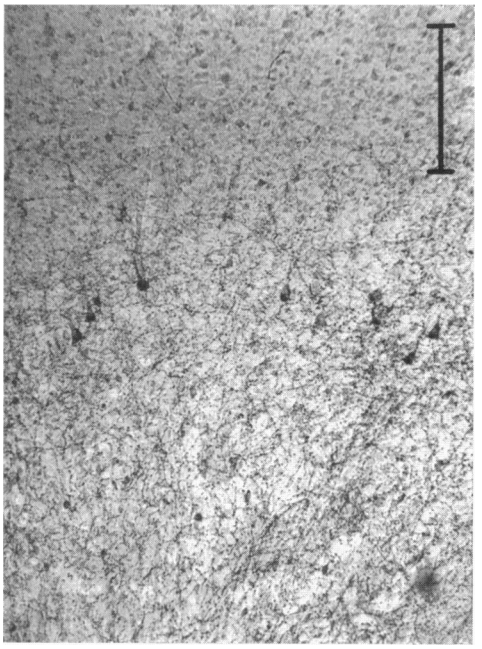


Fig. 19. Deep pyramidal cells near outer edge of zone of diffuse granular staining in marginal gyrus. Small polymorph cells are also visible in lower part of figure. There is no AChE activity in uppermost portion of the figure, which only shows cells counterstained with cresyl violet. Scale: 250 μm.

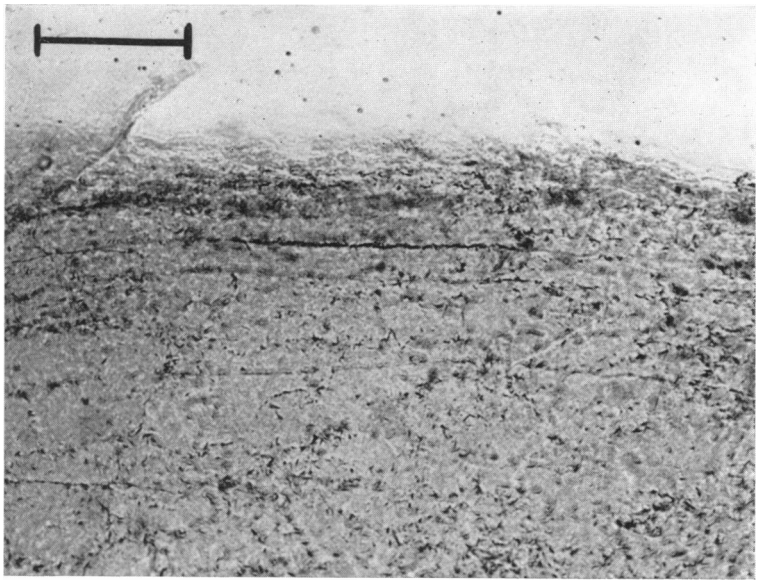


Fig. 20. Stained fibres in plexiform layer. Scale: 50 μm.

particularly in layer VI, but a few occur in some other layers (e.g. layer I). Cells of a similar character are sometimes seen in the U-formations beneath the cortex (Fig. 25).

AChE activity in the walls of a sulcus

Distribution in layers

Superficial. When compared with the adjacent gyral convexity, the outstanding peculiarity is the marked increase in superficial staining in the plexiform layer

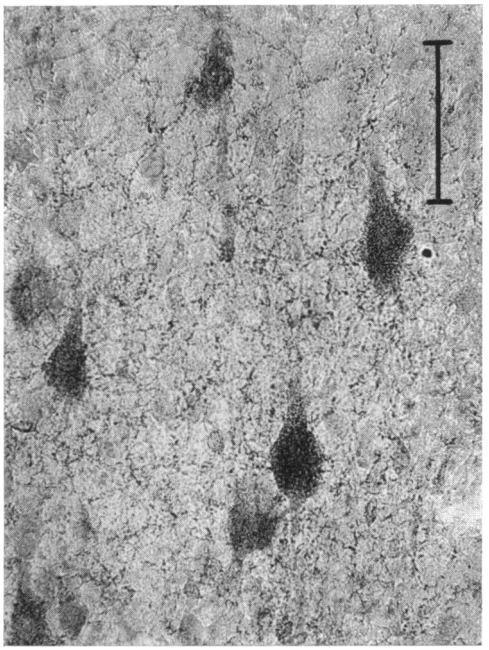


Fig. 21

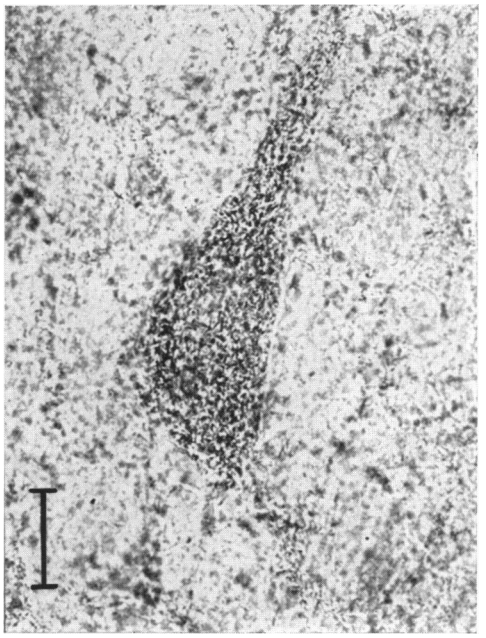


Fig. 22

Fig. 21. Pyramidal (Betz) cells of layer V in anterior sigmoid gyrus; note surrounding diffuse fibrillar network. Cellular staining does not extend into dendrites or axons. Scale: 100 μm.

Fig. 22. High-power view of stained Betz cell, with fibrillar 'endings' on its surface. Scale: 20 μm.

(Figs. 1, 26 and 48). There is a progressive widening of the stained zone near the bottom of the fissure (Fig. 26), presumably as a result of the well-known tendency for superficial layers to expand in this region. Although the superficial zone has a comparatively high level of activity, the strongest staining is often not at the surface itself, but somewhat deeper down at the boundary with layer II. The most superficial zone (about 0·2–0·5 mm thick) may stand out by contrast as a relatively clear crescent (Figs. 1, 3).

As in other parts of the cortex, the deeper zone of staining is outlined by the pale layer III, but this is somewhat narrower in the walls of the sulcus than elsewhere.

Deep. Near the margin of a sulcus the deep staining is comparable with that seen at the top of a gyrus; one finds a deep network of well-defined fibres extending from the white matter, and then, more superficially, a zone of comparatively diffuse staining, which includes layer V and probably layer IV. This band of deep staining becomes very attenuated near the bottom of the sulci, where most of the AChE activity is at the boundary between grey and white matter, mainly in the U-fibres arching around the sulcus. Because the cortex is thinner in the sulcal trough, while the superficial layers are relatively wide, the deeper stained layers (V and IV) are

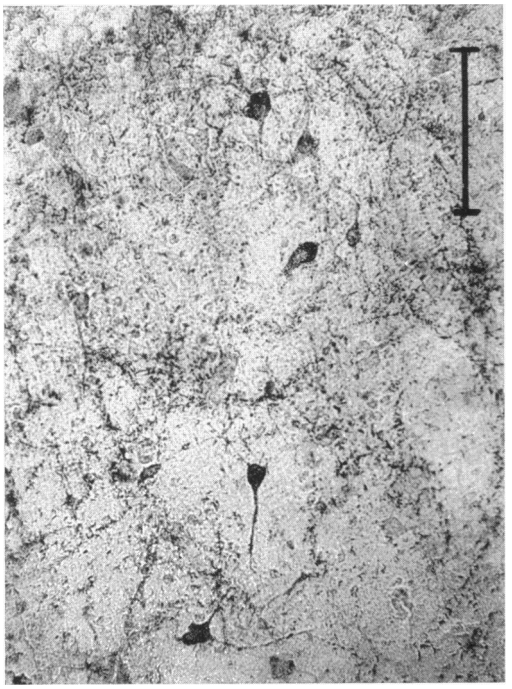


Fig. 23

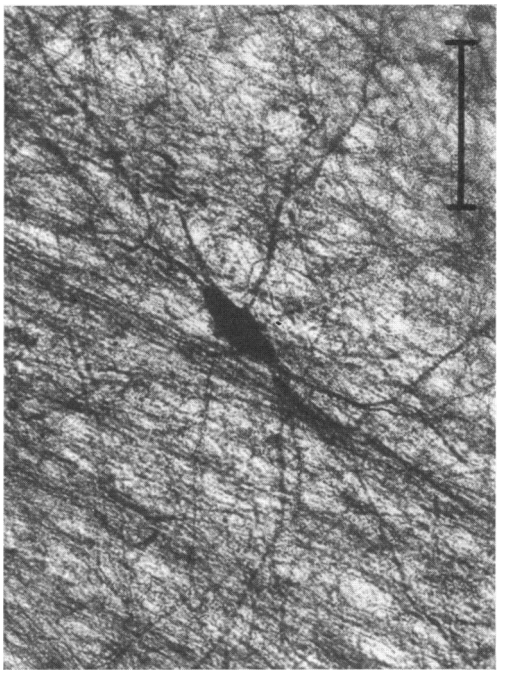


Fig. 24

Fig. 23. Small polymorph cells of layer VI in posterior sigmoid gyrus showing strong AChE activity extending into the neuronal processes. Scale: 100 μm.

Fig. 24. Intense AChE activity in cell in septum. Scale: 100 μm.

necessarily much reduced. These layers of staining are continuous all round the walls of a sulcus, and so they can be seen as concentric bands in a section cut across a sulcus (cf. Fig. 36).

Staining of fibres. In the walls of the sulcus the stained fibres are distributed in much the same way as in the rest of the gyrus. From the layer of fibres which runs alongside the core, many fibres or branches pass towards the buried cortex. In the deeper layers they form the usual network; this becomes more diffuse, with fewer clearly recognizable fibres, as it approaches the superficial layers. However, instead of gradually disappearing, the staining becomes more intense near the surface; many stained fibres are visible here, but the main AChE activity is diffusely distributed in profuse irregular deposits of crystals.

Under the floor of the sulcus, there is a radical change: the deep network is poorly defined, since only a few stained fibres pass into the grey matter; they mostly run together in an arc at or below the boundary between the grey and white matter (Figs. 3, 27, 30). They can be traced into each adjacent gyrus, where they are continuous with the system of stained fibres visible on either side of the relatively unstained core, already described above. These fibres evidently belong to the tangential system generally known as U-fibres or arcuate fibres. In a sulcus the

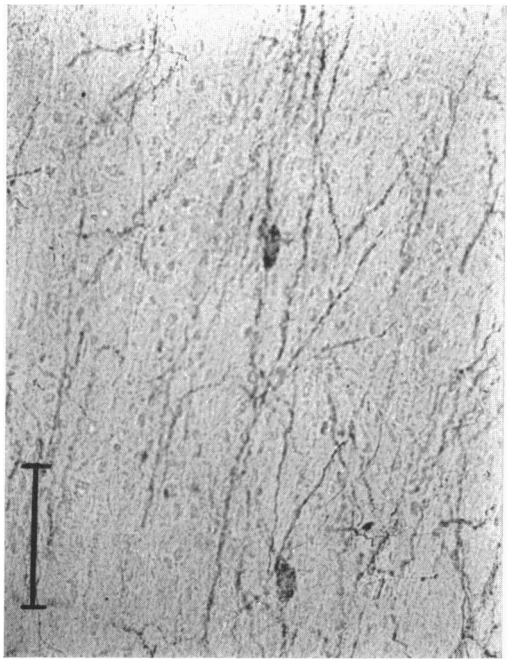


Fig. 25

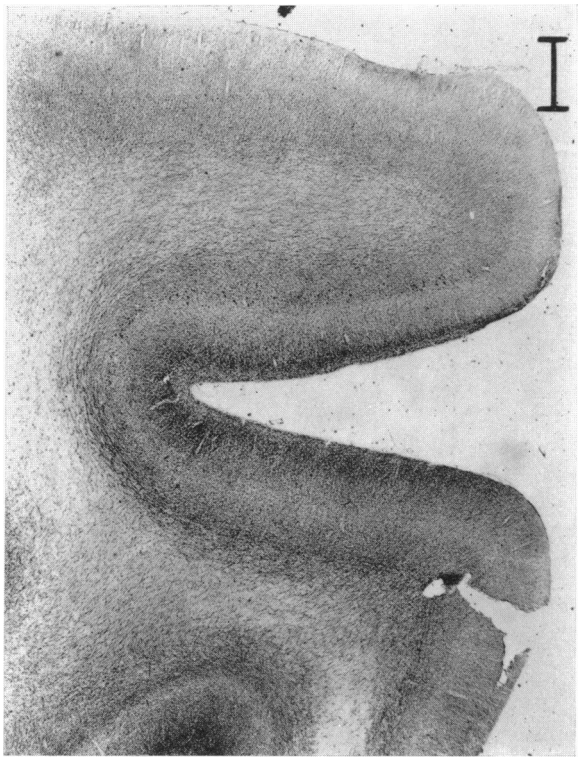


Fig. 26

Fig. 25. Small fusiform cells closely associated with U-fibres near base of suprasylvian gyrus. Scale: 50 μm.

Fig. 26. Frontal section through cruciate sulcus, showing clearly U-fibres and the deep and superficial zones of staining; the latter is particularly developed in the walls of the sulcus. Black dots at outer margin of deep zone are stained pyramidal cells. Scale: 1·0 mm.

superficial zone of strong staining is at its widest; when viewed under high power it reveals mostly thick deposits of crystals forming a rough kind of network but few definite fibres (Fig. 28). A relatively clear zone is sometimes present near the surface.

Cellular staining. In the walls of the sulcus stained cells are often seen, especially deep pyramidal cells which tend to be arranged in a continuous layer near the outer boundary of the zone of deep staining. Such cells are seen very clearly in the walls

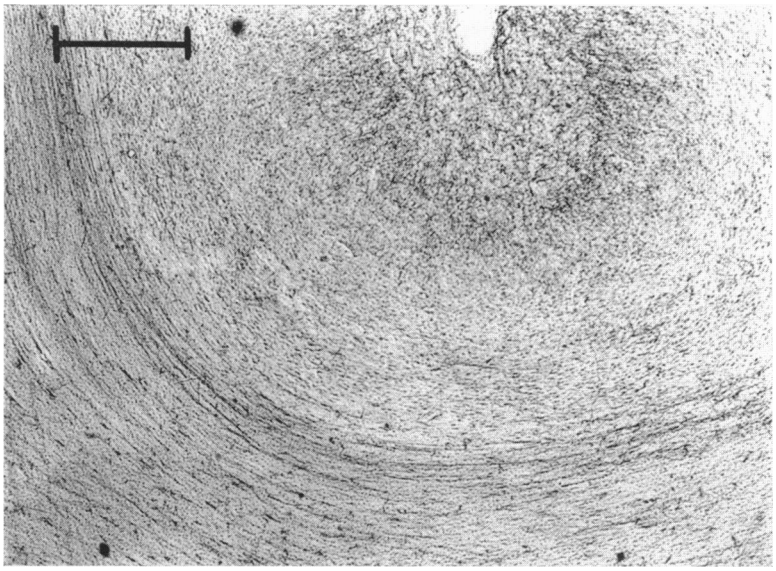


Fig. 27. U-fibres curving round splenial sulcus. Scale: 0·5 mm.

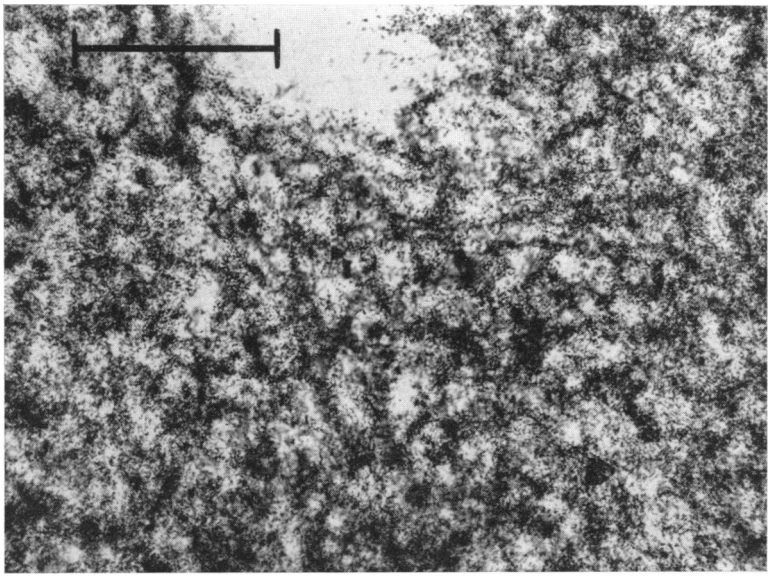


Fig. 28. High-power view of densely stained neuropil in plexiform layer at bottom of sulcus. Scale: 100 μm.

of the cruciate sulcus, where they are particularly large. For instance, in Fig. 26 they can be distinguished as black dots, just within the zone of deep staining.

At the bottom of the sulcus, there are fewer stained cells and these are usually rather small. They are found mostly just above the layer of U-fibres. Stained polymorph cells are also seen; some of them are fusiform and they bear a particularly close relation to U-fibres, like those illustrated in Fig. 25.

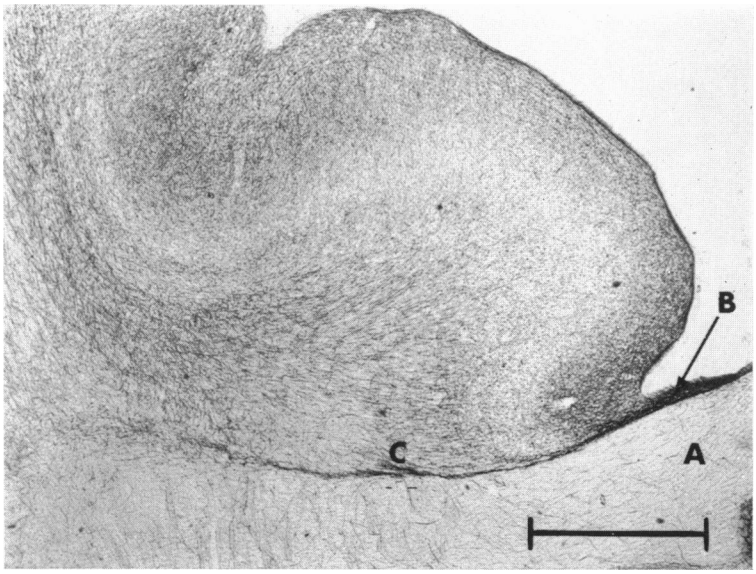


Fig. 29. Frontal section through limbic cortex showing numerous stained fibres in cingulum and supracallosal formation; these are joined by fibres which 'perforate' the corpus callosum. A, Corpus callosum; B, induseum griseum; C, cingulum. Scale: 1·0 mm.

AChE activity in different cortical areas. The above description applies typically to most areas of the neocortex, with only relatively minor variations between different areas of the outer aspect of the hemisphere, including the pericruciate region anteriorly, the wide expanse of parietal cortex around the lateral, suprasylvian and the upper parts of the ectosylvian and sylvian sulci, and the occipital region. There is a tendency for the staining to be particularly dense and the fibrillar network especially well developed towards the frontal pole and near the base of the hemisphere.

The most obvious atypical region is that of gyri which surround the corpus callosum, such as the greater part of the cingulate gyrus (Figs. 1, 29). The characteristic features are much stronger staining in superficial layers, even on the surface of the gyrus, and no clear central core, since this is occupied by stained fibres of the cingulum. Cellular staining is relatively inconspicuous in this region.

Interconnexions of stained areas

Within the neocortex. The staining has so far been described mainly as seen in isolated areas. To what extent are the stained elements joined in a continuous system?

It is evident that the stained U-fibres arching around sulci form a link between the stained fibrillar networks in the adjacent gyri. As already pointed out, when a sulcus is cut across (cf. Fig. 36), in whatever plane, a continuous layer of stained

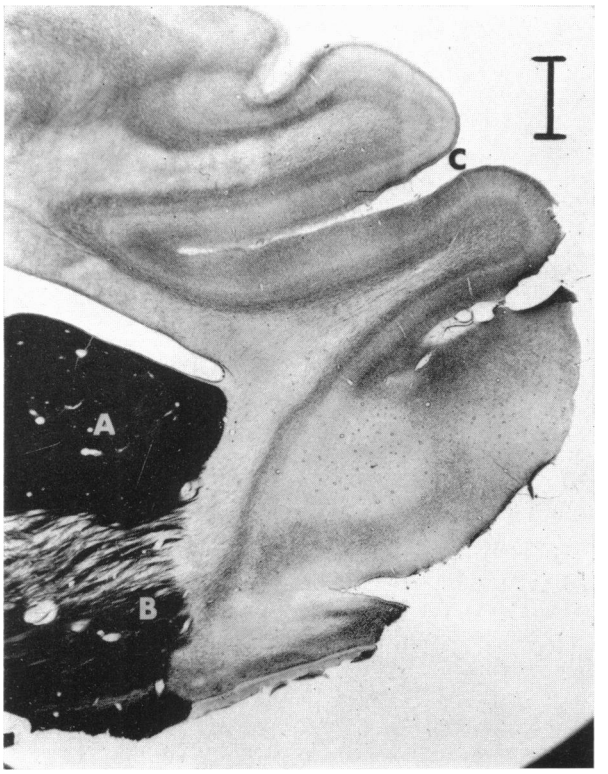


Fig. 30

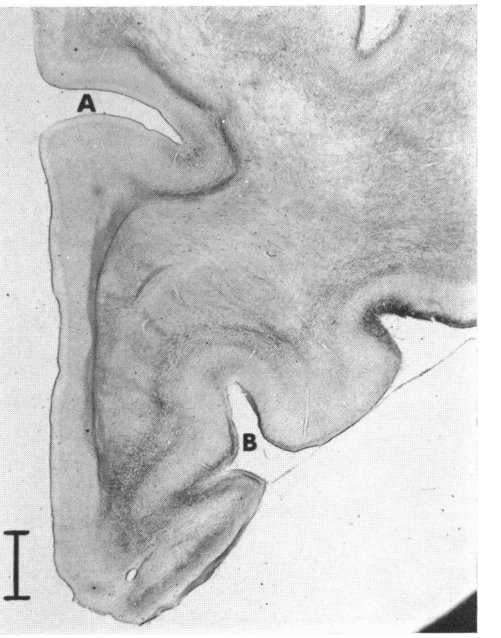


Fig. 31

Fig. 30. Parasagittal section showing continuous layer of staining around cruciate sulcus; from precruciate region it extends back to base of striatum, which is particularly strongly stained. A, Caudate nucleus; B, putamen; C, cruciate sulcus. Scale: 2·0 mm.

Fig. 31. Section through frontal pole, showing continuity of staining in pericruciate region, gyrus proreus and cortex around anterior rhinal fissure. A, Cruciate sulcus; B, anterior rhinal fissure. Scale: 2·0 mm.

fibres is visible around it. For instance, in Fig. 30, a parasagittal section through the cruciate sulcus, one can see that the stained pericruciate U-system (cf. Fig. 26) extends right round the cruciate sulcus, even its deepest portion. Another view of this region, cut in a frontal plane (Fig. 31) again shows the continuity of the staining around the cruciate sulcus, and its continuity with similar staining in gyrus proreus and gyrus orbitalis.

In this way a continuous layer of staining, associated more or less clearly with U-fibres, can be traced under the grey matter over the whole hemisphere. Of course, it does not follow that there are direct connexions between all the various areas. Since the plane of section is not necessarily parallel with the plane of the fibres, which usually appears to be at right-angles to the long axis of the sulcus, one cannot always determine the probable orientation of the fibres. Although the U-fibres can be seen around all sulci, they are consistently more evident in certain areas. For instance, those connecting the cingulate and splenial gyri in the medial wall of the

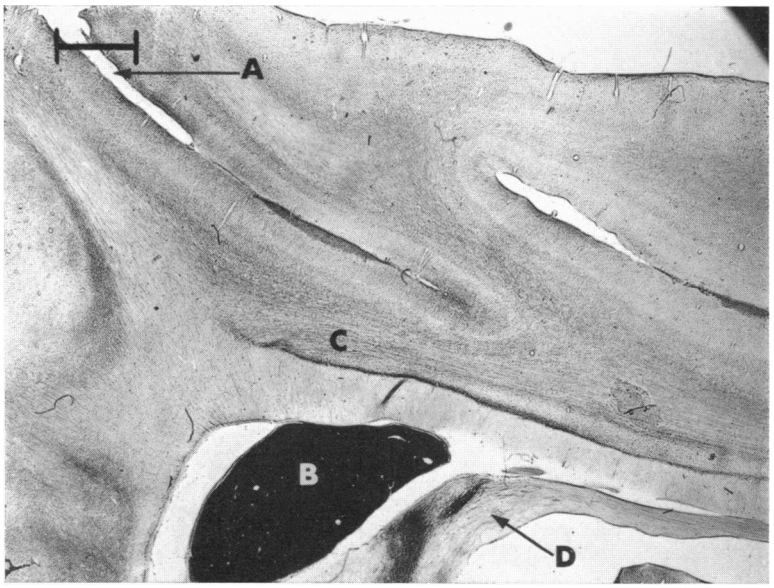


Fig. 32. Parasagittal section in region of cruciate sulcus. Note connexion between cingulum and pericruciate fibres. A, Cruciate sulcus; B, caudate nucleus; C, cingulum; D, septum and fornix. Scale: 2·0 mm.

hemisphere are always particularly distinct (Fig. 27); and, more laterally, the ectosylvian and suprasylvian gyri are usually linked by very substantial U-bundles. In contrast, the links between the marginal and suprasylvian gyri are often poorly defined.

Many stained fibres by-pass a given gyrus instead of following the predominant U-formation and then join U-fibres in the next gyrus. For instance, fibres from the cingulate region by-pass the suprasplenial gyrus towards the marginal, and other fibres from the ectosylvian region by-pass the suprasylvian gyrus to reach the marginal from the lateral side. Such fibres are not usually organized into clear bundles visible in frontal sections. Only in parasagittal sections does one see a substantial system of long connexions, in the medial wall of the hemisphere. These are the stained fibres of the cingulum, which are grouped in a thick tract extending over the full length of the corpus callosum; many of its fibres can be traced forward into the frontal pole and down into the precommissural septum; caudally they project into the region behind and below the splenium, some of them going as far

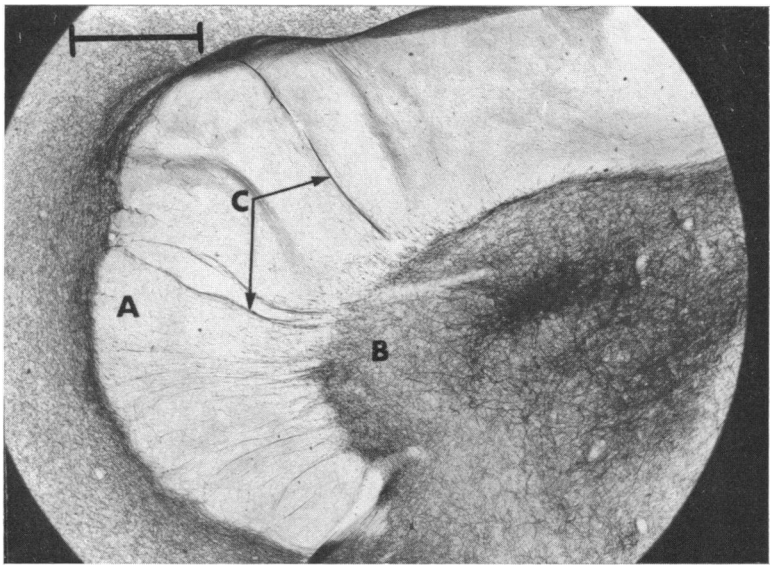


Fig. 33. Sagittal section through genu of corpus callosum. The strongly stained medial septal nucleus is linked with the supracallosal formation by 'perforating' fibres. A, Genu; B, septum; C, 'perforating' fibres. Scale: 1·0 mm.

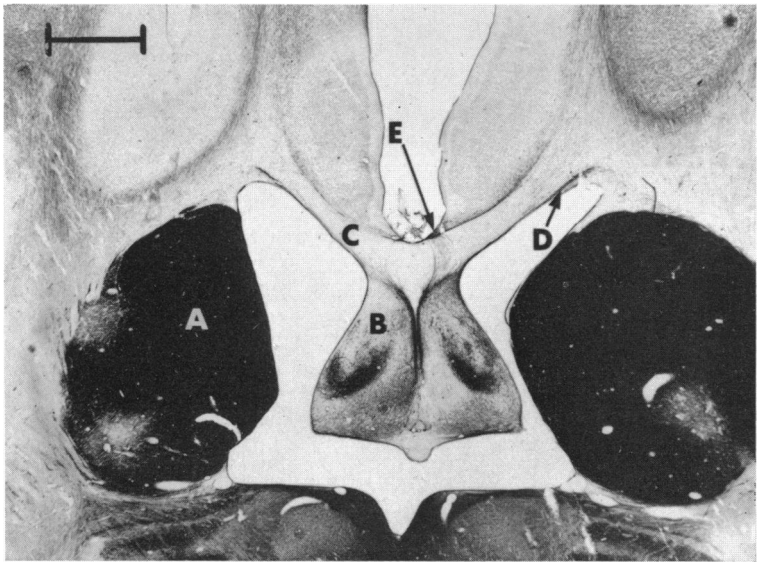


Fig. 34. Horizontal section of kitten's forebrain. Note intensely stained caudate nuclei and strong staining in septum. The latter is directly continuous with 'subcallosal band' and is linked by 'perforating' fibres with induseum griseum and other parts of supracallosal formation. A, Caudate nucleus; B, septum; C, corpus callosum; D, 'subcallosal band'; E, induseum griseum. Scale: 2·0 mm.

as the hippocampus. Rostral fibres intermingle with U-fibres curving round the medial portion of the cruciate sulcus, some joining the precruciate and others the postcruciate fibres (Fig. 32).

Connexions with structures outside the neocortex. Even from a cursory examination (cf. Fig. 1) it is clear that there are unlikely to be distant connexions through the main projection or commissural pathways. This is confirmed by a closer study of the white matter in the internal capsule, the corpus callosum (Figs. 33, 34) and the

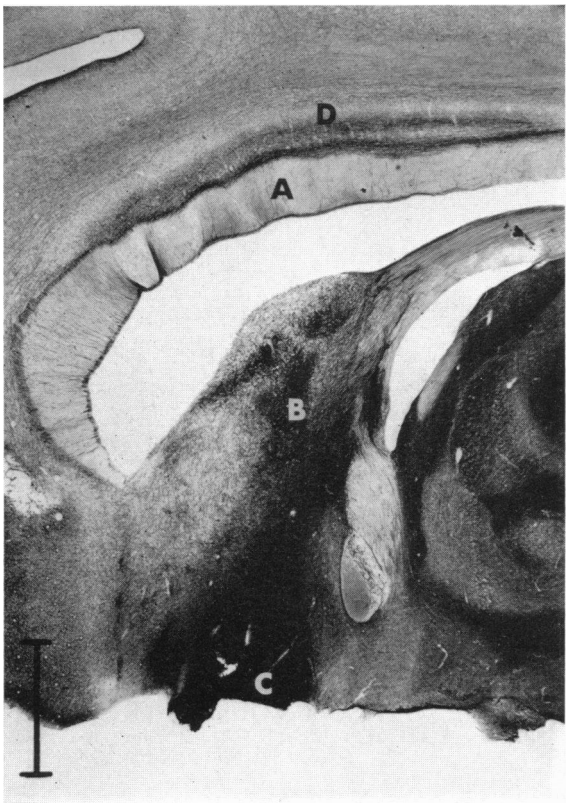


Fig. 35. Precommissural septum and olfactory tubercle at the base of the brain in sagittal section. A, Corpus callosum; B, septum; C, olfactory tubercle; D, cingulum. Scale: 2·5 mm.

anterior commissure (Fig. 1), all of which contain very few stained fibres. The latter are often obviously travelling in a different direction from that of the predominant elements, as in the corpus callosum (Figs. 29, 33).

The only substantial connexions between the neocortical staining and other regions seem to occur at the following points: in the limbic area along the medial margin of the hemisphere; in the temporal region by connexions with the striatum; by an intermediate route via the 'subcallosal band'; and, round the rhinal fissure, with the palaeocortex on the inferior surface of the hemisphere.

In the limbic area. The cortex of the marginal and suprasplenial gyri is linked with the limbic region by U-fibres around the splenial and cruciate sulci (Fig. 1). Medial neocortical fibres thus come into close relation with fibres of the cingulum and with the supracallosal formation. The latter, which consists of the induseum griseum and the corresponding longitudinal striae, is invariably very strongly stained (Figs. 1, 29, 34, 35), as was pointed out by Gerebtzoff (1959). It is generally believed to be the only remnant of the supracallosal component of the archicortex and can readily be

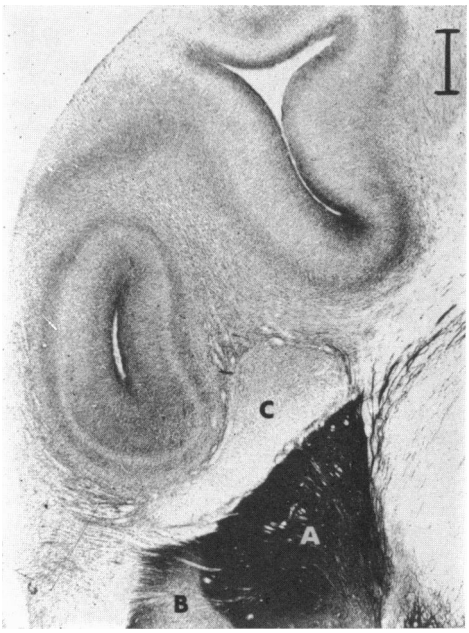


Fig. 36

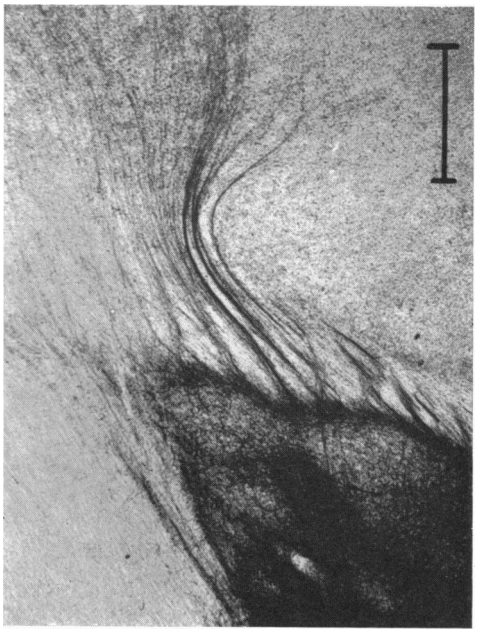


Fig. 37

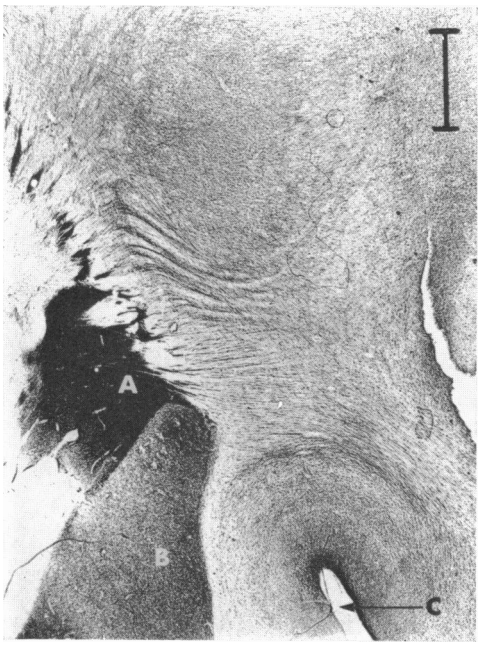
Fig. 36. Section through pallium overlying the stained putamen. There is also some weaker staining of the lateral amygdaloid nucleus at the left of the putamen but the claustrum is much paler. Two sulci have been cut and show pattern of AChE activity in concentric rings. A, Putamen; B, lateral amygdaloid nucleus; C, claustrum. Scale: 1·0 mm.

Fig. 37. Fibres in external capsule emerging from putamen and curving around claustrum (cat foetus just before term). Scale: 0·5 mm.

traced posteriorly around the splenium of the corpus callosum into the hippocampus. Anteriorly, it is continuous with strong staining in the precommissural septum. It is joined by a substantial number of 'perforating' fibres which travel through the corpus callosum (Figs. 29, 33, 34). The supracallosal region is thus connected with the dorsal fornix and the strongly stained septal nuclei (Figs. 33, 34). The staining of the induseum is continuous with the relatively well-developed superficial staining of the cortex in the medial wall (Figs. 1, 29). The cingulum, which runs in an antero-posterior direction in the white matter of the cingulate gyrus (Figs. 29, 32), appears to be linked, throughout its length, with the supracallosal formation and also with the U-fibres ascending in the medial wall.

Striatal links through the external capsule. The lower rostral and lateral areas of the neocortex, in the anterior sigmoid, lower coronal, anterior suprasylvian, lower ectosylvian and sylvian gyri show a continuous layer of staining which can be followed through the external capsule to the deeper portion of the striatum.

For instance, one can see strong AChE activity in the external capsule in Figs. 1



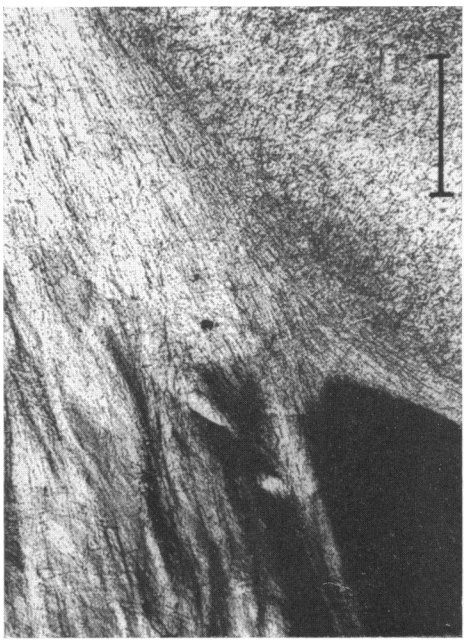


Fig. 38 Fig. 39

Fig. 38. Stained fibres from putamen radiating towards lateral cortex (frontal section). Note also, lateral amygdaloid nucleus below putamen showing some activity, and U-fibres around rhinal fissure linking neocortex and palaeocortex. A, Putamen; B, lateral amygdaloid nucleus; C, rhinal fissure. Scale: 0·5 mm.

Fig. 39. High-power view of stained fibres leaving rostral aspect of putamen. Scale: 0·5 mm.

and 36. At higher magnification (Fig. 37) it is evident that this is due to large numbers of fibres leaving the intensely stained mass of the putamen. Very few of them enter the overlying claustrum; instead they curve round it and are then projected forwards, backwards and upwards towards the cortex of the lateral aspect of the hemisphere (Fig. 38).

Many stained fibres can also be seen leaving the putamen and basal portion of caudate nucleus (Fig. 39) to form a stained band extending upwards and rostrally towards the anterior sigmoid gyrus, where they are continuous with the deep layer of staining (Figs. 2, 30, 40, 48). Most of these fibres do not travel along this band in a straight line, and therefore cannot be traced continuously from the striatum to the pallium, but evidence will be given below that many of them probably do reach the cortex (see description of the effects of chronic lesions). Similar fibres also pass into gyrus proreus and gyrus orbitalis.

'*Subcallosal band*'. In addition to the stained fibres which belong to the U-system, an appreciable number of isolated fibres come up from the deep white matter in an irregular manner, without forming an obvious tract (Fig. 41). The great majority can be traced to a band of staining under the callosum and the callosal radiation, close to the roof and the outer wall of the lateral ventricle (Figs. 1, 2, 34 and 41–43). We have called this the 'subcallosal band', because it bears an intimate relation to the

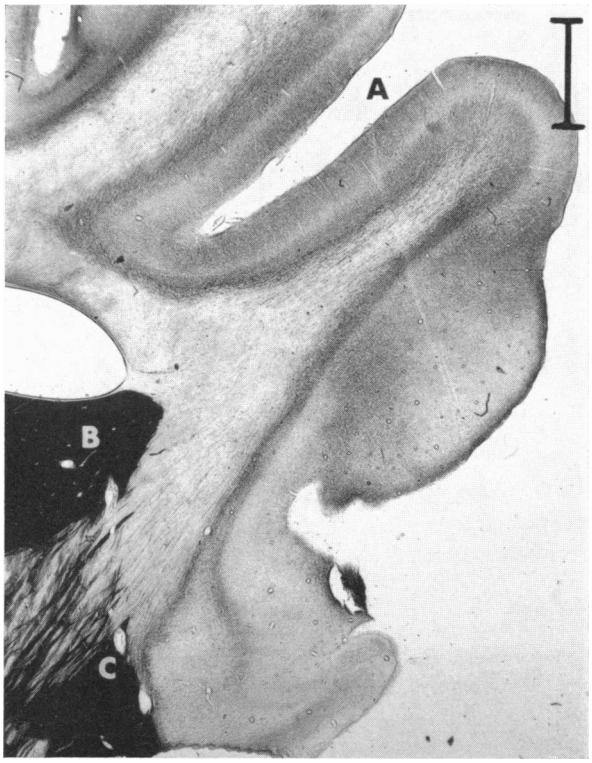


Fig. 40. Parasagittal section showing fibres from putamen passing towards anterior sigmoid region. A, Cruciate sulcus; B, caudate nucleus; C, putamen. Scale: 2·0 mm.

bundle of fibres usually known as the subcallosal or superior occipito-frontal fasciculus or stratum (Onufrowicz, 1887; Muratoff, 1893; Vogt & Vogt, 1902; Winkler & Potter, 1914).

The lateral portion of the 'subcallosal band' is sharply limited to the outer boundary of the crescent-shaped subcallosal bundle, where this caps the angle of the ventricle (Figs. 41–43). As can be seen in these figures, there is little or no staining in the subcallosal bundle itself.

The 'subcallosal band' can usually be followed round the outer angle of the lateral ventricle as far as the caudate nucleus (Fig. 42). But this lateral or striatal portion is less well developed than the medial, sometimes containing only a few detectable fibres, especially more rostrally, and in young animals. If traced medially, past the

crescent of the subcallosal bundle, the stained band is found near the ventricular surface, close to the ependyma. It extends below the corpus callosum, and thus reaches the dorsal fornix and septum with which it is continuous (Fig. 34).

All along its course the 'subcallosal band' is connected with the overlying cortex by numerous isolated fibres. Its medial portion is thus joined with the network of

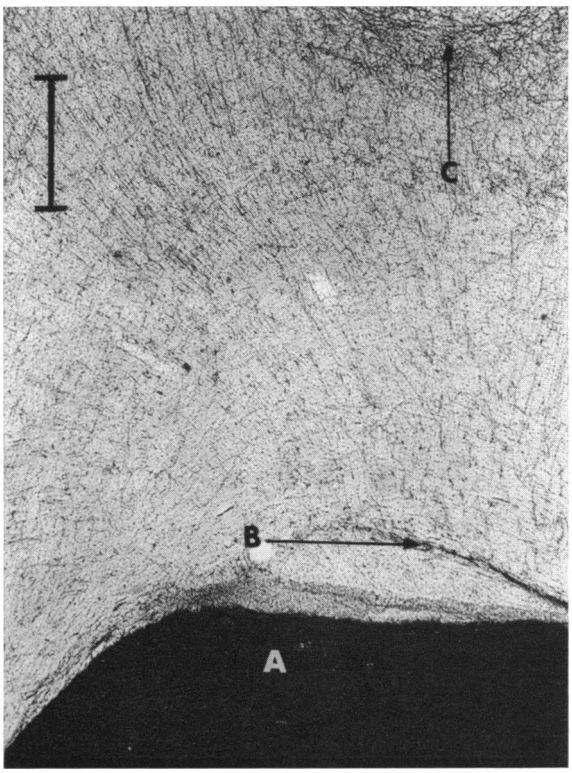


Fig. 41. Fibres passing from 'subcallosal band' towards the overlying (mainly suprasylvian) cortex; note lack of staining in the subcallosal bundle between the band and the black mass of the head of the caudate nucleus. A, Caudate nucleus; B, 'subcallosal band'; C, bottom of suprasylvian sulcus. Scale: 0·5 mm.

fibres in the cortex of the medial wall of the hemisphere, reaching as far as the marginal and even suprasylvian gyri on the outer surface. Fibres from the rostral part form links with the medial pericruciate region. Laterally, similar but fewer fibres connect with the gyri on the lateral aspect of the hemisphere. One can sometimes see a clear division at a point where all the more medial fibres appear to come from the fornix, and all the more lateral fibres from the caudate nucleus.

The 'subcallosal band' is also visible in sagittal sections along the lower margin of the callosal radiation, from its rostral end to the hippocampus (Fig. 2). The band therefore forms an extensive layer of fibres which spreads over the entire roof of the lateral ventricle. Some of these fibres may interconnect distant cortical areas, but most of them are likely to provide links with the septum and striatum.

Connexions with palaeocortex and archicortex. The general continuity of staining between the neocortex and palaeocortex on either side of the rhinal fissure is evident from Fig. 1. In the walls of this sulcus, the zone of superficial staining is particularly dense, and it is so wide that it includes nearly the full thickness of the cortex. In addition to this rich superficial network, deeper U-fibres are also present in the extreme capsule, providing a further link between the neocortex and the profuse staining in the pyriform and prepyriform region (Figs. 31, 38). In the same way, there is no break between the dense networks of stained fibres in the hippocampal formation and in the rest of the medial wall.

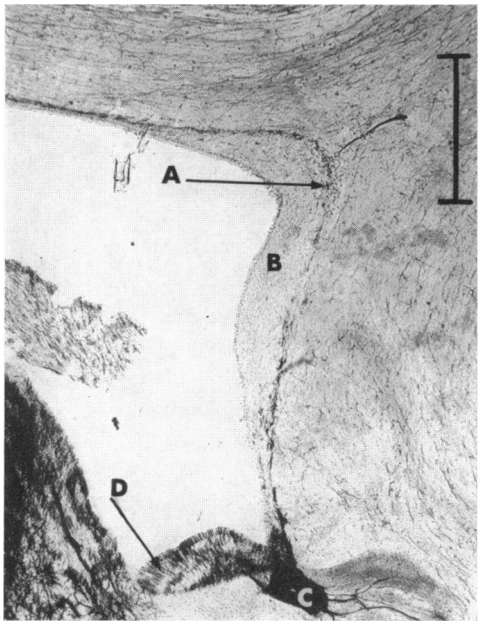


Fig. 42

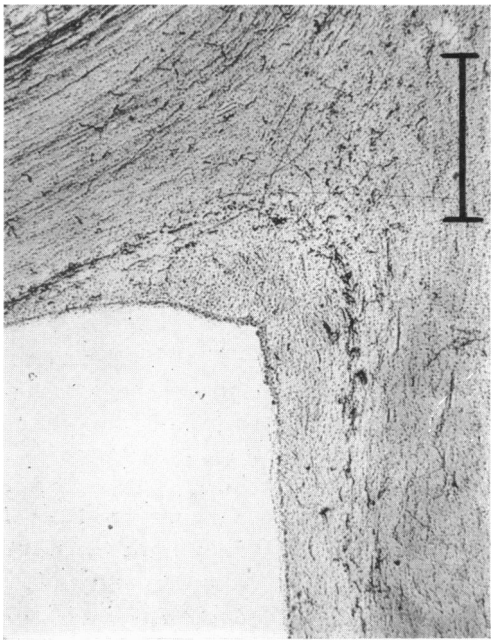


Fig. 43

Fig. 42. 'Subcallosal band' at outer margin of unstained subcallosal fasciculus, below callosal radiation and around lateral angle of ventricle; note fibres in its lower portion emerging from tail of caudate nucleus. Note also staining in adjacent stria terminalis. Frontal section. A, 'Subcallosal band'; B, subcallosal bundle (fasciculus); C, tail of caudate nucleus; D, stria terminalis. Scale: 1·0 mm.

Fig. 43. Higher-power view of 'subcallosal band' in outer angle of lateral ventricle. Scale: 0·5 mm.

AChE staining in other parts of the cat's brain.

As there is no general description in the literature of AChE staining in the cat's forebrain, it is pertinent to review quickly some of its principal features. In most respects the areas of high AChE activity are similar to those seen in other species (Koelle, 1954; Ishii, 1957; Gerebtzoff, 1959; Shute & Lewis, 1961, 1963; Okinaka & Yoshikawa, 1962).

Palaeocortex and archicortex. The palaeocortex of the basal rhinencephalon has a rich supply of stained fibres throughout all layers; there is no contrasting clear zone in the superficial half of the cortex, as in the neocortex, although narrow layers of unstained cells are often visible. On the other hand, an unstained layer is seen at the surface in areas covered by the lateral olfactory tract.

Staining in the amygdaloid complex is variable. The lateral nucleus shows relatively little AChE staining except along its outer edge, but the central and basal nuclei are strongly stained, especially the magnocellular portion of the latter; many fibres which link the putamen and the cortex on both sides of the rhinal fissure pass through the lateral nucleus and the external capsule (Figs. 36, 38).

Some of the most intense ChE activity to be found anywhere in the brain is that on the inferior surface below the striatum, in the region of the olfactory tubercle (Figs. 2, 35). As in the striatum there is so much staining that it is difficult to make out details of the structure, but many very strongly stained cells are readily visible. In the olfactory bulb, there are sharp zones of staining above and below the unstained mitral cells. Septal AChE activity is very strong (Figs. 1 and 33–35); it is found in cells and fibres connected with the cingulum, the dorsal fornix system, the 'subcallosal band' and the hippocampus. Below it is continuous with that of the olfactory tubercle (Fig. 35), the diagonal band of Broca and the pre-optic region, which contains many stained cells similar to those found in the septum and the pallidum.

The archicortex of the hippocampus (Fig. 2) is also richly supplied with stained fibres, many of which probably come from the fornix via the fimbria, as well as from the cingulum and the temporal cortex. The pyramidal cells of Ammon's horn and the granules in the dentate gyrus are unstained, but they are outlined by adjacent layers of strong staining (see also Shute & Lewis, 1963; Mathisen & Blackstad, 1964).

Basal ganglia. Most parts of the basal ganglia have an extremely high level of ChE activity and they form the most conspicuous area of staining in the brain (Figs. 1, 2 etc.). The only exception to this is the claustrum, which, by comparison, appears almost unstained (Figs. 36, 37). As suggested by Gerebtzoff (1959), this would seem to be evidence against the view that the claustrum is developed from the underlying striatum.

The highest activity is present in the caudate nucleus, the nucleus accumbens and the putamen, with somewhat weaker general staining of the pallidum; but the latter contains many very clearly stained cells, whereas the striatum shows much more diffuse staining and somewhat fewer stained cells. Large numbers of stained fibres run between the putamen and the pallidum, and similar fibres connect with the strongly stained entopeduncular nucleus, and the substantia nigra in the midbrain.

Diencephalon. There is a particularly high level of activity in the habenula and its main efferent pathway, the habenulopeduncular tract (fasciculus retroflexus of Meynert), which is probably the most strikingly stained bundle in the whole central nervous system (Fig. 44).

In the thalamus, there is a substantial, but variable, amount of staining affecting particularly the antero-ventral group of nuclei, the reticular nucleus and the lateral geniculate body, as well as the dorsal and medial portions in general. By contrast, the medial geniculate, the ventro-lateral region and the medial lemniscus show

relatively little activity; this does not support the suggestion that the second-order afferent neurones are cholinergic (Feldberg & Vogt, 1948).

There are scattered areas of strong staining in the hypothalamus, particularly in the supra-optic and paraventricular nuclei, and in the pre-optic region; the subthalamic nucleus and the substantia nigra are also consistently well stained.

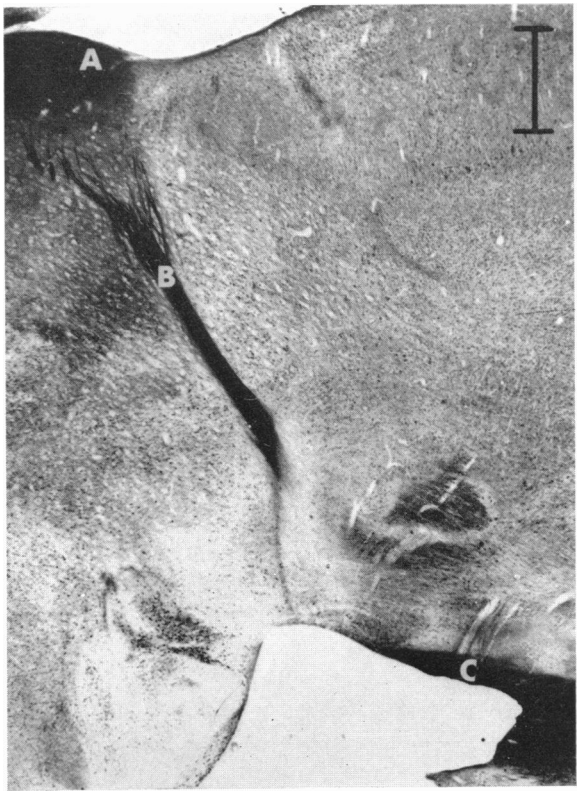


Fig. 44. Parasagittal section of midbrain in kitten showing habenulopeduncular tract. A, Habenula; B, habenulopeduncular tract; C, interpeduncular nucleus. Scale: 1·0 mm.

Cholinesterase staining in the monkey's cortex

There was a considerable variation in the overall amount of staining obtained in the brains of three rhesus monkeys. Since a detailed description of the findings cannot be given here, only the main features will be mentioned. In general, the pattern of staining is very similar to that found in cats. Most of the white matter shows only little AChE activity, the latter being concentrated in a subcortical band which follows the surface contour (Fig. 45), with variable extensions into the cortex. A zone of particularly marked staining is present in the anterior wall of the central sulcus (cf. observations on the human cortex by Okinaka *et al.* 1961).

Examination under higher power (Fig. 46) shows that, as in the cat, the central core of a gyrus tends to be largely unstained, but it is lined on either side by stained

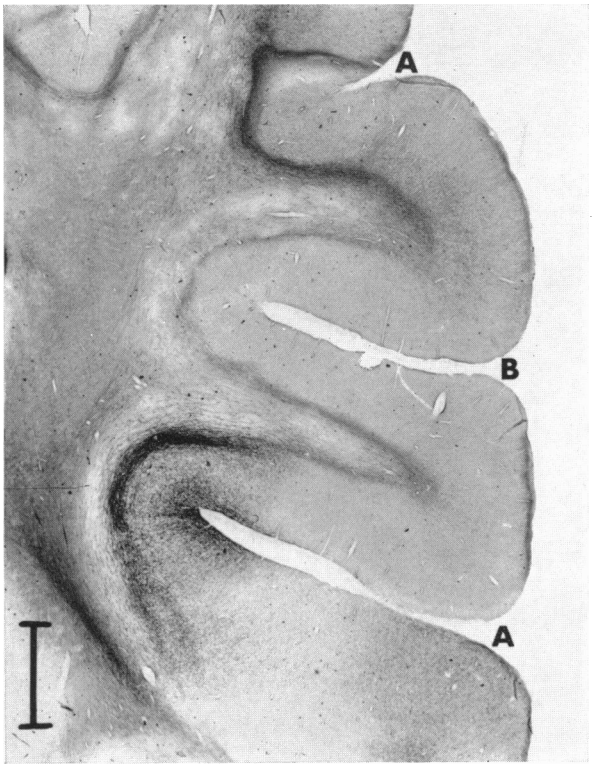


Fig. 45. Pattern of AChE activity in low-power view of frontal section of a rhesus monkey's brain. A, Inferior precentral sulcus; B, frontal sulcus. Scale: 2·0 mm.

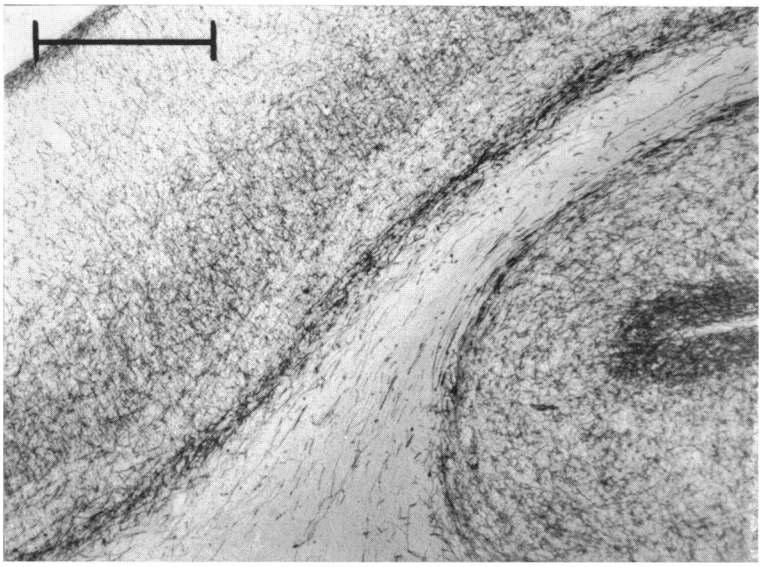


Fig. 46. Stained fibres in the base of a gyrus near occipital pole of monkey's brain. Scale: 1·0 mm.

fibres belonging to U-formations. From these fibres, a network extends into the deeper half of the cortex, but the superficial half of the cortex is relatively free of stain, except for the molecular layer. At the bottom of a sulcus, the network of fibres is continuous with a particularly wide zone of dense surface staining.

AChE activity in chronically isolated slabs of cortex (in cats)

One cannot easily deduce the origin of AChE-containing fibres in normal material, but the absence of very strongly stained cell bodies suggests that relatively few cholinergic cells are situated in the cortex. To obtain further evidence on this point small blocks of tissue in the pericruciate, marginal or suprasylvian regions of the cortex were undercut in fifteen cats. After a sufficient time (7–30 days) to allow for the degeneration of cut afferent fibres, the area of brain containing the lesion was examined for AChE activity (Hebb, Krnjević & Silver, 1963; Krnjević & Silver, 1963*b*). When AChE-containing pathways are cut, there are two main types of changes (Shute & Lewis, 1961): fibres in the distal segment rapidly lose their staining while the proximal segment shows an increased reaction, especially in the region close to the cut.

Effect of undercutting on staining of fibres

Complete or substantial isolation always leads to an almost total loss of fibre staining. For instance, if one compares the isolated anterior sigmoid gyrus of Fig. 47 with the similar area in the contralateral hemisphere (Fig. 48) it is evident that the usual pattern of staining has disappeared. In a higher-power view of the region of the lesion (Fig. 49), fibres are visible up to the cut, with particularly strong staining at the margin, but there is no staining in the isolated portion. A further direct comparison between isolated and normal cortex is shown in Figs. 51 and 52.

The gross accumulation of stain in fibres in the tissue proximal to the cut is visible at higher magnification in Fig. 50. The fibres are swollen irregularly and twisted into worm-like shapes. When they are traced back, some fibres may show characteristic changes of this type for a distance of a few mm, but it is unusual to see unmistakable changes much further away. Lesions affecting the anterior sigmoid gyrus thus cause typical alterations in fibres as far as the putamen, but not further back. This confirms the suggestion that most of the stained fibres in this region of the cortex come from the putamen, without however, giving any indication whether the cells of origin are in the corpus striatum or at a more caudal site in the brain stem. Similar distorted fibres can be traced from the medial pericruciate region to the subcallosal band.

In other areas, afferent pathways are also clearly shown by this procedure, since the proximal parts of cut fibres are usually particularly well stained. Many afferent fibres which do not travel in conspicuous bundles, and which may normally be easily overlooked, become much more prominent after cortical undercutting. It can then be seen that substantial numbers of AChE-containing fibres from the U-formations may by-pass a gyrus to join the next U-bundle or may travel relatively long distances in isolation through the white matter.

When a lesion is deep enough to leave a U-fibre formation intact, fibre staining

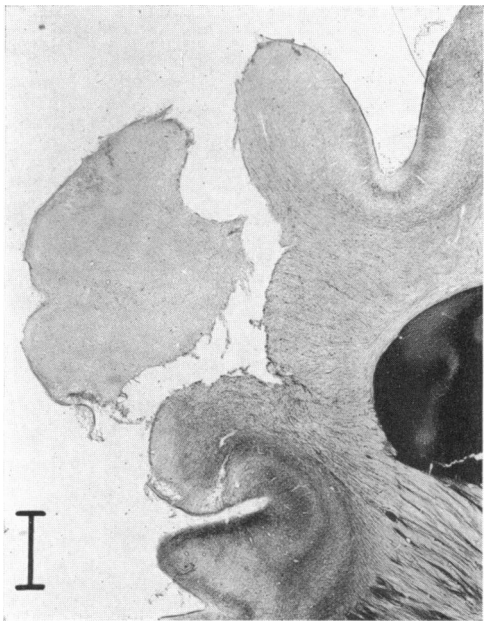


Fig. 47

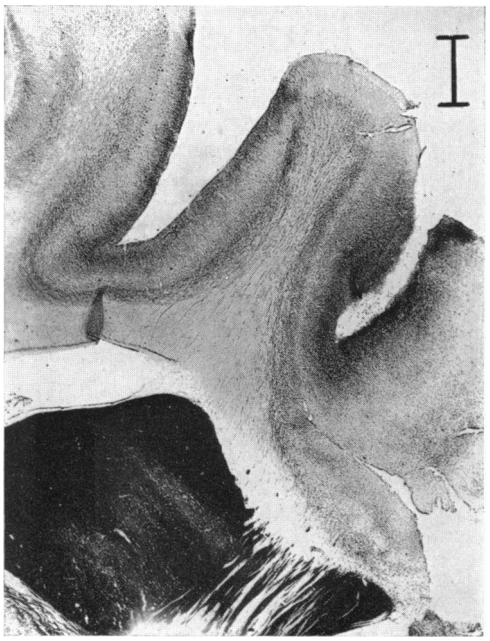


Fig. 48

Fig. 47. Parasagittal section of cat's brain through pericruciate region undercut 14 days earlier. Note loss of staining in isolated portion (cf. Fig. 48). Scale: 2·0 mm.
Fig. 48. Parasagittal section through normal pericruciate region (cf. Fig. 47). Scale: 2·0 mm.

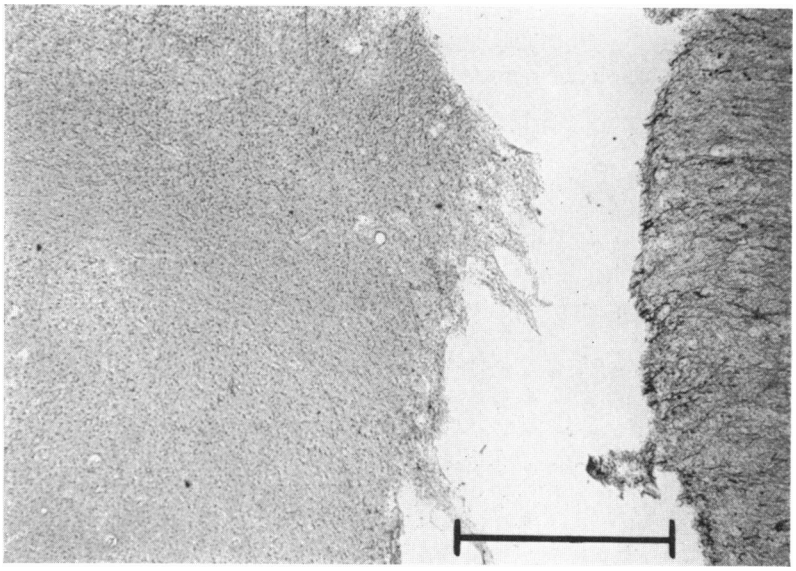


Fig. 49. Higher-power view of Fig. 47. Note accumulation of staining on proximal side of lesion. Scale: 1·0 mm.

is preserved, both around the sulcus and in the undercut gyrus. This is in agreement with the possibility that a substantial number of U-fibres have their origin in the adjacent cortex.

Partial lesions have more limited effects. For instance, a cut through the lateral side of the suprasylvian gyrus causes staining to disappear in the lateral part of the gyrus above the lesion (Fig. 53); below the cut, the U-fibres show particularly

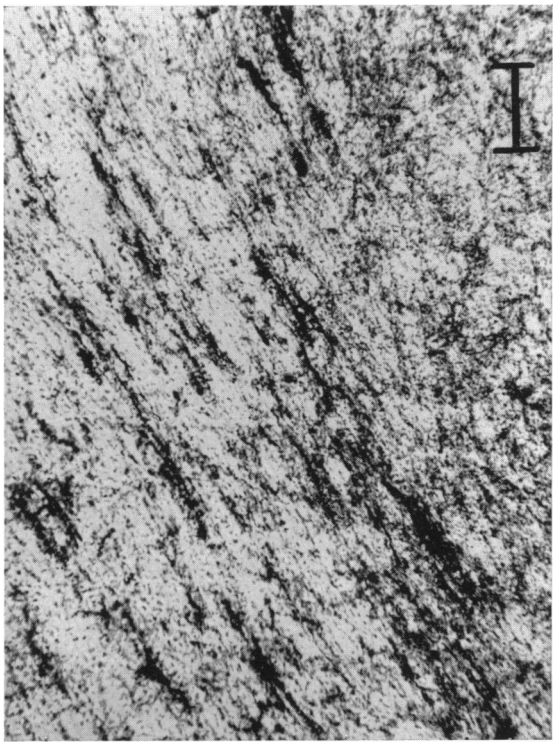


Fig. 50. Accumulation and distortion of staining in fibres emerging from rostral aspect of putamen. Same preparation as in Figs. 47 and 49 (cf. Fig. 39). Scale: 100 μm.

strong AChE activity, indicating that most of the U-fibres come from the lateral side of the cortex. The medial side of the gyrus remains unaffected. Various lesions thus show that whereas the lateral part of the suprasylvian is supplied by fibres coming mainly from the sylvian and ectosylvian regions, the medial part of the marginal gyrus is supplied mainly by fibres coming from the limbic region; but the lateral part of the marginal and the medial part of the suprasylvian, in the walls of the lateral sulcus, have a mixed supply of fibres from both the medial and lateral sides (as well as from the 'subcallosal band' below). This overlapping, double innervation makes a criss-cross pattern below the lateral sulcus, where, as already pointed out, U-fibres are relatively poorly developed (cf. Fig. 58).

Effects of undercutting on cellular staining

Deep pyramidal cells of layer V. The relatively diffuse pattern of fibrillar staining characteristic of this cortical layer also vanishes to a large extent. Fig. 54 gives an example of a typical large pyramidal cell of layer V in an isolated portion of precruciate cortex. Only a few fragments remain of the profuse staining seen on the normal side (Fig. 55) and there are hardly any of the stained terminal formations

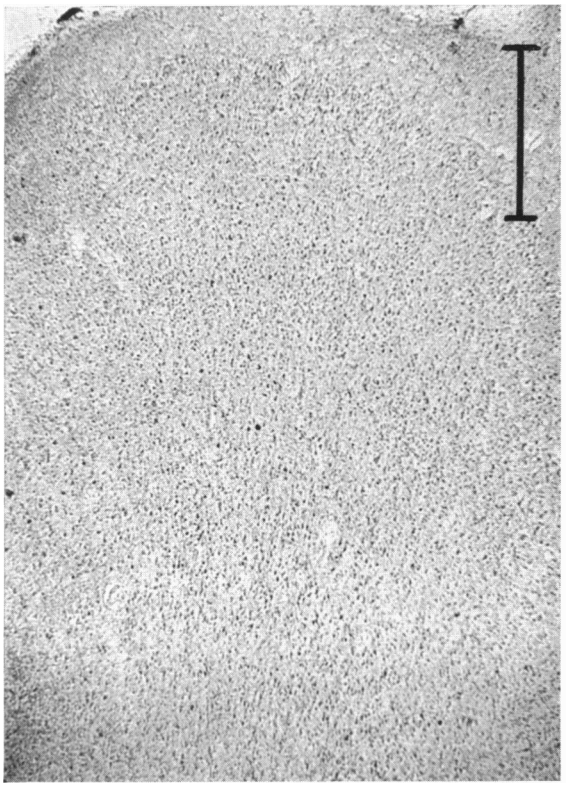


Fig. 51

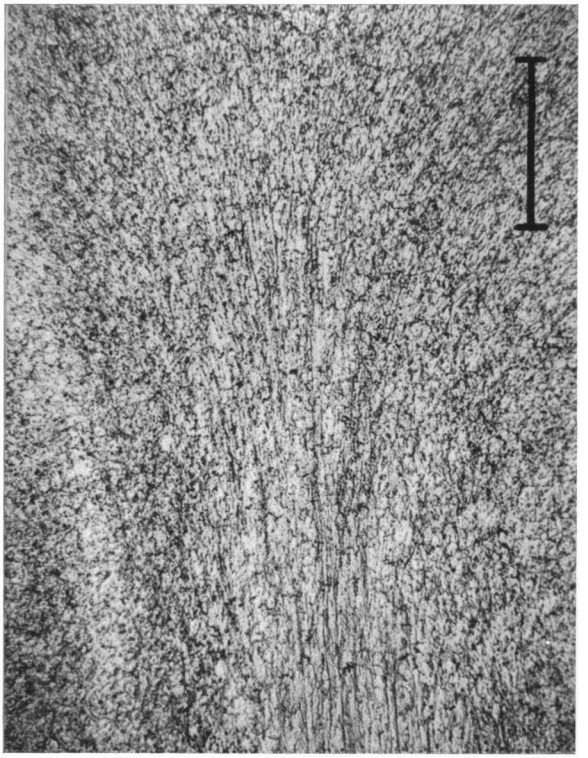


Fig. 52

Figs. 51, 52. Medium-power view of similar regions of isolated (Fig. 51) and normal (Fig. 52) anterior sigmoid gyrus, showing that undercutting causes fibre staining to disappear. Scales: 0·5 mm.

usually associated with such cells. However, although the shape of the cell is somewhat distorted, by chromatolytic changes, some of the diffuse intracellular staining has remained. In general, deep pyramidal cells are much less stained than usual, mainly because the surrounding fibrils and free endings disappear.

Polymorph cells of layer VI. In undercut areas these cells are often stained much more strongly than normally (Fig. 56). As usual the staining is intracellular and it extends along the dendrites and axons, although the latter, if they survive, do so only in a fragmented state, and they do not show a definite accumulation of AChE

near the lesion as do cut afferent axons. On one occasion we observed a somewhat comparable increase in staining of superficial cells close to a very large lesion. One cannot therefore dismiss the possibility that marked degenerative changes may be associated with a generalized increase in staining reaction.

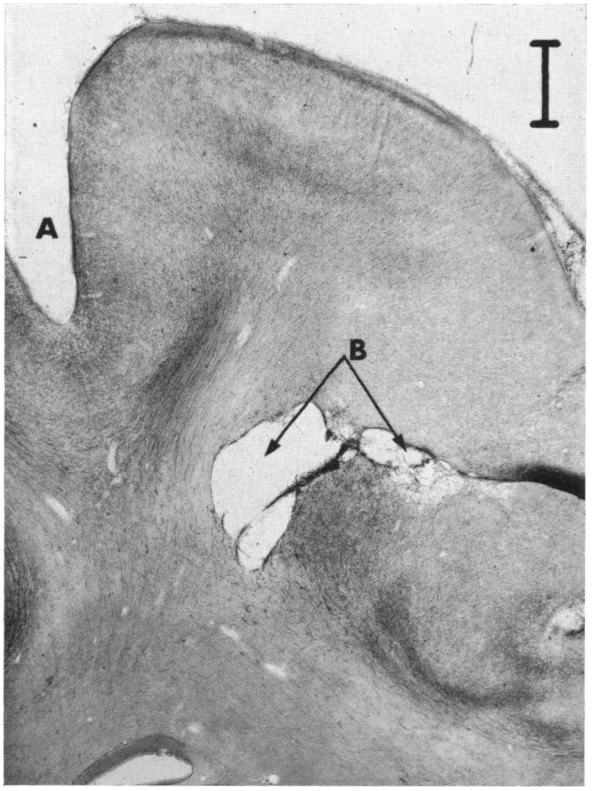


Fig. 53. Lesion through lateral part of base of suprasylvian gyrus. Note, 12 days after operation, loss of staining above cut, and accumulation in U-fibres from ectosylvian region below. A, Suprasylvian sulcus; B, lesion. Scale: 1·0 mm.

DISCUSSION

Selective nature of pallial AChE staining. When a section of the cat's brain treated to show AChE-containing fibres (e.g. Fig. 1) is compared with a section in which all fibres have been stained (cf. Vogt & Vogt, 1902; Winkler & Potter, 1914) it is clear that AChE staining reveals a very particular pattern of fibres. The areas of white matter which are conventionally the most strongly stained, the centrum semiovale, the internal capsule and the principal commissures, remain largely blank, and the main zone of substantial staining is in the subcortical and inner cortical regions, corresponding to the Vogts' relatively weakly stained zone of radiating fibres (cf. Vogt & Vogt, 1902, vol. I, table 5). Such a consistent pattern of staining, indicating a specific group of fibres, is likely to be of some significance. The relation

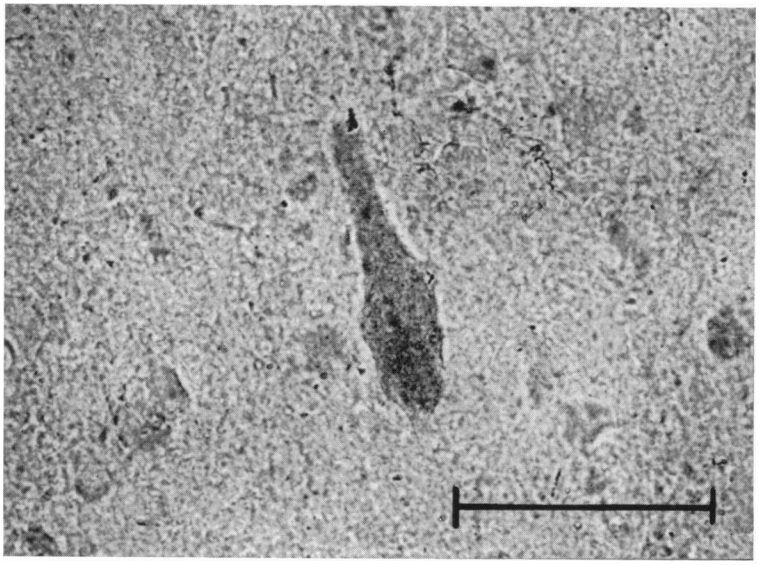


Fig. 54. High-power view of Betz cell in isolated pericruciate cortex. Usual surrounding pattern of diffuse, fibrillar staining (cf. Fig. 55) is largely absent. The cell shows chromatolytic changes but is still stained to some extent. Scale: 50 μm.

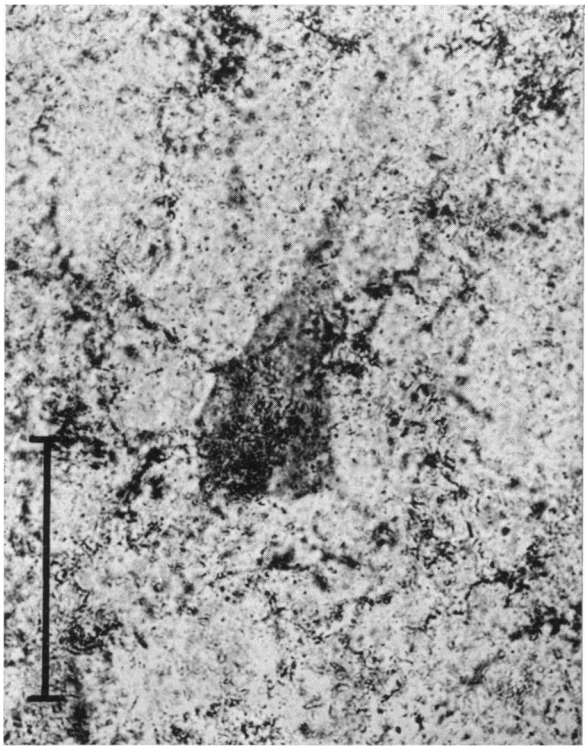


Fig. 55. AChE activity in and around Betz cell in intact pericruciate cortex. Scale: 50 μm.

of AChE-containing fibres to the various cellular layers in a typical gyrus is shown diagrammatically in Fig. 57.

Although there is some evidence that AChE activity is better seen in unmyelinated than in myelinated fibres (Bonichon & Gerebtzoff, 1958) the pattern of staining in the cerebral hemisphere does not correspond to a simple distribution of unmyelinated fibres; there are few if any truly unmyelinated fibres in the brain (van Crevel & Verhaart, 1963), and the main afferent and efferent pathways, which here are almost

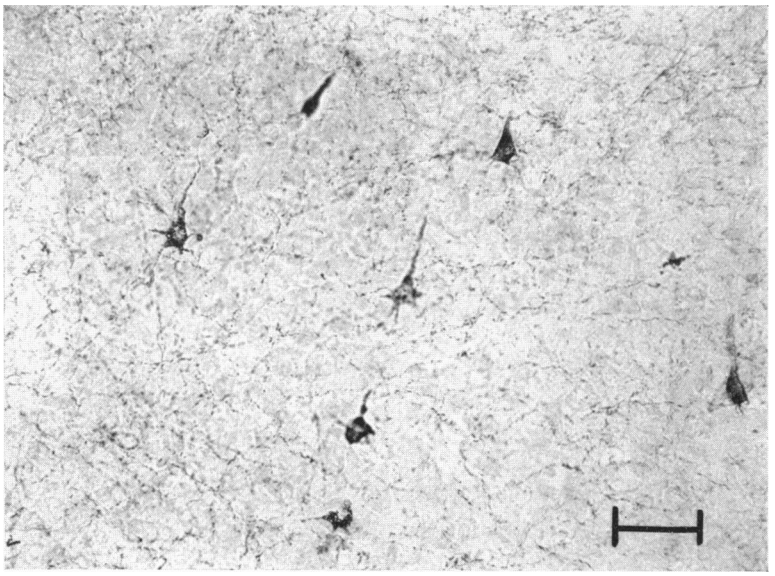


Fig. 56. Particularly strong staining of deep polymorph cells of suprasylvian gyrus partly undercut 31 days earlier. Scale: 100 μm.

completely unstained, are principally made up of very fine fibres. Moreover, in the foetal brain there is an even more striking contrast between the stained fibres and the unstained internal capsule (Krnjević & Silver, 1965), long before the onset of myelination (Vogt, 1900).

The cellular staining is also highly selective, affecting particularly certain types of cells; and even within a localized group, some cells are stained much more strongly than others.

Fibre staining in the forebrain. U-shaped links between many cortical areas have been known since the earliest days of brain anatomy. They were first described as *fibriae propriae* by Arnold (1851) and their presence was confirmed by all later authors who have studied the distribution of fibres in the cerebral cortex (e.g. Meynert, 1867; Dejerine, 1895; Campbell, 1905; Brodmann, 1914; Vogt & Vogt, 1919; Lorente de Nó, 1949). They were examined in particular detail by some authors who were especially interested in cortical association systems (Kaes, 1891; Mayendorf, 1919; Rosett, 1933).

It is generally agreed that these fibres and their intracortical branches form an

extensive system of horizontal connexions covering all parts of the cortex. According to Kaes (1891) and Mayendorf (1919) the branches break up into a rich network in the deeper half of the cortex and their terminals intermingle with those of the main afferent fibres, the outer edge of this network at the level of layer IV corresponding to the lines of Baillarger and Gennari; but Kaes (1891) pointed out that where the network is particularly well developed, some of its branches may extend to the surface. As this description of horizontal fibres agrees in every respect with the

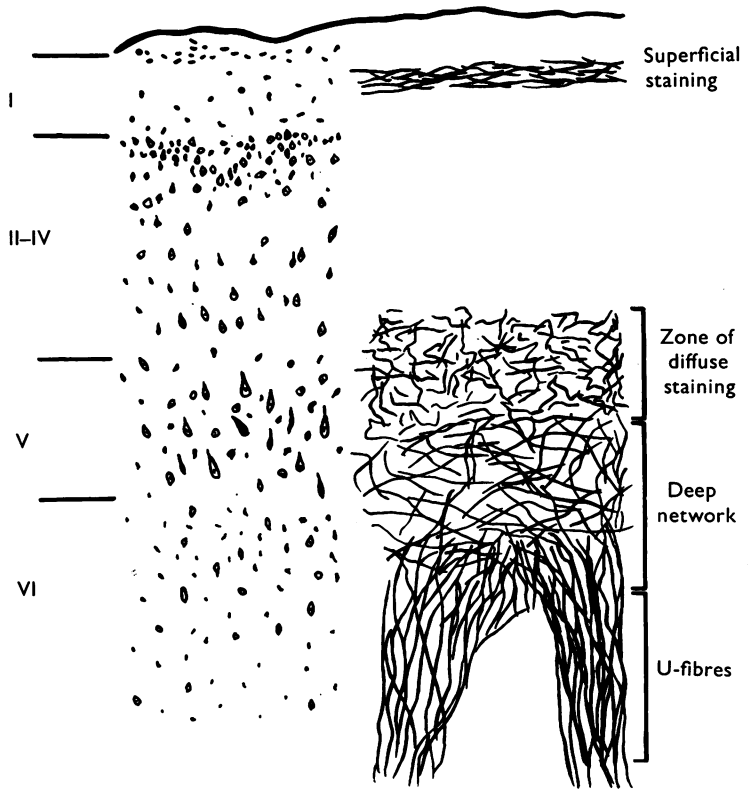


Fig. 57. A diagram illustrating the distribution, in a gyrus, of AChE-containing fibres (right), in relation to the various layers of cells (left).

distribution of stained fibres observed in our study, it is highly probable that the horizontal system consists largely (and perhaps exclusively) of AChE-containing fibres.

Cellular staining

Although a few clearly stained cells may be found anywhere in the cortex, they are predominantly in two main groups which have different characteristics.

Deep pyramids. The more superficial group is made up of large pyramidal cells, mainly of layer V, where they are in the outer half of the zone of fibrillar or diffuse staining. It seems very unlikely that these cells can be cholinergic neurones because

of their relatively weak intracellular staining, not extending into their axons or apical dendrites. The pyramidal tracts, which consist principally of efferent axons of pyramidal cells in the sensorimotor areas, contain very little ACh, AChE or choline acetylase (Feldberg & Vogt, 1948; Burgen & Chipman, 1951; Hebb & Silver, 1956).

The most likely explanation for the AChE activity associated with these cells is that it is the result of their being innervated by surrounding AChE-containing fibres. It is therefore particularly relevant that these cells are situated in the range of depth where units sensitive to ACh are especially likely to be found (Krnjević & Phillis, 1963*b*). Moreover, the pyramidal cells in the sensorimotor region which can be activated antidromically from the pyramidal tracts, and most of which in cats are ACh-sensitive, have a distribution in depth which does not differ significantly from the general distribution of ACh-sensitive cells (Krnjević & Phillis, 1963*b*).

Polymorphs of layer VI. The second main group of stained cells is found particularly in the deepest part of the cortex. They are readily distinguished from pyramidal cells by their small size, their variable shapes and their situation in the best-developed portion of the deep network of stained fibres. Their sharp staining is evidently intracellular and it often extends into various processes, including the axon. Several of these features suggest that at least some polymorph cells may be cholinergic neurones. This interpretation is supported by the fact that their staining, unlike that of the deep pyramids, is usually much increased in undercut areas.

Origin of cortical AChE-containing fibres. The marked reduction of AChE and choline acetylase activity (Hebb *et al.* 1963) produced by undercutting, and the absence of large numbers of obviously 'cholinergic' cells, make it unlikely that the observed network of stained fibres arises from cells within the same gyrus. Unfortunately the retrograde changes produced by cutting AChE-containing fibres do not extend sufficiently far back along the axons to show unequivocally their site of origin. Nevertheless, the available evidence points strongly to a subcortical origin for many of the fibres, but some may also come from adjacent or more distant areas of the cortex.

Fibres from subcortical regions. Although it is practically impossible in adult cats to trace the course of a given fibre from subcortical centres to the cortex, the general continuity of fibre staining between certain subcortical regions and the cortex cannot leave much room for doubt. For instance, isolated fibres can occasionally be followed from the stained 'subcallosal band' to the overlying cortex; since the 'subcallosal band' is continuous with similar fibres in the fornix and septum, such fibres may well arise from the strongly stained septal cells. The direct connexions from subcortical centres are confirmed by examination of the embryonic forebrain, in which AChE-containing fibres from the striatum and septum can be seen extending progressively through the hemisphere, towards the unstained pallium (Krnjević & Silver, 1964, 1965). Such direct connexions are also seen more readily in smaller animals such as rats (Shute & Lewis, 1963).

Extent of subcortical connexions

The apparent pattern of projections to the cortex is shown diagrammatically in Fig. 58.

On lateral side. Below the level of the suprasylvian sulcus, all parts of the cortex are relatively close to the external capsule and thus can be easily reached by ascending fibres. The lateral part of the suprasylvian gyrus, however, is mainly supplied by U-fibres from the sylvian and ectosylvian regions. Do these include striatal fibres? According to previous observers U-fibres do not merely link adjacent

Fig. 58. A diagram showing projections to the cortex, of AChE-containing fibres from subcortical regions.

gyri: some of them probably extend over several gyri (Campbell, 1905; Poliak, 1932); deeper fibres, especially, may pass below a particular gyrus to reach more distant areas (Mayendorf, 1919; Rosett, 1933). The deep network of fibres in the cortex is too irregular to allow one to follow stained fibres with any certainty around the surface of a gyrus into an adjacent U-formation. But in view of the evidence quoted above it seems quite likely that some striatal fibres may be present in the U-bundles binding the ectosylvian and suprasylvian gyri.

Other striatal fibres are also visible in the white matter below the suprasylvian gyrus; some coming from the lateral part of the 'subcallosal band' where it adjoins the caudate nucleus, and others, more diffusely still, from the general direction of the caudate–putamen. It is therefore likely that a substantial number of striatal

fibres travel upwards at least as far as the suprasylvian gyrus. Since fibres from the U-formation linking the ectosylvian and suprasylvian gyri can also be seen to by-pass the suprasylvian gyrus, crossing stained fibres coming from the medial side, some striatal fibres may even reach the marginal gyrus.

The presence of direct links between the striatum and the cortex is of interest. Such connexions have been described before (Bechterew, 1899; von Economo, 1918; Kodama, 1926, 1927, 1928; Coenen, 1927; Mettler, 1942; Papez, 1942; Carman, Cowan & Powell, 1963), but many authors have denied their existence (Dejerine, 1895; Wilson, 1914; Riese, 1924; Burandt, French & Akert, 1961; Laursen, 1963). Moreover, some direct links observed previously were said to consist exclusively of corticofugal fibres (e.g. Carman *et al.* 1963). Only a few, apparently corticopetal, fibres project from the caudate nucleus, via the 'subcallosal band', and they might well be missed. It is more surprising that the rich lateral projection from the putamen should have gone largely unnoticed; possibly because the relevant fibres are relatively poorly myelinated and therefore may not show clearly in conventional degeneration experiments.

On medial side. It is generally agreed that direct connexions exist between the septal region and the cingulate gyrus (Cajal, 1911; Cragg & Hamlyn, 1959; Valenstein & Nauta, 1959; Powell, 1963). The last authors in particular showed that septal efferent fibres project to the cingulate via the dorsal fornix, as well as to the hippocampus. In spite of some contrary opinion (MacLean, 1960), plenty of horizontal connexions have also been found between the cingulate gyrus and various adjoining areas of the neocortex (Showers, 1959; Kreiner, 1962).

Many stained fibres in the medial and superior part of the cortex come directly from the 'subcallosal band', which, like the adjacent subcallosal bundle, is evidently a lateral extension of the fornix system, as suggested originally by Onufrowicz (1887).

AChE-containing fibres in the medial wall thus probably come from the septum, either passing around the corpus callosum in the cingulum or as 'perforating' fibres of the fornix longus. Some of these fibres by-pass gyri in the medial wall to reach the marginal cortex, and, together with fibres ascending from the 'subcallosal band', a few reach as far laterally as the suprasylvian gyrus. In the marginal–suprasylvian region there is probably a substantial overlap of striatal and septal AChE-containing projections.

The 'subcallosal band'. This layer of stained fibres bears a close relation to the adjacent subcallosal or fronto-occipital fasciculus and it seems likely that at least some of its fibres have the same kind of distribution to the cortex. The present observations therefore confirm the descriptions of cortical connexions of the subcallosal fasciculus given by several previous authors (Onufrowicz, 1887; Muratoff, 1893; Bechterew, 1899; Kodama, 1926, 1928; Coenen, 1927; Mettler, 1942; Papez, 1942).

Exact origin of fibres in ascending projections. Most 'striatal' fibres emerge from the putamen but the relative absence of stained cells in the putamen itself suggests that most of the cells of origin may be in the globus pallidus or even further back, perhaps in the reticular formation of the midbrain and hypothalamus (cf. Shute & Lewis, 1963). Strongly stained fibres which link the corpus striatum with the substantia nigra may correspond to the AChE-containing ventral tegmental

projection of the midbrain reticular formation seen in rats by Shute & Lewis (1963), confirming earlier evidence of a nigrostriatal pathway (Ferraro, 1928; Kodama, 1928; Ranson, Ranson & Ranson, 1941; Fox & Schmitz, 1944; Rosegay, 1944).

Many fibres in the medial wall may arise from cells of the septal nuclei; but, as the latter are continuous with the olfactory tubercle, the pre-optic area and the region of the diagonal band of Broca (Elliott Smith, 1896; Fox, 1940; Valenstein & Nauta, 1959; Powell, 1963), it would be surprising if some fibres did not originate in these lower areas which also contain much AChE activity in fibres and cells. It is significant that the medial septal nucleus is continuous, via the nucleus of the diagonal band, with a ventral extension of the globus pallidus (Fox, 1940). This is in agreement with the hypothesis (Rose, 1927*a*, *b*) according to which the incomplete cortex of the septal region and the olfactory tubercle (cortex semiparietinus of Rose, or semicortex of Filimonoff, 1947) is developed from the same group of cells of the germinal layer as the corpus striatum. The general continuity of areas with intense AChE activity in the septum and the striato-pallidum is particularly clear in the embryonic brain (Krnjević & Silver, 1964, 1965). The main projections to the cortex therefore seem to come from basal telencephalic elements which have a common embryonic origin in the striatal ependyma.

The precommissural septum also has connexions with areas of strong AChE activity in the brain stem, such as the habenula and the medial forebrain bundle, as well as with the amygdaloid complex, via the diagonal band and the stria terminalis (Fox, 1940; Valenstein & Nauta, 1959; Powell, 1963). The central position of the precommissural septum and its wide connexions are thus fully in agreement with Broca's original description of this region as 'le carrefour de l'hémisphère' (Broca, 1879).

Although the cingulate region is generally said to receive a strong projection from the rostral thalamus, we have not been able to detect any definite ChE-containing bundles forming a direct connexion with the thalamus, not even one containing predominantly pseudocholinesterase (cf. Shute & Lewis, 1963).

Our evidence does not show whether any 'septal' fibres really originate even further caudally in the midbrain reticular formation, although it is compatible with Shute & Lewis's concept of a projection from the midbrain reticular formation to the cortex of the medial wall of the hemisphere; but our results suggest that this projection cannot be simply divided into a septal component travelling to the hippocampus, and a cingulate component projecting to the limbic neocortex, since the fibres of the cingulum probably extend back as far as the hippocampus, while numerous 'perforating' fibres from the septum and fornix clearly join the supracallosal system. It seems more likely that the whole medial wall, both neocortical and archicortical, is innervated by a single septal projection.

Intracortical cholinergic neurones. It was suggested above that some U-fibres probably have a subcortical origin. Most authors, however, have believed that many U-fibres actually arise in the cortex to reach adjacent areas. If these fibres are cholinergic, as seems likely, what are their cells of origin?

In view of previous evidence (Koelle, 1963), it is very unlikely that AChE-containing fibres could come from unstained cell bodies. The only cortical cells which consistently show intracellular AChE and which are situated in close relation

to U-fibres are the polymorph cells of layer VI. Meynert (1867), the first author to investigate the origin of U-fibres, believed that they came from spindle (or polymorph) cells of what is now called layer VI. Some later observers (e.g. Cajal, 1911; Mayendorf, 1919; Rosett, 1933) thought that U-fibres could arise from cells in all the cortical layers, except possibly layer I, but the present observations clearly support Meynert's view.

Other neurones which are similar to polymorph cells and show the same kind of AChE staining are present elsewhere in the brain; for instance in the pre-optic region and in the reticular nucleus of the thalamus. According to our observations on kittens (Krnjević & Silver, 1964, 1965), primitive stained cells appear in the subcortical white matter shortly before birth and they migrate into the cortex during the first few post-natal weeks. They seem to be closely associated with the developing U-fibres. It is therefore possible that AChE-containing polymorph cells of layer VI have a separate origin and that they migrate to the cortex to establish short intracortical, possibly cholinergic, links.

The presence of these neurones in the cortex is not contrary to our evidence that the cortical AChE and choline acetylase are much reduced by undercutting, since the greater part of their AChE content is likely to be in their profuse terminal branches rather than in the cell bodies, and the proximal part of the axons.

Staining in walls of sulci. The systematic difference between the staining of the gyral convexity and that of the walls of sulci must have some significance. It gives support to previous suggestions (von Economo, 1926; Kreiner, 1961) that the known structural characteristics of these parts are not merely the consequence of folding but that they reflect true differences in organization and function. According to Kreiner, horizontal, or association pathways are particularly well developed in the sulcal type of cortex, whereas vertical pathways (i.e. afferent and efferent connexions with deep nuclei) are established in the intervening areas. As the latter expand, fissures tend to be formed in the areas where the slower-growing horizontal connexions are concentrated. Such a scheme is clearly in good agreement with our observations.

The fibres in the zone of superficial staining, seen particularly well in sulci, are likely to be mainly independent of the deeper networks, though some interconnecting fibres are undoubtedly present. The superficial staining remains relatively continuous over the whole medial wall in the adult (Fig. 1), perhaps because the cortex in this region fails to develop very prominent afferent and efferent connexions.

The functional significance of the superficial AChE-containing network is not clear. Unlike the deep zones it is not associated with ACh-sensitive cells, which are strikingly deficient at this level (Krnjević & Phillis, 1963*b*). The possibility that superficial fibres innervate the apical dendrites of deeper cells might seem in keeping with the claim that only axodendritic cortical synapses contain AChE (de Lorenzo, 1961; Torack & Barrnett, 1962); recent observations by Lewis & Shute (1964) have, however, cast some doubt on the specificity of the histochemical method used in these studies of axodendritic synapses. Moreover, surface applications of ACh do not indicate any marked ACh-sensitivity of the superficial dendrites of deeper neurones (K. Krnjević, M. Randić & D. W. Straughan, unpublished observations). Szentagothai (1965) has recently shown that many horizontal fibres of layer I probably

come from cells in the deeper layers of the cortex. The AChE-containing horizontal fibres which we have observed in layer I may therefore be branches of the polymorph cells of layer VI.

Conclusions

The neocortex of the cat (and probably monkey) has, in addition to the well-known afferent, efferent and commissural pathways, another kind of innervation, which, though less well defined, appears to be mainly tangential in character, and is distinguished by its high content of AChE. The terminal network of these fibres is particularly closely related to deep pyramidal cells. Since many of the latter are readily excited by ACh, it seems probable that the AChE-containing tangential system provides a cholinergic innervation for these cells.

Several features of this diffuse system of fine fibres suggest that it is phylogenetically relatively ancient. In the cortex it is particularly well developed in the older regions of the archicortex and palaeocortex, while in the neocortex it is associated mainly with the infragranular layers, which are said to be concerned with a relatively primitive kind of function (Bolton, 1914). AChE-containing fibres in general are better developed in the older parts of the brain (Gerebtzoff, 1959) and in simpler animals (Shen, Greenfield & Boell, 1956). These fibres therefore correspond rather well to the primitive system of fine fibres which Herrick (1933) and Bishop (1961) described as a diffuse and non-specific activator of cortical cells.

Many fibres of this system probably belong to medial and lateral projections from the basal corpus striatum and the septal region, which have a common developmental origin and may therefore be considered as a single functional unit. If it is indeed the forebrain extension of the midbrain reticular formation, as suggested by Shute & Lewis (1963), the diffuse system of AChE-containing fibres may well be the final corticopetal link in the reticular ascending activating pathway, which neuroanatomists have hitherto failed to identify (Nauta & Kuypers, 1958; Droogleever Fortuyn, 1960). That this link should be cholinergic would be fully consistent with the pharmacology of cortical arousal (cf. Monnier & Romanowski, 1962; Ling, 1963). There is much evidence that the cortical arousal initiated by stimulating non-specific thalamic nuclei reaches the cortex by an indirect route (Schlag & Chaillet, 1963); the presumed cholinergic pathway described here may be activated from the thalamus by thalamo-striate fibres or by connexions with the midbrain reticular formation.

SUMMARY

1. The distribution of acetylcholinesterase in the cat's forebrain has been examined histochemically to obtain more evidence about cholinergic pathways in the cortex. A detailed description is given of the arrangement of stained elements in the neocortex.

2. In the convexity of a gyrus, stained fibres travel subcortically on either side of the unstained core of white matter. As they enter the cortex the fibres form a network in the deepest layer; more superficially, they pass into a zone of more diffuse fibrillar staining which extends through layers V and IV. Since the superficial cell layers contain few or no stained fibres, the outermost edge of this zone is seen as a relatively sharp line at a depth of 0·8–1·0 mm.

3. Cellular staining is mainly confined to pyramidal cells of layer V and polymorph cells of layer VI. The staining of the pyramidal cells is restricted to the region of the soma and it appears to be mainly at the surface of the cells. Polymorph cells show denser staining, which is clearly intracellular and extends into the various neuronal processes.

4. In the walls of a sulcus there is a marked increase in staining in the most superficial layers. Well-stained U-fibres travel round sulci at the border between the grey and white matter and their branches enter the network of fibres seen in the deepest cortical layer.

5. Staining in different parts of the neocortex and the connexions between stained areas are described. Within the neocortex connexions are mainly provided by the U-fibres. Some fibres probably come from the septal region, and they innervate the limbic cortex and other areas of the medial portion of the hemisphere, as far as the suprasylvian gyrus; other fibres, from the lenticular nucleus, travel to the lateral aspect of the hemisphere. A substantial number of stained fibres from the septum and striatum reach the cortex via the 'subcallosal band'.

6. The basic pattern of staining in the monkey's forebrain is similar to that in the cat.

7. Experiments in which parts of the cortex in cats were undercut showed that the stained fibres must arise either from distant cortical areas, possibly from polymorph cells of layer VI, or from subcortical cells.

8. It is concluded that AChE-containing fibres form a distinct, though relatively diffuse, tangential system, which may provide a cholinergic innervation of some deep pyramidal cells. This system appears to be under the control of striatal and septal cells; it may be the final corticopetal link in the ascending activating pathway from the midbrain reticular formation.

We are grateful to McGill University, Montreal, and the Medical Research Council of Canada, for financial assistance without which publication of this paper would not have been possible.

REFERENCES

ARNOLD, F. (1851). *Handbuch der Anatomie des Menschen*, vol. 2. Freiburg i.B.: Herder'sche Verlagshandlung.

BECHTEREW, W. V. (1899). *Die Leitungsbahnen im Gehirn und Rückenmark*, p. 554. Leipzig: Arthur Georgi.

BISHOP, G. H. (1961). The organization of cortex with respect to its afferent supply. *Ann. N.Y. Acad. Sci.* **94**, 559–569.

BOLTON, J. S. (1914). *The Brain in Health and Disease.* London: E. Arnold.

BONICHON, A. & GEREBTZOFF, M. A. (1958). L'acétylcholinestérase dans la cellule et la fibre nerveuse au cours du développement. 2. Relation entre la localisation synaptique de l'enzyme et la myélinisation de la fibre. *Annls Histochim.* **3**, 171–178.

BONNET, V. & BREMER, F. (1937). Action du potassium, du calcium, et de l'acétylcholine sur les activités électriques, spontanées et provoquées, de l'écorce cérébrale. *C. r. Séanc. Soc. Biol.* **126**, 1271–1275.

BORNSTEIN, M. B. (1946). Presence and action of acetylcholine in experimental brain trauma. *J. Neurophysiol.* **9**, 347–366.

BREMER, F. & CHATONNET, J. (1949). Acétylcholine et cortex cérébral. *Archs int. Physiol.* **57**, 106–109.

BROCA, P. (1879). Localisations cérébrales. Recherches sur les centres olfactifs. *Revue d'Anthropologie*, 2ième Série, **2**, 385–455.

BRODMANN, K. (1914). Physiologie des Gehirns. *Neue dt. Chir.* **11**, 85–426.

BURANDT, D. C., FRENCH, G. M. & AKERT, K. (1961). Relationships between the caudate nucleus and the frontal cortex in *Macaca mulatta*. *Confinia neurol.* **21**, 289–306.

BURGEN, A. S. V. & CHIPMAN, L. M. (1951). Cholinesterase and succinic dehydrogenase in the central nervous system of the dog. *J. Physiol., Lond.* **114**, 296–305.

CAJAL, S. R. (1911). *Histologie du système nerveux de l'homme et des vertébrés*, vol. II. Paris: A. Malvoine.

CAMPBELL, A. W. (1905). *Histological Studies on the Localisation of Cerebral Function*, p. 14. Cambridge University Press.

CARMAN, J. B., COWAN, W. M. & POWELL, T. P. S. (1963). The organization of cortico-striate connexions in the rabbit. *Brain*, **86**, 525–562.

COENEN, L. (1927). The connection of the cortex with the neostriatum and paleostriatum in rabbits. *Proc. K. ned. Akad. Wet.* **30**, 811–822.

CRAGG, B. G. & HAMLYN, L. H. (1959). Histologic connections and electrical and autonomic responses evoked by stimulation of the dorsal fornix in the rabbit. *Expl Neurol.* **1**, 187–213.

DALE, H. H. (1914). The action of certain esters and ethers of choline, and their relation to muscarine. *J. Pharmac. exp. Ther.* **6**, 147–190.

DEJERINE, J. (1895). *Anatomie des centres nerveux*, vol. I. Paris: Rueff.

DE LORENZO, A. J. D. (1961). Electron microscopy of the cerebral cortex. 1. The ultrastructure and histochemistry of synaptic junctions. *Johns Hopkins Hosp. Bull.* **108**, 258–279.

DEMPSEY, E. W. & MORISON, R. S. (1943). The electrical activity of a thalamocortical relay system. *Am. J. Physiol.* **138**, 283–296.

DROOGLEEVER FORTUYN, J. (1960). The contributions of cortex, telencephalon, diencephalon and midbrain to the state of sleep and wakefulness. In *Structure and Function of the Cerebral Cortex*, pp. 88–92. Eds. D. B. Tower and J. P. Schadé. Amsterdam: Elsevier.

ECCLES, J. C., FATT, P. & KOKETSU, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. *J. Physiol., Lond.* **126**, 524–562.

ELLIOTT SMITH, G. (1896). The morphology of the true 'limbic lobe', corpus callosum, septum pellucidum and fornix. *J. Anat. Physiol. Lond.* **30**, 185–205.

FELDBERG, W. (1945). Present views on the mode of action of acetylcholine in the central nervous system. *Physiol. Rev.* **25**, 596–642.

FELDBERG, W. & VOGT, M. (1948). Acetylcholine synthesis in different regions of the central nervous system. *J. Physiol., Lond.* **107**, 372–381.

FERRARO, A. (1928). The connections of the pars suboculomotoria of the substantia nigra. *Archs Neurol. Psychiat., Chicago*, **19**, 177–180.

FILIMONOFF, I. N. (1947). A rational subdivision of the cerebral cortex. *Archs Neurol. Psychiat., Chicago*, **58**, 296–311.

FOX, C. A. (1940). Certain basal telencephalic centers in the cat. *J. comp. Neurol.* **72**, 1–62.

FOX, C. A. & SCHMITZ, J. T. (1944). The substantia nigra and the entopeduncular nucleus in the cat. *J. comp. Neurol.* **80**, 323–334.

GEREBTZOFF, M. A. (1959). *Cholinesterases*. London: Pergamon Press.

HEBB, C. O. & SILVER, A. (1956). Choline acetylase in the central nervous system of man and some other mammals. *J. Physiol., Lond.* **134**, 718–728.

HEBB, C. O., KRNJEVIĆ, K. & SILVER, A. (1963). Effect of undercutting on the acetylcholinesterase and choline acetyltransferase activity in the cat's cerebral cortex. *Nature, Lond.* **198**, 692.

HERRICK, C. J. (1933). The functions of the olfactory parts of the cerebral cortex. *Proc. natn. Acad. Sci. U.S.A.* **19**, 7–14.

HURLEY, H. J., SHELLEY, W. B. & KOELLE, G. B. (1953). The distribution of cholinesterases in human skin, with special reference to eccrine and apocrine sweat glands. *J. invest. Derm.* **21**, 139–147.

HYDE, J., BECKETT, S. & GELLHORN, E. (1949). Acetylcholine and convulsive activity. *J. Neurophysiol.* **12**, 17–27.

INFANTELLINA, F. (1955). Azione dell'acetilcolina, dell'eserina e dell'atropina sul lembo isolato di corteccia cerebrale di gatto. *Archo Sci. biol., Bologna*, **39**, 209–232.

ISHII, Y. (1957). The histochemical studies of cholinesterase in the central nervous system. 1. Normal distribution in rodents. *Archvm histol. jap.* **12**, 587–611.

KAES, T. (1891). Die Anwendung der Wolters'schen Methode auf die feinen Fasern der Hirnrinde. *Neurol. Zentbl.* **10**, 456–459.

KENNARD, M. A. (1955). Effect of bilateral ablation of cingulate area on behaviour of cats. *J. Neurophysiol.* **18**, 159–169.

KODAMA, S. (1926). Über die sogenannten Basalganglien. *Schweiz. Arch. Neurol. Psychiat.* **18**, 179–246.

KODAMA, S. (1927). Über die sogenannten Basalganglien. *Schweiz. Arch. Neurol. Psychiat.* **20**, 209–261.

KODAMA, S. (1928). Über die sogenannten Basalganglien. *Schweiz. Arch. Neurol. Psychiat.* **23**, 179–265.

KOELLE, G. B. (1950). The histochemical differentiation of types of cholinesterases and their localizations in tissues of the cat. *J. Pharmac. exp. Ther.* **100**, 158–179.

KOELLE, G. B. (1951). The elimination of enzymatic diffusion artifacts in the histochemical localization of cholinesterases and a survey of their cellular distributions. *J. Pharmac. exp. Ther.* **103**, 153–171.

KOELLE, G. B. (1954). The histochemical localization of cholinesterases in the central nervous system of the rat. *J. comp. Neurol.* **100**, 211–235.

KOELLE, G. B. (1963). Cytological distributions and physiological functions of cholinesterases. In *Cholinesterases and Anticholinesterase Agents*, pp. 187–298. Eds. O. Eichler and A. Farah. Berlin: Springer.

KREINER, J. (1961). The myeloarchitectonics of the frontal cortex of the dog. *J. comp. Neurol.* **116**, 117–133.

KREINER, J. (1962). Myeloarchitectonics of the cingular cortex in dog. *J. comp. Neurol.* **119**, 255–267.

KRNJEVIĆ, K. & PHILLIS, J. W. (1961). Sensitivity of cortical neurones to acetylcholine. *Experientia*, **17**, 469.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963*a*). Iontophoretic studies of neurones in the mammalian cerebral cortex. *J. Physiol., Lond.* **165**, 274–304.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963*b*). Acetylcholine-sensitive cells in the cerebral cortex. *J. Physiol., Lond.* **166**, 296–327.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963*c*). Pharmacological properties of acetylcholine-sensitive cells in the cerebral cortex. *J. Physiol., Lond.* **166**, 328–350.

KRNJEVIĆ, K. & SILVER, A. (1963*a*). Cholinesterase staining in the cerebral cortex. *J. Physiol., Lond.* **165**, 3–4*P*.

KRNJEVIĆ, K. & SILVER, A. (1963*b*). The distribution of 'cholinergic' fibres in the cerebral cortex. *J. Physiol., Lond.* **168**, 39–40*P*.

KRNJEVIĆ, K. & SILVER, A. (1964). The development of acetylcholinesterase (AChE) staining in the forebrain of the cat. *J. Physiol., Lond.* **175**, 22–23*P*.

KRNJEVIĆ, K. & SILVER, A. (1965). Acetylcholinesterase in the developing forebrain. *J. Anat.* (In the press.)

LAURSEN, A. M. (1963). Corpus striatum. *Acta physiol. scand.* **59**, suppl. 211, 1–106.

LEWIS, P. R. (1961). The effect of varying the conditions in the Koelle technique. *Bibl. anat.* **2**, 11–20.

LEWIS, P. R. & SHUTE, C. C. D. (1964). Demonstration of cholinesterase activity with the electron microscope. *J. Physiol., Lond.* **175**, 5–7*P*.

LEWIS, P. R., SHUTE, C. C. D. & SILVER, A. (1964). Confirmation from choline acetylase analyses of a massive cholinergic innervation to the hippocampus. *J. Physiol., Lond.* **172**, 9–10*P*.

LING, G. M. (1963). EEG effects of some biogenic amines and psychoactive drugs. *J. Neuropsychiat.* **4**, 255–263.

LORENTE DE NÓ, R. (1949). Cerebral cortex: architecture, intracortical connections, motor projections. In *Physiology of the Nervous System*, 3rd ed., pp. 288–330. Ed. J. F. Fulton. New York: Oxford University Press.

MACINTOSH, F. C. (1941). The distribution of acetylcholine in the peripheral and the central nervous system. *J. Physiol., Lond.* **99**, 436–442.

MACINTOSH, F. C. & OBORIN, P. E. (1953). Release of acetylcholine from intact cerebral cortex. *Abstr. XIXth int. physiol. Congr.* pp. 580–581.

MACLEAN, P. D. (1960). Psychosomatics. In *Handbook of Physiology*. Section I, Neurophysiology, vol. III, pp. 1723–1744. Eds. J. Field, H. W. Magoun and V. E. Hall. Washington: Amer. physiol. Soc.

MATHISEN, J. S. & BLACKSTAD, T. W. (1964). Cholinesterase in the hippocampal region. *Acta anat.* **56**, 216–253.

MCCAMAN, R. E. (1963). Microdetermination of choline acetylase and its distribution in nervous tissue. *Fedn Proc. Fedn Am. Socs exp. Biol.* **22**, 170.

MAYENDORF, N. v. (1919). Die Assoziationssysteme des menschlichen Vorderhirns. *Arch. Anat. Physiol., Physiol. Abt.*, pp. 283–390.

METTLER, F. A. (1942). Relation between pyramidal and extrapyramidal function. *Res. Publ. Assoc. nerv. ment. Dis.* **21**, 150–227.

MEYNERT, T. (1867). Der Bau der Grosshirnrinde und seine örtlichen Verschiedenheiten nebst einem pathologisch-anatomischen Corollarium. *Vierteljahrsschrift für Psychiatrie*, pp. 198–252.

MILLER, F. R., STAVRAKY, G. W. & WOONTON, G. A. (1940). Effects of eserine, acetylcholine and atropine on the electrocorticogram. *J. Neurophysiol.* **3**, 131–138.

MITCHELL, J. F. (1963). The spontaneous and evoked release of acetylcholine from the cerebral cortex. *J. Physiol., Lond.* **165**, 98–116.

MONNIER, M. & ROMANOWSKI, W. (1962). Les systèmes cholinoceptifs cérébraux. Actions de l'acétylcholine, de la physostigmine, pilocarpine et de GABA. *Electroenceph. clin. Neurophysiol.* **14**, 486–500.

MURATOFF, W. (1893). Secundäre Degenerationen nach Zerstörung der motorischen Sphäre des Gehirns in Verbindung mit der Frage von der Localisation der Hirnfunctionen. *Arch. Anat. Physiol., Anat. Abt.*, pp. 97–116.

NAUTA, W. J. H. & KUYPERS, H. G. J. M. (1958). Some ascending pathways in the brain stem reticular formation. In *Reticular Formation of the Brain*, pp. 3–30. Ed. H. H. Jasper. London: Churchill.

OKINAKA, S. & YOSHIKAWA, M. (1962). Histochemical studies on the autonomic nervous system. In *Proceedings IV International Congress of Neuropathology*, vol. I, pp. 180–187. Ed. H. Jacob. Stuttgart: G. Thieme.

OKINAKA, S., YOSHIKAWA, M., UONO, M., MURO, T., MOZAI, T., IGATA, A., TANABE, H., UEDA, S. & TOMONAGA, M. (1961). Distribution of cholinesterase activity in the human cerebral cortex. *Am. J. phys. Med.* **40**, 135–145.

ONUFROWICZ, W. (1887). Das balkenlose Mikrocephalengehirn Hofmann. *Arch. Psychiat. NervKrankh.* **18**, 305–328.

PAPEZ, J. W. (1942). A summary of fiber connections of the basal ganglia with each other and with other portions of the brain. *Res. Publ. Assoc. nerv. ment. Dis.* **21**, 21–68.

POLIAK, S. (1932). *The Main Afferent Fiber Systems of the Cerebral Cortex in Primates*, p. 249. Berkeley: University of California Press.

POPE, A. (1952). Quantitative distribution of dipeptidase and acetylcholine esterase in architectonic layers of rat cerebral cortex. *J. Neurophysiol.* **15**, 115–130.

POWELL, E. W. (1963). Septal efferents revealed by axonal degeneration in the rat. *Expl Neurol.* **8**, 406–422.

RANSON, S. W., RANSON, S. W. & RANSON, M. (1941). Fiber connections of corpus striatum as seen in Marchi preparations. *Archs Neurol. Psychiat., Chicago*, **46**, 230–249.

RIESE, W. (1924). Beiträge zur Faseranatomie der Stammganglien. *J. Psychol. Neurol., Lpz.* **31**, 81–122.

ROSE, M. (1927*a*). Der Allocortex bei Tier und Mensch. I. Teil. *J. Psychol. Neurol. Lpz.* **34**, 1–111.

ROSE, M. (1927*b*). Die sogenannte Riechrinde beim Menschen und beim Affen. *J. Psychol. Neurol., Lpz.* **34**, 261–401.

ROSEGAY, H. (1944). An experimental investigation of the connections between the corpus striatum and substantia nigra in the cat. *J. comp. Neurol.* **80**, 293–321.

ROSENZWEIG, M. R., KRECH, D. & BENNETT, E. L. (1958). Brain chemistry and adaptive behaviour. In *Biological and Biochemical Bases of Behaviour*, pp. 367–400. Eds. H. F. Harlow and C. N. Woolsey. Madison: University of Wisconsin Press.

ROSETT, J. (1933). *Intercortical Systems of the Human Cerebrum.* New York: University of Columbia Press.

SCHLAG, J. D. & CHAILLET, F. (1963). Thalamic mechanisms inolved in cortical desynchronization and recruiting responses. *Electroenceph. clin. Neurophysiol.* **15**, 39–62.

SHEN, S. C., GREENFIELD, P. & BOELL, E. J. (1956). Localization of acetylcholinesterase in chick retina during histogenesis. *J. comp. Neurol.* **106**, 433–461.

SHOWERS, M. J. C. (1959). The cingulate gyrus: additional motor area and cortical autonomic regulator. *J. comp. Neurol.* **112**, 231–301.

SHUTE, C. C. D. & LEWIS, P. R. (1961). The use of cholinesterase techniques combined with operative procedures to follow nervous pathways in the brain. *Bibl. anat.* **2**, 34–49.

SHUTE, C. C. D. & LEWIS, P. R. (1963). Cholinesterase-containing systems of the brain of the rat. *Nature, Lond.* **199**, 1160–1164.

SJÖQVIST, F. (1963). The correlation between the occurrence and localization of acetylcholinesterase-rich cell bodies in the stellate ganglion and the outflow of cholinergic sweat secretory fibres to the fore paw of the cat. *Acta physiol. scand.* **57**, 339–351.

SNELL, R. S. (1961). The histochemical localization of cholinesterase in the central nervous system. *Bibl. anat.* **2**, 50–58.

SPEHLMANN, R. (1963). Acetylcholine and prostigmine electrophoresis at visual cortical neurons. *J. Neurophysiol.* **26**, 127–139.

SPEHLMANN, R. & KAPP, H. (1961). Die Wirkung lokaler Mikroelektrophorese von Acetylcholin auf einzelne Neurone des visuellen Cortex. *Pflügers Arch. ges. Physiol.* **274**, 37–38.

STONE, W. E. (1957). The role of acetylcholine in brain metabolism and function. *Am. J. phys. Med.* **36**, 222–255.

SZENTAGOTHAI, J. (1965). The use of degeneration methods in investigation of short neurons and neuronal connexions. In *Degeneration Patterns in the Nervous System.* Eds. M. Singer and J. P. Schadé. *Progress in Brain Research*, vol. 14. Amsterdam: Elsevier. (In the press.)

TORACK, R. M. & BARRNETT, R. J. (1962). Fine structural localization of cholinesterase activity in the rat brain stem. *Expl Neurol.* **6**, 224–244.

VALENSTEIN, E. S. & NAUTA, W. J. H. (1959). A comparison of the distribution of the fornix system in the rat, guinea pig, cat and monkey. *J. comp. Neurol.* **113**, 337–363.

VAN CREVEL, H. & VERHAART, W. J. C. (1963). The rate of secondary degeneration in the central nervous system. 1. The pyramidal tract of the cat. *J. Anat.* **97**, 429–449.

VOGT, C. (1900). *Étude sur la myélinisation des hemisphères cérébraux.* Paris: G. Steinheil.

VOGT, O. & VOGT, C. (1902). Zur Erforschung der Hirnfaserung. In *Neurobiologische Arbeiten*, vol. 1. Ed. O. Vogt. Jena: Fischer.

VOGT, C. & VOGT, O. (1919). Allgemeine Ergebnisse unserer Hirnforschung. *J. Psychol. Neurol., Lpz.* **25**, 1–462.

VON ECONOMO, C. (1918). Wilson's Krankheit und das Syndrôme du corps strié. *Z. ges. Neurol. Psychiat.* **43**, 173–209.

VON ECONOMO, C. (1926). Die Bedeutung der Hirnwindungen. *Allg. Z. Psychiat.* **84**, 122–132.

WINKLER, C. & POTTER, A. (1914). *An Anatomical Guide to Experimental Researches on the Cat's Brain.* Amsterdam: W. Versluys.

WILSON, S. A. K. (1914). An experimental research into the anatomy and physiology of the corpus striatum. *Brain*, **36**, 427–492.