

THE BASAL RHINENCEPHALIC STRUCTURES AND OLFACTORY

CONNEXIONS IN THE COYPU RAT (MYOGASTOR COYPUS)

A Thesis submitted for the degree of

Ph.D. (Neuro-anatomy)

by

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ABSTRACT

The main object of this work has been to study the basal rhinencephalic structures of a macrosmatic rodent (Myocaster corvus) which had not been investigated previously. It has included a study, using the method of experimental degeneration, of secondary olfactory connexions, and some preliminary observations on the distribution of cholinesterase.

So far as cytoarchitecture is concerned, the olfactory bulb is essentially identical with that of other macrosmatic mammals such as the rat. Secondary olfactory centres, defined as those regions which receive fibres originating in the main and accessory bulbs, are also of the usual pattern. They include the anterior olfactory nucleus, the olfactory tubercle, the frontal and temporal piriform cortex, the nucleus of the lateral olfactory tract and the cortical amygdaloid nucleus. The entorhinal area is well developed but differs from the paleo-cortical areas in the absence of secondary connexions from the bulb and in its more complex and very characteristic cytoarchitectural lamination.

The septum is well developed and its major sub-divisions can easily be recognised, but it would not be possible^e to justify, on the basis of the present material, a sub-division into as many different nuclei as those identified by some investigators. The amygdala is also well developed and a detailed study has been undertaken due to the growing interest in the "limbic system" of which the amygdala is a part.

The distribution of cholinesterase in the brain indicates a

general similarity between the coypu and other rodents and the interesting preliminary observations in the experimental specimen establish a case for more extensive study.

Lesions limited to the bulb, with or without involving the accessory bulb, lead to degeneration in the lateral olfactory tract only and is distributed to the secondary olfactory centres defined above. No fibres could be traced to the septum, hippocampal formation, entorhinal area and the greater part of the amygdala. Unequivocal degeneration in the olfactory commissural component is found only when the anterior olfactory nucleus is involved in the lesion unless this component or the anterior commissure is damaged directly. The experiments also suggest that bulbo-petal fibres arise from the basal areas of the brain as well as from the opposite anterior olfactory nucleus. There is definite reciprocal relationship between the amygdala and the hypothalamus which would appear to be of greater functional significance than the very scanty secondary olfactory connexions. (Conclusions are based mainly on Nauta-Gygax (1954) preparations.)

The use of the term "rhinencephalon" is discussed critically and the suggestion is given to sub-divide the structures previously included under this comprehensive term into a strictly "olfactory system" and a "limbic system" which is concerned mainly with visceral and behavioural mechanisms.

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INTRODUCTION

Recently considerable attention has been directed to the "rhinencephalon", and it has been carefully reviewed in the light of modern technical methods of investigation (see Gastaut & Lammers, 1961; Bargmann & Schade, 1963). The concept of a rhinencephalon was largely based on the accounts of early investigators who had to rely for their conclusions on macroscopic observations and the examination of Weigert or Nissl sections of normal material and occasionally Marchi preparations. The limitations of these methods are now widely recognised. The concept owed much to comparative neuroanatomical studies and the realisation that, in primitive vertebrates such as cyclostomes, the fore-brain has a primary relationship with olfactory function and at that evolutionary level the terms "rhinencephalon" and "prosencephalon" are almost interchangeable. It was of course recognised that in the more advanced vertebrates the fore-brain had taken on many other functions (the process of "prosencephalisation"), particularly in mammals, and that in some, e.g. man, olfaction played a relatively insignificant part so that they could be described as "microsmatic". However the term rhinencephalon has been retained for a complex of structures including not only those in the basal part of the hemisphere, such as the piriform cortex and olfactory tubercle, which have direct connexions with the olfactory bulb, but also the amygdala, the hippocampus and fornix together with certain related parts of the telencephalon and diencephalon.

Recently the absence of well established direct olfactory connexions with many parts of the rhinencephalon has been emphasised (see Goldby, 1961) and for example, the mammalian hippocampus and its related structures are no longer accepted as mainly olfactory in function (Brodal, 1947). There is also doubt whether the greater part of the amygdaloid complex can be concerned directly with smell (Breathnach & Goldby, 1954). In view of these uncertainties, it was decided to investigate the olfactory connexions in a macrosmatic mammalian brain using a silver staining method (the Nauta technique) which shows both axonal and preterminal degeneration as well as the classical Marchi method. The need for careful study of such structures as the amygdala has become more apparent owing to the widespread and growing interest in the "limbic system", an ill-defined term often used as synonymous with the "rhinencephalon", of which the amygdala is a part. This limbic system appears to be concerned with visceral and behavioural mechanisms, particularly those associated with the expression of emotional states, and possibly with memory and sexual functions (see Papez, 1937; and more recently MacLean, 1954).

For our research we chose the coypu rat (Myocastor coypus) a semi-aquatic rodent (some details of the natural history of the coypu are contained in a thesis recently published from this School - Kacker, 1964). Until recently it has not been used in the laboratory for animal experimentation although, due to its availability and reasonable size, interest has been

aroused in its use as an experimental animal (Butterworth, Shillito, Spencer & Stewart, 1962). The olfactory bulbs and the rhinencephalic structures generally are well developed (Fig. 2) so that the animal can be classed as "macrosmatic". The present work is limited however to the basal rhinencephalic structures of the telencephalon (the olfactory bulb, peduncle, piriform cortical area and olfactory tubercle, amygdala, septum and entorhinal area) and its general purpose can be summarized as follows:

- (i) To study the basal rhinencephalic structures in an animal not previously investigated from this point of view by the classical neurohistological methods.
- (ii) To report some preliminary observations on the distribution of acetylcholinesterase enzyme in these structures.
- (iii) To study the secondary olfactory connexions of the olfactory bulb and those of the anterior olfactory nucleus, using the method of experimental degeneration.

MATERIAL AND METHODS

N.B. Details of routine procedures, operative or histological are in Appendices I and II.

Animals

31 adult coypus were used in this investigation, 5 for the study of the normal brain, 2 as controls for certain staining techniques, and 24 for making experimental lesions in the olfactory bulb and other parts of the rhinencephalon. The weights of the animals ranged from 1.60 kg. to 6.75 kg., so that all were young, probably between 3 and 9 months of age. Both males and females were used without distinction. They had all been caught in the wild and appeared healthy and no sign of ill-health developed in the animal house or during the period of the experiments.

Normal material

Several whole brains were available and were used for the macroscopic observations. The following series of sections were also prepared for microscopic study; in all cases fixation was by perfusion through the aorta with 10% formalin.

- (1) A transverse series of 30 μ celloidin sections through the whole fore-brain. Every 10th section was stained with toluidine blue for cells and the section immediately following it with Loyez haematoxylin for myelinated fibres.
- (11) A sagittal series of the whole hemisphere stained similarly to the above series.

- (iii) A horizontal series stained in a similar manner to the above mentioned series.
- (iv) A number of sections were also cut from small blocks (Fig. 19, p. 57), usually in paraffin, for the study of special features, e.g. the histological features of parts of the cortex, which were cut in an unsatisfactory plane in the main series.
- (v) Two complete 10 μ series (transverse and sagittal) of the whole olfactory bulb and peduncle were cut in paraffin and stained mainly by toluidine blue. A few sections were stained by Holmes silver method.
- (vi) From one normal brain Nauta sections were prepared from all the regions relevant to this thesis to serve as controls for the experimental specimens.
- (vii) A series of transverse sections of the whole brain, 60-80 μ thick and 1 mm apart, were stained by Koelle's histochemical technique on frozen sections using Lewis (1961) modification. One albino rat brain was also stained by this method for comparison with the coypu.

Experimental material

All operations were performed under ether anaesthesia and with full aseptic precautions (for details see Appendix I). No sepsis nor unplanned deaths occurred during the post-operative period in any animal. Complete or incomplete destruction of the olfactory bulb was effected with an iridectomy knife, meibomian cyst scoop, thermo-cautery, or by

suction. In general the lesions fall into three groups:

- i) complete or incomplete lesions of the olfactory
bulb and accessory olfactory bulb ... 8 specimens
- ii) lesions involving anterior olfactory
nucleus 11 specimens
- iii) lesions involving anterior commissure 5 specimens

Details of the character of the lesion will be given when describing the actual specimens.

Degeneration resulting from the lesions was studied by the Marchi and Nauta-Oygax (1954) methods. A critical discussion of the criteria used in assessing degeneration will be found on pp. 128-139. Here it can be said that the Marchi technique was found to be far less satisfactory than the Nauta and was used only for two specimens with survival periods of three and four weeks respectively. In all 22 specimens were studied by the Nauta method for which survival periods varying between 7 and 56 days were used.

SOME MACROSCOPIC FEATURES OF THE
RHINENCEPHALON AND THEIR TERMINOLOGY

The term "rhinencephalon" has been used both in the title and introduction to this thesis. It means of course "smell brain" but to arrive at a more precise definition in morphological terms is difficult although the importance of doing so has been emphasised in a recent publication (Brodal, 1963). When one reads in a recent text-book (Elliot, 1963) that the rhinencephalon is a 'functional fabric, diffuse, and interwoven with other structures into which its extensions finally merge' it seems doubtful if a precise morphological definition is even possible. Nevertheless the term is used in nearly all neuroanatomical texts and usually as a synonym for Broca's limbic lobe (see Crosby, Humphrey & Lauer, 1962). The unsatisfactory nature of both terms is perhaps indicated by the fact that neither appears in the index of the classical "Comparative Anatomy of the Nervous System" published in 1936 (Kappers, Huber & Crosby).

Historically Owen (1868) seems to have been the first to use the term in a neuroanatomical sense and he limited it to the olfactory bulb and its peduncle. He considered the olfactory bulb as a 'primary vesicle' of the brain because in most macrosmatic mammals the lateral ventricle is continued into it; and so together with the olfactory crus it has all the essential features of a primary encephalic division. Turner (1890) extended its use to include a "lobus hippocampi",

the whole basal lobe of the hemisphere medial to the rhinal fissure. This corresponds to the piriform lobe of many authors (e.g. Elliot Smith, 1895) but it is not clear how much of the hippocampal formation proper (dentate gyrus, Ammon's horn and fimbria) Turner included in his "hippocampal lobe". Earlier, Broca (1878) had described a "great limbic lobe" present in all mammals, to which he ascribed a general olfactory function. This surrounds the hilum of the hemisphere and consists of the supracallosal gyrus continuous behind the splenium with the hippocampal gyrus and uncus (Broca's lobe of the hippocampus). The uncus receives the external olfactory tract of modern terminology, and the circuit round the hilum is completed by a medial olfactory root to the parolfactory area (septum) and anterior extremity of the supra-callosal gyrus. Elliot Smith (1895) in defining what he called the "true limbic lobe" excluded the supra-callosal gyrus which he assigned to the neopallium. He retained however the hippocampal vestiges in this situation. As a result the hilum is now completely surrounded by a distinct and well-defined ring of gray matter, phylogenetically the more ancient part of the cortex cerebri, completed anteriorly by the precommissural and septal areas that connect the anterior extremity of the hippocampus and the piriform lobe. Elliot Smith considered that the true limbic lobe is the only part of the hemisphere which is intimately associated with olfaction and that it is synonymous with Turner's "rhinencephalon". Modern authors on the other

hand have tended to return to Broca's conception and to include the cingulate (supra-callosal) gyrus.

The fornix as a continuation of the fimbria, is the most obvious macroscopic fibre tract of the rhinencephalon. Since it can easily be traced to the mammillary body, which is in turn connected by a large "column of Vicq D'Azyr" to the anterior thalamic nuclei, these parts of the thalamus and hypothalamus came to be included in the rhinencephalon by many authors. When, later, microscopical observations indicated that the stria medullaris had many connexions with the so-called "medial olfactory area" (see Crosby, Humphrey & Lauer, 1962, p.271, for references) the epithalamus (habenular ganglia) was also included. The maximum extent of this complex of rhinencephalic structures was illustrated by Allen (1940) and was adopted by Fulton in his well known "Physiology of the Nervous System" (1943) (see Fig. 1). In more recent publications, descriptions of the anterior thalamic nuclei and the mammillary bodies are usually omitted from accounts of the rhinencephalon although their connexions with the hippocampus and cingulate cortex are of course mentioned. The epithalamus (habenula) is usually retained (see Gray's Anatomy, 1962, 33rd edition).

Microscopic observations have naturally added much detail to our knowledge of the rhinencephalic structures. The differentiation for example, of an anterior olfactory nucleus in the peduncle by Herrick (1924); the division of the

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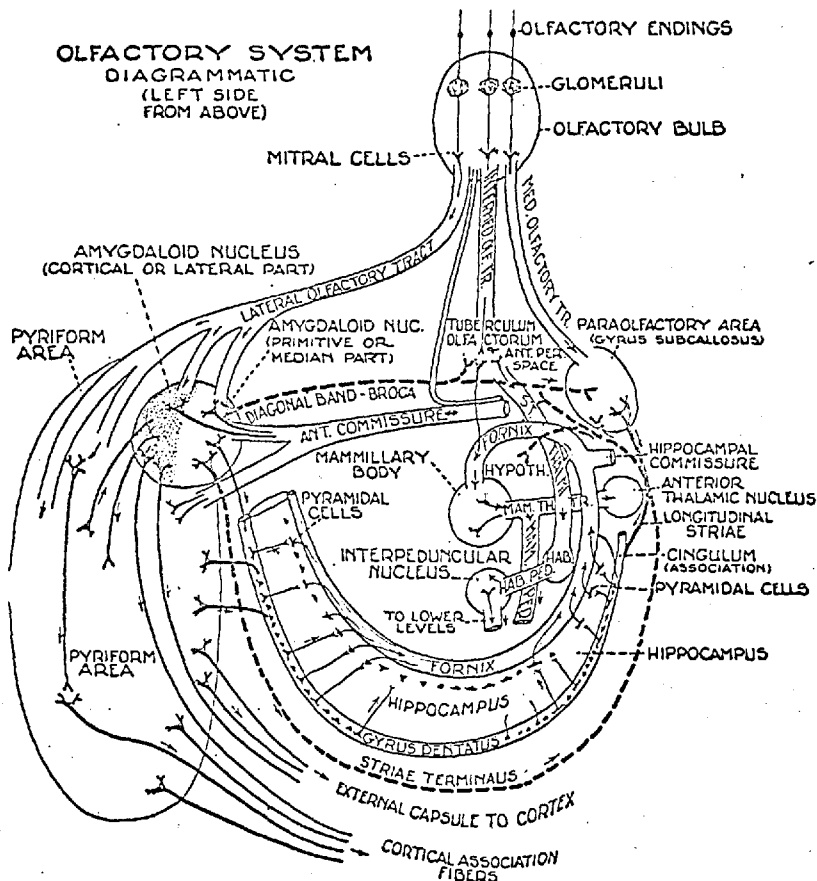


FIG. 77. A diagram of the principal connections of the olfactory system and rhinencephalon (W. F. Allen, *Amer. J. Physiol.*, 1940, 128, 767).

Fig. 1. A diagram of the extensive olfactory system as illustrated by Allen (1940) and adopted by Fulton and many others. (Reproduced from Fulton's Physiology of the Nervous System, 2nd ed., 1943.)

piriform cortical area into anterior and posterior parts the latter being the entorhinal cortex or area 28 of Brodmann; the recognition of a large number of nuclei in the parolfactory and amygdaloid regions; and many details of fibre connexions. This accumulation of descriptive detail has not made the problem of a general definition any easier, especially as the names applied have varied from author to author. Elliot's somewhat vague definition as a diffuse fabric interwoven and merging with other structures, quoted at the beginning of this section, seems about the best that can be given. It has also been suggested that the term "rhinencephalon" has outlived any usefulness it may once have had and should be abandoned (Goldby, 1961; Brodal, 1963; but see also Gastaut & Lacombe, 1961). This suggestion will be referred to again in the discussion.

It must be stated here that the present study deals only with certain parts of this extensive rhinencephalon. These are the olfactory bulb and structures on the basal surface of the hemisphere to which connexions from the olfactory bulb can be traced directly, and some others, so closely related to them, that participation in olfactory function is almost certain. The latter consist of the septal region and the entorhinal area. The hippocampal formation and some other parts of Broca's limbic lobe will not be considered. This is not because the hippocampus, and indeed many other parts of the brain may not have some relation to olfactory function. After stimulating the olfactory receptors or the bulb, action potentials have been

picked up from many regions (see for example, Berry, Hagamen & Hinsey, 1952) but to include them all would have involved a study of almost the whole fore-brain, and would destroy any precise meaning that the term "rhinencephalon" could have either in a functional or morphological sense.

It still remains to define briefly the parts to be studied, and to state the terminology which will be used for them in this thesis. This is necessary because of the variety of terms which have been applied to such structures by different authors but no attempt has been made to give all possible synonyms. While doing this the macroscopic features of these parts in the coypu brain will also be described shortly. They are illustrated in Figs. 2 and 3. Except in size they do not differ significantly from the similar features of the rat's brain so that a detailed description is not necessary.

Olfactory bulb and accessory olfactory bulb

The defining characteristic is the direct reception of the central processes of the receptor cells in the olfactory mucosa or in the vomero-nasal organ.

In the coypu the olfactory bulb is piriform in shape (see Fig. 4) and measures about 9 mm cranio-caudally. As the length of the cerebral hemisphere is 35 mm, the ratio of the length of the olfactory bulb to the hemisphere is about 1 to 4, thus indicating the macrosomatic nature of this brain. The bulb could be seen from the dorsal aspect of the brain projecting for about 6 mm in front of the frontal pole. Its dorsal surface is fairly smooth, particularly when compared with



Fig. 2. Photograph of the ventral surface of the coypu brain to show the basal rhinencephalic structures. Compare with Fig. 3. About 2.75 times natural size.

the shaggy rostral and ventral surfaces where most of the olfactory nerves enter the bulb. The accessory bulb invaginates the postero-dorsal aspect of the main bulb and is seen quite clearly as an indentation when the frontal pole, which covers it, is raised slightly.

Olfactory peduncle

This is the part of the brain by which the olfactory bulb is attached to the frontal lobe of the hemisphere. In the coypu the olfactory peduncle is short and stout, and is separated from the bulb by an obliquely running 'circular' sulcus lying more rostrally in the lateral side (Fig. 3). The olfactory peduncle extends from the circular sulcus to the cranial border of the olfactory tubercle where it joins the hemisphere. Between this joint and the bulb it lies in a deep ventro-medial depression in the frontal lobe.

Anterior olfactory nucleus

This is the gray matter of the peduncle which may extend for a short distance into the bulb rostrally and the frontal lobe caudally (see Herrick, 1924). In the coypu it is well developed and will be described in detail under the section dealing with microscopic features.

Olfactory tracts

These are secondary olfactory fibres arising from neurones in the bulb or accessory bulb and running superficially in the peduncle to other parts of the brain. Where the peduncle joins

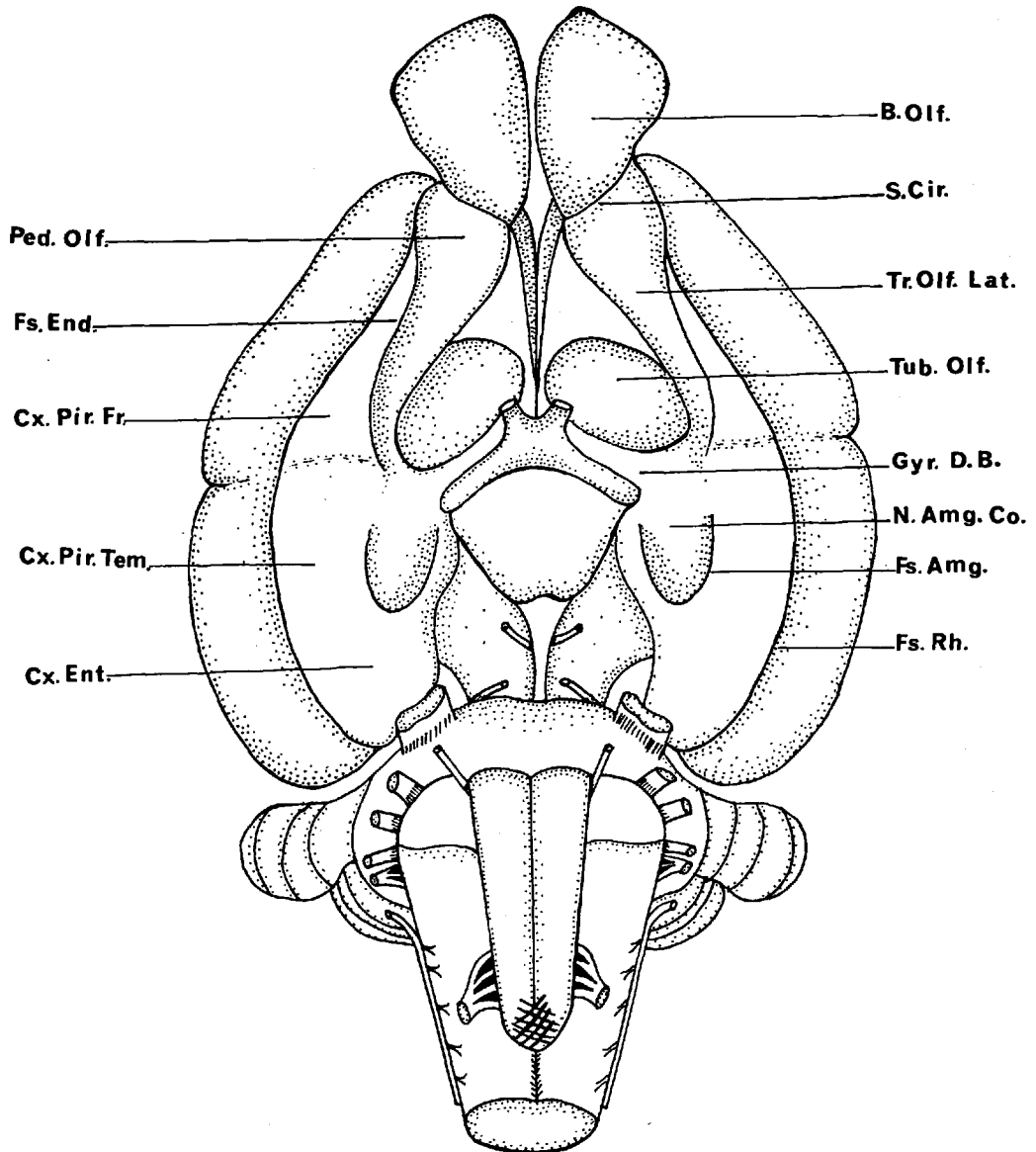


Fig. 3. A drawing of the ventral surface of the brain to show the different parts of the basal rhinencephalon. The groove between the frontal and temporal parts of the piriform cortex is occupied by the middle cerebral artery.

(For abbreviations in this and other figures see p.194.)

the frontal lobe they diverge to form tracts or striae to which the terms medial, intermediate and lateral are usually applied. For a description of the situation in the human brain see Klass (1928) who also describes medial and lateral accessory striae, and Erhart (1950). There is some lack of uniformity in the way these tracts or striae have been named, but only one of the variations needs to be mentioned here. Some authors (e.g. Lauer, 1945; Johnson, 1959) have used the term intermediate olfactory tract for fibres situated deeply in the peduncle beneath the gray matter of the anterior olfactory nucleus. These fibres run into the anterior limb of the anterior commissure, and, as will be seen, there is doubt if they arise from cells within the olfactory bulb. In this thesis such fibres will be referred to as an "olfactory commissural component" and the term "tract" limited to the superficial fibres, which can be classified as follows:

(a) Lateral olfactory tract. Fibres which diverge lateral to the olfactory tubercle or anterior perforated space to spread out over the surface of the piriform cortex and amygdala. They follow approximately the course of a shallow groove which can usually be identified at the medial boundary of the piriform cortex, the endorhinal fissure. In the human brain they have been identified as forming a lateral olfactory stria or gyrus.

In the coypu brain, the lateral olfactory tract stands out as a distinct shining white band which appears immediately caudal to the circular sulcus. It is broader cranially and

gradually tapers caudally towards the amygdala behind which it can no longer be identified.

(b) Intermediate or accessory olfactory tracts. Secondary olfactory fibres which spread superficially on the olfactory tubercle or anterior perforated substance. These do not form a very definite tract and can rarely be identified macroscopically. They are represented by the variable medial and lateral accessory striae described by Klass (1928) in the human brain. Erhart (1950) states that they are present in only 1 to 5% of human specimens. Such a tract cannot be identified macroscopically in the coypr material.

(c) Medial olfactory tract. Secondary olfactory fibres which appear to diverge from the peduncle medial to the olfactory tubercle and pass towards the septal or parolfactory region. As a macroscopic feature the medial olfactory tract is variable and it could not be identified clearly in the coypr. Erhart (1950) states that it cannot be seen in 20% of human brains.

Olfactory commissural component

A well defined bundle of fibres deeply situated in the peduncle which can be traced from the bulb to cross in the anterior commissure. These fibres are sometimes referred to as the intermediate olfactory tract (see above) or as coming from the medial or intermediate olfactory tracts (Crosby, Humphrey & Lauer, 1962) or from the medial olfactory tract alone

(Kappers, Huber & Crosby, 1936). It is sometimes called the anterior limb or interbulbar component of the anterior commissure (e.g. by Gurdjian, 1925).

In the coypu this component of the anterior commissure is well developed, deeply situated in the olfactory peduncle and lying entirely within its gray matter (anterior olfactory nucleus) throughout its extent. Posteriorly it is joined by a few fascicles coming from or going to the base of the brain and becomes embedded in the nucleus accumbens before turning medially to cross in the anterior commissure.

Olfactory Tubercle

An elevation on the base of the brain bounded laterally by the lateral olfactory tract, caudally by the diagonal band, and extending to the ventro-medial border of the hemisphere where it abuts on the septal region, where it may be bounded by a medial olfactory tract or gyrus in some mammals. It possesses a cortex of characteristic structure and lies immediately ventral to the head of the caudate nucleus and nucleus accumbens; it is perforated by small basal arteries, branches of the middle and anterior cerebral arteries. Prominent in macrosmatic mammals, it is represented by part of the anterior perforated substance or olfactory trigone in the human brain.

In the coypu brain, the olfactory tubercle is quite large and forms a prominent feature of the ventral surface of the brain. Its large size, as well as that of the bulb and piriform

lobe demonstrates clearly the macroscopic nature of this brain. The arcuate sulcus forms the boundary between the olfactory tubercle and the lateral olfactory tract.

Septum

A mass of nervous tissue on the medial aspect of the frontal lobe immediately anterior to the lamina terminalis and the anterior commissure. Above it is overhung by the genu of the corpus callosum and cranial to this is bounded by the anterior hippocampal rudiment. Ventrally the septum abuts on the olfactory tubercle anteriorly and the diagonal band posteriorly. Above it is continuous with the septum pellucidum, when this is present, between the corpus callosum and the fornix; the septum pellucidum may be regarded as formed by a stretching of the upper part of the septum between these two structures.

The septum has also been called the parolfactory region (Broca) and the precommissural or paraterminal body (Elliot Smith), and superficial features have received names such as the sub-callosal gyrus and anterior and posterior parolfactory gyri in the human brain. Although unsatisfactory in some respects the term septum has received general recognition and has been adopted here. In the coypu it is well developed (Fig. 4) and presents no unusual macroscopic features.

Gyrus diagonalis (diagonal band of Broca)

A slightly elevated ridge on the basal surface of the hemisphere, caudal to the olfactory tubercle and passing from the amygdala forwards and medially to the caudo-ventral part of

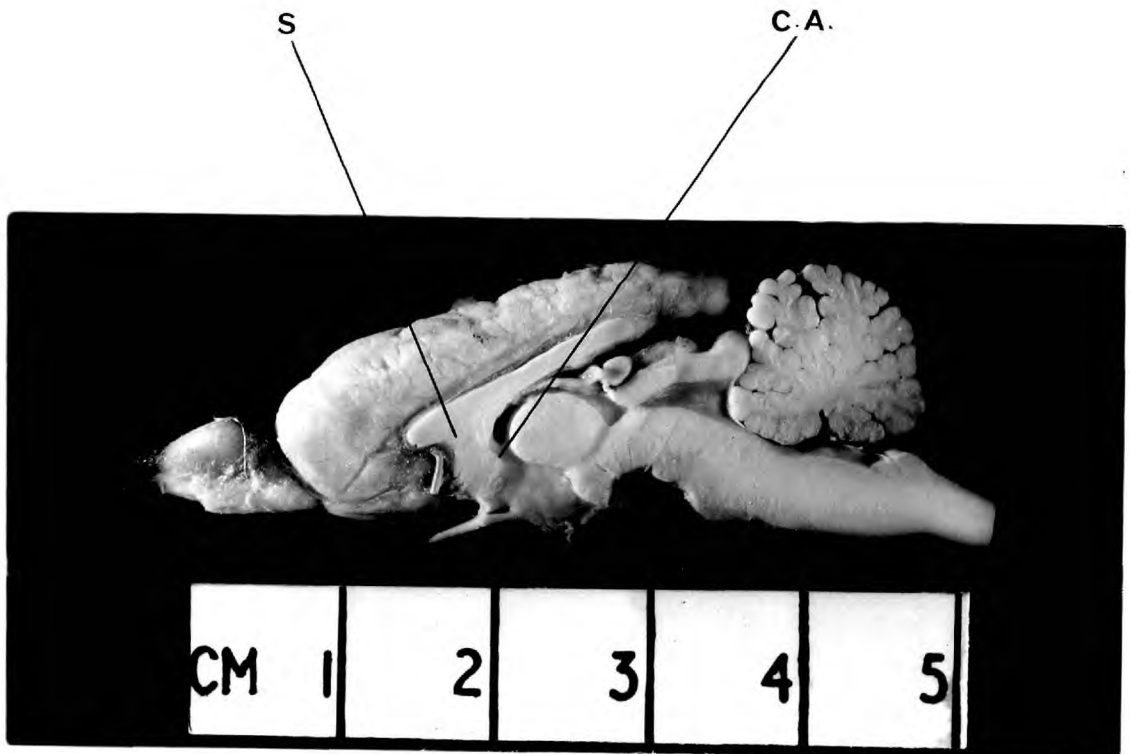


Fig. 4. Photograph of the medial surface of the coypu brain to show the shaggy ventro-medial surface of the olfactory bulb, the septal region (S) and the anterior commissure (C.A.).

the septum. It contains a tract of fibres as well as nerve cells.

In the coypu it forms a distinct and slightly elevated band at the caudo-medial boundary of the olfactory tubercle, and lies between it and the optic tract and chiasma.

Amygdala

This term, taken from human anatomy, applies to the group of nuclei on the medial side of the temporal lobe at the caudal end of the lateral olfactory tract and ventral to the main mass of the corpus striatum. It is bounded medially by the caudal termination of the hippocampal formation. It is more or less clearly outlined laterally by the amygdaloid fissure in most mammals, which is sometimes continuous with the endorhinal fissure rostrally, as in the opossum (Loo, 1930). It is covered by a cortical layer which may be called the cortical amygdaloid nucleus (or the periamygdaloid cortex of Rose, 1926 and 1927) which is continuous through a transitional area with the piriform cortex laterally. It is represented by the uncus in man, but it is doubtful if the sulci related to the uncus can be compared directly with the amygdaloid sulcus of more primitive mammals.

In the coypu, the amygdala forms a prominent surface bulge, and the superficial boundary of the cortical nucleus is clearly outlined by a U-shaped amygdaloid fissure (Figs. 2 and 3) which is a constant feature of this brain. Its lateral limb is not continuous with the endorhinal sulcus as in the opossum. The

nucleus of the lateral olfactory tract, usually classified in the amygdaloid group, is also a macroscopic feature immediately rostral to the cortical nucleus.

Piriform cortex

The cortical area on the base of the brain which is bounded laterally by the rhinal and medially by the endorhinal fissures. It extends from the olfactory peduncle, where it is continuous with the lateral part of the anterior olfactory nucleus posteriorly to the amygdaloid region. There are no anterior or posterior boundaries which can be seen macroscopically. It is often referred to as the prepiriform or anterior piriform cortex but these terms will not be used in this thesis. In the coypu the middle cerebral artery runs transversely across the middle of the piriform lobe and forms a convenient division between frontal and temporal parts of the piriform cortex.

Entorhinal cortex

The cortex of the temporal lobe which extends posterior to the amygdala, bounded laterally by the posterior end of the rhinal fissure and medially by the subicular cortex which separates it from the hippocampal formation. This is the posterior piriform cortex of some authors (e.g. Gray, 1924) but it differs markedly in structure from the rest of the piriform cortex and receives no secondary olfactory connexions from the bulb. It seems best therefore, to emphasise the difference by a separate name although there is no sulcus separating it from

the piriform cortex anteriorly or from the retrosplenial region of the neocortex caudally.

In the coypu, as in most other macromammals, the entorhinal cortex is less extensive than the piriform cortex, particularly when compared with the higher primates where the proportions are reversed. Its rostral and caudal boundaries are not marked by any macroscopic feature.

SOME MICROSCOPIC FEATURES OF THE
RHINENCEPHALON AND THEIR TERMINOLOGY

In this section the parts of the rhinencephalon to be studied are considered in turn. A brief summary of the condition in mammals generally, taken from the literature, is given for each part before the condition in the coypu is described. The microscopic features of the rhinencephalon in the coypu are so similar to those of comparable macrosmatic mammals (e.g. the rat) that separate treatment seemed unnecessary.

Olfactory bulb

The olfactory bulb is typically laminated and the lamination is very uniform in all mammals. Seven layers are normally recognised: 1. layer of olfactory nerves; 2. glomerular layer; 3. external plexiform layer; 4. mitral cell layer; 5. internal plexiform layer; 6. internal granular layer; and 7. ependymal or periventricular layer. The obliterated olfactory ventricle is usually represented by a mass of ependymal cells.

Our knowledge of the significance of these laminae dates from the work of Golgi (1875), who, with the aid of his new method of bichromate-silver impregnation demonstrated for the first time the finer structure of the glomerulus and some other parts of the bulb. However, his misinterpretation of some features of the connexions were due to his belief in the reticularist hypothesis (Gerlach) then current. Later Cajal (1890), van Gehuchten & Martin (1891), Calleja (1893) and others,

all using the chrome silver method of Golgi, made further contributions. However, it was Cajal (1890, 1901-2, translated 1911 and 1955) who provided the correct interpretation of the synaptic relationships within the glomeruli between the peripheral olfactory fibres and the dendrites of the mitral cells. His classical work forms the basis of our present idea of the mode of conduction through the bulb. It appears that the mitral cells are structurally and functionally polarised in the sense that the tufted endings of the dendrites in the glomeruli are receptive and their axons emissive. They therefore transmit impulses of olfactory origin to the olfactory tracts. The function served by the collateral branches of the axons of the mitral cells, by periglomerular cells, and the granule cells are not so clear but they are probably parts of an elaborate apparatus for spreading and stepping up the energy of transmission (avalanche conduction of Cajal). Many granule cells could be stimulated by the collaterals and the axons of the granule cells could then discharge to the outer plexiform layer or possibly the periglomerular cells or the glomeruli, leading eventually to the activation of a much larger number of mitral cells. Shepherd (1963) in electrophysiological experiments was unable to obtain evidence for the avalanche conduction of Cajal.

Since the observations of the above mentioned authors, the olfactory bulb of mammals has been described in detail by many observers. Among these are Winkler & Potter (1911 and 1914), Gardjian (1925), Young (1936), Humphrey (1936),

Crosby & Humphrey (1939), and more recently Allison (1953a), Johnson (1957), Pilleri (1961) and Lohman (1963). Allison (1953b) reviewed the previous work and drew particular attention to the presence in the external plexiform layer of many 'displaced mitral cells' or Cajal's 'tufted cells' (cellules à houppette). These are medium sized, ovoid or triangular cells peripheral to the mitral cell zone which stain less deeply than the mitral cells. They are most numerous in mammals, in which they are found either as supplementary to a highly developed mitral cell layer (as in the shrew), or as compensatory in forms such as the primates which show a marked diminution of the mitral cell layer itself (Crosby & Humphrey, 1939). Cajal (1911), Young (1936), Humphrey (1936), Allison (1953 a and b), and some other authors were of the opinion that the tufted cells give rise to fibres that interconnect with the opposite olfactory bulb. There has been, however, much controversy over the origin and termination of these commissural fibres and this question will be discussed later in the light of the experimental evidence.

Modern work on the olfactory bulb has been largely concerned with the relationship between the bulb and the olfactory mucosa and the problem of olfactory discrimination. The number of receptor cells is very large, 50,000,000 on each side in the rabbit (Clark, 1957) and Gasser (1956) showed that the fibres to which they give rise are very fine (0.1 μ or less in diameter). He made some computations of an

indirect nature from his electron micrograph preparations of the olfactory mucosa in the pig and arrived at the conclusion that there was a reasonably close correspondence between the number of nerve fibres and the number of receptor cells. De Lorenzo (1957; 1960) confirmed Gasser's observation of the very fine calibre of the olfactory nerve fibres and described their terminations where they pushed into the mitral cell dendrites in a glomerulus. The presynaptic terminals contain mitochondria and synaptic vesicles.

On the assumption of a one to one relationship between receptor cells and olfactory nerve fibres Allison & Warwick (1949) estimated that about 26,000 fibres must enter each glomerulus. A mitral cell has one main dendrite which distributes to one glomerulus only, each glomerulus being reached by the dendrites of about 24 mitral and 68 tufted cells. Allison and Warwick concluded that the glomerulus is an excellent mechanism for spatial summation.

As concerning the possible neural mechanism for olfactory discrimination, the matter is by no means settled. Adrian (1950) by stimulating small areas of the olfactory mucosa in the rabbit and recording the responses in the bulb was able to demonstrate some degree of topographical localisation for the projection of the olfactory mucosa on the bulb. The oral extremity of the mucosa projects mainly to the anterior and the aboral to the posterior part of the bulb. In 1955b he also showed, by recording potentials from intrabulbar electrodes,

that the sensitivity of the mitral cells to different smells is by no means uniform. So far, he has found it possible to divide the cells into at least 4 or 5 types, each particularly sensitive to one group of smells. This specificity of mitral cells may apply also to the glomeruli from which they receive their impulses, and it may be that the particular glomeruli concerned receive their fibres predominantly from groups of receptors which are themselves selectively sensitive to one or other chemical substance. However, Le Gros Clark (1957) gives a different explanation stating that the olfactory nerve plexus on the surface of the bulb: "serves precisely the opposite purpose of ensuring a complete randomization of fibres so that each glomerulus receives impulses from every type of receptor; in other words, that each glomerulus is in itself a functional unit concerned with the total range of olfactory discrimination".

The existence of a centrifugal fibre system entering the olfactory bulb from the brain, through which the activity of the bulb can be influenced by higher centres, was described originally by Cajal (1911) and more recent evidence will be discussed later.

Olfactory bulb of the cortex

From without inwards the layers are:

- (1) Layer of olfactory nerve fibres. This forms a dense plexus of unmyelinated fibres over the surface of the greater part of the bulb, most marked on the ventral, medial and anterior surfaces (Fig. 5); caudally, it disappears first from the dorsal, then the lateral and lastly the medial surface. In

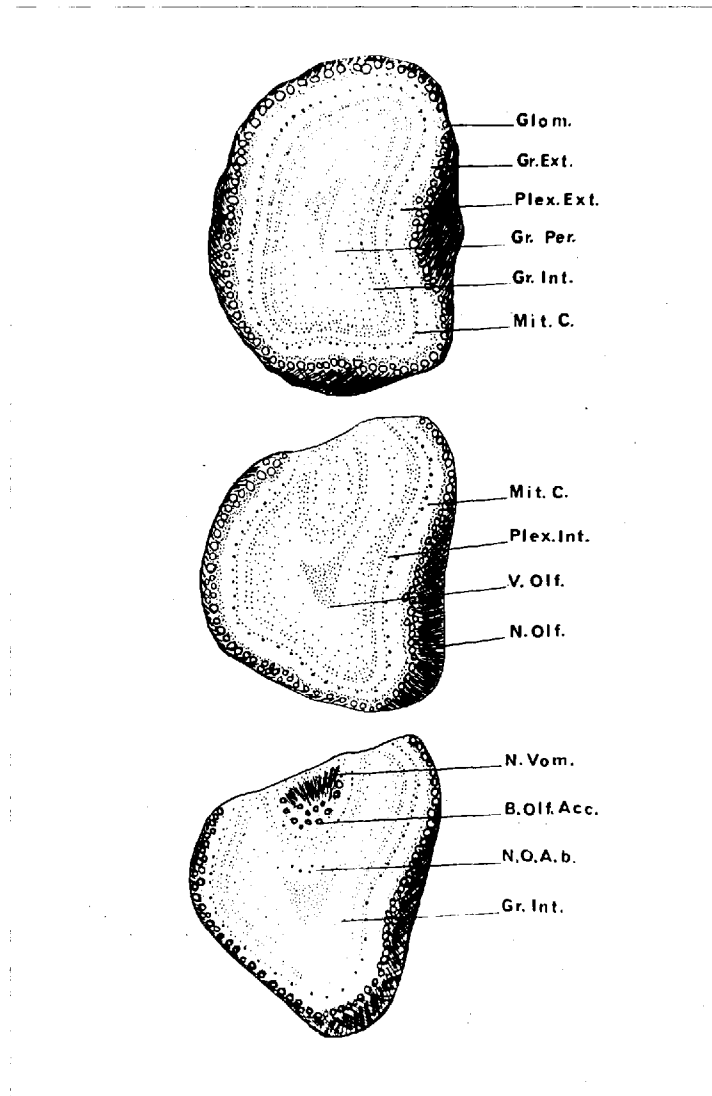


Fig. 5. Drawings of transverse sections of the olfactory bulb to show general features. Toluidine blue stain. X 7.

Holmes' silver preparations the fibres can be seen approaching the bulb in compact bundles which separate into smaller fasciculi and are interwoven for a short distance before terminating in the olfactory glomeruli.

(ii) Layer of glomeruli. The bulb is surrounded on all sides by the glomerular layer which corresponds in extent with the layer of olfactory nerve fibres. They form a fairly regular stratum, mostly one glomerulus thick. The glomeruli are easily seen with the low power as fairly large rounded structures almost completely free of cells and about 125 μ in diameter. They are circumscribed by well-defined capsules of periglomerular or outer granular cells, most of which are rounded, small, with little cytoplasm and very similar to the cells of the inner granular layer. Mixed with them are a small number of larger cells fusiform or triangular in shape and generally resembling the smaller tufted cells. The Loyez preparations show a number of fine myelinated fibres among the periglomerular cells. These could be afferent fibres from other parts of the bulb or cerebral hemisphere; they cannot be olfactory nerve fibres which are unmyelinated, but the possibility that they are axons of at least the larger periglomerular cells cannot be excluded.

(iii) External plexiform and tufted cell layer. A broad fibre layer composed partly of radially disposed fibres, most of which are the dendrites of the mitral cells. There are many finer fibres running obliquely or parallel to the surface, some of

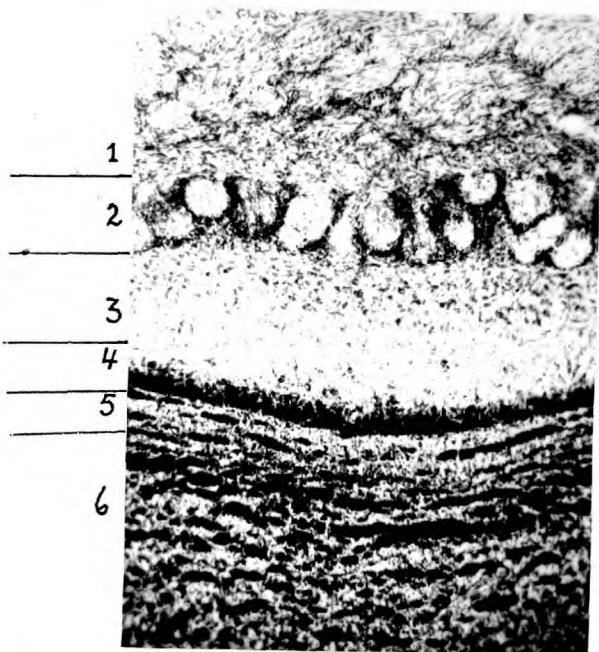


Fig. 6. Photograph of a transverse section to show the 6 layers of the main olfactory bulb. Toluidine blue, X 55.

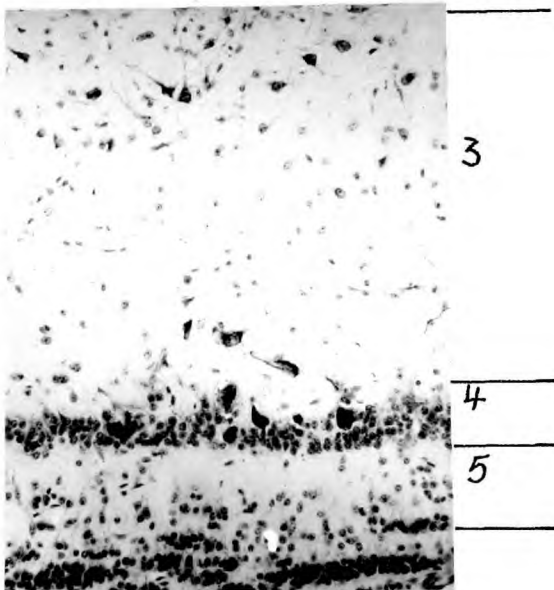


Fig. 7. Higher power view (X 140) of the external plexiform and mitral cell layers. Note the displaced mitral cells close to the latter and the tufted cells in the superficial part of the external plexiform layer. Toluidine blue.

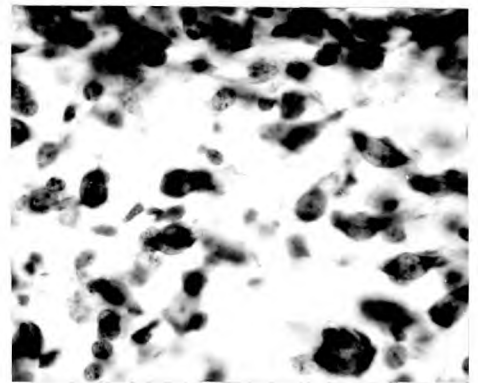


Fig. 8. High power view (X 360) of the tufted and periglomerular cells. Toluidine blue.

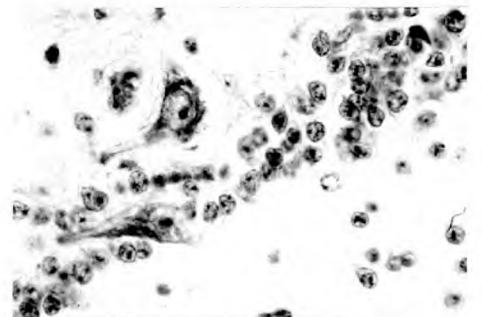


Fig. 9. High power view (X 360) of the mitral and associated granule cells. Toluidine blue.

which are myelinated. A few cells are seen against a rather clear background. These are the displaced mitral or tufted cells. They are pyramidal or multipolar in shape and although smaller than the typical mitral cells, are similar in appearance but slightly elongated. Near the glomeruli they are somewhat smaller than those nearer the mitral cell layer so that there may well be two types of cell - 'displaced mitral' cells near the mitral cell layer and more numerous and smaller 'tufted cells' near the glomeruli. (Fig. 7).

(iv) The layer of mitral cells. This layer is compact and clear cut, and besides the typical mitral cells contains many granule cells. The mitral cells are large and situated at the outer border of the layer (Fig. 7). They are the most characteristic cells of the olfactory bulb, commonly ovoid or triangular in shape, and with conspicuous Nissl bodies (Fig. 6). The main dendrite goes to the glomerulus as described above. The axons are fine but as they pass to the internal plexiform layer and turn caudally towards the olfactory tracts they become thicker and acquire myelin sheaths. Most of them appear to enter the lateral olfactory tract. The bases of the mitral cells are surrounded by densely packed granule cells.

(v) Internal plexiform layer. This is much narrower than the external plexiform layer and lies just deep to the mitral cells. It contains a few cells of the granule type. The fibres are probably mainly the collaterals of axons of tufted and mitral cells.

(vi) Internal granular layer. This, the main granule cell layer, contains small rounded cells closely packed in irregular laminae and clumps. Their nuclei usually contain two nucleoli and there is little visible cytoplasm. Neuroglial elements are also present. Myelinated fibres grouped into bundles (the axons of mitral and tufted cells) are found in this layer between and among the clumps of granule cells. The internal granular layer has no definite inner margin but gradually gives place to a region containing few cells (periventricular cells), mostly glial elements, and a loose network of fine myelinated fibres. These are probably the bulbo-petal fibres described by Cajal and others. Caudally an ill-defined concentration of small deeply staining nuclei is seen among which are a few clumps of epitheloid cells, some arranged as small cysts. These are the remnants of the olfactory ventricle.

Accessory olfactory bulb

The first author to describe the accessory bulb is von Gudden (1870) in the rabbit. Also in the rabbit it has been described by Cajal (1911), McCotter (1912), Winkler & Potter (1911) and Young (1936). McCotter (1912) followed the vomero-nasal nerve from Jacobson's organ to the accessory bulb and showed that its size in different species varies in direct proportion to that of the vomero-nasal organ. The accessory bulb has now been described for many mammals, such as marsupials (Herrick, 1924), rodents (Gurdjian, 1925), carnivores (Winkler & Potter, 1914), and in the insectivores (Le Gros Clark, 1933).

It is lacking in the bat (Humphrey, 1936) and is either vestigial or absent in higher primates including man (Kappers, Huber & Crosby, 1936).

In general it resembles the main bulb and shows the same type of lamination. Cajal (1911) pointed out that the most important difference is in the mitral cell layer. These cells (Young, 1936) are not characteristically mitral or triangular in shape and do not form a single stratum. They are uniformly distributed throughout most of the plexiform layer, and there are no tufted cells. Allison (1953a) showed that the glomeruli of the accessory bulb scarcely merit the name, being much smaller and less definitely circumscribed than in the main bulb. Most authors described the position of the accessory bulb as on the postero-dorsal aspect of the main bulb and slightly to the medial side.

Accessory olfactory bulb of the coypu

The accessory olfactory bulb of the coypu begins to invaginate the dorsal surface of the main bulb just anterior to the frontal pole which overlaps the greater part of it (Fig. 13) and ends a little rostral to the plane of the caudal extremity of the main bulb.

The outermost layer is formed by fibres of the vomero-nasal nerve which spread over its surface (Fig. 5) and turn inward to end in the glomeruli, which are smaller than those of the main bulb and less regularly arranged (Fig. 10). Their capsules of periglomerular cells are less definite and contain fewer cells.

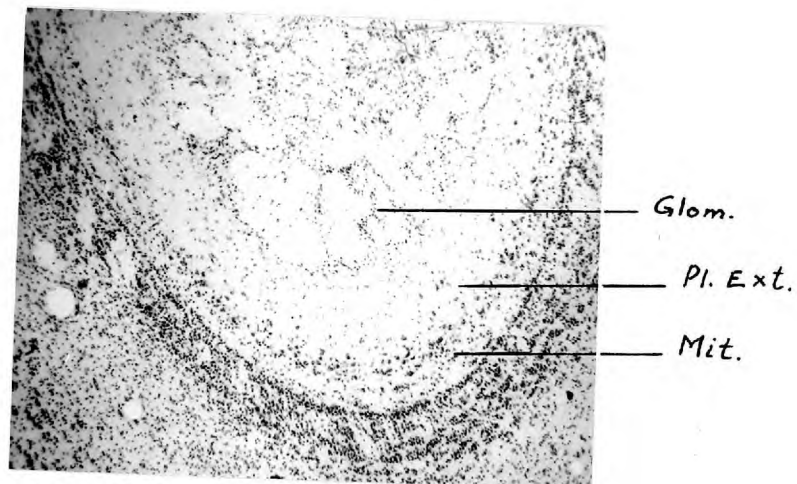


Fig. 10. Photograph of a transverse section of the accessory olfactory bulb. Toluidine blue, X 55.

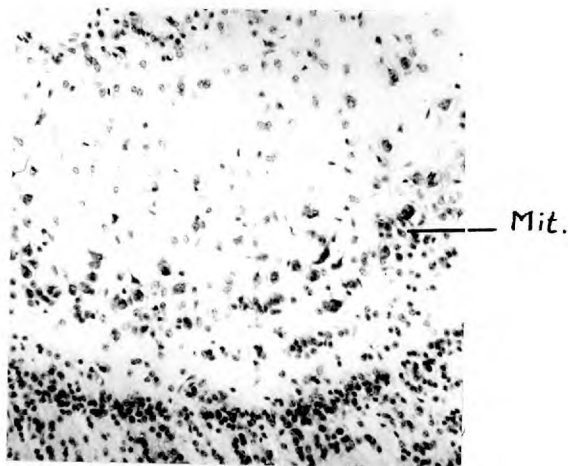


Fig. 11. Higher power view (X 140) of the external plexiform layer. Note that the mitral cells do not form a compact layer as is the case in the main bulb. Toluidine blue.

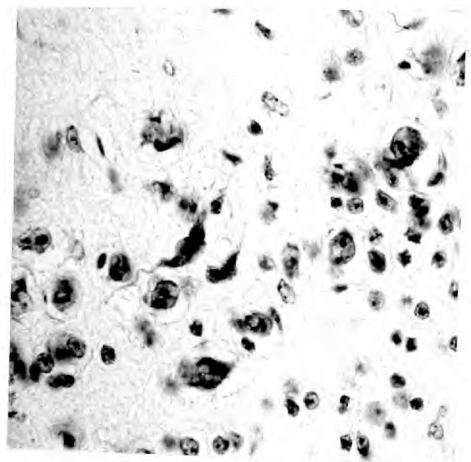


Fig. 12. Higher power view (X 360) of the mitral cells of the accessory bulb. Toluidine blue.

The external plexiform layer is much narrower than in the main bulb and contains fewer cells, probably none of them tufted cells. In its deeper part there is a broad, diffuse mitral cell zone, much less definite than in the main bulb and with much less admixture of granule cells. The axons of the mitral cells are very fine and it is difficult to follow them. They become medullated and join the bundles of axons from the main bulb which traverse the internal granular layer of the accessory bulb to reach the lateral olfactory tract. The internal plexiform layer of the accessory bulb is relatively broad. The internal granular is a very narrow layer of the granule cells similar to those of the main bulb, and particularly caudally, is broken up by the presence of medullated fibre bundles mentioned above (Fig. 14).

Anterior olfactory nucleus

This nucleus was first described by Herrick (1910 and 1924) in amphibian and marsupial brains. He defines it as "undifferentiated olfactory tissue of the second order that typically borders the bulbar formation, forming a ring or a cylinder of gray matter within the olfactory bulb or crus or adjacent part of the hemisphere". One understands Herrick's term "undifferentiated" as meaning a lack of clear differentiation into defined nuclei or laminae. It could also apply to the position of the cells close to the ventricular ependyma which may be regarded as primitive. He points out however, that this arrangement shows variations in each

vertebrate species depending to a great extent on the degree of development of the olfactory apparatus. The variations have been illustrated in several later studies, e.g. Young (1936), who pointed out that the use of the term "undifferentiated" could not be applied literally to the nucleus in the rabbit where it shows very considerable differentiation.

Herrick divides the nucleus into a pars bulbaris entirely within the olfactory bulb and a pars dorsalis, ventralis, medialis and lateralis in the olfactory peduncle. He also recognised a pars externa closely associated with the lateral olfactory tract. Caudal to the olfactory peduncle he described a pars posterior which merges into the medial wall of the hemisphere.

The pars externa of the anterior olfactory nucleus has attracted a great deal of attention. Herrick (1924), from his studies on the opossum, divides it into dorsal and ventral limbs, the former in close relation with the accessory bulb. He suggested that it forms a buffer between the main and accessory bulbar formations and the main body of the anterior olfactory nucleus. It is always interposed between the two. Obenchain (1925) states that the pars externa is probably an accessory olfactory nucleus which serves the function of reinforcing or stepping-up olfactory stimuli. She described it in Caenolestes and thought that the cells had migrated as a result of the neurobiotactic influence of the overlying olfactory tract fibres. Young (1936) accepted this explanation.

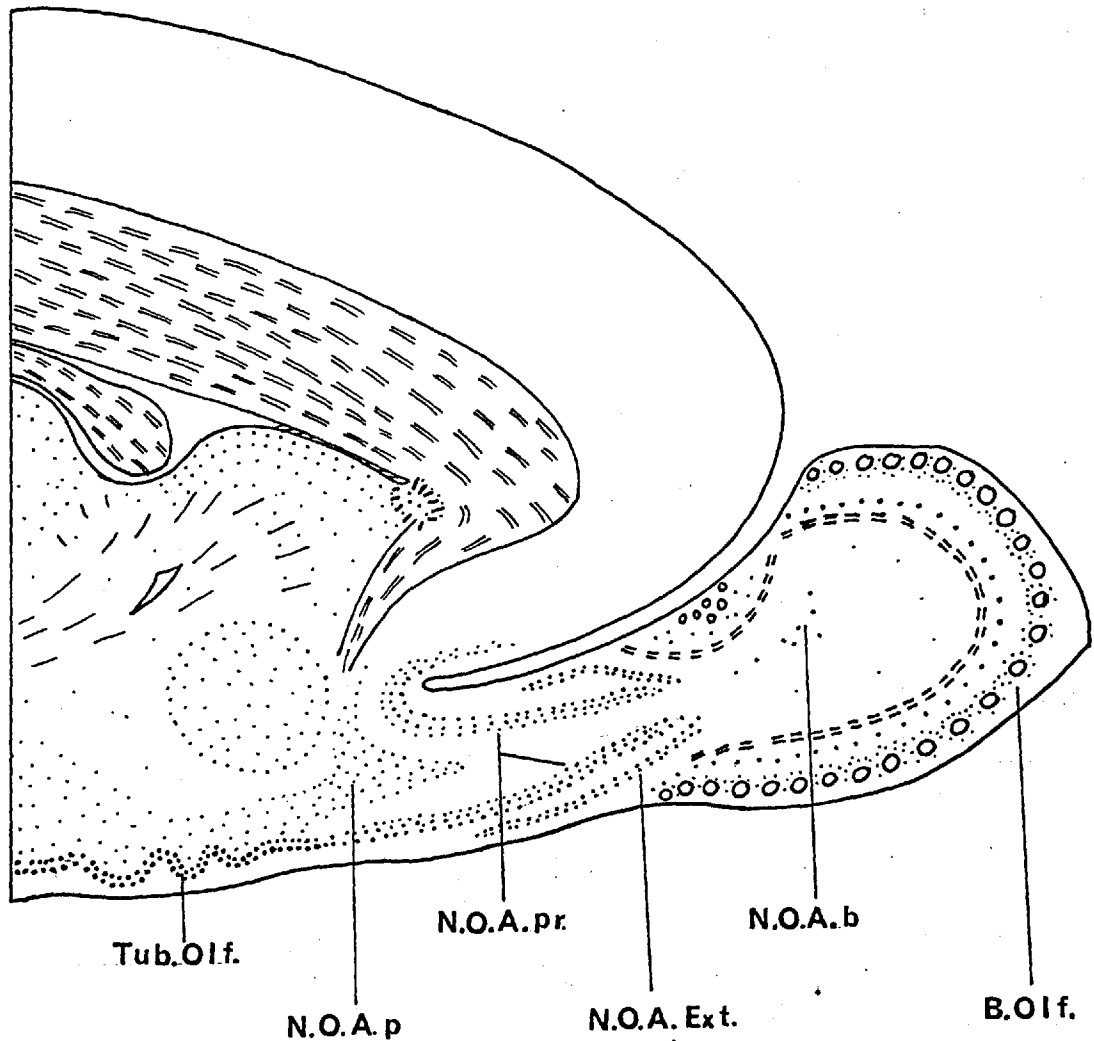


Fig. 13. A diagram illustrating the main features of the anterior olfactory nucleus as they would appear in a longitudinal section from a generalised mammal. (A list of abbreviations is given on p. 194.)

for the rabbit. He suggested that the division into dorsal and ventral limbs could also be the result of the aggregation of the particular fibres that exert this neurobiotactic influence into the dorsal and ventral parts of the lateral olfactory tract. The pars externa has also been described by Gurdjian (1925) for the rat, Humphrey (1936) for the bat, and more recently by Johnson (1957) and Lohmann (1963) for the guinea pig. Humphrey, however, found no division into dorsal and ventral limbs. She thought that this might be due to the absence in the bat of the accessory bulb, which, according to Herrick, is associated with the dorsal limb.

The most detailed account of the anterior olfactory nucleus is that given by Crosby & Humphrey (1939). They have studied it in a representative series of vertebrates comprising a large number of mammals, reptiles and birds. From this study and a review of the literature they concluded that a common pattern underlies its morphology, and that variation occurs only in the relative size and degree of differentiation of the component parts. They found that when the peduncle is short, as in many insectivores and bats, the parts of the nucleus tend to be telescoped to some extent into the bulb. At the opposite extreme, as in many primates and in man (Crosby & Humphrey, 1941), when the peduncle is long, the anterior olfactory nucleus is represented by scattered clumps of cells around the remains of the olfactory ventricle.

The diagram (Fig. 13) illustrates the main features of the

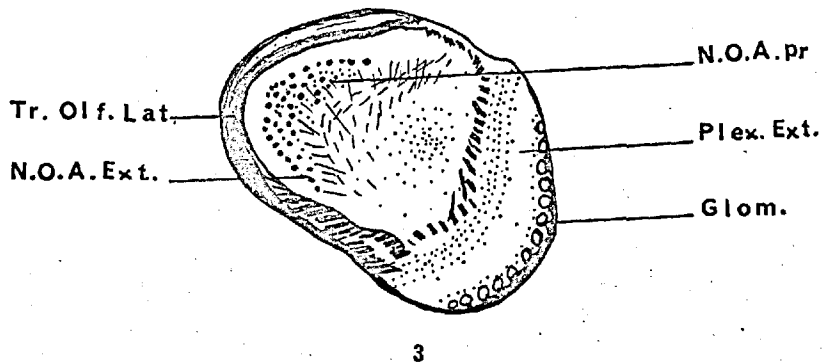
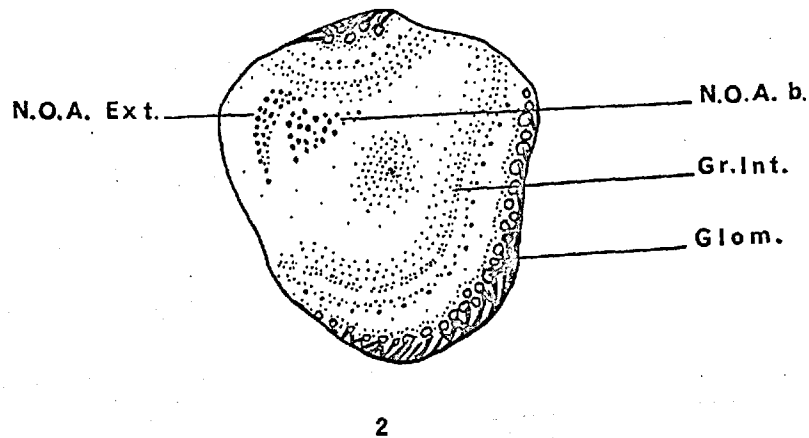
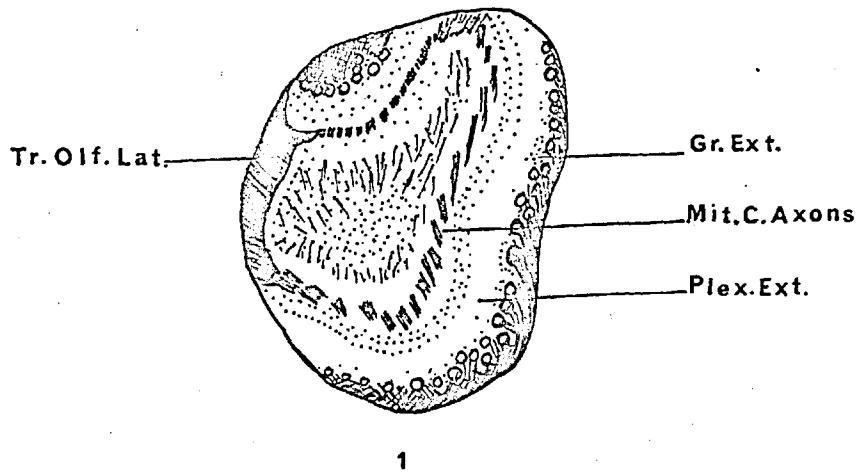


Fig. 14. Projection drawings of transverse sections of the caudal third of the olfactory bulb to show the most rostral end of the anterior olfactory nucleus. 1 and 3 are stained by Loyez method; 2 stained by toluidine blue. X 15.

anterior olfactory nucleus as they would appear in a longitudinal section from a generalised mammal. It consists essentially of the pars externa, close to the bulb and made up of small, deeply stained multipolar cells, and what may be called the 'pars principalis' in the form of a cylinder of cells surrounding the olfactory ventricle or its remnants in the peduncle. The latter is commonly described as possessing dorsal, ventral, medial and lateral parts, but these terms have little more than topographical significance and the parts do not show consistent cytoarchitectural differences. Caudally the anterior olfactory nucleus becomes continuous with the piriform cortex laterally and the frontal neocortex dorsally. Medially it is said to come in contact with some superficial cell groups in the septum representing a hippocampal rudiment. Ventrally it gives place to the olfactory tubercle but an extension in the form of scattered large cells between the cortex of the tubercle and the underlying striatum has been described as a pars posterior by some authors (e.g. Crosby & Humphrey, 1939).

Anterior olfactory nucleus of the cynom

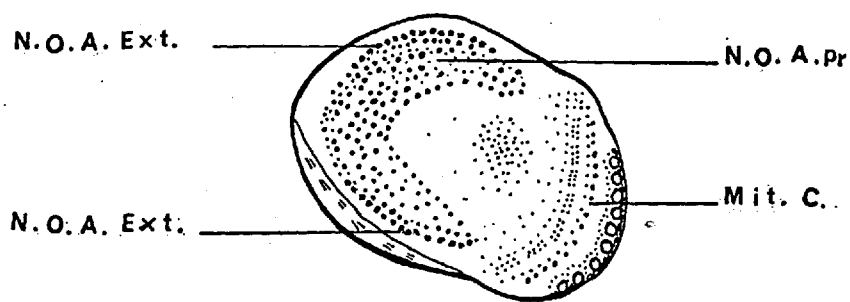
This nucleus is essentially similar to that described for other mammals, and to simplify the description we have divided it into three main parts: 1. pars externa; 2. pars principalis; and 3. pars posterior.

1. Pars externa, a horseshoe shaped mass of small deeply stained multipolar cells adjacent to and partly within the bulb,

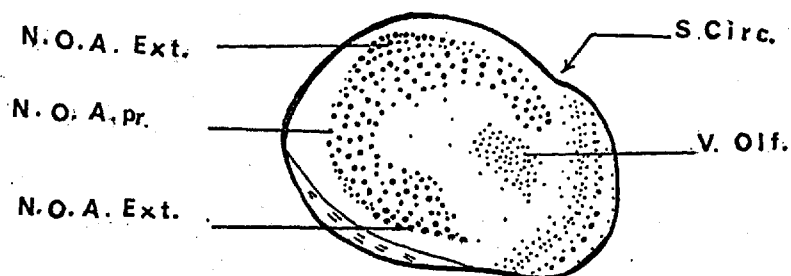
surrounding the peduncle obliquely except on its ventro-medial side. It is best developed dorso-laterally, where there is also a short caudal extension in dorsal and lateral tails (Fig. 15). The pars externa is interposed between the granule cell layer of the bulb externally and the pars principalis of the anterior olfactory nucleus more deeply. Its most rostral tip lies just deep to the caudal end of the accessory bulb, (Fig. 18)

2. Pars principalis, a thick ring of multipolar or pyramidal cells which surrounds the olfactory commissural component throughout the greater part of the olfactory peduncle (Figs. 15 and 16). Traced anteriorly its cells are replaced first on the medial side by olfactory bulb structure. Dorsally, ventrally, and laterally it extends forward within the bulb for a short distance. As the pars principalis is traced posteriorly it becomes differentiated laterally by the appearance of a definite, closely packed, superficial lamina continuous with the piriform cortex. This is probably the pars lateralis of other authors. Dorsally it is continuous with the neocortex of the frontal pole. Medially it gives place to some superficial, deeply stained, closely packed cells immediately anterior to the ventral part of the septum. These probably represent the anterior hippocampal rudiment, although they cannot be traced into continuity with the indusium.

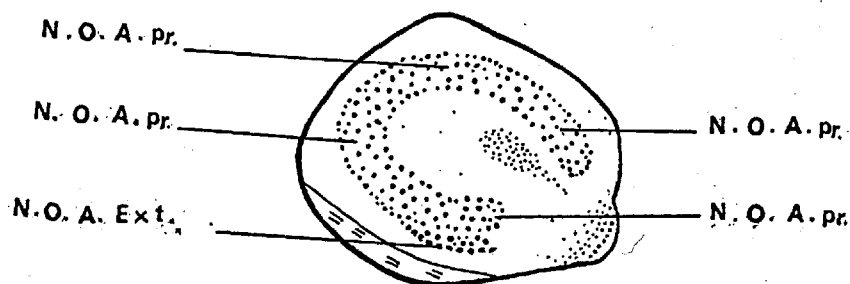
3. Pars posterior. Ventrally the pars principalis gives place to the structure of the olfactory tubercle but extends



1

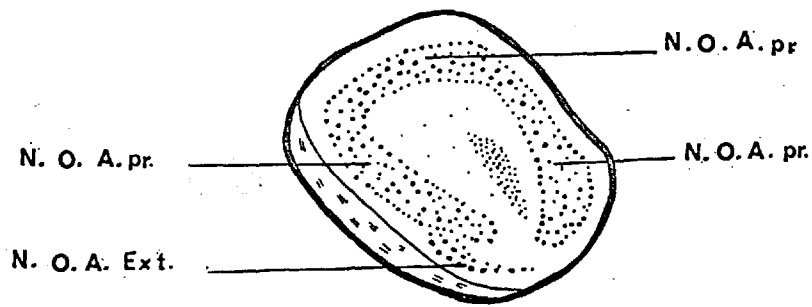


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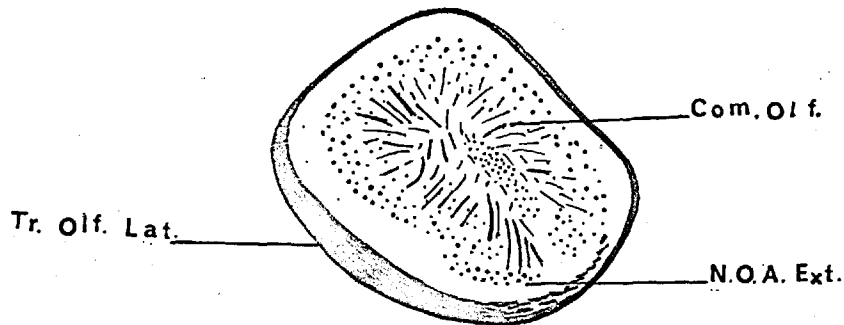


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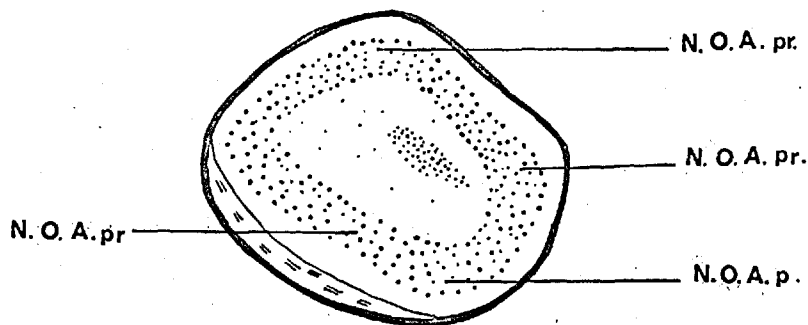
Fig. 15. Projection drawing of transverse sections of the most cranial end of the olfactory peduncle showing the pars externa and pars principalis of the anterior olfactory nucleus. The obliterated olfactory ventricle is represented by small ependymal cells. Toluidine blue stain. X 15.



1



2



3

Fig. 16. Projection drawings of transverse sections at the caudal third of the olfactory peduncle. Note how the pars principalis of the anterior olfactory surrounds the core of the peduncle completely. Section 2 is Loyez preparations; 1 and 3 are toluidine blue. X 15.

posteriorly as a conspicuous layer of rather large cells deep to the cortex of the tubercle as described by Crosby & Humphrey, 1939. Further caudally it gives place to scattered, irregularly arranged cells which lie around the ventral surface of the nucleus accumbens.

It should be noted that with the possible exception of the pars posterior the parts of the pars principalis are all regions in a cuff or tubular layer of cells surrounding the peduncle. Although on the basis of topographical position it is possible to designate a pars dorsalis, ventralis, lateralis and medialis, these parts are not clearly differentiated in cell type and arrangement or in fibre connexions. The recognition of a pars lateralis is probably justified by its special relationship with the lateral olfactory tract and piriform cortex. The whole anterior olfactory nucleus could be regarded as the cortex of the olfactory peduncle and the rostral extension of the much larger secondary olfactory area which includes the olfactory tubercle and the piriform cortex.

Piriform cortex

The extent and boundaries of this cortical area have already been described (p. 27) and are illustrated in Fig. 3. It differs markedly in structure from the neopallium or neocortex (Elliot Smith) which covers most of the surface of the hemisphere. It also differs, but less fundamentally, from other cortical formations (e.g. that of the tubercle)

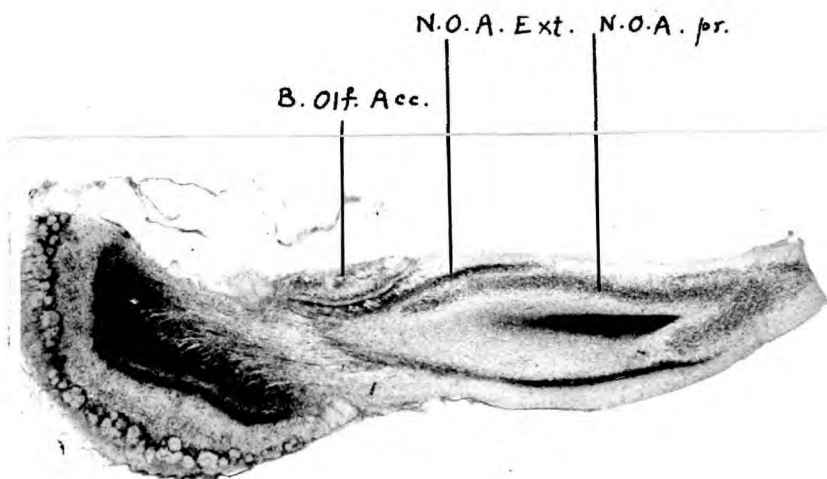


Fig. 17. Photograph of a sagittal section of the olfactory bulb and peduncle at the level of the middle of the accessory bulb to show the anterior olfactory nucleus. Toluidine blue, X 15.

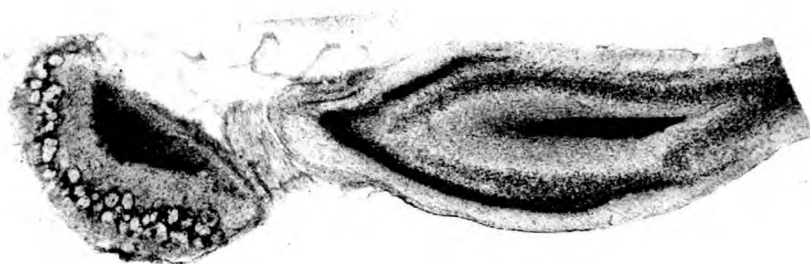


Fig. 18. A sagittal section of the olfactory bulb and peduncle just lateral to that of Fig. 17. Note the pars externa (darkly stained) and pars principalis (lightly stained) of the anterior olfactory nucleus. Toluidine blue, X 15.

found in the piriform lobe.

Various terms have been used to specify different cortical types in mammals so that the terminological situation is confusing (see Pribram & Kruger, 1954). On the basis of phylogenetic studies the cortex of the piriform lobe is often referred to as paleocortex, in contrast to the more highly differentiated archicortex of the hippocampal formation (cornu ammonis and dentate gyrus). Both palaeo- and archi-cortex differ, particularly in the number of cell laminae which can be distinguished, from the neo-cortex. The latter, which is non-olfactory in function, has become extensively and highly developed only in mammals, where it is considered to be the typical cortical formation. Its expansion and the growth of its main commissure (the corpus callosum) has been responsible for great modifications in the topography of both palaeo- and archi-cortical formations.

Brodmann (1909) in his classical studies of the mammalian cortex, introduced a different terminology. The extensive neo-cortex he called homo-genetic, characterised by the possession of six cell layers either in the adult or during ontogeny. When the six laminae are preserved in the adult the term "homotypic" is also used. The palaeo- and archi-cortex, which at no stage possess the six layers, he classes as "heterogenetic". Brodmann's terms homo- and hetero-genetic correspond closely with the more generally accepted terms isocortex and allocortex, later introduced by Vogt. Yet other

terms have been introduced by the cytoarchitectonic school, e.g. "semicortex" by Rose (1929) for the cortex of the piriform lobe, but most of these have never gained wide acceptance and are of little more than historical interest. The same may be said of certain other terms invented by Brodmann for subdivisions of the heterogenetic (allocortical) areas, e.g. cortex primitivus, rudimentarius and striatus.

Brodmann, the Vogts, Rose and many others worked with material stained only by the Nissl method and were therefore limited in their observation to the form, size and disposition in laminae or otherwise of the perikaryon of the neurone. Cajal, and later his pupil Lorente de Nó, O'Leary and others made far more detailed observation which took into account the dendritic plexuses and the afferent and efferent connexions of cortical areas as demonstrated by the Golgi and other metallic impregnation methods. Cajal, whose work is of fundamental importance, unfortunately used his own individual terminology for the parts of the piriform lobe, making comparison with the work of others difficult. The difficulty is increased by the fact that he used different terms for the human and the other mammalian species which he examined. He appears for example (Cajal, 1955), to regard the human hippocampal gyrus as the same as the piriform lobe of lower mammals, which is not of course the case. Most authors would include at least the uncus and anterior perforated space as parts of the piriform lobe. He also refers to the narrow

rostral extension of the olfactory cortex as 'frontal' a term which is more usually applied to the extensive neocortex of the whole frontal lobe. In Cajal's usage it clearly refers only to the cortex medial to the cranial end of the rhinal fissure and closely related to or covered by the lateral olfactory tract. He states that it receives collateral branches from the fibres of that tract. Further caudally, still medial to the rhinal fissure and about on a level with the amygdala, Cajal differentiated the 'sphenoidal' cortex (1901-2), a part of the piriform lobe which he found received terminal fibres from the lateral olfactory tract. This appears to be the same as the "écorce temporale olfactive" (his Fig. 450, 1911) or the "Region olfactive principale ou centrale de l'hippocampe" in the human brain.

Further caudally again, and also bounded laterally by the rhinal fissure, he differentiated the "écorce temporale postérieure ou supérieure" which is separated medially from the cornu ammonis of the hippocampal formation by the subicular areas. Caudally and dorsally it gives place to the retrosplenial areas of neocortex, usually with no macroscopic boundary. He also refers to this cortex as the "noyau angulaire" (Fig. 450, 1911). Lorente de Nó, who later studied it in great detail (1933), adopted the term 'entorhinal'. As Cajal pointed out, it receives no secondary olfactory connexions from the bulb and is more complex in its cell content and laminar structure than other parts of the piriform lobe.

Lorente de No considered however that it was simpler than the general neocortex and although possessing 6 laminae has fewer cell types. For this reason he regarded it as transitional between the allo- or palaeo-cortex of the piriform lobe and the isocortex or neocortex. It covers the greater part of the surface of the hippocampal gyrus in the human brain (Brodmann's area 28).

The entorhinal area is closely related to the hippocampal formation and connected to it by Cajal's "voie temporo-annonique". It is well developed in an anosmatic mammal, the porpoise (Breathnach & Goldby, 1954) and although, in other mammals, it receives tertiary olfactory connexions from other parts of the rhinencephalon (Cragg, 1961; Powell, Cowan & Raisman, 1963), it is doubtful if its primary function is olfactory.

Gray (1924) studied the cortex of the piriform lobe in the opossum (D. virginiana) a primitive mammal where the macroscopic features are more easily compared with those in the coypu. He differentiated: (i) an area piriformis anterior extending from the lateral part of the anterior olfactory nucleus along the lateral olfactory tract caudally to about the level of the rostral border of the olfactory tubercle; this corresponds with the frontal part of the piriform cortex of the coypu as defined on p. 27, (ii) an area piriformis medialis occupying the middle or intermediate part of the piriform lobe and adjacent to the caudal parts of the olfactory

tubercle and the amygdala, i.e. the temporal part of the piriform in the coypu (p. 27), and (iii) an area piriformis posterior which clearly corresponds with the entorhinal area of Brodmann, Rose, Lorente de Nó and others and is the term which is used in this thesis. Gray considered that it is in his area medialis that the piriform cortex attains its most characteristic differentiation; this is the region which according to Cajal receives the main terminals of the lateral olfactory tract and was named by him "sphenoidal" or "écorce temporal franchement olfactive".

The piriform cortex has been described under its various names in great detail by many authors and frequently subdivided (e.g. by Economo (1929) into areas piriformis FK and ID (lateral olfactory gyrus, insular area) and area piriformis temporalis TI, and by Rose (1929) into prepiriform 1, 2 and 3). It is not necessary to discuss the small cytoarchitectural differences on which these and other subdivisions are based. In general it is of relatively simple structure. Cajal (1911) and O'Leary (1937) on the basis of Golgi as well as Nissl preparations, distinguished five layers of which the fifth is the underlying white matter. In the Nissl stained coypu material there appears to be three basic layers - molecular, pyramidal and polymorphic (Fig. 20), but closer study shows that the pyramidal layer can be divided indistinctly into two, corresponding probably with Cajal's layers II and III. The following describes the layers in the

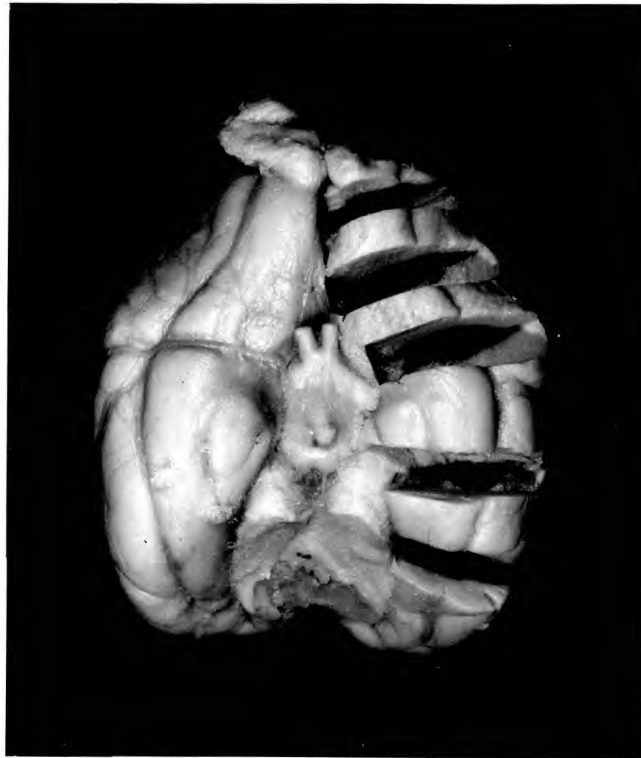


Fig. 19. Photograph of the ventral surface of the coypu brain from which some blocks were taken so as to supplement the original transverse series and used particularly for the cytoarchitectonic study of the anterior olfactory nucleus, frontal and temporal piriform cortex, olfactory tubercle and the entorhinal cortex, X 2.

frontal part of the piriform cortex of the coypu.

I. An outer molecular layer consisting of the dendrites and axons of underlying cells and also a few horizontal cells. To this may be added a "fibrillary layer" on its surface formed by the lateral olfactory tract fibres.

The presence of myelinated fibres in this lamina is a characteristic feature of the whole piriform cortex. In the coypu the fibrillary layer is made up medially of the massive lateral olfactory tract (Fig. 20) with fibres directed rostrally. Fine myelinated fibres spread laterally from the tract just beneath the pial surface into the wall of the rhinal fissure but do not extend to the neocortex beyond it. The deeper plexiform layer contains a few myelinated fibres and a few nerve cells, most small and fusiform. These are probably Cajal's horizontal cells but there is an occasional larger cell which appears to have been displaced from layer 2.

IIa. A fairly compact layer of small and medium pyramidal or polymorphic cells somewhat variable in size and often arranged in clumps. In the coypu the cells are mostly fusiform or multipolar rather than typically pyramidal but with the long axis perpendicular to the surface (Figs. 21 and 23).

Particularly dense clumps of cells are present deep to the lateral olfactory tract, giving this part of the cortex a corrugated appearance not seen where it extends into the wall of the rhinal fissure.

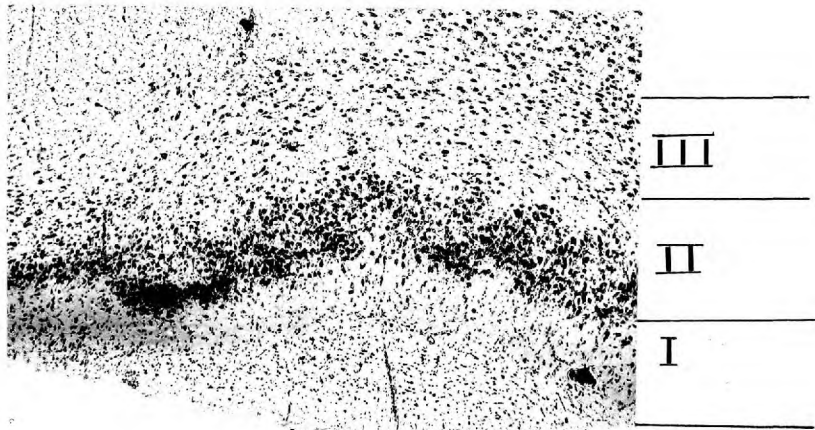


Fig. 20. A transverse section to show the frontal piriform cortex. Note the very wide molecular layer which contains the massive lateral olfactory tract in its outer (fibrillary) part. Toluidine blue, X 40.

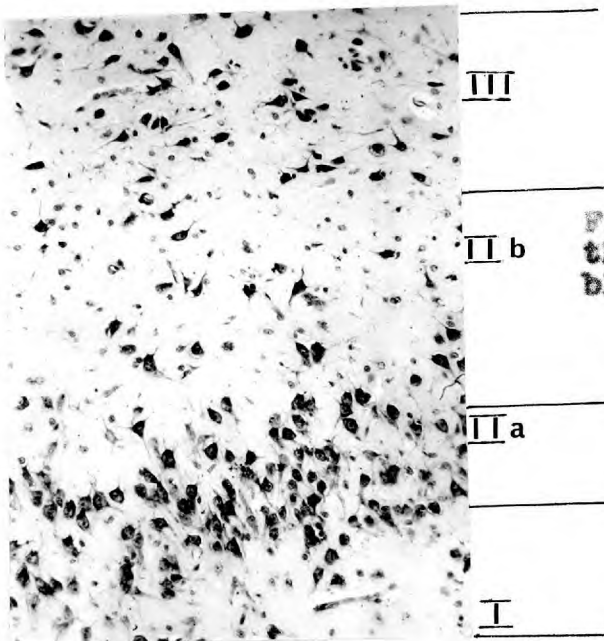


Fig. 21. Higher power view (X 140) of the compact outer pyramidal and the diffuse inner pyramidal layer.

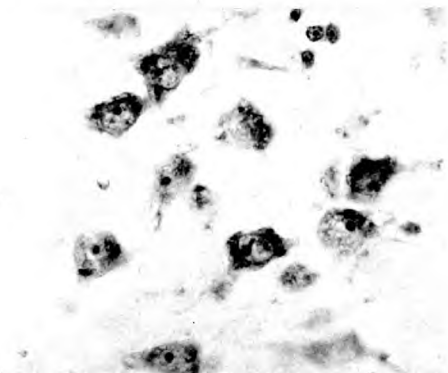


Fig. 22. High power (X 360) of the inner pyramids, toluidine blue.

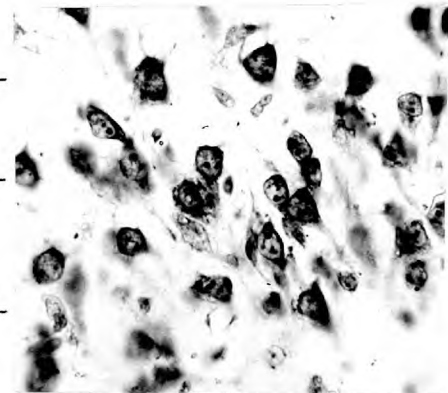


Fig. 23. High power (X 360) of the outer pyramids, toluidine blue.

IIb. A broad layer of deep pyramids in which the cells are slightly larger, and somewhat more scattered than in layer II^a. Cajal found that these pyramids have characteristic brushy dendrites, and they were called double pyramids by Kolliker (1893). In the coypu these cells stain less intensely than those of layer II^a (Fig. 23) and they are much scarcer in the frontal than in the temporal part of the piriform cortex.

III. A broad layer of scattered polymorphic cells which in the coypu are mostly rather small and multipolar. They lie among myelinated fibres approaching or leaving the cortex.

In the coypu the lateral boundary of the frontal piriform cortex is sharp at the bottom of the rhinal fissure where it gives place to neocortex. Rostrally the ventro-lateral part of the anterior olfactory nucleus has a practically identical structure, except for the absence of clumping of the cells in layer II^a, so that no clear boundary exists. Medially there is an equally indefinite transition to the cortex of the olfactory tubercle which contains the same basic layers but with certain special characteristics which will be described later.

The temporal part of the piriform cortex in the coypu is illustrated in Figs. 32 and 33. The same 3 basic layers are present. Layer I is thinner chiefly because of the shrinkage of the lateral olfactory tract, most of the fibres of which have ended at more rostral levels. Its superficial fibrillary part is recognisable only medially close to the

amygdaloid fissure, and laterally close to the rhinal fissure. Layer IIA contains cells of the same general type as before but more regularly arranged and with less clumping and few corrugations. Clumps are still seen however, chiefly in the medial wall of the rhinal fissure and it may be noted that similar clumps are quite numerous in the second layer of the neocortex beyond this fissure (Fig. 32). Layer IIB (the deep pyramids) seems more definite here, its cells being scattered in contrast to the closely packed superficial pyramids. They are difficult however to separate from the multipolar cells of the IIId or polymorphic layer, which are distinguishable chiefly because they lack the orientation perpendicular to the surface which is characteristic of Layer IIB. In turn the IIId layer cells are in some places not clearly separated from the underlying mass of cells which has been identified as belonging to the claustrum.

As before the neocortical boundary is fairly sharp. It lies at the bottom of the rhinal fissure where a narrow intermediate zone could be interpreted as a peri-rhinal area. Medially the boundaries are much less definite and there is a transition to the nucleus of the lateral olfactory tract and the cortico-amygdaloid nucleus. Caudally the transition to the multi-laminate entorhinal cortex is gradual.

It is clear that the piriform cortex of the coypu is relatively uniform and simple in structure. The three basic layers are present throughout but in Nissl stained preparations

it is difficult to decide definitely where the boundary between the IInd and IVM layers should be put. It is obvious that the lamination is less complex than in the entorhinal or much of the neocortex. The absence of granule cells is also a striking feature. The presence of myelinated fibres in layer I (forming its fibrillary part) is also characteristic. There is a strong presumption that these are lateral olfactory tract fibres most of which appear to end in the frontal region of the cortex. Caudally the remaining myelinated fibres are concentrated in two regions, medially close to the amygdala and laterally, close to the rhinal fissure.

While it would be possible on the basis of such features as the presence or absence of clumping in the IInd layer and the distribution of myelinated fibres in the Ist layer to subdivide the piriform area, it is doubtful if the subdivisions would have much significance or correspond at all closely with those made by others (e.g. Rose, 1929). There does seem some basis for comparison with Gray's description of an area piriformis anterior (frontal) and medialis (temporal) but to go much further than this would require a detailed study of cell types and fibre plexuses such as would be possible only in Golgi material. So far as ordinary cytoarchitectonic studies are concerned the outstanding characteristics of the piriform area are simplicity and uniformity.

Entorhinal cortex

The entorhinal cortex is much more complex and 6 laminae

can be identified although the schemes adopted by Cajal (1911) and Lorente de Nó (1934a, 1934b) differ slightly. Cajal actually described 8 laminae but these included the underlying white matter (the 8th) and an almost cell free deep plexiform layer (the 4th). In Nissl preparations he illustrates 6 cellular laminae.

Lorente de Nó gives many details of the intracortical plexuses and of the dendritic arborisations of cells and the orientation (in some cases the destination) of their axons. It is not necessary to discuss these details here because the methods used in the present study (chiefly the Nissl technique) do not demonstrate them so that no comparisons can be made. The 6 laminae seen in Nissl preparations can be identified in many different mammals and may be taken as characteristic for the mammalian entorhinal area in general. As Lorente de Nó pointed out however, identifications of laminae in Nissl preparations are all more or less subjective and different observers, even using the same material, may come to different conclusions. It may be noted that Rose (1926) defines only 4 cellular laminae in the entorhinal area. Partly for this reason it does not seem necessary to discuss the different subdivisions made by Rose and others, the significance of which depends essentially upon a knowledge of their connexions and of the arrangement of the fibre plexuses, axonal and dendritic.

In the coypu the situation of the entorhinal cortex is illustrated in Fig. 3, 24. It extends dorsally for a considerable distance on the caudal aspect of the hemisphere finally

giving place to the retrosplenial region of the neocortex. Its characteristics can best be demonstrated in sections horizontal to the hemisphere (Fig. 24). The 6 laminae are clear and correspond fairly closely with those defined by Lorente de No in the mouse. They are as follows:

- I. The plexiform or molecular layer is fairly wide. It contains occasional fusiform or multipolar cells representing the horizontal cells of Lorente de No.
- II. A narrow layer of rather closely packed medium sized polygonal or star cells, generally oriented perpendicular to the surface, and not sharply separated from the IIIrd layer. This layer is best marked medially (adjacent to the subiculum) and laterally (adjacent to the rhinal fissure). In both these situations the cells show a tendency to clumping. This layer is much less distinct in the intermediate region.
- III. A broad layer of fusiform or polygonal cells, more widely spaced, and corresponding to Lorente's superficial pyramids. Deep to the cells is a narrow almost cell free zone, Lorente's layer IIIa (Cajal's 4th), which contains the deepest part of a dense fibre plexus which extends throughout the IIIrd layer.
- IV. A thin layer of rather large fairly widely spaced polygonal cells (Fig. 25). They are the largest cells seen in this cortex and form Lorente's layer of deep pyramids or Cajal's "horizontal spindle cells".



Fig. 24. Photograph of a horizontal section to show the entorhinal cortex. Celloidin section 30 μ , stained by toluidine blue method, X 10.

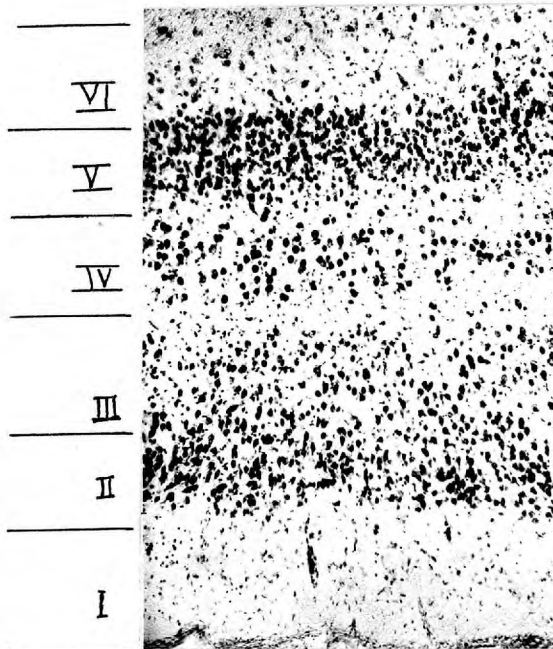


Fig. 25. Higher power view (X 95) of the entorhinal cortex to show its 6 layers. Toluidine blue preparation.

V. A broad layer of close-packed small cells, many resembling granule cells. These are Lorente's small pyramids with recurrent axons.

VI. An ill-defined layer of very widely spaced large or medium polygonal or polymorphic cells.

This highly characteristic cortical area is fairly uniform in structure but could be divided into medial, intermediate and lateral areas corresponding approximately to Lorente's Areas A, B and C on the basis of the cytoarchitectural differences in the IInd layer.

Olfactory tubercle

The size and differentiation of the olfactory tubercle is thought to bear some proportion to the development of the olfactory system, although macroscopically at least, it is a conspicuous feature of the anosmatic porpoise (Breathnach, 1953). One of the earliest studies was by Calleja (1893) who besides describing the 'islets' which are named after him, described the three main layers which have formed the basis of all subsequent descriptions viz: (i) outer or molecular, (ii) pyramidal (large and medium cells) and (iii) the plexiform or polymorphic layer. It was described for a large series of mammals by Beccari (1910) and by Cajal (1911) in the cat, dog, rabbit, mouse and man. Among later accounts are those of Gurdjian (1925, rat), Loo (1931, opossum), Young (1936, rabbit),

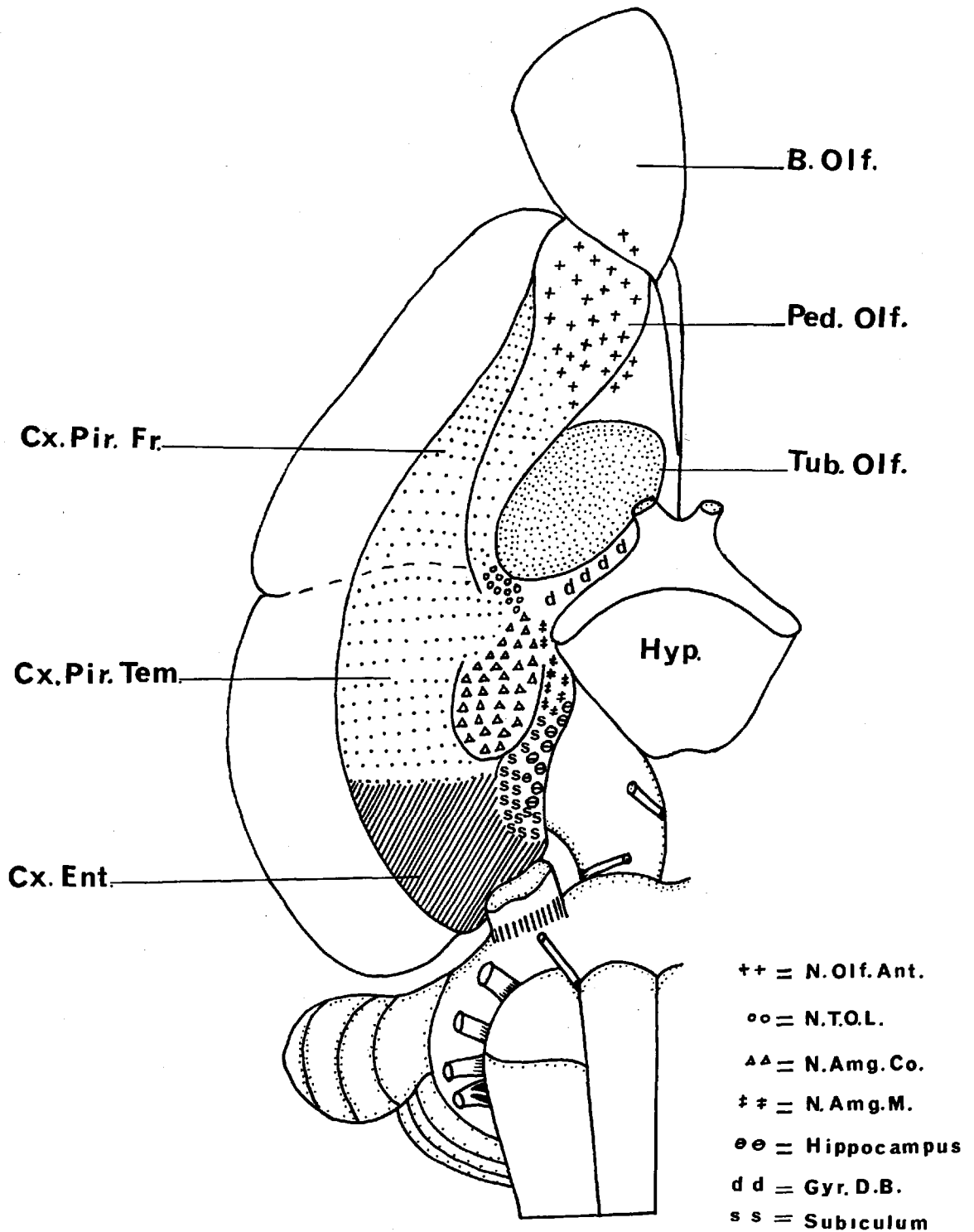


Fig. 26. A drawing of the ventral surface of the coypu brain to show the basal rhinencephalic structures; based on the cytoarchitectonic study of the brain. (For abbreviations see p. 194)

Humphrey (1936, bat) and Crosby & Humphrey (1941, man).

The islets of Calleja are its most striking feature and as Cajal (1911) pointed out, apart from them, the structure is very similar to that of the piriform cortex. Gurdjian counted 12 islets in the rat and there is often one which is particularly large on the medial side close to the nucleus accumbens (Loo, 1931; Crosby & Humphrey, 1941). Many are made up of small "granule" cells in which nothing but the nucleus can be seen in Nissl preparations but others contain small pyramidal or polymorphic cells. No-one has offered a satisfactory explanation for these extremely odd masses of cells; they vary greatly in shape and size and are so sharply defined and so different from their surroundings as to suggest pathological growths. The appearance of the smaller cells suggests localised proliferation of glial cells, but Cajal (1911) in Golgi preparation seems to have confirmed that they are neurones which discharge by fine axons into the underlying white matter.

Although the degree of differentiation varies in different mammals the molecular, pyramidal and polymorphic layers can always be distinguished. Slight histological differences (e.g. presence or absence of clumping and corrugation in the second layer) have enabled some authors to distinguish different regions, described as anterior, intermediate or posterior (Cajal, 1911) or medial, intermediate and lateral (Young, 1936). Humphrey (1936) describes an anterior less differentiated and a posterior more highly

differentiated region corresponding with Cajal's anterior and intermediate regions. Within the latter she distinguished medial, intermediate and lateral portions.

Beccari (1910) was doubtful if the tubercle received any secondary olfactory fibres, but most authors state that fibres from the lateral and sometimes intermediate olfactory tracts can be traced into the molecular layer of at least its antero-lateral part. It may be noted that it is just this antero-lateral part which, in the anosmatic porpoise, seems to have lost the characteristic structure of the olfactory tubercle (Breathnach, 1953). It is also connected to the septum, to the medial fore-brain bundle (Morin, 1950) and the olfactory commissural component of the anterior commissure (Humphrey, 1936; Fox, Fisher & Desalva, 1948).

The characteristic molecular pyramidal and polymorphic layers can be recognised in the coypu. They are continuous laterally with the corresponding layers of the piriform cortex, and are of a similar general character (Figs. 27 to 29). The cells of the pyramidal layer however are smaller, more rounded and stain less deeply. The nucleus accumbens is deep to the polymorphic layer and separated from it by the finely myelinated fibres of the medial fore-brain bundle (Fig. 29).

Two main regions can be recognised antero-lateral and postero-medial. In the former the pyramidal (2nd) layer is greatly corrugated and contains dense clumps of cells. Postero-medially cellular density is much more uniform and

there are no corrugations, so that it is much more like piriform cortex than the rest of the tubercle.

The islets of Calleja are very conspicuous and can be found in all the layers. Some extend to the pial surface very like the one illustrated by Cajal (1911) from a cat (his Fig. 465a). A particularly large island is usually seen close to the nucleus accumbens on the medial side. Although many of the islands consist of very small cells, superficially like the proliferated ependymal or glial cells which are found along the course of the obliterated olfactory ventricle, some are obviously composed of very small fusiform or polygonal neurones.

The Septum

In spite of confusion due to a varied terminology a survey of the literature shows a large measure of agreement concerning the cell masses and fibre connexions of the septum. The chief differentiations are a large celled medial nucleus, a small celled lateral nucleus and bed nuclei of the hippocampal and anterior commissures. Certain other nuclei not wholly included in the septum, such as the nucleus of the diagonal band and the nucleus accumbens, are usually described with this region, and sometimes the caudo-medial part of the anterior olfactory nucleus and the anterior hippocampal rudiment.

The medial nucleus is superficially placed in the rostral part of the septum (Humphrey, bat, 1936; Breathnach, porpoise, 1953 and Lohmann, guinea pig, 1963) where it is closely related

to or continuous with the medial part of the anterior olfactory nucleus and the anterior hippocampal rudiment. Caudally and ventrally it is more or less continuous with the nucleus of the diagonal band. It consists of large cells, although some authors divide it into large and small celled parts, the small cells being more rostrally situated (Loo, opossum, 1931; Fox, cat, 1940). Young (1936) in the rabbit found the larger celled part rostrally.

The lateral nucleus is usually larger and is bounded laterally by the ventricle and separated below from the nucleus accumbens by a cell free zone as described for the rabbit by Young (1936). Again some authors have subdivided this nucleus, usually into dorsal and ventral parts (Loo, 1931; Andy & Stephan, 1959 and 1961, in the Galago and in Soricidae). Its cells are small or medium in size and fusiform or polymorphic in character.

The bed nuclei of the commissures consist of small cells, rounded or fusiform, around and among the fibres of the commissures. They do not form very well defined nuclei. A nucleus triangularis (Cajal, 1911; Loo, 1931; Andy & Stephan, 1959) probably belongs to this category. It is a rather striking triangular mass of small cells seen in transverse sections ventral to the hippocampal commissure and between the columns of the fornix. Rostral to it a dorsal septal nucleus (Loo, 1931) or group of nuclei (Andy & Stephan, 1959) has been described in some forms but does not appear to have received

general recognition.

The nucleus accumbens septi is an extension from the head of the caudate nucleus below the ventricle into the septal region. Herrick (1924) and a number of other authors name it as a septal nucleus but it appears to be essentially a part of the corpus striatum (Goldby, 1934; Crosby et al., 1962).

The nucleus of the diagonal band was described by Broca in 1879 and has since been recognised by many authors (e.g. Johnston, 1923, rabbit; Craigie, 1925, rat; Loo, 1931, opossum; Breathnach, 1953, porpoise, and many others). Young (1936) describes it very clearly in the rabbit as a bow-shaped mass of cells beginning caudo-ventrally in the septum near the median plane and reaching forwards as far as a giant islet of Calleja close to the nucleus accumbens. It extends ventrally and laterally across the base of the brain behind the olfactory tubercle to the anterior amygdaloid area. It contains very large cells like those of the medial septal nucleus with which it is sometimes continuous (Breathnach, 1953), and is accompanied by a band or bundle of fine myelinated fibres throughout its course.

The anterior hippocampal rudiment generally consists of a layer of medium sized cells, sometimes broken into discontinuous groups, which extends from the anterior olfactory nucleus in a dorso-caudal direction to the genu of the corpus callosum to become continuous with the indusium. It may also extend for a short distance ventral to the genu as the septo-hippocampal nucleus (Rumphrey, 1936).

In the Primates and perhaps in other mammals with great neocortical expansion and a correspondingly large corpus callosum, the upper part of the septum is stretched between the fornix and the corpus callosum. This is the "septum pellucidum" of human anatomy (Elliot Smith, 1895). In this region the space in the median plane between the two hemispheres is the "cavum" of the septum pellucidum which may be open rostrally as in the cat and macaque or closed as in man (Thompson, 1932).

The cytoarchitectural differentiation in the septum of the coypu is not very marked. Its structure is clearly similar to that of other mammals although by no means all the smaller nuclei can be seen distinctly. Elaborate subdivisions such as those made by Andy & Stephan (1959) in *Galago* would be very difficult to justify.

Medial and lateral nuclei are certainly present, the latter much larger than the former (Fig. 29). The medial nucleus is definitely magno-cellular in type and appears rostrally and ventrally on the medial aspect of the nucleus accumbens, from which it is separated by islands of small cells resembling islets of Calleja. It is never clearly defined. It abuts ventrally on the olfactory tubercle and can be followed caudally on the surface to become continuous with a well developed nucleus of the diagonal band which is similar to that of other mammals. Its cells are multipolar and while generally large vary considerably in size. It is accompanied



Fig. 27. Transverse section showing the frontal piriform cortex at the level of the middle of the olfactory tubercle. Toluidine blue, X 5.5.

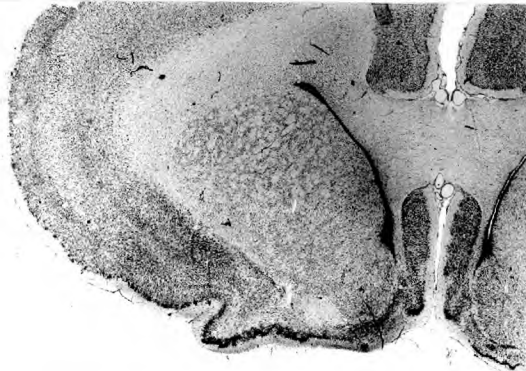


Fig. 28. Transverse section just rostral to the main part of the septum. Toluidine blue, X 5.5.

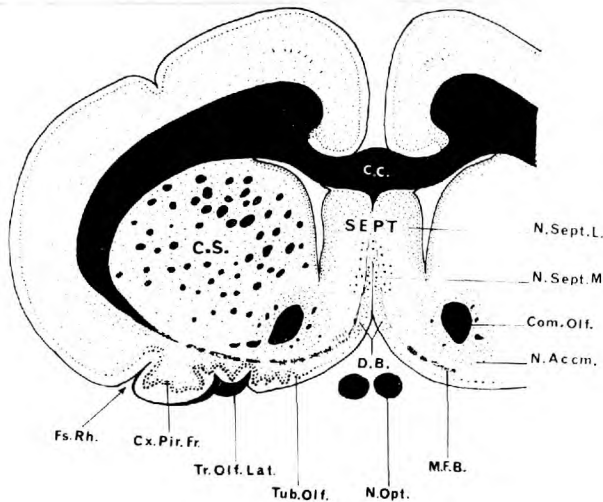


Fig. 29. Drawing of a transverse section in the middle of the septum showing the main nuclei. Adjacent toluidine blue and Loyez sections superimposed, X 6.5.

by the diagonal band fibres which are finely myelinated and stain poorly in the Loyez preparations. More rostrally the large cells of the medial septal nucleus are also accompanied by vertically oriented fibres which appear to run between the septum and olfactory tubercle.

The lateral nucleus forms the greater part of the septum and is situated dorso-laterally adjacent to the ventricle and above the nucleus accumbens. In front of the lamina terminalis it fuses over a considerable area with its fellow of the opposite side. A few larger cells in the median plane in this region may represent a caudal remnant of the medial nucleus or possibly the beginning of the nucleus of diagonal band. The cells of the lateral nucleus are medium in size except in its caudal part where they are smaller and more closely packed adjacent to the fimbria as the latter approaches the hippocampal commissure. This part may represent Loo's (1931) nucleus fimbrialis septi.

The bed nuclei of the commissures are poorly represented and indeed around the anterior commissure no concentration of cells can be made out. There is some concentration of small cells around the hippocampal commissure, most marked dorsally and ventrally. In the latter situation they extend down between the columns of the fornix and may represent a nucleus triangularis.

Rostrally and ventrally, the anterior olfactory nucleus comes into relation with some groups of medium sized cells at the caudo-ventral border of the neocortex of the frontal pole.

These cells can be traced with some difficulty along the border of the neocortex to the genu of the corpus callosum where they become continuous with the cells of the indusium griseum. They represent the anterior hippocampal rudiment. No clear septo-hippocampal nucleus ventral to the genu can be identified.

Amygdala

The position of the amygdala has been described (pp.26 & 27) and its surface extent is illustrated in Fig. 3. The classification and terminology of its nuclei is based on the work of Johnston (1923). Johnston thinks that many of the amygdaloid nuclei were formed by an ingrowth from the lower border of the piriform cortex in the region of the endorhinal sulcus (usually referred to as the amygdaloid fissure at this level), the cells which remained superficial medial to the sulcus forming a cortical amygdaloid nucleus. He thinks that this nucleus and the nucleus of the lateral olfactory tract, also superficial in position, and the more deeply situated central and medial nuclei are phylogenetically older than two other nuclei which he referred to as basal and lateral. Later workers (e.g. Crosby & Humphrey, 1941) refer to these nuclear groups as cortico-medial and baso-lateral respectively. The cortico-medial group appears to be more intimately related with the stria terminalis, and the baso-lateral group with the anterior commissure (Humphrey, 1936) but the question of their relative phylogenetic age is doubtful because it is difficult

to establish the homologies of the individual nuclei in sub-mammalian vertebrates.

Among the mammals the arrangement of the amygdaloid nuclei is remarkably uniform and no clear phylogenetic trends can be recognised. Crosby & Humphrey (1941) in their study of the human amygdala were able to identify the baso-lateral and cortico-medial group and to use the same terminology as for sub-primate mammals. It is usual also to include in the amygdala a third or anterior group (Fox, 1940) consisting of the nucleus of the lateral olfactory tract, an ill-defined anterior amygdaloid area and certain intercalated masses of cells. The nuclear subdivisions can now be described in terms of the groups specified.

Anterior group

Anterior amygdaloid area. This term was first suggested by Gurdjian (1928) for a diffuse region which he identified in the rostral extremity of the amygdaloid complex of the rat brain. Johnston (1923) apparently did not recognise it as distinct from the central nucleus in the human foetus. It does not appear to be present in the bat (Humphrey, 1936), has fair representation in the rabbit (Young, 1936), guinea pig (Johnson, 1957), and the cat (Fox, 1940), and has a considerable extent in the shrew and in man (Crosby & Humphrey, 1941, 1944). Lying just dorsal to this area and extending for a short distance caudally, two or three groups of small cells are found which Johnston (1923) named "intercalated masses". They have

been identified by many workers, e.g. Fox (1940) in the cat, Brodal (1947b) in the rat, and Johnson (1957) in guinea pig.

In the coypu brain this diffuse area is seen in the rostral extremity of the amygdala (AAA, Fig. 30) lying between the central nucleus dorsally and the lateral olfactory tract ventrally. It is bounded rostrally by the deep layers of the olfactory tubercle, laterally by the claustrum, medially by the diagonal band, and ventro-medially by the nucleus of the lateral olfactory tract. It is composed of scattered small cells which have not differentiated into the ~~well-defined~~ nuclear masses characteristic of the caudal part of the amygdala. Dorsomedially two groups of cells can be seen which represent the intercalated masses (Fig. 31). They lie just medial to the central nucleus (I in Fig. 30) and disappear caudally where the lateral nucleus begins.

The nucleus of the lateral olfactory tract, shows a wide range of variations in the mammalian series. Gurdjian (1928) described for the rat dorsal and ventral, and Young (1936) for the rabbit medial and lateral parts; Crosby & Humphrey (1941) described an extremely minute representative of this nucleus in the microsmatic human brain. Breathnach & Goldby (1954) were unable to find it in the anosmatic porpoise.

In the coypu it is formed by a well delimited group of deeply stained cells under the medial border of the lateral olfactory tract (Fig. 26) and lying between the diagonal band medially and the anterior amygdaloid area laterally (Fig. 30).

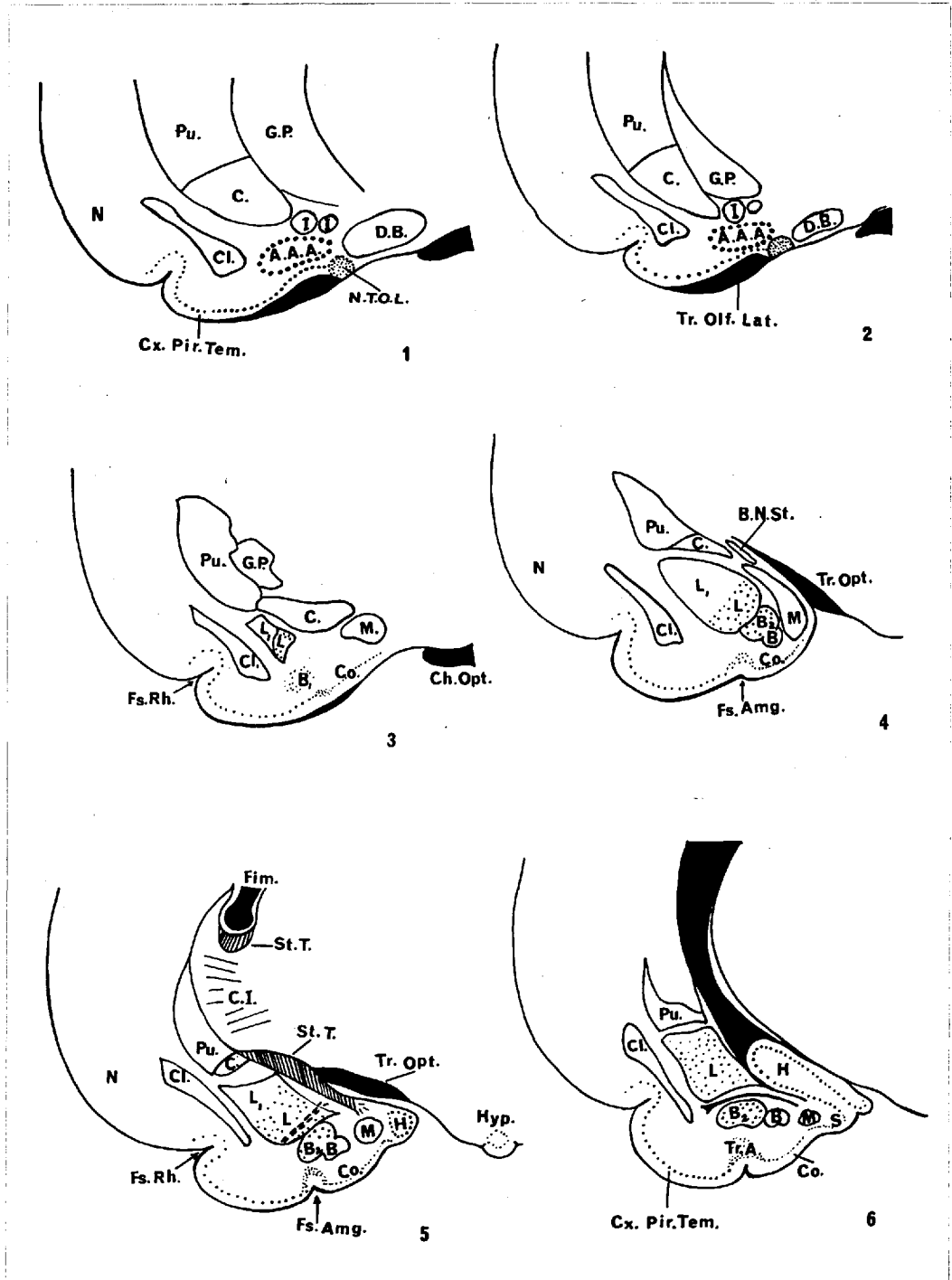


Fig. 30. Drawings prepared by projection from 6 transverse sections through the amygdala from the rostral (No. 1) to near its caudal end (No. 6). Levels 1, 3, 4 and 5 correspond to the photographs shown in Figs. 31, 32, 33 and 34 respectively. X 5.

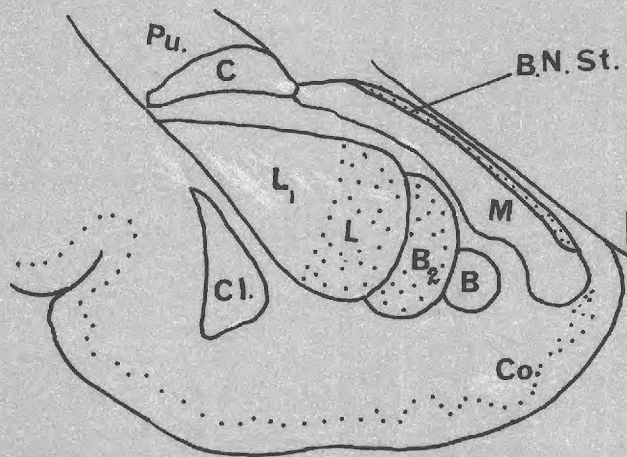
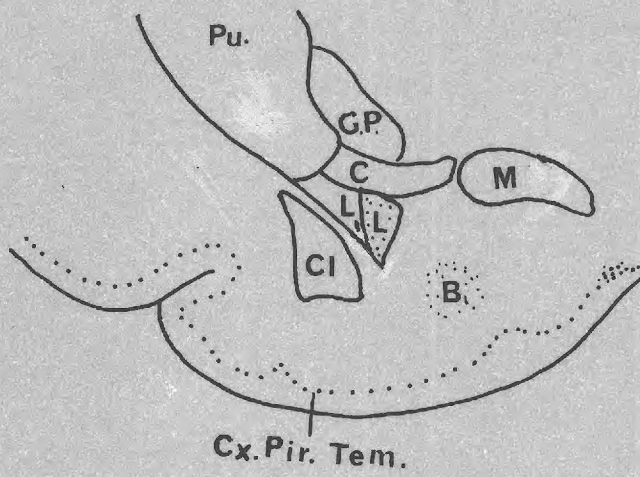
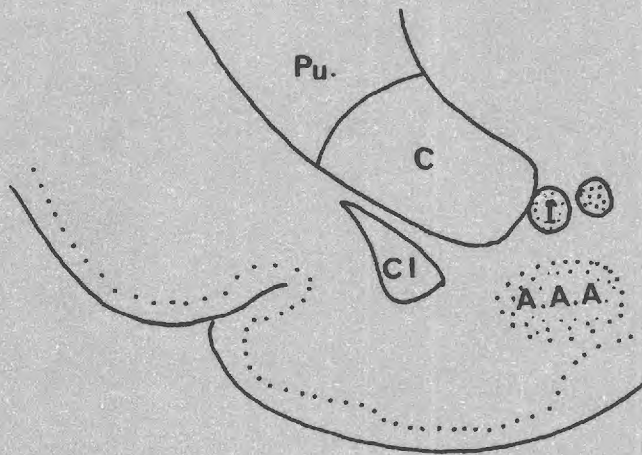
Sometimes two rounded masses instead of one, as described above by Gurdjian (1925) for the rat, can be identified. Rostrally it is continuous with some diffusely arranged cells beneath the lateral olfactory tract which become indistinguishable from the medial border of the piriform cortex or the lateral border of the olfactory tubercle. Caudally it is gradually replaced by the cortical amygdaloid nucleus.

Cortico-medial group

In this group are included the cortical nucleus, the cortico-amygdaloid transition area, the medial and central nuclei, and the bed nucleus of the stria terminalis.

The cortical nucleus was found to be well developed in all mammals examined including Man (Crosby & Humphrey, 1941) but least so in the bat (Humphrey, 1936). A molecular layer, a layer containing pyramidal cells and an underlying zone of scattered cells give justification for classification as a cortical formation. Laterally it is continuous through the cortico-amygdaloid transition area in the amygdaloid fissure with the piriform cortex.

In the coypu the cortical nucleus begins rostrally as a caudal continuation of the nucleus of the lateral olfactory tract, and is caudal to the nucleus of the diagonal band. Caudally it lies entirely within the U-shaped amygdaloid fissure (Fig. 36). Laterally lies the cortico-amygdaloid transition area which is a mass of intermingled pyramidal and polymorphic



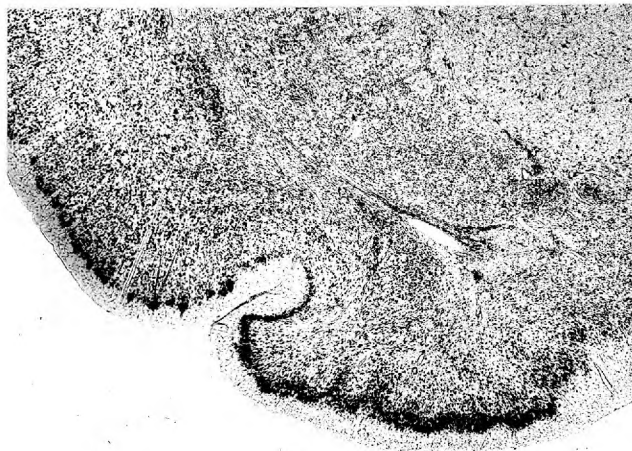


Fig. 31. Transverse section at the level of the rostral part of the temporal piriform cortex and showing the central amygdaloid nucleus and intercalated masses. Compare with Fig. 30(1). Toluidine blue, $\times 8.5$.

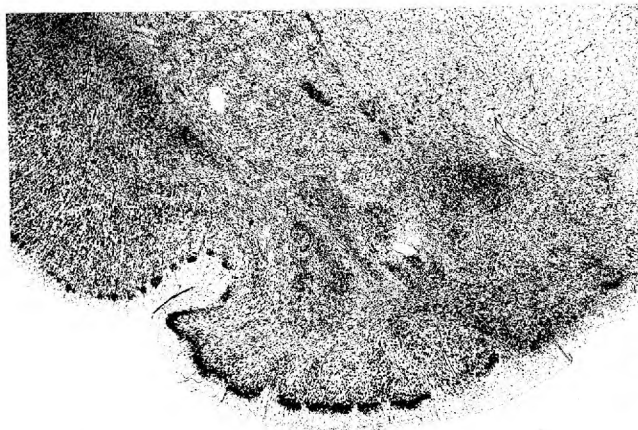


Fig. 32. Transverse section at a level caudal to that of Fig. 31 showing the central, lateral and cortical amygdaloid nuclei. Note the two parts of the lateral nucleus (compare with Fig. 30 (3)). Toluidine blue, $\times 8.5$.

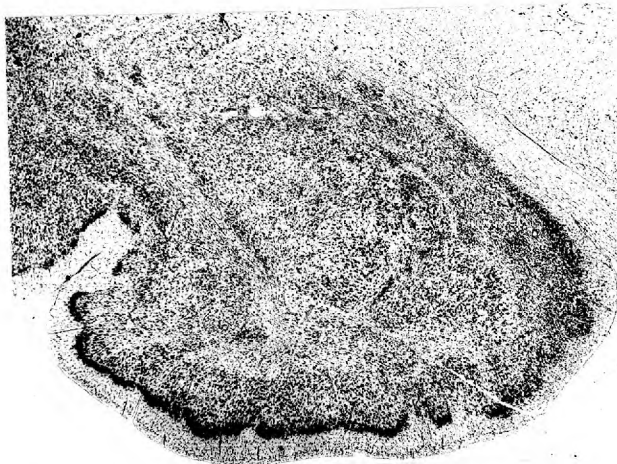


Fig. 33. Transverse section caudal to that of Fig. 32 to show the medial, lateral basal and cortical amygdaloid nuclei. Compare with Fig. 30 (4). Toluidine blue, $\times 8.5$.

cells in the amygdaloid fissure between the cortical nucleus and the piriform cortex. Medially it is separated by the medial limb of the amygdaloid fissure from the medial amygdaloid nucleus and the subiculum. It consists of large deeply stained cells arranged in three laminae more or less similar to those of the piriform cortex, except that the pyramidal cells are not so closely packed (Fig. 33). Dorsally the deeper layers of the cortical nucleus cannot be distinctly separated from the basal nucleus.

The medial nucleus forms the ventromedial border of the amygdala and has been described by all authors working on mammalian brains. Gurdjian (1928) describes it as extending throughout the whole antero-posterior length of the amygdala in the rat, and it has been found to be quite large in such mammals as the cat (Fox, 1940) and the bat (Humphrey, 1936). It is well differentiated in primates as described by Crosby & Humphrey (1941); however, Breathnach & Goldby (1954) found that in the porpoise it contains fewer cells than in most mammals.

In the coypu it is first seen just rostral to the level at which the cortical nucleus appears but it does not extend as far caudally. Its most rostral end is not clearly outlined as it fuses with the anterior amygdaloid area and the cortical nucleus (Fig. 31). Here it is very close to the pre-optic region which lies medial to it (Fig. 32). Caudally its outline becomes more definite and it can be distinguished from

the cortical nucleus because of the difference in cellular architecture (Fig. 33). The medial nucleus consists of densely packed lightly staining, medium sized multipolar cells. Dorsally it is close to the cells of the bed nucleus of the stria terminalis and the central nucleus. Laterally, the basal and lateral amygdaloid nuclei are found (Figs. 30 and 33). At more caudal levels it blends with the cells of the subiculum.

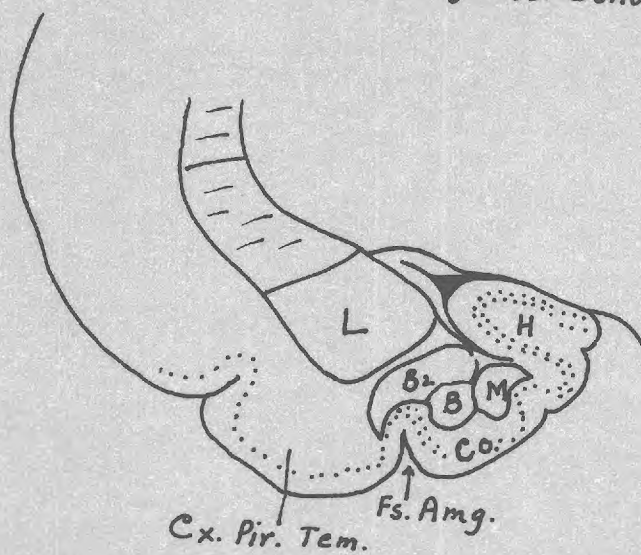
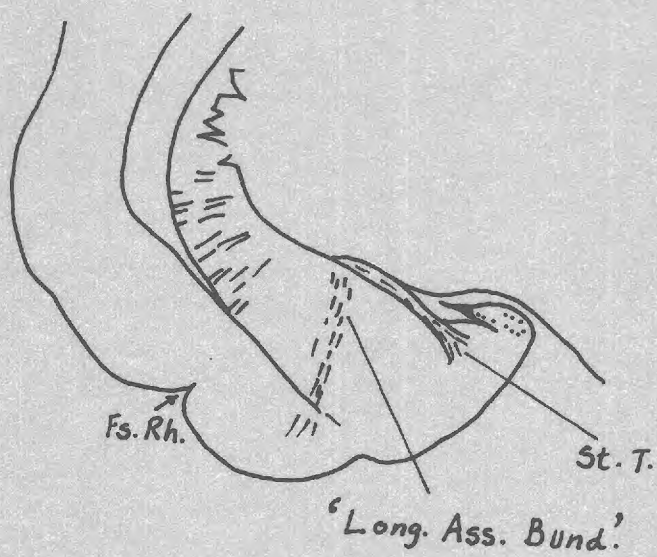
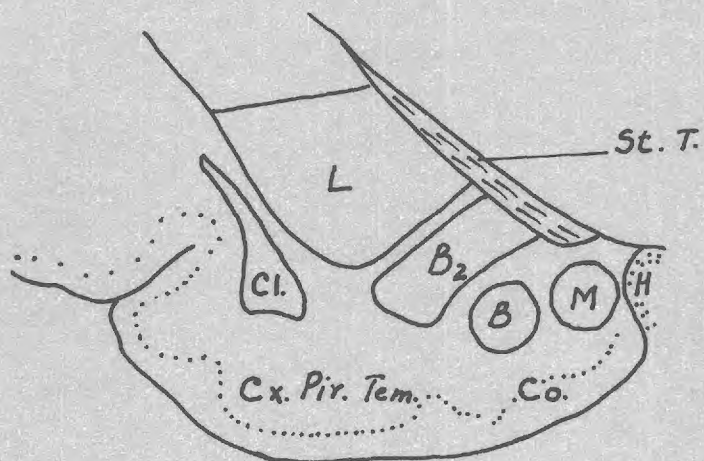
The central nucleus is deeply situated and begins at the most rostral extremity of the amygdala. It can be identified easily because of its characteristic relation to the putamen which lies dorsal to it. Medially the central nucleus is continuous with the caudal end of the bed nucleus of the stria terminalis as described by Johnston (1923). It has been seen in all mammals examined e.g. the rat by Gurdjian (1928) and Brodal (1947b), the cat by Fox (1940), and man by Crosby & Humphrey (1941). Breathnach & Goldby (1954) found that the central nucleus in the porpoise is perhaps larger than in primates. Its neurones resemble those of the putamen and the boundary between the two is difficult to define; the putamen however is richer in myelinated fibres.

In the coypu the central nucleus is large and easily recognised, applied to the ventral surface of the putamen in the characteristic manner. It is one of the first well defined nuclei to appear dorsal to the anterior amygdaloid area and intercalated masses (Figs. 30 and 31). Caudally it becomes

smaller and shifts medially as it is squeezed by the enlarging lateral nucleus ventro-lateral to it (Fig. 32). It disappears at about the level of the middle of the lateral nucleus. It is composed of medium and small sized cells like those of the putamen from which it is difficult to distinguish it rostrally. The putamen however, is broken up by many bundles of projection fibres which give it the characteristic appearance of the corpus striatum. Ventrally and medially it fuses gradually with the anterior amygdaloid area and the bed nucleus of the stria terminalis.

The bed nucleus of stria terminalis is the mass of gray matter which extends from the antero-medial fore-brain area to the amygdala, in which the stria is embedded. Johnston (1923) seems to have regarded it as one of the primitive olfactory centres. The thickening of the internal capsule in mammals has brought about a reduction of the middle part of this gray to very small dimensions. According to Johnston the medial and central amygdaloid nuclei develop from its caudal part. It has also been described in most other studies of the mammalian amygdala, e.g. by Gurdjian (1928), Loo (1931), Young (1936) and Humphrey (1936).

In sections of the coypu brain, the bed nucleus can be seen only for a short distance where the central and medial nuclei occupy the dorso-medial border of the amygdala (Fig. 33). It appears as a dorsal continuation of the medial nucleus, and in so close a relation with inner surface of the central nucleus



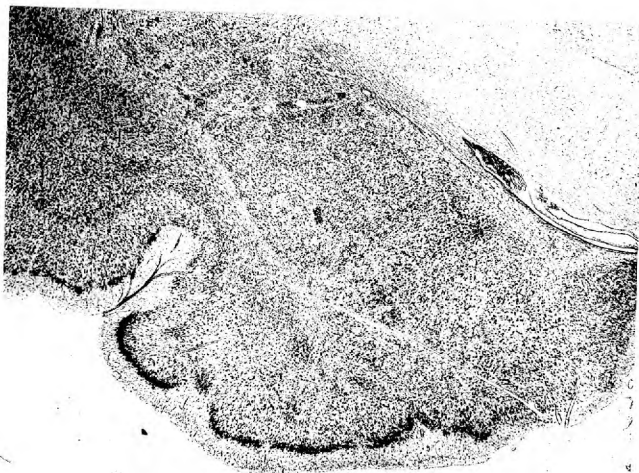


Fig. 34. Transverse section to show the lateral and basal amygdaloid nuclei. Compare with Fig. 35, and both figures should be compared with Fig. 30 (5). Toluidine blue, X 8.5.



Fig. 35. Transverse section just caudal to that shown in Fig. 34 to show the fibres of the 'longitudinal association bundle' and stria terminalis. Loyez stain, X 8.5.



Fig. 36. Transverse section to show the lateral, basal and medial amygdaloid nuclei. The cortical nucleus is bounded by the U-shaped amygdaloid fissure. Note the close relation between the small-celled basal and the medial nucleus. Toluidine blue, X 8.5.

that the arrangement at once recalls Johnston's interpretation mentioned above.

Baso-lateral group

The lateral nucleus is the largest and longest of the amygdaloid nuclei in the rat, rabbit and cat; and in most mammals it was found to be well developed and closely related with the more ventrally situated basal nucleus. It has been divided by Young (1936) for the rabbit into two parts, anterior (dorsal) and posterior (ventral). Brodal (1947b) for the rat, and Johnson (1957) for the guinea pig made a similar subdivision. In the rabbit, however, Young found that the anterior part contained large cells while the posterior part was small-celled; Brodal and Johnson found the reverse condition. There is some doubt in the minds of Gurdjian (1928) and Brodal (1947b) concerning the limit between the lateral and basal nuclei in the rat. Gurdjian mentions that a conspicuous transverse fibre bundle infiltrates the ventral part of the lateral nucleus. Brodal on the other hand takes these bundles to mark the boundary between the lateral and basal nuclei. Consequently Gurdjian's lateral nucleus is much larger than that described by Brodal who defines the caudal part of Gurdjian's nucleus as his lateral magno-cellular part of the basal nucleus. Fox (1940) in his description of the cat brain also points out that the lateral nucleus has a striate appearance due to the fibres running through it to assemble in the anterior commissure. In man and in the shrew

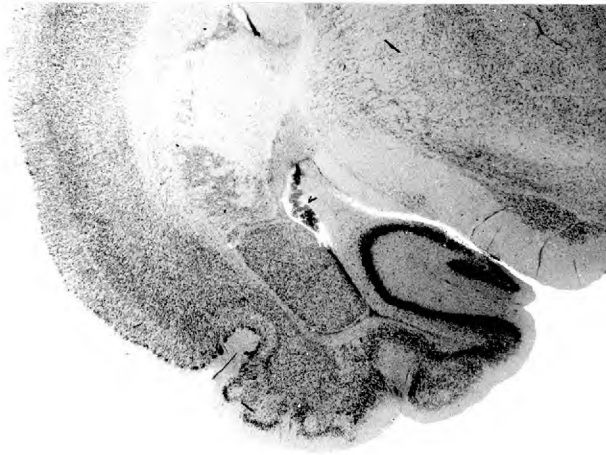


Fig. 37. A transverse section at the level of the caudal third of the amygdala. Note the quadrilateral appearance of the lateral nucleus; the largest, well delineated and most easily defined of all the amygdaloid. The cortico-amygdaloid transition area is clearly shown. Toluidine blue, X 8.5.

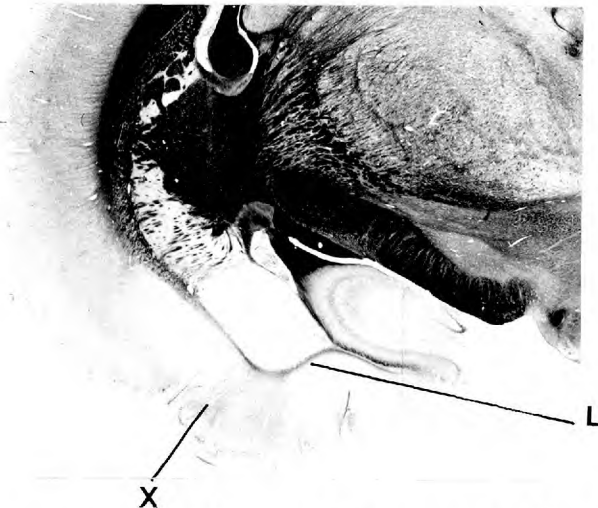


Fig. 38. A transverse section neighbouring that shown in Fig. 37 stained by Loyez (Weigert) method. The myelinated fibres marked (L) which lie between the lateral and basal amygdaloid nuclei join the external capsule and some reach the 'longitudinal association bundle'. Very fine fibres (X) can just be seen passing forward to reach this bundle. X 8.5.

Crosby & Humphrey (1941, 1944) find that the lateral nucleus merges cranially and caudally with the basal nucleus without any distinct transition.

In the coypu the lateral nucleus first appears at the level of the caudal part of the anterior amygdaloid area lying between the external capsule and claustrum laterally, and the central nucleus and putamen dorso-medially. It is the largest and best defined of all the amygdaloid nuclei. Caudally it becomes much larger, spreading out dorsally and medially and acquiring a quadrilateral outline (Figs. 30 and 36). As a result, the central and medial nuclei are squeezed dorsally and ventrally thus leaving the stria terminalis to form its medial boundary.

Two parts of the lateral nucleus can be recognised, a magno-cellular ventral part and a parvo-cellular dorsal part. The magno-cellular part extends throughout the whole length of nucleus and is often called posterior; it is intimately associated with the basal nucleus so that it is sometimes difficult to define the boundary between them (Fig. 33). The parvo-cellular part which is often called anterior is much smaller and its rostro-caudal extent is shorter than that of the magno-cellular part which forms the whole caudal extremity of the lateral nucleus. It is worth noting that the condition in the coypu so far as the size of the cells in the two parts is concerned, is similar to that in the guinea pig and rat and the reverse of that in the rabbit. In accord with

the findings of Fox and Gurdjian, the ventral part of the magnocellular division is infiltrated by transverse myelinated fibres that give it a striate appearance (Figs. 30 and 35). They probably represent the fibres which join the 'longitudinal association bundle' of Fox (1940).

The basal nucleus is divided into a larger-celled lateral part, close to and sometimes fused with the lateral nucleus, and a smaller-celled part which is medial in position. An accessory basal nucleus is described in detail by Humphrey (1936) in the bat where it seems to be quite large and extensive, occupying a position medial to the basal nucleus rather than lateral to it as in the opossum (Johnston, 1923). Appearing caudal to the putamen, in a position medial to the basal nucleus and dorsal to the cortical nucleus, it extends as far backwards as the caudal pole of the hemisphere. Large basal and accessory basal nuclei have been described for the macaque (Lauer, 1945) and for man (Crosby & Humphrey, 1941). The accessory basal nucleus is not well differentiated in the rat (Brodal, 1947b), and in the rabbit (Young, 1936), and is entirely lacking in the cat (Fox, 1940).

In the coypu the basal nucleus is first seen at about the level of the middle of the amygdala ventromedial to the lateral nucleus. It extends to the caudal limit of the amygdala. Throughout it is bounded medially by the medial nucleus and ventrally by the cortical nucleus (Fig. 36). Laterally it abuts on and partially fuses with the cortico-amygdaloid

transition area. Thus the basal nucleus, through the intermediation of the transition area, is in cellular continuity with the piriform cortex.

The basal nucleus can be divided into the two subdivisions described by other authors. A lateral magno-cellular part is in very close relation with the posterior magno-cellular part of the lateral nucleus, and can only ^{be} differentiated from it because of its slightly larger and darker cells. The medial part which is formed by medium sized cells that are widely dispersed cannot easily be limited from the cortical and medial nuclei ventrally. It is doubtful however, if cytoarchitecturally there is any real justification for including the parvo-cellular part of the basal nucleus in the baso-lateral group. It appears very similar and more closely related to the cortico-medial group (see Discussion). Medially it is very difficult to identify an accessory basal nucleus but there is a suggestion of one lying rostrally dorsal to the cortical nucleus (B₁ in Fig. 30).

SOME OBSERVATIONS ON THE DISTRIBUTION OF CHOLINESTERASE IN
BASAL RHINENCEPHALIC STRUCTURES

Apart from its interest in relation to the problems of synaptic transmission, methods for the histochemical localisation of acetylcholinesterase (AChE) have proved a useful technique for the differential staining and identification of parts of the central nervous system. Koelle (1954) gave the first comprehensive description of the distribution of the enzyme in the brain of a mammal, the rat. With some exceptions this work was confirmed by Shute & Lewis (1961). Similar observations were made by Ischii (1957) in a number of rodents, by Gerebtzoff (1959) and by Foldes et al. (1962) in man. The method has also been of value for the identification of groups of cells and fibres which are difficult to stain differentially by the classical techniques. For example, Shute & Lewis (1960) were able to identify the salivatory nuclei in the brain stem of the rat on account of their high AChE activity and their relationship to the cholinergic fibres of the nervus intermedius.

The power of this method has been greatly increased by the discovery that it can be applied in experimental material to determine the polarity of groups of fibres and therefore the direction in which they conduct. In 1957 Lewis & Hughes put forward the hypothesis that AChE is synthesised in the perikaryon and transported down the axon to be released at its terminals. Later they showed that after division of an axon

an accumulation of enzyme could be demonstrated proximal to the lesion (Hughes & Lewis, *Nature*, 1961). A fuller account of the use of this finding for determining some of the connexions of the amygdala and hippocampus was given by Shute & Lewis (1961) who emphasise not only the proximal accumulation but also the disappearance of AChE distal to a lesion. In 1963 the same authors published a description of the AChE containing systems of the rat's brain in which observations made in normal and experimental material were combined. In this way they were able to arrive at a comprehensive description of the fore-brain component of the reticular formation in this animal.

Before describing the distribution of AChE in the individual parts of the coypu brain some general features of the results should be pointed out.

The characteristics of the staining vary in different parts of the fore-brain. In some, such as the caudate-putamen, the staining is so dense that it is difficult to determine its exact localisation, that is, whether the staining is primarily in the perikaryon or in the surrounding neuropil. In other situations, e.g. the medial septal nucleus, localisation to the perikaryon and its processes is precise, so that the neurones stand out clearly from their surroundings.

The incubation time has a considerable effect on the results. It has been found (Shute & Lewis, 1960, 1961) that a short incubation ensures high selectivity for sites of high

enzyme concentration. They give periods of $1\frac{1}{2}$ to 4 hours as optimal for sections between 100 μ and 25 μ in thickness, the thicker sections requiring the shorter time. This was for the rat. For sections of the coypu brain ranging from 60 μ to 100 μ somewhat longer periods appeared to give optimal results, namely 4 to 7 hours incubation. Periods of 17 hours were also tried and this led to a very dark staining often rather diffuse. Nevertheless the main features of the distribution of the enzyme are sometimes shown very clearly and some of these long-incubation specimens have been used for illustration. It is obvious however, that in such specimens the negative observations will be more reliable than the positive.

Results are also complicated by the existence of true or specific acetylcholinesterase (AChE) and pseudo- or non-specific cholinesterase (ChE). Koelle's thiocholine technique which has been used in the work to be reported here, reacts with both, but the activity of the ChE can be prevented by the use of inhibitors (e.g. ethopropazine, Bayliss & Todrick, 1956, quoted by Shute & Lewis, 1963) which have not been used. However, ChE is usually thought to be located mainly in the blood vessels and glia so that it could be assumed that its presence will not affect the picture obtained of the distribution of AChE in the structures studied in this thesis to an important degree. It must be pointed out however, that Shute & Lewis (1963) have shown that both AChE and ChE

containing neurones do exist in the rat's brain. The situation may well be similar in the coypu but in the rat the ChE containing neurones were found principally in the thalamus and outside the field of this investigation. The only neurones of this kind relevant to the present work are the cells of the interstitial nucleus of the hippocampal commissure in the septum and of the islets of Calleja in the olfactory tubercle, both of which contain ChE in the rat (Shute & Lewis, 1963).

With these reservations in mind the results can now be described. It must also be realised however that they are based on the study of only two brains. One of these is completely normal and all the sections were incubated for 4 hours; in the other the left olfactory peduncle had been completely divided a little rostral to the olfactory tubercle 5 days before the animal was killed. There is considerable damage to the overlying frontal lobe. The right hemisphere of this specimen is not directly affected by the lesion. Sections from this experimental animal were incubated for varying times, 4, 7 or 17 hours.

Olfactory bulbs and peduncle

In the normal brain with 4 hours incubation all the sections from these regions were completely negative. Positive results were obtained from the experimental brain with incubation times of 7 and 16 hours. It is not possible to say how far these positive findings are the result of the operation or of the longer incubation times. It is probable that the

latter are more important and the reason for saying this is that the findings are similar to those of other investigators in the normal olfactory bulb of other rodents (Shute & Lewis, 1963; Ischii, 1957).

In the experimental brain on both right and left sides there is fairly deep staining in the glomerular layer and in the perikarya of the mitral cells. The superficial layer of olfactory nerve fibres is completely negative as were the cells of the inner granular layer. Moderate staining is present in the outer and inner plexiform layers and in the tufted or displaced mitral cells. The fibres from the olfactory commissural component in the central part of the bulb are negative. The distribution of stain in the accessory bulb is similar although the layers are less distinct.

In the peduncle there is a little staining in the neuropil of the anterior olfactory nucleus and in some of the fibres of the olfactory tracts on the surface. Although in a majority of the sections the staining is slightly heavier on the normal (right) side than on the left, the difference is not consistent in all sections and cannot be considered significant.

The remaining part of this description is based entirely on the sections from the normal brain incubated for 4 hours.

Piriform and entorhinal cortex

The frontal part of the piriform cortex is entirely negative but some very faint staining can be seen in the second



Fig. 39. A transverse section stained by Koelle technique. Note the densely stained olfactory tubercle, nucleus accumbens and corpus striatum. X 6 (section No. 17; 80 μ)



Fig. 40. A transverse section at the level of the middle of the septum showing intense staining of the diagonal band and the medial septal nucleus. The medial fore-brain bundle and olfactory commissural component do not stain. Koelle, X 4 (section No. 20; 100 μ)



Fig. 41. A transverse section caudal to that shown in Fig. 40. ~~Note that~~ the staining of the nucleus of the diagonal band and anterior amygdaloid area is in the perikaryon of the cells. The bed nuclei of the stria terminalis and anterior commissure also stain. Koelle, X 4.5 (section No. 21; 80 μ)

or pyramidal layer in the temporal region. The picture changes abruptly in the entorhinal area where the second layer, formed by closely packed polygonal cells, shows quite distinct but diffuse staining (Fig. 46). In the third layer (the layer of superficial pyramids) an occasional cell stains well; the fourth layer shows quite definite but somewhat diffuse staining. It is clear that the entorhinal cortex differs from the piriform not only in its cytoarchitecture but also in its AChE activity.

Olfactory tubercle

The olfactory tubercle shows staining which is comparable in intensity to that of the caudate putamen (Figs. 39 and 40). It is diffuse and in the neuropil rather than in the perikarya. The staining is particularly intense in the islets of Calleja, which on this account stand out very clearly. These are structures which may contain ChE instead of or as well as AChE (Shute & Lewis, 1963).

Septum and diagonal band

The large cells of the medial septal nucleus stain deeply and the stain is quite clearly located in the perikaryon and its processes. The nucleus of the diagonal band consists of similar cells which stain in the same way (Fig. 40). The fibres of the diagonal band also stain and in some cases it seems possible to trace them to the cells of the medial septal nucleus.

The lateral septal nucleus shows little or no staining and

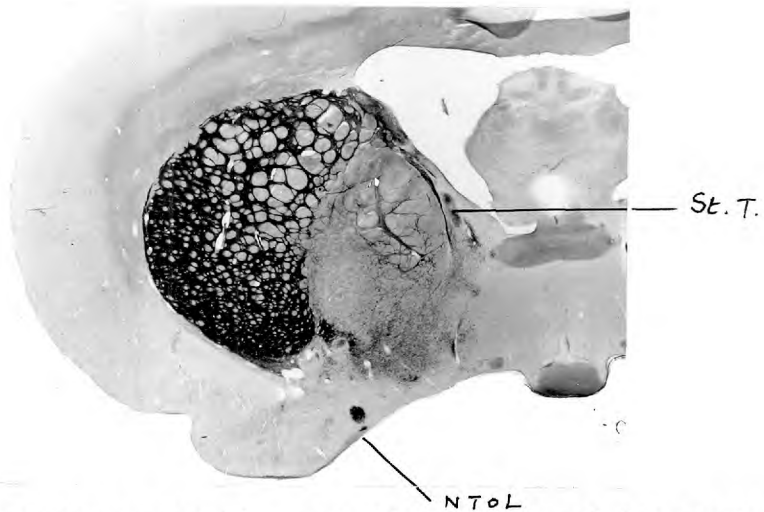


Fig. 42. A transverse section at the level of the anterior commissure (which does not stain by this method) showing staining in some fibres of the stria terminalis. Note intense staining of the nucleus of the lateral olfactory tract. Koelle technique. X 4.5 (section 22; 80 μ).

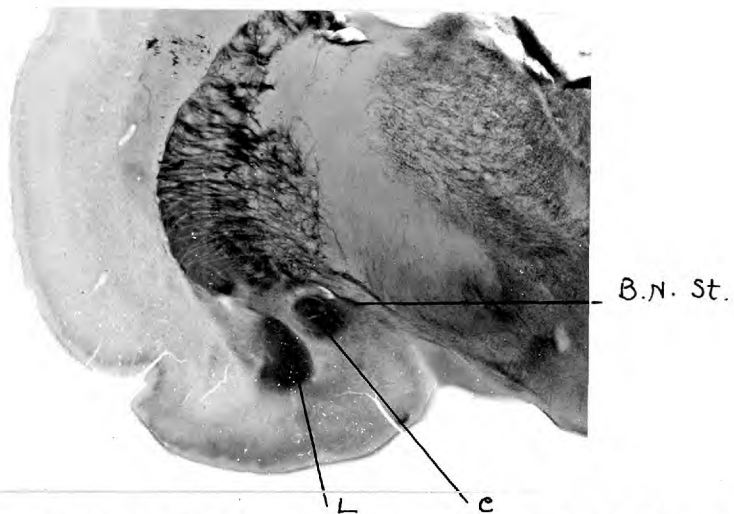


Fig. 43. A transverse section to show the staining of the bed nucleus of the stria and its closely associated central amygdaloid nucleus. Note the intense staining in the large-celled ventral part of the lateral nucleus. Koelle method. X 5.5 (section 25; 120 μ).

the same applies to the fibres of the precommissural fornix system. A little very faint staining can be seen in the region of the hippocampal commissure which may represent a positive reaction in its bed nucleus. This is doubtful however and enough sections are not available to study this region more fully.

The nucleus accumbens stains intensely like the other parts of the corpus striatum (Fig. 39). The stain appears to be mainly in the neuropil and not in the perikarya of the cells.

The amygdala

Anterior nuclei. The anterior amygdaloid area shows selective positive staining which is mainly intracellular and similar to that in the nucleus of the diagonal band, which lies medial to it. The nucleus of the lateral olfactory tract gives an intense reaction and appears as one or two small rounded masses, deeply stained, at the caudo-lateral border of the olfactory tubercle (Fig. 42). The staining is very similar to that of the islets of Calleja. The intercalated masses could not be distinguished and presumably have not stained.

Cortico-medial nuclei. The cortical nucleus shows slight staining in the superficial part of the molecular layer but is otherwise negative. The principal nuclei in this group - medial and central - both show moderate diffuse staining. The bed nucleus of the stria terminalis stains heavily and

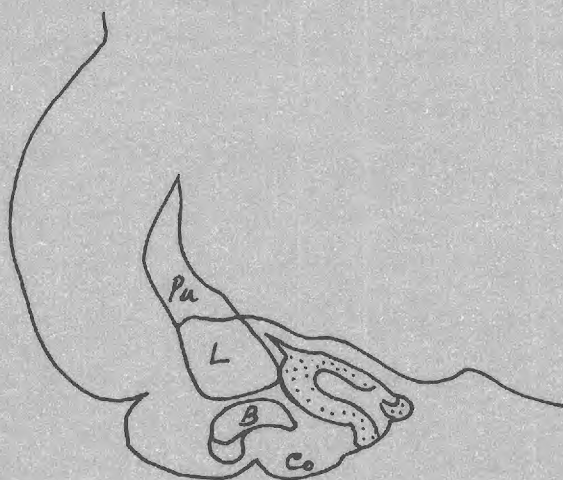
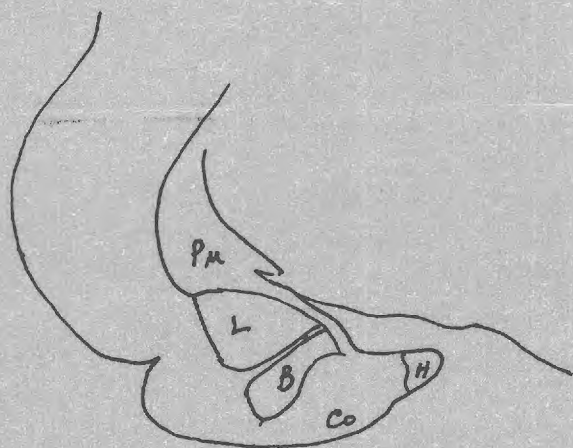




Fig. 44. A transverse section in the middle of the amygdala showing the lateral nucleus and the much darker basal amygdaloid nucleus. Koelle method. X 4.5 (section No. 27; 80 μ)



Fig. 45. A transverse section to show the lateral and basal amygdaloid nuclei which are more easily delineated by this method. Note the close relation between the basal nucleus and the cortico-amygdaloid transition area. Koelle. X 4.5 (section 28; 80 μ)

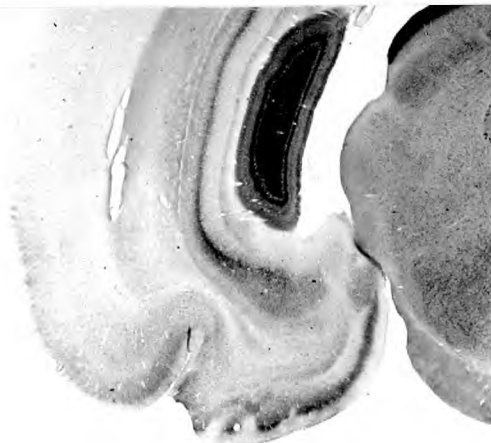


Fig. 46. A transverse section to show the entorhinal cortex which shows quite distinct laminar staining. Koelle method. X 5.5 (section No. 32; 80 μ)

many of the fibres of the stria itself show quite definite activity. A commissural, hypothalamic and lateral pre-optic component, could all be recognised on account of their positive staining. (The lateral pre-optic component was called the "lateral strial bundle" by Shute & Lewis, 1963, and they think that it projects in the opposite direction to that normally described, i.e., from the lateral pre-optic area to the amygdala.) In this series the stria terminalis does not appear to have stained throughout its course because in the most caudal sections at the level of the lateral and basal amygdaloid nuclei it could not definitely be identified.

Baso-lateral nuclei. The basal nucleus shows marked staining probably in the perikarya as well as in the surrounding neuropil. The staining is particularly intense in its ventro-lateral part where it is difficult to distinguish from the cortico-amygdaloid transition area. The latter however, does not appear to stain. The lateral nucleus stains moderately in its ventral part but rather less deeply dorsally. It shows its typical quadrilateral shape and is clearly outlined by the external capsule laterally (which does not stain) and by the basal nucleus ventrally which stains much more deeply than the lateral.

The lateral olfactory tract, the medial fore-brain bundle and all the components of the anterior commissure show a quite diffuse yellowish colouration which does not appear to indicate any significant reaction with the enzyme. It may be added

that the hippocampal formation (dentate gyrus and cornu ammonis) stains quite deeply and that the staining extends into the subiculum. The alveus contains some stained fibres but both fimbria and fornix are negative.

The experimental specimen M.13

It is appropriate to describe briefly at this point results obtained in the single experimental specimen. As already stated the left olfactory peduncle had been completely divided close to its junction with the cerebral hemisphere (Fig. 47) five days before the animal was killed. The results in the right olfactory bulb and peduncles were taken probably to represent a normal condition and have been described on pages 94 and 95.

In the hemispheres caudal to the lesion the only ^{apparent} differences from the staining in the normal brain were seen in the olfactory tubercle and the nucleus of the lateral olfactory tract. On the side of the lesion staining in the olfactory tubercle (Fig. 48) ~~and in the nucleus of the lateral olfactory tract (fig 49) appeared~~ ^{but closer} ~~reduced or absent as compared with the opposite side.~~ ^{to be} Examination showed that this appearance was due to obliquity of the sections so that different regions were being compared. ~~the nucleus of the lateral olfactory tract on the side of the lesion also shows complete failure to stain (Fig. 49). It could be seen very clearly~~ ~~on the opposite side.~~ There was no change in the staining reaction of other parts of the basal rhinencephalon on either side of this brain.

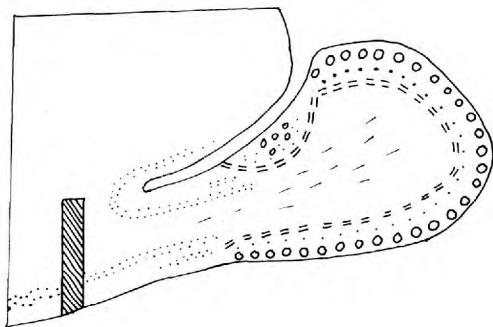


Fig. 47. Diagram of a sagittal section of the left olfactory bulb and peduncle to show the lesion made in specimen #.13 which was then processed by the Koelle method (see Figs. 48, 49).

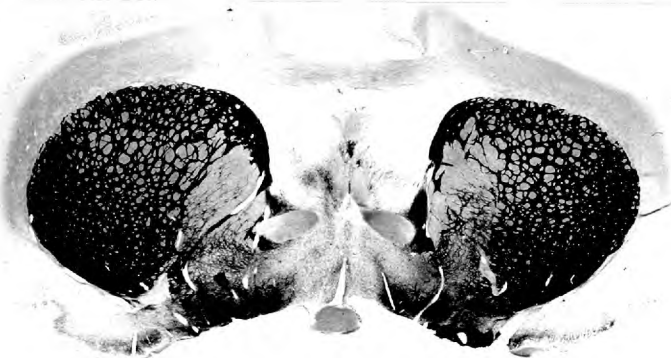


Fig. 48. A transverse section at the level of the olfactory tubercle. ~~that the tubercle is intensely stained~~
~~on the right side where the tubercle is intensely stained.~~
 Koelle. X 3.

on the
right side
k



Fig. 49. A transverse section just caudal to the level of the olfactory tubercle. ~~that the tubercle is intensely stained~~
~~on the right side where the tubercle is intensely stained.~~
 Koelle method. X 3.

While no conclusions can be based on an examination of a single specimen, especially as only one normal specimen was available for comparison, it seemed worthwhile to record these observations which will later be discussed shortly in relation to the more extensive observations made by the classical neurohistological methods.

FIBRE TRACTS AND CONNEXIONS IN THE BASAL RHINENCEPHALON

For an account of the literature relevant to the fibre tracts and their connexions, it is necessary to take note not only of the observations made in normal material but also observations made on the basis of experimental degeneration. It is only by the use of the latter that the precise origin, termination and polarity of fibres can usually be determined. It is true that the Golgi technique, by staining the whole neurone and its processes will give some information of this kind in normal material, but it rarely allows an axon to be traced beyond the limits of a single section and is mainly useful when short-axon neurones (Golgi's Type 2) are being studied.

Olfactory tracts

The lateral olfactory tract. It is generally agreed that the fibres of this tract arise from the mitral cells of the olfactory bulb (see p.30 et seq.) and this has been confirmed experimentally by the observation of retrograde degeneration after lesions of the tract (Allison, 1953a). The fibres in the tract are thought to be supplemented by the axons of neurones in the anterior olfactory nucleus (Loo, 1931 and others). It is also possible that the axons of tufted cells pass into it but this is disputed and will be discussed fully in connexion with the olfactory commissural component. It is said however that both mitral and tufted cells can be driven antidromically after stimulation of the lateral olfactory tract (Green, Mancía

and Baumgarten, 1962; Shephard, 1963). Some authors describe inhibition of the mitral cells and it is not certain that the fibres concerned are centripetal (Phillips, Powell & Shephard, 1963; Yamamoto, Yamamoto & Iwama, 1963). Followed caudally the secondary olfactory fibres form a large lateral olfactory tract, and the most medial end superficially in the antero-lateral part of the tubercle, although this connexion was not found by Cajal (1911). Throughout the length of the tract many fibres diverge laterally into the molecular layer of the piriform cortex and some can be followed to but not beyond the rhinal fissure. In Golgi-stained material, Cajal demonstrated that they establish an axo-dendritic contact with the pyramidal cells in the plexiform layer of the piriform cortex. This finding was confirmed experimentally, also in Golgi material, by Jones & Thomas (1962). Some fibres also discharge into the nucleus of the lateral olfactory tract and to the cortical amygdaloid nucleus or periamygdaloid cortex. Even in normal material, as described by Humphrey (1936) and Cajal (1911), the entorhinal area appears to receive no fibres directly from the lateral olfactory tract and this has been confirmed experimentally.

With the exception of a possible contribution from the tufted cells the above results have been obtained by many investigators, using different histological and experimental techniques; they can be said to be firmly established and almost certainly to represent common features of the olfactory

system in all mammals. Another finding, although less often reported, is almost equally reliable and equally probable a common mammalian feature. This is the connexions from the bulb to the anterior olfactory nucleus. It is indeed probable that all parts of this nucleus receive terminations or collateral branches of secondary fibres from the bulb and that the contacts are again predominantly axo-dendritic. This would be consistent with the character of this nucleus as an extension into the peduncle of the olfactory cortex of the base of the brain.

The reports of a number of other features of the secondary olfactory connexions in mammals are less uniform and often in direct conflict with each other. These conflicting reports concern chiefly the possibility of connexions with the medial and central amygdaloid nuclei and the bed nucleus of the stria terminalis. Because of the varied potentiality of the histological methods used in the discussion which follows (Marchi, Glees and Nauta), the results obtained are grouped according to the technique.

The first to be considered are those studies in which the classical Marchi method has been used (e.g. by Cajal, 1911). After ablation of the olfactory bulb no fibres showing Marchi degeneration have ever been traced to the deeper amygdaloid nuclei and the bed nucleus of the stria terminalis either by way of the lateral olfactory tract or any other pathway. This is of course not conclusive. The fibres in question might have lost their myelin sheaths some distance from their endings, or

have become too fine and scattered for demonstration by this method.

The Glee method, by staining degenerating terminal and preterminal fibres would be expected to provide precise evidence for the sites of termination of secondary olfactory fibres. The first study, using this method (Clarke & Meyer, 1947, in the rabbit) reports very definite terminal degeneration in the cortico-medial and central amygdaloid nuclei and in the bed nucleus of the stria terminalis; this was confirmed by Meyer & Allison (1949) with the same method in the macaque and the baboon. The degeneration is found after unilateral lesions or ablation of the olfactory bulb and is seen bilaterally in the central amygdaloid nuclei and the bed nucleus of the stria terminalis. The fibres do not appear to have been traced in serial sections throughout their length so that they need not lie in the lateral olfactory tract and it is presumed that the degenerating terminals on the side opposite the lesion must belong to fibres which have crossed in the anterior commissure. Adey (1953) in the phalanger made similar observations but commented that the degeneration seen deep in the amygdaloid region was very small in amount. Since similar conditions have been found in such widely different mammals including primates and a marsupial, it is natural to conclude that they represent a condition common to all mammals.

The presence, however, of such degenerating fibres in the central nucleus, and the bed nucleus of the stria, has not

been confirmed by nearly all authors using another silver impregnation method (the Nauta method). The Nauta studies were carried out mainly in the rabbit, rat, guinea pig and cat, and the possibility of a species difference cannot be ruled out absolutely, but for the reason given above, can be considered unlikely and will not be discussed further.

Johnson (1959, in the guinea pig) is the only author to use this method who claims to have confirmed the findings obtained by Clark & Meyer with the Glee's technique unequivocally. He reports, however, only one specimen (case III-3) in which the lesion is confined to the bulb. In this specimen degeneration appears to be limited to the lateral olfactory tract. He states that at caudal levels the most medial fibres of the tract end in the cortico-medial amygdaloid nuclei and it is not clear if the degenerated fibres were traced beyond the molecular layer of the cortical nucleus. In a second experiment with a lesion in the peduncle involving the lateral olfactory tract and also the anterior olfactory nucleus and olfactory commissural component, he finds that the distribution of degenerated fibres caudal to the lesion is essentially similar. However, Johnson does not describe his material in detail. Lammers (1959), using the Nauta technique, is quoted by Lohman (1963) as also confirming (in the cat) Clark & Meyer's findings. Lammers however, seems to have revised his opinion concerning the projection system of the olfactory bulb because in a later paper with Lohman (Lohman & Lammers, 1963) it is stated "In the course

of our preliminary investigations into the olfactory connexions (Lohman & Lammers, 1961) we got the impression that the projection system of the olfactory bulb as described by Le Gros Clark et al. is not fully acceptable". Lohman & Lammers (1963) also state that in a large number of experiments on cats, rabbits, monkeys and particularly on the guinea pig, their findings are in accordance with those published in a paper by Lohman alone in 1963 and dealing with the guinea pig. In this work Lohman found that after lesions limited to the bulb no degeneration could be found in the olfactory commissural component, the medial amygdaloid nucleus, the central amygdaloid nuclei nor in the bed nuclei of the stria terminalis. Degeneration is limited to the lateral olfactory tract, which distributes fibres only to the olfactory tubercle, the piriform cortex and the antero-lateral part of the cortical amygdaloid nucleus. Other investigators who failed to find a projection to the deeper nuclei of the amygdala and using the Nauta technique are Cragg, 1961 (rabbit, rat and cat), Saunders-Woudstra (1961) in rat, White (1962) in the rat and Powell et al. (1963) in the rat.

Electrophysiological studies also give fairly comparable results concerning the projection of olfactory fibres to the amygdala. Following single shock stimulation of the olfactory bulb in the cat Fox, McKinley & Magoun (1944) recorded potentials from the nucleus of the lateral olfactory tract, from the surface of the tubercle, and from that part of the piriform cortex that surrounds the

cortical amygdaloid nucleus. They were unable, however to record potentials from the amygdaloid nuclei, septum, diagonal band and hippocampus. Rose & Woolsey (1943) reported a response from the peri-amygdaloid region but not from the deeper parts of the amygdala.

Centrifugal fibres. Until recently the existence of centrifugal fibres in the lateral olfactory tract was doubtful. Cajal (1911) first described the presence of such fibres which reach the olfactory bulb from the basal part of the brain. Allison (1953a) reported that two months after transection of the lateral olfactory tract in both rabbit and newborn rat the axons on the bulbar side of the lesion exhibited very little difference from those on the opposite normal side of the brain. He concludes therefore that if lateral olfactory tract contains any bulbo-petal fibres, they must be very few. Recently however, Cragg (1962) demonstrated massive centrifugal degeneration leading to the granule cells and to the perimeter of the glomeruli in Nauta preparations of the bulb after lesions in the anterior olfactory areas. He thinks that the coarser degenerating centrifugal fibres run in the lateral olfactory tract. Finer fibres, which reach no further than the inner granular layer run in the olfactory commissural component. Powell & Cowan (1963) with lesions which are limited to the lateral olfactory tract confirmed Cragg's finding. From their material these authors were also able to exclude the amygdala and adjacent part of the piriform cortex,

together with the hypothalamus, as possible sites for the origin of these fibres. However, they point out that all experiments in which the olfactory tubercle is damaged result in degeneration of the coarse centrifugal fibres running in the lateral olfactory tract.

Electrophysiological studies also suggest the existence of centrifugal fibres in the lateral olfactory tract to the bulb. Kerr & Hagbarth (1955) showed that excitation of the brain caudal to the bulb exerted a profound depressive influence on its electrical activity. Other workers such as Green, Mancia & Baumgarten (1962), Phillips et al. (1963), Shepherd (1963) and Yamamoto et al. (1963) have obtained excitation in mitral and some tufted cells from stimulation of the lateral olfactory tract. This has generally been thought to be due to antidromic conduction but it is difficult in work of this kind always to distinguish orthodromic from antidromic effects.

Intermediate and medial olfactory tracts. The intermediate olfactory tract is a variable feature of the mammalian brain (see p. 22) and appears to consist of fibres which enter the superficial aspect of the olfactory tubercle. Such fibres have been seen by many authors and are often described as coming from the medial side of the lateral olfactory tract (e.g. by Elliot-Smith, 1909, in *Oryzeteropus* and Allison, 1954, in Man). Cajal, in Marchi preparations failed to confirm their presence, but almost all the more recent experimental studies have demonstrated fibres of this kind limited to the

anterior and lateral areas of the tubercle (e.g. Clark & Meyer, 1947, and Lohman, 1963). Since they appear to come from the lateral olfactory tract there seems no need to give them a separate name, especially as the term "intermediate tract" has also been applied to a quite different fibre bundle (see p. 22).

Most authors dealing with normal material describe a medial olfactory tract running to the anterior hippocampal nucleus and septum but there is much disagreement over its origin and distribution. Humphrey (1936) for example, states that in the bat most of its fibres probably arise from the anterior olfactory nucleus rather than from the bulb. Young (1936) traces these fibres in the rabbit caudally along the medial and ventromedial aspect of the peduncle and into the medial septal area. He describes the contribution of this tract to the precommissural hippocampus, the medial septal nucleus and the medial aspect of the olfactory tubercle. Brodal (1947a) states that if the medial olfactory tract is present in mammals at all, its significance is negligible. Allison (1953b) suggested that it is not an efferent pathway from the olfactory bulb in mammals, but an afferent tract from the septum.

That fibres are present in this situation is undoubted but the experimental evidence for their origin in the bulb is uniformly negative. Clark & Meyer (1947), from their Gleeper preparations, emphasised that in none of their experiments were degenerating fibres or terminals seen in septum, anterior

hippocampal nucleus or in any part of the hippocampal formation and later work with the Nauta technique has confirmed this. There is no doubt therefore that the medial olfactory tract does not consist of secondary olfactory fibres and is not comparable to the lateral olfactory tract. It may well consist of fibres from or to the anterior olfactory nucleus or between the tuberculum and the septum, but not of projection fibres from the bulb. A terminology which suggests they are the latter is unsatisfactory.

Anterior commissure

The anterior commissure of mammals is generally described as consisting of two limbs, anterior or interbulbar and posterior or intertemporal (e.g. Loo, 1931, in opossum). In Kappers et al. (1936) the posterior limb is divided into inter-temporal and stria terminalis components, and many more components are defined in the older literature (for example 5 by Loo, 1931, and 8 in the bat by Humphrey, 1936). This thesis is mainly concerned with the anterior limb so that a detailed discussion of all these components will be unnecessary.

The anterior limb or the olfactory commissural component of this thesis, is a bundle of well myelinated fibres (Fig. 50) which can be traced from the core of the bulb throughout the peduncle to cross in the antero-inferior part of the commissure. There has been much disagreement over the precise origin and termination of these fibres. Van Gehuchten & Martin (1891), following Golgi (1875) (quoted from Allison, 1953b), believed that the axons of the tufted

cells like those of the mitral cells pass into the lateral olfactory tract. From Golgi studies Cajal (1911) thought they probably arose from tufted cells in one bulb and terminated on the granule cells in the opposite bulb. Humphrey (1936) thinks that it contains also fibres from the anterior olfactory nucleus which probably pass to the corresponding contralateral regions. Earlier, Herrick (1924) had made a similar suggestion for the opossum and this is in accord with Young's (1936) description for the rabbit.

All these authors were working on normal material. The first experimental workers to investigate this problem used the Marchi method and discrepancies soon appeared. Loewenthal (1897) reported that after bulbar lesions Marchi degeneration was confined to the lateral olfactory tract and that the olfactory ^{Commissural} component was not affected unless the lesion extended into the peduncle, where presumably it caused damage to the anterior olfactory nucleus. When this occurred degeneration could be traced to the opposite bulb and peduncle. Van Gehuchten (1904), and later Young (1941, 1942), also using the Marchi technique, obtained results similar to those of Loewenthal. These findings were recently confirmed by Lohman (1963), Lohman & Lammers (1963), and White (1962, 1964) all using the Nauta technique. Other authors, however, report that ablation of the bulb alone is enough to cause degeneration not only in the lateral olfactory tract but also in the olfactory commissural component. These are Probst (1901) and Cajal (1901, 1911) in Marchi preparations, and

Clark & Meyer (1947) and Adey (1953) in Glee's preparations. Clark & Meyer, however, pointed out that only very few degenerating fibres were seen in the commissure "and it was hardly possible to say with certainty how far the anterior olfactory nucleus may have been indirectly affected in these experiments by trauma". Cragg (1961) and Powell et al. (1963) report that after bulbar lesions, fine degenerated fibres can be traced, in Nauta preparations, through the olfactory commissural component to ipsilateral terminations among deeper cells of the piriform cortex and tubercle. Cragg also points out that his lesions are at the posterior end of the olfactory bulb and that it is very probable that the anterior part of the anterior olfactory nucleus was also destroyed. It does appear therefore, that all of these workers could have included some part of the anterior olfactory nucleus in their bulbar lesions. This point was clearly explained by Fox & Schmitz (1943) who said that the oblique attachment of the bulb to the crus with the resultant extension of the anterior olfactory nucleus in the bulbar region makes it difficult to destroy any sizeable portion of the bulb without involving some of the anterior olfactory nucleus. Accordingly it would be possible to conclude that the fibres of the olfactory commissural component originate almost entirely from retro-bulbar structures. It is worth noting that Baumgarten et al. (1962) found that antidromic impulses evoked by stimulation of anterior commissure fail to invade the somata of tufted cells.

As concerning the terminations of these fibres there seems to be general agreement. Fox & Schmitz (1943) and Fox, Fisher & Desalva (1948) studied the connexions of the anterior commissure in Marchi preparations of the cat and monkey after lesions in the mid-line or slightly lateral. They confirmed that commissural fibres do run to the bulb, anterior olfactory nucleus, piriform cortex, and to the olfactory tubercle. Brodal (1948) and Allison (1953a) by using the modified Gudden method demonstrated retrograde cell changes in the pars dorsalis of the anterior olfactory nucleus after transection of the commissural fibres. Allison also demonstrated, in Glees preparations, terminal degeneration in the contra-lateral anterior olfactory nucleus, but he emphasised that he always found more degeneration in the ipsilateral bulb than in the contra-lateral. He thus concludes that the bulbo-petal fibres arise from the ipsilateral basal areas of the hemisphere, as well as from the contra-lateral bulb. Similar findings and conclusions were reported recently by Cragg (1962) in Nauta preparations.

One would conclude from these studies that the olfactory commissural component arises at least mainly in the anterior olfactory nucleus, crosses in the anterior commissure and that some of its fibres end in the opposite bulb and anterior olfactory nucleus. Some may also end in the olfactory tubercle and piriform cortex, both in the ipsi- and contra-lateral sides (see Fig. 109). According to Powell et al. (1963) the fibres with the ipsilateral endings may come from the bulb.

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Fig. 50. A transverse section showing the heavily myelinated olfactory commissural component and the stria terminalis just dorsal to it. Loyez method. X 4.5.

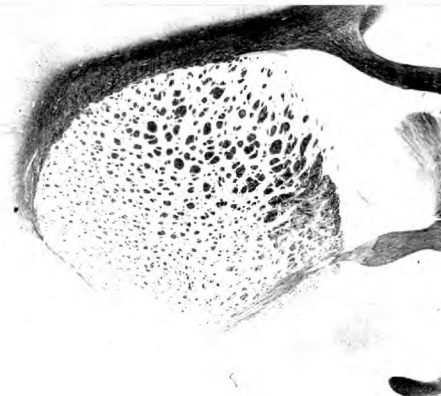


Fig. 51. A transverse section showing the intertemporal component of the anterior commissure passing laterally just ventral to the corpus striatum to reach the external capsule. Loyez. X 7.



Fig. 52. A transverse section showing the intertemporal component and the heavily myelinated olfactory commissural component of the anterior commissure. Loyez. X 7.

The posterior or intertemporal limb of the anterior commissure crosses in the mid-line in its postero-superior segment (Fig. 52). In macrosmatic mammals, e.g. cat (Fox, 1940) this component is much smaller and less heavily myelinated than the anterior or olfactory commissural component. In the microsmatic monkey (Fox et al., 1948) however, the opposite is true. Investigators working on normal material (e.g. Young, 1936; Humphrey, 1936) generally believe that the fibres of this component interconnect the piriform cortex and some of the amygdaloid nuclei of the two sides. The corpus callosum has no separate existence in the opossum, and Loo (1931) thinks that all neopallial commissural fibres also cross in this limb. It is generally accepted, however, that a neopallial component becomes progressively smaller during the phylogenetic ascent of mammals. Fox et al. (1943, 1948) were doubtful in Marchi preparations, of any connexions with the amygdala after transecting the commissure in the mid-line. After a similar lesion Brodal (1948) could not confirm the commonly described connexion (e.g. by Humphrey, 1936) from the baso-lateral amygdaloid nuclei, but found retrograde changes in the nerve cells of the anterior group of amygdaloid nuclei and the cortical amygdaloid nucleus.

The medial forebrain bundle

Earlier workers (e.g. Young, 1936) regard this as an interconnection between the medial and ventral areas of hemisphere including the olfactory tubercle, on the one hand,

and hypothalamic and possibly the tegmental region of the mid-brain on the other. Young regards a tuberculo-hypothalamic tract as one of its major constituents, the septohypothalamic its largest component, and that both may carry ascending as well as descending fibres. Loo (1931) describes a similar condition in the opossum, but thinks that the most rostral part of the bundle arises in the olfactory bulb and anterior olfactory nucleus. More recently Valverde (1963a), who studied this bundle in Golgi preparations of the mouse, considers that it comprises fibres originating in the septal nuclei, the piriform area and from some giant pyramidal cells of the frontal cortex.

No evidence can be found in the experimental literature for the alleged contribution of secondary olfactory fibres to the medial fore-brain bundle. Neither Morin (1950) in guinea pig, Saunders-Woudstra (1961) in the rat, nor Lohman (1963) in the guinea pig could find degeneration after bulbar lesions. Lohman however agrees with Young that the pars medialis of the anterior olfactory nucleus projects by way of the medial fore-brain bundle to the lateral hypothalamic area. Morin finds no contribution from the bulb or peduncle, but describes some from the olfactory tubercle.

From the foregoing description one can conclude that if the medial forebrain system is influenced by olfactory impulses, this influence must be of an indirect nature. It is worth noting the similarities between this fibre system, the stria terminalis and fornix fibre systems. All appear to be

interconnecting the secondary olfactory areas (anterior olfactory nucleus, piriform cortex, olfactory tubercle) and their closely related structures (septum, amygdala, hippocampus) with the hypothalamus.

Septal connexions (including the diagonal band).

Many fibre systems are connected with or pass through the septal region; the most obvious of these are the precommissural fornix fibres which run chiefly in the lateral nucleus. The diagonal band of Broca connects with the amygdala, and there are many fibres between the septum and olfactory tubercle. Some fibres of the medial fore-brain bundle run to or arise in the septum (see above). As mentioned earlier, there is much experimental evidence that no secondary olfactory fibres reach the septum and the so-called medial olfactory tract may consist of fibres which come from or go to the anterior olfactory nucleus or olfactory tubercle. It is worth noting that in the anosmatic porpoise the septal structures are certainly not retrogressive (Breathnach, 1953).

Some recent experimental work has thrown light on the connexions and functions of the septum. Daitz & Powell (1954), following fimbrial section in the rat, rabbit and monkey, report complete cell loss in all the cells of the medial septal nucleus and partial cell degeneration in the nucleus of diagonal band suggesting the presence of many septo-hippocampal fibres. In degeneration studies in the cat Nauta (1958) describes septal fibres joining the precommissural

fornix and the medial fore-brain bundle which distributes to the entire lateral hypothalamic area. There are rich connexions between the septum and amygdala via the stria terminalis dorsally and the ventral amygdalo-fugal fibre system (see below). These structural relationships between the septum, hippocampus, amygdala and hypothalamus are leading many authors to suggest the possibility of a close functional relationship.

Stria terminalis and other amygdaloid connexions

The question of secondary olfactory connexions to the amygdala has already been discussed.

The stria terminalis includes fibres which connect the amygdala with the pre-optic and hypothalamic regions and probably also the septum. Transverse sections in the middle of the amygdala show the stria in the medial wall of the hemisphere as a lightly myelinated band. The various components converge on the caudo-ventral border of the internal capsule and from here swing caudally to reach its dorsal aspect. They then continue forward in the floor of the lateral ventricle to turn medially and ventrally in the plane of the anterior commissure and break up into the various components. Detailed description in mammals has been given by Johnston (1923) and Van der Sprenkel (1926). Johnston identified five components in the opossum: commissural, hypothalamic, infracommissural, supracommissural and a stria medullaris component. Similar components have been described by Gurdjian (1925, 1928) for the

rat, by Young (1936) for the rabbit, and by Humphrey (1936) for the bat. The hypothalamic component of Johnston is homologous with the pre-optic component of Gurdjian, Young and Humphrey and all these authors describe it as arising entirely in the cortico-medial group of nuclei. The commissural bundle is unusually distinct in the bat and arises entirely in the nucleus of the lateral olfactory tract (Humphrey, 1936). More recently Valverde (1956⁶³a) studied the termination of the stria terminalis in the mouse by means of the Golgi method; his results were in agreement with previous studies and he was able to identify the 5 components described by Johnston.

Klingler & Gloor (1960) studied the connexions of the amygdala in human brains with a dissection technique. They say that the amygdala projects to the septum and to the anterior hypothalamus via two fibre systems: (i) a dorsal system, the stria terminalis, (ii) a larger ventral system consisting of an amygdalo-hypothalamic tract and an amygdalo-septal tract (diagonal band of Broca). These ventral tracts are probably similar to the 'longitudinal association bundle' so named by Johnston (1923) and Fox (1940).

Although much is known concerning the composition of the stria, the direction of conduction in its fibres is somewhat uncertain. Most authors agree that it is a major discharge path from the amygdala but there is evidence that some of its fibres are amygdalo-petal (Shute & Lewis, 1963; Powell et al., 1963). Lesions placed dorsally in its course

led to Marchi degeneration directed forward from the lesion and never caudally to the amygdala (Fox, 1943). Fox thinks that a large portion of the stria originates in the basal nucleus and projects to the septum, pre-optic region and vicinity of the paraventricular nucleus of the hypothalamus. Tadayasu et al. (1959) also using the Marchi technique, studied the fibre connexions in the rabbit and found that the most easily traceable descending pathways from the amygdala are the stria terminalis and the 'longitudinal association bundle'. They believe that fibres of the stria originate from nearly all the amygdaloid nuclei except the lateral. The 'longitudinal association bundle' they showed to originate from the magno-cellular parts of both lateral and basal amygdaloid nuclei, and to pass forward to reach the anterior amygdaloid area and nucleus accumbens.

Nauta (1961), using the Nauta-Gygax method, confirmed the existence of two major fibre systems; a diffuse ventral amygdalo-fugal pathway and a compact stria terminalis dorsally. The former he thinks is the more massive projective system and it connects with the lateral pre-optic and hypothalamic regions, the nucleus of the diagonal band, and the olfactory tubercle. A prominent component also terminates in part of the dorso-medial thalamic nucleus, and another in the gyrus fornicatus. The stria terminalis, on the other hand, originates mostly in the caudal half of the amygdaloid complex and forms a prominent pre-optic-hypothalamic component.

Wlverde (1963b) obtained similar results. Nauta also found evidence of fibres emerging through the lateral and ventral sides of the amygdala and distributing to the rostral part of the temporal gyri, insula and caudal orbito-frontal cortex. The existence of such amygdalo-temporal fibres and amygdalo-insular fascicles have been seen by dissection in man (Klingler & Gloor, 1960).

It is of importance to note that Powell et al. (1963) found after lesions of the piriform cortex very similar results to those described by Nauta after lesions of the amygdala. They have stressed the fact that their lesions never penetrated deeper than the subcortical white matter, and they were very careful not to injure the amygdala. When they placed lesions in the amygdala itself, the degenerating fibres in the ventral amygdalo-fugal pathway (longitudinal association bundle) were exactly similar to those after lesions in the piriform cortex. Accordingly it was difficult to determine whether this degeneration was entirely due to interruption of fibres arising in the piriform cortex and passing through the amygdala, or whether the amygdala also contributed to this system. These authors produced experimental anatomical evidence for a reciprocal relationship between the amygdaloid region and the hypothalamus. Caudally directed fibres were not only seen in the stria terminalis (as described by Nauta, 1958 and by Shute & Lewis, 1963), but also in the ventral amygdalo-fugal pathway.

Many reports have appeared lately in the electrophysiological literature concerning the connexions of

the amygdala. These are very varied and cannot be described fully within the limits of this thesis. Various behavioural and visceral effects have been obtained from stimulation of the amygdala (for summary see Gloor, 1960). Physiologically it appears that two different portions can be distinguished. For example Kaada (1951) and Kaada, Anderson & Jansen (1954) distinguish an anteromedial division (phylogenetically old) which responds with autonomic and somatomotor effects to stimulation, and a lateral division (phylogenetically younger) which on stimulation yields behavioural changes which indicate anxiety, sometimes fear and anger. They are similar to effects obtained by stimulating the hippocampus, the medial prefrontal, limbic and hippocampal gyral regions. The authors think that these similarities indicate functional as well as structural relationships between all these areas. Other behavioural changes which follow lesions restricted to the amygdala are relative docility and a state of chronic hypersexuality (Shreiner & Kling, 1956). Finally it is of interest to mention that in the surgical treatment of temporal lobe seizures Baldwin et al. (1956) routinely explored the medial temporal region with a gentle electrical current. Quite frequently this stimulation was followed by movements of the ipsilateral face and jaws. These motor responses occurring when the tip of the electrode is in or very near to the amygdala.

Entorhinal connexions

Recent experimental observations show that the entorhinal

area receives tertiary olfactory connexions from the piriform cortex (Cragg, 1961; Powell et al., 1963). The entorhinal area has many connexions with other near-by structures, particularly with the hippocampus. In normal material Cajal (1911) described two distinct afferent pathways to the hippocampus; the large perforant, or direct temporo-ammonic tract arising from the lateral part, and a superficial, or alvear, pathway originating from the medial part. These tracts received full description in the elaborate study by Lorente de No (1934a) and their presence has recently been confirmed in experimental animals (Adey & Meyer, 1952). The last authors have also shown that following an extensive lesion of the superior temporal gyrus and insular cortex in the monkey, considerable fibre degeneration could be seen entering the deep layers of the entorhinal cortex.

EXPERIMENTAL OBSERVATIONS IN THE COYPU

The experiments to be reported were all designed to demonstrate by the method of experimental degeneration secondary connexions of the olfactory bulb, accessory olfactory bulb and anterior olfactory nucleus and with particular reference to the olfactory tracts and to the olfactory commissural component.

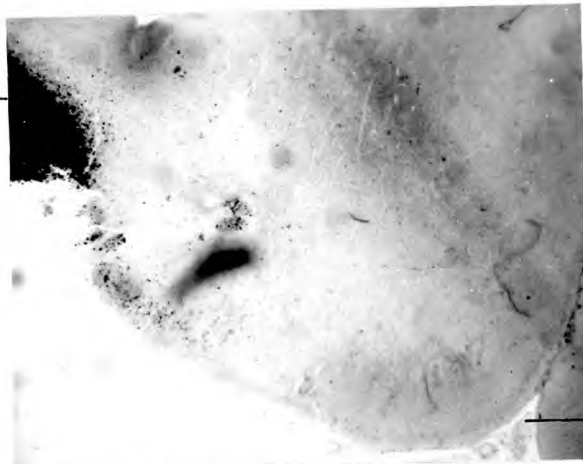
Criteria of degeneration

The Marchi technique was used only in two specimens. It has well known limitations. In the first place it stains only the degeneration products of myelin and cannot therefore demonstrate preterminal or terminal degeneration where no or very little myelin is present. Non-myelinated fibres are not stained anywhere on their course. Pseudo-reactions, i.e. diffuse staining of lipoidal droplets having no relation to the distribution of the fibres injured by the lesion, are common, and may lead to difficulties in interpretation. In the two coypu specimens pseudo-reactions are not in fact very marked and the degeneration of myelinated fibres in major tracts, such as the lateral olfactory tract, is clearly demonstrated (Fig. 77). Degeneration in fine myelinated fibres, probably approaching their termination, is not shown satisfactorily however. Such fibres are present in the 1st or molecular layer of the piriform cortex and degeneration in this situation is much better shown by the Nauta technique. This can be seen by comparing the two photographs (Figs. 53 and 54) of sections taken from the same level of specimens having similar lesions and survival times,

stained by the Marchi and Nauta techniques respectively. Since the Nauta technique is almost as satisfactory for demonstrating degeneration in the major tracts and the main purpose of this work is to demonstrate the sites of termination of fibres, no further use was made of the Marchi method.

The Glees method (Glees, 1946) has been claimed to be the most satisfactory for demonstrating degeneration in terminal arborisations and end-bulbs (see Evans & Hamlyn, 1956, who compared it with the Nauta method) but on this material the results were not very successful. Some preliminary observations were made in sections of the spinal cord in regions in which a previous study (Goldby & Kacker, 1963) had shown, by the Nauta method, that terminations of the cortico-spinal tract are numerous. The Glees method stained only an occasional end-bulb in these situations. In the basal rhinencephalon of two animals which had survived bulbar lesions for 7 and 14 days respectively the Glees method demonstrated fragmentation of fibres in the outer or molecular layer of the piriform cortex and the cortical amygdaloid nucleus, but it was not seen so clearly as in the corresponding Nauta preparations. No end-bulbs, normal or degenerate, could be found in any part of the basal rhinencephalon. Whether, in our hands, the technique has failed, or whether it is unsuitable for this material, cannot be decided definitely. Further experience might have led to better results. Other workers however have found the Glees method unsatisfactory. Cowan & Powell (1956) in normal

Tr. Olf. Lat.



Fs. Rh.

Fig. 53. A transverse section (from specimen M.1) stained by the Marchi technique to show degeneration in the lateral olfactory tract after bulbar lesion. Note the limited lateral spread which reaches less than half the way across the frontal piriform cortex. X 55. (compare with Fig. 54)



Fs. Rh.

Fig. 54

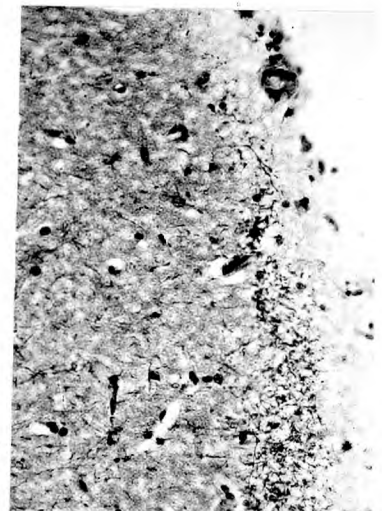


Fig. 55

Fig. 54. A transverse section (from specimen 172, section C) stained by the Nauta method to show the degeneration in the lateral olfactory tract spreading as far laterally as the rhinal fissure. X 55. (Extent of lesion and survival time is similar to that of M.1.)

Fig. 55. Higher power view (X 140) to show the degeneration in the bottom of the rhinal fissure. Nauta stain.

monkey and human brains found that it gave evidence of terminal degeneration (assumed to be an artefact or pseudo-reaction) bilaterally not only in parts of the hypothalamus but also in regions such as the septum and the bed nucleus of the stria terminalis which are of importance for this thesis. It is true that Adey, Rudolph, Hine & Harritt (1958) failed to confirm Cowan & Powell's observations. Nevertheless one must at least conclude that the technique gives inconsistent results in different hands. It is also true that the more controversial of the findings by this method which have been reported in the rhinencephalon are bilateral (e.g. Clark & Meyer, 1947) so that the best form of control, comparison with the normal side in a single preparation, was impossible. For these reasons, and because of the disappointing results we obtained ourselves, the method was not used further.

The Nauta technique was developed to bring out the pathological or degenerative change in axons more or less selectively and to suppress the staining of normal fibres (Nauta & Gyax, 1954; Glees & Nauta, 1955; Nauta, 1957). This suppression is rarely complete. Nauta (1957) states that "it is common to find a variable percentage of the normal fibres impregnated black" and this is certainly our experience in the coypu. Degenerative debris is always black, but so are a large number of the finer normal fibres. Coarser normal fibres are generally a brown or golden colour. The most complete suppression was achieved in a specimen which had had very

prolonged fixation in formalin (18 months) before processing (Fig. 56). Nauta (1957) says that excessive length of fixation may be detrimental but there is no evidence that staining in any of the degenerated fibres has been lost in this specimen. It must be emphasised however that periods longer than 18 months could be detrimental (as has been shown in one of our specimens which was fixed for 24 months). Most of the specimens were processed after 2 to 6 weeks fixation and gave reasonably satisfactory results, and a fair degree of suppression of the staining of normal fibres was achieved by increasing the proportion of ammonia in the ammoniacal silver nitrate solution (see Appendix II).

Normal fibres can be recognised by their generally uniform calibre and lack of fragmentation (Fig. 58). Earlier investigators (e.g. Cajal, Glees) working on silver preparations stated that the first signs of axonal degeneration are argentophilia and varicosities, but these alone are not dependable criteria in Nauta preparations. In longitudinal sections of the olfactory bulb and peduncle from a completely normal brain intense argentophilia and varicosities are a common feature of the fine fibres of the olfactory commissural component (Fig. 60). The only dependable criterion is drop-like disintegration of the axons (Figs. 57, 59 and 63). Mere irregularity in calibre or beading without definite fragmentation cannot be taken as conclusive evidence of degeneration in the coypu olfactory system. Glees & Nauta

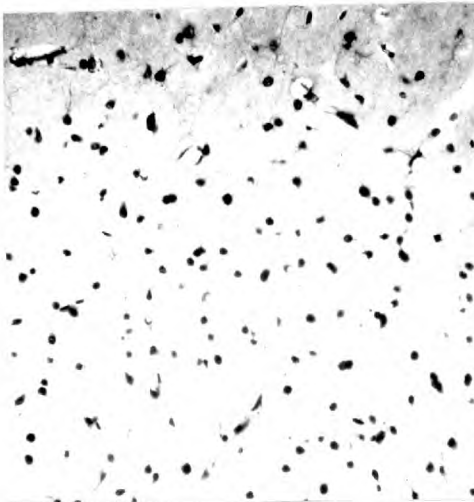


Fig. 56. A transverse section of the normal lateral olfactory tract stained by Nauta method after 18 months fixation showing complete suppression of normal fibres. X 140 (M.1, section 3)

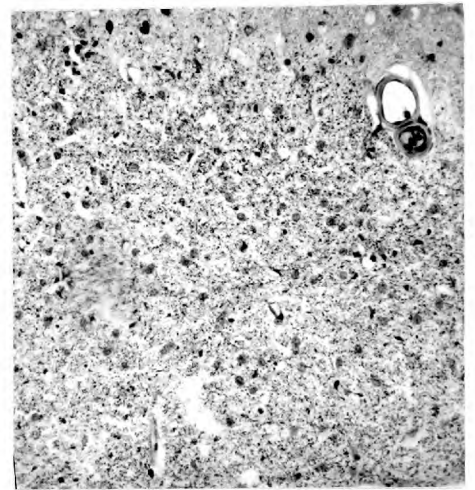


Fig. 57. The degenerating olfactory tract from the same section as in Fig. 56 for comparison. Nauta method. X 140.

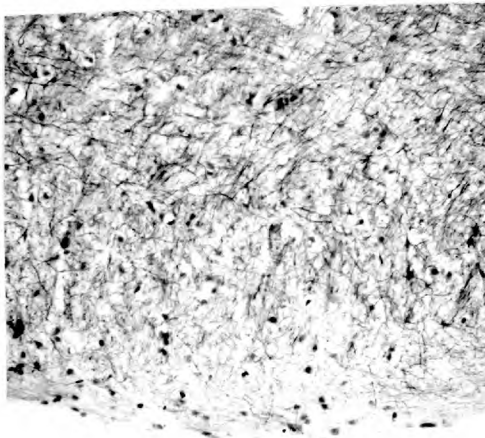


Fig. 58. A transverse section of the normal olfactory tract stained by Nauta method after 3 months fixation showing only very slight suppression of normal fibres. Compare with Fig. 56. X 140 (174, section 2)

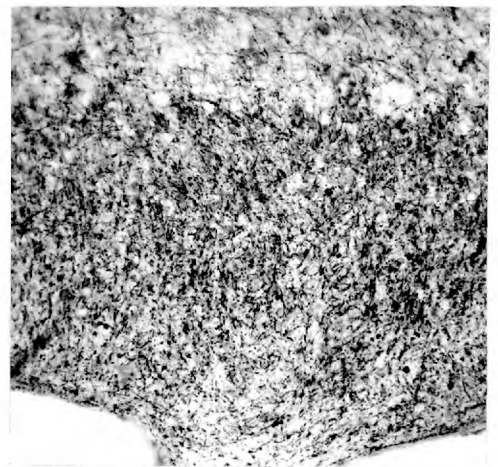


Fig. 59. The degenerating olfactory tract from the same section as in Fig. 58 for comparison. Nauta method. X 140.

(1955) have drawn attention to the fact that "the most important and persistent source of error is represented by artificial deformities of normal axons, a phenomenon which was rather frequently observed following silver impregnation". In our experience artefacts or pseudo-reactions with an apparent fragmentation of fibres, in regions which could not have been affected by the lesion are also found occasionally. They were seen for example in the optic chiasma and tracts where they are possibly the result of manipulation during the removal of the brain. These artefacts are generally coarse in appearance (Fig. 62) and unlike the typical drop-like fragmentation of true degeneration (Fig. 63) but because of the difficulty of recognising their rigorous control by comparison with the normal side or with sections from a completely normal brain is always necessary.

In addition to degeneration in the course of a fibre tract (where the debris of both axon and myelin sheath may be stained (Evans & Hamlyn, 1956)) the Nauta method will also demonstrate degeneration in fibres very close to their termination. This "pre-terminal" degeneration takes the form of very fine argentophilic granules close to the perikaryon of a cell or to its dendrites. The actual end-bulbs are not usually demonstrated (Glees & Nauta, 1955; Bowsher, Brodal & Walberge, 1960) and were certainly not seen in this material. What has been interpreted as preterminal degeneration however is seen in the nucleus of the lateral olfactory tract and

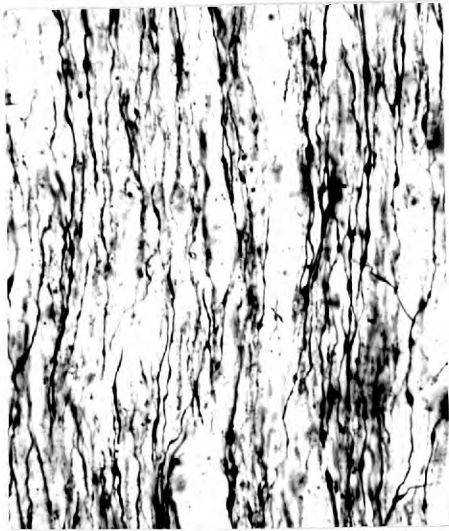


Fig. 60. A longitudinal section of the olfactory peduncle of a normal brain stained by the Nauta method showing some varicosities and deformities. X 360. (section A₁-9)



Fig. 61. True degeneration in the olfactory commissural component just caudal to the lesion (M.2) at the same level as that shown in Fig. 60 (for comparison). Nauta stain. X 360.

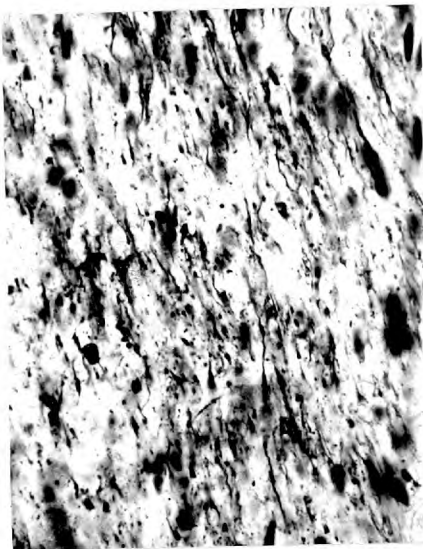


Fig. 62. A longitudinal section of the optic tract stained by Nauta method showing pseudo-reaction. Note the coarse fragments and compare with Fig. 63. X 360. (M.11, E4).



Fig. 63. Photomicrograph of the lateral olfactory tract stained by Nauta method showing true degeneration. Note the fine drop-like disintegration. X 360 (M.2, D2).

anterior amygdaloid area (Figs. 64 and 65) where it appears to indicate axosomatic endings, and in the molecular layers of cortical formations (Fig. 80) and the granular layer of the olfactory bulb when endings are probably axo-dendritic.

Because of the granular nature of the degeneration products these may simulate a normal fibre in a thin transverse section. Conversely a thin section of a normal fibre may look very like a degeneration granule. Most sections were cut at 15-25 μ and at this thickness the presence or absence of fragmented fibres can usually be judged with reasonable confidence. In one specimen (M.8) such sections showed unequivocal fragmentation in transverse sections of the olfactory commissural component which was practically unrecognisable in some 8 μ sections from the same specimen cut in a cryostat. This difficulty does not arise in longitudinal sections where considerable lengths of the fibres can be seen.

The rate of development of recognisable degeneration probably varies in different fibre tracts and in different animals. According to Crevel & Verhaart (1963) even apparently identical fibres in a single tract may show very great differences in the time required for degeneration, some being resistant for as long as 6 months. Goldby & Kacker (1963) using the Nauta technique in the coypu thought that they could demonstrate degeneration of all the fibres in the pyramidal tract after 3 weeks but shorter survival periods (7 to 14 days) are often used with the Nauta technique. In

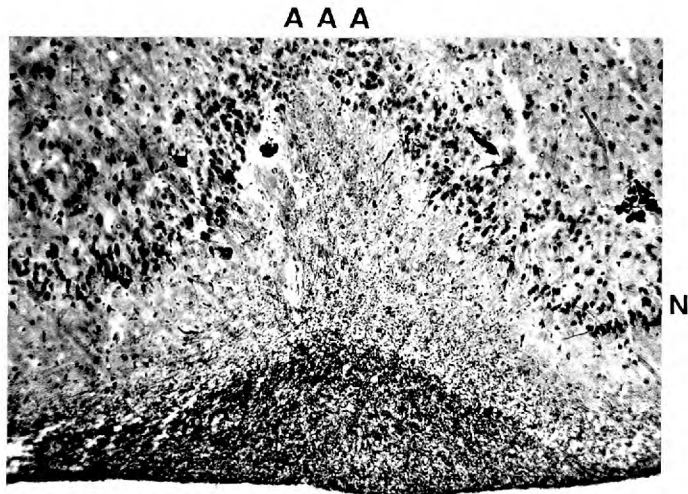


Fig. 64. A transverse section stained by the Nauta method to show degenerating fibres passing deeply from the lateral olfactory tract to reach the cells of the anterior amygdaloid area (AAA) and the nucleus of the lateral olfactory tract (N). X 55 (specimen 176, section 4).

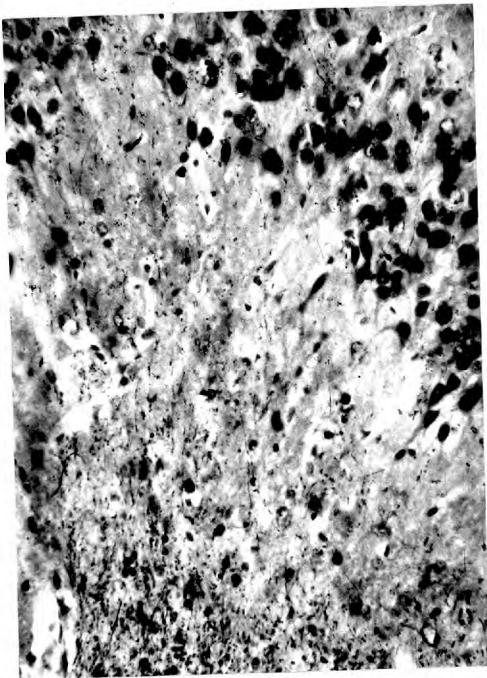


Fig. 65. Higher power view (X 140) to show the degenerating fibres reaching the perikarya of the cells of the anterior amygdaloid area. Nauta stain.

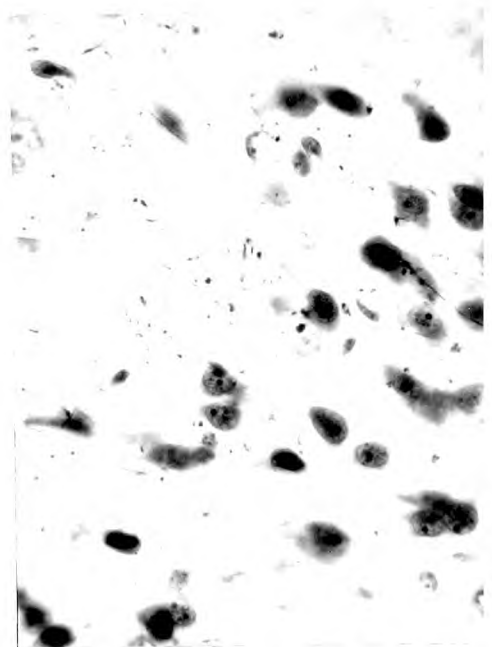


Fig. 66. Higher power view of the nucleus of the lateral olfactory tract stained by Nauta method showing the axosomatic type of synapsis. X 360.

one of our specimens degeneration could be seen clearly over the piriform cortex after 7 days (Fig. 67). It was more marked in a 14 day specimen, but we have taken 3 weeks as being optimal for the demonstration of degeneration (Fig. 68). After 3 weeks there is no evidence for the removal of degeneration debris and in one specimen with 56 days survival much debris is still present although a little seemed to have disappeared (Fig. 69).

In interpreting histological evidence of degeneration it is always necessary to consider whether the changes seen are orthograde or retrograde. Retrograde degeneration is generally assumed to develop more slowly than orthograde, but Cragg (1962) has interpreted degeneration in the lateral geniculate body of a rabbit as early as 8 days after a cortical lesion, as being retrograde in character. Powell & Cowan (1964), also using the Nauta method, found that after lesions of the cingular cortex, typical orthograde preterminal degeneration is seen after 7 days survival but that after 10-21 days a distinctly different granularity is seen. It lacks a pericellular arrangement and appears to be related to the break-up of cell-bodies and dendrites because it is found only in areas showing retrograde cell degeneration in Nissl preparations. Because of the rather long survival periods, some of the degeneration seen in the coypu brain may have been retrograde, but this is unlikely. In specimen M.9 the olfactory peduncle had been severed caudal to the anterior olfactory nucleus (see Fig. 73). Sagittal sections of the



Fig. 67. A transverse section at the level of the temporal piriform cortex showing only very faint staining of the degenerating lateral olfactory tract fibres 7 days after bulbar lesions. Nauta method. X 140 (M.11, section E4).



Fig. 68. A transverse section showing intense staining of the degenerating fibres 21 days after bulbar lesions. Section at level of temporal piriform cortex from specimen M.2 section D1. Nauta stain. X 140.

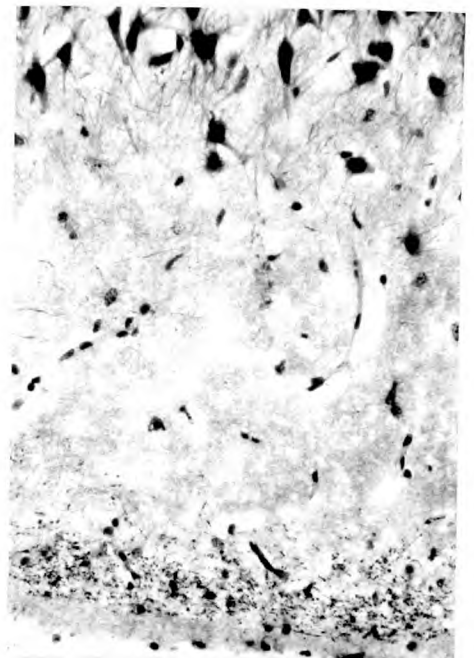


Fig. 69. A transverse section of the temporal piriform cortex to demonstrate the persistence of the degenerating debris even after 56 days survival. Nauta stain. X 140.

bulb and peduncle show only very few (if any) fragmented fibres confined to the deeper part of the lateral olfactory tract rostral to the lesion and this was after 3 weeks survival. It was also noted that in specimens with complete division of the anterior commissure never more than about half of the fibres show degeneration on each side of the lesion, and one of these specimens had survived 43 days. For this reason it has been assumed that all degeneration seen, whether in the lateral olfactory tract or elsewhere, is orthograde although it must be admitted that this is not certain in all situations.

Degeneration after lesion of the olfactory bulbs

The results will be reported for groups of specimens defined by the situation and extent of the lesion (see p.11) which is always on the left side. Operative details and details of the histological methods are given in Appendices I and II.

The first group of specimens with lesions in the olfactory bulb has been divided into those with lesions confined to the main bulb (6 specimens) and those with lesions involving both main and accessory bulbs (2 specimens). The extent of the lesions is shown in Figs. 75 and 82 and has been estimated partly on the basis of macroscopic examination and partly from sections. The estimates are approximate only because of the difficulty of excluding the possibility that additional damage was caused in removing the bulbs from the skull. One specimen (170) is also illustrated by a photograph (Fig. 70).

The second group with lesions involving the peduncle and anterior olfactory nucleus are illustrated in Figs. 83, 84 and 85 including photographs in four cases (Figs. 71, 72, 73 and 74).

The third group with lesions of the anterior commissure is illustrated only by the photograph (Fig. 101) from one of the specimens, M.14. It shows the general type of the lesion, an incision through the whole depth of the hemisphere slightly lateral to the median plane. Two of the remaining specimens in this group are similar and in the last two the incision is in the median plane.

Further details of the lesions are given in the description of the specimens.

Lesions confined to the main bulb are all incomplete because attempts to remove the whole bulb inevitably led to damage of the accessory bulb and/or the rostral extremity of the anterior olfactory nucleus. Two specimens (M.1 and 173) were stained mainly by the Marchi technique but also with some Nauta sections, and four (170, 171, 172 and M.12) were prepared by the Nauta method only.

The two Marchi specimens will be described first. In specimen 173^(Fig. 75) only about $\frac{1}{2}$ of the anterior part of the main bulb is destroyed, and it is only in this specimen that sections of the remaining part of the bulb and the peduncle are available.

In the bulb Marchi staining is present among the periglomerular cells. This could be a pseudo-reaction in



Fig. 70 (specimen 170)



Fig. 71 (Specimen M.8)



Fig. 72 (specimen M.15)

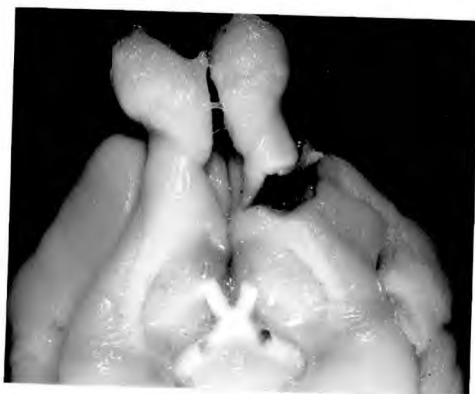


Fig. 73 (specimen M.9)



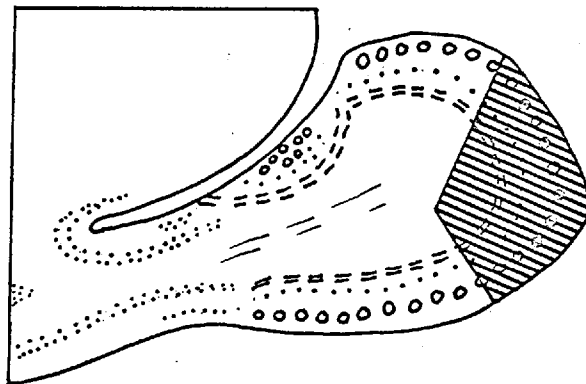
Fig. 74 (specimen M.4)

Figs. 70, 71, 72, 73 & 74 are photographs of the coypu brain (X 2) showing the extent of the surgical lesion in some of the experimental specimens. Fig. 70 is representative of a lesion which is strictly confined to the bulb. All the other 4 figures demonstrate different lesions of the anterior olfactory nucleus.

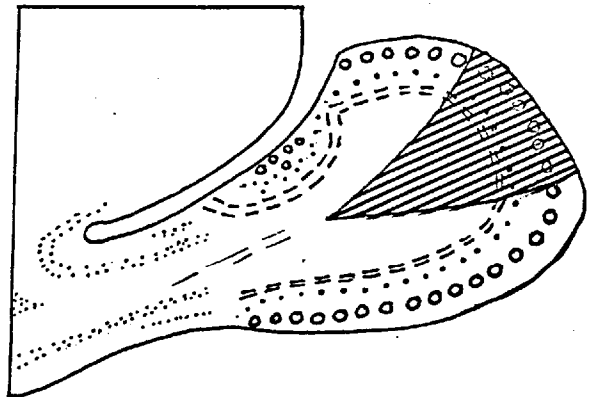
a region inevitably damaged in removing the brain. It could however, represent degeneration in bulbo-petal fibres, a matter which will be discussed later. Degeneration is marked among the cells of the inner granular layer and can be traced beneath the accessory bulb into the peduncle (Fig. 76). In the peduncle the degenerating fibres are concentrated ventrally and laterally in the molecular layer on the anterior olfactory nucleus, but a few are present, more deeply situated, ventro-medially. The latter could not be traced beyond the anterior olfactory nucleus. Superficial degeneration in this situation which could represent a medial olfactory tract is not present.

Caudal to the peduncle there is marked degeneration in the lateral olfactory tract (Fig. 77) which is lost just rostral to the amygdala. Laterally degeneration spreads from the tract into the superficial part of the molecular layer of the piriform cortex but reaches only about half way towards the rhinal fissure. There is a similar spread medially for a short distance into the antero-lateral part of the olfactory tubercle. In specimen M.1 the lateral olfactory tract degeneration is similar but rather more intense. In neither specimen could degenerated fibres be traced with confidence into the amygdala.

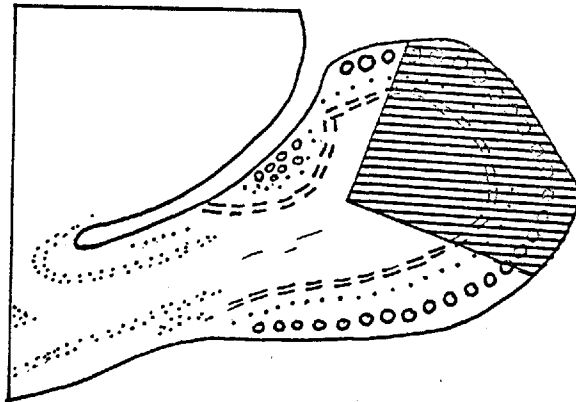
The commissural component in both specimens shows slight Marchi stippling near the bulb; probably a pseudo-reaction because more caudally and in the commissure itself practically no staining can be detected and certainly no more than is seen in the adjacent white matter of the frontal pole, which is



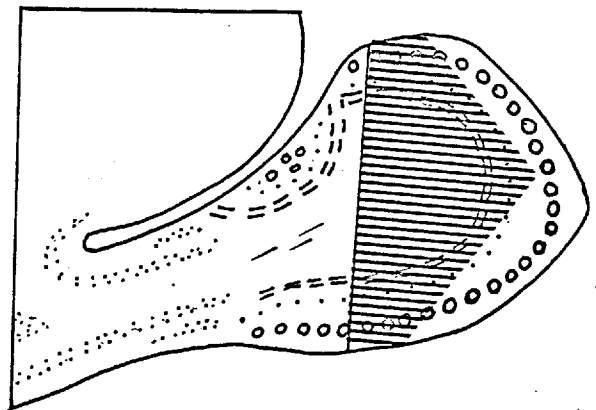
M 12 (14)



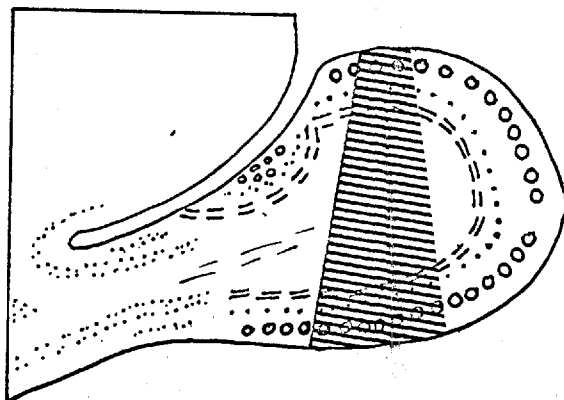
171 (14)



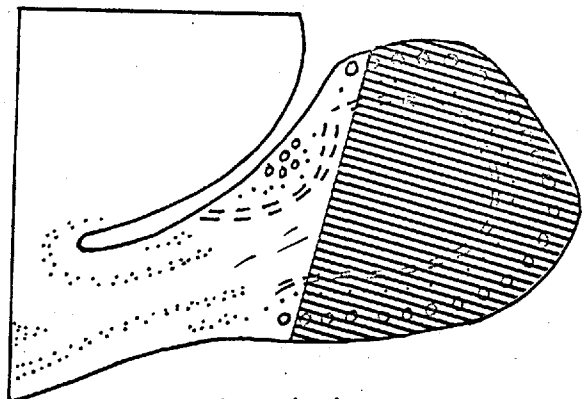
173 (28)



M 1 (21)



172 (21)



170 (10)

Fig. 75. Diagrams showing the different bulbar lesions drawn on a sagittal view of the bulb and peduncle. The specimen numbers followed by the number of days survival between brackets.

unaffected by the lesion.

In specimen 173 the right bulb (normal) was also examined but no satisfactory evidence of degeneration was seen.

Nauta sections are available for both these specimens and the superiority of the Nauta technique for demonstrating degeneration of fine fibres over the piriform cortex has already been referred to. The method shows quite clearly that these fibres extend to the rhinal fissure, where they end abruptly. Degeneration could also be traced further caudally in the molecular layer of the temporal piriform cortex and along the lateral border of the amygdaloid fissure, although none could be traced medially to the cortical amygdaloid or deeper amygdaloid nuclei. Over the temporal piriform cortex degeneration is most marked close to the lip of the rhinal fissure and a few fibres can be traced into the region of transition to the entorhinal cortex, some of which may reach the entorhinal cortex itself. In the peduncle, the olfactory tubercle and the olfactory commissural component the findings are quite similar to those in the Marchi sections but there is some suggestion of the termination of a few fine fibres in the region of the anterior olfactory nucleus.

The remaining four Nauta specimens show very similar results. In all degeneration in the lateral olfactory tract spreads over the piriform cortex to the bottom of the rhinal fissure laterally, and over about the antero-lateral third of the olfactory tubercle medially. Even when the lesion is small enough to leave a considerable part of the bulb undamaged



Fig. 76. A transverse section of the olfactory bulb just caudal to the lesion (specimen 173). Degenerating fibres are seen passing in the granular layer of the accessory bulb on their way to the lateral olfactory tract. Marchi stain. X 55.



Fig. 77. A transverse section at the level of the caudal end of the olfactory peduncle from the same specimen as that of Fig. 76 to show Marchi degeneration in the lateral olfactory tract. X 55



Fig. 78. A transverse section showing the normal olfactory tract from the same section as that shown in Fig. 77 for comparison. Marchi stain. X 55.

(M.12 and 171) degeneration can be traced to the anterior amygdaloid area, nucleus of the lateral olfactory tract and the antero-lateral border of the cortical amygdaloid nucleus (Figs. 80 and 81). It can also be traced over the cortical amygdaloid transition area to a concentration lateral to the bottom of the amygdaloid fissure. No degeneration is found in any of the other amygdaloid nuclei. In the piriform cortex (frontal and temporal) degeneration does not differ from that described in the first two specimens.

The olfactory commissural component is completely negative in specimen 172 and almost certainly so in specimen 170 and possibly in M.12. The remaining specimen (171) provides only the same unconvincing evidence of degeneration limited to a very small proportion of the fibres as was seen in the Marchi specimens. It was observed however, that even this faint appearance always disappeared caudally particularly where the olfactory component approaches the mid-line to cross in the anterior commissure. In specimen M.12, with the smallest lesion, degeneration is seen in fibres among the periglomerular cells and in the inner plexiform and granular layers of the undamaged part of the bulb, as in the Marchi preparations. Specimen 170 showed what appeared to be terminal degeneration in tract fibres on the dorsal aspect of the anterior olfactory nucleus (Fig. 79).

One can summarise the results from these specimens, in which the lesions were strictly confined to the main olfactory bulb, as follows. In the bulb secondary olfactory fibres

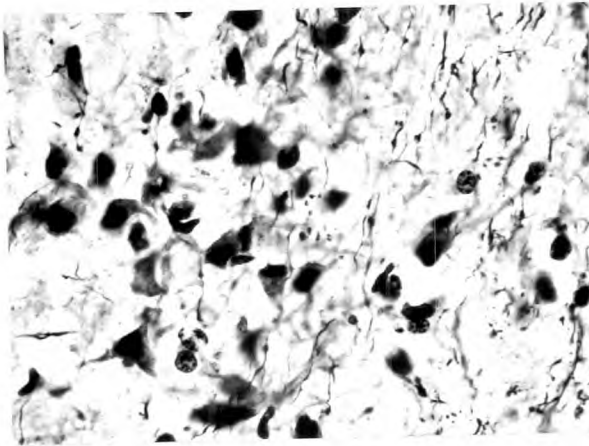


Fig. 79. Photomicrograph of the anterior olfactory nucleus of specimen 170 just caudal to the bulbar lesion to show preterminals of some of the degenerating olfactory tract fibres amongst the cells of this nucleus. Nauta stain. X 360.

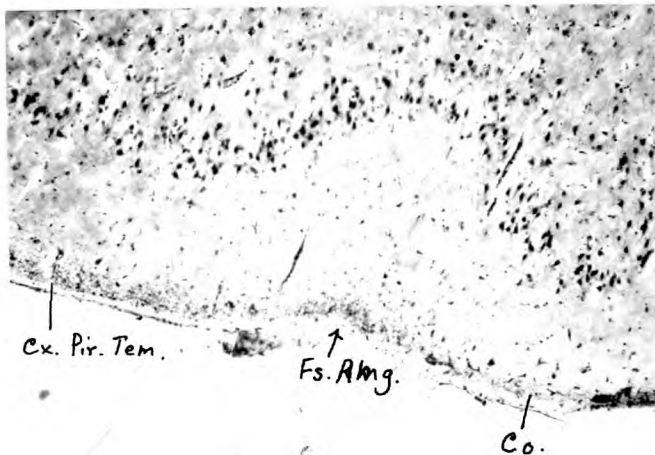


Fig. 80. A transverse section at the level of the most rostral end of the amygdaloid fissure to show the degenerating olfactory tract fibres passing medially in the molecular layer of the cortical amygdaloid nucleus. Nauta stain. X 55 (specimen 171)

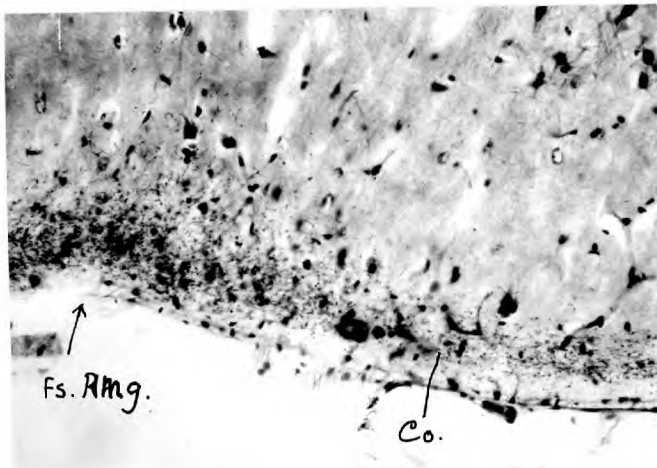


Fig. 81. A higher power view (X 140) of the cortical amygdaloid nucleus. Note the medial spread of fibres in the molecular layer of the cortical nucleus. Nauta stain.

accumulate in the inner plexiform and granular layers and can be traced into the peduncle where they are concentrated laterally and ventrally. Probably some connect with the anterior olfactory nucleus, particularly dorsally. Posterior to the peduncle, the lateral olfactory tract distributes fibres to the outer part of the molecular layer of the antero-lateral region of the olfactory tubercle and throughout the piriform area. In the temporal piriform cortex the fibres tend to be concentrated near the lip of the rhinal fissure, and a few extend into the region of transition to the entorhinal cortex and possibly to a very small extent to the entorhinal cortex itself. They also pass to the nucleus of the lateral olfactory tract (where preterminal degeneration is seen close to the perikarya), to the anterior amygdaloid area, to the cortical amygdaloid transition area and to the antero-lateral border of the cortical amygdaloid nucleus (although this last point is not demonstrated clearly in all the specimens).

It should be noted that except in the nucleus of the lateral olfactory tract the degenerating fibres are all situated very superficially in the outer part of a molecular layer. This suggests that the connexions are all axodendritic, although one cannot exclude the possibility that the finest terminal or preterminal fibres have failed to stain. In the Nauta preparations this is unlikely.

The only other positive finding which needs discussion is the degeneration in the glomerular layer of the bulb in one of

the Marchi and one of the Nauta specimens. The fibres affected appear to be the myelinated fibres seen among the periglomerular cells in normal preparations. It has already been suggested that this finding may be an artefact resulting from damage caused in removing the brain. It is possible, however, as the original lesion in both cases extended into the central parts of the bulb, that these are afferent fibres to the glomeruli degenerating as a result of the lesion. This seems a more probable interpretation in view of some findings which will be reported later in specimens with lesions affecting the peduncle without direct damage to the bulb.

It is important also to emphasise certain negative findings. No medial olfactory tract consisting of secondary olfactory fibres running to the septal region has been found in any specimen. Similarly, no degeneration has been found in any of the amygdaloid nuclei except the nucleus of the lateral olfactory tract, amygdaloid transition area, and the antero-lateral part of the cortical nucleus. The olfactory commissural component is almost completely normal in all the specimens. Very few, if any of its fibres can arise in the main bulb. The slight and rather doubtful degeneration seen in the olfactory component near the bulb in some specimens cannot be detected more posteriorly or in the commissure itself. If these are true degenerating fibres they could represent a very small ipsilateral discharge from the main bulb to the hemispheres but being so few and diffuse they cannot be followed to their terminations in this material.

Attempts to destroy the accessory olfactory bulb without damaging other parts of the olfactory apparatus were unsuccessful. Two specimens were obtained however (M.6 and M.7) in which the accessory bulbs were completely or almost completely destroyed (by thermo-cautery) together with much of the main olfactory bulb, but with no significant damage to the anterior olfactory nucleus. If secondary fibres from the accessory olfactory bulb differ in their distribution from those of the main bulb, one would expect these two specimens to show differences from all those in the group already described, where the lesion is limited to the main bulb.

The approximate extent of the lesion is shown in Fig. 82 and it will be seen that in M.6 a few cells have been lost from the cranial part of the anterior olfactory nucleus. In M.7 the damage to the accessory bulb although extensive, is slightly less than in M.6 and the anterior olfactory nucleus appeared quite unaffected. Both specimens were prepared by the Nauta technique.

No satisfactory sections were available from the olfactory peduncle, but more caudally degeneration in the lateral olfactory tract is well marked and can be traced to the same regions as in those specimens with lesions confined to the main bulb, i.e. to the piriform cortex, the olfactory tubercle, the nucleus of the lateral olfactory tract, anterior amygdaloid area, and the ^{rostral} cranial part of the cortical amygdaloid nucleus. In specimen M.6 an axosomatic type of connexion is clearly demonstrated in the anterior amygdaloid

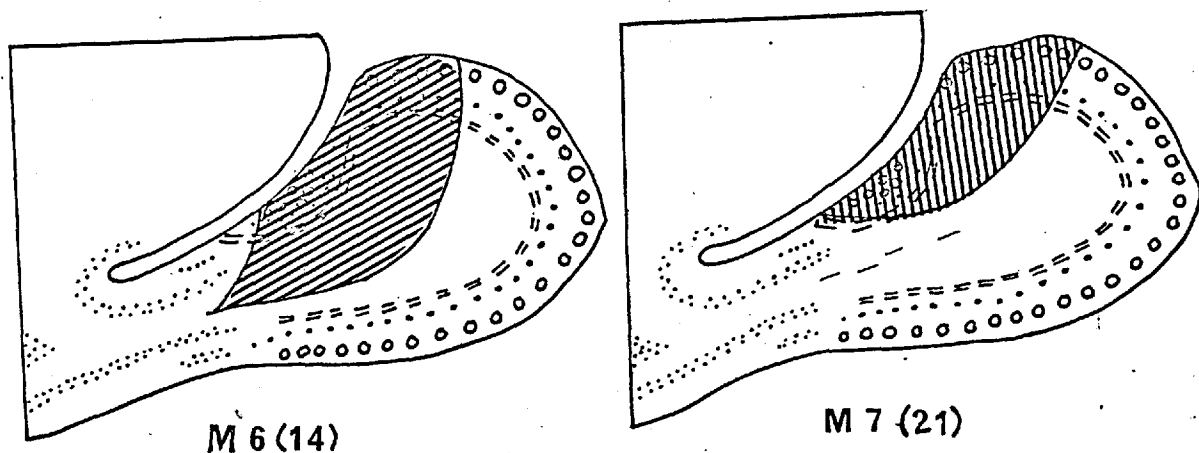


Fig. 82. Diagrams showing the lesion in the main and accessory bulbs drawn on a sagittal view of the bulb and peduncle.

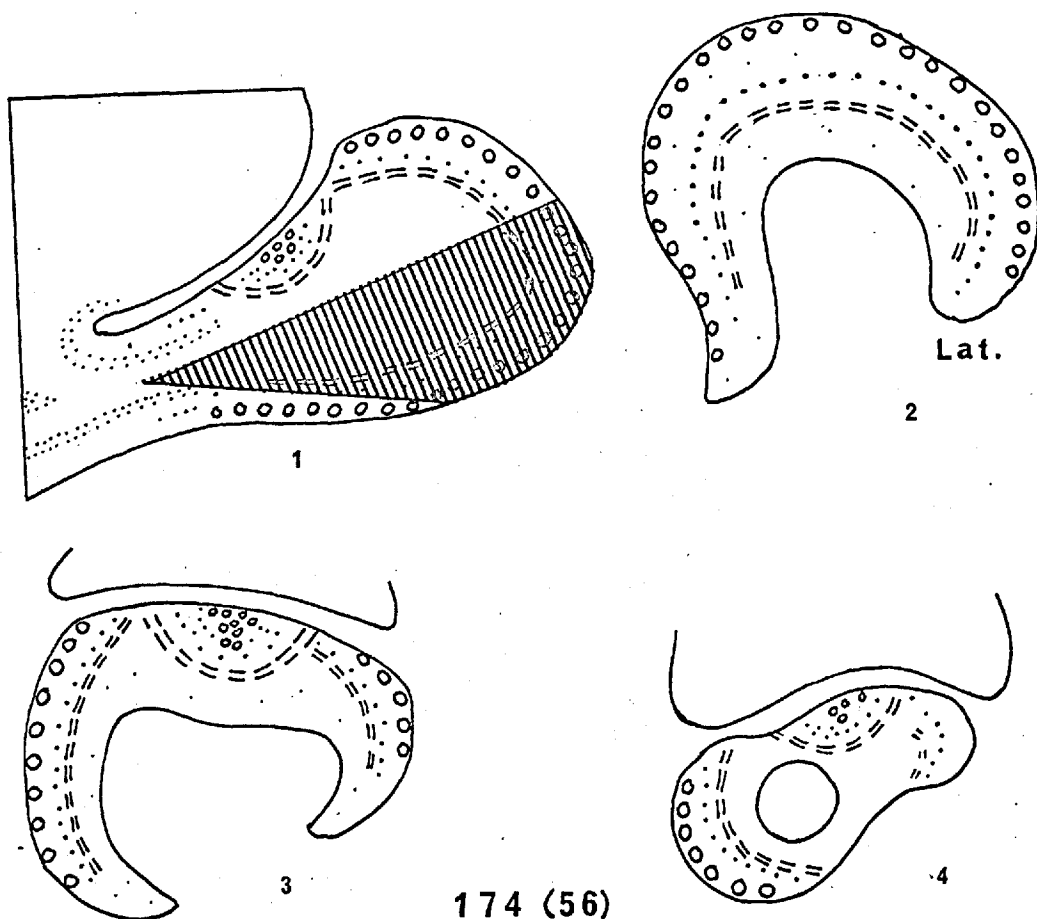


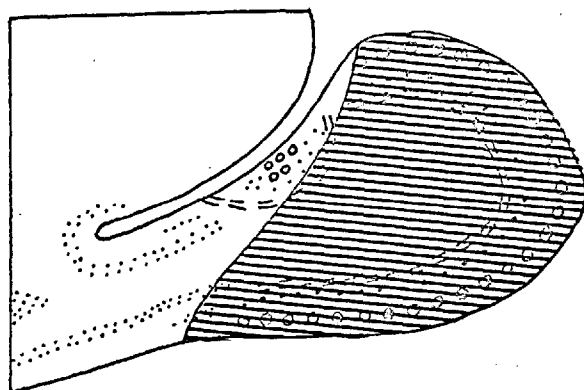
Fig. 83. Diagrams showing the lesion in the ventro-lateral surfaces of the main bulb and the rostral part of the anterior olfactory nucleus of specimen 174.

area and nucleus of the lateral olfactory tract. The olfactory commissural component showed slight and unconvincing evidence of degeneration in a few fibres.

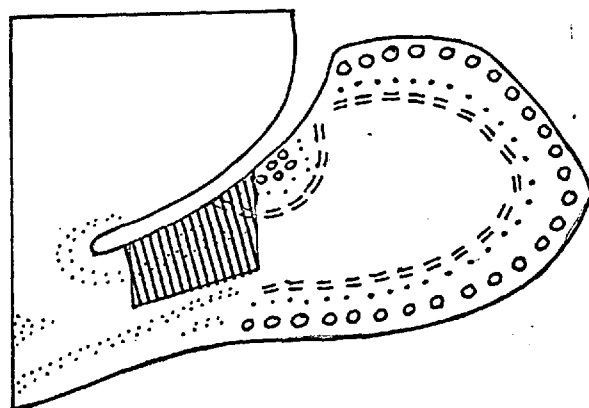
One must conclude that secondary fibres from the accessory olfactory bulb are not distributed outside the regions which also receive secondary fibres from the main bulb. It may be of course that not all of these regions receive fibres from the accessory bulbs, i.e. that their distribution is less extensive than that of fibres from the main bulb. The rather small amount of material examined did not suggest that this was the case, although it did not exclude the possibility.

Lesions involving the anterior olfactory nucleus

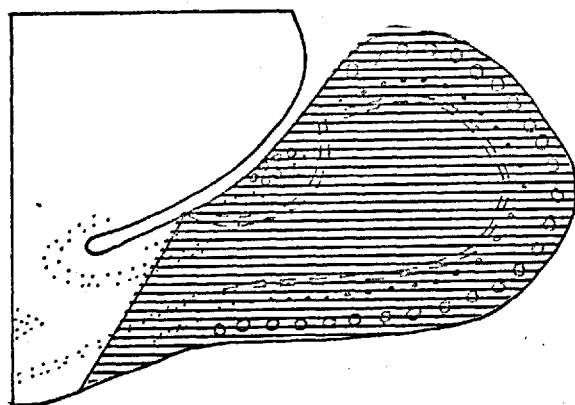
Eight specimens are available in which the lesion ranges from virtually complete destruction of the whole peduncle (specimens M.4 and M.9) to relatively trivial damage either at the cranial extremity of the nucleus or to the core of the nucleus with little direct effect on its cellular layers (specimens 174, M.11 and 176). In M.2 the lesion involved the middle part of the peduncle only. In five specimens the bulb is destroyed or damaged and in all of them the connexions of the bulb are more or less completely divided. In most the anterior pole of the hemisphere is also damaged so that there is degeneration in the commissural and association fibres of this part of the cortex. This is not relevant to the matters under investigation and will not be described in



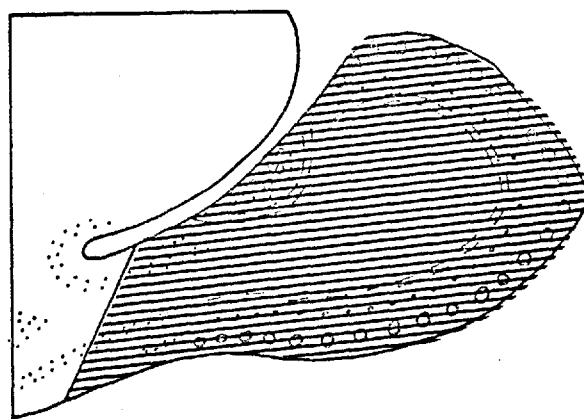
M 11 (7)



176 (14)

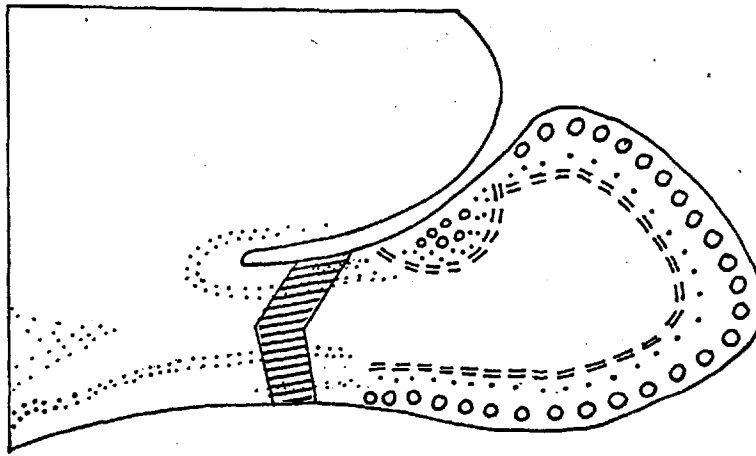


M 15 (12)

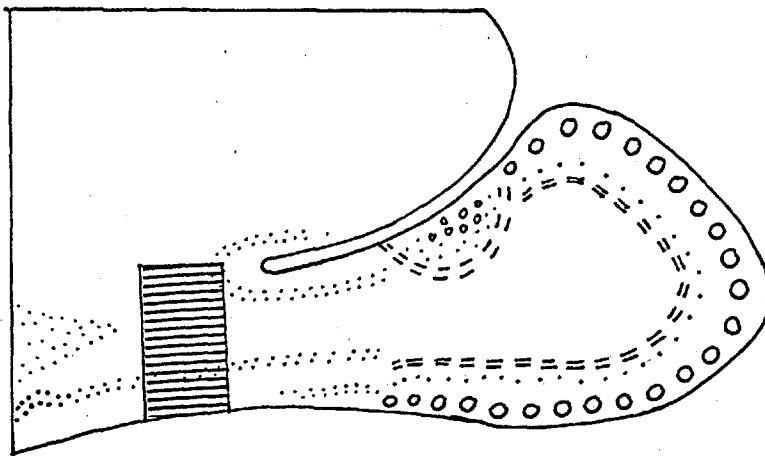


M 8 (21)

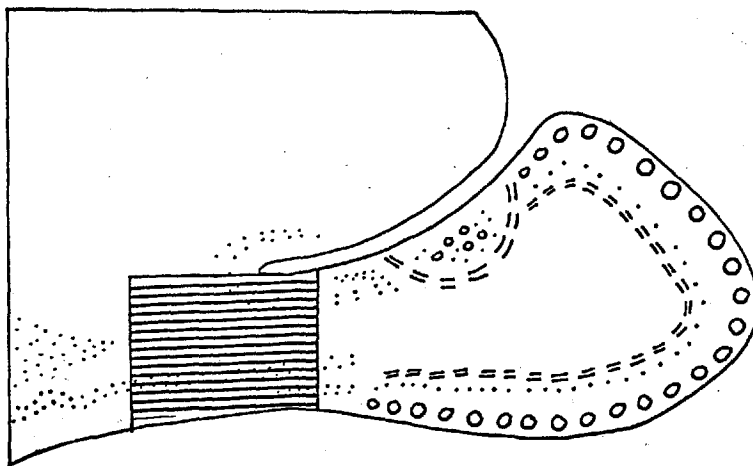
Fig. 84. Diagrams showing the lesion in the different parts of the anterior olfactory nucleus and bulb drawn on a sagittal view of the bulb and peduncle. Photographs of specimens M.8 and M.15 are shown in Figs. 71 and 72 (p.142).



M2 (21)



M9 (21)



M4 (21)

Fig. 85. Diagrams showing the extent of the lesion in the anterior olfactory nucleus. Compare with Figs. 73 and 74.

detail; it has been allowed for in interpreting the results. All these specimens were stained by the Nauta technique.

Owing to damage to the secondary olfactory connexions (see Figs. 83, 84 and 85), degeneration is found in all the basal regions of the hemisphere in which it was found after bulbar lesions only. In this respect there is little significant difference from the specimens previously described. Some (M.9, 176) showed perhaps a more definite spread of degeneration in the molecular layer of the cortical amygdaloid nucleus, but as before, the specimens are not uniform in this respect and variations seem to bear no relation to the extent of involvement of the anterior olfactory nucleus. It was noticed with most of these specimens, particularly M.2, M.4 and M.9, that some preterminal degenerating fibres in the lateral olfactory tract are found close to the perikarya of pyramidal cells in the piriform cortex (Fig. 68) and amygdaloid transition area. With lesions limited to the bulb degenerating fibres are seen only in the plexiform layer of these regions and the synapses formed are presumably axodendritic. The fibres which end more deeply must have degenerated as a result of the lesion in the anterior olfactory nucleus and presumably arose there.

The chief differences from the previous specimens are found in the state of the olfactory commissural component in which, in the specimens where the bulb is undamaged (M.2, M.4, M.9 and 176), degeneration can be studied both rostral and caudal to the lesions. It is only in these specimens that

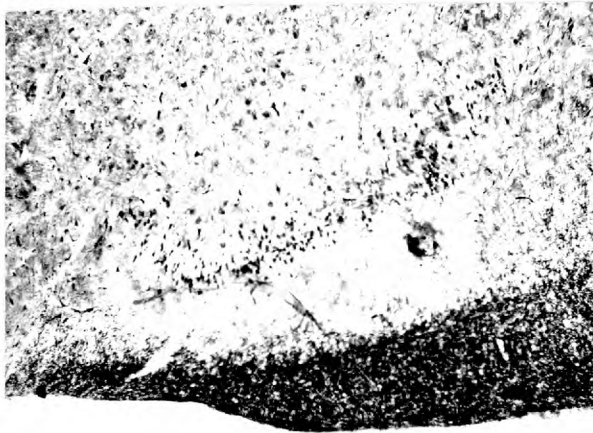


Fig. 86. A transverse section showing intense argentophilia and fragmentation of the degenerating olfactory tract fibres. Note that some degenerating fibres pass deeply to affect axo-somatic contact with the cells of the anterior amygdaloid area. Nauta stain. X 55 (M.8-B)

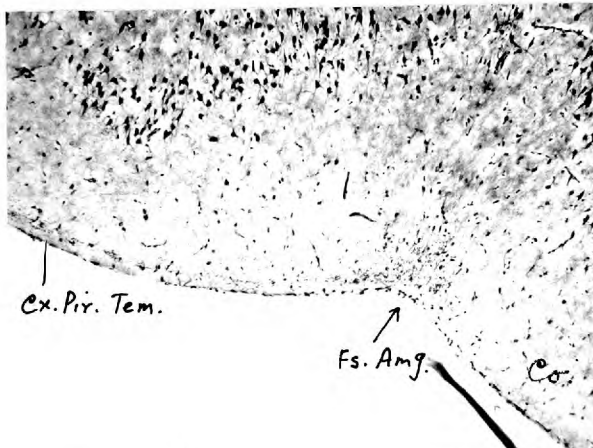


Fig. 87. A transverse section at the level of the middle of the amygdaloid fissure showing the aggregation of degenerating fibres in the fissure. Nauta stain. X 55. (M.8-D)

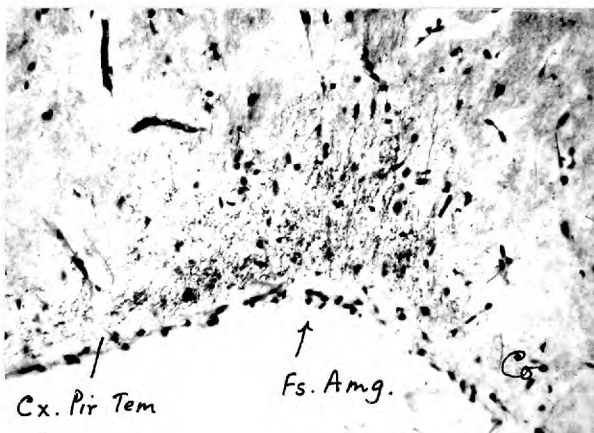


Fig. 88. Higher power view of Fig. 87 to demonstrate the limited medial spread of olfactory tract fibres into the cortical amygdaloid nucleus. Some degenerating fibres pass deeply in the fissure to reach the cell of the amygdaloid transition area. Nauta stain. X 140.

bulbo-petal fibres can be studied on the side of the lesion, and it must be pointed out that these need not necessarily be confined to the olfactory commissural component but could run more superficially in the olfactory tracts. Crossed bulbo-petal fibres can also be studied on the contra-lateral side, but only if they arise in or ^{rostral} cranial to the lesion.

Bulbo-petal fibres are well illustrated in specimen M.9. On the operated side in sagittal sections many degenerated fibres can be followed cranially in the core of the peduncle (Fig. 91) to the inner granular layer of both main and accessory bulbs, some continuing through the plexiform layers to ramify among the periglomerular cells. There are also ^a very few degenerated fibres in the deeper part of the lateral olfactory tract (Fig. 92), so that there are two bulbo-petal pathways, superficial as well as deep. Degenerated fibres are also found among the cells of the pars externa and pars principalis (particularly dorsally) of the anterior olfactory nucleus (Fig. 90) and some of them probably end in these situations.

In the bulb on the unoperated side degenerated fibres are also present in the core of the peduncle and bulb and in the inner granular layer. They are less numerous than on the operated side and none can be traced to the periglomerular cells. These fibres must have arisen in the anterior olfactory nucleus of the opposite side because no degeneration is seen in the bulb after lesions confined to the opposite bulb;

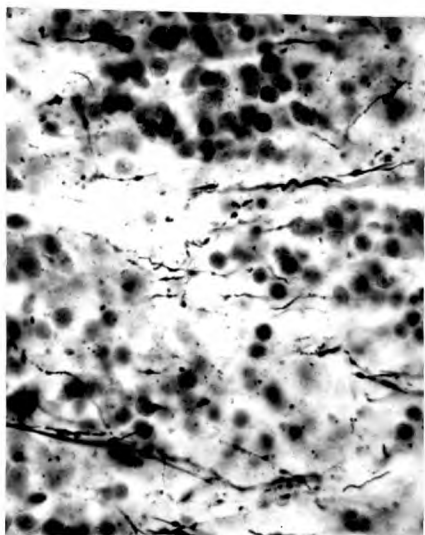


Fig. 89. Photomicrograph showing degeneration in the internal granular layer of the bulb on the ipsilateral side of the lesion (specimen M.9). Nauta stain. X 360.



Fig. 90. Photomicrograph showing intense degeneration dorsally and close to the pars externa of the anterior olfactory nucleus of the ipsilateral side of the lesion (M.9). Nauta stain. X 360.

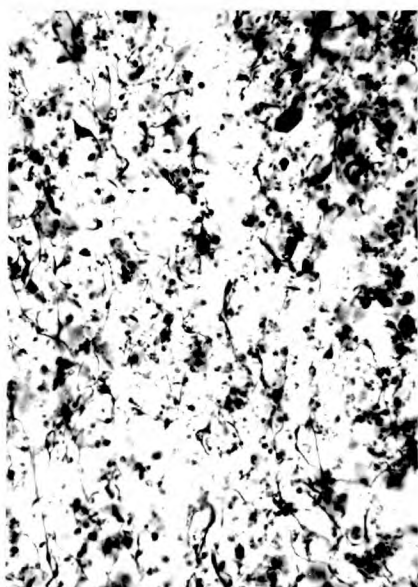


Fig. 91. Photomicrograph showing very intense degeneration in the olfactory commissural component in the core of the peduncle proximal to the lesion (M.9). Nauta stain. X 360.



Fig. 92. Photomicrograph of the lateral olfactory tract from the same section and at the same level of that in Fig. 91 to show the presence of an occasional degenerating fibre amongst a great majority of normal fibres. X 360.

and it can also be said that bulbo-petal fibres cannot come exclusively from the opposite anterior olfactory nucleus. If this were the case the bulbar degeneration should be equal on the two sides, which it is not.

Specimen M.4, with more complete destruction of the anterior olfactory nucleus, shows very similar features. In this specimen however, it is possible to trace degeneration not only to the core of the contra-lateral bulb but to a small extent to the anterior olfactory nucleus and to the granular and glomerular layers of the bulb on that side, although the amount reaching the glomerular layers is very small indeed. In M.2, the findings are practically identical with those in M.4: that is to say, substantial degeneration reaching to the internal granular and glomerular layers on the operated side (Figs. 93-96) and similar but much less degeneration on the unoperated side. The fact that the degeneration in bulbo-petal fibres on the operated side is only slightly greater than in M.9, where the lesion is in the caudal part of the anterior olfactory nucleus, suggests that few of these fibres can arise in the ipsilateral nucleus. In specimen 176, with a much smaller lesion in the anterior olfactory nucleus the findings are again similar although the degeneration, as expected, is much less intense. Fewer fibres reach the periglomerular cells on the operated side than in the other specimens and this may be due to the sparing of most of the fibres of the lateral olfactory tract (see Cragg, 1962).

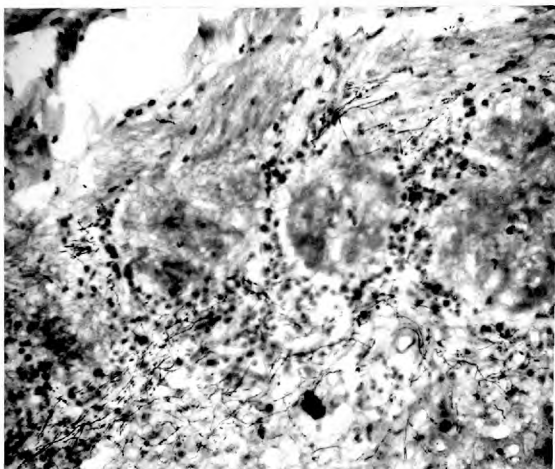


Fig. 93

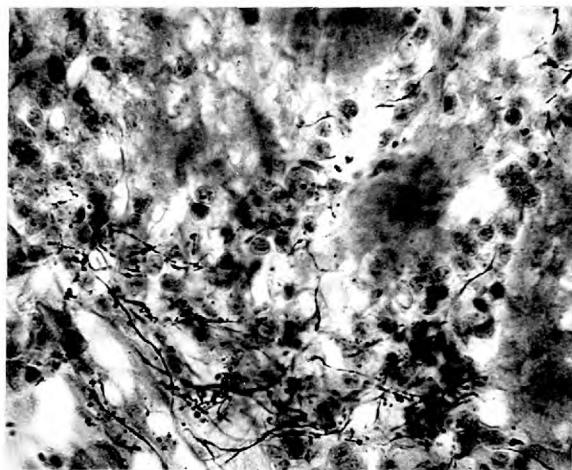


Fig. 94

Figs. 93, 94. Photomicrographs showing preterminal degeneration amongst the periglomerular granules on the same side of the lesion (specimen M.2). Nauta stain. Low power (X 140) and high power (X 360) respectively.

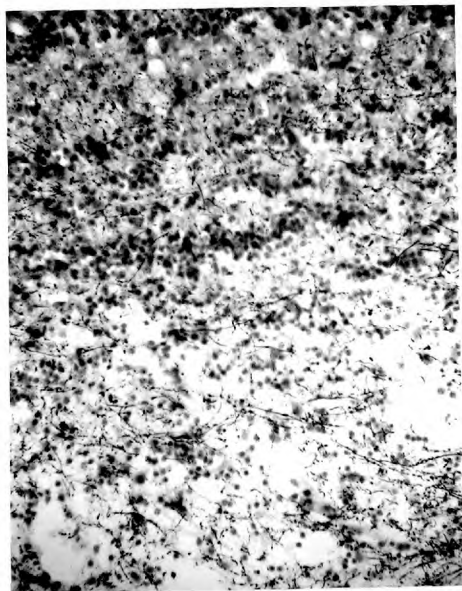


Fig. 95

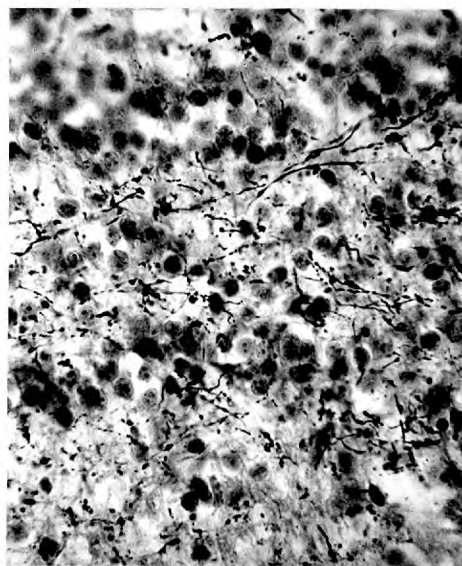


Fig. 96

Figs. 95, 96. Photomicrographs showing intense degeneration in the internal granular layer of the bulb proximal to the lesion from the same section as that shown in Fig. 93. Low power (X 140) and high power (X 360) respectively.

In specimens M.15 and M.8 the left bulb is completely destroyed. In the right bulb (the unoperated side) degeneration is again of a similar character but in neither specimen could degenerated fibres be seen in the external plexiform layer or among the periglomerular cells. Degeneration is also present in the dorsal part of the pars principalis (M.15 and M.8) and in the pars externa (M.15 only) of the anterior olfactory nucleus. In the remaining 2 specimens (174 and M.11) the damage to the anterior olfactory nucleus is slight and degeneration in the bulbs has not been studied. Degeneration in the commissural component is very slight and in fact the condition does not differ much from that found in the specimens with the lesion confined to the bulb.

In normal material it was seen that the olfactory commissural component is joined on its lateral side by a few fascicles which appear to come from or go to the basal surface of the hemisphere in the region of the olfactory tubercle and frontal piriform cortex. In three of the experimental specimens (M.2, M.4 and M.9) these fascicles are almost completely degenerated on the operated side and very slightly contra-laterally. They could not be traced with confidence to their terminations but appear to lead mainly to some diffuse degeneration deep to the frontal piriform cortex and olfactory tubercle. They are probably fibres which have arisen in the anterior olfactory nucleus and end in the basal rhinencephalic structures, mainly ipsilaterally but partly contra-laterally (Figs. 97^{to}_L100).

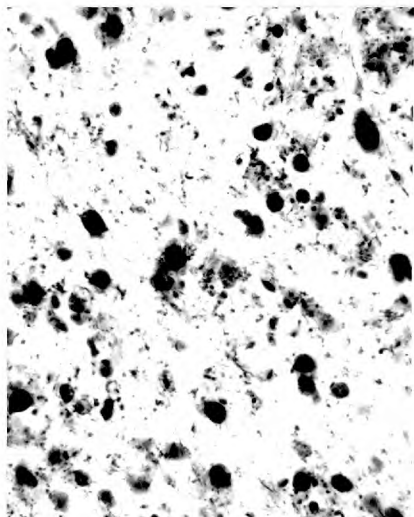


Fig. 97. A transverse section showing marked degeneration in the olfactory commissural component on the same side of the lesion (specimen M.4; with lesion of anterior olfactory nucleus). Nauta stain. X 360. (section 2)



Fig. 98. A transverse section showing slightly less degeneration in the contralateral olfactory component at the same level of that in Fig. 97. (M.4). X 360.



Fig. 99

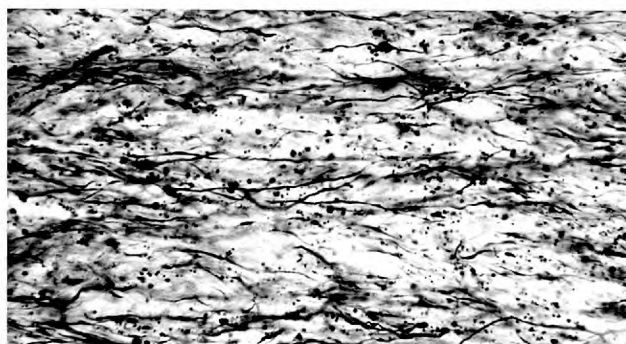


Fig. 100

Figs. 99, 100. Low power (X 140) and high power (X 360) photographs showing the olfactory commissural component crossing in the mid-line (from same specimen as that of Figs. 97 and 98). Nearly equal number of degenerating and normal fibres are seen. Nauta method.

Finally in one specimen (W.4) a few degenerating fibres are seen to enter the medial fore-brain bundle and can be followed caudally to the lateral hypothalamic area.

One may summarise the interpretation of the findings in these specimens, all with a unilateral lesion of the anterior olfactory nucleus, as follows:

- (i) the anterior olfactory nucleus contributes a few fibres to the lateral olfactory tract which end axo-somatically in the piriform cortex and amygdaloid transition area.
A small contribution may be made to the medial fore-brain bundle, but the evidence for this is derived from one specimen only; it is not confirmed in the others.
- (ii) Bulbo-petal fibres reach the inner granular and periglomerular cells of the bulb by way of the olfactory commissural component (and to a small extent in the deeper part of the lateral olfactory tract). Those in the olfactory commissural component are both crossed and uncrossed and few if any of the crossed fibres reach further than the inner granular layer.
- (iii) Some of the commissural fibres connect the anterior olfactory nucleus on one side with that of the other, their terminations being mainly in the dorsal part of the pars principalis and in the pars externa.
- (iv) Some fibres from the anterior olfactory nucleus end among diffusely scattered cells deep to the olfactory tubercle and piriform cortex both on the same side and after crossing in the anterior commissure. The evidence for

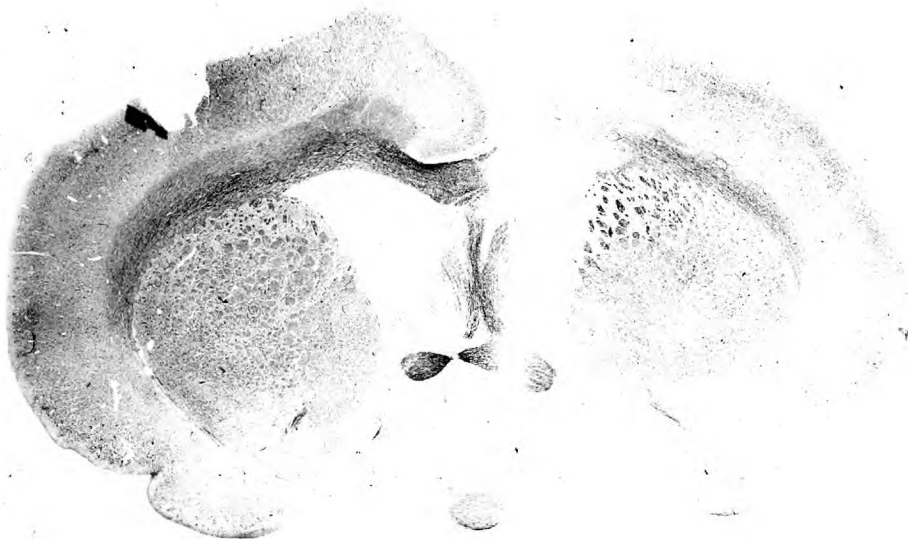
this is strong but not conclusive (see Fig. 109).

- (v) The fibres mentioned in sections (ii) to (iv) constitute the entire olfactory commissural component and the great majority arise in the anterior olfactory nucleus.

Some of the bulbo-petal fibres could arise in other parts of the hemispheres, ipsi- or contra-lateral, but if so, these parts have not been identified in the present study.

Lesions of the anterior commissure

To obtain further evidence about the commissural connexions of the basal rhinencephalon, 5 specimens were prepared in which the anterior commissure was cut in or close to the median plane. In one (179) only the intertemporal component had been divided, but in the other four specimens (178, 180, M.10 and M.14) all components of the commissure are divided completely. In all cases there is considerable damage in such regions as the corpus callosum, the hippocampal commissure and sometimes the cranial extremity of the hippocampus, the septum and its associated fibre systems including the stria terminalis, the anterior amygdaloid area, the lateral pre-optic region and the medial fore-brain bundle. For this reason degeneration is widespread in many parts of the brain, but only that which is relevant to the commissural connexions of the basal rhinencephalon will be described. Specimen M.9 described earlier (p. 158), can be taken as an example of a lesion of the olfactory commissural component without involvement of the intertemporal component.



Coypu W.14 (section C2)

Fig. 101. A transverse section of the coypu brain (W.14) to show the exact site of the surgical lesion involving all components of the anterior commissure just lateral to the mid-line. Note the damage which was inflicted during operation to other structures such as the corpus callosum, fornix and stria terminalis. Frozen H&E section. X 5.

In specimen 179 with an intact olfactory component but complete division of the intertemporal component, no degeneration could be found in the olfactory bulb or anterior olfactory nucleus of either side. In the olfactory component itself, near the median plane, varicosities and possibly fragmentation could be seen in a few fibres. This has been a common finding in previous specimens with lesions limited to the olfactory bulb and does not differ significantly from the condition seen in quite normal control specimens. One may conclude from this specimen that there is no passage of fibres from the intertemporal to the olfactory component of the commissure, at least in more than trivial numbers. It should be noted that this conclusion is based on the evidence of one specimen only. However, it could be said that the reverse condition is also true because in K.9, with a lesion of the olfactory component only, no evidence of degeneration is seen in the intertemporal component.

In the intertemporal component of specimen 179 degeneration is heavy and the contrast between it and the normal olfactory component is shown clearly where they meet near the median plane (Figs. 102 and 103). As one would expect in a commissure divided in this situation, degeneration is equally marked but incomplete on both sides. It can be traced laterally in bundles of fibres passing through the corpus striatum to the external capsule. In the corpus striatum the amount of degeneration is surprisingly large but it is less

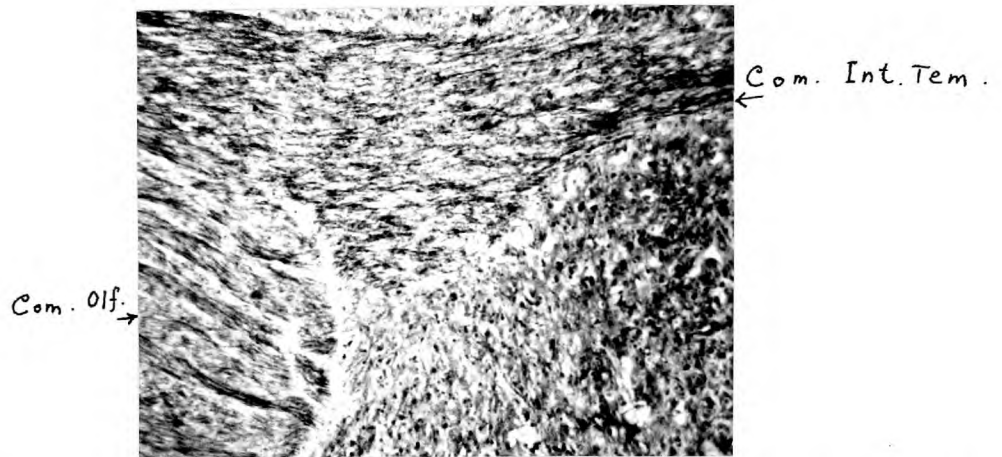


Fig. 102. A transverse section showing some degeneration in the inter-temporal component of the anterior commissure which is meeting the olfactory component in the mid-line (specimen 179 with inter-temporal component lesion). Note that all the fibres of the olfactory component are normal. Nauta stain. X 55.



Fig. 103. Higher power view (X 140) of the same section shown in Fig. 102, showing a mixture of degenerating and normal fibres in the inter-temporal component of the anterior commissure.

marked than expected in the external capsule, suggesting that many fibres have ended in and ventral to the corpus striatum. Some degeneration, probably pre-terminal, is also found in the baso-lateral amygdaloid nuclei on both sides. In sections of the stria terminalis at the level of the amygdala some degeneration is seen bilaterally but only in a minority of the fibres on the side opposite to the lesion. It is difficult to say whether this degeneration resulted from the lesion in the commissure or from direct damage to the stria itself or to such regions as the septum, lateral pre-optic or subthalamic regions. That the amount of degeneration in the stria and amygdala is much greater on the side of the lesion, suggests that it must be due to direct damage to the structures mentioned.

The degeneration is seen clearly in the stria terminalis as it lies dorsal and ventral to the internal capsule and there appears to be pre-terminal degeneration in the bed nucleus, the medial, the central and the small-celled part of the basal amygdaloid nucleus. It follows that the stria terminalis contains at least a proportion of amygdalo-petal fibres. Fibres in the cortical nucleus are particularly well stained and all appear normal. Degeneration is also present in the 'longitudinal association bundle' between the lateral and basal amygdaloid nuclei. This again indicates that the bundle carries amygdalo-petal and not only amygdalo-fugal fibres (see p.124). Degeneration in this bundle can be traced ventro-laterally to the external capsule and for a short

distance in the deeper layers of the temporal piriform cortex.

In the four specimens in which both components of the anterior commissure are divided, the distribution of degeneration in the corpus striatum, stria terminalis, 'longitudinal association bundle', external capsule and amygdala is similar. None is present in these situations in M.9 where the lesion affected the olfactory commissural component only. It follows that none of these degenerated fibres can have come from the olfactory commissural component.

In the four specimens (178, 180, M.10 and M.14) the olfactory component is heavily degenerated on both sides, but again as expected, about half the fibres are normal. Degeneration can be traced forwards in both hemispheres into the core of the olfactory peduncle (Fig. 107) and of the bulb without any difficulty. In three of them (180, M.10 and M.14) there is some preterminal degeneration in the pars externa of the anterior olfactory nuclei and in M.14 it is also found in the dorsal part of the pars principalis (Fig. 106). The majority of degenerated fibres enter the bulb and are found in great numbers among the cells of the internal granular layer (Fig. 105). Their abundance in this situation contrasts strongly with the very small amount seen in the contra-lateral bulb of experiments with peduncular lesion (e.g. M.2, M.4 and M.9).

It will be seen that there are two important differences between specimens with an anterior commissure lesion near the median plane and those such as M.9 with a lesion in the



Fig. 104. Photomicrograph showing only very few degenerating granules amongst the periglomerular cells (specimen M.14 with lesion in the anterior commissure). Compare with Fig. 94 which shows much more degenerating fibres probably reaching it via the lateral olfactory tract. Nauta stain. X 360.

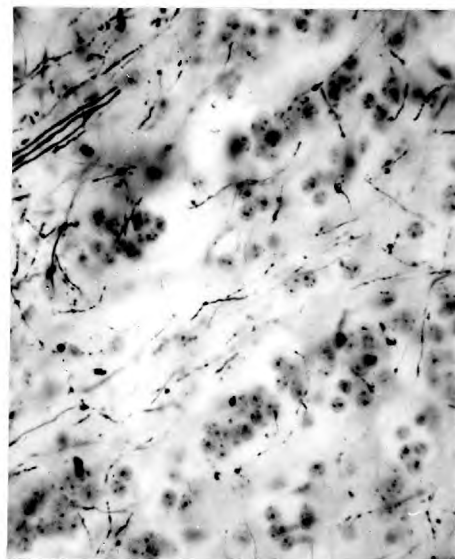


Fig. 105. Photomicrograph to show degenerating fibres in the internal granular layer of the bulb, from the same section as that of Fig. 104. X 360.

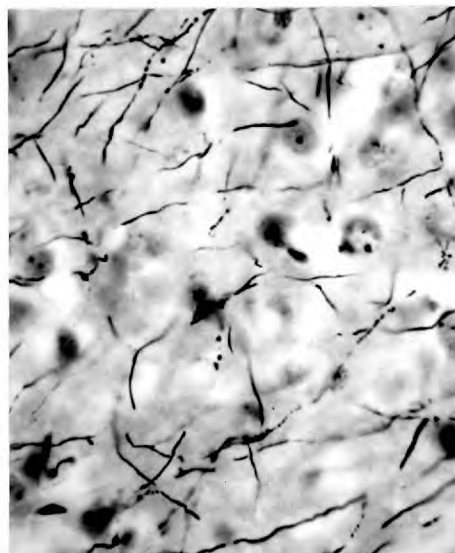


Fig. 106. Photomicrograph showing degenerating pre-terminals amongst cells of the anterior olfactory nucleus (same section as Fig. 104). X 360.



Fig. 107. Photomicrograph showing intense degeneration in olfactory commissural component in the core of the peduncle (longitudinal section; same as in Fig. 104). X 360.

olfactory peduncle. Firstly, after the median-plane lesion, the degeneration is equally abundant in both bulbs and comparable to that in the bulb on the side of the lesion when this is in the peduncle. Secondly, after median plane lesions, very few of the degenerated fibres reach the periglomerular cells in either bulb. These differences can be explained if:

(1) the fibres ending among the periglomerular cells are predominantly uncrossed; they might well be represented by the bulbo-petal fibres of the lateral olfactory tract as suggested by Cragg (1962) and Powell & Cowan (1963).

(ii) some fibres join the commissural component and cross to the opposite bulb from regions of the hemisphere caudal to the peduncle. These might come from the olfactory tubercle or piriform cortex but the present work does not provide any evidence for their site of origin.

The only other observation to be reported from these specimens is that in M.14 some degeneration is seen among scattered cells deep to the olfactory tubercle on both sides which may indicate a connexion with this structure.

The findings in this group of specimens are generally confirmatory of the interpretations based on specimens with lesions of the peduncle which are summarised on p. 164. In particular they reinforce the conclusion that the fibres which reach the periglomerular cells are predominantly ipsi-lateral and that the olfactory commissural component must

contain both crossed and uncrossed fibres. They show that some of the crossed fibres must arise in the hemisphere caudal to the peduncle, possibly from the olfactory tubercle. They show also that there is no passage of fibres from the olfactory to the intertemporal component of the commissure, the two components being quite independent. Because of the widespread damage to many structures precise interpretation of much of the degeneration seen is not possible, but it does show that both the stria terminalis and the 'longitudinal association bundle' carry amygdalo-petal as well as amygdalo-fugal fibres.

DISCUSSION AND CONCLUSIONS

The main object of this work has been to record the macroscopic and microscopic features in the basal rhinencephalic structures of a macrosmatic rodent which had not been investigated previously. It has included a study, using the method of experimental degeneration, of the secondary olfactory connexions, and some observations on the distribution of AChE, which cannot be regarded as more than preliminary, have also been recorded.

It may first be pointed out that the coypu has proved a very suitable animal for a laboratory investigation of this kind. It tolerates anaesthesia well and remains in good health during post-operative periods which extended up to 56 days. No mortality and no sign of infection occurred in any of the 24 experimental specimens. The tissues of the nervous system react well with the neurohistological methods employed for the study of cytoarchitecture, fibre connexions and the distribution of acetylcholinesterase. In short, the coypu is a very convenient laboratory animal particularly when its greater size, as compared with the rat, is of importance. A similar conclusion was reached by Goldby & Kacker (1963) in a study of the pyramidal motor system of this animal.

The most obvious conclusion that can be drawn from the observations recorded is that in its structure and connexions the basal rhinencephalon of the coypu resembles that of

comparable macrosmatic mammals such as the rat, very closely indeed. The few small points of difference which have been noted do not call for extensive discussion.

So far as cytoarchitecture is concerned, the primary olfactory receptive centres in the main and accessory olfactory bulbs are essentially identical with those of other macrosmatic mammals. Secondary olfactory centres, defined as those regions which receive fibres originating in the main and accessory bulbs, are also of the usual pattern. They include the anterior olfactory nucleus, the olfactory tubercle, the frontal and temporal piriform cortex, the nucleus of the lateral olfactory tract and the cortical amygdaloid nucleus. It should be pointed out that in spite of differences in terminology, all these regions, with the possible exception of the nucleus of the lateral olfactory tract, can be classified as cortical in type. They are all fundamentally similar in presenting three main laminae - molecular, pyramidal and polymorphic - and in all of them the specific afferents from the olfactory bulb lie in the molecular layer. Synaptic contacts are almost certainly axodendritic in type. Together they form an extensive paleo-cortical area which reaches into the olfactory peduncle as the anterior olfactory nucleus. In spite of differences such as the presence of islets of Calleja in the olfactory tubercle, the common features provide ample justification for classifying all these structures together.

The only other cortical area which has been studied is

the entorhinal. This is well developed in the coypu but differs from the paleo-cortical areas in the absence of secondary connexions from the bulbs and in its more complex and very characteristic cytoarchitectural lamination. These features may justify its classification as intermediate between paleo- and neo-cortex.

The other structures considered, the septum medially and the amygdala laterally, are nuclear in character. The septum is well developed and its major sub-divisions, as described in other mammals, can easily be recognised but it would not be possible to justify, on the basis of the present material, a sub-division into as many different nuclei as those identified by Andy & Stephan (1959) in the galago. It is very difficult, however, in making comparisons, to say how far differences may be due to different subjective preconceptions of how marked the variations in cell type and density of packing must be before they justify identification as separate nuclei.

The amygdala is also well developed and can certainly be described in the same terms as are used for other mammals. The major sub-division into cortico-medial and baso-lateral groups of nuclei is easily established. The finer sub-divisions run up against the difficulty that many of the described nuclei are extremely ill-defined and often blend with surrounding structures. It is suggested that the cortico-amygdaloid nucleus and the transition area to the piriform

cortex are more naturally classed with the other paleo-cortical structures of the basal rhinencephalon and possibly the same can be said of the nucleus of the lateral olfactory tract. Of the remaining amygdaloid nuclei only the lateral nucleus is clearly defined cytoarchitecturally, although the magno-cellular part of the basal nucleus is closely associated with it and could easily form its ventral part. The small celled, so-called medial part of the basal nucleus is so different from the magno-cellular part and so closely related to the medial and cortico-amygdaloid nuclei that it could reasonably be classified with the latter rather than in the baso-lateral group. The central nucleus is very difficult to separate from the overlying putamen and the name "anterior amygdaloid area" clearly indicates the lack of clear-cut boundaries in this region. The conventional classification of the amygdaloid nuclei is based on Johnston's embryological and comparative anatomical observations, but recently Koikegami et al. (1955) and Koikegami (1963) have pointed out that this may be unsatisfactory from a functional point of view. They agree that the large-celled part of the basal nucleus should be grouped with the lateral nucleus because it has autonomic and extra-pyramidal functions. The small-celled medial part on the other hand, they consider should be classed with the medial and cortical nuclei which carry out autonomic functions only. In their view, the baso-lateral amygdaloid nuclei (the striatal part of the amygdala of Holmgren) form the amygdala proper. They

accept, however, Holmgren's definition of the cortico-medial nuclei but including the small-celled part of the basal nucleus, as a sub-pallial part of the amygdala.

There is also the question of the relationship of the amygdala to olfactory function. It is generally stated that this is located mainly and perhaps exclusively in the cortico-medial group of nuclei. Some doubt is thrown on this by the observations of Breathnach & Goldby (1954) that these nuclei are well developed in the anosmatic porpoise. There is in fact little correlation between the relative size of the nuclear group as a whole and the abundance or absence of olfactory tract connexions. When such connexions are present, as is the case in all but anosmatic mammals, some reach the cortical nucleus but the spread to the cortico-medial group as a whole is questionable and will be discussed later.

The varied distribution of acetylcholinesterase in the basal rhinencephalon makes possible some further distinctions among the structures concerned. In the paleo-cortical regions, all of which receive secondary olfactory connexions, only the olfactory tubercle and the nucleus of the lateral olfactory tract are strongly positive for the enzyme and both these regions can be regarded as specialised from the cytoarchitectural point of view. The nucleus of the lateral olfactory tract is less cortex-like than any other part of the

paleo-cortex and the tubercle contains the very characteristic islets of Calleja. The concentration of AChE is an additional reason for separating them from other paleo-cortical areas. A further distinction between the entorhinal and piriform cortices is also established by the concentration of AChE in the second layer of the former and its almost complete absence from the whole of the latter. In the amygdala the AChE distribution gives some justification for separating the basal from the lateral nucleus, in that the basal is much more strongly positive for the enzyme than the lateral.

~~In the experimental species it may be noted that after a section of the peduncle very little evidence of a primary of the enzyme is found proximal to the lesion, although its disappearance from the nucleus of the lateral olfactory tract and olfactory tubercle distal to the lesion is striking. Staining in the amygdala nuclei shows no change. These findings might indicate some special significance for the secondary olfactory system of both the basal and the lateral olfactory tract and the tubercle, but clearly require further investigation in additional specimens before they can be regarded as more than suggestive.~~

It may be concluded that these preliminary observations ~~in the present and experimental specimens~~ indicate a general similarity between the coypu and other rodents so far as the distribution and behaviour of AChE is concerned and at least establish a case for more extensive study.

The fibre connexions which have been studied can be discussed under three main headings, all with reference to the olfactory bulb:

1. The olfactory tracts.
2. The olfactory commissural component, and
3. The connexions of the amygdala.

1. The olfactory tracts. Lesions limited to the bulb, with or without involving the accessory bulb, lead to degeneration in the lateral olfactory tract only. These fibres are all distributed within the molecular layer of the paleo-cortical formations described above, although they were traced only to the antero-lateral parts of the olfactory tubercle and the cortical amygdaloid nucleus. None can be traced to the septum, none to the hippocampal formation, and although a few may reach the anterior margin of the entorhinal cortical area, their number is so small that they can hardly be considered significant. Terminations of secondary olfactory fibres in these situations have been confirmed by all other investigators whether using the Marchi, Gies or Nauta histological techniques. It should be noted that all experimental investigations also agree in finding no evidence for the presence of a medial olfactory tract carrying secondary olfactory fibres to the septum or related structures. Whether some or all of the fibres running to the olfactory tubercle should be called an intermediate or accessory olfactory tract is merely a matter of terminology. There

seems no need to introduce such a term at least in the coypu.

An important point is the absence of significant degeneration from the olfactory commissural component when the lesion is confined to the bulb. Attempts at complete bulbar ablation almost inevitably cause some damage to the anterior olfactory nucleus, the rostral part of which extends into the bulb and it is only when this structure is damaged that degeneration appears in the olfactory commissural component. All efferent fibres from the bulb, whether coming from the mitral or the tufted cells, run superficially in the lateral olfactory tract (Fig. 108). In this conclusion the present work is in agreement with the early observations of van Gehuchten (1904) and the more recent studies of Young (1941), both using the Marchi method. It also agrees with those of White (1964), Lohman (1963) and others who used the Nauta technique. It appears therefore that in studies in which degeneration has been found in the olfactory commissural component after what are claimed to be pure bulbar lesions, this result is due to associated damage to the anterior olfactory nucleus which has not been allowed for. This point will be discussed further in the next section.

2. The olfactory commissural component. Unequivocal degeneration in the olfactory commissural component is found only when the anterior olfactory nucleus is involved in the lesion (with or without involvement of the

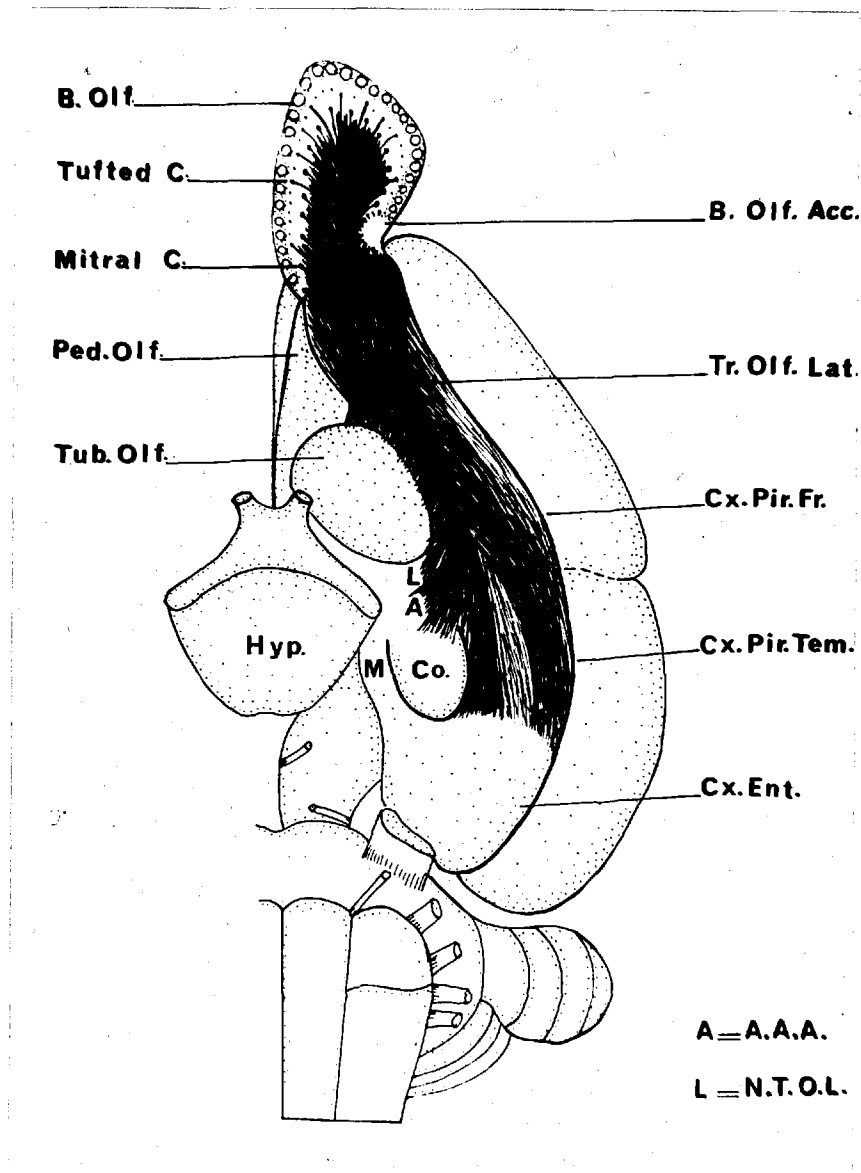


Fig. 108. A drawing of the ventral surface of the coypu brain to show the secondary olfactory connexions.

olfactory bulb) unless this component or the anterior commissure is damaged directly. Its fibres cannot therefore come either from mitral or tufted cells. This conclusion does not agree with that of Cajal (1911) and later Allison (1953a) who thought that two relatively independent pathways for bulbo-fugal connexions could be demonstrated: a mitral cell-olfactory tract system, and a tufted cell-anterior commissure system. Cajal, however, admitted that the axons of the tufted cells are fine and difficult to follow through the maze of fibres in the inner layers of the bulb and the retrograde changes in tufted cells after lesions of the anterior commissure, reported by Allison, are not very marked. Allison emphasised the variable appearance of these cells even in normal preparations, and in a later paper points out that "the occurrence of retrograde atrophy rather than complete cellular degeneration can presumably be attributed to the existence of well-developed collaterals in the bulb, which remain in a partially functional condition after the main axons are destroyed" (Allison, 1953b). The balance of evidence is now strongly against an origin of fibres in the anterior commissural component from tufted cells in the bulb.

The fibres of the olfactory commissural component are distributed to the deeper layers of the tubercle and piriform cortex on the same side, and, after crossing, to the same regions on the opposite side and to the contra-lateral bulb. In this conclusion the present work agrees with that of Fox & Schmitz (1943), Cragg (1961) and Powell et al. (1963). The

fibres which reach the opposite bulb and mainly in the internal granular layer but also, as described by others, in pars externa and pars principalis of the anterior olfactory nucleus, particularly dorsally (see Fig. 109). The present experiments also suggest that bulbo-petal fibres arise from the basal areas of the brain and not only from the opposite anterior olfactory nucleus. Such fibres terminate mainly in the internal granular layer but a few can be traced to the external plexiform layer and to the periglomerular cells. The material indicates that the majority of bulbo-petal fibres travel with the olfactory commissural component but a few, probably those reaching the periglomerular layer, may travel in the deeper layers of the lateral olfactory tract as demonstrated by Cragg (1962) and Powell & Cowan (1963). The exact origin of the bulbo-petal fibres that arise from the base of the brain cannot be determined from the present work but the observations of Powell et al. (1963) suggest an origin mainly in the olfactory tubercle and the frontal piriform cortex.

All these observations show that the olfactory component of the anterior commissure is not a simple interbulbar commissure as described by Cajal. Like the sub-cortical white matter elsewhere it is a complex of associational, commissural and decussating fibres which interrelate the different parts of the basal rhinencephalon on both sides of the brain. The fibres which end in the bulbs may represent the 'feed-back'

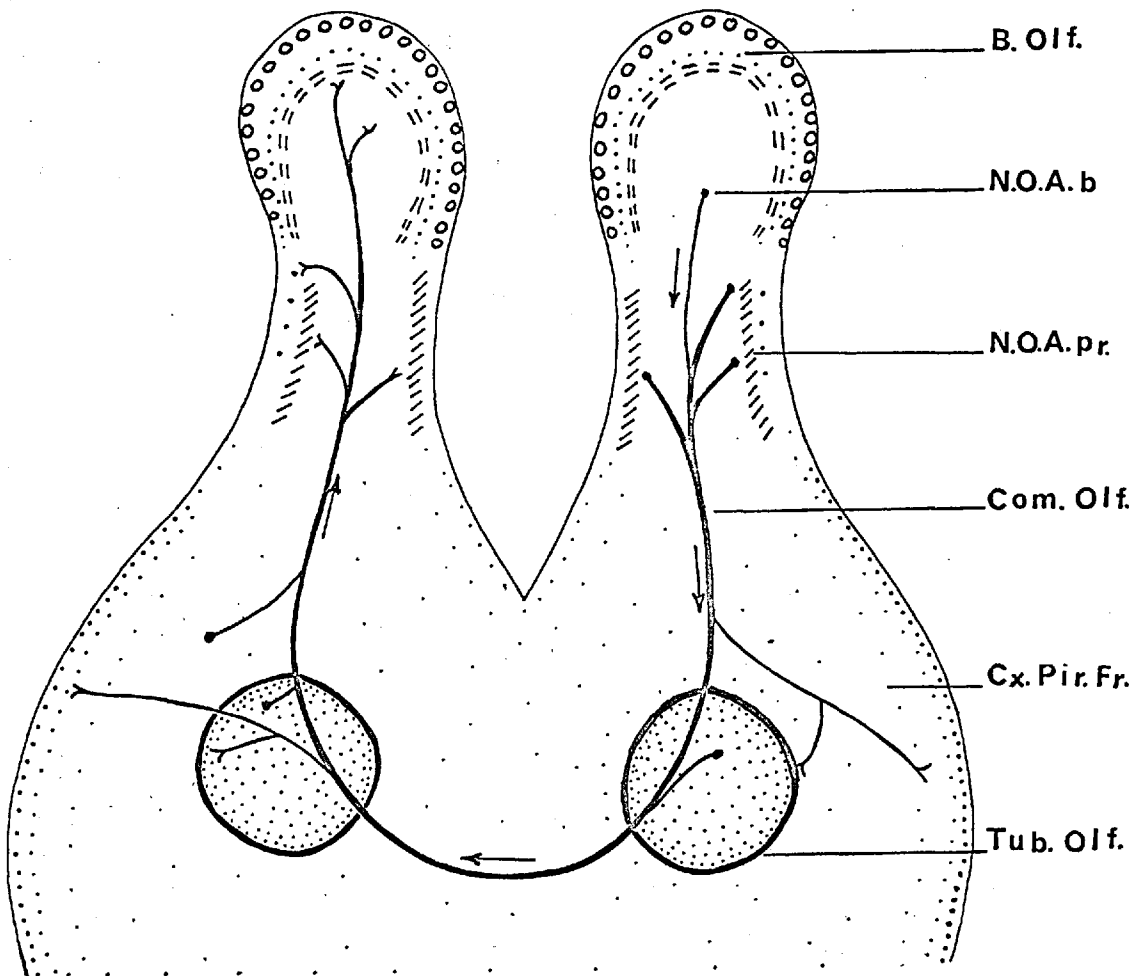


Fig. 109. A semi-diagrammatic illustration of a horizontal section of the brain to show the olfactory commissural component. It originates mainly from the anterior olfactory nucleus, crosses in the midline and terminates in the opposite anterior olfactory nucleus and internal granular layer. Few fibres are also distributed to the olfactory tubercle and piriform cortex both ipsi- and contra-laterally. The experimental findings suggest an origin of some of the fibres from these latter structures.

mechanism postulated previously by Allison (1953b) and by Kerr & Hagbarth (1955) on electrophysiological evidence.

3. The amygdaloid connexions. The distribution of secondary olfactory fibres to the amygdala will be discussed more fully because of the difference in our findings from those obtained with another silver method (the Glees technique). The distribution of lateral olfactory tract to the nucleus of the tract, the anterior amygdaloid area and the amygdaloid transition area has been described, generally by all other investigators and a few fibres spread medially in the antero-lateral part of the cortico-amygdaloid nucleus. There is no evidence of connexions in any other part of the amygdala of the coypu. The endings are mostly axo-dendritic in type.

The absence of degeneration from the baso-lateral amygdaloid nuclei is also in agreement with the findings of all other investigators. This is not so with the absence of degeneration in the central, medial and the greater part of the cortico-amygdaloid nucleus, and in the bed nucleus of the stria terminalis. Workers using the Glees silver method have found secondary olfactory terminations in all these situations, notably Le Gros Clark & Meyer (1947) in the rabbit and to a less extent Allison & Meyer (1949) in the monkey, and Adey (1953) in the phalanger. It is not likely that the negative findings in the coypu can be explained as the result of a technical failure to stain degenerating fibres by the Nauta technique. False positives are commoner than false negative

results with this method. Our specimens were all consistent, and were studied after survival periods which varied from 7 to 56 days and in other situations all showed clear cut and characteristic evidence of degeneration. If fibres from the bulbs ran in significant numbers to the central and medial nuclei and the bed nucleus of the stria terminalis, it is extremely unlikely that their degeneration products would have remained unstained. It has been emphasised earlier that the Glee's method tends to give inconsistent results in different hands and recently it was particularly stressed by Powell et al. (1963) that it tends to show pseudo-degeneration bilaterally in certain regions of the brain which are pertinent to this study. Moreover, negative findings as in the coypu have been reported in recent years by nearly all authors using the Nauta technique in different mammals ranging from rodents to primates. The balance of the evidence is therefore very strongly against the termination of any secondary olfactory fibres in the deeper nuclei of the amygdala.

The experiments in which the inter-temporal component of the anterior commissure has been transected just lateral to the mid-line gave interesting results concerning some of the amygdaloid connexions. It carries some inter-amygdaloid fibres (as shown by Humphrey, 1936), but only a few are connected with the baso-lateral nuclei, as pointed out by Brodal (1948). Most of the amygdaloid connexions are found in the stria terminalis and the 'longitudinal association

bundle'. In the older literature and in a fairly recent description (Fox, 1943) the stria terminalis was considered to be the major pathway from the amygdala. The present work shows that it also contains amygdalo-petal fibres in accord with the observations of Nauta (1958) and Shute & Lewis (1963). Caudally directed fibres of the 'longitudinal association bundle' in the coypu are even more numerous (as mentioned by Powell et al., 1963) and they appear to terminate mainly in the large-cell parts of the lateral and basal amygdaloid nuclei; a few pass laterally to the deeper layers of the temporal piriform cortex. Whether the rostrally directed fibres of this bundle contain fibres from the amygdala only (as described by Nauta, 1961) or also fibres from the piriform cortex as well as the amygdala (as described by Powell et al., 1963) cannot be said definitely from the coypu material available. What becomes clear, however, from all these observations, is that there is a very definite and profuse reciprocal relationship between the amygdala and the hypothalamus. This would appear to be of much greater functional significance than the very scanty secondary olfactory connexions, and would be in accord with the persistence of a well developed amygdala in the anosmatic porpoise (Breathnach & Goldby, 1954) and in microsmatic man (Crosby & Humphrey, 1941). It is interesting to note here that in one of the experiments with retro-bulbar lesions cholinesterase activity disappeared only from the ipsi-lateral

nucleus of the lateral olfactory tract and was unchanged in other amygdaloid nuclei.

The above mentioned findings are also in accord with the neurophysiological observations of Fox et al. (1944) who report no potentials from the amygdaloid nuclei after stimulation of the olfactory bulb. They have, however, recorded potentials from the nucleus of the lateral olfactory tract. Finally it can also be added that rats (Swann, 1934) and dogs (Allen, 1941) with bilateral destruction of the amygdala still retain the faculty of olfactory discrimination.

Stimulation of the amygdala does not give consistent results concerning the localisation of function in the cytoarchitectonically defined sub-nuclei. It appears however that the antero-medial division responds with autonomic and somatomotor effects, and the lateral division with various behavioural changes. Recently Green, Clemente & De Groot (1961) demonstrated that lesions confined to the amygdala did not alter sexual behaviour and that a state of hypersexuality occurs only when the lesion involves the piriform cortex as well. Powell et al. (1963) have shown that the piriform cortex sends a substantial projection to the hypothalamus, thus providing an anatomical basis for the well-known relationship between olfaction and reproductive behaviour. These authors have also shown that the piriform cortex sends some tertiary olfactory fibres to the amygdala.

A detailed study of the fibre connexions of the septum of

the coypu brain has not been undertaken and the few findings can be summarised as follows. In one experiment, with a lesion in the anterior olfactory nucleus, some degenerating fibres were seen passing in the medial fore-brain bundle to reach the lateral hypothalamic area, as described previously by Young (1936) and Lohman (1963). On their way they run very close to the nucleus accumbens septi and the nucleus of the diagonal band, and there is an indication that some may end amongst the cells of these nuclei. The few experiments in which the septal region was damaged directly also indicated the presence of septo-amygdaloid connexions via the diagonal band of Broca and the 'longitudinal association bundle' as described by Powell et al. (1963). Although no secondary olfactory fibres have ever been traced to the septum it appears to receive some tertiary olfactory fibres from the anterior olfactory nucleus and probably from the olfactory tubercle. The close structural relationships between the septum, amygdala, hippocampus and hypothalamus is leading many authors to suggest the possibility that they form an integrated mechanism which is not necessarily dominated by olfactory function.

The term "rhinencephalon" and the function of smell

It has been mentioned in the introductory pages of this thesis that if the term "rhinencephalon" is to be used at all, it is important that it should be defined precisely in morphological terms as emphasised in a recent publication by Brodal (1963). A possible morphological criterion would be

the direct reception of secondary olfactory fibres from the bulb. The only structures which undoubtedly receive such fibres in the coypu and other mammals are the anterior olfactory nucleus, olfactory tubercle, piriform cortex, nucleus of the lateral olfactory tract and the antero-lateral part of the cortical amygdaloid nucleus. They, together with the bulb could form a precisely defined rhinencephalon which would exclude the septum and most of the amygdala and of Broca's limbic lobe. Even so, the term "olfactory system" rather than "olfactory brain" would be more appropriate since the latter term includes embryological implications.

It is relevant here to refer to the following statement by Elliot Smith (1897): 'the rhinencephalon certainly fulfils other functions than that of a ^esmall centre, since parts of it exist in anosmatic cetaceans. If we use this term, therefore, we will be met by the paradoxical statement that smell-less animals still possess a "smell brain" '. The term is still widely used however and in a recent publication on the anatomy of the rhinencephalon Gastaut & Lammers (1961) defend this usage on the practical ground that it is understood even if inexact and perpetuating a very old error. It should however be stated that the concept of a rhinencephalon has been greatly modified in the recent years, owing to the evidence for restricting the olfactory centres to certain parts of the base of the brain. The olfactory centres no longer include the great limbic lobe of Broca and its sub-cortical cell stations (amygdala, septum, hypothalamus, anterior thalamic

nuclei and perhaps the epithalamus). Recently MacLean (1952), who has elaborated the theory of Papez (1937), maintained that the limbic lobe of Broca and its sub-cortical cell stations forms a system which is concerned with a variety of emotional and visceromatic reactions, and he named it the "limbic system". Earlier he referred to it as the "visceral brain" and this was done 'to help dispel the notion it had primarily to do with the sense of smell' (MacLean, 1954). All the recent experimental, anatomical and physiological data are in favour of MacLean's ideas. Accordingly it is suggested here that the structures previously included under the comprehensive term rhinencephalon can be sub-divided to form a strictly "olfactory system" as defined above and a "limbic system" which is concerned mainly with visceral and behavioural mechanisms. The structures which make up the latter system are found in all mammals whether they be macrosomatic, microsomatic or even anosomatic, some being actually better developed in higher mammals with a much reduced olfactory system. The amygdala for example reaches its greatest development in the microsomatic primates and Brodal (1947a) also emphasises the non-olfactory function of the hippocampus by referring to Rose (1936). Rose stated that if comparisons are made between the size of the olfactory bulb and the hippocampus in various mammalian species, the extreme position is occupied by Man, who has relatively (and absolutely) the largest hippocampus. From his cytoarchitectonic study Rose concludes that in man the gyrus hippocampi (including the entorhinal area) is no rudimentary

structure but, so far as certain sub-divisions are concerned, even a specific human organ. In conclusion it must be emphasised however, that all these structures are very closely related to the secondary olfactory centres and the majority receive tertiary olfactory connexions. It can be said that a somewhat attenuated olfactory influence reaches them, to be correlated with the varied information which comes from other sources. Berry et al. (1952) showed that action potentials may be recorded from such structures as the amygdala and hippocampus when the olfactory bulb of the cat is stimulated with long pulses. The long conduction time, however, suggests a multisynaptic pathway. It must also be stated that similar potentials have been recorded from such widely distributed projection fields as the corpus striatum and even the cerebellum and other regions which have never been considered as parts of a rhinencephalon. If the term is to be applied to all parts of the brain which may be influenced, however indirectly, by olfactory function, it would seem to have little value. An arbitrary limitation must be adopted and defined, and it is suggested that a limitation to the primary and secondary olfactory centres in the fore-brain would be the most appropriate.

ABBREVIATIONS

A.A.A.	Area amygdaloidea anterior
B.	Nucleus amygdalae basalis (parvocellular)
B.1.	" " " (accessorius)
B.2.	" " " (magno-cellular)
B.N.St.	Bed nucleus of the stria terminalis
B. Olf.	Bulbus olfactorius
B. Olf. Acc.	Bulbus olfactorius accessorius
C.	Nucleus amygdalae centralis
C.C.	Corpus callosum
Ch. Opt.	Chiasma opticum
C.I.	Capsula interna
Cl.	Clastrum
Co.	Nucleus amygdalae corticalis
Com. Olf.	Olfactory commissural component
C.S.	Corpus striatum
Cx. Ent.	Cortex entorhinalis
Cx. Pir. Fr.	Cortex piriformis frontalis
Cx. Pir. Tem.	" " temporalis
D.B.	Nucleus of the diagonal band of broca
Fim.	Fimbria
Fs. Amg.	Fissura amygdalae
Fs. End.	Fissura endorhinalis
Fs. Rh.	Fissura rhinalis
Glom.	Lamina glomerularis
Gr. Ext.	Lamina granularis externa
Gr. Int.	" " interna
G.P.	Globus pallidus
H.	Hippocampus
Hyp.	Hypothalamus
I.	Massa intercalata
L.	Nucleus amygdalae lateralis (magno-cellular)
L.1.	" " " (parvocellular)
M.	Nucleus amygdalae medialis
M.F.B.	Medial fore-brain bundle
N.	Neocortex

N. Accm.	Nucleus accumbens
N. Ang. Cort.	Nucleus amygdalae corticalis
N. Ang. Med.	" " medialis
N. Olf. Ant.	Nucleus olfactorius anterior
N.O.A.b.	" " " pars bulbaris
N.O.A. Ext.	" " " pars externa
N.O.A.p.	" " " pars posterior
N.O.A.pr.	" " " pars principalis
N.Olf.	Nervii olfactoria
N.Opt.	Nervus opticus
N.Sept.L.	Nucleus septalis lateralis
N.Sept.M.	" " medialis
N.T.O.L.	Nucleus tractus olfactorius lateralis
N. Vom.	Nervous vomeronasalis
Ped. Olf.	Pedunculus olfactorius
Per. Gr.	Periventricular granules
Plex. Ext.	Lamina plexiformis externa
Pl. Int.	" " interna
Pu.	Putamen
S.	Subiculum
S. Circ.	Sulcus circularis
St.T.	Stria terminalis
Tr. A.	Cortico-amygdaloid transition area
Tr. Olf. Lat.	Tractus olfactorius lateralis
Tr. Opt.	Tractus opticus
Tub. Olf.	Tuberculum olfactorium
V. Olf.	Ventriculus olfactorius (obliterated)

APPENDIX I
(Surgical procedure)

Anaesthesia

Ether by inhalation was the anaesthetic used in all the experiments. The animal is picked up by the tail and placed in a wooden box through which air, saturated with ether vapour, is pumped by means of a foot-bellows. After the animal is transferred to the operating table, the ether anaesthesia is then maintained throughout the operation by means of a conical mask placed over the snout. Premedication with atropine or nembutal was not necessary and the animals did not show signs of excessive mucous secretions in all operations which sometimes extended up to 1½ hours. Recovery from anaesthesia was very quick and no post-operative complication nor death occurred in any of them.

Operative techniques

Lesions in the bulbs and anterior olfactory nucleus

The skull is exposed by a mid-line dorsal incision extending from the fronto-nasal to the fronto-parietal sutures. The periosteum is incised in the mid-line and detached to the left. When the olfactory bulb is to be ablated cranial to the accessory bulb then a small opening is made in the skull, by means of a dental drill, half-way between these two sutures slightly to the left of the mid-line. This point lies almost exactly over the middle of the olfactory bulb as studied from a sagittal section of the head of the coypu. Fig. 110 shows the relationship of the cranial sutures to the different parts of the brain. The dura is then opened and the excision of the bulb is made by an iridectomy knife and then the bulb scooped by means of a meibomian cyst scoop. Sometimes the ablation was made by thermo-cautery, and occasionally suction was used. Haemorrhage following the removal of the bulb was slight and readily controlled. By using bone wax or a piece of temporal muscle, haemorrhage from bone during drilling

was controlled without any difficulty. For making a localised lesion in the accessory bulb thermo-cautery was used and the site of drilling is shown in Figure 110. For making lesions in the anterior olfactory nucleus the drilling was made slightly caudally (Fig. 110) and the olfactory peduncle was approached through the frontal pole.

Transection of the anterior commissure

This was done by inserting a narrow knife blade either in the mid-line or a few millimeters laterally at the level of the commissure, i.e. about the level of the fronto-parietal suture of the skull (Fig. 110). The knife was inserted to a depth of 15 mm. from the outer surface of the skull, a distance which would carry its point a little ventral to the level of the commissure. An antero-posterior cut was then made through a distance of about 5 mm.

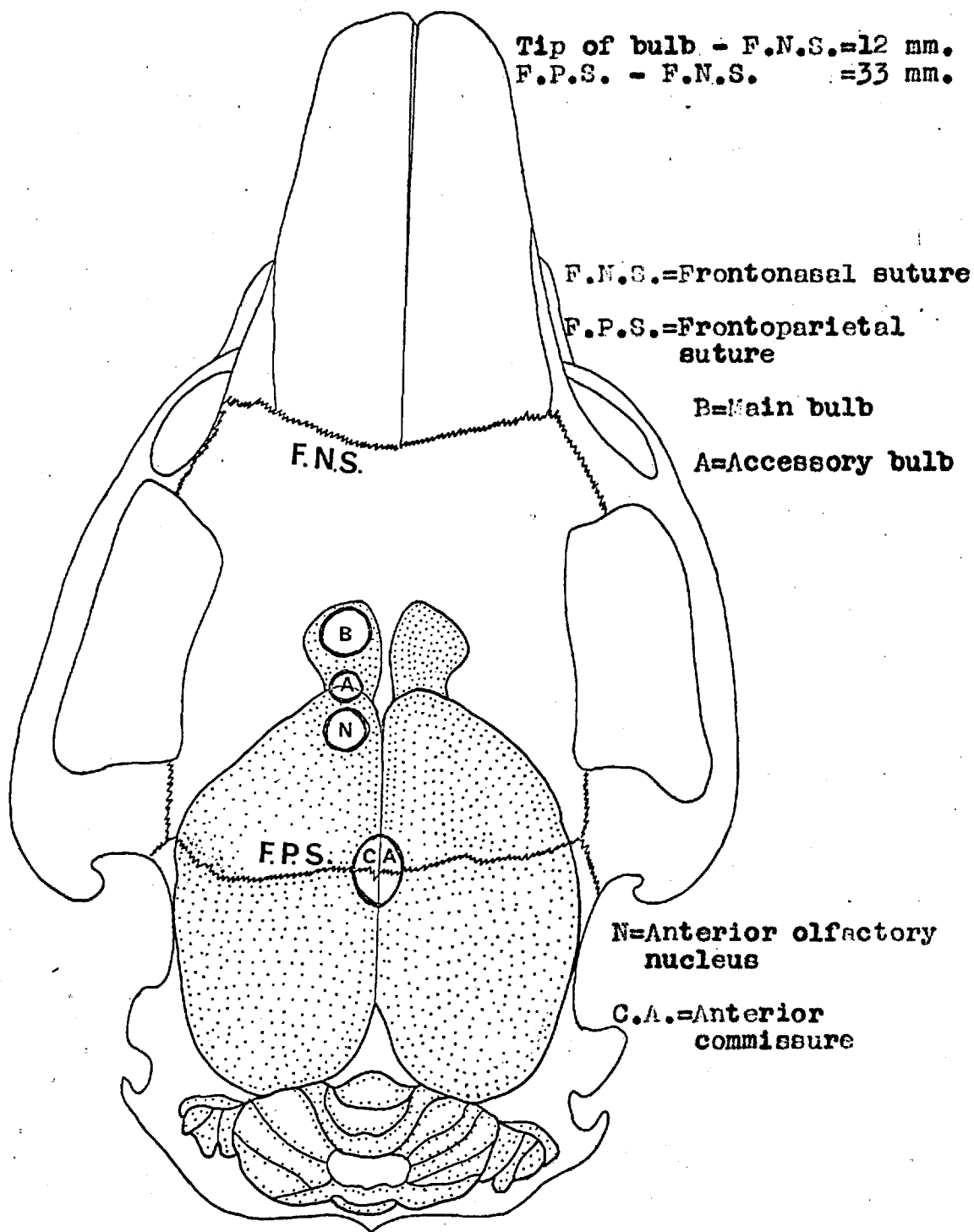


Fig. 110. A scale drawing showing the dorsal surface of the brain superimposed on a drawing of the skull to show the exact site where drilling was made for performing the surgical lesions. X 2.

APPENDIX II
(Neurohistological techniques)

Toluidine blue method (modified Nissl staining)

1. Fix tissue in 10% formalin (or acid alcohol).
2. Make celloidin section 30 μ thick (or paraffin 10 μ sections).
3. Put sections in 1% toluidine blue and 1% borax ($\frac{1}{2}$ -1 minute).
4. Rinse rapidly in water then transfer to 70% alcohol (2 seconds).
5. Differentiate for 1 second in Gothard's differentiator (pure creosote 50 ml., Cajuput oil 40 ml., xylene 50 ml., absolute alcohol 150 ml.) + chloroform when using celloidin.
6. Back to 70% alcohol and so on until properly differentiated.
7. Transfer to absolute alcohol + chloroform for a few seconds.
8. Clear in xylol.
9. Mount in balsam.

(Nissl bodies blue against unstained cytoplasmic background; nuclei nearly colourless - except for the nucleoli)

Loyez modification of Weigert method (for staining myelin sheath)

1. Fix material in 10% formalin.
2. Cut celloidin section (20-40 μ) and store in 70% alcohol.
3. Bring sections down to water and mordant them in saturated iron alum for 1 hour at 55°C.
4. Wash thoroughly in distilled water and stain for $\frac{1}{2}$ hour at 55°C in: 10% alcoholic haematoxylin 10 cc; distilled water 90 cc and saturated lithium carbonate 1 cc.
5. Pass through water and differentiate in saturated iron alum solution.
6. Wash well in water and differentiate again in: borax 2.0 g., potassium ferricyanide 2.5 g., distilled water 200 cc.
7. Wash thoroughly in distilled water, dehydrate, clear and mount.

(Blue myelin sheath against a colourless background.)

Lewis (1961) modification of Koelle technique for cholinesterase

1. Fix by perfusion with 10% formal in isotonic sodium sulphate followed by 7 hours in the same fixative at 4°C.
2. Immerse the fixed tissue in 20% aqueous alcohol overnight so as to improve the section-cutting.
3. Pipette about 1 ml of filtered incubation medium into consecutive cups of a large 'Perspex' tray. The incubation medium is made up as follows: dissolve 100 mg. acetylthiocholine iodide in 4 ml. distilled water.

Precipitate with 7 ml. of M/10 CuSO_4 . Add the initial portion a drop at a time shaking after each addition. Allow to stand for 10 minutes after completing addition of CuSO_4 . Centrifuge at 2,000 r.p.m. for 15-20 minutes.

Pipette off 10 ml. of supernatant and add to it 62 mg. glycine. When the glycine has dissolved add 2.5 ml. M/1 sodium acetate to give a pH of 5. Make up to a total volume of 50 ml. with distilled water.

4. Cut frozen sections 25-100 μ thick and transfer each section with a brush to the appropriate cup.
5. Keep the tray at room temperature. The incubation time could be varied between 3 to 6 hours (thicker sections need less time).
6. Remove incubation medium with a pipette and wash twice.
7. Transfer sections to a clean tray and wash with distilled water.
8. Replace the water with sodium sulphide solution prepared by dissolving 2.5 g. sodium sulphide in 110 ml. N/5 acetic acid to give a final pH of 5-6.
9. After 2 minutes pipette off the sulphide solution and refill the cups with distilled water.
10. Mount sections on albumenized slides dry, then store overnight at 37°C. Take them directly to absolute alcohol, xylene and then cover in neutral balsam.

(Brown deposit of copper sulphide in cholinergic neurones.)

Nauta-Gygax technique

1. Fix the brain in 10% neutral formalin for 2 weeks to 6 months.
2. Cut frozen sections 15-25 μ .
3. Mount sections on gelatinized slides and flatten them.
4. Wash briefly in distilled water, and soak sections in 0.5% phosphomolybdic acid for 30 minutes.
5. Without washing, transfer sections to 0.05% potassium permanganate for 6 $\frac{1}{2}$ min.
6. Assemble sections in distilled water and decolourize in a mixture of equal parts of 1% hydroquinone and 1% oxalic acid, 2 minutes.
7. Wash thoroughly in 3 changes of distilled water and soak sections for 15 min. in 1.5% silver nitrate.
8. From here on transfer sections individually. Quick wash in distilled water, and transfer sections to a mixture of: silver nitrate 4.5%, ethanol 10 ml., strong ammonia 2.3 ml., sodium hydroxide 1.5 ml, 2.5% aq. Only $\frac{1}{2}$ min. in this solution.
9. Transfer the sections to the reducing fluid (distilled water 400 ml., pure ethanol 45 ml., 10% formalin 13.5 ml., 1% aqueous solution of citric acid 13.5 ml.) 10-15 min (a brown colour will develop within 1 min.)

10. Wash briefly, then pass the sections through 1% sodium thiosulphate for 10-15 min.
Wash in 3 changes of distilled water, up the alcohols to xylol, and mount in balsam.

* In the original technique the sections are mounted after staining.

(The degenerating axons are stained black and show typical drop-like disintegration with the normal axons staining brown.)

Swank-Davenport (1935) modification of Marchi technique

1. Perfuse the brain with 10% formalin then carefully remove the brain.
2. Fix in 10% formalin for 24-48 hours and then cut small blocks (not more than 3 mm thick).
3. Place these thin blocks for 7-10 days in the following solution: 1% potassium chlorate 60 cc, 1% osmic acid 20 cc, glacial acetic acid 1 cc, commercial formalin 12 cc.
Put glass-wool at the bottom of the container to ensure uniform impregnation.
4. Wash in running water for 24-36 hours.
5. Dehydrate thoroughly in graded alcohols.
6. Embed in celloidin after two changes in absolute alcohol.
7. Cut sections at 25 μ and store them in 70% alcohol.
8. Dehydrate individual sections in changes of 96% and then absolute alcohol.
9. Clear in xylol and mount in Canada balsam.
10. Dry the slides overnight in the oven at 37°C.

BIBLIOGRAPHY

- ADEY, W.R. (1953). An experimental study of the central olfactory connexions in a marsupial (Trichosurus vulpecula). Brain, 76, 311-330.
- ADEY, W.R. & MEYER, M. (1952). Hippocampal and hypothalamic connexions of the temporal lobe in the monkey. Brain, 75, 358-383.
- ADEY, W.R., RUDOLPH, A.F., HINE, I.F. & HARRITT, N.J. (1958). Glee's staining of the monkey hypothalamus: a critical appraisal of normal and experimental material. J. Anat., Lond., 92, 219-235.
- ADRIAN, E.D. (1950). Sensory discrimination, with some recent evidence from the olfactory organ. Brit. med. Bull., 6, 330-332.
- ADRIAN, E.D. (1955). Potential oscillations in the olfactory organ. J. Physiol., 128, 21P.
- ALLEN, W.F. (1940). Effects of ablating frontal lobes, hippocampi and occipito-parieto temporal (excepting piriform areas) lobes on positive and negative olfactory conditioned reflexes. Am. J. Physiol., 128, 754-771.
- ALLEN, W.F. (1941). Effect of ablating the piriform-amygdaloid areas and hippocampi on positive and negative conditioned reflexes and on conditioned olfactory discrimination. Am. J. Physiol., 132, 81-92.
- ALLISON, A.C. (1953a). The structure of the olfactory bulb and its relationship to the olfactory pathways in the rabbit and rat. J. comp. Neurol., 98, 309-355.
- ALLISON, A.C. (1953b). The morphology of the olfactory system in the vertebrates. Biol. Rev., 28, 195-244.
- ALLISON, A.C. (1954). The secondary olfactory areas in the human brain. J. Anat., Lond., 88, 481-488.
- ALLISON, A.C. & WARWICK, R.T.T. (1949). Quantitative observations on the olfactory system of the rabbit. Brain, 72, 186-197.
- ANDY, O.J. & STEPHAN, H. (1959). The nuclear configuration of the septum of Galago Demidovii. J. comp. Neurol., 111, 503-545.
- ANDY, O.J. & STEPHAN, H. (1961). Septal nuclei in the Soricidae (Insectivora). Cytoarchitectonic study. J. comp. Neurol., 117, 251-273.

- BALDWIN, M., FROST, L.L. & CHARLES, D.W. (1956). Investigation of the primate amygdala: movements of the face and jaws. 2. Effect of selective cortical ablation. *Neurology (Minn.)*, 6, 288-293.
- BARGMANN, W. & SCHADE, J.P. (1963). The rhinencephalon and related structures. *Progress in Brain Research*, Vol. 3. Amsterdam, Elsevier Publishing Co.
- BAUMGARTEN, R. von, GREEN, J.D. & MANCIA, M. (1962). Recurrent inhibition in the olfactory bulb. II. Effects of antidromic stimulation of commissural fibres. *J. neurophysiol.*, 25, 489-500.
- BECCARI, N. (1910). Il lobo paraolfattorio nei mammiferi. *Arch. Ital. di Anat. e di Embryol.*, 9, 173-220.
- BERKELBACK van der SPRENKEL, H. (1926). Stria terminalis and amygdala in the brain of the opossum. *J. comp. Neurol.*, 42, 211-254.
- BERRY, C.M., HAGAMEN, W.D. & HINSEY, J.C. (1952). Distribution of potentials following stimulation of olfactory bulb in cat. *J. neurophysiol.*, 15, 139-148.
- BOWSER, D., BRODAL, A. & WALBERG, F. (1960). The relative values of the Marchi method and some silver impregnation techniques. *Brain*, 83, 150-160.
- BREATHNACH, A.S. (1953). The olfactory tubercle, prepiriform cortex and precommissural region of the porpoise (*Phocaena phocaena*). *J. Anat., Lond.*, 82, 96-113.
- BREATHNACH, A.S. & GOLDBY, F. (1954). The amygdaloid nuclei, hippocampus and other parts of the rhinencephalon in the porpoise (*Phocaena phocaena*). *J. Anat., Lond.*, 88, 268-291.
- BROCA, P. (1878). Anatomie comparée des circonvolutions cérébrales. I. Le grand lobe limbique et la scissure limbique dans la série des mammifères. *Rev. d'Anthropol.* 2nd series, 1, 385-498.
- BRODAL, A. (1947a). The hippocampus and the sense of smell. *Brain*, 70, 179-222.
- BRODAL, A. (1947b). The amygdaloid nucleus in the rat. *J. comp. Neurol.*, 87, 1-16.
- BRODAL, A. (1948). The origin of the fibres of the anterior commissure in the rat. Experimental studies. *J. comp. Neurol.*, 88, 157-205.

- BRODAL, A. (1963). The rhinencephalon and related structures. In: Progress in Brain Research. (Ed. Bargmann & Schädé) Amsterdam, Elsevier Publishing Co.
- BRODMANN, K. (1909). Vergleichende Localisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues. Barth, Leipzig.
- BUTTERWORTH, K.R., SHILLITO, K.E., SPENCER, K.E.V. & STEWART, H.C. (1962). The laboratory use of Myocaster corpus (the coypu). J. Physiol., 162, 36P and 37P.
- CALLEJA, C. (1893). La region olfactoria del cerebro. Madrid N. Moya.
- CAJAL, S.R. (1911). Histologie du système nerveux de l'homme et des vertébrés. Paris, A. Maloine.
- CAJAL, S.R. (1955). Studies on the cerebral cortex (limbic structures). Translated by L.M. Kraft. London, Lloyd-Luke Ltd.
- CLARK, W.E. LE GROS (1957). Inquiries into the anatomical basis of olfactory discrimination. The Ferrier Lecture. Proc. Roy. Soc., 146, 299-319.
- CLARK, W.E. LE GROS & MEYER, M. (1947). The terminal connexions of the olfactory tract in the rabbit. Brain, 70, 304-328.
- COWAN, W.M. & POWELL, T.P.S. (1956). A note on terminal degeneration in the hypothalamus. J. Anat., Lond., 90, 188-192.
- CROGG, B.G. (1961). Olfactory and other afferent connections of the hippocampus in the rabbit, rat and cat. Exp. Neurol., 3, 588-600.
- CROGG, B.G. (1962). Centrifugal fibres to the retina and olfactory bulb, and composition of the supraoptic commissures in the rabbit. Exp. Neurol., 5, 406-427.
- CRAIGIE, E.H. (1925). An introduction to the finer anatomy of the nervous system based upon that of the albino rat. Philadelphia, Blakiston's Son & Co.
- CREVEL, H. Van & VERHAART, W.J.C. (1963a). The rate of secondary degeneration in the central nervous system. I. The pyramidal tract of the cat. J. Anat., Lond., 97, 429-450.

- CREVEL, H. Van & VERHAART, W.J.C. (1963b). The rate of secondary degeneration in the central nervous system. II. The optic nerve of the cat. *J. Anat., Lond.*, 97, 451-464.
- CROSBY, E.C. & HUMPHREY, T. (1939). Studies on the vertebrate telencephalon. I. Nuclear configuration of olfactory and accessory olfactory formations and of the nucleus olfactorius anterior of certain reptiles, birds and mammals. *J. comp. Neurol.*, 71, 121-213.
- CROSBY, E.C. & HUMPHREY, T. (1941). Studies on the vertebrate telencephalon. II. The nuclear pattern of the anterior olfactory nucleus, tuberculum olfactorium and amygdaloid complex in adult man. *J. comp. Neurol.*, 74, 309-352.
- CROSBY, E.C. & HUMPHREY, T. (1944). Amygdaloid complex in the shrew (Blarina brevicauda). *J. comp. Neurol.*, 81, 285-306.
- CROSBY, E.C., HUMPHREY, T. & LAUER, E.W. (1962). Correlative anatomy of the nervous system. New York, Macmillan.
- DAITZ, H.M. & POWELL, T.P.S. (1954). Studies on the connexions of the fornix system. *J. Neurol. Psychiat.*, 17, 75-82.
- DE LORENZO, A.J. (1957). Electron microscopic observation of the olfactory mucosa and olfactory nerve. *J. biophys. biochem. Cytol.*, 3, 839-850.
- DE LORENZO, A.J. (1960). Electron microscopy of the olfactory and gustatory pathways. *Ann. Otolaryng., Rhin. Laryng.*, 69, 410.
- ECONOMO, C.V. (1929). The cytoarchitectonics of the human cerebral cortex. London, Oxford University Press.
- ELLIOT, H.C. (1963). Textbook of Neuroanatomy. Philadelphia & Montreal, Lippincott.
- ELLIOT SMITH, G. (1895). Morphology of the true "limbic lobe", corpus callosum, septum pellucidum and fornix. *J. Anat. & Physiol.*, 30, 157-167; 185-205.
- ELLIOT SMITH, G. (1897). Notes on the Olfactory Centre. Proceedings of the Intercolonial Medical Congress of Australasia, 4th session, Dunedin.
- ELLIOT SMITH, G. (1901). Notes on the natural subdivisions of the cerebral hemisphere. *J. Anat. & Physiol.*, 35, 431-454.

- ELLIOT SMITH, G. (1909). The tuberculum olfactorium. Anat. Anz., 34, 200-206.
- ERHART, E.A. (1950). Olfactory and parolfactory regions in brains of whites and negroes (external function). J. comp. Neurol., 93, 297-311.
- EVANS, D.H.L. & HAMLYN, L.H. (1956). A study of silver degeneration methods in the central nervous system. J. Anat., Lond., 90, 193-203.
- FOLDES, F.F., ZSIGMOND, E.K., FOLDES, V.M. & ERDOS, E.G. (1962). Distribution of cholinesterase in the human brain. J. Neurochem., 9, 559-572.
- FOX, C.A. (1940). Certain basal telencephalic centers in the cat. J. comp. Neurol., 72, 1-62.
- FOX, C.A. (1943). Stria terminalis, longitudinal association bundle and precommissural fornix fibers in cat. J. comp. Neurol., 79, 277-296.
- FOX, C.A., FISHER, R.R. & DESALVA, S.J. (1948). The distribution of the anterior commissure in the monkey (Macaca mulatta). Experimental studies. J. comp. Neurol., 89, 245-278.
- FOX, C.A. & SCHMITZ, J.T. (1943). A Marchi study of the distribution of the anterior commissure in the cat. J. comp. Neurol., 79, 297-314.
- FOX, C.A. MCKINLEY, W.A. & MAGOUN, H.W. (1944). An oscillographic study of the olfactory system of cats. J. Neurophysiol., 7, 1-16.
- FULTON, J.F. (1943). Physiology of the nervous system. Oxford University Press.
- GASSER, H.S. (1956). Olfactory nerve fibers. J. Gen. Physiol., 39, 473-496.
- GASTAUT, H. & LAMMERS, H.J. (1961). Anatomie du rhinencéphale. Paris, Masson et Cie.
- GEHUCHTEN, A. van. (1904). Contribution à l'étude des voies olfactives. Le Nevraxe, VI, 193-200.
- GEHUCHTEN, A. van & MARTIN, I. (1891). Le bulbe olfactive chez quelques mammifères. La Cellule, 7, 205-237.
- GEREBTZOFF, H.A. (1959). Cholinesterases. Pergamon Press, London.

- GLEES, P. (1946). Terminal degeneration within the central nervous system as studied by a new silver method. *J. Neuropath. exp. Neurol.*, 5, 54-59.
- GLEES, P. & NAUTA, W.J.H. (1955). A critical review of studies on axonal and terminal degeneration. *Mechr. Psychiat. Neurol.*, 129, 74-91.
- GOLDBY, F. (1934). The cerebral hemispheres of Lacerta viridis. *J. Anat., Lond.*, 68, 157-215.
- GOLDBY, F. (1961). Recent Advances in Anatomy. Ed. Goldby & Harrison. London, Churchill. Chapter 10 - "Rhinnencephalon".
- GOLDBY F. & KACKER, G.N. (1963). A survey of the pyramidal system in the coypu rat (Myocaster coypus). *J. Anat., Lond.*, 97, 517-531.
- GOLGI, C. (1875). Sulla fina struttura dei bulbi olfactorii. Reggio-Emilia.
- GRAY, P.A., Jr. (1924). The cortical lamination pattern of the opossum, Didelphys virginiana. *J. comp. Neurol.*, 37, 221-263.
- GREEN, J.D., CLEMENTE, C.D. & DE GROOT, J. (1961). In: *Activites du rhinnencephale*. Paris, Masson.
- GREEN, J.D., MANCIA, M. & VON BAUMGARTEN, R. (1962). Recurrent inhibition in the olfactory bulb. I. Effects of antidromic stimulation of the lateral olfactory tract. *J. Neurophysiol.*, 25, 467-488.
- GURDJIAN, E.S. (1925). Olfactory connections of the albino rat, with special reference to the stria medullaris and anterior commissure. *J. comp. Neurol.*, 38, 127-163.
- GURDJIAN, E.S. (1928). The corpus striatum of the rat. Studies on the brain of the rat. *J. comp. Neurol.*, 45, 249-281.
- HERRICK, C.J. (1910). The morphology of the forebrain in amphibia and reptilia. *J. comp. Neurol.*, 20, 413-547.
- HERRICK, C.J. (1924). The nucleus olfactorius anterior of the opossum. *J. comp. Neurol.*, 37, 317-359.
- HUGHES, A.F.W. & LEWIS, P.R. (1961). Effect of limb ablation on neurones in *Xenopus* larvae. *Nature*, 189, 333-334.

- HUMPHREY, T. (1936). The telencephalon of the bat. I. The non-cortical nuclear masses and certain pertinent fiber connections. J. comp. Neurol., 65, 603-711.
- ISHII, T. (1957). The histochemical studies of cholinesterase in the central nervous system. I. normal distribution in rodents. Arch. Hist. Jap., 12, 587-612.
- JOHNSON, Th.N. (1957). Studies on the brain of the guinea pig. I. The nuclear pattern of certain basal telencephalic centre. J. comp. Neurol., 107, 353-369.
- JOHNSON, Th.N. (1959). Studies on the brain of the guinea pig. II. The olfactory tracts and fornix. J. comp. Neurol., 112, 121-140.
- JOHNSTON, J.B. (1923). Further contribution to the study of the evolution of the forebrain. J. comp. Neurol., 35, 337-481.
- JONES, W.H. & THOMAS, D.B. (1962). Changes in the dendritic organization of neurons in the cerebral cortex following deafferentation. J. Anat., Lond., 96, 359-375.
- KAADA, B.R. (1951). Somato-motor, autonomic and electrographic responses to electrical stimulation of 'rhinencephalic' and other structures in primates, cat and dog. Acta Physiol., Scand., 24 (Suppl. 83), 1-285.
- KAADA, B.R., ANDERSEN, P. & JANSEN, J. Jr. (1954). Stimulation of the amygdaloid nuclear complex in unanaesthetised cats. Neurology 4, 48-64.
- KACKER, G.N. (1964). An experimental investigation of the pyramidal motor system in the coypu rat (Myocaster coypus). A Thesis submitted for the Degree of M.Sc. (Anatomy), London University.
- KAPPERS, C.U.A., HUBER, G.C. & CROSBY, E.C. (1936). The comparative anatomy of the nervous system of vertebrates, including man. 2. New York, Macmillan.
- KERR, D.I.B. & HAGBARTH, K.E. (1955). An investigation of olfactory centrifugal fiber system. J. Neurophysiol., 18, 362-374.
- KOELLE, G.B. (1954). Histochemical localization of cholinesterases in the central nervous system of the rat. J. comp. Neurol., 100, 211-236.

- KLASS, M. (1928). A note on the olfactory striae in man. *J. comp. Neurol.*, 47, 171-188.
- KOIKEGAMI, H. (1963). Amygdala and other related limbic structures, experimental studies on the anatomy and function. I. Anatomical researches with some neurophysiological observations. *Acta Med. Biol.*, 10, No. 3, 161-277.
- KOIKEGAMI, H., FUSE, S., YOKOYAMA, T., WATANABE, T. & WATANABE, H. (1955). Contributions to the comparative anatomy of the amygdaloid nuclei of mammals with some experiments of their destruction or stimulation. *Folia Psychiat. Neurol. Jap.*, 8, 336-370.
- LAUER, E.W. (1945). Nuclear pattern and fibre connections of certain basal telencephalic centres in the Macaque. *J. comp. Neurol.*, 82, 215-254.
- LEWIS, P.R. (1961). The effect of varying the conditions in the Koelle technique. *Bibl. Anat.*, 2, 11-20.
- LEWIS, P.R. & HUGHES, A.F.W. (1957). The cholinesterase of developing neurones in *Xenopus laevis*. In: Metabolism of the nervous system. London, Pergamon Press.
- LOEWENTHAL, S. (1897). Ueber das Riechhirn der Säugethiere. Festschrift zur LXIX Versammlung Deutscher Naturforscher Aerzte. Braunschweig (pp. 213-220).
- LOHMANN, A.H.M. (1963). The anterior olfactory lobe of the guinea pig. An experimental anatomical study. *Acta Anatomica*, 53, suppl. 49, 1-109.
- LOHMANN, A.H.M. & LAMMERS, H.J. (1961). An experimental study of the secondary olfactory connexions in the guinea pig and the cat. *Acta Morph. Neurol. Scand.*, 14, 285.
- LOHMANN, A.H.M. & LAMMERS, H.J. (1963). On the connections of the olfactory bulb and anterior olfactory nucleus in some mammals. An experimental anatomical study. In: Progress in Brain Research. Amsterdam, Elsevier Publishing Co.
- LOO, Y.T. (1930). The forebrain of the opossum Didelphis virginiana. I. Gross anatomy. *J. comp. Neurol.*, 51, 13-64.
- LOO, Y.T. (1931). The forebrain of the opossum Didelphis virginiana. II. Histology. *J. comp. Neurol.*, 52, 1-148.

- LORENTE de NO, R. (1934a). Studies on the structure of the cerebral cortex. I. The area entorhinalis. *J. Psychol. Neurol.*, 45, 381-438.
- LORENTE de NO, R. (1934b). Studies on the structure of the cerebral cortex. II. Continuation of study of ammonic system. *J. Psychol. Neurol.*, 46, 113-177.
- MacLEAN, P.D. (1952). Some psychiatric implications of physiological studies on fronto-temporal portion of the limbic system (visceral brain). *E.E.G. & Clin. Neurophys.*, 4, 407-418.
- MacLEAN, P.D. (1954). Studies on the limbic system (visceral brain) and their bearing on psychosomatic problems. In: Recent developments in psychosomatic medicine. Ed. R.A. Cleghorn & E. Wittkower. Philadelphia, Lippincott Co.
- McCOTTER, R.E. (1912). The connection of the vomero-nasal nerves with the accessory bulb in the opossum and other mammals. *Anat. Rec.*, 6, 299-318.
- MORIN, F. (1950). An experimental study of hypothalamic connections of the guinea pig. *J. comp. Neurol.*, 92, 193-213.
- NAUTA, W.J.H. (1957). Silver impregnation of degenerating axons. In: New Research Techniques of Neuroanatomy. Ed. W.F. Windle. Illinois, Springfield.
- NAUTA, W.J.H. (1958). Hippocampal projections and related neural pathways to the midbrain in the cat. *Brain*, 81, 319-340.
- NAUTA, W.J.H. (1961). Fibre degeneration following lesions of the amygdaloid complex in the monkey. *J. Anat.*, Lond., 95, 515-531.
- NAUTA, W.J.H. & GYGAX, P.A. (1954). Silver impregnation of degenerating axons in the central nervous system: a modified technique. *Stain Technol.*, 29, 91-93.
- OBENCHAIN, J.B. (1925). The brains of the South American marsupials *Caenolestes* and *Orolestes*. *Field Mus. Nat. Hist. Publ.* 224, Zool. Ser. 14, 175-232.
- O'LEARY, J.L. (1937). Structure of the primary olfactory cortex of the mouse. *J. comp. Neurol.*, 67, 1-31.
- OWEN, R. (1868). On the anatomy of vertebrates. Vol. 3. Mammals. London, Longmans, Green & Co.

- PAPEZ, J.W. (1937). A proposed mechanism for emotion. Arch. Neurol. Psychiat., 38, 725-743.
- PHILLIPS, C.G., POWELL, T.P.S. & SHEPHERD, G.M. (1963). Responses of mitral cells to stimulation of the lateral olfactory tract in the rabbit. J. Physiol., 168, 65-88.
- PILLERI, G. (1961). Beiträge zur vergleichenden morphologie des nagetiergehirnes. 7-10 Beitrag. Acta Anat., 44, Suppl. 42, 1-82.
- POWELL, T.P.S. & COWAN, W.M. (1963). Centrifugal fibres in the lateral olfactory tract. Nature, 199, 1296-1297.
- POWELL, T.P.S. & COWAN, W.M. (1964). A note on retrograde fibre degeneration. J. Anat., Lond., 98, 579-585.
- POWELL, T.P.S., COWAN, W.M. & RAISMAN, G. (1963). Olfactory relationships of the diencephalon. Nature, 199, 710-712.
- PRIEBRAM, K.H. & KRUGER, L. (1954). Functions of the "olfactory brain". Ann. N.Y. Acad. Sci., 58, 109-138.
- PROBST, M. (1901). Zur kenntniss des faserverlanges des temporallappens, des bulbus olfactorius, der vorderen commissur und des fornix nach entsprechenden extirpationen und Durchschneidungsversuchen. Arch. Anat. Physiol. Anat. Abt., 338-356.
- ROSE, J.E. & WOOLSEY, C.N. (1943). Potential changes in the olfactory brain produced by electrical stimulation of the olfactory bulb. Fed. Proc. exp. Biol., 2, 42.
- ROSE, M. (1926). Der allocortex bei tier und Mensch. I. J. Psychol. Neurol., 34, 1-111.
- ROSE, M. (1927). Der allocortex bei tier und Mensch. II. Die gog. riechrinde beim mensch und affen. J. Psychol. Neurol., 34, 261-401.
- ROSE, M. (1929). Cytoarchitektonischen atlas der grosshirnrinde der saus. J. Psychol. Neurol. 40, 1-51.
- SANDERS-WOUDSTRA, J.A.R. (1961). Experimenteel anatomisch onderzoek over de verbindingen van enkele basale telencephale hersengebieden bij de albino rat. Thesis. Groningen.
- SCHREINER, L. & KLING, A. (1956). Rhinencephalon and behaviour. Am. J. Physiol., 184, 486-490.

- SHEPHERD, G.W. (1963). Neuronal systems controlling mitral cell excitability. *J. Physiol.*, 168, 101-117.
- SHUTE, C.C.D. & LEWIS, P.R. (1960). The salivatory centre in the rat. *J. Anat., Lond.*, 94, 59-73.
- SHUTE, C.C.D. & LEWIS, P.R. (1961). The use of cholinesterase technique 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*, 199, 1160-1164.
- SWANK, R.L. & DAVENPORT, H.A. (1935). Marchi's staining method. *Stain Tech.*, 10, 45-52.
- SWANN, H.G. (1934). The function of the brain in olfaction. II. The results of destruction of olfactory and other nervous centres upon the discrimination of odours. *J. comp. Neurol.*, 59, 175-201.
- TADAYASU, B. & OMUKAI, F. (1959). Experimental studies on the fibre connections of the amygdaloid nuclei in the rabbit. *J. comp. Neurol.*, 113, 245-280.
- THOMPSON, M. (1932). On the cavum septi pellucidi. *J. Anat., Lond.*, 67, 59-77.
- TURNER, W. (1890). Convolutions of the brain. *J. Anat. & Physiol.*, 25, 105.
- VALVERDE, F. (1963a). Studies on the forebrain of the mouse. Golgi observations. *J. Anat., Lond.*, 97, 157-180.
- VALVERDE, F. (1963b). Amygdaloid projection field. "The rhinencephalon". In: Progress in Brain Research. Amsterdam, Elsevier Publishing Co.
- WHITE, L.E. (1962). Terminal and preterminal degeneration pattern following olfactory bulb lesions in the rat. *Anat. Rec.*, 142, 333.
- WHITE, L.E. (1964). Personal communications.
- WINKLER, C. & POTTER, A. (1911). An anatomical guide to experimental researches on the rabbit's brain. Amsterdam, W. Versluys.
- WINKLER, C. & POTTER, A. (1914). An anatomical guide to the experimental researches on the cat's brain. Amsterdam, W. Versluys.

- YAMAMOTO, C., YAMAMOTO, T. & IWANA, K. (1963). The inhibitory systems in the olfactory bulb studied by intracellular recording. *J. Neurophysiol.*, 26, 403-415.
- YOUNG, M.W. (1936). The nuclear pattern and fibre connections of the non-cortical centers of the telencephalon in the rabbit (Lepus cuniculus). *J. comp. Neurol.*, 65, 295-401.
- YOUNG, M.W. (1941). Degeneration of the fibre tracts following experimental transection of the olfactory bulb. *Anat. Rec.*, 79, 65-66.
- YOUNG, M.W. (1942). Further studies on the interbulbar fibres. *Anat. Rec.*, 82, 413.