# AN EXPERIMENTAL STUDY OF THE SECONDARY OLFACTORY CONNEXIONS IN *TESTUDO GRAECA*

By H. J. GAMBLE

*Department of Anatomy, St Mary's Hospital Medical School,
Paddington, London, W. 2*

## INTRODUCTION

Secondary olfactory connexions have received much attention in recent years, and experimental studies have shown that they are less extensive in mammals than had been thought (Clark & Meyer, 1947; Meyer & Allison, 1949). Experimental investigations of this problem in one reptilian species (*Lacerta viridis*—Goldby, 1937; Gamble, 1952a) have indicated that here also secondary olfactory connexions are of very much more limited extent than commonly been supposed. It has been shown, moreover (Gamble, 1952a) that secondary olfactory fibres in *Lacerta* cross via the habenular commissure to the opposite hemisphere, and that the anterior commissure in this species is in no way concerned with the secondary olfactory pathways. It seemed desirable that these findings, the one so similar to, the other so markedly different from, the findings in mammals, should be investigated in another and less specialized reptilian species, and for this *Testudo graeca* was chosen.

Despite such obvious specialization as the carapace and plastron, the Chelonia are the only living reptiles which have preserved a skull form at all closely resembling that of the primitive anapsid reptiles from which all later amniotes have been derived. It might reasonably be expected that the Chelonian brain would show features characteristic of early reptiles in general; certainly the published descriptions of Chelonian brains (Johnston, 1915; Schepers, 1948) suggest that specialization is little advanced.

## MATERIALS AND METHODS

Specimens of *T. graeca* of both sexes and averaging about 6 inches in length were used. Both before and after operation the animals were kept in a cage heated to approximately 30° C.

### Operative technique

Ether being apparently valueless, chloroform anaesthesia was used, and the addition of one to two drops of ammonia, apparently acting as a respiratory stimulant, facilitated the onset of anaesthesia to a sufficient depth. The skull was opened with a dental drill and the dura incised. Lesions in the brain were made with a cystotome or small curette. The wound was then filled with penicillin and sulphathiazol crystals and sealed with a solution of Perspex in acetone which dried rapidly to form a hard covering.

The lesions consisted in the removal of, or damage to, the main olfactory bulb, or the main and accessory olfactory bulbs, both unilaterally and bilaterally. Damage

to the accessory olfactory bulb alone was also attempted. The extreme shortness of the olfactory peduncle, and the situation of the accessory bulb (inset, as it were, into the dorsum of the main bulb), made lesions precisely limited to one or other bulb virtually impossible. Mortality was low (1 in 12) and, when it occurred, death appeared to be due to subdural bleeding. In none of the animals was any change in behaviour noted; feeding was often resumed within an hour of consciousness being regained.

*Histological methods*

(i) Nissl series were prepared from normal specimens. The brains were fixed in formol-acetic-alcohol and complete series were cut in the three primary planes after paraffin embedding. The sections were stained with cresyl violet or with a mixture of cresyl violet and thionone.

(ii) Silver impregnated series were prepared by Nonidez's (1939) technique, and sections cut at 7·5 and 10 $\mu$. The normal series consisted of one in a transverse and one in a horizontal plane. The experimental material consisted of eleven brains from which satisfactory serial sections were obtained and in which the lesion, survival time, etc., were as follows:

(*a*) Unilateral damage to or removal of main olfactory bulb: one specimen, surviving 8 days.

(*b*) Unilateral damage to or removal of main and accessory olfactory bulbs: seven specimens, surviving 5, 6, 7, 9, 10, 11 days.

(*c*) Unilateral damage to accessory olfactory bulb: one specimen surviving 7 days.

(*d*) Bilateral damage to, or removal of, main and accessory olfactory bulbs: three specimens, two surviving 10 days, the other for 8 days.

*The olfactory and related systems in normal material*

Certain features of the brain of *T. graeca* were described briefly by Goldy (1936); much longer accounts of the brain of other Chelonians are also available, notably those by Johnston (1915, 1923) for *Cistudo carolina* and by Schepers (1948) for *Testudo geometrica*. From these accounts it appears that both *Cistudo carolina* and *Testudo geometrica* resemble *T. graeca* very closely in cerebral morphology. Johnston and Schepers, however, were largely concerned with evolutionary problems and with the institution of homologies, often of a very detailed and somewhat speculative character, which are not relevant to the present investigation. It seemed desirable, therefore, to summarize the main features of the Chelonian forebrain, as seen in *T. graeca*, with particular emphasis on those parts most directly concerned with olfaction.

The olfactory bulb is relatively very large and is attached to the hemisphere by a short thick peduncle as in primitive mammals; the accessory bulb is small and is situated on the dorsal and posterior aspect of the main bulb. The hemisphere is elongated, its posterior pole overlapping the anterior part of the midbrain, and is marked by two longitudinal fissures: a dorso-lateral fissure in the anterior half or two-thirds of the hemisphere which, because of its relation to underlying cortical areas, has been homologized with the rhinal fissure of mammals, and a ventro-lateral fissure. The latter, as it is followed caudally, veers towards the basal surface

of the hemisphere, and again because of its relationship to underlying structures has been identified as the endorhinal fissure anteriorly and the amygdaloid fissure posteriorly (Johnston, 1915).

The cell masses of the hemisphere can be described briefly as follows. An ill-defined cellular zone surrounding the root of the olfactory peduncle forms the anterior olfactory nucleus, very detailed accounts of which were given by Crosby & Humphrey (1939) for several chelonians. The general arrangement in all is similar to that seen in *Testudo*. Behind this nucleus the hemisphere may be divided into basal and pallial (dorsal) regions. The pallial region contains the cortical areas of which there are three, hippocampal, dorsal and pyriform, arranged as longitudinal strips, situated medially, dorsally and laterally respectively. The basal region consists of the paraterminal body or septum medially and a ventro-lateral cell mass commonly referred to as the 'strio-amygdaloid' complex. It will be noted that this terminology, as well as that of the cortical areas, is largely based on generally accepted mammalian homologies; it should be noted also that the lateral boundary between pallial and basal structures is not very definite, since the ventro-lateral edge of the pyriform cortical area encroaches on the region occupied by the strio-amygdaloid complex.

The cortical area chiefly relevant to this investigation is the pyriform. It consists of a cell-lamina in which many of the cells are fusiform in shape and arranged perpendicular to the surface, though many other cells, less easily classified, are present. It can be divided into a dorsal part where the cells are large and compactly arranged and a ventral part where they are smaller and more scattered (Text-figs. 1, 2). Posteriorly this ventral part forms the nucleus of the lateral olfactory tract, and becomes continuous with certain basal structures to be described later.

Little need be said here of the other two cortical areas. Both are poorly differentiated in comparison with many other reptiles, and some of their cytoarchitectural features can be seen in Text-figs. 1–6. The hippocampal area shows the usual division into large- and small-celled parts (Text-fig. 4), although neither is very extensive or clearly defined. The contrast with such reptiles as *Anolis* and the geckos among Lacertilia and with many snakes is very marked. The dorsal cortical area is the least well defined, and its cells for the most part remain in the primitive position close to the ependyma (Text-figs. 2–5); laterally it is separated from the pyriform cortex over most of its extent by a cell-free gap, which coincides with the overlying rhinal fissure. Medially it is continuous with the large-celled part of the hippocampus, but differences in cell size and arrangement enable a distinction to be made. Anteriorly the three cortical areas all blend with the anterior olfactory nucleus, posteriorly with an undifferentiated sheet of cells covering the posterior pole.

Of the basal structures the paraterminal body or septum forms the whole thickness of the medial wall of the hemisphere below the hippocampal cortex and anterior to the interventricular foramen. It is comparatively small and shows only very indistinct nuclear differentiation in the tortoise; its connexions are thought to be largely with the hippocampal cortex, the hypothalamus and the olfactory tubercle, but so far as the present work is concerned the most significant bundle of fibres related to it is the so-called 'anterior olfacto-habenular tract' which is described below.

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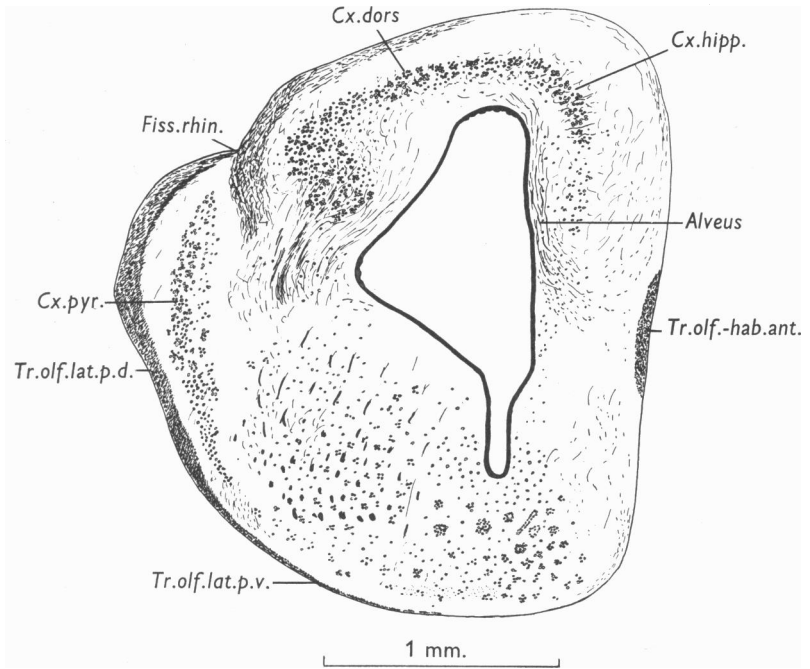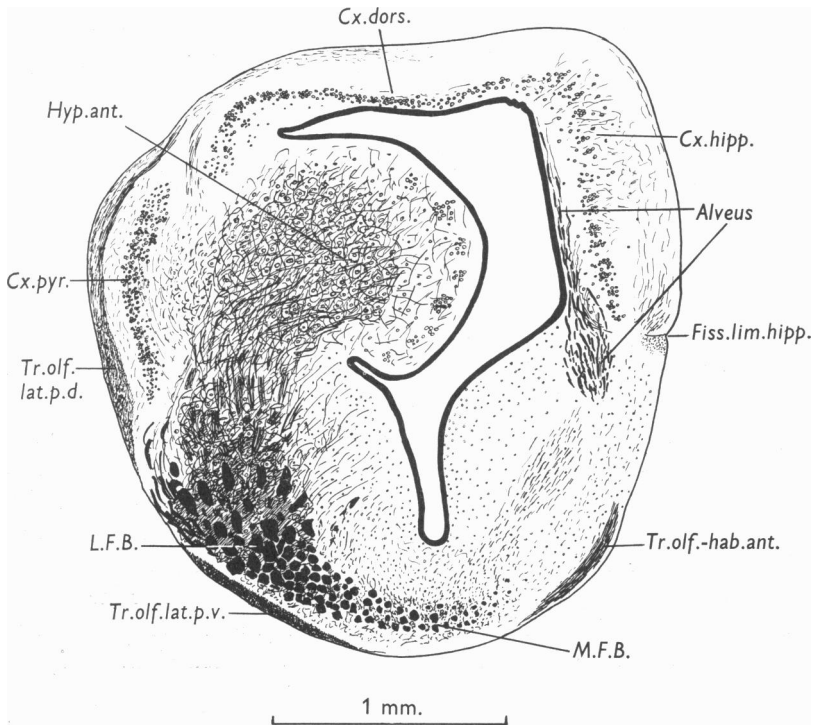
Text-fig. 1



Text-fig. 2

The main element in the basal structures of the hemisphere is the laterally situated strio-amygdaloid complex. On the basis of cell type and distribution three components can be recognized in it. A collection of mainly small and clumped cells extends from the anterior olfactory nucleus to the level of the lamina terminalis; it is bounded dorsally by a deep ventricular sulcus and extends medially round the ventral angle of the ventricle into relation with the paraterminal body (Text-figs. 1, 2). This is the palaeostriatum, or 'olfacto-striatum' as it has sometimes been
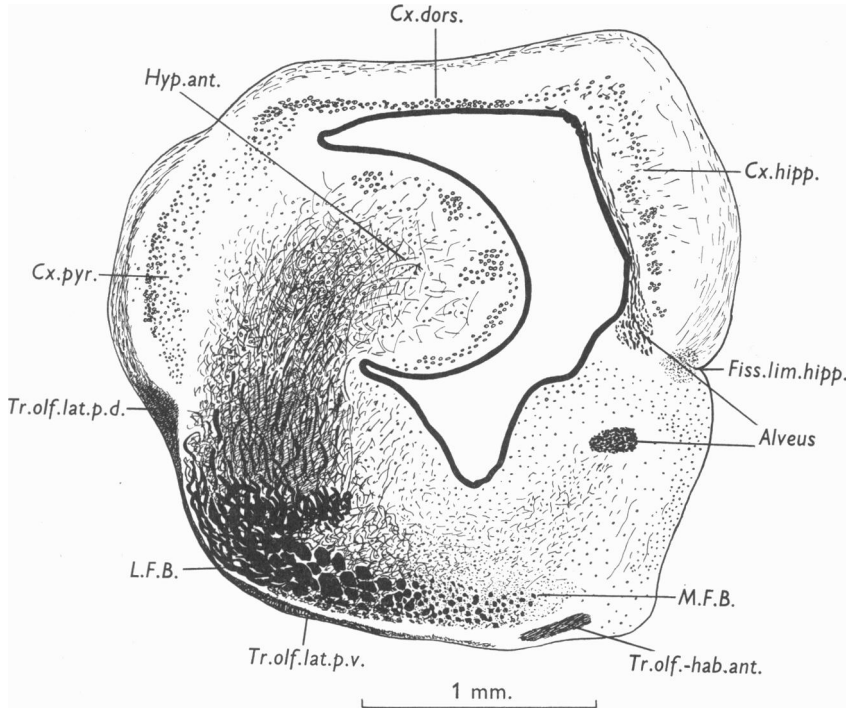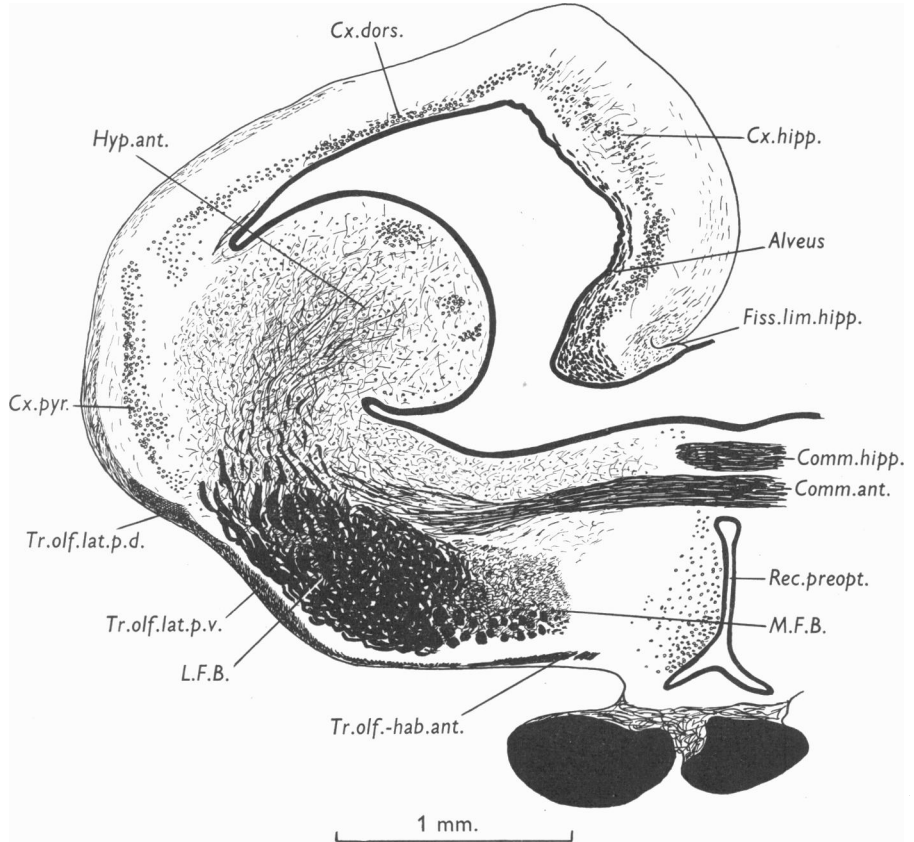


Text-fig. 3

called. Ventrally it bulges slightly from the surface and is covered by a very ill-defined layer of cells, the 'olfactory tubercle' of common usage. The whole palaeo-striatum is permeated by the basal forebrain bundle system of fibres, and like this system, may be divided, somewhat arbitrarily, into medial and lateral parts.

A massive projection into the ventricle dorsal to the palaeostriatum (Text-figs. 2–6) extends throughout the length of the ventro-lateral cell mass. Its cells form a thick lamina close to the ependyma and tend to be arranged in clumps. Posteriorly these cells are in apparent continuity with those of the pyriform cortex (Text-fig. 5), but anteriorly there is continuity with neither pyriform nor dorsal cortices. The concavity of the main cellular lamina contains smaller, scattered cells. The whole structure is known as the hypopallium and can be subdivided, on the basis of its appearance in silver impregnated sections, into anterior and posterior

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parts. The anterior part contains a dense plexus of fibres which fan out beneath the cellular lamina and appear to join the the lateral forebrain bundle (Text-fig. 2). Posteriorly these connexions are lacking, and instead bundles of fibres are found lying next to the ependyma and apparently running to or from the anterior commissure. Some of these fibres reach laterally to the deep surfaces of the dorsal and pyriform cortices (Text-figs. 5, 6).
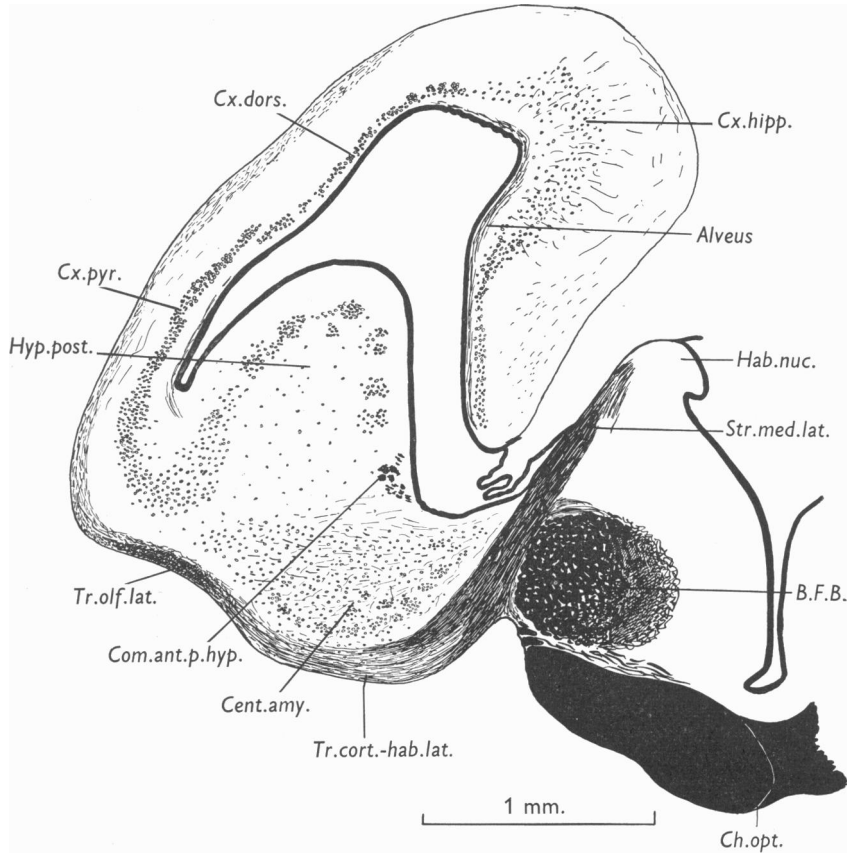


Text-fig. 4

Behind the palaeostriatum, and below the posterior part of the hypopallium, lies a large but rather ill-defined collection of cells, which has a tenuous continuity with the nucleus of the lateral olfactory tract. Fibres of the lateral olfactory tract lie on its surface where they appear to end, while its deeper cells are reached by or give origin to fibres of the anterior commissure and stria terminalis. By its situation and apparent connexions this structure exhibits the major characteristics of an amygdaloid nucleus, and is referred to hereafter as the central amygdaloid mass.

Of the fibre tracts only those relevant to the present investigation will be described. The most important of these are the secondary olfactory fibres which stream posteriorly from between the mitral and granule cell layers of the bulb. It is not possible to distinguish main from accessory bulbar fibres. All these fibres collect

ventro-laterally in the peduncle, and after passing the anterior olfactory nucleus they spread to form a broad layer over the hemisphere extending from the rhinal fissure above to the olfactory tubercle below (Text-fig. 1). Where they cover the anterior part of the pyriform cortex and the lateral part of the olfactory tubercle they are arranged in a plexiform manner, running in all directions, suggestive of
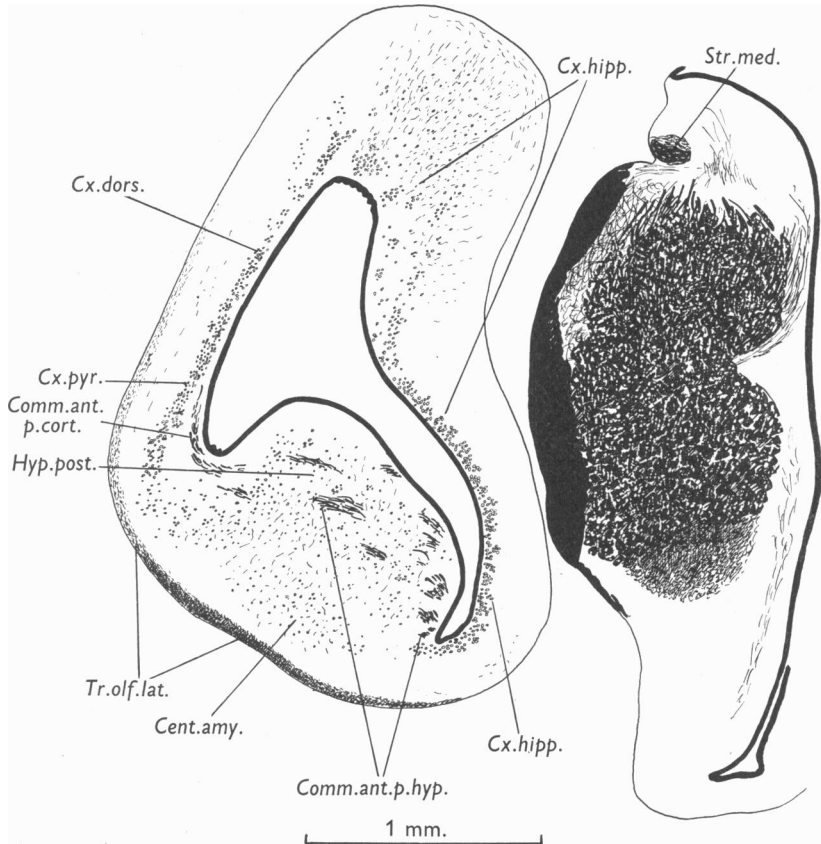


Text-fig. 5

connexions being effected with the underlying cells. A little posterior to this level (Text-fig. 2) fibres of the lateral forebrain bundle reach the surface, and the stream of secondary olfactory fibres is separated into dorsal and ventral parts for a short distance, but these parts soon come together (Text-fig. 4) so that the broad continuity of the tract is regained. At this and at more posterior levels only a few fibres are found superficial to the cells of the pyriform cortex, most being aggregated on the surface of the nucleus of the lateral olfactory tract. With this nucleus they pass to the central amygdaloid mass (Text-figs. 5, 6).

The fibres so far described form the lateral olfactory tract, which is particularly large in Chelonia. So far as normal material is concerned it is impossible of course to say whether the more posterior fibres have come directly from the mitral cells of the

bulb, or whether there have been intermediate relays, for example, in the anterior olfactory nucleus. The more ventrally situated fibres which spread on to the olfactory tubercle probably represent the medial olfactory tract described by Johnston (1915). According to Johnston, some of these fibres extended even more medially into the paraterminal (his paraolfactory) region and to the hippocampus. This did not appear to be the case in *Testudo graeca*, and one may add also that nothing was found resembling the so-called olfactory component of the anterior commissure present in many other reptiles.



Text-fig. 6

Experimental degenerations in *Lacerta viridis* (Gamble, 1952a) have shown that a considerable part of a system of fibres known as the stria medullaris consisted of secondary olfactory fibres. In *Testudo* the stria medullaris is a composite bundle on the anterior and dorsal aspects of the thalamus (Text-fig. 6), extending from the habenular commissure (where most of its fibres cross) into the basal part of the hemisphere below the interventricular foramen (Text-fig. 5). In this region the stria is joined by three sets of fibres. From the lateral side it is joined by fibres which appear to stream medially from two sources: (i) from the ventral part of the lateral

olfactory tract (Text-fig. 5), and (ii) from the surface of the posterior part of the pyriform cortex, the 'lateral cortico-habenular tract', which has been widely recognized in other reptiles and in amphibia (Herrick, 1948). The third contribution to the stria medullaris is the anterior olfacto-habenular tract, a compact bundle which can be followed forwards and dorsally near the surface of the paraterminal body (Text-figs. 3, 1 and 2 and Pl. 1, fig. 4) until it is lost over the anterior pole of the hemisphere. Here it comes into relationship with the hippocampal and dorsal cortical areas and the anterior olfactory nucleus. It should be noted that the terms 'cortico-habenular' and 'olfacto-habenular' both indicate that the fibres concerned run caudally to end in the habenular ganglia. This, of course, is uncertain, since the methods which have been used to demonstrate these tracts, at least in reptiles, can show only their topographical position and course. In one reptile, *Lacerta viridis*, (Gamble, 1952*a*), it has been shown by experimental degeneration that the 'olfacto-habenular tract' in fact runs in the opposite direction, that is from the region of the habenular ganglia cranially, and that it consists of secondary olfactory fibres which have crossed in the habenular commissure and probably have no functional connexion with the habenular ganglia. It was partly with the purpose of finding whether the olfacto-habenular tract in another and very different reptile was of a similar nature that the present investigation was undertaken.

Many other fibre tracts in the forebrain could of course be described, such as the stria terminalis, forebrain bundles, etc. The details are not relevant to the degeneration experiments to be reported, and the general position and characteristics of such tracts are sufficiently indicated in the Text-figs. 1–6.

### *Characteristics and distribution of experimental degeneration*

It is well known that various morphological changes can be demonstrated by silver impregnation methods along the course and at the termination of degenerating axons. The character of these changes depends on a large number of factors; the species of the animal, the particular tract of fibres which is being studied, the length of time allowed for degeneration and the technical method employed, are among the most important of these. In the tortoise, and using the Nonidez technique, the most conspicuous changes were found in secondary olfactory fibres about 10 days after removal of the bulb (Pl. 1, figs. 2, 3). At this time affected fibres are fragmented, leaving a considerable quantity of argyrophilic granular debris along their course, which is easily recognizable and gives an appearance strikingly different from that of the normal tract (cf. Pl. 1, figs. 1, 2). It is probable that even at this stage some debris has been removed, and in specimens which have survived a few days longer this process has gone so much further that loss of fibres is the most conspicuous feature in the affected tracts (Pl. 1, fig. 4). It is obvious that this loss of fibres will be observable only if at least a considerable proportion are affected, and even then it will be necessary to control the observation by comparison with the normal condition. Clearly the most convenient and satisfactory control will be comparison with the normal tract on the opposite side of the same animal, but this will be possible only when the effects of the lesion are unilateral so far as the tract under consideration is concerned. After shorter survival times (5–8 days) degenerat-

ing olfactory tract fibres in the tortoise show numerous irregular varicosities, but little fragmentation. Since these varicosities are not found in the normal brain, and also since their situation corresponds exactly with that of the axonal debris and loss of axons which can be observed after longer periods of survival, there seems no doubt that they constitute reliable evidence of degeneration in the conditions of these experiments. 'Terminal degeneration', whether in pericellular arborizations or in end bulbs, has been frequently reported, and when seen can provide most valuable and precise information about fibre connexions. In the present experiments, however, nothing was seen which could be interpreted with confidence as terminal degeneration. This does not mean that they provide no information about the connexions of fibre tracts. If axonal debris or any other visible result of degeneration is traced until a point is reached where it can no longer be seen, it is a reasonable inference that the affected fibres end in synaptic junctions near that point, although the synapses themselves may not be observed directly. Such inferences, though generally reliable, lack the detailed precision which clear-cut terminal degenerative changes can provide.

The experimental lesions were all limited to the olfactory bulbs, except in two cases where some damage, apparently due to interference with blood vessels, was found in the ventral and anterior parts of the palaeostriatum and anterior olfactory nucleus. Since the degeneration in these two specimens did not differ in its distribution from that in the others, this additional damage was clearly not extensive enough to cause demonstrable degeneration. In general, the results in all animals were similar in the distribution of degeneration, although the amount naturally varied according to the extent of the lesion. Owing to the proximity of the accessory to the main bulb in most specimens both received damage. Damage appeared to be limited to the main bulb in one case only, where almost inevitably it was very incomplete. The experiments, therefore, allowed no distinction to be made between the connexions of the main and accessory olfactory bulbs.

According to the survival time, axonal debris or axonal varicosities and small bulbous swellings could be found throughout the extent of the lateral olfactory tract described above. It was widespread and abundant in the dorsal part of the tract over about the anterior half of the pyriform cortical area. Posteriorly, behind the level of the lamina terminalis where the tract is reduced in size and limited to the region of the nucleus of the lateral olfactory tract along the ventral border of the pyriform cortex, degeneration was similarly limited in its distribution. It could be traced further posteriorly to become widespread again over the central amygdaloid mass, and, to a smaller extent, to penetrate into its substance. In two cases (in both of which the lesion had included the accessory olfactory bulb) a few axonal varicosities and bulbous swellings were found in the posterior part of the hypopallium, but such changes could not be traced into continuity with the lateral olfactory tract, and can be regarded as no more than suggestive evidence that secondary olfactory fibres reach this part of the brain. It should be noted that no evidence of degeneration was found over about the posterior half of the pyriform cortex in any specimen.

The degeneration mentioned so far was limited to the operated side in specimens where the lesion was unilateral so that comparison of the two sides could be used as a control. The more interesting findings, however, were made in secondary olfactory

fibres which crossed to the opposite side. The ventral part of the lateral olfactory tract, which spreads over the olfactory tubercle, showed extensive degenerative changes, strongly suggestive of connexions with the cells of the tubercle. The great majority of these degenerating axons, however, pass posteriorly to enter the stria medullaris where they could be followed through the habenular commissure into the opposite stria. From here they separated into two major pathways: (i) through the length of the 'anterior olfacto-habenular tract' to its termination on the surface of the anterior extremities of the hippocampal and dorsal cortices and the anterior olfactory nucleus, and (ii) laterally across the ventral surface of the hemisphere behind the olfactory tubercle to the surface of the pyriform cortex in about the middle of its extent and to the more anterior part of the central amygdaloid mass. This second pathway corresponds closely to the 'lateral cortico-habenular tract' described by many authors. Very extensive axonal destruction in the stria medullaris from a specimen in which both olfactory bulbs were removed is shown in Pl. 1, figs. 2 and 3, and compared with the appearance of the stria in a normal brain (Pl. 1, fig. 1). Pl. 1, fig. 4, illustrates loss of fibres from the contra-lateral anterior olfacto-habenular tract of a specimen which survived unilateral bulbar ablation for 11 days.

From these findings one may conclude that the olfactory tract fibres described in the first part of this paper consist predominantly of axons which have had their origin in the main and accessory olfactory bulbs, presumably from the mitral cells, and that these fibres run to the anterior half of the pyriform cortex, the nucleus of the lateral olfactory tract and the amygdala, all on the same side. That they end in these structures is almost certain, although unequivocal 'terminal' degeneration has not been seen. The olfactory tubercle can be added to these structures as receiving homolateral secondary olfactory fibres, and possibly the posterior part of the hypopallium. The evidence for the last connexion is not entirely convincing, and in any case involves only a few fibres. The homo-lateral connexions described have all been inferred from an examination of normal material, and the experimental evidence is merely a confirmation of the inferences. Negatively, the absence of experimental confirmation for the presence of direct bulbar connexions in the posterior pyriform cortex is of some importance, and a similar absence of homo-lateral bulbar connexions for the hippocampal cortex and paraterminal body may be pointed out. It must be admitted, however, that the presence of a few scattered degenerated fibres might easily be missed, so that the apparent absence of bulbar connexions from these regions may be relative only.

The presence, however, of widespread hetero-lateral bulbar connexions could not be inferred from an examination of normal material with the techniques usually employed. The degeneration experiments make it clear that the stria medullaris, anterior olfacto-habenular tract and lateral cortico-habenular tract consist largely of secondary olfactory fibres which have crossed or are about to cross, and that the habenular commissure is an olfactory commissure. Since some fibres persist in all these situations after bilateral ablation all the fibres concerned are not of this nature, though probably the majority are.

## DISCUSSION

These results may be compared with those in other reptiles from the point of view of the characteristics of the degeneration itself, and also from the point of view of the anatomical conclusions which can be reached.

So far as the degeneration itself is concerned, in *Lacerta viridis* (Gamble, 1952a) it was found that maximal formation of axonal varicosities was demonstrable in the olfactory pathways after 2 to 3 days' survival, and that granular axonal debris was never conspicuous and practically completely removed a few days later. This rapid and abrupt course of degeneration contrasted strongly with Armstrong's (1950) findings in the visual pathways of *L. vivipara* when survival periods of 2 weeks provided maximal evidence of axonal degeneration, and where debris persisted for as long as 2 months. Similar experiments in *Natrix natrix* (Armstrong, 1951) gave evidence of the persistence of axonal debris for as long as 4 months in some specimens. Obviously no general conclusions can be based on these observations; they emphasize, however, the variability, particularly in the time relationships, of degeneration in different species and different fibre systems.

The experimental degenerations which confirm inferences made from the study of normal material call for little comment, and this applies to almost all the homolateral findings. The absence (or relative absence) of bulbar connexions in the posterior pyriform cortex may be significant in relation to the observation that such connexions are also absent in the posterior pyriform or entorhinal area of mammals (Clark & Meyer, 1947; Meyer & Allison, 1949). The crossed connexions, however, are in a different category. They have previously been described only in *Lacerta viridis* (Gamble, 1952 a, b); and although in other reptiles the stria medullaris, anterior olfacto-habenular and lateral cortico-habenular tracts exist and pursue similar courses, that they should be composed of secondary olfactory fibres seemed, on the face of it, so unlikely that experimental investigation of their nature in at least one other reptile was desirable. Direct evidence that they do consist of crossing secondary olfactory fibres as in *Lacerta*, seems now to be adequate, and greatly strengthens the case for believing that such pathways are common to all reptiles.

The possible relationship between the habenular-crossing fibres of *Lacerta* and similar though differently named tracts in Amphibia has been discussed already (Gamble, 1952 a, b) and needs no further elaboration. Possible relationships with similar tracts in mammals were also discussed, but two points may now be considered further.

It was noted that the 'stria terminalis 5' component of the stria medullaris of the opossum (Loo, 1931) very closely resembled the anterior olfacto-habenular tract of *Lacerta*; equally it resembles that of *Testudo*. Other less striking comparisons may also be made. The 'anterior olfacto-habenular tract' and the 'lateral cortico-habenular tract' of the opossum differ from the similarly named tracts of *Testudo* only slightly. In the opossum both join the stria by passing medial to the internal capsule, while in *Testudo* they join it by passing lateral to the lateral forebrain bundle. It is also true that Loo's 'anterior olfacto-habenular tract', as such, extends only to the olfactory tubercle, but it is so intermingled with his 'tractus olfacto-hypothalamicus ventralis' that its precise extent could not be elucidated in normal material. It is certainly possible that the condition found in *Lacerta* and *Testudo*

may exist in the opossum, and the similarity in the arrangement of the components of the stria suggests a probability that this is so.

The secondary olfactory connexions in the phalanger have been investigated experimentally by Adey (1953). In general his findings accorded closely with those reported in the rabbit (Clark & Meyer, 1947) and in the monkey (Meyer & Allison, 1949), but with one exception possibly relevant in the present context. He described axonal degeneration in one component of the stria medullaris. This component, the 'periventricular olfacto-habenular tract' is the one which is least like the crossing olfactory components of the stria of *Testudo*, but the presence of any component of bulbar origin is of interest. It would be interesting to know whether or not similar degeneration occurred also on the opposite side and in the habenular commissure.

The finding of widespread and extensive terminal degeneration in the bed nucleus of the stria terminalis and certain of the amygdaloid nuclei of the unoperated hemisphere has been common to all recent experimental investigations of secondary olfactory connexions in mammals so that the problem of where crossing occurs is raised. The axons whose terminals have been seen to degenerate have not themselves been identified throughout their course. Axonal degeneration has, it is true, sometimes been reported in the anterior commissure; but in the phalanger 'the anterior commissure forms an exceedingly large bundle, but degenerating fibres are very few' (Adey): and in the rabbit, when the lesion definitely excluded the anterior olfactory nucleus 'relatively few fibres of the anterior commissure were found to be degenerated' (Clark & Meyer). In the monkey, although the anterior commissure is said to be the pathway used by the crossing fibres, no evidence of this has been presented (Meyer & Allison, 1949). Indeed the only evidence directly bearing on this problem is the result of one experiment in the rabbit described by Allison (1953) where terminal degeneration was reported bilaterally in the bed nucleus of the stria terminalis following transection of the anterior limb of the anterior commissure. Even here possible damage to the olfactory tract cannot be excluded with certainty, and some degeneration was also present in the outer layers of the prepyriform cortex. While a crossing in the anterior commissure may be the likeliest pathway for these secondary olfactory fibres, it can hardly be considered proven, and the present findings suggest that a course through the habenular commissure should be considered.

In reptiles which have been experimentally investigated, the 'olfactory component of the anterior commissure' either does not exist, e.g. *Testudo*, or is wholly unaffected by removal of the olfactory bulbs, e.g. *Lacerta* (Gamble, 1952b). Equally, in both species there is no evidence that interbulbar fibres exist. Both these features appear to be mammalian characteristics, and to be related to the presence of characteristically 'tufted' cells in the mammalian olfactory bulb (Allison, 1953). Crossing olfactory fibres which undoubtedly exist in reptiles must, in the absence of 'tufted' cells, be the axons of mitral cells and they cross in the habenular commissure. It is reasonable to suppose that similar pathways may exist in mammals in addition to new interbulbar fibres in the anterior commissure; the constancy of the pattern formed by the components of the stria medullaris in reptiles and mammals could alone provide a *prima facie* case for such a hypothesis.

## SUMMARY

Degenerative changes in secondary olfactory fibres following experimental lesions in the olfactory bulbar apparatus of *Testudo graeca* have been traced in serial silver impregnated sections. An essentially similar pattern to that described in *Lacerta viridis* (Gamble, 1952 *a*) has been demonstrated. Its possible relevance to the pattern of secondary olfactory fibres in mammals has been discussed, with particular emphasis on those components which transverse the stria medullaris and cross in the habenular commissure.

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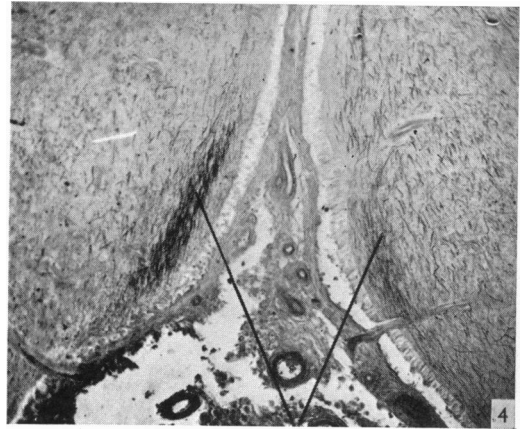
Stria med. lat.      Lat. forebrain bundle
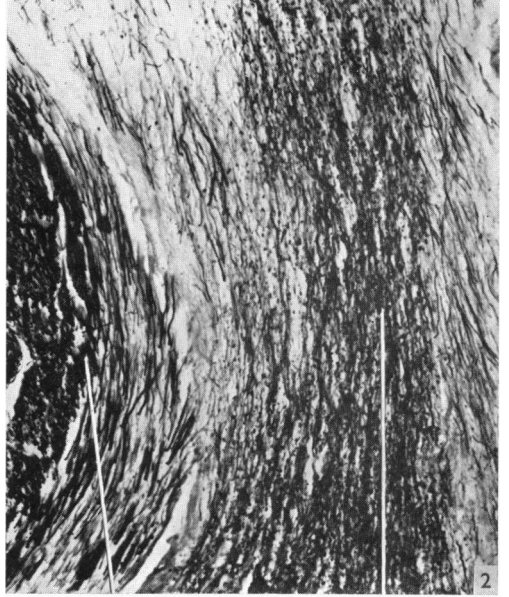


Lat. forebrain bundle      Stria med. lat.
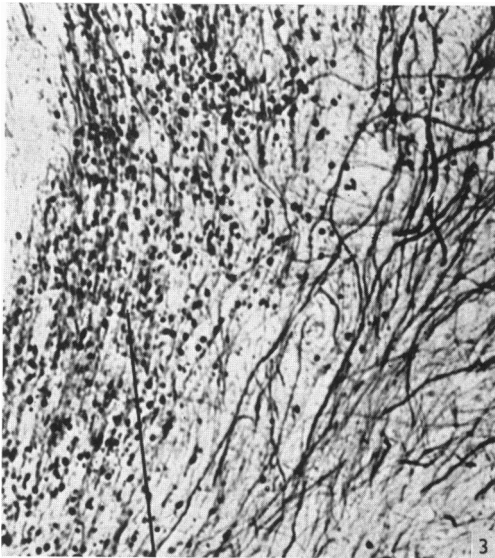


Stria med. lat.



Ant. olf.-hab. tr.

GAMBLE—SECONDARY OLFACTORY CONNEXIONS IN *TESTUDO GRAECA*

## *List of Abbreviations used in Text-figures*

| | | | |
|---|---|---|---|
| *B.F.B.* | Basal forebrain bundle | *Hab.nuc.* | Habenular nuclei |
| *Cent.amy.* | Central amygdaloid mass | *Hyp.ant.* | Hypopallium anterior |
| *Ch.opt.* | Optic chiasma | *Hyp.post.* | Hypopallium posterior |
| *Comm.ant.* | Anterior commissure | *L.F.B.* | Lateral forebrain bundle |
| *Comm. ant. p. cort.* | Anterior commissure, cortical component | *M.F.B.* | Medial forebrain bundle |
| *Comm.ant.p.hyp.* | Anterior commissure, hypopallial component | *Rec.preopt.* | Preoptic recess |
| | | *Str.med.* | Stria medullaris |
| *Comm.hipp.* | Hippocampal commissure | *Tr.cort.-hab.lat.* | Lateral cortico-habenular tract |
| *Cx.dors.* | Dorsal cortex | *Tr.olf.lat.* | Lateral olfactory tract |
| *Cx.hipp.* | Hippocampal cortex | *Tr.olf.lat.p.d.* | Lateral olfactory tract, dorsal part |
| *Cx.pyr.* | Pyriform cortex | *Tr.olf.lat.p.v.* | Lateral olfactory tract, ventral part |
| *Fiss.lim.hipp.* | Fissura limitans hippocampi | | |
| *Fiss.rhin.* | Rhinal fissure | *Tr.olf.-hab.ant.* | Anterior olfacto-habenular tract |

## EXPLANATION OF PLATE

Fig. 1. Transverse section through the normal brain in a plane similar to that shown in Text-fig. 5. The stria medullaris is normal and is cut longitudinally. ( × 220.)

Fig. 2. Tranverse section in a plane similar to that in fig. 1, 10 days after bilateral removal of the olfactory bulb. Axonal debris is present and very conspicuous in the stria medullaris, although some normal fibres persist. ( × 220.)

Fig. 3. Section passing longitudinally through the stria medullaris from the same specimen as in fig. 2. The axonal debris is clearly shown. ( × 340.)

Fig. 4. Transverse section through the septal region of the brain 11 days after unilateral bulbar ablation. The anterior olfacto-habenular tract is much reduced on the unoperated side. ( × 95.)