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The efferent connexions of the olfactory bulb in the frog: a study of degenerating unmyelinated fibres

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INTRODUCTION

The secondary olfactory pathways in the frog (*Rana pipiens*) are described in this paper as they were revealed by a Cajal on-the-slide method applied to the staining of experimentally induced degeneration of the olfactory tracts. Previously, only the reptilian and mammalian olfactory connexions had been studied by the experimental method. Degeneration of the frog's lateral olfactory tract was being studied in this laboratory in an effort to determine which of various silver-staining methods is best suited for demonstrating the degeneration of unmyelinated axons in the central nervous system (the frog's lateral olfactory tract contains very few myelinated axons). Being able to demonstrate degenerating unmyelinated fibres is important because many major fibre systems in the brains of the lower vertebrates are composed of unmyelinated axons and because the terminal branches of many mammalian fibre systems may be unmyelinated.

MATERIALS AND METHODS

Data were obtained from the brains of forty-one male leopard frogs (*Rana pipiens*) in which lesions were inflicted in the olfactory bulb, the accessory olfactory bulb, or the lateral wall of the hemisphere or in which larger parts of the hemisphere were removed. In addition, one sham-operated frog was prepared. After the lesions were inflicted the frogs were allowed to survive from one day to several weeks. They were then killed and their brains taken for histological examination of the lesion and of the distribution of degeneration.

Surgery was performed under ether anaesthesia. The brain was approached either dorsally, by opening the frontal bone, or laterally, by enucleating the eye and opening the medial wall of the orbit. The latter approach facilitated access to the ventrocaudal end of the bulb and lateral wall of the hemisphere. The lesions were inflicted with small steel knives or knives fashioned from small glass flakes. The latter were capable of cutting easily into the soft, yielding brain tissue. The relevant bits of tissue were removed by aspiration after they were excised. The lesions were placed with the aid of a stereoscopic, dissecting microscope. Finally, the skin was loosely closed with suture thread. The animals survived for 24 h in a damp terrarium and were afterwards transferred to an aquarium in which they could either remain in the water or sit upon floats. The temperature of the laboratory air and water varied with the seasons from a water temperature as low as 12 °C to air temperature as high as 28 °C. Accordingly, the frogs survived under seasonally varying conditions of temperature (see Table 1).

The frogs were killed by perfusion with saline under ether anaesthesia. The saline was quickly followed by 10% limestone-buffered formalin. The brains were removed, stored in the fixative less than 6 months, and embedded in high-grade gelatin for frozen sectioning. Sections were taken at 25–30 μm and stored in serial order. One brain was embedded in paraffin for Holmes (1943) staining. Three brains were stained in the block by Nonidez's method (1939) without perfusion or formalin fixation.

Table 1. *Lesion and survival data**

Survival		
Temperature	No. of days	Lesion description
Low	1, 2, 2, 3, 5, 6, 6, 6	Olfactory bulb transected transversely
High	5, 5, 5, 5, 5, 5, 6, 7, 7, 7, 7, 11, 28	
High	6, 6, 6, 7, 7	Olfactory bulb transected obliquely
Low	6	Accessory olfactory bulb selectively destroyed
High	7, 8	
Low	5, 5, 6	Lateral olfactory tract transected
High	1, 3, 5, 5, 5, 22	
High	4, 6	Anterior half of hemisphere removed
Low	5	Entire hemisphere removed
Low	5	No lesion (sham operated)

* The number of days of survival for each frog used is indicated on the left. The number of frogs used can be obtained by counting the numerical entries. The kind of lesion inflicted is indicated on the right. Whether the frog survived at relatively high or low temperature is also indicated. Low = 25 °C or less. High = more than 25 °C.

Sections from the formalin-fixed brains were stained with thionin, with two forms of the Nauta–Laidlaw technique (Nauta, 1957), with a Cajal stain for frozen sections (Romeis, 1948), or with the Holmes stain for axons. The Nauta stain was used either with phosphomolybdic acid, as usual, or with uranyl nitrate instead (5% for 20 min or overnight as recommended by Nauta in personal communication). The Cajal stain (described in Romeis) was modified slightly and used in the following way when the best results were obtained:

(1) Sections were affixed to slides with albumin and dried.

(2) Slides were rinsed in water and immersed in 2% silver nitrate (132 ml), 100% ethanol (66 ml), and pyridine (4·2 ml). Ten slides were stained together in the dark for 5 h.

(3) Slides were washed with agitation for 15–20 s in 100% ethanol.

(4) They were transferred to: hydroquinone, 0·6 g; concentrated formalin, 40 ml; acetone, 30 ml; distilled water, 140 ml; in which they remained *without agitation* for 3 min.

(5) Slides were washed once in distilled water and transferred to 1% sodium thiosulfate for 1 min.

(6) They were finally washed very well, dehydrated, cleared, and covered, using permount.

Good sections have a pale yellow to rust coloured background which is free from precipitate and dendritic processes. The nuclei of the nerve cells stain well but the perikarya are faint. Unmyelinated axons are black or brown. Myelinated axons are brown to yellow and may stain indistinctly or be speckled with silver. The perikarya and dendrites may be stained more strongly by increasing the temperature at which step 2 is carried out and by decreasing the pyridine concentration of the silver bath. The effect is not consistent, however, and the background may become speckled and dark.

Several series of unoperated brains were available for comparison with the experimental material. Weil-, Nissl-, and Cajal-stained material was examined.

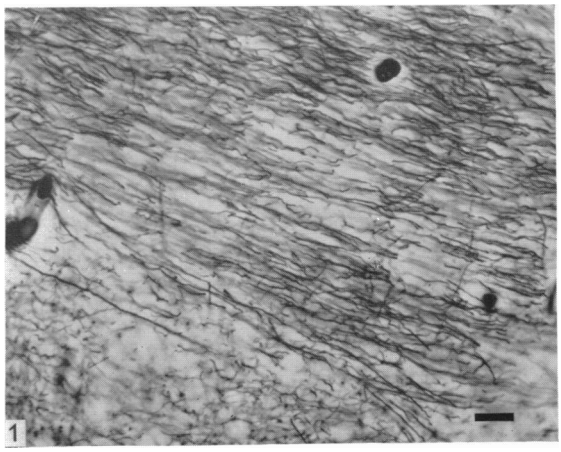
NOMENCLATURE

Nomenclature for the frog brain was taken largely from Hoffman (1963), with the following exceptions. The term 'lateral olfactory tract' is used in place of Hoffman's 'dorsolateral olfactory tract', in order to facilitate comparison between the frog and mammals, which possess a lateral olfactory tract. Hoffman's 'lateral olfactory tract' which is consistent with Herrick's (1921) terminology, includes both the dorsolateral olfactory tract and the ventrolateral olfactory tract. The latter is thought to connect the accessory olfactory bulb to the amygdala and it follows a course near the ventricle; accordingly, a mammalian homologue for it is not known. The terms 'lateral pallium' and 'dorsal pallium' are used in place of Hoffman's terms 'primordium piriform area' and 'primordium general pallium' in order to avoid the homology implied by the last term. The terms 'retrobulbar pallium', 'extragranular plexiform layer', and 'nucleus of the hemispheric sulcus' belong to the present authors. The last structure was drawn by Herrick (1921, fig. 7) but it was not named. 'Retrobulbar pallium' was adapted from Rose's (1931) 'retrobulbar region' and is used in order to avoid using the term 'anterior olfactory nucleus' (see Discussion). The other two terms do not appear in other literature. The region labelled 'nucleus of the hippocampal commissure' corresponds to the region so named in Herrick's papers (1927, 1933) on urodeles and does not correspond to Hoffman's bed nucleus of the hippocampal commissure. Finally, the term 'eminentia postolfactoria' is taken from Gaupp (1899) and Rothig (1926).

RESULTS

Appearance of the degeneration

The lateral olfactory tract of the frog is well suited to the study of unmyelinated axons in the central nervous system for the following reasons. It is a superficial structure accessible to surgical approach. The tract is relatively straight and its fibres run in parallel array. The axons, which are 0·2–0·5 μm in diameter, are individually visible. The proportion of myelinated axons, as revealed by the Weil stain, is very small in the anterior part of the tract and drops off to zero posteriorly. Figure 1 shows a longitudinal section through the anterior part of the tract which is stained by the Cajal method. Individual Cajal-stained fibres may be seen. Figure 2 shows the same level in a Weil stain. The normal, unmyelinated fibres were stained not only by the Cajal method but by the Nonidez, which is an in-the-block Cajal stain, by the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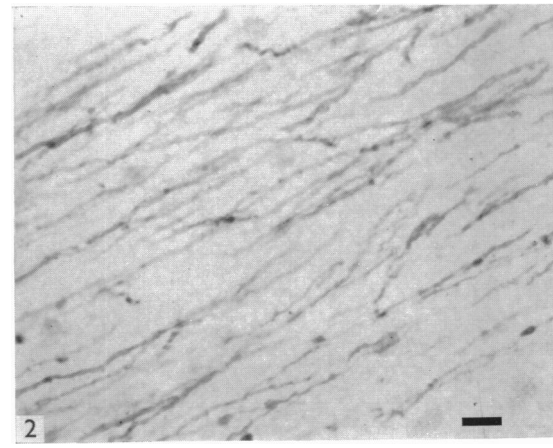
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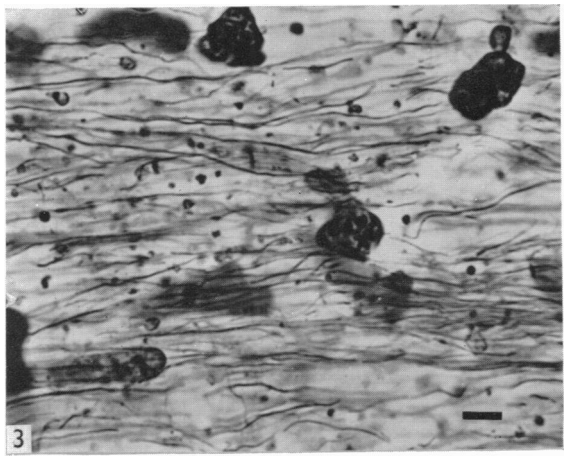
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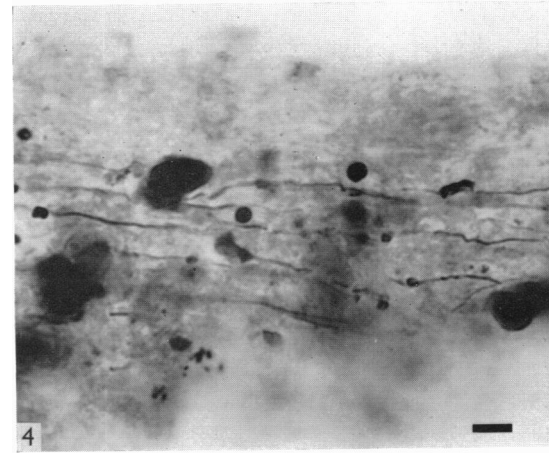
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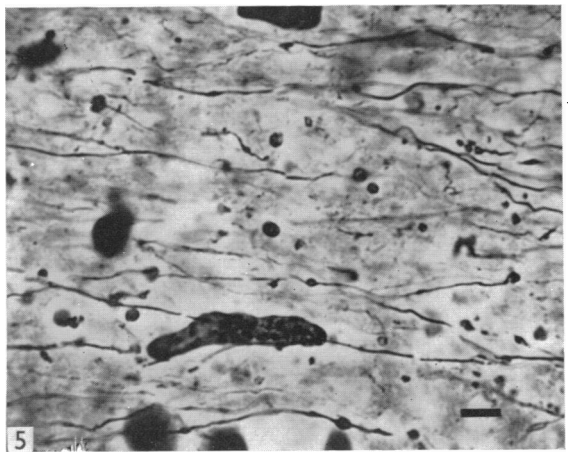
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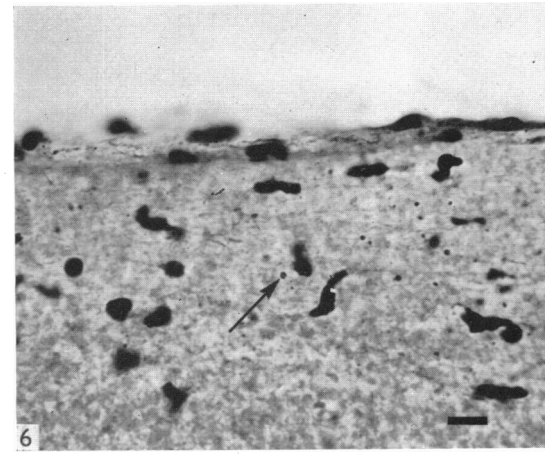
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Holmes, and by the Nauta–Laidlaw method when uranyl nitrate was substituted for phosphomolybdic acid. The normal, unmyelinated fibres did not stain with the phosphomolybdic acid method even when the potassium permanganate treatment was attenuated and formalin was added to the reducer. Such procedures caused strong staining of normal, myelinated fibres in the olfactory tract and in other parts of the brain.

The earliest sign of degeneration due to olfactory bulb lesions was an increase in number and staining intensity of small, ringlike objects which are variably present in normal brains. The rings, which were observed in Cajal preparations, were present in the zone of finer fibres internal to the lateral olfactory tract and in the tract just beneath the pia. Some rings were found among the fibres of the olfactory tract posteriorly, where it was less dense. Some of the rings were circular, some were elliptical, and some were angular. They occurred free, with a tail, or having two tails at opposite poles as though interrupting a fibre. The rings largely disappeared from those animals surviving 5 d or more in relatively warm conditions although with other kinds of lesions larger rings have been observed among other degenerative signs after many weeks survival.

A more conspicuous and reliable sign of degeneration, because it did not appear in normal animals, the sham operated animal, nor in the three animals which sustained a selective lesion of the accessory bulb, was the occurrence of a dispersion of moderately large opaque spheres among the fibres of the olfactory tract (Figs. 3, 5). The spheres varied in diameter from 1 to 3 μm. The impression was gained that the spheres increase in size with the age of the lesion, the largest spheres occurring after 7 d survival. With increased size there often occurred decreased opacity. The smallest spheres stained black in Cajal sections, the colour of the associated axons, while many of the larger spheres stained brown to amber but retained a darkly stained, sometimes irregular border. The earlier occurring, smaller spheres were rarely seen attached to a tract fibre but were dispersed among the fibres. The older, larger spheres, however, were often attached to the end of a fibre segment. Accompanying the appearance of the spheres was a reduction in the number of olfactory tract fibres. Both fibres and spheres disappeared after the longer survival periods. In one animal which sustained a complete olfactory bulb lesion and survived 11 d in the warmth neither axons nor spheres were present in the position of the affected lateral olfactory tract.

Fig. 1. Anterior end of lateral olfactory tract photographed in longitudinal section. Cajal. Bar = 11 μm.

Fig. 2. Same region as in Fig. 1. Weil. Bar = 11 μm.

Fig. 3. Degeneration in posterior, totally unmyelinated part of lateral olfactory tract after 4 d postoperative survival. Cajal. The large dark objects are cell nuclei. Bar = 5 μm.

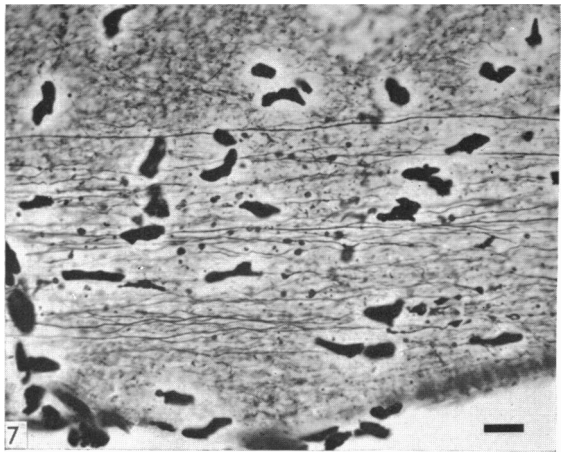
Fig. 4. Same region as in Fig. 3 after 6 d survival. Bar = 5 μm. Nauta stain using uranyl nitrate.

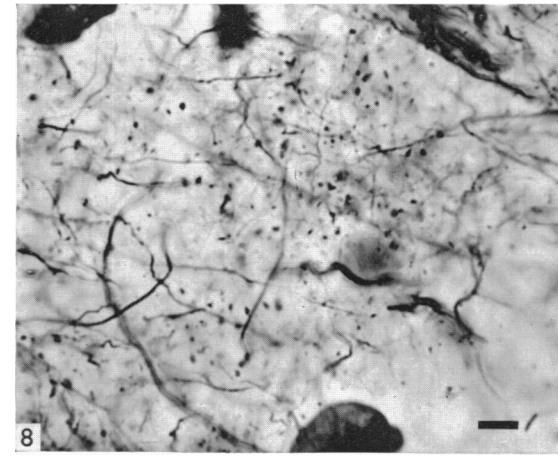
Fig. 5. Section adjacent to that in Fig. 4. Bar = 5 μm. Cajal stain.

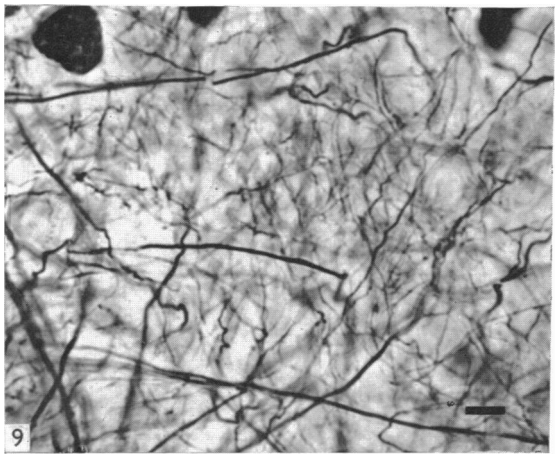
Fig. 6. Same region as in Fig. 4 after 7 d survival. Bar = 11 μm. Nauta stain using phosphomolybdic acid. Arrow points at a degeneration-sphere.

Another type of spherular degeneration product was always amber in colour in the Cajal sections. It appeared in the plexiform layer (extragranular plexiform zone) of the olfactory bulb between the mitral cells and the granule cells. This is a zone rich in dendritic and fine axonal branches.

Degeneration spheres were observed among still other groups of degenerating unmyelinated fibres. Smaller spheres than those appearing within the degenerating lateral olfactory tract occurred in the zone of fine fibres deep to the tract. Still smaller droplets, spindle-shaped forms, and angular granules were observed in the optic tectum. (It should be recalled that in some frogs one eye was excised in order to gain







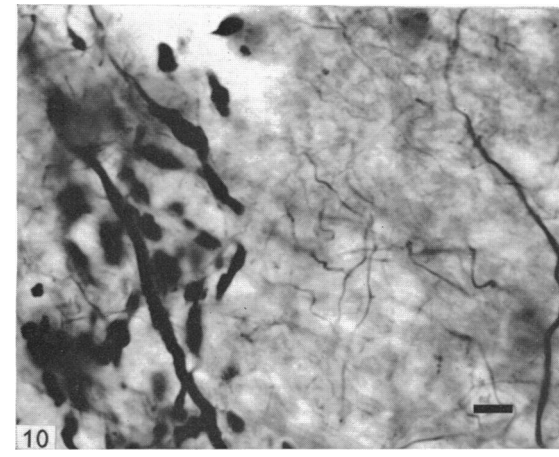


Fig. 7. Section adjacent to that in Fig. 6. Bar = 11 μm. Cajal stain.

Fig. 8. Degeneration in optic tectum 6 d after enucleation. Cajal stain. Bar = 5 μm.

Fig. 9. Normal optic tectum. Cajal stain. Bar = μm.

Fig. 10. Degeneration in optic tectum from section adjacent to that used in Figs. 8 and 9. Nauta stain using uranyl nitrate. Bar = 5 μm.

access to the lateral wall of the hemisphere.) The very tiny droplets appeared in the tectum in place of the loose plexus of extremely fine, unmyelinated fibres which normally occupy the plexiform zones (Figs. 8, 9) between the layers of relatively more coarse myelinated fibres of retinal origin which ramify in the tectum.

The larger spherular products of degeneration were stained by the Nonidez technique, the Holmes method, and by the Nauta–Laidlaw method using uranyl nitrate (Fig. 4), but the smallest spheres and granules were observed only in the Cajal preparations. Surprisingly, while the normal, unmyelinated axons of the lateral olfactory tract were never observed with the Nauta method using phosphomolybdic acid, it was possible to stain the spherular degeneration products with this method in the posterior, totally unmyelinated part of the olfactory tract (Fig. 6). The impregnation was faint, however, even when the reducer was strengthened with additional formalin, and the smaller spheres did not stain (compare with Fig. 7). Neither the Nauta–Laidlaw method with phosphomolybdic acid nor the same with uranyl nitrate revealed degeneration corresponding to the fine debris in the optic tectum which was stained by the Cajal method (see Fig. 10).

The effect of survival temperature on the degenerative response cannot be assessed independently of the possible effect of seasonal variation in the intensity of the response. Survival at low temperatures in the winter depressed the rate of degeneration to the extent that some animals bearing lesions were otherwise indistinguishable from normals after 5 d survival. Animals were not prepared which survived at low temperatures during the summer or high temperatures during the winter.

It might be suggested that the degeneration observed in this study is traumatic degeneration rather than secondary (orthograde) degeneration because it appeared after very short survival times. In that case the entire course of the affected olfactory tracts would not be revealed to the observer. It is characteristic of the mammalian degenerative response that a relatively short segment of affected axon degenerates in both directions soon after the axon is severed (Cajal, 1959). This process, known as traumatic degeneration, precedes secondary degeneration by a number of days (Cajal, 1959). It is during secondary degeneration that the entire axon peripheral to the point of damage undergoes dissolution. The following experiment was performed in order to determine whether traumatic or secondary degeneration was being observed. Small incisions were made in the lateral olfactory tract in the middle of its anterior-posterior extent in a number of animals. In every case spherular degeneration was found in the posterior half of the tract only; and the degeneration occurred throughout the length of that segment. The results demonstrate that secondary degeneration of the unmyelinated fibres produced a spherular product.

The olfactory pathways

Two kinds of olfactory bulb lesions were inflicted: (1) transections made in a coronal plane, the larger of which damaged the dorsal retrobulbar region but often spared the ventral, caudal part of the bulb, and (2) transections made in an oblique plane. The latter usually spared the retrobulbar zone but involved the ventral, caudal bulb. The lesions were sometimes limited in the sections by a border of scar-like tissue and were always bounded by a sharp transition to the region of normally staining cells.

The degenerating olfactory pathways will be described as observed in a typical specimen sustaining the second type of lesion. The results were similar in both groups but the pattern of degeneration was more nearly complete where the lesion was oblique. The text description is illustrated semi-diagrammatically by Figs. 11–24. This particular frog survived 5 d in the warmth (above 25 °C) and sustained a moderately large, oblique lesion of the olfactory bulb which destroyed most of the mitral cell layer and part of the region of granule cells. The retrobulbar pallium and accessory olfactory bulb were spared. The lesion was inclined caudally and ventrally in order

Figs. 11–24. These are drawings of transverse sections of a celloidin-embedded frog brain. The nuclear masses are represented by dotted outlines. The major fibre systems are drawn on the right as they appear in corresponding frozen sections stained by the Cajal method. Coarse black lines represent myelinated fibres. The degeneration observed in corresponding Cajal stained frozen sections from an experimental brain is represented on the left by the large dots. The lesion sustained by the particular experimental animal is represented on the right by the blackened areas. For supplementary illustrations, Herrick's (1921) and Hoffman's (1963) articles may be consulted.

LIST OF ABBREVIATIONS

a.	amygdaloid nucleus
a.b.	accessory olfactory bulb
a.o.t.	anterior olfactohabenular tract
d.o.t.	dorsal olfactory tract
d.p.	dorsal pallium
e.p.	eminentia postolfactoria
h.	rostral end of ventral habenular nucleus
h.c.	hippocampal commissure
i.m.	interbulbar mass
l.c.t.	lateral corticohabenular tract
l.f.b.	lateral forebrain bundle
l.f.b.+d.	lateral forebrain bundle and decussation of lateral forebrain bundle
l.o.t.	lateral olfactory tract
l.p.	lateral pallium
m.f.b.	medial forebrain bundle
m.o.t.	medial olfactory tract
n.h.	nucleus of the hemispheric sulcus
n.h.c.	nucleus of the hippocampal commissure
n.s.	nucleus of the septum
o.d.	optic degeneration
o.n.d.	degenerated optic nerve
o.n.n.	normal optic nerve
p.a.	preoptic area
p.h.	primordium hippocampi
p.r.	preoptic recess
r.p.	retrobulbar pallium
s.m.	stria medullaris
st.	striatum
st.d.	dorsal subdivision of the striatum
st.v.	ventral subdivision of the striatum
v.	ventricle
v.o.t.	ventrolateral olfactory tract
v.n.	vomeronasal nerve
z.gl.	glomerular zone of olfactory bulb
z.gr.	granular zone of olfactory bulb
z.m.	mitral cell zone of olfactory bulb
z.p.	extragranular plexiform zone of olfactory bulb

to achieve maximal damage of mitral cells and minimal damage of the retrobulbar pallium. Part of the lesion crossed into the contralateral bulb. That imperfection did not create a problem, however, because the resulting degeneration was clearly limited.

Amber-coloured degeneration spheres occurred at the levels illustrated in Figs. 11–15, corresponding to the rostrocaudal extent of the olfactory bulb. The degeneration was present in the interbulbar mass, in the plexiform zone external to the granular layer, and in the ventromedial corner of the hemisphere at the level of Fig. 15. Elsewhere, the degeneration was stained dark.

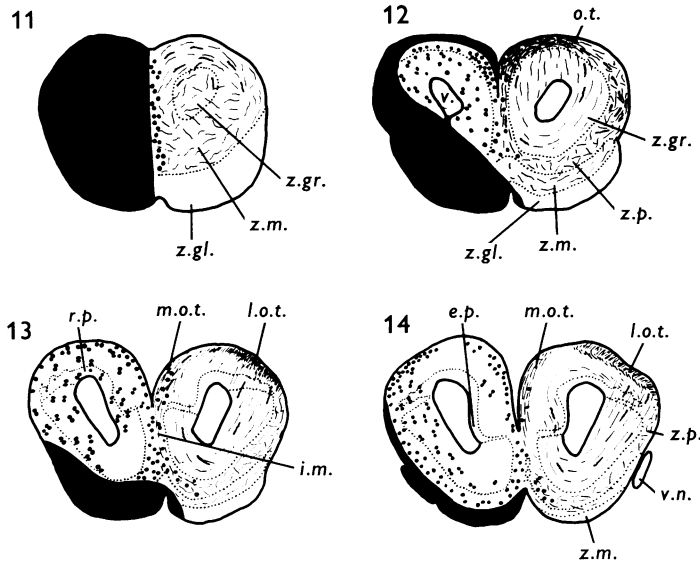


Fig. 11. Section through the conjoined olfactory bulbs anterior to the ventricles.

Fig. 12. Section through the anterior ends of the olfactory ventricles. The beginning of the olfactory tract may be seen dorsomedially. The mitral cell formation has moved ventrally.

Fig. 13. Section through the rostral retrobulbar end of the cerebral pallium. The medial olfactory tract is beginning to appear separate from the lateral olfactory tract, which has begun to move laterally.

Fig. 14. Section through the rostral end of the eminentia postolfactoria. The medial and lateral olfactory tracts are separated.

Degenerating fibres gained a position in the olfactory tract by passing first through the granular layer of the bulb, although some passed through the posterior, dorsal margin of the extragranular plexiform layer. Since the rostral end of the pallium forms a partial cap over the granular layer, it too is penetrated by fibres on their way to the olfactory tract. The tract appears first in a dorsomedial position just anterior to the rostral end of the pallium. As the tract enlarges by the addition of fibres, it comes to occupy the whole dorsal aspect of the retrobulbar region, where it is termed the dorsal olfactory tract; then it moves laterally, taking up a position on the lateral wall of the hemisphere, where it is termed the 'lateral olfactory tract'. It continues to gain fibres on its ventral margin until a level (Fig. 18) just posterior to the accessory olfactory bulb is reached.

Degeneration on the medial side of the hemisphere occurred in the medial olfactory tract. Much of its fibre content is derived from the dorsomedial margin of the dorsal olfactory tract but some fibres apparently enter from the medial, posterior margin of the extragranular plexiform layer. Degeneration in the medial olfactory tract occurred over the eminentia postolfactoria (Figs. 12–15), the rostral end of the primordium hippocampi (Fig. 15), and the cell formations of the ventromedial angle of the hemisphere, including the lower anterior parts of the septum. Although the medial olfactory tract appears to be continuous with the anterior olfactohabenular tract over an arbitrary and undefined border, the degeneration spheres could not be traced into the latter.

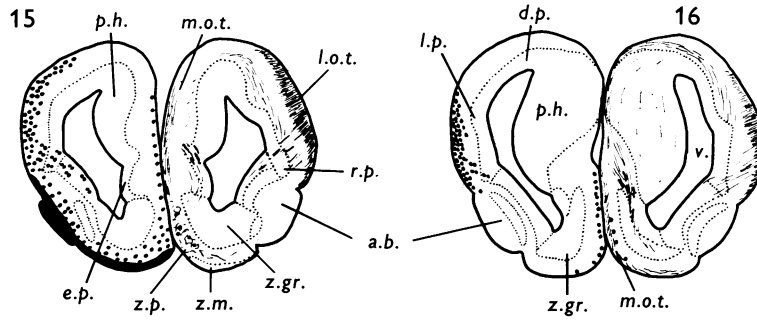


Fig. 15. Section through the rostral end of the primordium hippocampi and caudal end of olfactory bulb. The accessory olfactory bulb is beginning to appear.
Fig. 16. Section through the middle of the accessory olfactory bulb.

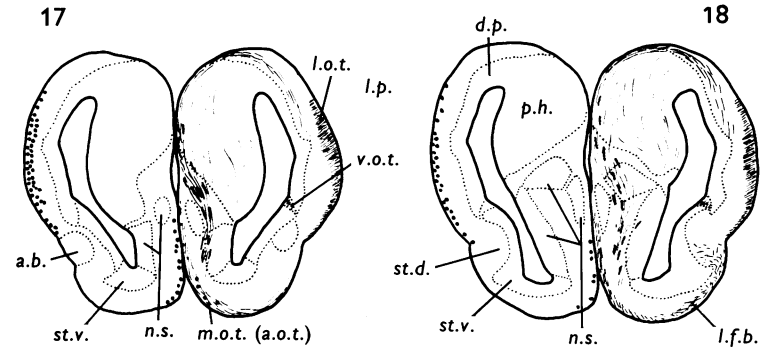


Fig. 17. Section through the caudal end of the accessory olfactory bulb.
Fig. 18. Section through the rostral end of the striatum.

At the level of Fig. 18 the lateral olfactory tract and the degeneration it contained was spread over the surface of the hemisphere from near the lateral margin of the dorsal pallium to a position overlapping the dorsal margin of the striatal region. The majority of fibres lay over the lateral pallium. There is a zone of fine degenerating fibres just internal to the lateral olfactory tract in the molecular layer of the hemisphere. Some of the fibres are radially or obliquely oriented and may therefore be derived from the overlying olfactory tract fibres. But the majority of the finer fibres are arranged parallel to the lateral olfactory tract, and therefore it cannot be said

whether they arise from the olfactory bulb or from the olfactory tract. The fine fibre zone accompanies the olfactory tract throughout its journey except for the part of its course within the stria medullaris.

Degenerated fibres continued to be present along the surface of the lateral wall of the hemisphere as far caudally as the posterior pole. They were in superficial relationship to the lateral pallium for as long as it persisted, with the rostral end of the amygdaloid region (Fig. 22), and with the dorsal part of the striatum. At the level shown in Fig. 23, the amygdala has receded into the telencephalon medium and is confluent with the preoptic area. Many of the degenerated fibres turned medially and entered the lateral corticohabenular and anterior olfactohabenular components of the stria medullaris at this level. In doing so, the fibres penetrated through a nucleus interstitial to those roots of the stria. It is termed here the nucleus of the hemispheric sulcus. (The nucleus is again referred to in the legend for Fig. 23.) Some degenerated fibres could be followed into the stria medullaris proper and were related to the rostral end of the ventral subnucleus of the habenular complex in this brain, but did not appear to reach the dorsal subnucleus or the commissure. A variable amount of degeneration was present in the stria medullaris near the habenular region in different brains, but with a single exception, not even in brains sustaining radical destruction of the hemisphere was degeneration found within the habenular nucleus or in the habenular commissure. The exception sustained a bilateral lesion of the dorsal parts of the olfactory bulbs and retrobulbar pallium. In that brain, which was stained by the method of Nonidez, degeneration was well developed in the *commissure* but sparse in both lateral olfactory tracts. In other Nonidez brains, which carried unilateral, right or left, olfactory bulb lesions, the ipsilateral olfactory tract was well degenerated but the habenular commissure was normal.

Not all the fibres in the lateral corticohabenular and anterior olfactohabenular tracts at the level of Fig. 23 arise in the olfactory bulb or in the dorsal retrobulbar pallium. Evidence for that assertion was found in one animal which survived for 11 d following a lesion of the latter structures. In that animal there was severe fibre loss in the lateral olfactory tract (the degeneration was largely resorbed) but the roots of the stria medullaris were only slightly affected.

Although degeneration was not present in the commissural systems in the brain illustrated, degeneration was found contralaterally in relation to the nucleus of the hemispheric sulcus, the rostral (hemispheric) amygdaloid region, the lower posterior edge of the lateral pallium and a small part of the dorsal subdivision of the striatum (Figs. 20–23). The degeneration was not continuous in a rostral direction with that illustrated in the medial olfactory tract of that side. Neither was it continuous in a caudal direction with the rostralmost contingent of degenerating optic fibres illustrated in Fig. 24. Further evidence that the degeneration is neither of optic origin nor crosses the midline through the interbulbar bridge comes from two brains in which the lateral olfactory tract of one side was incised but optic enucleation was performed in one case only. In these animals the contralateral degeneration was present in essentially the same position as described above, but no degeneration was present in the interbulbar mass. Furthermore, the degeneration in question was not present in two brains having lesions of the accessory olfactory bulb in animals one eye of which was excised, although the optic degeneration was well developed.

The contralateral degeneration was not present in all brains having olfactory bulb lesions however. It was present in those brains in which degeneration was also found in the medial olfactory tract but not present if the medial olfactory tract did not degenerate or if the degeneration products had disappeared as a result of long survival time. Apparently, the fibres having a contralateral destination arise from the part of the olfactory bulb which also contributes to the medial olfactory tract. Degeneration occurred in the medial olfactory tract only in those brains in which the olfactory bulb lesion was medially placed.

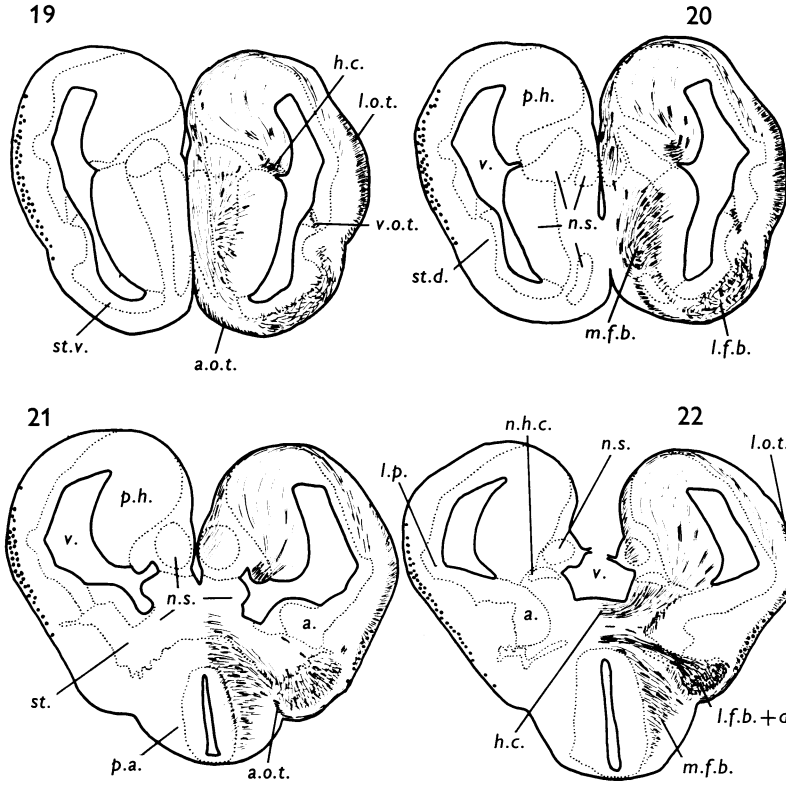


Fig. 19. Section through the middle of the septal region.

Fig. 20. Section through the rostral end of the commissural ridge.

Fig. 21. Section through the rostral end of the preoptic area. The amygdaloid nucleus has begun to appear on the right. Notice degeneration depicted on the right.

Fig. 22. Section through the transverse segment of the hippocampal commissure. Degeneration is illustrated bilaterally.

It should be pointed out that in no case of olfactory bulb lesion were degenerating fibres observed in the medial forebrain bundle, preoptic area, or hypothalamus.

There is a small tract located in the triangular-shaped boundary zone between the lateral pallium and the striatum. This fibre group, the ventrolateral olfactory tract (v.o.t.) of Herrick (1921), contains extremely fine fibres which barely stain in the

Cajal sections. It runs longitudinally between the region of the accessory olfactory bulb and the amygdaloid region (Figs. 17–20). Herrick believed it to be the efferent fascicle of the accessory bulb. It was named ventrolateral olfactory tract by Herrick to distinguish it from his dorsolateral olfactory tract (the lateral olfactory tract of this paper). In no case among the present experiments was degeneration observed in the ventrolateral tract. Among the specimens examined were those having lesions of the accessory olfactory bulb and lesions of both main and accessory bulbs. Nothing more can be said about the efferent connexions of the accessory olfactory bulb from the results of this study because neither degeneration nor fibre loss was observed in either of the two brains in which the accessory bulbs were selectively damaged.

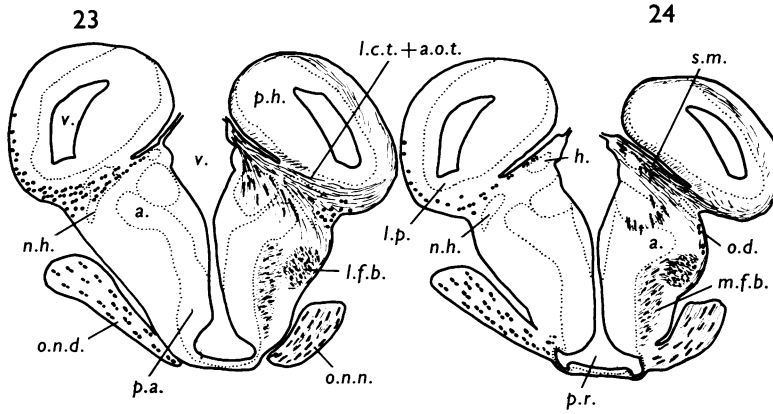


Fig. 23. Section caudal tc commissural ridge. The nucleus of the hemispheric sulcus appears between the cerebral hemisphere and the caudal part of the amygdala. It is traversed by fibres of the lateral corticohabenular tract and the anterior olfactohabenular tract, both of which are roots of the stria medullaris. The optic nerves appear at this level. The nerve on the left is degenerated because the ipsilateral eye was removed at the time the olfactory bulb lesion was inflicted.

Fig. 24. Section just rostral to the optic chiasma. The posterior poles of the cerebral hemispheres will be fully detached from the brain stem at a slightly more posterior level. The olfactory degeneration continues posteriorly into the pole of the hemisphere. A few degenerating optic fibres appear on the right just dorsal to the lateral forebrain bundle.

DISCUSSION

The degeneration of unmyelinated axons

The appearance of the degenerating olfactory tract fibres as observed in this study agrees with that observed by Gamble (1956) in the olfactory tract of the turtle. Gamble used the method of Nonidez (1939). Spherular products of degeneration among degenerating mammalian nerve fibres were also illustrated by Cajal (1959). The spherular degeneration observed among the unmyelinated olfactory tract fibres is quite different in form from the degeneration usually found in myelinated tracts during orthograde degeneration as revealed by silver stains. The authors have had the opportunity to observe the degeneration of myelinated axons both during work done with the Nauta stain on other problems and in Cajal and Nauta stained sections

from frogs which were used in this study and had sustained optic lesions. Although isolated darkly stained spheres may be found in degenerating myelinated fascicles in Nauta or Cajal stained sections, the predominant feature of the degeneration is the arrangement of bead-like, fusiform, or sausage-like fragments in straight or curvilinear series. The fragments can be disconnected or partially connected, irregular or smooth in outline, vacuolated or opaque but characteristically retain the path of the undamaged fibre. By contrast, the degeneration-spheres in the olfactory tract were largely unconnected and dispersed among the remaining fibres. In addition to differences in form there is a difference in the time required for resorption of the degeneration; the damaged unmyelinated fibres degenerated and disappeared within 2 weeks but the products of degeneration of myelinated fibres may be expected to remain in the tissue for months (Riss, Knapp & Scalia, 1963; Evans & Hamlyn, 1956; Liu & Chambers, 1958) whether they be in amphibian or mammalian material.

Although there is no direct evidence for it, the possibility should not be overlooked that the spherical degeneration products are degenerating synaptic sites rather than fragments of the axons *per se*. An affirmative argument could be constructed from the dispersed appearance of the droplets, their early appearance, and their early disappearance (Clark & Meyer, 1947; Heimer, 1967). If all of the droplets were degenerating *boutons en passage*, then the degeneration of the axons *per se* was not observed; the axons would merely have disappeared with the same time course as the boutons. On the other hand, the rapidity with which the degeneration products disappeared could explain the dispersed appearance of the droplets.

The distribution of the olfactory tracts

According to Herrick (1924*b*, 1931), there is a variety of cell located along the posterior margins of the mitral cell layer of the olfactory bulb of tailed amphibians whose form is intermediate between that of the mitral cell and that of the cells of the anterior olfactory nucleus. These were termed 'transitional cells'. The axon, which is thinner than that of the mitral cell of the olfactory bulb, enters the olfactory tracts, gives off collaterals to the anterior olfactory nucleus, and continues posteriorly for some distance. It is clear, from Herrick's description, that many of the transitional cells do not make contact with the incoming primary olfactory fibres. Since they are said to receive collaterals from the mitral cells, many of them are therefore tertiary olfactory neurons as are those of the anterior olfactory nucleus. The latter cell group is described by Herrick as an undifferentiated mass of neurons located between the olfactory bulb and the more differentiated regions posteriorly. In some of his illustrations (Herrick, 1910, his fig. 10, for example) it is clear that Herrick has labelled parts of the retrobulbar pallium in the amphibian as anterior olfactory nucleus. In this he is consistent with his description of the nucleus in the opossum (Herrick 1924*a*) in which it is a part of the retrobulbar region distinctly separate from the olfactory bulb proper, and most of it resembles cerebral cortex. Cells resembling large mitral cells and having a larger share of Nissl substances were observed in Nissl preparations in this laboratory in a position in the frog's olfactory bulb corresponding to that described for the transitional cells by Herrick.

Hoffman (1963) has reported that in the frog the anterior olfactory nucleus is morphologically inseparable from the granular layer of the bulb (which is distinctly

separate from the retrobulbar pallium in the preparations examined in this laboratory). The cells of the nucleus were recognized in his Nissl preparations by cytological characteristics which distinguished them from the granules. The distribution of the characters was such that the anterior olfactory nucleus formed the outer circumference of the posterior part of the granular layer. An anterior olfactory nucleus conforming to Hoffman's description could not be found in the material examined for this study.

Given that tertiary olfactory neurons of long axon exist in the frog's olfactory bulb, in particular the transitional cells of Herrick (Hoffman, 1963, also used the term 'transitional cell' but referred to a different entity) and the cells of the anterior olfactory nucleus of Hoffman, the lesions inflicted for this study destroyed tertiary neurons since the transitional cell region and the granular layer were involved in most cases. Nothing was learned, however, about the connexions of the transitional cells or the anterior olfactory nucleus of Hoffman in this study because differences in the distribution of the degeneration could not be correlated with differing specific involvement of the tertiary olfactory neurons.

Since fibre endings or synaptic terminals were not readily distinguishable from fine products of axon degeneration in this study, the synaptic relations of the degenerating olfactory fibres can be only indirectly appreciated. It will be assumed that since the dendrites of the periventricular cell masses in the frog's cerebrum extend outward toward the periphery, the olfactory-tract fibres come into synaptic relation with the dendrites of the cell masses over which they pass. Therefore, secondary olfactory terminations of the lateral olfactory tract are probably made in the retrobulbar pallium, the lateral pallium, the dorsal segment of the striatum, the rostral end of the amygdaloid nucleus, and the ventral habenular region. Terminations probably occurred contralaterally in the amygdaloid region and in the posterior, adjoining parts of the dorsal striatum and lateral pallium. Terminations were probably made also with the nucleus of the hemispheric sulcus because the degeneration infiltrated through the nucleus bilaterally. The medial olfactory tract did not pass posterior to the ipsilateral anterior hippocampal and septal regions and can therefore be expected to have synapsed in those zones. Its fibres passed through the superficial layer of the eminentia postolfactoria and probably synapsed with dendrites belonging to that nucleus.

Information on the olfactory pathways of the frog obtained from the study of normal material may be found in papers by Herrick (1921), Rothig (1926) and Hoffman (1963). The olfactory connexions in tailed amphibians were described in other writings of Herrick (1924*b*, 1927, 1931, 1933, 1948). According to Herrick (1948, p. 54), Rothig, and Hoffman, the olfactory bulb of amphibians projects to the primodium hippocampi and septal region, although Herrick is not definite about that fact in all of his writings. That connexion has been verified in the present study only in the limited sense that the rostral extremes of the hippocampal and septal regions were reached by the degenerating medial olfactory tract. Herrick supposed that olfactory connexions were made with the striatum and with the preoptic area and hypothalamus via the medial forebrain bundle. A minimal connexion to the striatum was observed in this study but no contribution to the preoptic area or hypothalamus was found. Nor were substantial olfactory connexions to the habenular

nucleus as suggested by Herrick and described by Rothig confirmed. Only a few degeneration spheres were related superficially to the rostral end of the ventral habenular nucleus. Rothig described a connexion between the olfactory bulbs occurring through the habenular commissure in the frog. This was not observed in the present study. Of course, the possibility that the connexions in question are accomplished by fibres of finer calibre than can be demonstrated by the Cajal technique should not be overlooked. Finally, the nucleus of the hemispheric sulcus was not previously described in the amphibian brain.

Finding degeneration in the contralateral amygdaloid region in this study presents an interesting problem. Degeneration could not be found in the habenular commissure of the frog in the present study even after radical hemispherectomy or after a lesion was placed in the stria medullaris except in one case of bilateral olfactory bulb ablation stained by Nonidez's method. Two other brains stained by that method and having unilateral olfactory bulb ablations (one left, one right) showed no commissural degeneration. Yet, in a number of animals having olfactory bulb lesions (including one of the unilateral Nonidez brains) and in the animal sustaining a lesion in the stria medullaris, though degeneration was not observed in the commissure, degeneration was found in the contralateral hemisphere in the stria medullaris. The contralateral degeneration was, of course, not present in normal brains, nor was it found in some of the operated brains. Unless it is supposed that fibres can degenerate in sympathy with primarily degenerating fibres because of some relationship other than direct continuity, the hypothesis must be made that the ipsilateral and contralateral degeneration was that of continuous fibres whose commissural portion either did not degenerate or did not stain. While the value of the single instance of degeneration in the habenular commissure is limited by its isolation, the fact that the commissural degeneration was well developed in an animal whose lateral olfactory tracts were just beginning to degenerate could be interpreted to mean that different segments of a fibre may degenerate and resorb out of synchrony. This is not a new idea. It has been noticed in Glees and Nauta studies that degeneration of main axons and terminal branches may be observed after different survival times (Clark & Meyer, 1947, and Hayhow, 1958, for example).

The present findings substantially agree with those of Gamble (1956) on the turtle, but there are important differences. According to Gamble, the olfactory tract in the turtle distributes over the ipsilateral lateral pallium, the external surface of the paleostriatum, and the external surface of the amygdaloid region. A commissural pathway enters the stria medullaris and crosses in the habenular commissure. After crossing it is again located in the stria medullaris. Some olfactory fibres distribute to the contralateral amygdaloid region and the neighbouring part of the lateral pallium; others continue rostrally in the anterior olfactohabenular tract, travel over the lower, rostral part of the septum, and finally distribute over the rostral end of the precommissural hippocampal cortex, the dorsal pallium, and the anterior olfactory nucleus. If one does not attend to differences in symmetry, the olfactory connexions in the turtle and those adduced for the frog are practically identical, the major features being a unilateral contribution to the rostral, medial wall of the hemisphere, a unilateral contribution to the lateral pallium and part of the striatum, and a bilateral contribution to the amygdaloid region and limited parts of the lateral

pallium and striatum. The contributions to the medial wall of the hemisphere are not symmetrical, however, but ipsilateral in the frog and contralateral in the turtle. Apparently, homology can exist between systems which are crossed in some species and uncrossed in others.

SUMMARY

Axonal degeneration was induced in the olfactory tracts of male leopard frogs (*Rana pipiens*) by surgically removing an olfactory bulb. The degeneration was demonstrated in sections of their brains by silver impregnation after the frogs had survived relatively short periods (5–7 d). Degeneration was no longer present in the sections after 11 d postoperative survival. Since few myelinated axons appear in the olfactory tracts in Weil-stained sections, the degeneration observed was that of unmyelinated axons, which are stained in profusion in the normal olfactory tracts by some silver methods. The product of the degeneration of the unmyelinated fibres was a multitude of opaque spherules dispersed randomly among the remaining tract fibres. The spherular degeneration is different from the degeneration of myelinated fibres in the frog. Myelinated axons degenerated into linearly arranged droplets or fragments of irregular shape. The spherular degeneration was stained by the Nonidez, Holmes, and Nauta–Laidlaw techniques but the finest degenerative particles were revealed only by a Cajal-on-the-slide method.

Degeneration was present in lateral and medial olfactory tracts. The latter projected to the anterior end of the hippocampal and septal primordia. The lateral olfactory tract distributed to the lateral pallium, amygdala, and dorsal paleostriatum and to the ventral habenular region via a communication with the stria medullaris. Although degeneration was not found in the habenular commissure or in the anterior commissure, degeneration was found in the contralateral amygdaloid region.

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REFERENCES

CAJAL, S. RAMON Y (1959). *Degeneration and Regeneration of the Nervous System.* New York: Hafner.

CLARK, W. E. LE GROS & MEYER, M. (1947), The terminal connections of the olfactory tract in the rabbit. *Brain* **70**, 304–328.

EVANS, D. H. L. & HAMLYN, L. H. (1956). A study of silver degeneration methods in the central nervous system. *J. Anat.* **90**, 193–203.

GAMBLE, H. J. (1956). An experimental study of the secondary olfactory connexions in *Testudo graeca*. *J. Anat.* **90**, 15–29.

GAUPP, E. (1899). *Anatomie des Frosches*, Abt. 2. Braunschweig.

HAYHOW, W. R. (1958). The cytoarchitecture of the lateral geniculate body in the cat in relation to the distribution of crossed and uncrossed fibers. *J. comp. Neurol.* **110**, 1–63.

HEIMER, L. (1967). Silver impregnation of terminal degeneration in some forebrain systems: a comparative evaluation of current methods. *Brain Res.* **5**, 86–108.

HERRICK, C. J. (1910). The morphology of the forebrain in Amphibia and Reptilia. *J. comp. Neurol.* **20**, 413–546.

HERRICK, C. J. (1921). The connections of the vomeronasal nerve, accessory olfactory bulb and amygdala in Amphibia. *J. comp. Neurol.* **33**, 213–280.

HERRICK, C. J. (1924*a*). The nucleus olfactorius anterior of the opossum. *J. comp. Neurol.* **37**, 317–359.
HERRICK, C. J. (1924*b*). The amphibian forebrain. II. The olfactory bulb of *Amblystoma*. *J. comp. Neurol.* **37**, 373–396.
HERRICK, C. J. (1927). The amphibian forebrain. IV. The cerebral hemispheres of *Amblystoma*. *J. comp. Neurol.* **43**, 231–326.
HERRICK, C. J. (1931). The amphibian forebrain. V. The olfactory bulb of *Necturus*. *J. comp. Neurol.* **53**, 55–70.
HERRICK, C. J. (1933). The amphibian forebrain. VI. *Necturus*. *J. comp. Neurol.* **58**, 1–288.
HERRICK, C. J. (1948). *The Brain of the Tiger Salamander*. Chicago: The University of Chicago Press.
HOFFMAN, H. H. (1963). The olfactory bulb, accessory olfactory bulb, and hemisphere of some anurans. *J. comp. Neurol.* **120**, 317–368.
HOLMES, W. (1943). Silver staining of nerve axons in paraffin sections. *Anat. Rec.* **86**, 157–187.
LIU, C. N. & CHAMBERS, W. W. (1958). Intraspinal sprouting of dorsal root axons. *Archs Neurol. Psychiat., Lond.* **79**, 46–61.
NAUTA, W. J. H. (1957). Silver impregnation of degenerating axons. In *New Research Techniques of Neuroanatomy*, pp. 17–26. Ed. W. F. Windle. Springfield: Thomas.
NONIDEZ, J. F. (1939). Studies on the innervation of the heart. *Am. J. Anat.* **65**, 361–412.
RISS, W., KNAPP, H. D. & SCALIA, F. (1963). Optic pathways in *Cryptobranchus allegheniensis* as revealed by the Nauta technique. *J. comp. Neurol.* **121**, 31–44.
ROMEIS, B. (1948). *Mikroscopische Technik*, p. 416, no. 1806. München: Leibniz Verlag.
ROSE, M. (1931). Cytoarchitektonischer Atlas der Grosshirnrinde des Kaninchens. *J. Psychol. Neurol.* **43**, 353–440.
ROTHIG, P. (1926). Beiträge 10. Über die Faserzüge in Vorder- und Zwischenhirn der Anuren. *Z. mikrosk.-anat. Forsch.* **5**, 23–58.