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Electron Microscopic Evidence for a Retinohypothalamic Projection to the Suprachiasmatic Nucleus of *Passer domesticus**

H. G. Hartwig **

Department of Anatomy and Cytobiology, Justus Liebig University, Giessen, Federal Republic of Germany

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Summary. The possibility of a direct retinohypothalamic projection was reinvestigated in *Passer domesticus* by electron microscopy following left unilateral retinectomy. To avoid misinterpretation of non-specific degeneration, the course of degenerative changes was observed at intervals of 6, 12, 24, 48 and 96 hours after operation. Of the hypothalamic areas examined in only one, the contralateral suprachiasmatic nucleus, was it possible to identify reliable indications of secondary anterograde degeneration comparable to those observed in the contralateral optic tectum. Single dark profiles within the supraoptic nucleus and the basal infundibular (tuberal) nucleus showed neither internal changes in structure nor an increase in number per unit area in retinectomized birds. Since photoperiodically induced gonadal growth occurs in totally blinded birds the functional significance of the retinohypothalamic projection to the suprachiasmatic nucleus is open to discussion.

Key words: Retinohypothalamic projection — *Passer domesticus* — Suprachiasmatic nucleus — Electron microscopy.

Introduction

Experimental investigations have shown that photic stimuli provide an important cue for neuroendocrine regulations in birds (for reviews see Farner, 1973; van Tienhoven and Planck, 1973). The avian hypothalamus itself appears to be photosensitive since photoperiodically induced gonadal growth occurs in totally blinded birds (Benoit, 1938; Benoit and Assenmacher, 1959; Menaker, 1969; Menaker, 1971; Menaker *et al.*, 1968, 1970; Gwinner *et al.*, 1971). However, in the intact bird the possibility of hypothalamic stimulation also via the lateral eyes should not be excluded (cf. Gwinner *et al.*, 1971; Oliver and Baylé, 1973).

This concept, however, requires demonstration of an anatomical connection, mono- or polysynaptic, between the retina and at least one nuclear area of the hypothalamus. In birds several negative findings (Cowan *et al.*, 1961: *Columba livia*; Hirschberger, 1971: *Melopsittacus undulatus*; *Amadina fasciata*, *Taeniopygia guttata*, domestic fowl, *Coturnix coturnix*, *Excalfactoria chinensis*, *Tyota alba*,

Send offprint requests to: Dr. H.-G. Hartwig, Zentrum für Anatomie und Cytobiologie, Justus Liebig-Universität, D-6300 Giessen, Friedrichstraße 24, Federal Republic of Germany.

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Strix aluco) as well as some positive findings have been reported (Blümcke, 1961: domestic fowl; Bons and Assenmacher, 1969: *Anas platyrhynchos*) (See Nauta *et al.*, 1969, Benoit and Assenmacher, 1973, and Oksche, 1970, 1973, for reviews). Technical difficulties in the light microscopic demonstration of degenerating fibers and synaptic terminals may explain the contradictory results. Following interruption of retinal axons, silver impregnation methods fail to demonstrate unmyelinated intrahypothalamic optic fibers and fine terminals.

In several species of birds (Bons and Assenmacher, 1969: *Anas platyrhynchos*; Hartwig, 1970: White-crowned Sparrow, *Zonotrichia leucophrys gambelii*; Hirschberger, 1971, see above) a group of fibers leaves the rostral and anterior portion of the optic tract and penetrates lateral to the ventricular wall and parallel to the optic tract into a basal anterior hypothalamic area. According to the results of Hartwig (1970), obtained with different silver methods in the White-crowned Sparrow, these fibers seem to be *en passant* elements. Bons and Assenmacher (1969) suggest that in the duck these fibers terminate within the supraoptic nucleus, whereas Hartwig (1970, White-crowned Sparrow) and Hirschberger (1971, species see above) have been able to trace them to the most rostrally situated primary optic nucleus, the *nucleus lateralis anterior*, and in addition also to the suprarotund nucleus (Hirschberger).

In the present study the problem of a direct retinohypothalamic pathway is reinvestigated in the House Sparrow *(Passer domesticus)* by means of electron microscopy. The findings reported are concerned with the above-mentioned rostral suprachiasmatic and basal anterior hypothalamic area, the lateral division of the magnocellular supraoptic nucleus and the basal infundibular (tuberal) nuclei.

Material and Methods

Twenty young male House Sparrows *(Passer domesticus)* captured in July were anesthetized with Nembutal (Arnall, 1961). The retina of the left eye was removed in 16 birds under aseptic conditions; the eviscerated eyeball was filled with sulfonamide containing gel (Aristoplomb, Nordmark) to avoid postoperative wound infection and to prevent hemorrhage. Compaired with enucleation or intraorbital transection of the optic nerve this operation avoids mechanical traction of the optic chiasma and subsequent lesions in adjacent hypothalamic structures (see Sousa-Pinto and Castro-Correia, 1970). In 4 animals, after incision of the peripheral cornea, only the eye lens was removed without damage of retinal structures.

Six, 12, 24, 48, and 96 hours after surgery 3 retinectomized and 1 sham-operated animal were sacrificed in each group under Nembutal anesthesia and immediately perfused for 20 sec via the left cardiac ventricle with a fluid containing 8.0 gm NaCl, 0.2 gm KCl, 0.2 gm $CaCl_2$, 0.1 gm $MgCl_2$, 1.0 gm sucrose, and 15000 USP units heparine per liter, followed by an isotonic 0.1 m cacodylate buffered formaldehyde (4%) and glutaraldehyde (2.5%) containing solution (pH 7.2) to which sucrose (27.6 gm) and 0.422 gm $CaCl_2$ per liter had been added. The perfused brains were sectioned in slices 0.5–1.0 mm and retained in the perfusion fluid for a total fixation time of 2 hours. They were post-fixed with cacodylate-buffered 1% OsO_4 for a period of 2 hours.

Corresponding bilateral suprachiasmatic, lateral supraoptic, tectal, and basal infundibular areas were isolated by trimming the slices and embedded after dehydration in graded alcohols via propyleneoxide in Durcupan (Fluca). Final diagnosis of the nuclear areas was accomplished in semithin sections (Fig. 1B). Ultrathin sections (Porter — Blum MT 1 ultramicrotome, supported with glass knives) were collected on both coated (Formvar, Serva) and uncoated copper grids and then stained with lead citrate and uranyl acetate. A Philips M 201 electron microscope was used for ultrastructural observations. In order to obtain comparable results in all analyzed specimens only an area approximately 50–100 μm distant from the

optic tract fibers was examined in the left and right suprachiasmatic, lateral supraoptic and lateral tectal area of each animal. In addition to these areas the contralateral basal infundibular nucleus was examined for degenerative synaptic structures.

Results

Sham-operated Animals

No certain signs of degenerative changes were observed in electron micrographs of the hypothalamic and tectal areas in the sham-operated animals sacrificed 6, 12, 24, 48, and 96 hours after removal of the left lens. Furthermore, careful examination of the corresponding left and right nuclear areas did not show any differences. In all regions examined, synaptic structures characterized by (a) dark cytoplasmic matrix, (b) decreased number of presynaptic vesicles, and (c) swollen mitochondria were only occasionally seen (none or one per section). Within the rostral hypothalamus such altered profiles were often covered by sheaths of glial lamellae, structures lacking in the optic tectum. Terminal fibers of this type most probably belong to elements that have undergone unspecific degeneration (cf. Cohen and Pappas, 1969).

Retinectomized Animals

Ipsilateral Tectal Area

The characteristic features of avian optic terminals were described in the pigeon optic tectum by Cuénod *et al.* (1970) and Akert *et al.* (1971). The synaptic structures observed in the ipsilateral tectal areas of retinectomized House Sparrows and also in the optic tectum of sham-operated animals are in agreement with this description. Except for a very few elements bearing signs of unspecific degeneration there was no evidence for degenerative phenomena in the ipsilateral optic tectum of retinectomized animals. Like in sham-operated animals these terminals did not show any postoperative changes. In the optic tectum 4–6 dendrites were often arranged around one single optic terminal (see Fig. 3D). These profiles displayed a relatively dark cytoplasmic matrix containing large numbers of densely-packed presynaptic vesicles and numerous mitochondria.

Contralateral Tectal Area

Six Hours after Operation. The large-size presynaptic optic elements showed a slight swelling of their synaptic vesicles. Also some postsynaptic membrane thickenings appeared darker than in sham-operated animals.

Twelve Hours after Operation. All large-size optic terminals were characterized by slight swelling of synaptic vesicles and neurofibrillar hyperplasia accompanied by a distinct darkening of the postsynaptic membrane thickening (Fig. 3C, D). Some smaller, apparently optic terminals had not yet undergone degenerative changes by this time.

Twenty-four Hours after Operation. Nearly all optic terminals exhibited enlarged synaptic vesicles, slightly swollen mitochondria and neurofibrillar hyperplasia. At this postoperative stage glial lamellae had been formed, and in some cases they started to isolate the degenerating structures from the surrounding neuropil.

Fourty-eight Hours after Operation. The pattern of structural degeneration resembled that observed in the 24-hours group. However, there was a distinct darkening of the cytoplasmic matrix of the large-size terminals and an increase in mitochondrial swelling.

Ninety-six Hours after Operation. The optic tectum displayed signs of a fully developed secondary structural degeneration. Nearly all optic terminals were characterized by a dark cytoplasmic matrix and ghosts of synaptic vesicles (Fig. 3 E). The glial processes in juxtaposition with the degenerating terminals appeared to be richer in filaments than in control animals.

Ipsilateral Suprachiasmatic Area

In the House Sparrow the neuronal perikarya of the suprachiasmatic region are secretory (see Oksche *et al.*, 1973, 1974) with a well-developed granular endoplasmic reticulum and a Golgi complex forming dense-core vesicles 100–200 nm in diameter. In the present material different types of synapses were observed in the suprachiasmatic neuropil. In contrast to the tectum, the dendrites were never aggregated around single terminals. Some of the presynaptic elements that occured within the circumscribed area of the suprachiasmatic nucleus (Fig. 1; cf. Crosby and Showers, 1969; Oksche *et al.*, 1974), were characterized by a slightly darker cytoplasmic matrix and a high density of clear synaptic vesicles resembling the smaller optic terminals of the tectum (Fig. 2 F). Other synaptic profiles were probably monoaminergic, containing dense-core vesicles of 100 nm in diameter. The third type of endings had a mixed population of clear and dense-cored vesicles. In the suprachiasmatic nucleus most of the synapses were axo-dendritic. In the ipsilateral suprachiasmatic nucleus retinectomy never produced definite degenerative changes at any of the postoperative stages examined.

Contralateral Suprachiasmatic Area

In unilaterally retinectomized House Sparrows degenerating terminals of the type described in the contralateral tectum also occurred in the circumscribed suprachiasmatic area of the suprachiasmatic nucleus (Fig. 1). The number of such degenerating nerve endings was, however, relatively small, and many synapses did not show any signs of degeneration. Nevertheless, the number of dark profiles visualized in the suprachiasmatic nucleus of the House Sparrow increased 96 hours after removal of the contralateral retina to 15–20 degenerating synapses per analyzed section. In sham-operated animals as well as in the ipsilateral suprachiasmatic nucleus of retinetcomized birds only 0–2 degenerating terminals per section were seen; no postoperative changes were observed.

The degeneration process is first detected between 6 to 12 hours after retinectomy. In accordance with the findings in the optic tectum the first signs of degeneration were enlarged synaptic vesicles, darkening of the postsynaptic membrane thickenings and occasional neurofibrillar hyperplasia (Fig. 2). At 96 hours after removal of the contralateral retina nearly all degenerating optic terminals within the suprachiasmatic nucleus were covered by glial lamellae which encompassed the dendritic process (Fig. 3 A, B). Only terminals with clear vesicles degenerated. In a few cases one or two additional dense-core vesicles

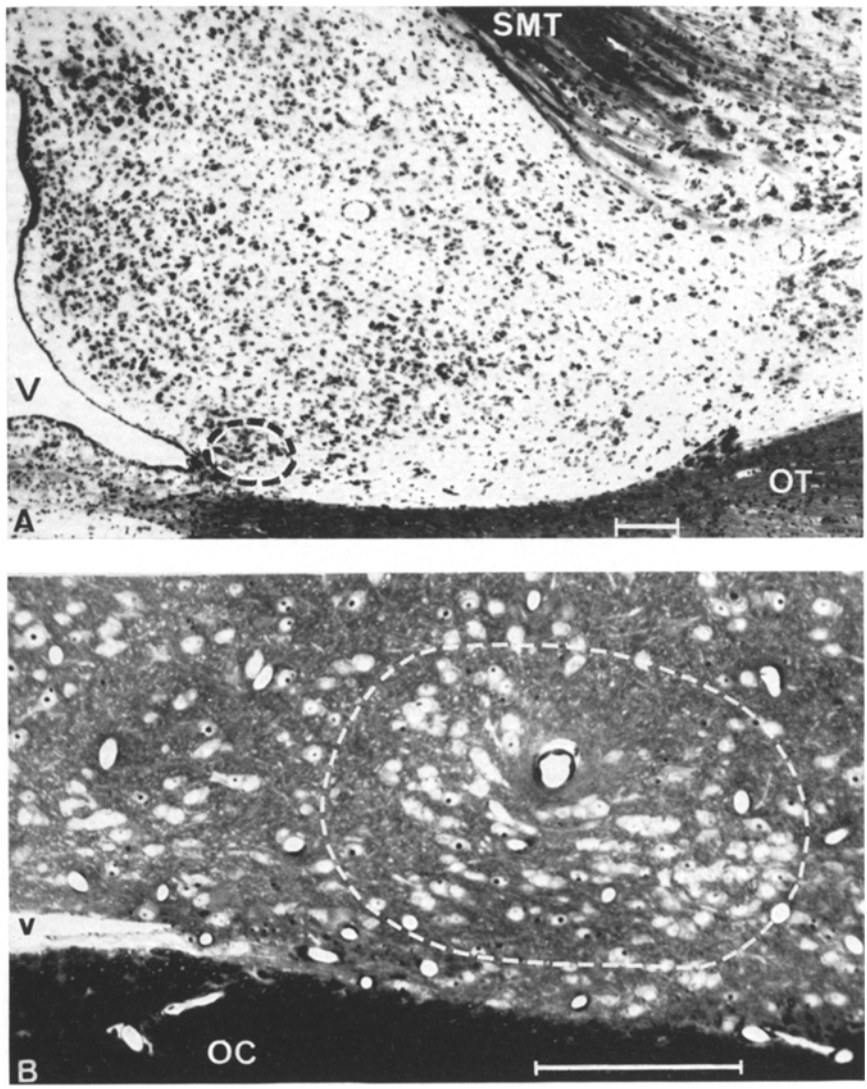

Fig. 1 A and B. *Passer domesticus*. Anterior hypothalamus, frontal section. (A) Untreated control animal. Paraffin-embedded material, stained according to Klüver-Barrera. (B) Animal 6 hours after contralateral retinectomy (see also Fig. 2 A). Durcupan-embedded material, semithin section, stained according to Rüdeberg (1967). *Encircled region:* suprachiasmatic nucleus; *OC* optic chiasma; *OT* optic tract; *SMT* septomesencephalic tract; *V* third ventricle. Scale markers: 100 μm

were identified (Fig. 2 A). Terminals bearing exclusively dense-core vesicles or a mixed population with a high number of dense-core vesicles were not affected by removal of the retina.

Myelinated nerve fibers are completely lacking within the area of the suprachiasmatic nucleus.

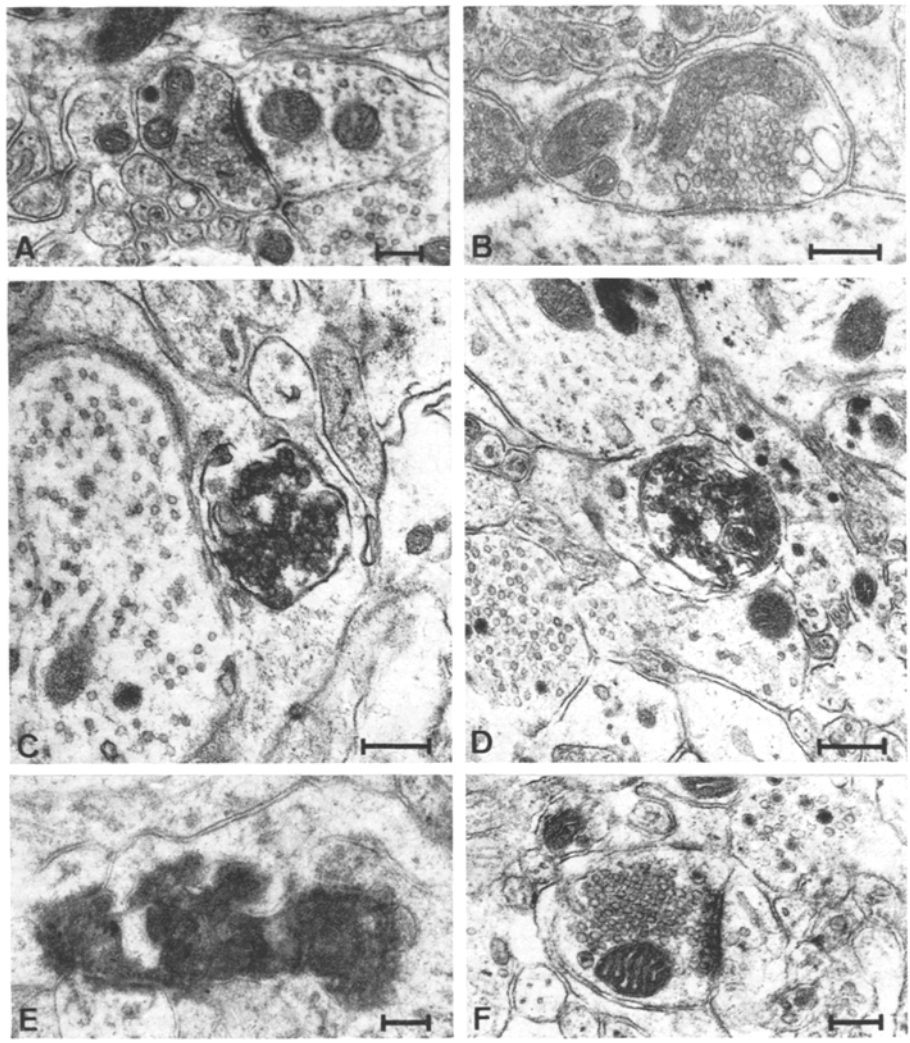

Fig. 2A—F. *Passer domesticus*. Degenerating terminals within the area of the suprachiasmatic nucleus 6 (A), 12 (B), 48 (C, D), and 96 (E) hours after contralateral retinectomy. Electron micrograph (A) was taken from the encircled region in Fig. 1B. Electron micrograph (F) shows a synaptic ending with structural characteristics of an optic terminal in a sham-operated animal. Scale markers: 200 nm

Lateral Part of the Magnocellular Supraoptic Nucleus

At no stage of the experiment were degenerating optic terminals or synapses observed in the lateral part of either the ipsi- or contralateral magnocellular supraoptic nucleus. On the other hand, beginning at 48 hours after retinal injury, numerous degenerating myelinated nerve fibers appeared at the basis of the lateral magnocellular supraoptic nucleus.

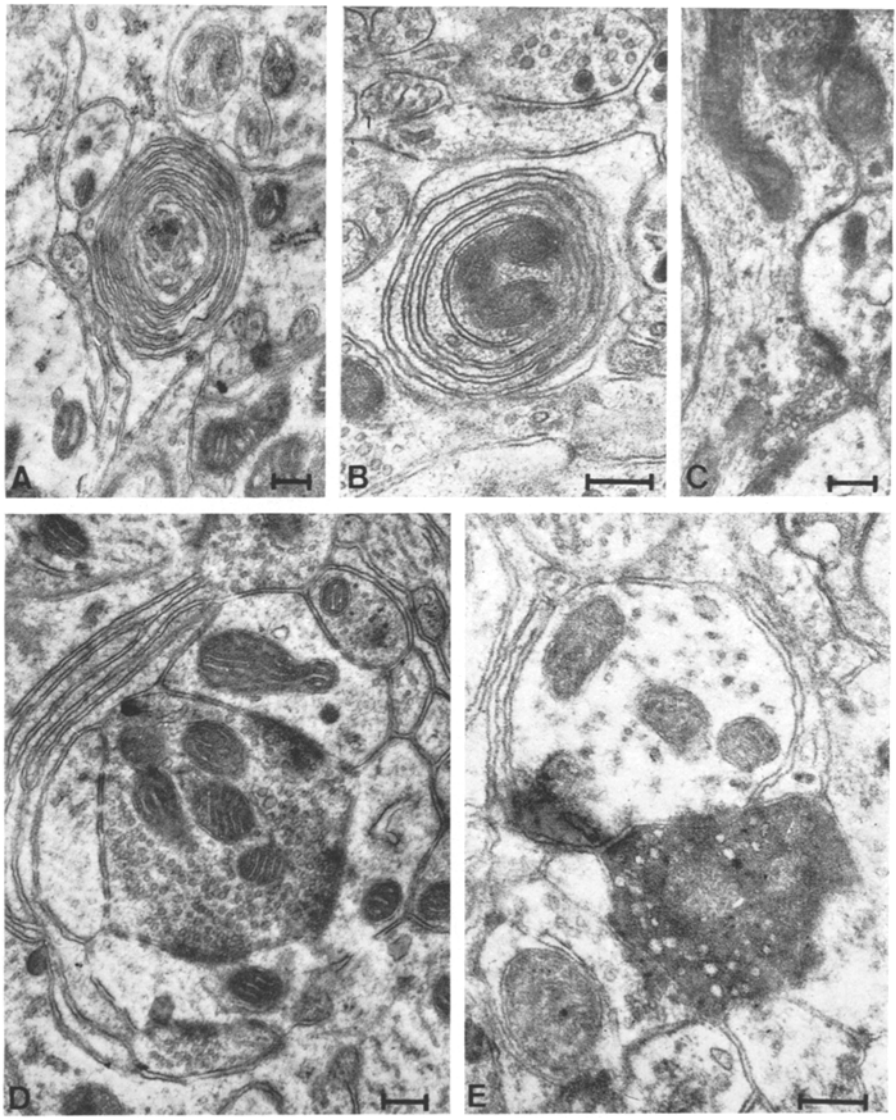

Fig. 3 A—E. *Passer domesticus.* Degenerating terminals within the suprachiasmatic nucleus (A, B) and the optic tectum (C, D, E). A and B, 96 hours after contralateral retinectomy. C and D, 12 hours after contralateral retinectomy. (Note neurofibrillar hyperplasia in C). E, 96 hours after contralateral retinectomy. Scale markers: 200 nm

Basal Infundibular (Tuberal) Nucleus

Systematic examination of this homologue of the mammalian arcuate nucleus gave no indication of degeneration comparable to that in the contralateral optic tectum or in the contralateral suprachiasmatic nucleus (see also Oksche, 1970; Hartwig and Oksche, 1972). A few dark fiber profiles were found in experimental as well as in sham-operated animals. These structures, however, did not show the characteristic feature of terminal optic degeneration.

Discussion

The ultrastructural changes in the avian visual system after surgical lesion of afferent retinal fibers are well known since the studies of Gray and Hamlyn (1962) with the optic tectum of the domestic fowl. Enlargement of synaptic vesicles of optic terminals is one of the first indications of secondary anterograde ultrastructural degeneration in the pigeon optic tectum following interruption of the axoplasmic flow by mechanical (Akert *et al.*, 1971) or pharmacological procedures (Cuénod *et al.*, 1972, colchicine). However, Cohen and Pappas (1969) noticed dark profiles within the ventrobasal and lateral thalamus of normal (untreated) cats and rats. Such degenerating structures were demonstrated in all analyzed untreated specimens, and they did not depend on the fixation procedures applied (immersion or perfusion fixation fluids of different composition and osmolarity). They "were morphologically indistinguishable from those seen in tissue from the same area of cats with lesions of the dorsal column nucleus of two or four day's duration" (Cohen and Pappas, 1962; see also Lin and Ingram, 1972).

In view of these findings the observation of additional parameters is necessary for the electron microscopic identification of central nervous pathways in degeneration experiments. In the present study attention was given to the time course of degeneration as well as to the number of degenerating profiles per unit area. The development and progress of degeneration within the optic tectum of the House Sparrow is in good agreement with the above-mentioned findings of Akert *et al.* (1971) in the pigeon. They form a basis for the interpretation of the findings in some functionally significant hypothalamic areas.

In only one of these hypothalamic areas, the suprachiasmatic nucleus (the apparent homologue of the mammalian suprachiasmatic nucleus, cf. Crosby and Showers, 1969), the degenerative changes observed resemble the well-investigated degeneration process in the optic tectum. Other rostral hypothalamic nuclei are free of degenerative changes. Although the number of degenerating profiles per unit area of this circumscribed suprachiasmatic area is low in comparison to the optic tectum, the enlargement of synaptic vesicles accompanied by neurofibrillar hyperplasia and the subsequent darkening of the presynaptic cytoplasmic matrix, indicate a retinohypothalamic projection to the suprachiasmatic nucleus of *Passer domesticus*.

The course by which retinal fibers reach the suprachiasmatic nucleus is not quite clear. The well known *en passant* optic fibers of the anterior hypothalamus (see introduction) leave the optic tract slightly lateral to the suprachiasmatic nucleus. According to light microscopic observations in semithin sections all fibers of this group possess a well developed myelin sheath. Myelinated fibers are completely lacking within the circumscribed area of the suprachiasmatic nucleus. The degenerating presynaptic profiles in the suprachiasmatic nucleus of *Passer domesticus* may therefore be (a) terminals of the very sparse unmyelinated optic fibers (see Oksche, 1970) or (b) terminals of unmyelinated axon collaterals of myelinated optic fibers, or (c) terminals of optic fibers which have lost their myelin sheath after penetration into the anterior hypothalamus.

A critical reevaluation of silver-impregnated serial sections (Nauta-Fink-Heimer, Bielschowsky, Bodian) of the visual system of the White-crowned Spar-

row, *Zonotrichia leucophrys gambelii*, (Hartwig, 1970) did not produce any new positive evidence for the existence of a direct retinohypothalamic connection with the rostral hypothalamus in this species. On the other hand, Karten (see Van Tienhoven and Planck, 1973) was able to demonstrate, 3–4 days after unilateral blinding, degenerating boutons in the contralateral suprachiasmatic nucleus of pigeons (method by Fink-Heimer). Recently Meier (1973) reported successful labeling of contralateral anterior hypothalamic areas in the pigeon *(Columba livia)* and in the Jackdaw *(Coleus monedula)* after unilateral intraocular injection of tritiated leucine and proline. Although Meier (1973) did not identify precisely the nuclei of the labeled anterior hypothalamic region, the diagrammatic representation of his findings shows them in the basal suprachiasmatic and supraoptic region. In view of the present ultrastructural findings the highly labeled Zones 1 and 2 of Meier (1973) may correspond to the suprachiasmatic nucleus. The more laterally situated, weaker labeled and narrower Zone 3 may belong to the marginal lateral region of the magnocellular supraoptic nucleus. In the House Sparrow this nucleus is free of electron-microscopically detectable degenerating terminals. Although some topographical and neuroanatomical aspects are still open to discussion the findings in the House Sparrow are not in principal contradiction to observations of Bons (1974) in unilaterally blinded (transection of the optic nerve) domestic mallards. The problem of direct retinohypothalamic connections has still to be reviewed very critically (cf. Lin and Ingram, 1972; Laties, 1973a, b; Lin, 1973). Further comparative studies are necessary to define clearly the topography of the anterior hypothalamic nuclei of birds and their fiber connections (Oksche *et al.*, 1974).

Results from studies with stereotaxic lesions indicate that the anterior (rostral) hypothalamus of birds plays an important role in ovulation mechanisms. Ralph and Fraps (1959a, b) observed an immediate cessation and prolonged interruption of ovulation in adult laying hens bearing electrolytic lesions in a circumscribed ventromedian preoptic region which includes the suprachiasmatic nucleus.

In mammals the anterior hypothalamus seems to have comparable functions. Clattenburg *et al.* (1972) observed ultrastructural changes in the suprachiasmatic nucleus of rabbits following induced ovulation. On the other hand, the suprachiasmatic nucleus of mammals might be the only hypothalamic region receiving direct retinal inputs (Moore and Lenn, 1972; Hendrickson *et al.*, 1972; Moore, 1973). Furthermore, this nucleus seems to play an important role in generating circadian rhythmicity (Moore *et al.*, 1974).

Little is known with respect to the efferent anatomical connections of the suprachiasmatic nucleus in mammals and birds. Ralph (1959) concluded from stereotaxic lesions experiments with adult laying hens, that the participation of the medial basal preoptic hypothalamus in regulating ovulation terminates 6 hours before the expected time of ovulation. One might speculate that a neuroendocrine agent of the suprachiasmatic nucleus (and also other anterior basal hypothalamic neurons) is transported to the median eminence or to one of the tuberal neuroendocrine effectors within this time interval.

After critical consideration of different experimental and neuroanatomical aspects it is suggested that the reported ultrastructural findings in *Passer domesticus* indicate the existence of a direct projection of the retina to the contralateral

suprachiasmatic nucleus. Since photoperiodically induced gonadal growth occurs in totally blinded birds, the functional significance of this connection is open to discussion.

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