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A Contribution to the Study of the Afferents to the Pigeon Optic Tectum

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Summary. The origins of several afferent pathways to the pigeon's tectum were studied using the horseradish peroxidase tracing technique. The results confirm the presence of several previously described afferents, and add further data on intertectal and hyperstriatofugal projections. Two new sources of afferents to the tectum, located in the hypothalamus and the septum, were identified. The latter is described in greater detail.

Key words: Optic tectum – Septum – Wulst – Brain stem – HRP method – Avian brain

Introduction

The optic tectum of birds is a neural structure with one of the most complex of organizational patterns. In recent years increased efforts have been made to elucidate its functional anatomy. The origins of the afferents to the tectum are naturally of major interest in this respect. The massive input from the contralateral retina is well documented, but the tectum is thought to receive afferents from a number of other sources as well. Our interest in tectal afferents was stimulated by the results of a recent immunohistochemical study which revealed that the optic tectum of the pigeon receives peptidergic innervation with terminals containing vasotocin and neurophysin (Weindl et al. 1981). This suggested the existence of a hypothalamo-tectal projection. In order to obtain more information in this respect, experiments using the HRP method were designed. The application of HRP microinjections into several parts of the tectum revealed, in addition to the hypothalamo-tectal projection (Mestres, Weindl, submitted), the existence of a number of important afferents arising from the nuclei of the brain stem, the septum and the wulst. These are the topic of the present contribution.

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Materials and Methods

14 adult pigeons (*Columba livia*) of both sexes weighing approximately 450 g were used for these experiments. The birds were anaesthetized with a chloralhydrate-pentobarbital-magnesiumsulfate mixture.

0.2, 0.3 or 0.5 µl of a 50% solution of horseradish peroxidase (HRP, Sigma Type VI), dissolved in physiological saline, were injected by pressure over a period of 15–30 min using a glass micropipette with a tip diameter of approximately 60 µm inserted into the right optic tectum through a trephined opening in the skull. The micropipette penetrated to a depth of 0.7–0.9 mm from the surface. After the injection and before the removal of the pipette an interval of 10 min was allowed to elapse. The survival period varied between 16 and 20 h. The birds were then fixed by intravascular perfusion with a buffered 1.5% glutaraldehyde, pH 7.35, solution. Immediately after fixation the brain was dissected and frontally sectioned into 3–4 mm thick slices, which were then postfixed in fresh aldehyde solution. 30–40 µm thick sections were cut with the freezing microtome and were treated with diaminobenzidene according to the method of Malmgren and Olsson (1977).

The sections were air dried and then dehydrated with ethanol, cleared with xylol and mounted in Cedax.

Results

The injection sites were all located in the lateral part of the tectum, varying slightly in antero-posterior and ventro-dorsal position. The HRP solution filled the pipette channel and diffused from there into the surrounding neuropil. The diffusion area had a diameter of 1.0–1.2 mm. In nearly all cases the tip of the pipette reached Cajal's (1911) layers 11–13 (Fig. 1a, b). Apart from neurons in these layers, periventricular pericytes, microglia and some nerve fibers in the immediate vicinity of the injection site also appeared labeled. Endings as well as numerous nerve fibers showing a positive HRP reaction were found in layers 2–8 of the tectum, into which the retinal pathway projects.

In the midbrain we also found labeled cells in the nucleus isthmi pars parvocellularis (Ipc), particularly its lateral "head", and in the nucleus spiriformis lateralis (SpL) (Fig. 2a, b). Many HRP-labeled neurons were discovered in the stratum griseum centralis (SGC) above the ventricle of the contralateral portion of the optic tectum. Their axons could be followed into the tectal commissure (TC). No marked cells were found in the remaining portions of the SGC (Fig. 3a, b).

HRP-filled neurons were also observed in the following hypothalamic nuclei: n. preopticus medialis, n. preopticus paraventricularis magnocellularis, n. supraopticus, n. periventricularis magnocellularis, the anteromedial area of the hypothalamus, as well as in some ventromedial parts of the lateral hypothalamus. These observations will be discussed in detail elsewhere (Mestres and Weindl, submitted).

A cluster of densely stained neurons is located within the nucleus septalis lateralis (SL), both ipsilateral and contralateral to the injection site (Fig. 4a). Most of these cells are bipolar, resembling neuroblastoid cells (Fig. 4b). One process is thicker than the other and can be identified as a dendrite. The thin process may possibly be an axon, although this cannot be determined with certainty. Between heavily marked perikarya one finds small

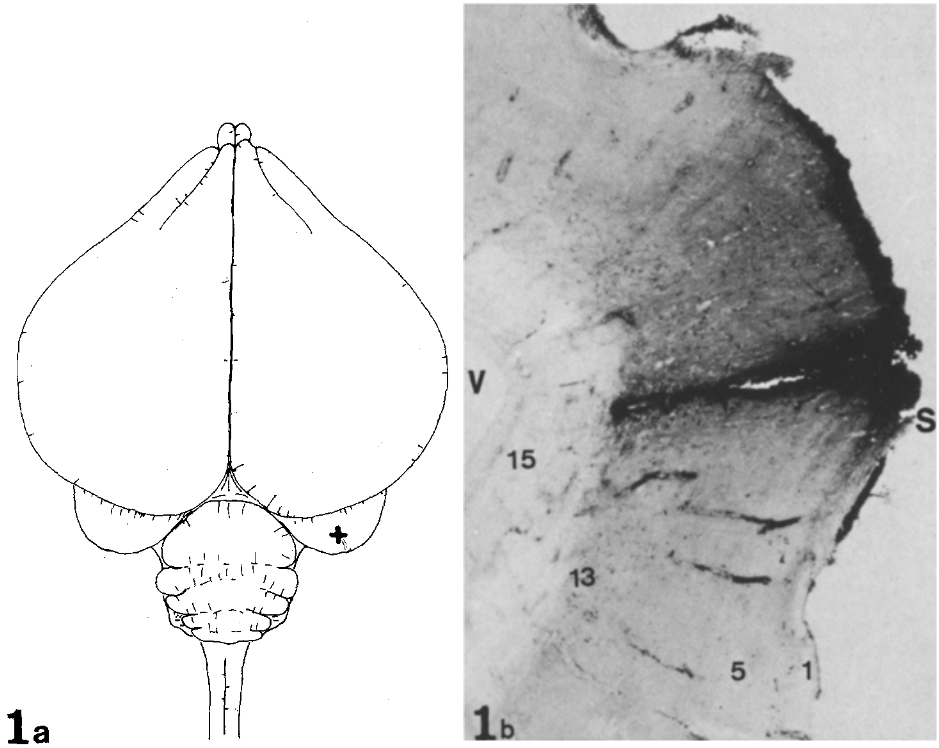

Fig. 1. **a** Diagram. Dorsal view of pigeon brain illustrating localization of HRP injection into the optic tectum (+). **b** Micrograph of injection channel, DAB method. The discrete gray toning on either side of the channel marks the area of HRP diffusion. A few labeled neurons are visible. For orientation, some of Cajals layers are indicated. Ventricle (*V*); pial surface (*S*). ×45

HRP-granulated cells that probably correspond to microglia. The cluster only partially occupies the region labeled SL in the Karten and Hodos (1967) atlas. This region lies immediately next to the lateral ventricle at level A 8 of this atlas. More marked cells could be observed in the ipsilateral than in the contralateral SL.

A large number of HRP-filled neurons were located in the so-called wulst of the telencephalon (Fig. 5a). Most of these cells were arranged in clusters or groups located either closer to the pial surface or nearer the lateral ventricle (Fig. 5a). Between these more or less distinct zones, however, there are numerous scattered neurons. The zones close to the surface of the hyperstriatum accessorium (HA) extend laterally as far as the valecular groove, in some places even beyond it, and medially down into the telencephalic septum along its midline. The zone adjoining the wall of the telencephalic ventricle occupies the more lateral parts of the septum, and fuses with the other, juxtapial zone more ventrally, but without reaching the previously mentioned cell group in the septum lateralis (SL).

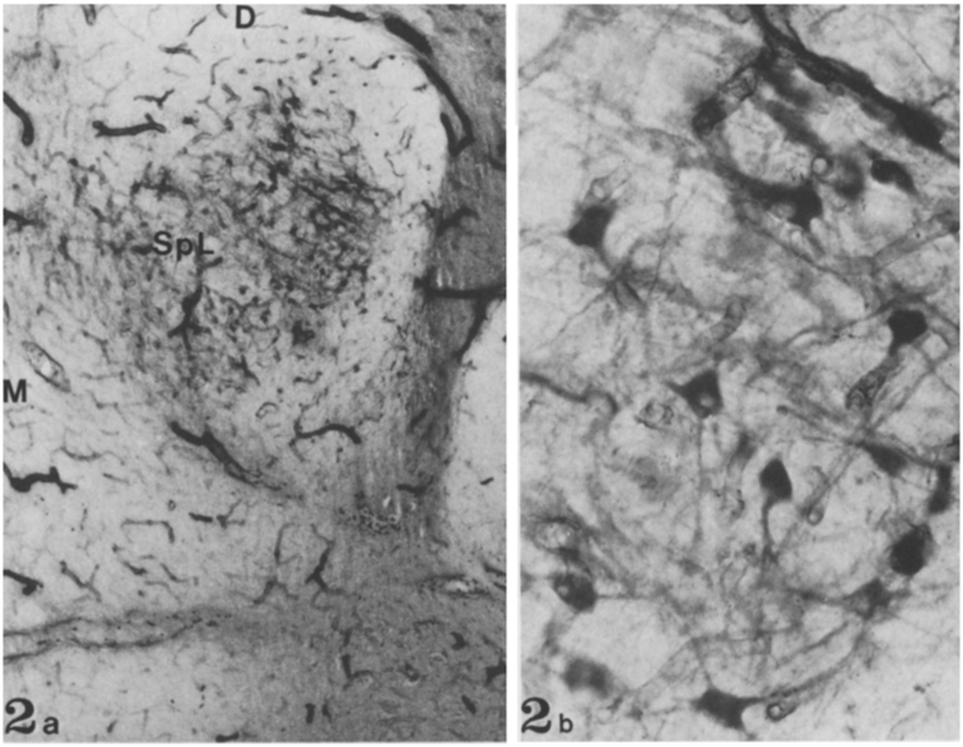

Fig. 2. **a** Nucleus spiriformis lateralis (*SpL*) on the right side. *M* medial; *D* dorsal. Perfusion with saline was short and HRP reactivity therefore also appears in blood vessels. ×40. **b** Detail of HRP-positive neurons in the SpL. ×450

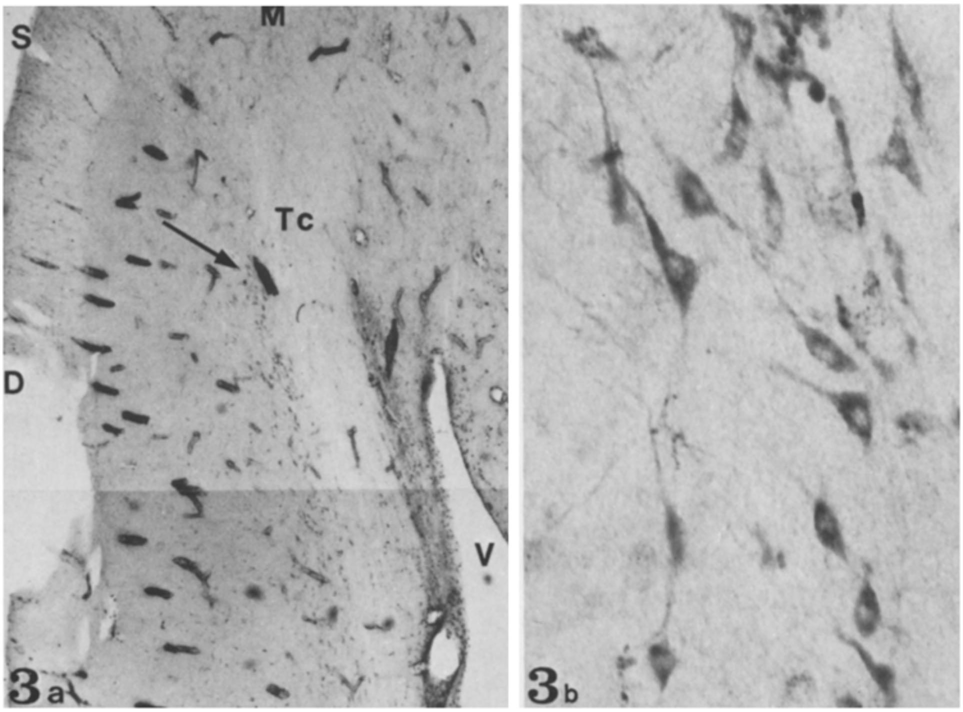

Fig. 3. **a** Frontal section of left optic tectum. *Tc* tectal commissure; *V* optic recess; *S* pial surface of medial part of tectum; *arrow* neurons in Stratum griseum centralis. ×35. **b** Detail of HRP-positive neurons shown in Fig. 3a. ×450

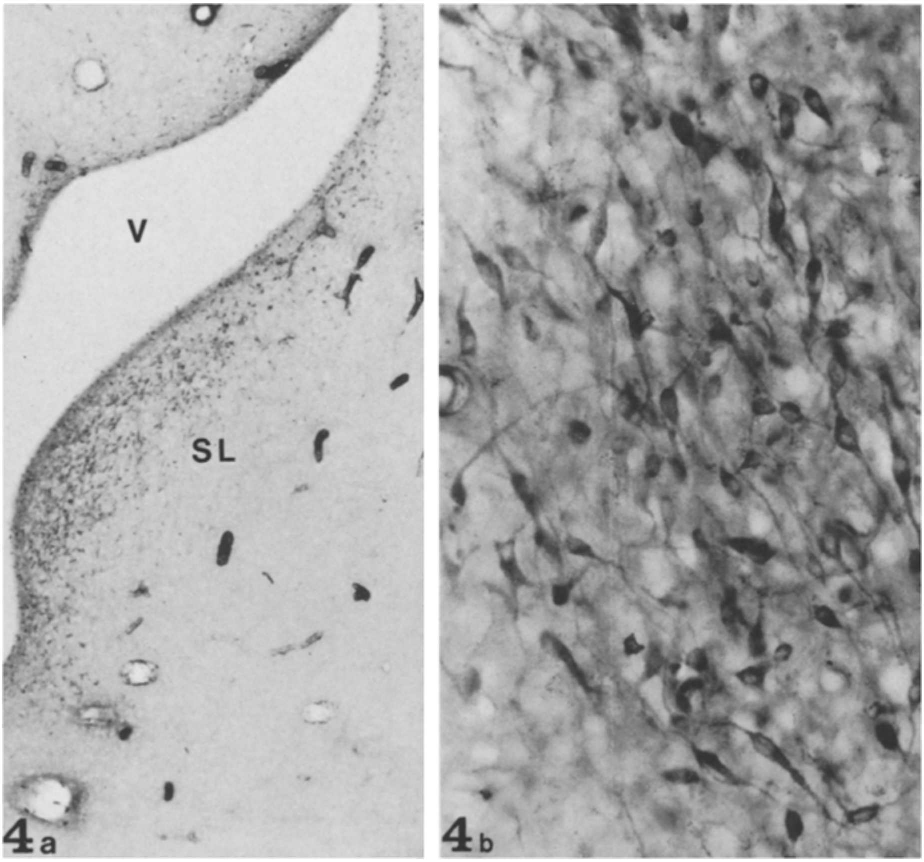

Fig. 4. **a** Nucleus septalis lateralis (*SL*) on the left side. *V* lateral ventricle. ×60. **b** Detail of HRP-positive neurons in the SL. ×450

At a post-injection survival time of 16 h the labeling of neurons is homogenous and it is possible to follow the axons from the perikarya to the superifcial stratum alveus, where they join the tractus septomesencephalicus (TSM) (Fig. 5b, c). A similar distribution of marked cell bodies is found both on the ipsi- and contralateral sides, but their density is again noticeably higher on the side homolateral to the injection site. Antero-posteriorly the area with stained neurons extends from approximately plane A 11 to plane A 8 of the Karten and Hodos (1967) atlas.

Figure 6 summarizes the afferents to the optic tectum considered in this study.

Discussion

The afferents to the optic tectum of mesencephalic origin which we describe confirm certain results of earlier studies. Brecha et al. (1976) injected HRP

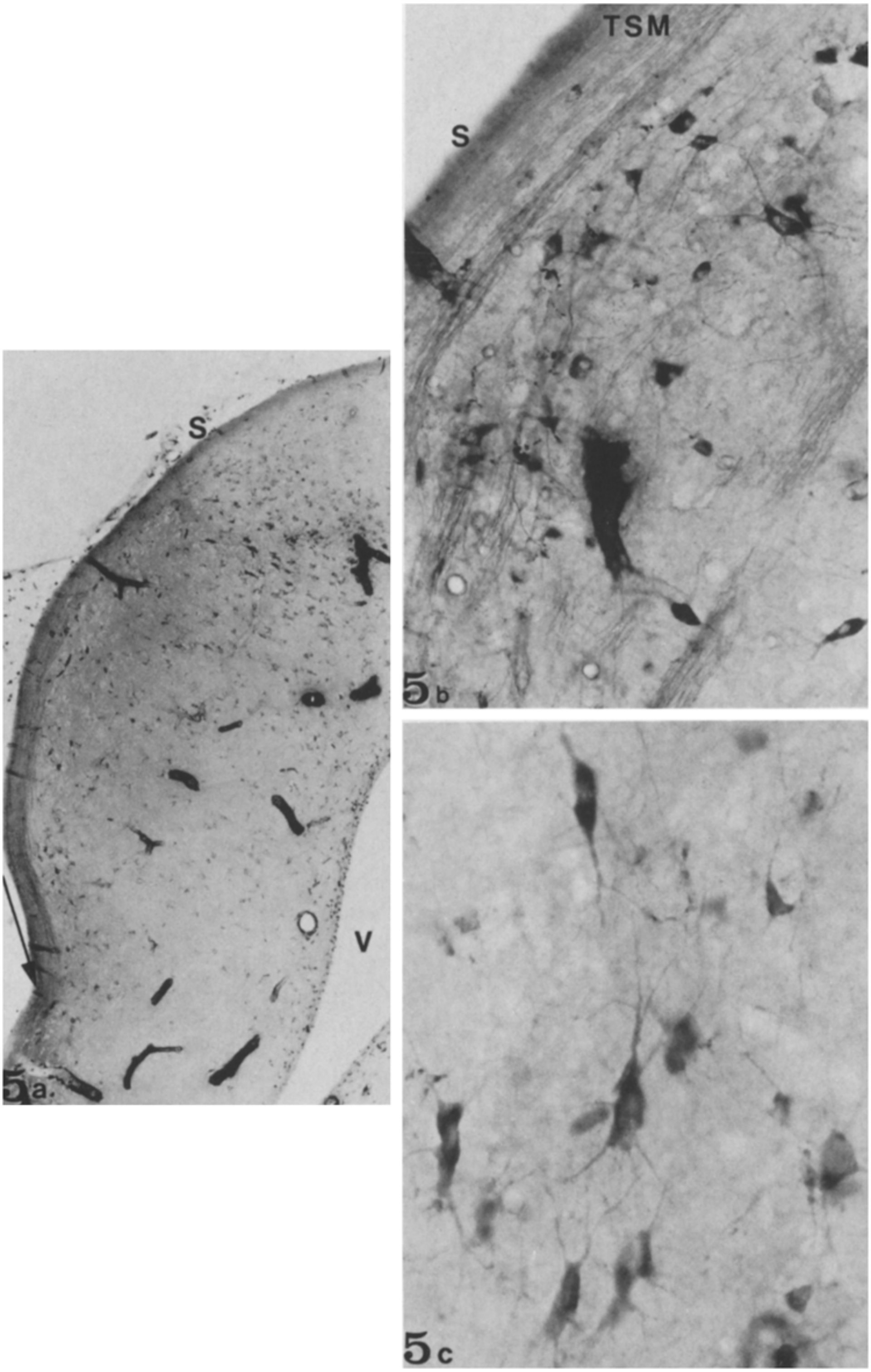

Fig. 5. **a** Wulst of the telencephalon on the right side. Note distribution of small groups of HRP-positive neurons between pial surface (*S*) and ventricle (*V*). *Arrow:* positive axons in the tractus septomesencephalicus (TSM). ×45. **b** Detail of HRP-positive neurons close to the fibers of the TSM in the hyperstriatum. ×220. **c** Detail of HRP-positive neurons in deeper parts of the hyperstriatum. Note homogenous labeling of cytoplasm and cell processes. ×450

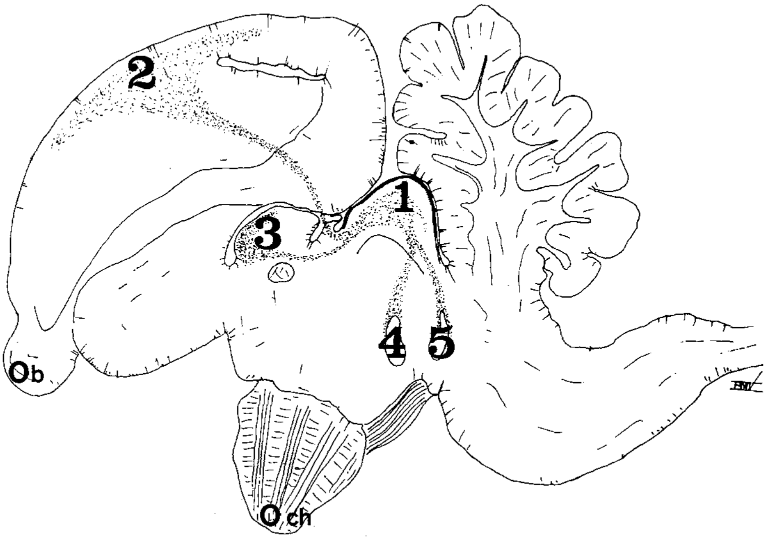

Fig. 6. Diagram illustrating connections observed between the optic tectum (*1*) and wulst (*2*), septum lateralis (*3*), nucleus spiriformis lateralis (*4*) and nucleus isthmi pars parvocellularis (*5*). *Och* optic chiasma; *Ob* olfactory bulb

into the tectum and already identified the ipsilateral nuclei isthmus pars parvocellularis (Ipc) and spiriformis lateralis (SpL), as well as a number of further ipsilateral nuclei, as sources of pathways to the tectum. That these additional nuclei were not found marked in our material is most likely due to the restricted range of post injection survival times used. Minelli et al. (1979), using the retrograde cell degeneration technique, also found the SpL and some other mesencephalic nuclei projecting to the ispilateral tectum, but interestingly enough, not the Ipc.

La Vail and La Vail (1974), using techniques based on the axonal retrograde transport for HRP, described the pathway originating from the deep layers of the contral-lateral tectum which has also been detected with anterograde methods (Voneida and Mello 1975; Hunt and Künzle 1976). Although some of these studies, as does ours, induce the idea that one tectum is topographically represented upon the other, a more detailed investigation is needed to clarify this issue. The problem is of functional interest in relation to the integration of the ocular inputs in species that, by virtue of eye position, have only a restricted field of binocular vision.

The origin of the wulst-tectal pathways has recently been described by Bagnoli et al. (1980), who also used the retrograde HRP method. The existence of such a pathway had already been demonstrated by Adamo (1967), Karten et al. (1973) and Miceli et al. (1979) with anterograde techniques. Our results correspond closely with those of Bagnoli et al. (1980), and it is thus now certain that both the ipsilateral as well as the contralateral superficial hyperstriatum accessorium and also the telencephalic septum project upon the tectum, an issue that was disputed in earlier accounts. It is important to note that the origin of this pathway only partially overlaps the termination of the visual thalamofugal afferents that reach deeper hyperstriatal layers (Hunt and Webster 1972; Karten et al. 1973; Meier et al. 1974; Miceli et al. 1979). Whether there is partial coincidence with the anterior terminations of the tractus infundibuli (Benowitz and Karten 1976) or the as yet anatomically undefined somatosensory projection to the frontal wulst (Delius and Benetto 1972) remains uncertain. The more precise localization of the source of the hyperstriato-tectal pathway now avaiable will undoubtedly aid the current functional studies suggesting that it has a regulatory influence on tectal inhibitory mechanisms (Britto 1978; Bagnoli et al. 1979; Nau and Delius 1981).

The bilateral projections of the nucleus septalis lateralis (SL) upon the tectum appears to be a new discovery. Krayniak and Siegel (1978), who examined the septal afferents in the pigeon with an anterograde technique, describe various pathways to the di- and mesencephalon, but none to the optic tectum. Inasmuch as knowledge of the SL is limited to the fact that some fibers of archistriatal origin terminate there (Zeier and Karten 1971), it appears premature to assign a precise functional role to this projection from the septum into the tectum.

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