Efferents from the Optic Tectum to the Brain Stem in the Japanese Quail (**Coturnix japonica**). Anterogradely Biocytin Method

By

Shoei SUGITA, Nobuhisa FUJIKAKE, Kunio SUGAHARA, Katsuhiko FUJIWARA and Naomi WADA

Department of Animal Science, Faculty of Agriculture, Utsunomiya University, 350 Minemachi, Utsunomiya, Tochigi 321, Japan and * Department of Veterinary Physiology, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753 Japan.

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**Summary:** Efferents from the optic tectum to the brain stem in the Japanese quail (*Coturnix japonica*) were studied with the anterogradely biocytin method. After injection of biocytin into the ipsilateral optic tectum, labeled terminals were seen in the rotund nucleus (Rt), neuropil part of the ventral lateral geniculate nucleus (GLnv), principal part of the dorsal lateral geniculate nucleus, lateral part of the dorsolateral thalamic nucleus, triangular nucleus (T), superficial parvocellular nucleus (SPC), pretectal nucleus, pretectal area (PA), subpretectal nucleus, central gray matter (GC), isthimo optic nucleus (ION), magnocellular and parvocellular parts of the isthimo nuclei (Imc and Ipc), semilunar nucleus (SLu), lateral and medial pontine nuclei and reticular formation (FRM) of the medulla, ipsilaterally. Labeled fibers were seen in the septomesecephalic tract nucleus, FRM, interstitio-paraoecto-subpraetectal nucleus, and the dorsal and ventral tectoreticular tracts (TRd and TRv). In the contralateral brain stem, labeled terminals were seen in the Rt, T, FRM, PA and paramedian nucleus. The contralateral terminals were remarkably fewer than those of the ipsilateral side. The present findings of the labeled terminals of the SPC and the GC at the level of the mesencephalic nucleus of the trigeminal nerve (MnT), and the topographic projection from optic tectum to the Rt in the thalamus, were original observations in the avian. The labeled terminals in the GLnv, Ipc, Imc and ION showed topographical projections from the optic tectum. Pathways to the contralateral brain stem were via the commissure posterior, ventral supraoptic decussation, and the predorsal bundle. The present results suggest that tectofugal impulses in the quail relate to various functions with special relation to the function of the GC at the level of the MnT as well as a visual function.

In the avian the majority of retinal efferents terminates within the optic tectum of the midbrain (Cowan et al., 1961; McGill et al., 1966; Crossland et al., 1979) and the optic tectum projects to many area of the brain stem nuclei (Hunt and Kunzle, 1976). The tectum is a highly differentiated structure in which there are six major layers (Lavail and Cowan, 1971). Retinal efferents form the most superficial layer I and terminate within the outer sublaminae of layer II (Crossland et al., 1973; Hunt and Webster, 1975). Many studies of the efferents of the tectum were performed in several species of mammals (Altman and Carpenter, 1961; Graham, 1977; Stein et al., 1982, 1984). These reports suggested that visual information was related to various functions in the brain stem. Therefore, it is important to understand where secondary visual information from the optic tectum is distributed in the brain stem of the avian as well as mammals.

There, unfortunately, are few reports on the efferents of the tectum and there are still disagreements in the distribution of the efferents from the tectum in the avian brain stem. Hunt and Kunzle (1976) reported the efferents of the tectum with the autoradiographic method. The autoradiographic method, however, remains a problem because it does not differentiated between small terminals and the background of silver grains. On the other hand, Wild (1989) reported that the location of the projection neurons of the tectum to the brain stem was identified with a retrogradely horseradish peroxidase method. His experiment, however, was performed in the thalamus. Reiner and Karten (1982), furthermore, showed that only the distribution of the efferent

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ALL CORRESPONDENCE TO: Shoei Sugita Ph.D. Department of Animal Science, Faculty of Agriculture, Utsunomiya University, Minemachi, Utsunomiya, Tochigi 321, Japan.
neurons of the tectum is to the lower brain stem. No complete description has been published. In the present study, therefore, we analyzed the tecto-recipient nuclei of the quail brain stem with an anterogradely biocytin method that clearly showed anterograde terminals.

Materials and Methods

Seventeen female Japanese quail (Coturnix japonica), weighing 80–120 g, were used in the present study. The animals were anesthetized with sodium pentobarbital (1.3 mg/30 g), and the brain was exposed by removing the skull. A 2.5% biocytin in physiological saline was injected into several parts of the ipsilateral optic tectum by an iontophoretic method with direct currents of 5–10 μA for 10 minutes. After twenty-four hours, the animals were anesthetized again and perfused transcardially with 200 ml of Ringer’s solution at 40°C, followed by 500 ml of fixative containing 2% paraformaldehyde and 2% glutaraldehyde in a 0.1 M phosphate buffer (pH7.4), and finally with 10% sucrose in the same buffer. The brain was cut serially in a coronal plane with a freezing microtome at 50 μm. The histochemical study to visualize biocytin was performed in following order. After the sections were washed for three times with a 0.3% in Triton-X in phosphate buffer, sections were incubated in 0.1% avidin-D HRP complex (Sigma) overnight at 4°C. After washing the sections two times in Triton-phosphate buffer, each section was treated with diamino benzidine with nickel ammonium and counterstained with neutral red. The nomenclature used here is that of Karten and Hodos (1967), and of Hunt and Kunzle (1976).

Results

After the injection of biocytin into the unilateral optic tectum (Fig. 1c, 3a), many labeled terminals were seen in the thalamus, pretectum, mesencephalon, pons and medulla in the ipsilateral brain stem. A considerable number of labeled terminals, however, was also found in the contralateral brain stem (Fig. 1).

a) Tecto-thalamic projections

Many terminals were seen in the ipsilateral rotund nucleus (Rt) and triangular nucleus (T) and fewer were seen remarkable within the lateral part of the dorsolateral thalamic nucleus (DLL) (Fig. 1a, 3b–c). Considerable numbers of labeled terminals were also found in the neuropil part of the ventral lateral geniculate nucleus (GLvn), principal part of the dorsal lateral geniculate nucleus (GLdp) (Fig. 1a, 3d, e). Weakly labeled terminals and fibers were found in the superficial parvocellular nuclei (SPC) (Fig. 1a, 3f). A sparse number of labeled axons was found in the nucleus of the tractus septomesencephalicus and tractus occipitomesencephalicus (Fig. 1a, 4a). A significant number of labeled fibers crossed the midline at the ventral supraoptic decussation (DSV) (Fig. 1a, 4b) and terminated in the contralateral Rt and T (Fig. 1a). Although many of these tecto-recipient nuclei showed a non-topographical organization, the distribution pattern of labeled terminals in the GLvn and the Rt presented topographical connections with the optic tectum (Fig. 2). After the injection of biocytin into the caudal tectum, labeled terminals were seen in the medial part of the caudal half of the GLnv. Labeled terminals were found in the rostral or medial parts of the GLnv after injection of a tracer into the rostro-dorsal tectum. Furthermore, labeled terminals were found in the middle part of the GLnv after the injection of biocytin in the middle tectum. The caudo-lateral parts of the GLnv were labeled after the injection of biocytin into the rostroventral tectum (Fig. 2). In the Rt, the lateral and dorsal parts were labeled after the injection of biocytin into the rostro-dorsal and middle optic tectum, respectively (Fig. 2). The ventral and medial parts of the Rt were labeled after the injection of the tracer into the caudal and rostro-ventral optic tectum, respectively (Fig. 2).

b) Tecto-pretectal projections

Heavily and lightly ordered projections were seen in the regions of the ipsilateral prepectal area. Heavily labeled terminals were seen in the ipsilateral prepectal nucleus (Pt) (Fig. 1b, 4c). More ventrally the pathways separated from the brachium of the colliculus superior (BC) and their component parts terminated in the subprepectal nucleus (SP) (Fig. 4c). Many fibers extending to the PT were seen in the interstitio-paraecto-subprepectal nucleus (IPS) (Fig. 4c). Few labeled terminals were seen in the bilateral prepectal area (Fig. 1b) and the crossing fibers at this level wherein the commissure posterior (CP) (Fig. 4d).

c) Tecto-mesencephalic projections

Labeled axons run between the isthmic nuclei and terminate heavily within the ipsilateral parvocellular (Ipc) and magnocellular (Imc) part of the isthmic nuclei and semilunar nucleus (SLu). The major portion of the tectal projection was the isthmo-optic nucleus (ION) (Fig. 1, 4e, 4f, 5a). Distributions of labeled terminals were localized in the particular...
Fig. 1. Drawings of a series of the frontal sections from the thalamus to the medulla in a case of injection site is middle tectum. Labeled terminals and the pathing fibers are represented by dots and wave line, respectively. Injection field of the tectum is shown as the closed black area in (c). Calibration bar: 1 mm. Abbreviations. FLM: medial longitudinal fasciculus, FRM: reticular formation, FRL: mesencephallic lateral reticular formation, MnT: mesencephalic nucleus of the trigeminal nerve, OL: olivary nucleus, OM: occiptomesencephalic tract, OMN: oculomotor nucleus, OT: optic tract, PA: pretectal area, Pam: paramedian nucleus, PM: medial pontine nucleus, PV: posteroventral thalamic nucleus, SPI: spiriform nucleus, TRv: ventral part of the recto-recticular tract, II: layer II, V: ventriculus, others follow Fig. 3–5.
regions of the Ipc, Imc, SLu and ION (Fig. 1c–d, 4e, 4f, 5a). Terminal buttons were easily seen in the central gray matter (GC) at the level of the mesencephalic nucleus of the nerve trigemini (MnT) (Fig. 5b) and the ventral GC at the level of the oculomotor nuclei (Fig. 1d). Terminal axons to the GC at the levels of the MnT and OM were separated from the components of the pathing fibers of the CP and the ventral part of the tecto-reticular tract, respectively (Fig. 1). Labeled fibers were also seen in the mesencephalic lateral reticular formation (FRL), occipitomesencephalic tract (OM) and dorsal and ventral tecto-reticular tracts (TRd and TRv) (Fig. 1d, 5c). Furthermore, retrogradely labeled neurons were seen in the Ipc and Imc, ipsilaterally shown in (Fig. 5d).

d) Tecto-pontine and medulla projections
Considerable numbers of labeled terminals were found ipsilaterally in the lateral and medial pontine nuclei (Fig. 1d, 5d). Axons of these terminals run through the FRL (Fig. 5d). Some fibers travelling within the dorsal FRM cross the midline and travel caudally within the paramedian FRM as the predorsal bundle (PB) (Fig. 1d, 5e) and terminate in the contralateral FRM of the medulla and paramedian nucleus (Fig. 1d, e). The ventral tecto-reticular pathway extends profusely into all parts of the ipsilateral pontine FRM (Fig. 1d).

e) Intrinsinc projections of the tectum
Labeled terminals were found in the ipsilateral tectal-cortex which was separated from the injection site in many cases (Fig. 1b, 1c, 5f). Labeled fibers extending from the injection sites travelled horizontally and then vertically and terminated in layer II of the ipsilateral tectal-cortex at a point somewhat distant from injection site.

f) Pathways of the projection to the contralateral brain stem.
Three pathways to the contralateral brain stem were identified as the CP, DSV and PB. Labeled fibers through the DSV were most dense among the three commissural fibers (Fig. 1a, 4b). Labeled fibers through the CP and PB terminated in the contralateral mesencephalic nuclei and the lower brain stem, respectively (Fig. 1b, d). On the other hand, the labeled fibers via the DSV terminated in the contralateral thalamic nuclei such as the rotund and triangular nuclei. Labeled fibers of the commissure posterior cross the midline and terminate in the contralateral pretectal area (Fig. 1b). The crossing fibers of the PB in the lower brain stem terminated in the contralateral reticular formation in the medulla (Fig. 1d).

Discussion
Projections from the optic tectum were studied by Hunt and Kunzle (1976) with the autoradiographic technique in the pigeon. The present results on the quail showed that the tectal projections were very similar to the map of those in the pigeon (Hunt and Kunzle, 1976). The terminals in the GC at the level of the MnT and SPC, and the topographical projections to the Rt were first seen in the present study. The large visual receptive field of the Rt neurons would clearly demand an afferent input from widely disparate areas of the optic tectum. However, there were differences in the projection pattern from the tectum to the Rt in previous reports. Hunt and Kunzle (1976) reported that there was no evidence of the topography within the tecto-Rt projections. On the other hand, Karten and Revzin (1966) showed that there was a possibility of the topographical projections from tectum to the Rt with a degeneration method. The present results from the biocytin method show that the distribution of terminals from the optic tectum to the Rt is not
homogeneous. The dorsal and ventral parts of the Rt had rich and poor terminations, respectively, when the injection site was restricted to the medio-dorsal part of the tectum. In contrast to this result, the rostral Rt and the lateral Rt were labeled more heavily than the caudal and ventral Rt after injection of biocytin into the caudo-ventral tectum. It seems to be that tecto-Rt projections result in have topographical distributions. Lesions of the tectum and subtectum (Hodos and Karten, 1974) or Rt (Hodos and Karten 1966, Hodos 1969) have been shown to result in deficiency in visual discrimination, while denervation of the DSV through which crossing tecto-Rt fibers run results in a subsequent failure of the interocular transfer of visual information (Meier, 1971). Thus, it could be considered that tecto-Rt projections function in visomotor control with a combination of information from the bilateral optic tectum and those neuronal connections should have topography. The tecto-geniculate projection, in the present study, was arranged as in previous studies (Crossland et al., 1973). On the other hand, it is known that the retino-geniculate projection also has a topographical connection the same as the retinotectal projection (Crossland and Uchwat, 1979). Therefore, retino-geniculate and -tectal, and tecto-geniculate projections should be closely interrupting each other. In the dorsal thalamus, there were few labeled terminals in the DLL as shown by Hunt and Kunzle (1976). In the present study, however, there existed labeled fibers in the SPC. Hunt and Kunzle did not describe the tectal projection fibers in the SPC. The difference between the present study and their results should be coming from the difference of the method because it is difficult to identify the small labeled axons or terminals with the autoradiographic technique.

The isthmic nuclei share certain anatomical features which may point to a role in the control of incoming visual information. Furthermore, a physiological study showed that isthmic nuclei moderate the visual information in the tectum (Wang et al., 1995). Therefore Ipc, Imc and ION receive the highly topographically organized tectal projections (McGill et al., 1966, Karten, 1967). Efferents from the tectal cells to the GLnv laid in layer II of the tectum (Benowitz and Karten, 1976, Reiner and Karten, 1982) and it was suggested that layer II rise the topographic tectogeniculate projections as well as the topographic projection to the Ipc (Hunt and Kunzle, 1976) and to the ION (Uchiyama and Watanabe, 1985). There exist reciprocal projection between the tectum and Ipc (Hunt and Kunzle 1976; Hunt et al., 1977). Although the present study did not focus on an analysis of the retrogradely labeled cells, both labeled neurons and terminals were seen in the Ipc. Previous reports (Hunt and Kunzle, 1976 and Hunt et al., 1977) support the present study. Tecto-ION projections were also observed in chicks and pigeons by various techniques. Uchiyama and Watanabe (1985) showed that tecto-ION neurons are distributed within the sublayer h of the layer II with the retrogradely HRP method. Among the many tecto-recipient nuclei in the brain stem, it is known that DLL receives the tectofugal information from layer III and ION from layer II. There should be a laminar topographical projection from the tectum to the brain stem nuclei, however, the present study did not show the laminar projection to each tecto-recipient nuclei because it was impossible to inject the tracer into the each layer.

In the present study, it was easy to find terminals originating from the optic tectum in the GC at the level of the MnT. According to our knowledge, there is no report presenting the projection from the optic tectum to the GC at the level of the MnT in the bird. It is well known that tectal projections to the GC in mammals terminate closely to the oculomotor nucleus in the ventral part of the GC (Stein et al., 1984; Altmann and Carpenter, 1961). Therefore, this projection has been considered as a visomotor integration. It was pointed out that the GC is connected intimately to some regions belonging to the sensory system, such as the posterior column of the spinal cord and the nucleus of the spinal tract of the trigeminal nerve (Tiwari and King, 1974). In the bird, on the other hand, tecto-GC terminate in both the lateral part of the GC at the level of MnT and the ventral part of the GC at the level of the oculomotor nucleus. Then tectal projections to the GC in the bird might be related to both the oculomotor system and other sensory information from the lower brain stem, on the GC at the level of the MnT.

Projections from the tectum to the lower brain stem such as medulla and pons are similar to previous results in the pigeon (Hunt and Kunzle, 1976). Intrinsic connections within the tectal-cortex should moderate visual information which are received in the different areas and deeper layers of the tectal-cortex.

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Explanation of Figures

Plate I

Fig. 3. Photomicrographs showing the injection site of the tectum (a), labeled terminals in the tectorecipient nuclei and pathing fibers in the brain stem (b–f). b: lateral part of the dorsal lateral thalamic nuclei (DLL), c: rotund nucleus (Rt) and triangular (T) nuclei, d: neuropil part of the ventral lateral geniculate nucleus (GLvn) and ventral supraoptic decussation (DSV), e: dorsal principal part of the lateral geniculate nucleus (GLdp), f: superficial parvocellular nucleus (SPC). Calibration bars: 100µm in (b), (d–f), 200µm in (c) and 400µm in (a).
Plate II

Fig. 4. Photomicrographs showing the labeled terminals in the tectorecipient nuclei and pathing fibers. a: tractus septomesencephalic nucleus (nSMT), b: pathing fibers in the DSV, c: pretectal nucleus (Pt), subpretectal nucleus (SP), interstitio-paraetecto-subprectectal nucleus (IPS), brachum of the superior colliculus (BSC), d: crossing fibers in the commissure posterior (CP), e: magnocellular and parvocellular parts of the isthmic nuclei (Imc and Ipc), f: isthimo optic nucleus (ION). Calibration bars: 200\(\mu\)m in (a), (b), (c) and (f), 400\(\mu\)m in (b) and (c).
Plate III

Fig. 5. Photomicrographs showing the labeled terminals in the tectorecipient nuclei and pathing fibers. a: semilunar nucleus (SLu), b: central gray matter (GC) at the level of the MnT, c: dorsal part of the tecto-reticular tract (Trd), d: lateral pontine nucleus (PL), e: crossing fibers in the predorsal boundle (PB), f: tectal cortex. Calibration bars: 200μm in (a)–(e). 100μm in (f).