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The mapping of the visual field onto the dorso-lateral tectum of the pigeon (*Columba livia*) and its relations with retinal specializations

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Abstract

Most of the physiological studies of the pigeon retino-tectal visual pathway have investigated the *accessible tectum*, a small dorso-lateral tectal section that can be easily accessed by a simple craniotomy. However, at present we lack a detailed study of the topographical arrangement between the visual field, the retina and the accessible tectum. In particular, it is not known which section of the visual field is mapped onto the accessible tectum, and which of the specialized retinal areas mediates this projection. Here we determined, using local field potential (LFP) recordings and reverse retinoscopy, the shape, size and position in the visual space of the portion of the visual field mapped onto the accessible tectum (called here the *accessible visual field*, or AVF). Using this data and the mapping of Nalbach et al. [Vis. Res. 30 (4) (1990) 529], the retinal area corresponding to the AVF was determined. Such retinal area was also directly delimited by means of retrograde transport of DiI. The results indicate that the AVF is a triangular perifoveal zone encompassing only 15% of total visual field. The retinal region corresponding to the AVF has the shape of an elongated triangle that runs parallel to the visual equator and contains the fovea, the tip of the pecten, a perifoveal region of the yellow field and a small crescent of the red field. In agreement with this anatomical heterogeneity, visual evoked potentials measured in different parts of the accessible tectum present steep variations in shape and size. These results are helpful to better design and interpret anatomical and physiological experiments involving the pigeon's visual system.
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1. Introduction

Birds' retinae feature several well-defined specialized regions, which differ in the density and composition of their cellular elements. In pigeons, the most common model for visual studies among birds, these regions are: the *red area*, a section of greater cellular density encompassing the dorso-temporal retina, which looks at the lower nasal part of the visual field; the shallow and cellularly dense *central fovea*, located near the projection of the optic axis, which looks at the centro-lateral visual field; and the more cellularly sparse *yellow field*, encompassing the inferio-temporal retina, which looks at the superior-anterior visual field. Extensive literature (Hahmann and Güntürkün, 1993; Nalbach et al., 1990) indicates that these areas have different visual properties and may serve different visual functions.
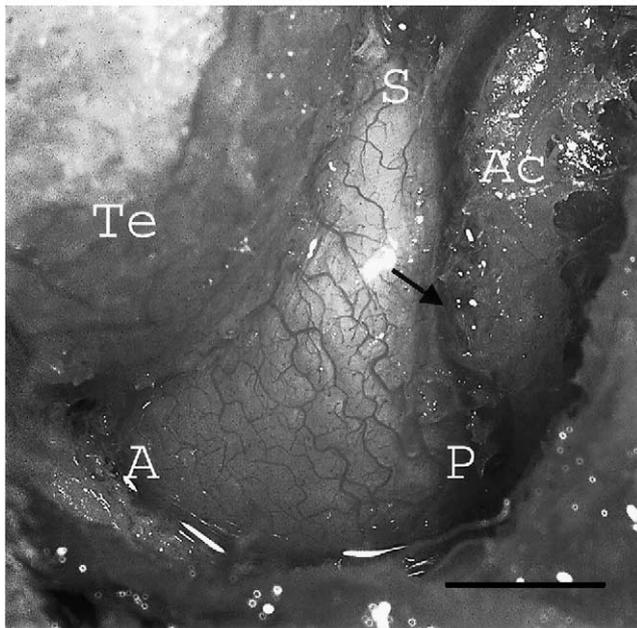
The main retinorecipient structure in the brain of birds and most non-mammalian vertebrates is the optic tectum. In pigeons, the tectum receives a direct projection from the contralateral eye, consisting of about two million axons, representing at least 90% of ganglion cells (Mpodozis et al., 1995). The retino-tectal projection follows a topographic arrangement that has been coarsely investigated with anatomical and electrophysiological techniques (Clarke and Whitteridge, 1976; Cowan et al., 1961; Hamdi and Whiteridge, 1954). According to these studies, the dorsal retina projects to the ventral tectum, while the anterior retina projects to the posterior tectum, producing a double inversion along the dorso-ventral and the anterior-posterior axes. However, none of these studies has described in detail how the different retinal areas map onto the tectum.

From an experimental point of view, most of the tectum is not directly accessible, as it is covered by the telencephalon, the anterior semicircular canal, or it lies directly on top of the bone forming the floor of the skull. Because of
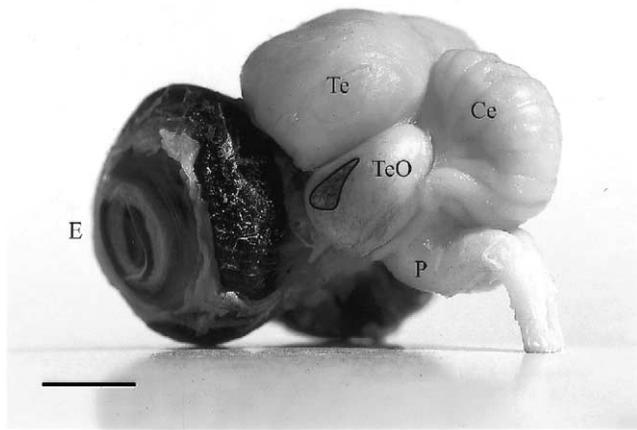
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this anatomical situation, the great majority of electrophysiological studies in pigeons have investigated the *accessible tectum*, the small dorso-lateral section that can be easily accessed by a simple craniotomy. The important experimental advantage of the accessible tectum is that it permits microelectrode penetrations under visual control (Fig. 1) (Frost and DiFranco, 1976; Frost et al., 1988; Hughes and Pearlman, 1974; Jassik-Gerschenfeld and Hardy, 1981).

At present, we lack a precise description, in a standard reference system, of the mapping of the retina and the visual field onto the accessible tectum in pigeons. In particular, it is not known where and to what extent the specialized retinal areas project upon the accessible tectum. Evaluation of the results from past experiments and the planning and interpretation of new experiments would benefit greatly from this knowledge. It would allow for more precise planning and interpretation of experiments involving tectal injections of neural tracers, or tectal lesions restricted to certain well-delimited zones of the retino-tectal projection (i.e. the foveal representation). Similarly, experiments assessing physiological differences between tectal responses elicited from different regions of the visual field would be facilitated in both execution and interpretation.

Here, we present a detailed map of the visual field of the accessible tectum (which we will call the *accessible visual field* or AVF), together with a determination of the retinal section that corresponds to it. We found that the AVF is a small triangular perifoveal zone encompassing only 15% of the total visual field. Its corresponding retinal section has the shape of an elongated triangle that runs parallel to the visual equator. This retinal region is not homogeneous, as it contains the fovea, the tip of the pecten, a pericentral portion of the yellow field and a small crescent of the red field. As expected from this anatomical heterogeneity, evoked potentials measured in different parts of the accessible tectum present steep variations in shape and size, indicating that the anatomical asymmetries of the retino-tectal projection are indeed reflected at physiological levels.

2. Materials and methods

2.1. Anesthesia

Adult feral pigeons ($N = 7$) of both sexes, obtained from a local dealer and kept in an animal facility for at least 2 weeks, were anesthetized with an intramuscular dose of a mixture of ketamine (40 mg/kg) and xylazine (12 mg/kg) injected into the pectoral muscles. This initial dose was supplemented by 13 mg/kg ketamine and 4 mg/kg xylazine every 2 h or when the heart frequency indicated a change in the level of anesthesia. The body temperature and the instantaneous heart beat frequency were continuously monitored by an on-line data acquisition system. The body temperature was maintained in the 39–41 °C. The ethics committee of the Universidad de Chile's Facultad de Ciencias approved all experimental procedures.

2.2. Eye and head immobilization

During each experiment the pigeon's head was mounted in a stereotaxic holder specially designed to allow maximum

(A)

(B)

Fig. 1. (A) Craniotomy exposing the accessible tectum (dorso-lateral aspect) in the pigeon. The accessible tectum has a triangular shape (A: anterior, S: superior, P: posterior). The anterior border (line AS) is delimitated by the telencephalon (Te), the posterior border (line SP) by a blood vessel (black arrow) running in parallel with the anterior semicircular canal (AC) and the horizontal border (line PA) is defined by a horizontal line passing just above the auditory meatus. The patterns of blood vessels change from animal to animal (scale bar: 2 mm). (B) Macroscopic anatomy of the pigeon brain. The optic tectum (TeO) is the largest visual structure in the pigeon's brain. The accessible tectum (area delimitated by a dark trace) is only a small fraction (15%) of the overall tectum. The accessible tectum was marked with black China ink. Note the relative size of the eye with respect to the brain (E: eye, Te: telencephalon, Ce: cerebellum, P: pons; scale bar: 5 mm).

exposition of the pigeon's the visual field. This stereotaxic instrument oriented the animal's head in accordance with orientations defined by the pigeon-brain stereotaxic atlas (Karten and Hodos, 1967). Once the pigeon was placed in the stereotaxic frame, a craniotomy exposing the dorsal aspect of the left tectum was performed. The dura over the tectum was also removed just prior to microelectrode insertion.

Eye immobilization was achieved either by using D-tubocurarin (10 mg/kg) coupled with mechanical ventilation (two experiments) or by using surgical glue (Histoacryl, Barun-Melsungen AG) (five experiments). The merit of the latter procedure, compared with the normal application of curare, was quantified by mapping the projection of the pecten using reverse opthalmoscopy.

2.3. Reverse retinoscopy and mapping of receptive fields

The precise mapping of receptive fields and retinal landmarks (pecten and optic axis) was achieved by using a translucent plastic, vertically-oriented hemisphere (diameter: 57 cm) centered on the pigeon eye in conjunction with inverse retinoscopy. This hemisphere was used as a screen to represent the pigeon's visual world as centered in the right eye. The hemisphere was attached to hinges that permitted it to swivel into position.

An ophthalmoscope attached to a floor stand with a flexible arm was used to visualize the tip of the pecten from the right eye. Once the tip of the pecten was correctly visualized at the center of the pupil, the ophthalmoscope was left in position and the eye was covered with a small piece of opaque plastic. Next, a low-power laser beam (0.1 mW), mounted in a moving stand and attached to a universal joint, was aligned until its beam passed through the opening of the ophthalmoscope and reached the eye of the pigeon. Then, the hemisphere was swiveled into its working position. The point defined by the interception of the laser beam with the hemisphere defined the projection of the pecten tip into the visual field. The same procedure was repeated with two points in the anterior side of the pecten and two points in the posterior side. Fortunately, the pecten contains many folds that provide easily defined landmarks to apply reverse ophthalmoscopy. These five points together defined the pecten profile as it was projected into the visual field represented by the plastic hemisphere.

The mapping of the projection of the optic axis was done similarly. The ophthalmoscope, attached to the floor stand and the flexible arm, was moved until the corneal reflection was centered on the pupil. To determine the centering, a 40× dissecting microscope was aimed at the pupil. Once the reflection from the ophthalmoscope was in the center of the pupil, the ophthalmoscope was left in place and the laser beam was again positioned to pass through the ophthalmoscope opening and to reach the pigeon's eye. Once the laser beam was correctly positioned, the translucent hemisphere was put in place and the point where the beam intercepted the hemisphere, which represents the projection of the optic axis, was marked onto the hemisphere. During each experiment the position of the optic axis and the profiles of the pecten projection were determined. In experiments where surgical glue was used to immobilize the eye, the pecten profile was also measured at the end of the experiment to assess the degree of eye immobilization.

2.4. Neural recordings

To map the projection of the visual world onto the dorsal tectum, tungsten microelectrodes (1–2 MΩ at 1 kHz, Frederick Haer) were lowered 150 μm, under visual control, into the accessible tectum using a hydraulic advancer. At the beginning of each experiment a photograph of the accessible tectum was made using a macro lens mounted on bellows. This photograph was used to define the succession of recording sites along the border of the accessible tectum. Depending on the anatomical morphology of each pigeon, 6–9 recording positions were selected. In each recording site, multiunitary tectal activity was recorded and fed into an audio monitor. The center of the associated receptive field was found by flashing small (0.2°) light spots onto the translucent hemisphere and the typical ON/OFF response associated with retinal fibers was detected. The point of highest response was defined as the center of the receptive field and its position was marked onto the translucent hemisphere.

To document regional differences in visual processing, flash-evoked local field potentials (LFP) were recorded at different tectal regions in three pigeons. Flashes (2.5°) were delivered to the outside of the translucent hemisphere exploring a 7.5 × 7.5° grid, centered in the receptive field under study. LFPs were sampled at 2000 samples/s with a data acquisition program written in IGOR (http://www.wavemetrics.com).

2.5. Pathway tracing

To trace the projection between the retina and the accessible tectum, a solid microinjector (Marin et al., 2001) was used to deposit small crystals (less than 100 μm) of DiI. These crystals were applied to seven points along the border of the accessible tectum inside the tectal retinorecipient layers. After 5 days of survival time (in other studies we have used between 5 and 20 days of survival time (Marin et al., 2001)), the pigeon was perfused and the contralateral retina was flat mounted for inspection with an epifluorescent microscope (Olympus).

2.6. Analysis of spatial data

Once the projection of the pecten and of the optic axis were marked on the hemisphere, an equidistant polar projection with the optic axis as the pole was drawn. The

spherical coordinates of the receptive fields as well as the retinal landmarks were plotted in the coordinate system introduced by Nalbach et al. (1990), which defined the position of various retinal landmarks in terms of ocular space and head orientation in relation with the eye-bill axis.

3. Results

3.1. The accessible tectum

Fig. 1A shows a typical craniotomy over the left tectum where only a small spherical triangular section, corresponding to the accessible tectum, is exposed. This triangular section is delimited anteriorly by the telencephalon and posteriorly by a major blood vessel that runs parallel with the anterior semicircular canal. The base of the triangle is delimited by bone forming the floor of the tectum. The triangle has an average base 3.3 mm wide, with a range between 2 and 4.3 mm. This wide range reflects age and anatomical differences in the conformation of the auditory meatus and the skull. In terms of the pigeon stereotaxic atlas (Karten and Hodos, 1967), the craniotomy exposes only the dorsal aspects of the sections located between plates APs 5.9 and 1.6. To assess the size and relative position of the accessible tectum in one animal, its outline was marked with black ink after perfusion. Fig. 1B shows the outline of the accessible tectum in relation to the overall tectum. We estimate that the accessible tectum represents 15% of the tectal surface.

3.2. The accessible visual field

The AVF outline was determined by mapping the receptive field loci of points chosen alongside the border of the accessible tectum. The AVF profile looks like an elongated triangle with a finger protruding in the dorso-posterior visual field and exhibits a great consistency among birds. Fig. 2A shows the orientation of the average AVF expressed in the coordinate frame of the pigeon atlas. In all cases it was found that the optic axis and the area surrounding the tip of the pecten mapped inside the AVF.

3.3. The projection from the retina

To assess the position and shape of the retinal region that project upon the accessible tectum, we retrogradely labeled its boundary with small DiI crystals deposited alongside the border of the accessible tectum. It was found that the marked retinal region has an elongated triangular shape and contains the fovea, a small portion of the red field, and the tip of the pecten (Fig. 2B). The shape of this retinal region, as well as the position of the pecten, are almost identical to the position and shape determined by visual mapping (compare Fig. 2A with B).

3.4. Regional variation of local field potentials across the accessible tectum

As the retinal region projecting to the accessible tectum contains a heterogeneous mixture of retinal areas (perifoveal area, dorso-lateral area, and a peripheral portion of the red field, see above), we recorded LFPs evoked by small localized flashes to search for regional variation across the accessible tectum. A consistent pattern was found in that dorsal LFPs have larger amplitudes than ventral ones (Fig. 3). All LFPs, independent of the recording site, exhibited an initial negative (N) wave (Holden, 1968; Letelier et al., 2000), but the amplitude of this initial negative phase as well as the complete temporal profile were found to be position dependent. In all pigeons studied ($n = 4$), the N wave is systematically larger (by a 3:1 ratio) in the dorsal part than near the representation of the horizon Fig. 3B and C compare traces in positions H and G with traces in position C). Also the temporal structure of LFPs showed clear variations with respect to tectal position.

4. Discussion

Our results show that the AVF represents only a small, pericentral fraction of the total visual field of the pigeon. The retinal area that looks at the AVF, and therefore projects onto the accessible tectum, is also small, but very heterogeneous, as it contains several specialized regions. This anatomical heterogeneity is physiologically reflected in the steep variations in shape and size presented by visual evoked potentials measured in different parts of the accessible tectum.

The position of the AVF in the visual space, as well as its corresponding retinal region, becomes immediately evident once the AVF profile is expressed in the reference frame of Nalbach et al. (1990) (Fig. 4A). The AVF corresponds to a small (15% of total) elongated triangular portion of the monocular visual field that lies mostly in the strip between the horizontal meridian and 30° of dorsal eccentricity. Extensive regions of the visual space, such as the binocular crescent and most of the dorsal area, are excluded. The visual field of the lateral fovea, which in pigeons serves to mediate lateral fixations (Maldonado et al., 1988) falls at the center of the AVF, while its ventral anterior pole contains the visual field of a pericentral corner of the red area. The AVF also contains the projection of the optic axis, located 10° below the foveal area. This spatial result perfectly matches the anatomical tracing of the retinal region projecting to the accessible tectum. The coherence between the anatomical (Fig. 2B) and spatial (Fig. 4A) determinations is such that the retinal region projecting to the accessible tectum and the AVF can almost be superimposed (compare Fig. 2B with 4A, also see Fig. 5).

In a first approximation, the shapes of the accessible tectum and the AVF were found to be almost congruent. If the AVF outline is transformed using simple geometrical
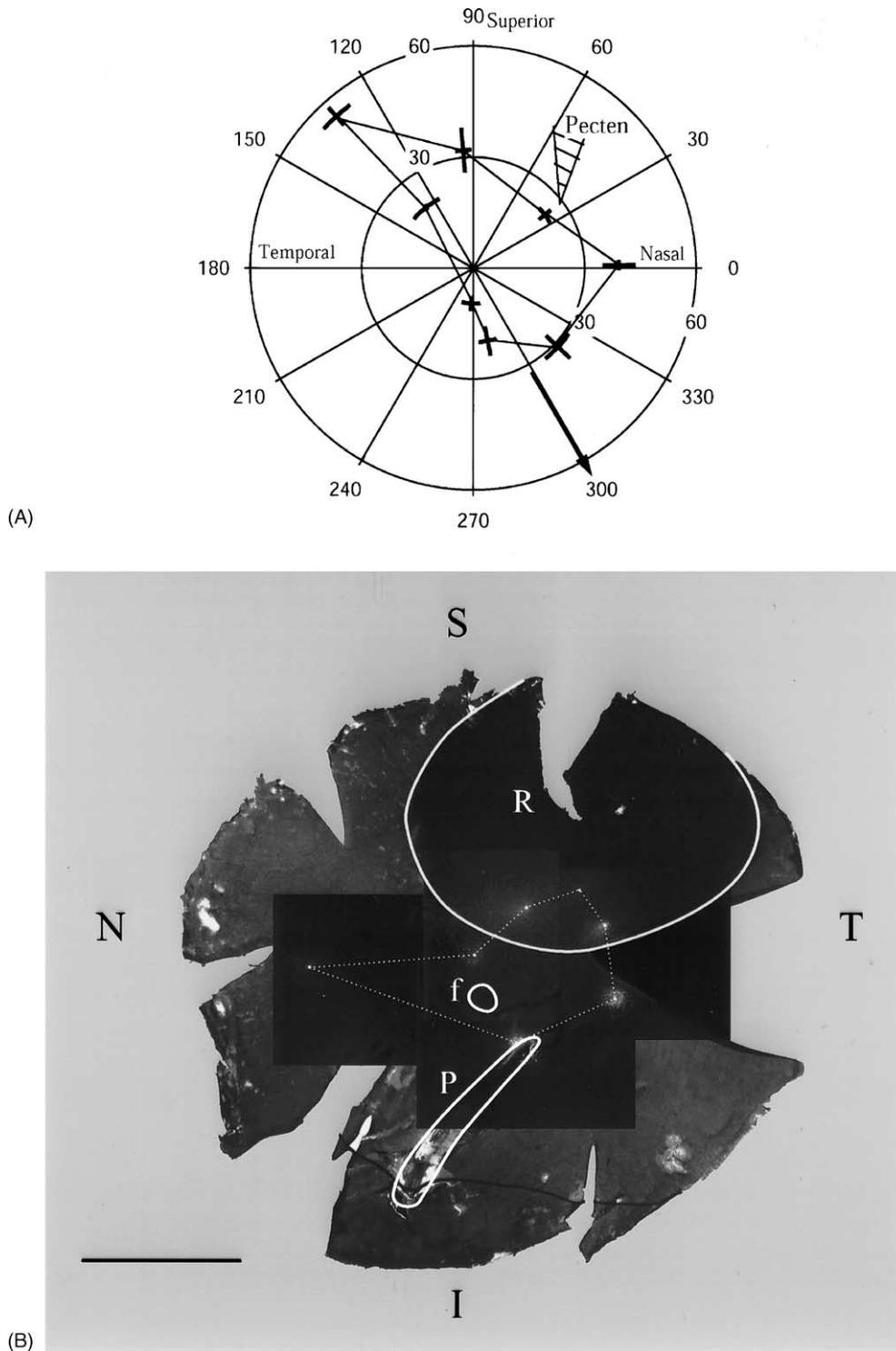
Fig. 2. (A) Average profile of AVF projecting to the accessible tectum (seven cases). This profile represents, for the right eye, the average AVF projecting into the accessible tectum expressed in an equidistant polar coordinate system with the optic axis as the north pole (Nalbach et al., 1990). Crosses represent the standard deviations for the elevation and azimuth. A polygonal line approximated the average profile. The figure uses the head inclination defined in the stereotaxic atlas (Karten and Hodos, 1967). The pecten projection was similar in all pigeons. The arrow defines the "beak axis" (i.e. the relative orientation of the beak with respect to the horizontal meridian). (B) Retinal flat mount. The polygonal figure defined by the bright (white) spots corresponds to the retinal region marked after injecting seven small DiI crystals alongside the border of the accessible tectum. The shape of this retinal region is congruent with the AVF shape (compare with A). The darker rectangular sections correspond to the fluorescent images superimposed onto the bright-field picture of the entire retina. This retinal region contains the fovea (f), the tip of the pecten (p: pecten) and the border of the red field (R: red field, N: nasal, T: temporal, S: superior, I: inferior; scale: 5 mm).
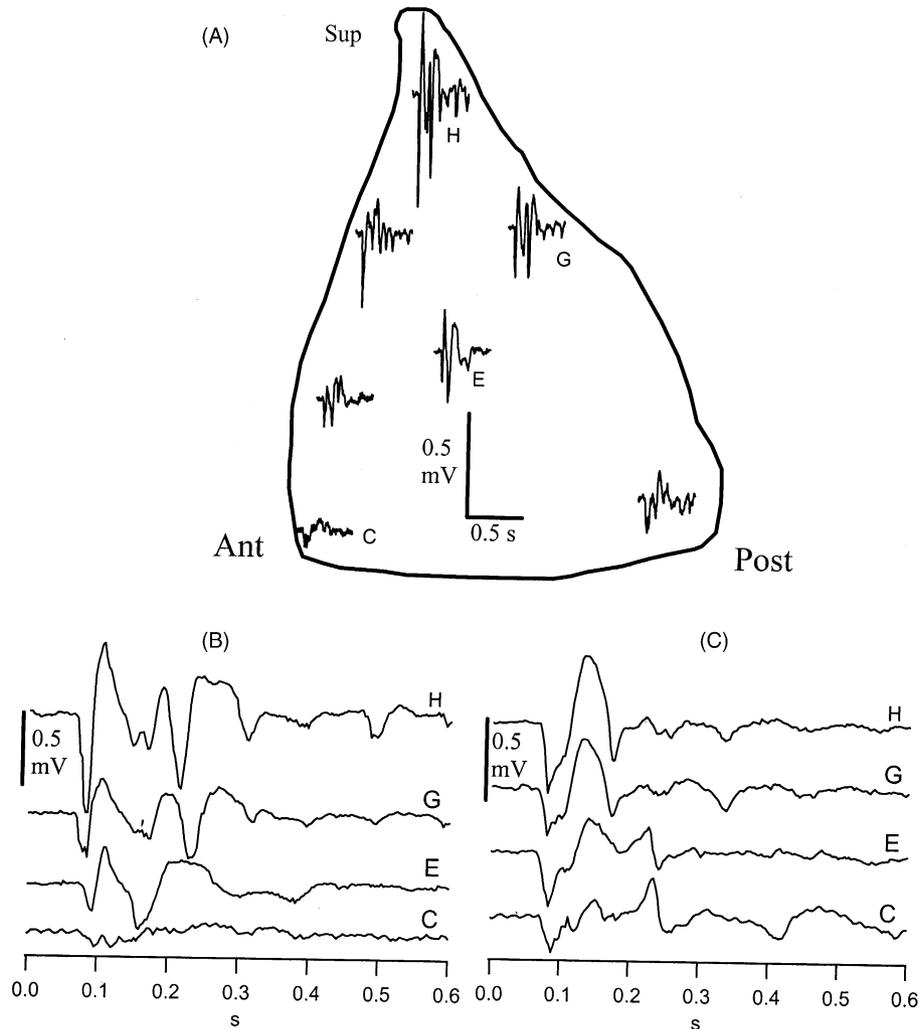
Fig. 3. (A) Regional variation in LFP in the accessible tectum (four cases). The LFPs elicited by small flashes (2°) in the center of the receptive field were recorded for different tectal loci in the accessible tectum. LFPs exhibited a consistent amplitude variation between the dorsal and ventral positions. Dorsal LFPs (position H) have larger amplitude than ventral LFPs (position C) (see Fig. 1B, Ant: anterior, Post: posterior, Sup: superior). (B) LFP traces at higher temporal resolution. Traces from (A) at selected positions. (C) LFP profiles from another pigeon at the same positions. Note how, in both cases, the traces corresponding to position H are greater than at position C. Also notice how the temporal profile changes from bird to bird.

operations (like reflection, rotation, and uniform stretches in the *x* and *y* dimension), its transformed outline and the outline of the accessible tectum are very similar (Fig. 5). Both shapes represent elongated triangles, with a very strong congruence in the ventral part, where they are almost identical. This congruence degrades towards the dorsal tectum.

In most physiological studies, the optic tectum is presented as a homogenous structure. Nevertheless, our LFP recordings show that even a tectal section as small as the accessible tectum shows a dramatic physiological heterogeneity. Inside this tectal portion, tectal loci receiving afferences from different retinal areas show steep differences in their LFP profiles. Tectal LFPs are mostly a manifestation of the synaptic currents triggered by the volley of incoming retinal action potentials, and their profiles reflect the collective operation of large populations of synapses (Holden, 1968; Letelier et al., 2000). Therefore, differences in LFP profiles, like those described in this study (Fig. 3), will result either

from: (a) differential properties and density of retinal ganglion fibers in the stimulated retinal areas, (b) differences in the physiology and density of the postsynaptic elements in the tectum, or (c) a combination of both these factors. Our results directly show that the anatomical heterogeneity of the retinal projection onto the accessible tectum is indeed reflected at the physiological level. Thus, marked differences should be expected in visual synaptic physiology between tectal regions in receipt of afferences from the different retinal specializations, in particular, between the dorsal (yellow field recipient) and ventral (red field recipient) tectum. In a recent study (Gu et al., 2000), the visual properties of tectal receptive fields, in fact, have been described as differing between these tectal regions.

The physiological regionalization described here is especially relevant in the case of studies involving current source density (CSD) analysis. This technique, which allows synaptically active neuropiles to be localized deep within a
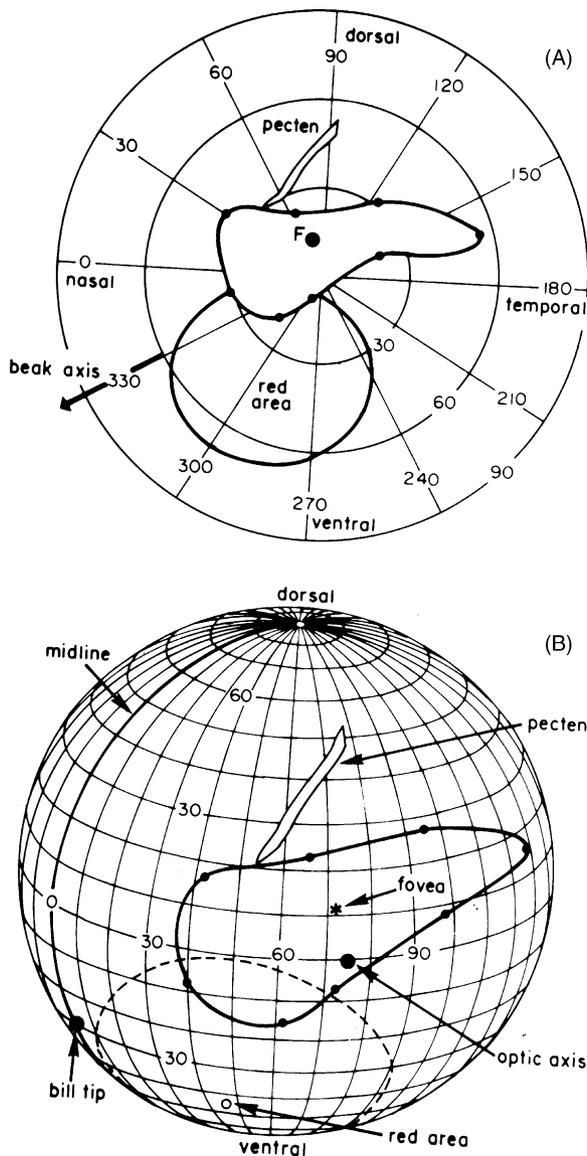
Fig. 4. (A) AVF profile, and retinal landmarks, in standardized coordinates. The average position of AVF was plotted in relation with the positions of retinal landmarks using the reference frame defined by Nalbach et al. (1990). In this representation, which corresponds to the head orientation of a walking bird, the AVF is mostly a superior perifoveal band that contains the projections of the optic axis and the fovea. Only a small fraction of the red field is located inside the AVF. The fovea (asterisks) is always located inside the AVF. The red field is indicated by a thick black line. The pecten position and the extent of the red field were obtained from Nalbach et al. (1990). (B) 3D representation. Position of the average AVF plotted in a sphere representing the left visual field. Data from (A) plotted in 3D according to Hahmann and Güntürkün (1993).
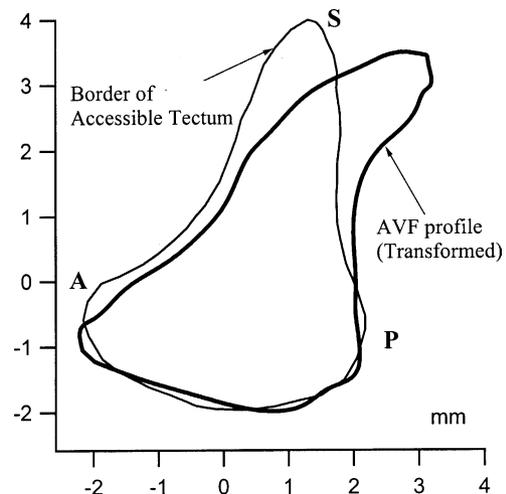

Fig. 5. AVF and the accessible tectum are almost congruent. The AVF profile when subjected to simple geometrical transformations (a reflection followed by an 18° rotation, and magnified 7 times in the *x* dimension and 4.5 times in the *y* dimension) is similar to the accessible tectum. Both areas are congruent in the ventral part, but this congruence degrades dorsally, where the superposition is less perfect than ventrally (S: superior, A: anterior, P: posterior).

5. Conclusions

We have found that the accessible tectum, although a mere 15% of the total tectum, is not homogeneous, as it receives afferences from a section of the retina containing several specialized areas. Accordingly, the accessible tectum presents clear regional differences in visual physiological processing. These results, along with recent results concerning functional and structural asymmetries of the tecto-fugal pathway (Gu et al., 2000; Karten et al., 1997), show that the physiological exploration of this pathway must take into account the conspicuous regionalization of the retino-tectal projection in birds. The data presented here allow tectal physiological results to be related to anatomical specializations of the retino-tectal projection.

Acknowledgements

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multilayered structure, is based on the synchronic collection of LFPs at different depths. Several studies have performed CSD analysis in the accessible tectum of the pigeon, under the assumption that LFPs are homogeneous across tectal surface structures (Letelier et al., 2000; Nakagawa et al., 1997). Our data indicate that this is not the case, suggesting that LFP and CSD studies should be reevaluated.

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