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Bilateral and Ipsilateral Ascending Tectopulvinar Pathways in Mammals: A Study in the Squirrel (*Spermophilus beecheyi*)

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Abstract

The mammalian pulvinar complex is a collection of dorsal thalamic nuclei related to several visual and integrative processes. Previous studies have shown that the superficial layers of the superior colliculus project to multiple divisions of the pulvinar complex. Although most of these works agree about the existence of an ipsilateral tectopulvinar projection arising from the stratum griseum superficiale, some others report a bilateral projection originating from this same tectal layer. We investigated the organization of the tectopulvinar projections in the Californian ground squirrel using cholera toxin B (CTb). We confirmed previous studies showing that the caudal pulvinar of the squirrel receives a massive bilateral projection originating from a specific cell population located in the superficial collicular layers (SGS3, also called the “lower SGS” or “SGSL”). We found that this projection shares striking structural similarities with the tectorotundal pathway of birds and reptiles. Morphology of the collicular cells originating this projection closely corresponds to that of the bottlebrush tectal cells described previously for chickens and squirrels. In addition, we found that the rostral pulvinar receives an exclusively ipsilateral projection from a spatially separate population of collicular cells located at the base of the stratum opticum, deeper than the cells projecting to the caudal pulvinar. These results strongly support, at a structural level, the homology of the pathway originating in the SGS3 collicular cells upon the caudal pulvinar with the tectorotundal pathway of nonmammalian amniotes and contribute to clarifying the general organization of the tectopulvinar pathways in mammals.

INDEXING TERMS

vision; pulvinar; tectum; superior colliculus; rotundus; tectofugal; parallel visual pathways

The optic tectum, a laminated structure located in the roof of the midbrain, is the main retinal target in most vertebrates. In turn, the tectum is the origin of several efferent ascending and descending visual pathways that arise from specific cell populations located in different tectal layers. Among these routes, the tectofugal pathway (tectum-dorsal-thalamic nucleus-pallial telencephalon) is the main ascending visual pathway in most nonmammalian vertebrates.

In nonmammalian amniotes (birds and nonavian reptiles), the thalamic relay of the tectofugal pathway is the nucleus rotundus thalami (Rt). The Rt receives a bilateral and nontopographic input from the tectum originating exclusively from the tectal ganglion cells

(TGCs) located in layer 13 (Hodos and Karten, 1966; Karten and Revzin, 1966; Hunt and Kunzle, 1976; Benowitz and Karten, 1976; Mpodozis et al., 1996; Luksch et al., 1998; Marín et al., 2003). TGCs are of distinctive morphology, featuring very large dendritic arbors, each covering a circular area of about 1–3 mm in diameter of tectal surface, with characteristic bottlebrush endings distributed in specific retinorecipient tectal laminae (Mpodozis et al., 1996; Luksch et al., 1998). The Rt in turn sends its axons topographically to a pallial, nonlaminar structure called the “entopallium” located within the dorsal ventricular ridge (Karten and Hodos, 1970; Benowitz and Karten, 1976; Nixdorf and Bishof, 1982; Laverghetta and Shimizu, 2003; Krütfeldt and Wild, 2005; Fredes et al., 2010). This bilateral projection arising from tectal TGCs to the nucleus rotundus can be considered a diagnostic trait of the tectofugal pathway, because it has been described in amniotes as phylogenetically distant as granivorous birds (Benowitz and Karten, 1976; Mpodozis et al., 1996; Marín et al., 2003), turtles (Rainey and Ulinski, 1982; Reiner, 1994), caimans (Pritz, 1975), snakes (Dacey and Ulinski, 1983), and diurnal lizards (Dávila et al., 2002).

In mammals, the stratum griseum superficiale (SGS) of the mesencephalon is considered homologous to the optic tectum of nonmammalian vertebrates. This region is contiguous with several underlying divisions of the mesencephalon, and collectively forms the superior colliculus (SC). Although several different collicular projections to the dorsal thalamus have been described over the years, the identity of the components of the mammalian tectofugal pathway remains a controversial issue. Benowitz and Karten (1976) first proposed that part of the pulvinar complex could be homologous to the avian rotundus. Evidence supporting this hypothesis first appeared in 1975, when Trojanowski and Jacobson reported for primates (rhesus monkey) the presence of a bilateral projection from the collicular SGS to the caudal pulvinar. This finding was later confirmed by Benevento and Rezak (1976) in rhesus monkey and by Robson and Hall (1977) in squirrels. However, other studies have failed to describe such bilateral projection, reporting instead the presence of tectopulvinar projections that are only ipsilateral (Graham and Berman, 1981; Donnelly et al., 1983; Cadusseau and Roger, 1985; Abramson and Chalupa, 1988). Thus, at present, not only the identity of the mammalian nucleus rotundus but more generally the organization of the tectopulvinar projections require further clarification (Puelles et al., 2000; Aboitiz and Montiel, 2007).

The present work used modern neuronal tracers to investigate the collicular connectivity of the pulvinar complex in the Californian ground squirrel, a diurnal ground-dwelling sciurid that exhibits well developed retinotectal projections (Michael, 1973; Major et al., 2000) a large and well-laminated SC (Kaas and Collins, 2001), and a large pulvinar complex (Robson and Hall, 1977). In a recent study of the squirrel Baldwin et al. (2011) showed further details regarding the existence of two tectopulvinar projections: a bilateral one, directed to the caudal pulvinar, and an ipsilateral one, directed to the lateral and medial divisions of the lateral rostral pulvinar. The present study confirmed these previous results and extended them, showing that these two segregated tectopulvinar pathways originated from different collicular cell populations with distinct morphological features and laminar locations. The present results strongly support the structural homology of the bilateral tectopulvinar pathway with the tectofugal pathway of nonmammalian amniotes and help to clarify the general organization of the tectopulvinar pathways in mammals.

MATERIALS AND METHODS

Twenty-eight adult California ground squirrels (*Spermophilus beecheyi*), weighing 400–650 g and including both males and females, were trapped in accordance with state permits and used in accordance with guidelines set forth by the UCSD Animal Care and Use Committee

and by the Science Faculty's Ethics Committee (Comité de Ética de la Facultad de Ciencias, Universidad de Chile).

The animals were anesthetized with a mixture of ketamine (50 mg/kg IM) and xylazine (10 mg/kg IM) and given additional doses of 10% of the initial amount every 15 minutes during the surgical procedure. Once anesthetized, the animals were weighed and mounted in a stereotaxic apparatus designed for rodents (Kopf Instruments, Tujunga, CA). The posterior dorsal aspect of the skull was exposed by means of a skin incision, and the muscles obstructing the area were retracted. Depending on the experiment, a craniotomy was performed to expose the cortex overlying the SC or the lateral thalamus. To identify the structure to be injected, a tungsten microelectrode (1 M Ω) was attached to a hydraulic micromanipulator and stereotaxically positioned and introduced into the brain. The physiological signals were amplified (AM Systems model 1800) and monitored on an oscilloscope (Tektronix model 5113) coupled to an audio monitor. Visual stimuli were generated with a hand-held laser pointer or small flashlight. Once the target structure was identified by its response to visual stimulation, a glass micropipette previously filled with native purified cholera toxin B subunit (CTb; List Biological Laboratories, Campbell, CA; 0.5% in phosphate buffer 0.1 M) was lowered into the brain based on the coordinates obtained from the physiological recordings. The tracer was then pressure injected, applying a train of regulated pressure pulses by means of a Picospritzer II (General Valve) connected to a tank of compressed nitrogen. The wound was closed by means of sterile surgical clips, and the animal was returned to its cage. During surgery and recovery, all wounds and pressure points were treated with lidocaine gel 5%, and the body temperature was maintained by using a warm water bag approximating the normal body temperature. Every effort was made to avoid animal discomfort.

After survival periods of 5–7 days, the squirrels were deeply anesthetized with an overdose of the same mixture of ketamine and xylazine as used for the surgery and perfused through the heart with approximately 500 ml saline (0.9%), followed by 500–800 ml cold 4% paraformaldehyde in PB.

After removal of the skull, each brain was postfixed overnight in the fixative solution and then transferred to a solution of 30% sucrose in PB until it sank. The brains were cut in the coronal plane at 40 μ m and processed with immunohistochemical techniques to reveal CTb distribution using the avidin-biotin peroxidase method. Details of these procedures have been described elsewhere (Shimizu et al., 1994; Rodman and Karten, 1995). Briefly, to reveal CTb, the sections were incubated with antibodies against CTb (List Biological Laboratories; polyclonal antibody raised in goat against the native purified CTb subunit, at a dilution of 1:2,000), followed by biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA; anti-goat IgG, made in rabbit, at a dilution of 1:200). Avidin-coupled peroxidase (Vector ABC kit) was then used with diaminobenzidine as the final chromogen. Brain tissue from uninjected animals that had undergone the same immunohistochemical procedure produced no detectable labeling. One of the six series of sections was reacted for CTb and was counterstained with Giemsa (Iñiguez et al., 1985) to reveal the cell bodies. Another series was left uncounterstained. Thus, one of every three sections was reacted and examined for CTb labeling. Alternate series of sections were stained for Nissl substance. Whole sections were drawn in Adobe Photoshop (Adobe Systems, Mountain View, CA) from low-power pictures of the tissue taken with a dissection scope (Olympus SZX10) coupled to a digital camera. Selected histological material was photographed with a conventional microscope (Olympus BX61) coupled to a digital camera (Spot digital camera; Diagnostic Instruments, Sterling Heights, MI). Image contrast adjustments, annotations, photomontage, and figure assembly were performed in Adobe Photoshop.

RESULTS

Two different pathways to the pulvinar complex arise from the SC

In a first set of experiments we sought to confirm the previously reported overall pattern of the SC projections upon the pulvinar complex (Robson and Hall, 1977; Baldwin et al., 2011). For that purpose, in two cases we placed small CTb injections, centered in the superficial layers of the SC, although some tracer diffusion to the most dorsal aspect of the deep layers was unavoidable (Fig. 1A). These injections were located laterally and in the middle of the rostrocaudal axis of the structure. They made up no more than 10% of the total surface of the SC.

At rostral thalamic levels, these injections into the SC resulted in ipsilateral anterograde labeling of a dense plexus of axonal terminals in two different nuclei: the third layer of the dorsal lateral geniculate and the rostral division of the pulvinar complex. In both structures, the label appears as well-defined patches of terminals limited to restricted domains within each of them. The intensity of the label indicates that these structures receive a high density of collicular innervation. Also, in both structures, the location of the labeled patches was different between our two cases, suggesting a topographic arrangement of the collicular afferents (Figs. 1B, 2) that is in agreement with the results of Baldwin et al. (2011).

At more posterior levels, the caudal division of the pulvinar complex appears to be bilaterally labeled by a distinct plexus of axonal processes and terminals. In both cases, the intensity of the label was higher in the ipsilateral caudal pulvinar than in the contralateral one. The density of labeled axons following small injections appeared less than that observed in the dorsal lateral geniculate and rostral pulvinar (Fig. 1B). It is striking to note that, in spite of the small area covered by the injections and their different locations, in these cases the labeled processes were found throughout the whole extent of the nucleus, clearly delimiting its borders on both sides (Fig. 3A). Furthermore, efferent axons from the SC could be followed in continuity into the ventral supraoptic decussation (VSOD) and to their termination in the contralateral caudal pulvinar. These observations are in accordance with the earlier report of Robson and Hall (1976) and indicate that the tectopulvinar projection follows a highly divergent, nontopographic pattern.

In one additional case the tracer injection was confined to the deep collicular layers (below SGS), with a minimal spread to most superficial layers and optic tract and possibly to the nucleus of the optic tract (Fig. 4A). After this injection, with the exception of the terminals in the ipsilateral nucleus of the optic tract, no other structure showed evidence of labeling at the level of the caudal pulvinar (Fig. 4B). In contrast, the examination of more rostral sections showed a patch of anterogradely labeled terminals ending within the rostral division of the pulvinar (Fig. 5). As in the previous cases (Fig. 1), these labeled terminals were localized and restricted to a small area within the nucleus.

In addition, after these tectal injections, the nucleus of the optic tract (NOT), the ipsilateral parabigeminal nucleus (PbG), the dorsal division of the lateral geniculate (LGN), and the ventral division of the lateral geniculate (GLV) nuclei were labeled by a distinct plexus of labeled processes and terminals. In the case of the dorsal division of the LGN, other layers in addition to the tectorecipient layer 3 appear to have a light anterograde labeling, possibly resulting from tracer diffusion to the optic tract. Furthermore, retrogradely labeled cell bodies were evident in the contralateral parabigeminal nucleus and layer 5 of cortical area 19. Further details of these cortical projections will be described in a subsequent article. Finally, we found no evidence of retrogradely labeled cells in any division of the pulvinar following any of the tectal injections.

Laminar origin of the tectopulvinar pathways

The previous experiments indicated the existence of two segregated pathways from the SC to the pulvinar complex: a topographic one, directed toward the ipsilateral rostral pulvinar, and a bilaterally projecting nontopographic one, targeting the caudal pulvinar. The results also suggest that these two pathways originate from two distinct cell populations, located within different collicular layers. Thus, with the hope of identifying the morphology and laminar distribution of the collicular cells from which these projections originate, we made unilateral injections of CTb into the caudal or the rostral divisions of the pulvinar.

We attempted to place restricted unilateral CTb injections into the caudal pulvinar in five different instances. Only one of these cases resulted in a deposit unequivocally confined within this nucleus, with no significant spread to any of the surrounding structures (Fig. 6). In this case, the main deposit of CTb encompassed about 50% of the total volume of the nucleus and was concentrated mostly in its lateral portion, with some diffusion to its more medial half (Fig. 6). As a result of this injection, some retrogradely labeled cell bodies were scattered bilaterally through the upper portion of the stratum opticum (SO), but the vast majority of the labeled cells at the SC were located bilaterally and confined exclusively to one of the retinorecipient superficial layers, the stratum griseum superficiale 3 (SGS3), also often designated as the “lower stratum griseum superficiale” or “SGSL” (Figs. 7–9). The labeled somata were distributed symmetrically within the SGS3 on both sides. However, this distribution was not homogenous across the tectum; the density of the labeled cells appeared to increase from the rostrolateral to the medial aspect of the SC (a proportion of 3:1 labeled cells can be observed between the medial and the ventrolateral aspect in both hemispheres; Fig. 7). Furthermore, the SGS3 ipsilateral to the injection site exhibited a larger number of labeled cell bodies, in a proportion of 1.5:1 (Fig. 7).

The labeled cells in SGS3 were of very distinctive morphology, featuring polygonal cells bodies of roughly homogeneous size (20–25 μm major axis) and several very long primary dendrites directed obliquely toward the collicular surface (Figs. 7, 9, 14). These dendritic processes can be followed until they fuse in a very conspicuous and continuous narrow band of labeling ($\sim 35 \mu\text{m}$ width) of dust-like appearance, located immediately below the collicular surface immediately internal to the stratum zonale (SZ), the most superficial zone of the optic tectum. This band of labeling very likely corresponds to the dendritic terminal specializations of the labeled SGS3 cells (the “bottlebrush endings”) as previously described by Major et al. (2000).

Remarkably, the injections of CTb into the caudal pulvinar also resulted in the labeling of a mesh of axonal processes and terminals in the lateral moiety of the contralateral caudal pulvinar. These labeled processes appear to form a mirror image of the deposit of tracer at the injected side (Fig. 6). This result was observed in all four cases in which the tracer compromised the caudal pulvinar, irrespective of which hemisphere (left or right) was injected. Furthermore, in all of these cases, there were no indications of labeled cells in the caudal pulvinar contralateral to the injected one. Thus, one can conclude that these labeled processes originated from collaterals of the collicular SGS3 cells projecting to the injected caudal pulvinar, because only such cells were bilaterally labeled after these injections. This same pattern of “mirror contralateral labeling” was described for pigeons after CTb injections into the tectorecipient nucleus rotundus (Mpodozis et al., 1996; see Discussion).

We next sought to characterize the morphology and laminar distribution of the collicular cells that project upon the rostral pulvinar (Fig. 10). To accomplish this, in three instances we attempted to place restricted unilateral CTb injections into this nucleus. In all of these cases, the resulting tracer deposit encompassed most of the nucleus, diffusing minimally to the caudal pulvinar (Fig. 11). These injections resulted in the retrograde labeling of cell

bodies only in the ipsilateral SC (Fig. 12). The labeled cells were located almost exclusively on the ventral margin of the SO, intermingled with the afferent and efferent axonal bundles that characterize this layer, even when a few of them were observed in the upper portion of the SGI (Figs. 12, 13). These labeled cells formed a homogeneous population of medium-sized neurons, exhibiting characteristic fusiform cells bodies (15–20 μm major axis) and a few long primary dendrites tangentially oriented toward the collicular surface (Fig. 14). Furthermore, a weak labeling, granular or dust-like in appearance, was observed throughout the SGS3 (Fig. 12). This labeling may correspond to dendritic terminations of the labeled SO cells.

In contrast to the results of the injections in the caudal pulvinar, no evidence of labeled processes was seen in the rostral pulvinar contralateral to the injected one. Anterograde and retrograde labeling in visual cortical areas and other telencephalic targets was also observed after injections positioned in the rostral and caudal pulvinar. These results will be presented in a subsequent article.

In summary, these experiments show that both the caudal and the rostral pulvinar nuclei receive projections from the SC. These afferents originate from clearly segregated sets of neurons: the caudal pulvinar is innervated by cells located in the SGS3 extending their primary dendrites obliquely to the tectal surface. In contrast, the cells projecting to the rostral pulvinar were located in the ventral portion of the SO and show a more fusiform shape. Furthermore, the caudal pulvinar receives a bilateral projection, whereas the rostral pulvinar receives only an ipsilateral projection from the SC. The topographic arrangement also seems to be different between these two projections, in that SC injections result in a restricted labeling pattern in the rostral pulvinar whereas the anterograde labeling in the caudal pulvinar spans the whole structure bilaterally (Fig. 15).

DISCUSSION

Two segregated tectopulvinar pathways

Projections from the SC to the pulvinar complex in the squirrel were first studied by Robson and Hall (1977). These authors described the existence of two major pathways: a bilateral one directed to the caudal pulvinar division and an ipsilateral one targeting the rostral pulvinar division. They also showed that both of these pathways originate from cells located in the region of the SGS3 and upper SO. Subsequently, Lugo-Garcia and Kicliter (1987) confirmed the tectal location of the tectopulvinar cells, and, more recently, the detailed study by Baldwin et al. (2011; see below) confirmed the existence of these two segregated pathways. Whether these two pathways originate from the same or different tectal populations remained unclear until now.

Our present results confirm these previous findings and clarify this latter point, identifying the distinctive characteristics and the specific laminar location of the collicular populations of cells that form the two tectopulvinar projections. We found that tectal cells projecting to the caudal pulvinar (which we will call “TCP cells”) were located mainly at the SGS3 tectal layer, whereas tectal cells projecting to the rostral pulvinar (which we will call “TRP cells”) were located at the lower margin of the SO tectal layer. TCP-pulvinar projections are bilateral and nontopographically organized, whereas TRP-pulvinar projections are ipsilateral and seem to follow a topographic arrangement. TCP cells extend their dendrites well into the retinorecipient zones (see below), whereas TRP cells extend their dendrites no farther than the SGS3. This seems to imply for the TCP cells a monosynaptic relationship and for the TRP cells a polysynaptic (or none) relationship with the retinal terminals.

Given that TRP and TCP cells very likely differ in synaptic relationship with the retinal afferents, our results suggest that these two tectopulvinar pathways mediate different visual properties. This is an important notion since previous work has shown that the rostral pulvinar subdivisions and caudal pulvinar nucleus are reciprocally connected with different visual cortical areas (Robson and Hall, 1977; Baldwin et al., 2011). Combined electrophysiological and anatomical studies will be necessary to disclose the possible differential involvement of these pathways in visual processing (see last paragraph).

TCP cells are bottlebrush dendritic ending cells

Langer and Lund (1974) described five different cell types in the superficial layers of the rat's SC. One type, "wide-field vertical cells," have their soma located in the SGS and exhibit three or four primary dendrites directed obliquely to the surface of the tectum. The dendritic fields span a circular or ovoid area larger than 1 mm in diameter, and their termination zone is in the most superficial layer of the SGS. Later, Major et al. (2000) described for the squirrel two types of SGS3 cells, both showing large dendritic trees (more than 2,000 μm in diameter) consisting of long ascending dendrites displaying characteristic bottlebrush dendritic endings (BBEs): type I cells with cell bodies located externally in the SGS3 and BBEs located in the most superficial layer of the SGS (SGS1) and type II cells with somata located in the SGS3 internal sublamina and in the upper margin of the SO and BBEs located at the SGS intermediate layers. It is very likely that the wide-field vertical cells of Langer and Lund (1974) correspond to the type I cells of Major et al. (2000) because of their strikingly similar morphology.

Remarkably, SGC type I and type II cells have also (and initially) been described from the optic tectum of birds (Cajal, 1911; Hunt and Kunzle, 1976; Karten et al., 1997; Luksch et al., 1998; Hellmann and Güntürkün, 2001). Both cells types featured long primary dendrites obliquely directed to the tectal surface, crowned with characteristic BBEs and forming dendritic terminal domains spanning large elliptic areas of up to 2 mm in the major axis (Luksch et al., 1998). Type I cells have their somata located at the most external sublamina of tectal layer 13 (SGC), and display BBEs at the level of the superficial retinorecipient layers (mainly upon layer 5b). Type II cells, in turn, have their somata located more internally in layer 13, and their dendrites reach mainly layer 9, but no farther superficially than tectal layer 8 (Karten et al., 1997). Projections of each cell type bilaterally terminate within different subdivisions of the thalamic nucleus rotundus in a symmetrical, highly convergent, nonretinotopic fashion (Marín et al., 2003). In pigeons, after a CTb injection into the ventral supraoptic decussation, retrogradely labeled cell bodies can be seen bilaterally in layer 13, whereas a band of a fine-grained (or dusty) labeling, corresponding to the BBEs of these cells, can be seen covering the whole extent of tectal layer 5b (Mpodozis et al., 1996; Karten et al., 1997; Luksch et al., 1998; Marín et al., 2003).

In summary, type I and type II cells of birds and squirrels are characterized by several common features, such as large to medium-sized polygonal cell bodies, large ascending dendrites with BBE specializations located at or near the superficial retinorecipient layers, and very large dendritic domains. For birds it has been shown that these cells project bilaterally, via the supraoptic decussation, to a dorsal thalamic nucleus, namely, the nucleus rotundus. The collicular TCP cells that we describe here also project bilaterally via the supraoptic decussation to a dorsal thalamic nucleus, the caudal pulvinar, and share with the BBE cells all main morphological and cytoarchitectonic characteristics (but see also Chomsung et al., 2008). The conspicuous band of labeling of dusty appearance that can be seen bilaterally in the SGS1 after the CTb injections in the caudal pulvinar constitutes strong indirect evidence of the presence of BBEs in the dendrites of the TCP cells, because TCP cells are the only tectal cells bilaterally labeled after these injections.

Unfortunately, our collection of cases was not diverse enough to permit evaluation of possible differential projections of the different BBE cell types to a particular section or subdivision of the caudal pulvinar, such as that observed in studies of birds (Karten et al., 1997; Hellman and Güntürkün, 2001). Localized injections within small areas of the caudal pulvinar will be necessary to determine this.

Subdivisions of the pulvinar complex

Recently, Baldwin et al. (2011) studied the organization of the pulvinar complex in the gray squirrel by means of differential expression of several neurochemical markers and anterograde projections from the SC. Their findings strongly support the existence of four different pulvinar subdivisions: caudal (C), rostromedial lateral (RLl), rostromedial medial (RLm), and rostromedial (RM). Of these, only the first three receive tectal innervations. Interestingly, our caudal division corresponds closely with the same nucleus identified by Baldwin et al. and in other previous studies (Robson and Hall, 1977; Lugo-Garcia and Kicliter, 1987). Baldwin et al. also confirmed that the caudal division is in receipt of a bilateral diffuse projection from the superficial tectal layers, even when they report a weak tectal contralateral projection, probably resulting from the different sensitivity of the tracers employed in these studies (mostly fluoro-ruby by them vs. only CTb in the present work). On the other hand, our rostral pulvinar nucleus corresponds well to the region defined jointly by the tectorecipient rostral subdivisions of Baldwin et al. (2011), RLl and RLm. Our rostral pulvinar injections embrace most of these subdivisions, so we can propose that tectal afferents to them originate from the TRP cells characterized in the present work. In this scenario, the same pool of tectal TRP cells could be the source of projections to both subdivisions. Alternatively, TRP cells could be composed of at least two different cellular subpopulations, each of them projecting selectively to the RLl or RLm. Alternative tracer injection techniques capable of generating very small and restricted injection sites (Marín et al., 2000, 2003; Fredes et al., 2010) could be applied to explore more precisely the number of parallel pathways in the tectopulvinar system.

Finally, a notable discrepancy between the present study and that of Baldwin et al. (2011) is worth mentioning. Baldwin et al. reported that some of their cases of CTb collicular injections resulted in the retrograde labeling of a small number of cells at the caudal pulvinar. These scant labeled cells appear scattered through the dorsal aspect of the nucleus, after injections in the caudal portion of the tectum (compare their Figs. 8, 10, and 11). In clear contrast, we do not find in our material any evidence of such pulvinotectal projections. Unfortunately, from their report it is difficult to assess whether and to what extent structures other than the tectum might have been compromised by their injections. It can be noted, however, that the injections that we describe here were located in the center of the rostrocaudal axis of the tectum. Thus, their results may be pointing to the existence of a small pulvinotectal projection directed toward the caudal portion of the tectum. To sustain this speculation and to clarify this discrepancy will require a careful reassessment of these studies.

TCP cells collateralize to the ipsilateral and contralateral caudal pulvinar

One of the more remarkable results of the present study was the observation, in the contralateral caudal pulvinar, of a plexus of labeled axons and terminals shaped as a “mirror image” of the tracer deposit in the injected caudal pulvinar. To our knowledge, no records of this kind of labeling are available in the mammalian literature. However, Mpodozis et al. (1996) and later Marín et al. (2003) demonstrated a very similar situation in the thalamic nucleus rotundus of pigeons. They showed that localized CTb injections in different regions of the nucleus rotundus resulted in a plexus of labeled axonal terminals that resemble, in form and location, a mirror image of the injection site in the contralateral rotundus.

Evidently these results are not explainable by tectotectal projections, considering the nontransynaptic properties of the tracer employed. Mpodozis et al. (1996) demonstrated that the mirror image is the result of what they described as “retrograde-anterograde collateral branching”: They suggested that tracer taken up by the tectal cell axons in the ipsilateral side was transported retrogradely to the cell body in the tectal layer 13 but was also transported anterogradely by a collateral of the same cell directed to the contralateral rotundus via the supraoptic decussation (that appears clearly labeled) in a homotopic fashion. Our results with the squirrel are very comparable to those with the pigeon, and a similar explanation for the mirror image in the contralateral caudal pulvinar could apply. In our case, the TCP cells in the SGS3 would send a main axonal branch to the ipsilateral caudal pulvinar and a collateral branch to the contralateral caudal pulvinar, with both branches ending in homotopical loci inside their targets.

Topography in the tectopulvinar projection

Marín et al. (2003) described in detail the topography of the tectorotundal projection in the pigeon. The authors performed small and very restricted injections of fluorescent liposoluble tracers in their crystalline form into the different subdivisions of the nucleus rotundus. These experiments resulted in bilateral labeling of cell bodies in layer 13 spanning the whole tectum, regardless of the localization of the crystal within the rotundus. This observation was consistent with earlier observations that even small injections in restricted parts of the optic tectum resulted in extensive labeling in all divisions of the nucleus rotundus (Karten et al., 1997; Marín et al., 2003). Remarkably, however, the injection of two different tracers into the same rotundal subdivision, though slightly separated from each other, resulted in the labeling of two intermingled cell populations distributed across the whole tectal surface on both sides. This intermingled, arrangement, which was described as “interdigitating topography,” could explain the very large receptive fields found in the rotundal cells (Morenkov and Pivovarov, 1975; Wang et al., 1993).

Our results provide three lines of evidence that suggest that the tectocaudal pulvinar pathway could be organized in a nontopographical fashion similar to that of the pigeon tectorotundal projection. First (and in agreement with Baldwin et al., 2011), even when our CTb injections in the SC were small and restricted, they resulted in a bilateral anterograde labeling of the caudal pulvinar throughout its whole extent. Second, our CTb injections in the caudal pulvinar that were restricted to definite regions in the nucleus always resulted in the bilateral labeling of cell bodies, which spanned the entire colliculus. Third, the resulting mirror image of the injection site in the contralateral caudal pulvinar, made up of labeled axonal collaterals from TCP origin, indicate that even a small locus within the caudal pulvinar receives bilateral afferents from a particular subset of TCPs broadly distributed throughout the whole extent of the TCP population of neurons.

Evidence consistent with this proposition for other mammals comes from the work of Luppino and collaborators (1988), who placed small horseradish peroxidase injections into the SC of the tree shrew (*Tupaia glis*) and found two types of anterogradely label patterns within the pulvinar, a specific or topographic projection to the rostral division and a diffuse or nontopographic projection over the caudal division of the pulvinar. This nontopographic pattern was characterized by a labeling of the caudal pulvinar division in its whole extent with a diffuse web of fibers, regardless of the location of the unilateral injections within the tectal surface (Luppino et al., 1988). Collectively, this body of data supports our hypothesis of a nontopographic arrangement in the projection from the SGS3 cells to the caudal pulvinar in the squirrel and suggests that other mammals, including primates (see below), have conserved this organization in the tectopulvinar pathway.

Other projections of the TCP cells

Major et al. (2000) have suggested that their squirrel type I and II cells could contribute to the tectopretectal projection. However, our experiments did not show any indications that TCP cells might have synaptic targets other than the caudal pulvinar. Nevertheless, we cannot dismiss the possibility that other cell populations with the same laminar distribution may be the source of projections to other brain targets. For the cat, Kubota et al. (1989) found that the SGS3 cells and the dorsal portion of the SO project topographically to the nucleus of the optic tract and posterior pretectal nucleus (Kubota et al., 1989). Lugo-Garcia and Kicliter (1987) demonstrated a projection from the SGS to the anterior and posterior pretectal nuclei in the squirrel. Anterograde tracing studies in the cat (Graham, 1977; Behan and Appell, 1992) and in primates (Benevento and Fallon, 1975) are consistent with these data.

On the other hand, our injections in the superficial layers of the SC generated a retrograde and anterograde label in the parabigeminal nucleus. The cells of SGS3 probably do not project to the parabigeminal nucleus, because the cells projecting to this structure are located in the more superficial layers of the SGS (Lugo-Garcia and Kicliter, 1987). Furthermore, studies on the morphology of the tectoparabigeminal cells show that these cells have small dendritic trees and a horizontal orientation (Moschovakis et al., 1988).

Our injections in the superficial layers of the SC also generated an anterograde label in the dorsal and ventral lateral geniculate nuclei. The projections to the former have been attributed to cells with narrow dendritic fields in the superficial layers of the SGS (Robson and Hall, 1976; Lugo-Garcia and Kicliter, 1987 [squirrel]; Albano et al., 1979; Graham and Casagrande, 1980 [tree shrew]; Kawamura et al., 1980; Kubota et al., 1989 [cat]; Graham and Berman, 1981 [rabbit]; Benevento and Standage, 1983 [macaque monkey]). The projections to the latter have been attributed to cells with narrow dendritic fields located also in the superficial layers of the SGS (Raczkowski and Diamond, 1978 [galago]; Albano et al., 1979; Graham and Casagrande, 1980 [tree shrew]) or in the whole SGS (Harrell et al., 1982 [cat]; Lugo-Garcia and Kicliter, 1987 [squirrel]). Neither the laminar distribution nor the morphology of the type I/TCP cells are consistent with a projection to these nuclei.

General organization of the SC efferents to the pulvinar complex

Although numerous articles studying the tectopulvinar connectivity have been published over the years, a clear picture of the general organization of these projections is still lacking. In particular, the presence of a bilateral projection from the colliculus to the pulvinar complex, even though demonstrated in different species, is still controversial, because not every study has described this trait. Studies performed in diurnal and visually active animals such as the squirrel (Robson and Hall, 1977; Lugo-Garcia and Kicliter, 1987; Baldwin et al., 2011), degu (Kuljis and Fernandez, 1982), and tree shrew (Luppino et al., 1988) have described the presence of both bilateral and ipsilateral tectopulvinar projections (but see the study of Albano et al., 1979). On the other hand, most studies in nocturnal-crepuscular mammals such as rabbit (Graham and Berman, 1981) and rat (Cajal, 1911; Donnelly et al., 1983; Cadusseau and Roger, 1985; Masterson et al., 2009) have described only an ipsilateral tectopulvinar projection. Interestingly, Masterson et al. (2009) showed that in the rat this ipsilateral projections originated from collicular cells that closely resemble, in morphology and laminar origin, the TRP cells described in the present article. The primate literature is also ambiguous regarding this issue; most studies claim that cells in the SGS3 and the dorsal portion of the SO project ipsilaterally to the inferior pulvinar (Raczkowski and Diamond, 1978; Benevento and Standage, 1983; Huerta and Harting, 1983; Stepniewska et al., 1999), but others have shown the presence of a bilateral projection from the deep SGS and upper

SO to the caudal division of the inferior pulvinar (Trojanowski and Jacobson, 1975; Benevento and Rezak, 1976).

In this context, it is interesting to note that nocturnal mammals, with rod-dominated retinas, exhibit modest superior colliculus volumes and therefore reduced collicular lamination compared with those of diurnal mammals with cone enhanced retinas (Van Hooser and Nelson, 2006; Vega-Zuniga et al., in preparation). It is therefore possible to expect that the visually driven collicular projections may appear decreased in nocturnal animals compared with diurnal ones. In support of this view, Taylor et al. (1986) found in the hooded rat that, after a large injection of HRP encompassing the whole ipsilateral tectum, a small bilateral projection from the SC to the dorsal part of the lateral-posterior nuclei (pulvinar) becomes evident (Taylor et al., 1986). The fact that only after injections affecting the whole tectum in rats, the bilateral projection to the lateral posterior nucleus is revealed suggests that the cells projecting to this structure are very restricted in number and/or unevenly distributed within the tectal extension.

Thus, the segregated tectopulvinar pathways that we show here for the squirrel may constitute a generalized condition across mammalian species. More precise and accurate injections of sensitive neural tracers into different regions of the inferior pulvinar in primates and other mammals will be necessary to determine this.

It is there a mammalian nucleus rotundus?

Despite other suggestions to the contrary found in the literature (Bruce and Neary, 1995; Dávila et al., 2003; Guirado et al., 2005), we cannot but conclude that, at least in sciurid rodents, the caudal pulvinar should be considered as the mammalian homologue of the nucleus rotundus of nonmammalian amniotes. Our data agree well with the evidence gathered previously in other mammals, including primates (as discussed in the previous section), so we believe that we can generalize this conclusion to all mammals.

In the context of the present debate about the evolutionary origin and homology of the mammalian neocortex (Aboitiz et al., 2003; Wang et al., 2010; Aboitiz, 2011; Butler et al., 2011), this conclusion becomes highly significant, because the identity of the mammalian rotundus is a necessary reference point to clarify the identity of the mammalian counterpart of the sauropsid DVR (the telencephalic target of the rotundal projections). In fact, several other studies have shown that the main telencephalic target of the caudal pulvinar projections is the temporal cortex (Kaas et al., 1972; Robson and Hall, 1977; Wong et al., 2008). Thus, according to our hodologic results, the sauropsid DVR appears to be evolutionarily related to the mammalian temporal cortex and not to the claustrum-mygdalar complex as has been proposed by several authors (Holmgren, 1925; Bruce and Neary, 1995; Puellas et al., 2000; Dávila et al., 2002; Guirado et al., 2005).

Motion sensitivity: a general property of the tectofugal pathway in amniotes?

In birds, the tectofugal pathway is the main route mediating the discrimination of several visual properties, such as brightness, color, pattern, spatial frequency, and size stimuli (Hodos and Karten, 1966, 1970; Bessette and Hodos, 1989; Wang et al., 1993; Nguyen et al., 2004; Xiao et al., 2006). In particular, it has been repeatedly stated that this pathway has a prominent role in motion detection. In fact, motion sensitivity is a characteristic feature of tectorotundal cells (Jassik-Gerschenfeld and Guichard, 1972; Frost and DiFranco, 1976; Frost and Nakayama, 1983; Frost et al., 1988). Most rotundal units also exhibit complex, higher order motion sensitivity properties, such as two-dimensional and in-depth motion selectivity (Wang and Frost, 1992; Wang et al., 1993), that result from the convergence of a large set of tectal inputs upon their dendritic trees (Luksch et al., 1998; Marín et al., 2003).

In turn, nearly all units of the entopallium, the pallial target of the rotundal efferents, also exhibit motion-sensitive responses, which are driven by the activation of retinotectal synapses (Gu et al., 2002; Marín et al., 2011).

Given the close morphological and hodological similarities between the avian BBE cells and the mammalian TCP cells, one can expect that both TCP cells and their caudal pulvinar targets will exhibit “avian-like” movement-sensitive properties. In fact, several studies in rodents and primates have reported that collicular SGS3 and SO cells exhibit complex movement-sensitive units (Humphrey, 1968; Straschill and Hoffman, 1969; Kadoya et al., 1971; Schiller and Koerner, 1971; Cynader and Berman, 1972; Albano et al., 1979; Graham et al., 1981; Mooney et al., 1985). Furthermore, for cats it has been shown that many units in the tectorecipient region of the lateral posterior/pulvinar complex exhibit higher order motion-sensitive properties, comparable to those of the rotundal units. However, such properties have been attributed not to the tectal afferences but mainly to the corticopulvinar feedback (Casanova and Savard, 1996; Merabet et al., 1998; Dumbrava et al., 2001). Thus, at present, the role of the mammalian tectofugal pathway in motion processing requires further clarification. In light of the present results, we think that the question of a possible motion sensitivity of TCP cells and, more generally, the role of the superior colliculus in motion sensitivity becomes worthy of further research.

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Figure 1.

Anterograde labeling of the rostral nucleus of the pulvinar complex after a CTb injection into the superior colliculus. Microphotographs of CTb-reacted, giemsa-counterstained sections. **A:** CTb injection in the lateral aspect of the superior colliculus (SC). Approximate SC layering is indicated at the contralateral side. Note that the main deposit of tracer concentrates in the superficial SC layers. **B:** Anterograde labeling of the rostral pulvinar nucleus (Pulr) and dorsal lateral geniculate (Gld) as a result of the injection shown in A. Note the patchy appearance of the terminals in the ipsilateral rostral pulvinar. Contralateral rostral pulvinar appears free of label. SZ, stratum zonale; SGS, stratum griseum superficiale; SO, stratum opticum; SGI, stratum griseum intermedium. Scale bar = 1 mm.

Figure 2. Serial reconstruction of the pattern of labeled processes resulting from a CTb injection in the superior colliculus (same case as in Figs. 1, 3). Drawings are oriented from caudal (C) to rostral (R). Lighter and darker gray shadowing indicates the intensity of the label. The injection site is represented in solid black. The caudal pulvinar appears bilaterally labeled through all its rostrocaudal extension. NOT, nucleus of the optic tract; Hab, medial habenula; GLv, ventral geniculate nucleus. Other abbreviations as in Figures 1 and 3. Scale bar = 1 mm.

Figure 3.

Anterograde labeling of the caudal nucleus of the pulvinar complex as a result of the CTb injection shown in Figure 1. **A:** CTb-reacted, giemsa-counterstained coronal section at the level of the tectal commissure. Note the bilateral anterograde labeling in the caudal pulvinar (Pulc). **B:** CTb-reacted-only coronal section at relatively the same level as in A. The labeled processes span the whole extent of the nucleus at both sides, but the ipsilateral side presents a denser plexus of fibers. MG, medial geniculate nucleus. Scale bar = 1 mm.

Figure 4.

CTb injection into the deep laminae of the superior colliculus. Microphotographs of CTb-reacted, giemsa-counterstained coronal sections at the level of the injection site (**A**) and rostral to it (**B**). The caudal pulvinar appears free of label at both sides. Note the very dense labeling at the ipsilateral nucleus of the optic tract (NOT). Other abbreviations as in Figures 1 and 3. Scale bar = 1 mm.

Figure 5.

Anterograde labeling of the rostral nucleus of the pulvinar complex after the CTb injection shown in Figure 4. Microphotograph of a CTb-reacted, giemsa-counterstained coronal section. Only a restricted area of the ipsilateral rostral pulvinar is labeled. A localized plexus of labeled processes is also evident at the ipsilateral dorsal geniculate nucleus. Scale bar = 1 mm.

Figure 6.

CTb injection site into the caudal pulvinar and resulting labeling of the contralateral caudal pulvinar. Microphotograph of a CTb-reacted, giemsa-counterstained section. Note that the injection site is restricted to the caudal pulvinar, with no diffusion of tracer to any other contiguous structures (**A**). At the contralateral caudal pulvinar, a dense plexus of labeled terminals made up a “specular image” of the injection site (**B**).

Figure 7.

Bilateral retrograde labeling of tectal cells after the CTb injection shown in Figure 6. Microphotographs of a CTb-reacted coronal section at the level of the tectal commissure (**A**). Retrogradely labeled cell bodies can be seen through the whole extent of the colliculus at both sides. Note that the density of labeled cell bodies is greater in the ipsilateral side than in the contralateral one. **B,C**: Higher magnifications of the boxed areas in **A**. Note that most of the cells exhibit two or more primary dendrites directed obliquely toward the collicular surface. Note also the superficial band of label indicated by the arrowhead. Scale bars = 1 mm in **A**; 500 μ m in **C** (applies to **B,C**).

Figure 8.

Serial reconstruction of the pattern of labeled processes resulting from a CTb injection into the caudal pulvinar. Drawings are displayed from caudal (C) to rostral (R). Gray shadowing indicate labeled processes. Black dots indicate labeled cell bodies. Solid black represented the injection site. Note that the labeled processes at the contralateral side form a specular image of the injection site. Note also that the retrograded labeled cell bodies span bilaterally through all levels of the superior colliculus. NOT, nucleus of the optic tract; Hab, medial habenula; GLv, ventral geniculate nucleus. Other abbreviations as in Figures 1 and 3. Scale bar = 1 mm.

Figure 9. Laminar location of the collicular cells projecting to the caudal pulvinar. **Inset:** Microphotograph of a CTb-reacted, giemsa-counterstained section at the level of the oculomotor nuclei showing the retrograde labeled cells after the CTb injection shown in Figure 6. **Top:** Higher magnification photograph of the boxed area in the inset. Most of the labeled cells are located in the SGS3 layer, but some of these cells are located in the SO layer as well. Note also the dense band of at the SGS1. Abbreviations as in Figure 1. Scale bars = 1 mm in inset; 100 μ m in top portion.

Figure 10. CTb injection into the rostral pulvinar. Microphotograph of a CTb-reacted, giemsa-counterstained section at the rostral pulvinar level showing the main CTb deposit into the nucleus. Note that there is a minimal diffusion of the tracer to the adjacent medial regions. Abbreviations as in Figure 1. Scale bar = 1 mm.

Figure 11.

Serial reconstruction of the pattern of labeled processes and cells resulting from a CTb injection into the rostral pulvinar. Drawings are displayed from caudal (C) to rostral (R). Solid black shading represents the injection site. Gray shading indicates diffusion of the tracer. Black dots represented retrogradely labeled cell bodies. Note the cells are present only in the ipsilateral tectal half. No labeling or tracer diffusion can be observed in the caudal pulvinar. Scale bar = 1 mm.

Figure 12.

Laminar location of the collicular cells projecting to the rostral pulvinar. **Inset:** Microphotograph of a CTb-reacted, giemsa-counterstained coronal section at the level of the oculomotor nuclei showing the retrograde labeled cells resulting from the injection shown in Figure 10. **Top:** Higher magnification photograph of the boxed area in the inset. Most of the labeled cells are located in the SO layer, but some of the cells are located in the upper SGI sublamina as well. Note also that no labeled cells can be seen in the more superficial layers. Abbreviations as in Figure 1. Scale bars = 1 mm in inset; 100 μ m in top portion.

Figure 13.

Comparison of the laminar location of the tectal cells projecting to the pulvinar complex. High-power photographs of the tectal cells projecting to the caudal (**A**) and rostral (**B**) pulvinar. The cells were retrogradely labeled after CTb injections in the corresponding nuclei. Note that the cells projecting to the caudal pulvinar are located mainly in the SGS3 layer, although some cells can also be seen in the SO layer. In contrast, most of the cells projecting to the rostral pulvinar are located in the SO layer (only very few of them appear located in the SGI layer). Note also the band of labeling at the SGS1 above the cells projecting to the caudal pulvinar (arrows). Scale bar = 100 μ m.

Figure 14.

Camera lucida reconstruction of tectal cells projecting to the pulvinar. **A:** Camera lucida reconstruction of a cell retrogradely labeled after a CTb injection in the ipsilateral caudal pulvinar. **B:** Camera lucida reconstruction of two cells retrogradely labeled after a CTb injection in the contralateral caudal pulvinar. Note that, in both cases, cells show two primary dendrites running oblique to the tectal surface. **C:** Camera lucida reconstructions of three cells retrogradely labeled after a CTb injection into the ipsilateral rostral pulvinar. Note the more fusiform shape and the smaller size of these cells compared with the cells depicted in A and B. The relative positions of the reconstructed cells are indicated within the corresponding tectal schema. Circles indicates the relative position of all retrogradely labeled cells. They do not represent actual numbers. Scale bars = 1 mm at left (applies to all tectal schema); 50 μ m at right (applies to all reconstructed cells).

Figure 15.

Summary of the organization of the tectopulvinar pathways in the squirrel. Two parallel pathways arise from different tectal populations. Tectal ganglion cells with bottlebrush dendritic endings (black; Major et al., 2000) located in the layer three of the stratum griseum superficiale 3 project bilaterally to the caudal pulvinar in a nontopographic manner (red). The contralateral projection arises from axonal collaterals that cross the midline by the supraoptic decussation. A different population of cells, located in the lower margin of the stratum opticum (SO), projects exclusively ipsilaterally to the lateral subdivisions of the rostral pulvinar. The medial division of the rostral pulvinar is devoid of tectal afferents (gray; Robson and Hall, 1977; Baldwin et al., 2011). At upper left, the cortical connectivity of the pulvinar divisions is shown based on the literature (Kaas et al., 1972; Robson and Hall, 1977; Wong et al., 2008). Most of the retinal ganglion cells (green) project topographically to the contralateral superior colliculus entering by the SO tectal layer with terminations in the three layers of the stratum griseum superficiale (Major et al., 2003). Note that the bottlebrush dendritic endings of the caudal pulvinar projecting cells colocalize with the retinal terminals in the stratum griseum superficiale 1.