# The architecture of an inhibitory sidepath within the avian tectofugal system

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The tectofugal system dominates vision in most avian species. A key component of this pathway is the projection from the optic tectum onto the nucleus rotundus and the nucleus subpretectalis. Since subpretectalis has inhibitory projections onto rotundus, it constitutes a modulatory tectofugal sidepath to the tectorotundal system. We analyzed the connections and the immunocytochemical pattern of the subpretectalis in pigeons and show that it receives afferents from some tectal celltypes and from the nucleus pretectalis. Subpretectalis-neurons project non-topographically onto pretectalis and the rostrolateral rotundus. In addition, our immunocytochemical data make it likely that the cells of the subpretectalis receive glutamatergic and GABAergic input. These data provide evidence that the tectofugal sidepath over the subpretectalis could be involved in two major functions: The first is a modulation of attentional shifts from one eye to the other, while the second is a temporal fine-tuning of rotundal units. *NeuroReport* 14:879–882 © 2003 Lippincott Williams & Wilkins.

**Key words**: GABA; Parvalbumin; Pigeon; Rotundus; Subpretectalis; Tectum opticum

## INTRODUCTION

In the majority of avian species visual analysis is dominated by the tectofugal system, that is equivalent to the extrageniculocortical pathway of mammals [1]. Within the tectofugal system, visual input is transferred via retinal ganglion cells to the contralateral tectum. Different classes of tectal layer 13 cells project onto the thalamic n. rotundus (Rt) and the n. triangularis (T), from where output arises to the telencephalic ectostriatum [2]. Although retinal input is nearly completely crossed and consequently unilateral, each hemisphere receives input from both eyes, realized by bilateral projections from the tectum to the Rt [3–5]. During the integration of bilateral visual input at rotundal level, information from the ipsilateral eye is selectively inhibited by GABAergic fibers from a cluster of nuclei, collectively called bed nuclei of the tecto-thalamic tract: n. subpretectalis (SP), n. interstitio-petecto-subpretectalis (IPS), n. subpretectalis-caudalis (SPcd), n. posteroventralis thalami (PV) and n. of the tectothalamic tract (nTT) [2,6–8]. Detailed electrophysiological analyses of rotundal units revealed that these GABAergic systems not only regulate bilateral integration but also enable the occurrence of complex computational properties required by a system that processes movement analyses and performs detailed feature perceptions based on coarse-coding principles [9–12].

The bed nuclei of the tecto-thalamic tract receive a sidebranch of the tectorotundal projection and seem to project exclusively onto Rt and T [2,7]. Due to the extreme difficulty to selectively inject into one of these inhibitory nuclei, their connectional properties were only deduced based on rotundal or tectal tracer injections. Obviously, this constitutes an important limitation to our understanding of the avian tectofugal system. We therefore concentrated on the SP, the largest subnucleus of the bed nuclei of the tectothalamic tract, and performed a series of tracer injections into various SP subregions. In addition, we used immunocytochemistry to unreveal possible transmitter- and receptor-specific interactions within the tecto-SP-rotundal pathway. In summary, the present study aimed to clarify (a) the pattern of the tecto-SP projection, (b) the regionalization and topography of the SP-Rt projection, (c) an analysis of possible further connections of the SP, (d) some of the chemoarchitectural features of SP.

## MATERIALS AND METHODS

All experiments were carried out according to the specifications of German law for the prevention of cruelty to animals.

For tracing experiments 12 adult pigeons (*Columba livia*) that had received successful injections of the tracer cholera toxin subunit B (CtB; Sigma, six birds) or of biotinylated dextran amine (BDA; 10 000 mol.wt; lysine-fixable; Molecular Probes, nine birds) into the SP were analyzed. Due to the essentially ipsilateral organization of the system, five pigeons with bilateral injections were used. Prior to surgery, pigeons were anesthetized with equithesin (0.31 ml/100 g)

and a glass micropipette (outer tip diameter 20 μm) mounted onto a Nanoliterinjector (WPI) was stereotaxically inserted into SP. CtB (23–75 nl; 1% in distilled water) or BDA (10% in 2% DMSO) was slowly injected over 20 min. After 3–5 days birds received 200 units sodium heparin, were deeply anesthetized with equithesin (0.55 ml/100 g) and perfused intracardially with 100 ml 0.9% (w/v) NaCl and 800 ml ice-cold 4% paraformaldehyde in 0.12 M phosphate buffer (PB), pH 7.4. Brains were cut frozen in the frontal plane at 35 μm and were reacted free-floating with the immuno-ABC-technique as outlined by Hellmann and Güntürkün [2].

For the immunohistochemical demonstration of various antigens, pigeons were perfused as described for tracing. However, with the exception of the parvalbumin series, primary and secondary fixatives additionally contained 0.2% glutardialdehyde. Free-floating sections were placed for 35 min in 1% $H_2O_2$/50% ethanol to reduce endogenous peroxidase activity. After washing, sections were incubated for 36 h at 4°C in the primary antibody solution in 0.12 M PB after the addition of 2% NaCl (w/v), 0.3% Triton X-100 + 0.1% sodium-azide (w/v) and 5% normal serum from the host of the secondary antibody. After being rinsed, the sections were incubated for 60 min at room temperature in the biotinylated secondary antibody (1/200 in 0.12 M PB, 2% NaCl, 0.3% Triton-X-100) and then processed with the immuno-ABC-technique. Control experiments with omission of the primary antibody revealed no specific labeling. Based on previous observations, antibodies against following antigens were chosen: glutamate decarboxylase (GAD; Chemicon, 1:2000), β-subunit of $GABA_A$-receptors (Boehringer, 1:50), Parvalbumin (Sigma, 1:500), glutamatergic AMPA-receptor subunits 1, 2/3, and 4 (GluR1, GluR2/3, GluR4, Chemicon, 1:5000). To estimate the relative proportion of neurons being labeled with these antibodies, the number of labeled neurons within a single section were counted at levels A 4.25, A 4.50, A 4.75, A 5.00, and A 5.25 (according to the pigeon brain atlas [13]) and compared with the number of cells at the same level in cresyl violet sections.

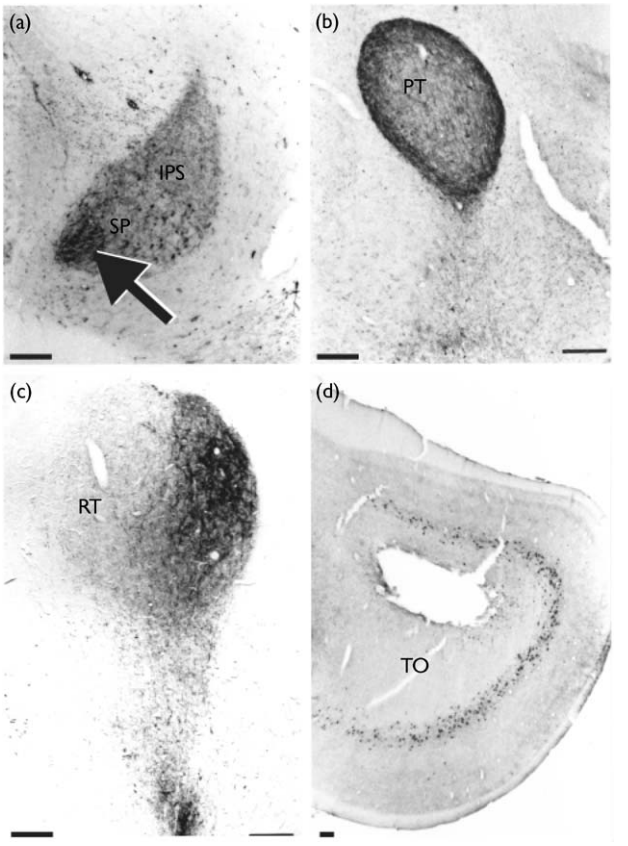


**Fig. 1.** Connectivity of the n. subpretectalis (SP). SP-injections (arrow) (**a**) result in anterograde and retrograde labeling of the n. pretectalis (PT) (**b**), anterograde labeling of rostral and lateral n. rotundus (Rt) (**c**), and retrograde labeling of cells in tectal lamina I3 (**d**). Dorsal is upward and lateral is to the right. Further abbreviations: n. interstitio-petecto-subpretectalis (IPS), tectum opticum (TO). Bar = 500 μm.

## RESULTS

The reconstruction of the CtB- and BDA-injection sites revealed that in six birds tracer spread was completely restricted to the SP (Fig. 1a). In the others, some medial or lateral leakage was visible. SP injections always resulted in somatic and neuropil labeling in the neighbouring IPS. Since IPS is directly above the injection site it is likely that this labeling pattern results from tracer leakage and/or labeling of traversing fibers. SP injections also resulted in somatic labeling in lamina 13 of the ipsilateral tectum opticum (TO; Fig. 1d). We did not observe any retrogradely filled cells in the contralateral tectum. In half of all cases more labelled cells were visible in the ventral tectum than the dorsal one, while no dorsoventral difference could be revealed in the other half. Generally, the most superficial extent of lamina 13 beared only few labeled somata. SP-injections consistently resulted in a pattern of labeled fibers and terminals in the complete extent of the n. pretectalis (PT; Fig. 1b). The circular band around PT was particularly heavily filled with labeled fibers. CtB-positive somata were only visible in the core of PT. A few scattered cells were also present ventral to PT, in and around the n. spiriformis lateralis (SpL).

All SP injections also resulted in a dense anterograde labeling within Rt (Fig. 1c). In all cases the rostral part of Rt was densely covered with fibers and terminals. Moving caudally to A 5.50, labelled terminals were no longer visible in the most dorsomedial portion of Rt and in the complete extent of T. Moving even further caudally, the labelled portion of the dorsomedial Rt was reduced until only a thin band of label remained in the most ventrolateral aspect at about A 4.75. The most caudal portion of Rt was completely devoid of labeled fibers. To analyze a possibly topographical connection, the SP injections were divided into dorsal, lateral, ventrolateral, and ventral cases. This analysis revealed no topographical variations within Rt labels. Additionally, no CtB- or BDA-positive somata were found within Rt.

Antibodies against GluR1 and Glu2/3 labeled no cellular processes within SP. GAD, $GABA_{A\beta}$, parvalbumin, and GluR4, however, labeled a homogeneous distribution of large somata within SP (Fig. 2a–d). Only using GluR4 was neuropil staining lacking. The proportion of labeled neurons relative to the number of cells counted with cresyl violet was

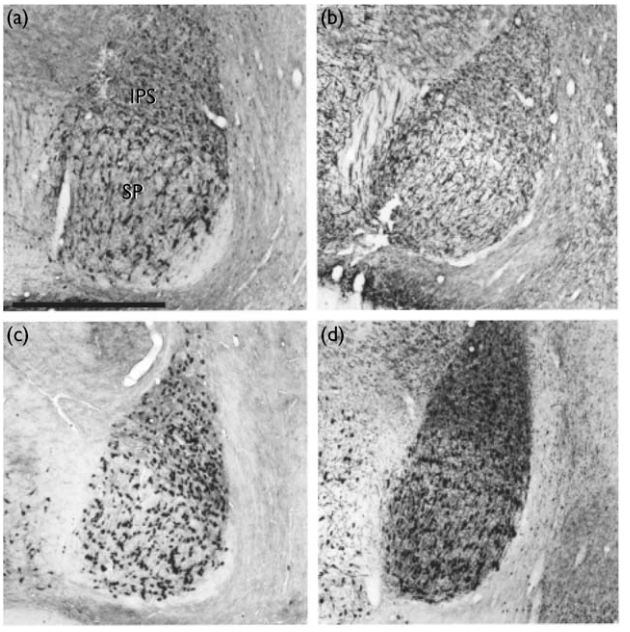


**Fig. 2.** Immunocytochemical studies revealed within the n. subpretectalis (SP) a dense somatic labeling with glutamate decarboxylase (**a**), GABA$_{A\beta}$ (**b**), glutamate AMPA receptor subunit 4 (GluR4) (**c**), and parvalbumin (**d**). Dorsal is upward and lateral is to the right. Abbreviations: n. interstitio-petecto-subpretectalis (IPS). Bar = 500 μm.

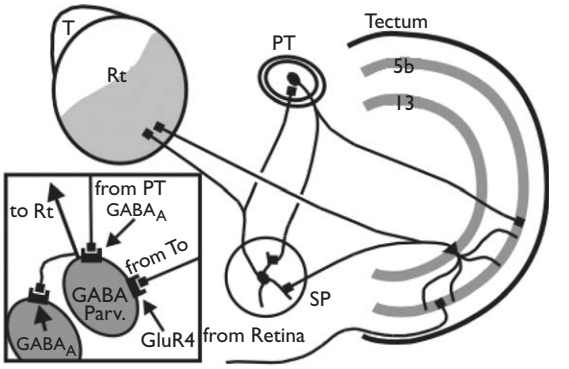


**Fig. 3.** Schematic description of the connectivity pattern of the n. subpretectalis (SP). The inset shows a hypothetical circuit within SP as proposed in the present study. Abbreviations: γ-aminobutyric acid receptor type A (GABA$_A$), glutamate AMPA receptor subunit 4 (GluR4), parvalbumin (Parv.), n. rotundus (Rt), n. triangularis (T), tectum opticum (To). Additionally, tectal laminae 5b and 13 are depicted.

> 90% for parvalbumin. With the exception of the most caudal SP, this was also true for GluR4. GAD and GABA$_{A\beta}$ labeled on the average 72% of the neurons.

## DISCUSSION

The present study shows that SP neurons receive a massive and presumably glutamatergic projection from certain classes of tectal lamina 13 neurons and constitute a GABAergic and non-topographical projection onto PT and rostrolateral Rt. These data make it likely that the mesothalamic component of the tectofugal system involves several inhibitory feedback loops which modulate tectofugal processes.

Our anatomical results show that neurons in tectal lamina 13 are not the only, but the most important source of afferents to the SP. In pigeons, cells of lamina 13 can be subdivided at least into five distinct classes with each class having a specialized intratectal circuitry and a distinct rotundal termination pattern [2]. Based on the somatic position within lamina 13 and the presence of a dorsoventral tectal density gradient in our material, it is likely that mainly tectorotundal celltypes I, II, and IV have collaterals to SP. Types III and V can probably be excluded since we did not observe a higher presence of labeling in the dorsal tectum (types III and V) or a dense labeling in the most superficial portion of lamina 13 (type III) [2]. If this conclusion is correct, tectal collaterals to SP would indirectly modulate the same rotundal subregions which are also directly activated by the tectorotundal pathway.

The reciprocal connection of SP with PT is one of the most important results of the present study. Similar to SP, also PT receives collaterals of tectal lamina 13 neurons which project to Rt [2]. PT neurons not only project back onto SP but also onto layer 5b of the optic tectum [14]. Layer 5b receives massive afferents from the contralateral retina and is the input layer of class I tectorotundal neurons which by themselves are the main source of projections to Rt, SP, and PT [2,15,16]. Thus, neurons of PT modulate SP by direct as well as by indirect projections over the tectum. According to this pattern, the mesothalamic component of the tectofugal system is constituted by multiple modulatory sidepaths which not only alter tectal and rotundal circuits but also modulate processes within the modulatory structures SP and PT (Fig. 3). Within this circuitry, the SP-projection onto Rt and PT is regionally specific [7], but not topographic. Rt contains a homogenous network of GABAergic axons and terminals [17,18], such that an activation of SP would induce a widespread inhibition within Rt.

Our immunocytochemical data show that most, albeit not all, SP cells were positive for GAD, GABA$_{A\beta}$, GluR4, and parvalbumin. Although we do not know if the lack of a complete labeling of all SP cells is due to technical limitations or due to the presence of a small number of neurons with different antigen-combinations, it is likely that most SP neurons are positive for all four antibodies in use. The GAD labeling is in line with previous studies [7,19] showing that the absolute majority of SP neurons are GABAergic. As often the case for GABAergic neurons, these cells are also positive for the calcium-binding protein parvalbumin [20]. Since SP cells are also positive for GluR4 and do not express GluR1 and GluR2/3, it likely that SP cells form homomeric glutamate-R4 receptors with calcium permeability. This observation strengthens the assumption of previous studies that the tectorotundal projection is constituted by glutamate-positive neurons [9,21,22]. The presence of GABA$_A$-receptors in most SP neurons is probably due to collaterals of rotundofugal axons terminating on neighbouring cells. Indeed, Tömböl *et al.* [19] observed local axonal arborizations with terminals within SP.

These observations provide important hints to specify the role of the SP within the tectofugal system (Fig. 3). As a

component of the bed nuclei of the tecto-thalamic tract, SP participates in an ensemble of widespread inhibitory projections onto the ipsilateral Rt. Since input from both eyes converge first at rotundal level [3–5], the balance between the information streams representing the two eyes and thus the two visual halffields has to be controlled. It is likely that the bed nuclei of the tecto-thalamic tract take a key role in this function [12]. Possibly, the role of the SP in this scenario is to modulate mainly [19] (this study), but not exclusively [3] the afferents from the contralateral visual field. Together, the bed nuclei of the tecto-thalamic tract could shift the balance of the attentional resources between the two visual half fields.

However, the tecto-SP-rotundal projection could do more than that. Since SP receives collaterals from tectorotundal axons, the inhibitory output of SP could arrive within Rt shortly after rotundal units were activated from the tectum. As first suggested by Mpodozis *et al.* [7], this would provide a sharp offset of activity patterns within Rt. Indeed, type I neurons of the tectal lamina 13 respond with a temporally highly precise sequence of bursts to a moving spot, whereby burst frequency is a linear function of object speed [11]. A system with such high demands on temporal accuracy would need a temporally precise offset for the Rt units to enable a disambiguation from subsequent input. The inhibitory input from SP would perfectly suit this function. However, to allow rotundal cells to rapidly process also the next wave of tectorotundal activation, the inhibition by the SP has also to be quickly terminated. Two of our findings could explain how this is achieved. First, SP neurons are endowed with $GABA_A$-receptors which are probably activated by collaterals of neighbouring SP cells such that every volley of SP output should result in a fast inhibition of SP neurons. Second, the inhibitory input from the GABA-positive PT [23] would be activated by collaterals of the rotundally projecting SP axons, serving as a second dampening signal with a slight temporal offset to the first. All together, the inhibitory sidepath over SP would provide a mechanism which allows global shifts of activation between the input from the two eyes as well as fast temporal structuring of rotundal information processing (Fig. 3).

## CONCLUSION

Our analysis of the connections and the immunocytochemical pattern of the nucleus subpretectalis (SP) in the pigeon thalamus revealed a number of features which hint to its functional role. First, SP receives mainly ipsilateral, and probably glutamatergic, tectal input. Since the different substructures of the bed nuclei of the tecto-thalamic tract probably receive a mixture of contra- and ipsilateral tectal inputs, their GABAergic projections onto the nucleus rotundus (Rt) could serve to shift attention from one eye to the other. Second, since the SP receives collaterals of the tectorotundal axons and projects onto Rt, its activation may terminate rotundal spike trains after a short time interval, enabling disambiguation from subsequent tectal input. Collaterals from neighbouring neurons as well as a reciprocal interaction with the GABAergic nucleus pretectalis could at the same time terminate SP activity patterns shortly after their onset. All together, these inhibitory feedback loops may transform tectofugal activity patterns into fast sequences of distinct activity trains which are able to integrate tectal activity patterns with high temporal resolution.

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