Acta Biologica Hungarica 53 (1–2), pp. 23–31 (2002)

ULTRASTRUCTURE OF NEUROPEPTIDE-Y IMMUNOREACTIVE ELEMENTS IN THE SUPERIOR COLLICULUS OF CAT*

Zs. Borostyánkői-Baldauf**

Laboratory of Neurobiology, Department of Anatomy, Semmelweis University, Budapest, P. O. Box 95, H-1450 Budapest, Hungary

(Received: September 30, 2001; accepted: November 17, 2001)

Whereas the presence of neuropeptide-Y (NPY) in the superior colliculus (SC) has been established, its participation in the ultrastructural organisation of the neuronal networks in the SC has not been studied. Accordingly, in the present paper light and electron microscopic NPY immunohistochemical studies were performed on the SC of cat. NPY fibres were found to be present predominantly in the superficial grey layer (SGL) of the SC, though a few small NPY cells were found in both the deeper and the upper layers. Ultrastructural observations revealed that the NPY nerve endings establish almost exclusively axo-dendritic synaptic contacts in the SGL of the SC. Thus, the presumably inhibitory impact of the NPY terminals is exerted through the dendrites of the SGL neurons, and not directly to the retinal axons, as thought previously.

Keywords: Axo-dendritic synapse – pretectum – electron microscopy – immunocytochemistry

INTRODUCTION

Neuropeptide-Y, which is abundantly distributed in the central nervous system, exerts a number of modulatory roles in different structures of the brain [28]. NPY can be found in every visual structure of mammals [3, 21]. Its presence in the superior colliculus (SC) has been demonstrated in anuran [10, 16], avian [4, 25], rodent [5], carnivore [3, 6] and primate [2] species.

The tectal NPY fibres have been shown to originate from the pretectum in frog [15], chicken [25] and cat [3]. The effect of NPY in the tectum is inhibitory [23], and a Y2-receptor mechanism of NPY-mediated inhibition of retinotectal information transfer has been described in toad [24]. The GABAergic nature of pretecto-tectal projection has been confirmed in cat [1], which may correspond to the submammalian NPYergic pretecto-tectal projection [15, 25]. However, no data are available on the postsynaptic elements of the collicular NPY fibres, through which the pro-

*Dedicated to Professor József Hámori on the occasion of his 70th birthday.

**E-mail: borostyz@ana.sote.hu

posed inhibition is produced, neither in submammalians nor in mammals. The aim of the present study, therefore, was, to determine the postsynaptic elements of collicular NPY fibres in cat.

MATERIALS AND METHODS

Under deep pentobarbital anaesthesia, 4 adult cats were perfused with phosphate-buffered saline (PBS), followed by 2 litres of fixative solution (4% paraformaldehyde, 0.05% glutaraldehyde and 0.2% picric acid in 0.1 M PS, pH = 7.4). Perfusion was conducted in accordance with the Hungarian health regulations, which conform to the NIH's "Principles of laboratory animal care" (1985). The perfused brains were removed from the skull and cut into small blocks, and 70 μm sections were made with a Vibratome.

The free-floating sections were immersed overnight in PB containing 20% sucrose, then frozen and thawed twice. The endogenous peroxidase activity was blocked with 1% H_2O_2 dissolved in PB for 30 min, and the sections were next treated with 10% normal goat serum dissolved in PB and incubated for 48 h at 4 °C with a polyclonal antibody against NPY (raised in rabbits, generously provided by Dr. T. J. Görcs), at a working dilution of 20,000. The specificity of the antibody used has been reported previously [7]. The sections were incubated overnight at 4 °C with goat-anti-rabbit IgG (Vector) diluted 1 : 300, then incubated with ABC (Vector) for 2 h and developed by the standard DAB (Sigma) method. A 3×15 min wash in PB was carried out after each.

For electron microscopy, the sections were treated with osmium tetroxide, dehydrated in ethanol, block-stained with 1% uranyl acetate in 70% ethanol and embedded in Durcupan ACM (Fluka).

RESULTS

In the superficial layers of the SC a dense NPY-immunoreactive (-ir) fibre network was observed, while only some NPY-ir fibres were found in the deeper layers (Fig. 1). The fibres mostly ran horizontally, though some transversally coursing fibres were also observed. Morphologically, the fibres were of varicose type, giving rise to numerous *en passant* boutons in the SGL. A few very small (5–10 μm in diameter) NPY-ir perikarya too were seen, both in the SGL and in the deeper layers of the SC. In every lamina, the perikarya were mostly round and emitted one or two principal dendrites (Figs 1B, D, F).

Ultrastructurally, numerous boutons measuring 0.5–1 μm filled with round or sometimes flattened vesicles were present in the superficial layers of the SC. Occasionally, a few dense-core vesicles were also observed (Figs 2 and 3). The NPY-ir boutons established exclusively symmetrical synaptic contacts, primarily on vesicle-free dendrites with various diameters (Fig. 3). Axo-somatic synapses were also

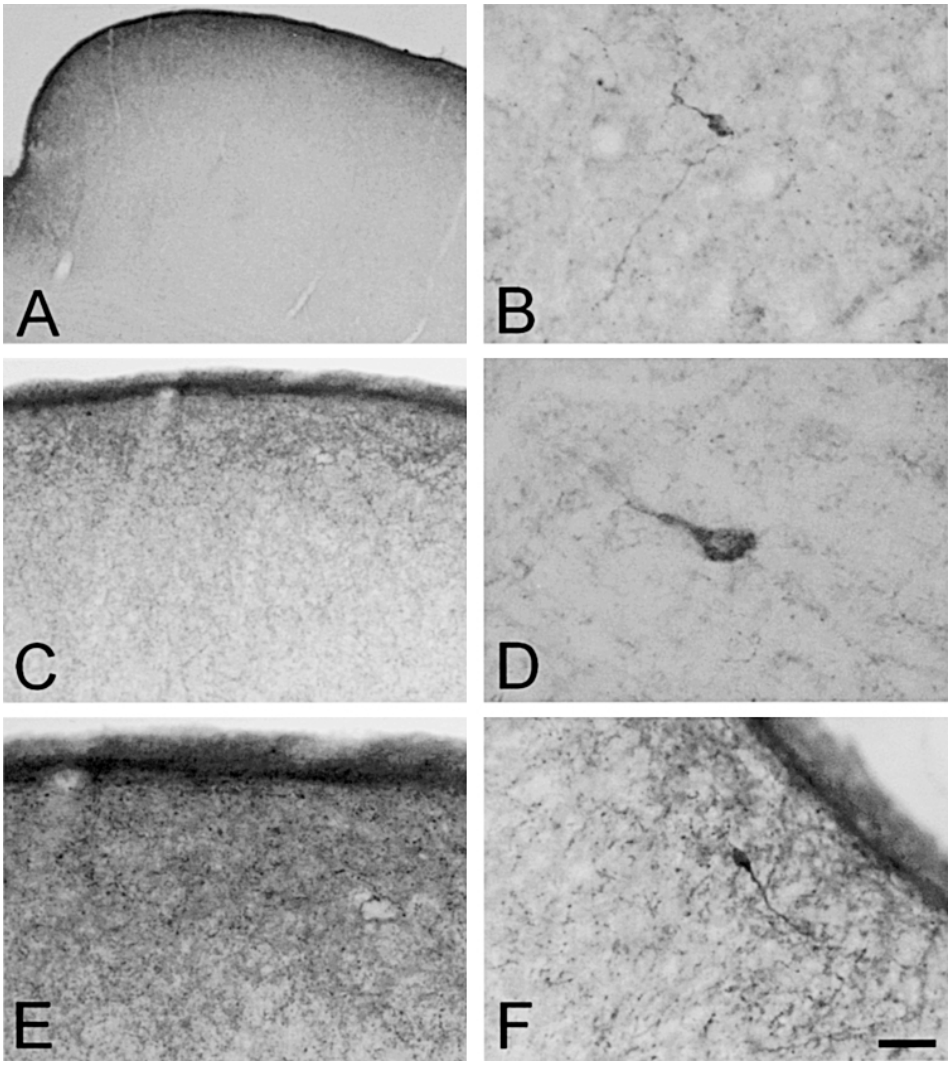

Fig. 1. NPY immunoreactivity in the cat superior colliculus. The dense NPY fibre network is shown in the SGL (A), and at higher magnifications (C, F). A few NPY perikarya can be recognised in deeper layers of the SC (B, D), and also in the SGL (F). The (presumably) axons of an NPY cell can be followed for a long distance (B). Scale bar: 500 µm (A), 100 µm (C), 50 µm (E), 33 µm (B, F), 20 µm (D)

found at times (Fig. 2), and the NPY-ir boutons occasionally synaptised at the origin of a large primary dendrite.

The types of the synaptic connections were estimated in two cats. Of the 105 synaptic contacts of NPY-ir terminals counted in cat No 1, 99 boutons established axo-dendritic synapses (96.2%), while 4 boutons established axo-somatic synapses

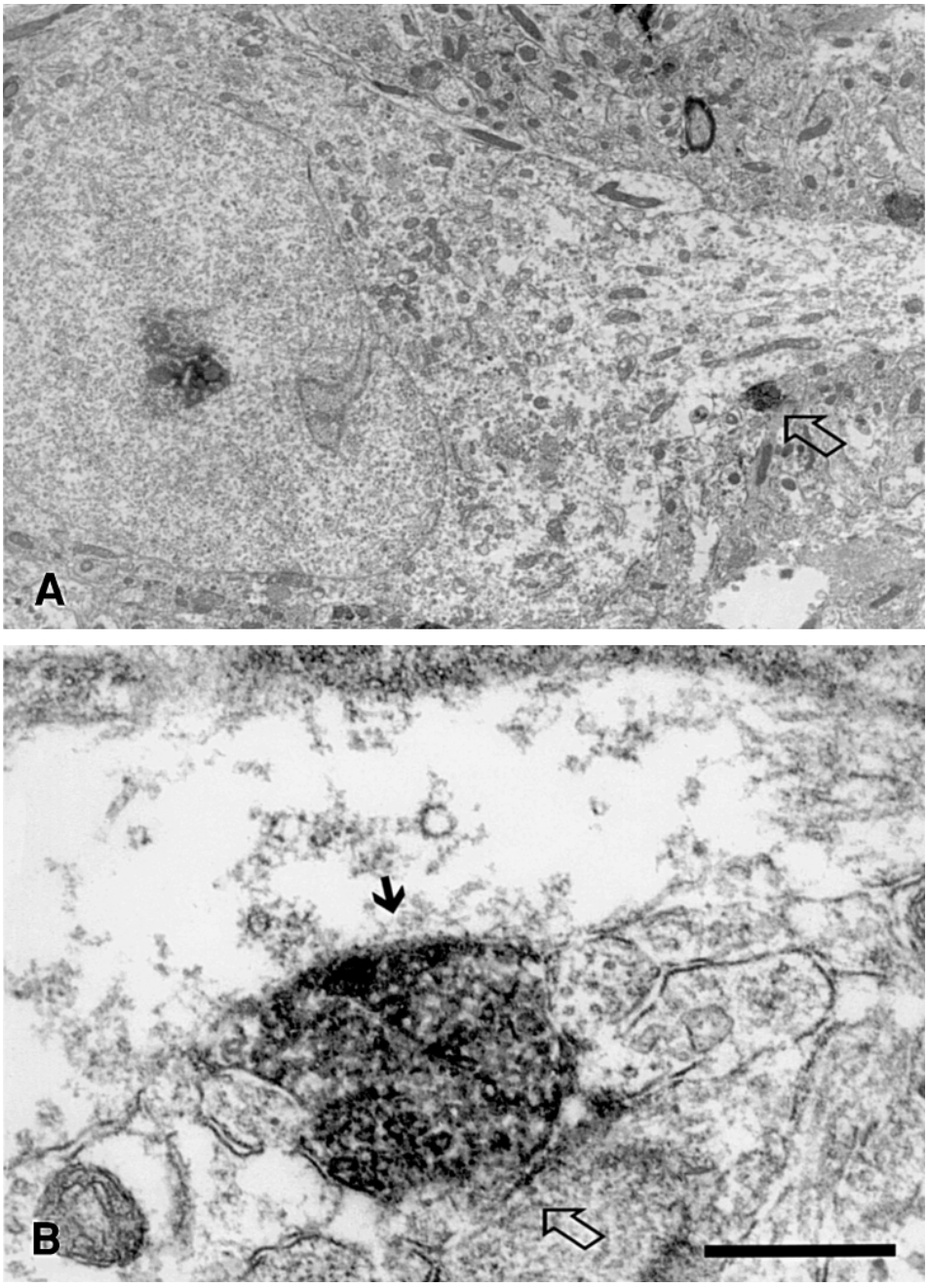

Fig. 2. Ultrastructure of NPY elements in the SGL of the cat SC. An NPY bouton (empty arrow) contacts the initial segment of a principal dendrite (A), and at higher magnification the symmetrical synapse (arrow) is seen (B). Scale bar: 2 μm (A), 200 μm (B)

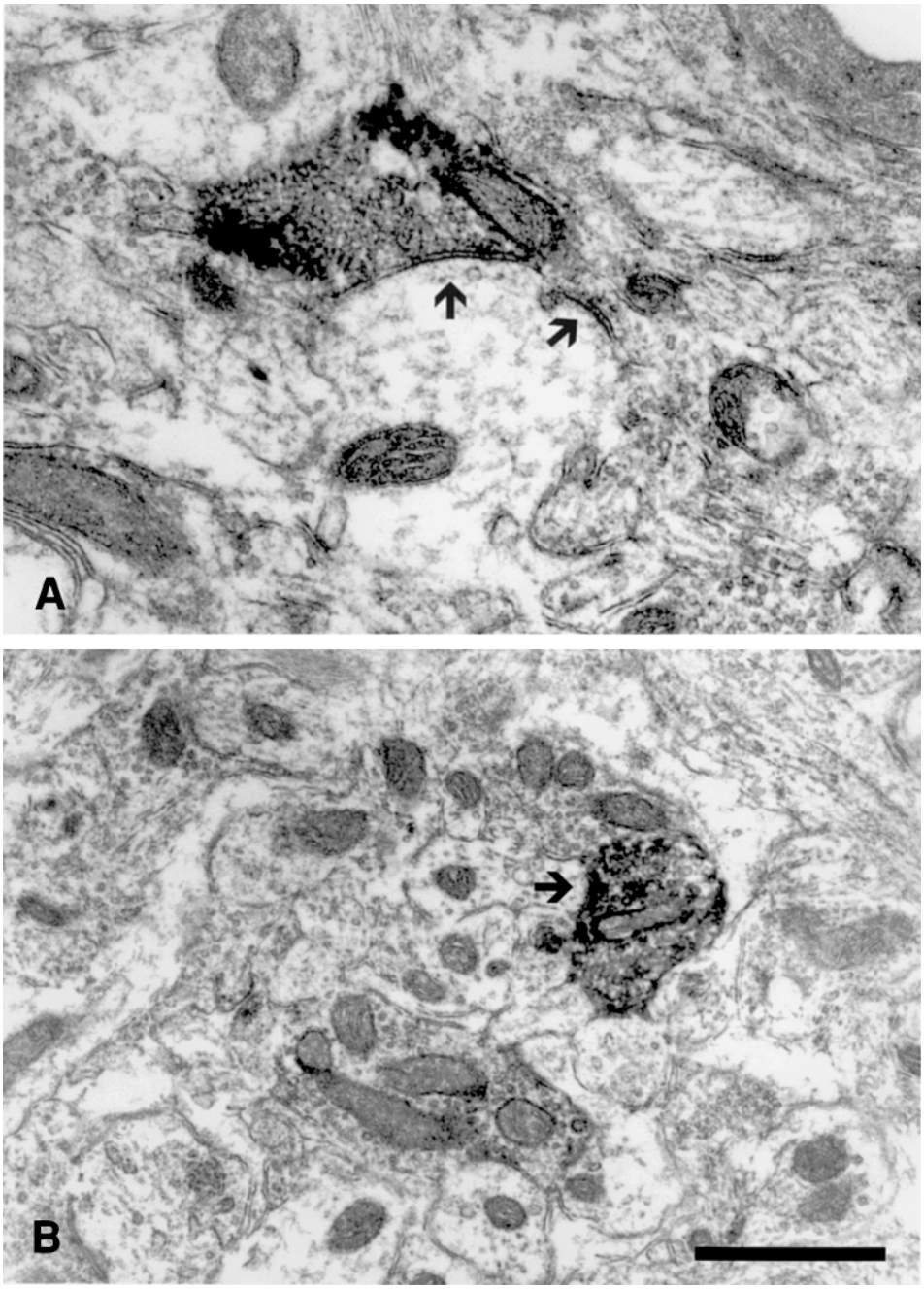

Fig. 3. NPY boutons contact vesicle-free dendrites with different calibres (A, B). The synapses are indicated by arrows. Scale bar: 500 µm (A), 300 µm (B)

(3.8%). In cat No 2, 116 synapses were analysed, and proved to be axo-dendritic in 113 cases (97.4%), and axo-somatic in 3 cases (2.6%). The nuclei of the cells contacted by NPY boutons were always round, and not intruded.

DISCUSSION

We reported earlier that there is a strong NPY fibre network in the SGL of the cat SC [3]. Some NPY cells located exclusively in the SGL were described previously [6], but a morphological description concerning the shape of the neurons has not been published. It is reported here that the NPY cells in the cat SC are small and either fusiform or unipolar-like. These NPY cells are located not only in the SGL, as described previously [6], but also in the deeper layers.

The origin of the NPY fibres in the SC is presumably the retina [14], the pretectum [3, 15, 25], the ventral lateral geniculate nucleus (vLGN) [22], or the intrinsic collicular neurons [6, present report]. Nevertheless, following enucleation the distri-

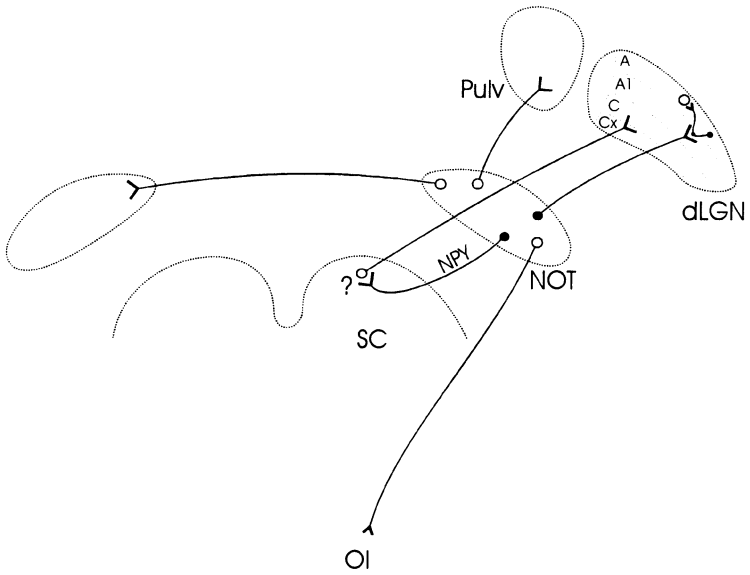

Fig. 4. Summarizing drawing of the different NOT neuron populations in the cat. Both the SC and the NOT give rise to a projection to the dLGN, where they end on different laminae in different manners. While the pretecto-geniculate pathway is GABAergic, which inhibits geniculate interneurons (disinhibition of relay cells) predominantly in A laminae, the tecto-geniculate pathway presumably has an excitatory action, which terminates on C laminae. In addition, the NOT sends a GABAergic, and possibly NPYergic pathway to the SC. It is hypothetised here that the pretecto-tectal projection may end on tecto-geniculate neurons, inhibiting the collicular impact on the dLGN during the function of the pretecto-geniculate pathway. Moreover, three additional excitatory neuron populations of the NOT project to the pulvinar (Pulv) [Wang and Bickford, personal communication], the inferior olive (OI) [13], and the contralateral NOT [26]. (Filled perikarya represent inhibitory neurons, and empty perikarya excitatory ones)

bution of NPY fibres in the SC remained unchanged [3, 25]. Moreover, the small number of intrinsic collicular NPY perikarya is not likely to give rise to such a rich, and extensive fibre network in the SC. However the vLGN projects not to the SGL, but to the deeper stratum opticum [1]. In contrast to the above-mentioned possibilities, convincing evidence has been presented for the collicular projection of pretectal NPY cells [3, 15]. Since the NPY is localised most frequently in GABAergic neurons, these NPYergic pretectal cells might be identical with the feline GABAergic pretecto-tectal projecting neurons [1].

In contrast to the proposed axo-axonic connection of NPYergic fibres on the retinal terminals in the tectum of amphibians [19], the NPY boutons in the cat SGL contact almost exclusively dendrites, and in a few cases somata. The labelled boutons in the SC of cat establish mostly symmetrical synapses, confirming the previously proposed inhibitory role of the NPYergic nerve endings in the anuran tectum [23]. These results do not prove such a direct inhibition of retinal information transfer in the cat SC, but rather suggest a more complex inhibitory mechanism. Such complex interplay for the pretecto-tectal pathway has also been suggested in amphibians [18]: it was proposed that either retinal afferents or tectal interneurons could be the postsynaptic elements of the pretectal afferents in salamanders, and that the nature of the pretectal impact on the tectum is modulatory rather than inhibitory. However, in cat, the dendrites and cell bodies have been proved to be contacted by the NPY fibres, originating presumably from the pretectum (Fig. 4).

Both the SGL of the SC and the pretectum project to the dorsal LGN (dLGN) modulating its function [20] (Fig. 4). While the pretecto-geniculate pathway innervates predominantly the A laminae [9], the tecto-geniculate projection terminates almost exclusively on the C laminae [12]. The pretectal fibres inhibit dLGN-interneuron dendrites [8, 9, 27] and therefore inhibit relay cells (disinhibition) resetting their activity at the end of saccadic eye movements [11, 17]. However, the tectal fibres transmit W-like visual information to the small W cell-containing tiers of the dLGN [12]. It seems plausible to suppose that the pretectum might inhibit the SC during its impact on the dLGN through the GABA/NPYergic pretecto-tectal pathway (Fig. 4). In addition, the few contacted perikarya in the SC possess a round nucleus, suggesting their excitatory (probably projecting), nature. These postsynaptic cells of the presumably pretectal NPY boutons can indeed be such collicular geniculate-projecting neurons.

In conclusion, the pretectal effect on the SC in cat could be realised through tectal neurons, and not via retinal afferents. Dissimilar to amphibians, which do not have such complex eye movements and visual functions as cat, the pretecto-tectal connection seems inhibitory rather than modulatory.

ACKNOWLEDGMENTS

Special thanks are due to Ms. Andrea Németh for her excellent technical assistance in the electron microscopy. This work was supported by the Office of Supported Research Groups, Hungarian Academy of Sciences (MTA-TKI).

REFERENCES

1. Appel, P. P., Behan, M. (1990) Sources of subcortical GABAergic projections to the superior colliculus in the cat. *J. Comp. Neurol. 302,* 143–158.
2. Bons, N., Mestre, N., Petter, A., Danger, J. M., Pelletier, G., Vaudry, H. (1990) Localization and characterization of neuropeptide Y in the brain of Microcebus murinus (Primate, Lemurian). *J. Comp. Neurol. 298,* 343–361.
3. Borostyánkői, Zs., Görcs, T. J., Hámori, J. (1999) Immunocytochemical mapping of NPY and VIP neuronal elements in the cat subcortical visual nuclei, with special reference to the pretectum and the accessory optic system. *Anat. Embryol. 200,* 495–508.
4. Boswell, T., Millam, J. R., Li, Q., Dunn, I. C. (1998) Cellular localization of neuropeptide Y mRNA and peptide in the brain of the Japanese quail and domestic chicken. *Cell. Tissue Res. 293,* 31–38.
5. Chronwall, B. M., DiMaggio, D. A., Massari, V. J., Pickel, V. M., Ruggiero, D. A., O'Donohue, T. L. (1985) The anatomy of neuropeptide-Y-containing neurons in rat brain. *Neuroscience 15,* 1159–1181.
6. Coveńas, R., Aguirre, J. A., de León, M., Alonso, J. R., Narváez, J. A., Arévalo, R., González-Barón, S. (1990) Distribution of neuropeptide Y-like immunoreactive cell bodies and fibers in the brain stem of the cat. *Brain Res. Bull. 25,* 675–683.
7. Csiffáry, A., Görcs, T. J., Palkovits, M. (1990) Neuropeptide Y innervation of ACTH-immunoreactive neurons in the arcuate nucleus of rats: a correlated light and electron microscopic double immunolabeling study. *Brain Res. 506,* 215–222.
8. Cucchiaro, J. B., Bickford, M. E., Sherman, S. M. (1991) A GABAergic projection from the pretectum to the dorsal lateral geniculate nucleus in the cat. *Neuroscience 41,* 213–226.
9. Cucchiaro, J. B., Uhlrich, D. J., Sherman, S. M. (1993) Ultrastructure of synapses from the pretectum in the A-laminae of the cat's lateral geniculate nucleus. *J. Comp. Neurol. 334,* 618–630.
10. Danger, J. M., Guy, J., Benyamina, M., Jegou, S., Leboulenger, F., Cote, J., Tonon, M. C., Pelletier, G., Vaudry, H. (1985) Localization and identification of neuropeptide Y (NPY)-like immunoreactivity in the frog brain. *Peptides 6,* 1225–1236.
11. Fischer, W. H., Schmidt, M., Stuphorn, V., Hoffmann, K.-P. (1996) Response properties of relay cells in the A-laminae of the cat's dorsal lateral geniculate nucleus after saccades. *Exp. Brain Res. 110,* 435–445.
12. Harting, J. K., Huerta, M. F., Hashikawa, T., van Lieshout, D. P. (1991) Projection of the mammalian superior colliculus upon the dorsal lateral geniculate nucleus: Organization of tectogeniculate pathways in nineteen species. *J. Comp. Neurol. 304,* 275–306.
13. Horn, A. K. E, Hoffmann, K.-P. (1987) Combined GABA-immunocytochemistry and TMB-HRP histochemistry of pretectal nuclei projecting to the inferior olive in rats, cats and monkeys. *Brain Res. 409,* 133–138.
14. Hutsler, J. J., White, C. A., Chalupa, L. M. (1993) Neuropeptide Y immunoreactivity identifies a group of gamma-type retinal ganglion cells in the cat. *J. Comp. Neurol. 336,* 468–480.
15. Kozicz, T., Lázár, Gy. (1994) The origin of tectal NPY immunopositive fibres in the frog. *Brain Res. 635,* 345–348.
16. Lázár, Gy., Maderdrut, J. L., Trasti, S. L., Liposits, Z., Tóth, P., Kozicz, T., Merchentaler, I. (1993) Distribution of proneuropeptide Y-derived peptides in the brain of *Rana esculenta* and *Xenopus laevis*. *J. Comp. Neurol. 327,* 551–571.
17. Lee, D., Malpeli, J. G. (1998) Effects of saccades on the activity of neurons in the cat lateral geniculate nucleus. *J. Neurophysiol. 79,* 922–936.
18. Luksch, H., Kahl, H., Wiggers, W., Roth, G. (1998) Connectivity of the salamander pretectum: An in vitro (whole-brain) intracellular tracing study. *Cell. Tissue Res. 292,* 47–56.
19. Luksch, H., Roth, G. (1996) Pretecto-tectal interactions: Effects of lesioning and stimulating the pretectum on field potentials in the optic tectum of salamanders in vitro. *Neurosci. Lett. 217,* 137–140.
20. Lysakowski, A., Standage, G. P., Benevento, L. A. (1986) Histochemical and architectonic differentiation of zones of pretectal and collicular inputs to the pulvinar and dorsal lateral geniculate nucleus in the macaque. *J. Comp. Neurol. 250,* 431–448.

21. Morin, L. P., Blanchard, J. H. (1997) Neuropeptide Y and enkephalin immunoreactivity in retinorecipient nuclei of the hamster pretectum and thalamus. *Vis. Neurosci. 14,* 765–777.
22. Morin, L. P., Blanchard, J. H. (1998) Interconnections among nuclei of the subcortical visual shell: The intergeniculate leaflet is a major constituent of the hamster subcortical visual system. *J. Comp. Neurol. 396,* 288–309.
23. Schwippert, W. W., Ewert, J.-P. (1995) Effect of neuropeptide-Y on tectal field potential in the toad. *Brain Res. 669,* 150–152.
24. Schwippert, W. W., Rottgen, A., Ewert, J.-P. (1998) Neuropeptide Y or fragment NPY 13–36, but not NPY 18–36, inhibit retinotectal transfer in cane toads *Bufo marinus*. *Neurosci. Lett. 253,* 25–36.
25. Székely, A. D., Csillag, A., Görcs, T. J. (1992) Neuropeptide Y innervation of retinorecipient layers of chick optic tectum. *J. Neurocytol. 21,* 148–156.
26. Vargas, C. D., Volchan, E., Hokoç, J. N., Pereira, A., Bernardes, R. F., Rocha-Miranda, C. E. (1997) On the functional anatomy of the nucleus of the optic tract-dorsal terminal nucleus commissural connection in the opossum (*Didelphis marsupialis aurita*). *Neurosci. 76,* 313–321.
27. Wahle, P., Stuphorn, V., Schmidt, M., Hoffmann, K.-P. (1994) LGN-projecting neurones of the cat's pretectum express glutamic acid decarboxylase mRNA. *Eur. J. Neurosci. 6,* 454–460.
28. Yamano, M. (1998) The neuropeptide Y system. In: Tohyama, M., Takatsuji, K. (eds), *Atlas of Neuractive Substances and their Receptors in the Rat*. Oxford University Press, Oxford–New York–Tokyo, pp. 294–301.