UNIVERSITE DU QUÉBEC

MÉMOIRE
PRESENTÉ A
L'UNIVERSITÉ DU QUÉBEC À TROIS-RIVIERES
COMME EXIGENCE PARTIELLE
DE LA MAÎTRISE EN PSYCHOLOGIE

PAR
CHRISTINE BERTRAND

IMMUNORÉACTIVITÉ ChAT, NOS ET GABA DANS LE NOYAU ISTHMO-OPTIQUE ET LES NEURONES ECTOPIQUES DU SYSTÈME VISUEL CENTRIFUGE ET DES PROJECTIONS AFFÉRENTES CHEZ LE PIGEON

AOÛT 1997

Ce document est rédigé sous la forme d'un article scientifique, tel qu'il est stipulé dans les règlements des études avancées (art. 16.4) de l'Université du Québec à Trois-Rivières. L'article a été rédigé selon les normes de publications d'une revue reconnue et approuvée par le comité d'études avancées en psychologie. Le nom du directeur de recherche pourrait donc apparaître comme co-auteur de l'article soumis pour publication.

Sommaire

La présente étude a examiné la présence d'une immunoréactivité(-ir) pour le ChAT, le NOS et le GABA au niveau des neurones du noyau isthmo-optique (NIO) et des neurones ectopiques (EN) dans le système visuel centrifuge (SVC) chez le pigeon. Ces différents messagers chimiques ont également été recherchés au niveau des neurones afférents aux cellules centrifuges, dans les couches 9/10 du tectum optique (TO), l'aire ventralis-Tsai (AVT), la zone péri-NIII (ZpNIII) et la formation réticulée pontique (PRF), incluant la zone péri-NVI (Zp-n.NVI). Cette technique a été combinée avec le marquage des cellules centrifuges après l'injection intraoculaire du traceur fluorescent Rhodamine ß-isothiocyanate (RITC) et le marquage des cellules afférentes au SVC soit, 1) par transport rétrograde du traceur FG après son injection dans le NIO/EN; soit 2) par transport transneuronal du traceur RITC suivant son injection intraoculaire. Les résultats de double-marquage démontrent que la majorité des cellules centrifuges du NIO, ainsi qu'un grand nombre de EN présente une immunoréactivité pour le ChAT et pour le NOS, mais pas pour le GABA. Seulement quelques petits interneurones GABA-ir sont observés dans la zone neuropillaire du NIO. Au niveau des afférences aux cellules centrifuges, de nombreux neurones afférents des couches 9/10 du TO sont NOS-ir et à un moindre degré ChAT-ir. Par ailleurs, plusieurs cellules GABA-ir sont distribuées à

travers les couches 9/10 du TO, mais ces dernières ne se projettent pas aux cellules centrifuges. Un grand nombre d'afférences extra-tectales aux cellules centrifuges sont ChAT-ir et NOS-ir, particulièrement dans le ZpNIII et à un niveau moins élevé dans l'AVT. Dans la PRF, plus spécifiquement le Zp-n.NVI, des cellules qui se projettent aux cellules centrifuges démontrent une immunoréactivité pour le NOS, mais pas pour le ChAT. Aucune immunoréactivité pour le GABA n'est observée à travers les différents neurones afférents du ZpNIII, de l'AVT et de la PRF. Ces résultats indiquent que l'influence des cellules centrifuges du NIO et des EN sur les cellules cibles rétiniennes, ainsi que des structures afférentes aux cellules centrifuges est excitatrice et que plusieurs de ces voies cholinergiques utilisent également le NO. Ces données amènent ainsi à discuter les interactions inhibitrices et excitatrices des différentes composantes du SVC et des systèmes afférents visuels et non visuels, ainsi que du rôle possible que ce système joue dans la modulation de l'attention visuelle.

Table des matières

Remerciements

L'auteur désire exprimer sa reconnaissance à son directeur de mémoire monsieur Dom Miceli, Ph.D., pour l'assistance à l'élaboration de ce manuscrit. Remerciements au CRSNG pour leur support financier octroyé afin de mener ce projet de recherche. L'auteur remercie vivement Nadine Mayotte du Laboratoire de Neuropsychologie expérimentale et comparée, et Michel Bélanger de Info-image pour leur précieuse aide technique, ainsi que monsieur Michel Volle, Ph.D, madame Manon Lebel des études avancées et monsieur Sylvain Gagnon, Ph.D. pour leur support.

Partie I - Résumé de l'article en français

Contexte théorique

Le but de la présente étude a été de déterminer les propriétés neurochimiques du système visuel centrifuge (SVC) du pigeon, en particulier de certaines voies visuelles et non visuelles qui composent ce système. L'identification des propriétés neurochimiques est un moyen permettant d'éclaircir le rôle joué par le SVC dans le comportement optiquement guidé. Malgré l'existence de plusieurs études portant sur ce système, son rôle fonctionnel n'a pas encore été clairement élucidé, favorisant ainsi la formulation d'hypothèses extrêmement diverses (cf. Repérant, Miceli, Vesselkin, & Molotchnikoff, 1989; Uchiyama, 1989 ; Ward, Repérant, & Miceli, 1991). Sur la base des projections préférentielles du tectum ventral au noyau isthmo-optique (NIO) et des projections centrifuges à la rétine ventrale, Holden (1990) a postulé que le SVC jouait un rôle dans l'attention comportementale pendant la recherche de nourriture ("searchlight hypothesis"). Différentes études ont également appuyé l'implication du SVC aviaire dans le picorage (Shortess & Klose, 1977 ; Weidner, Desroches, Repérant, Kirpitchnikova, & Miceli, 1987 ; Hahmann & Güntürkün 1992). Woodson, Shimizu, Wild, Schimke, Cox, & Karten, (1995) ont suggéré que le SVC rehaussait la

stabilisation rétinienne du regard fixe afin d'améliorer la précision du picorage de petits objets et ce, à travers la projection centrifuge sur les cellules ganglionnaires déplacées afférentes au système optique accessoire (Nickla, Gottlieb, Marin, Rojas, Britto, & Wallman, 1994). Enfin, il a également été postulé que le SVC produirait une augmentation sélective de la sensibilité rétinienne à divers stimuli significatifs (objets alimentaires, détection de prédateurs, etc.) dans le champ visuel (" highlighting hypothesis " : Uchiyama, 1989 ; Miceli, Repérant, Rio, & Medina, 1995).

Le système visuel centrifuge (SVC), impliquant une afférence allant du cerveau jusqu'à la rétine, a été décrit chez tous les groupes de vertébrés, incluant l'homme. Le SVC aviaire est particulièrement bien adapté pour les études anatomiques étant donné sa large projection rétinopète du tegmentum mésencéphalique, plus spécifiquement à partir du noyau isthmo-optique (NIO), fortement développé et différentié, et de ses neurones ectopiques (EN) associés plutôt dispersés (Cowan, 1970 ; Hayes & Webster, 1981). Ainsi, les granivores, tel que les pigeons, possèdent un NIO particulièrement bien développé qui comprend environ 10000 neurones auxquels et il faut ajouter 2000 à 2500 neurones ectopiques (Cowan, 1970 ; Cowan & Powell, 1963 ; Hayes & Webster, 1981 ; O'Leary & Cowan, 1982 ; Wolf-Oberhollenzer, 1987). Le NIO, situé dans la partie dorsale du mésencéphale postérieur, présente un aspect laminaire convoluté. Ce dernier est également constitué de neurones répartis en deux

couches séparées par un neuropile distinct. Les EN dispersés ventralement au NIO, sont des cellules plus grandes que celles du NIO et forment une population hétérogène de cellules multipolaires, dont la forme est triangulaire, ovale ou allongée (Miceli, Repérant, Marchand, & Rio, 1993). Des études en microscopie électronique consacrées à l'analyse synaptologique de la région neuropilaire du NIO du pigeon (Angaut & Repérant, 1978) et du poulet (Crossland, 1979) ont mis en évidence trois type de terminaisons axonales, dont 60% d'entre elles sont d'origine tectale. D'ailleurs, la seule afférence bien établie allant au NIO a longtemps été cette projection majeure du tectum optique (TO), spécifiquement à partir des couches 9/10. À la suite des travaux de Mc Gill, Schmidt, & Weinberg (1966a, b), basés sur des lésions expérimentales de quadrants rétiniens et de différentes régions du toit optique, il a été démontré que le NIO constituait la structure-clé de la boucle de rétroaction rétine → tectum optique → NIO → rétine.

Les informations fournies par la recension des écrits concernant les autres afférences au NIO ont longtemps été fragmentaires et souvent contradictoires (cf. Repérant & al., 1989 ; Ward & al., 1991). Cependant, plus récemment, à l'aide d'une technique de transport transneuronal du traceur fluorescent Rhodamine ß-isothicyanate (RITC) (Miceli & al, 1993), il a été possible de mettre en évidence différentes structures extra-tectales se projetant sur les cellules centrifuges visuelles, dont notamment l'aire ventralis-Tsai (AVT), la

zone péri-NIII (ZpNIII) et la formation réticulée pontique (PRF), incluant la zone péri-NVI (Zp-n.NVI) (Miceli, Repérant, Bavikati, Rio, & Volle, 1997). Cette dernière technique a donc permis de mieux comprendre l'organisation anatomique du SVC et de ses afférences. Par ailleurs, de l'information sur les propriétés neurochimiques des différentes composantes de cette organisation pourrait donner des indications sur la contribution du SVC à la fonction visuelle. Cependant, très peu d'informations existent à ce sujet.

Immunoréactivité GABA

Différentes études ont démontré l'absence d'immunoréactivité pour l'anticorps anti-GABA dans les neurones rétinopètes (NIO et EN) (Domenici, Waldvogel, Matute, & Streit, 1988 ; Granda & Crossland, 1989 ; Miceli & al., 1995). Cependant, Veenman et Reiner (1994) ont observé dans le NIO, quelques neurones immunoréactifs à l'anticorps contre l'acide glutamique décarboxylase (GAD) ; l'enzyme de synthèse pour le GABA. Certains indices morphologiques, tels que la petite taille de ces neurones et la présence de vésicules synaptiques dans les dendrites, ont permis d'assimiler l'immunoréativité GABA aux interneurones (Somogyi, 1989, pour revue). Ces données ont également été confirmées par Miceli et al. (1995) qui ont démontré qu'il s'agissait d'interneurones GABAergiques situés dans la zone interpillaire du NIO. Par ailleurs, il a été démontré que parmi les huit types de boutons différents présents dans le NIO, trois d'entre eux

exprimaient une immunoréactivité au GABA (P_{2a}, P_{2c} et P_3) et représentaient plus de 47% de la population des profils contenant des vésicules synaptiques (Miceli & al., 1995). Sur la base de critères purement morphologiques, les profils du type P_{2a} (plus de 26%) appartiendraient vraisemblablement aux interneurones. Ces données impliquent ainsi que ces derniers seraient fortement collatéralisés et exerceraient un rôle inhibiteur sur les neurones visuels centrifuges, du moins en ce qui concerne le NIO (Miceli & al., 1995), car il est connu que le GABA est un important neurotransmetteur inhibiteur dans le système nerveux central (Enna & Gallager, 1983 pour revue; Mugnaini & Oertel, 1985; Ottersen & Storm-Mathisen, 1998). De plus, de nombreuses terminaisons GABAergiques ont été trouvées dans le NIO, ainsi que des axones GABA immunoréactifs (-ir) myélinisés, suggérant également la présence d'afférences GABAergiques extranucléaires (Miceli et al., 1995).

Dans la mesure où, d'une part, près de la moitié des terminaisons axonales présentes dans le NIO sont GABA-ir et que, d'autre part, le NIO reçoit principalement des projections du toit optique (Cowan & Powel, 1963 ; Mcgill & al., 1966a, b ; Crossland & Hugues, 1978 ; Uchiyama, 1989 ; Woodson, Reiner, Anderson, & Karten, & al., 1991 ; Miceli & al., 1993), il est possible que ces fibres aient pour origine les couches 9/10 du tectum optique. Les études en microscopie électronique ont également démontré que, d'une part, les terminaisons GABAergiques du NIO présentent un profil synaptique

caractérisé par des vésicules rondes (Miceli & al., 1995) et que, d'autre part, ce même type de terminaisons est retrouvé au niveau des cellules tectales afférentes au SVC (Crossland, 1979 ; Angaut & Repérant, 1978). De même, Hunt et Künzle (1976b) ont observé que des cellules situées dans les couches 9/10 du TO chez le pigeon utilisaient le GABA. Cependant, la présence de terminaisons synaptiques GABAergiques dans le NIO contenant, non pas des vésicules rondes mais pléomorphes, suggère que ces dernières pourraient également avoir une origine extra-tectale (Miceli & al., 1995). De plus, il a été démontré qu'une proportion de terminaisons axonales, contenant des vésicules rondes, survivent suite à des lésions tectales (Crossland, 1979), ce qui pourrait impliquer que certains de ces profils de terminaisons sont d'origine extra-tectale.

Immunoréactivité ChAT

Les données concernant la choline acétyltransférase (ChAT), enzyme de synthèse de l'acétylcholine (Ach), dans les cellules centrifuges présentent certaines contradictions. En effet, Bagnoli, Fontanesi, Alesci, et Erichsen (1992) ont mis en évidence la présence de cellules ChAT-ir dans le pôle médial du NIO, ainsi que dans de nombreuses cellules ectopiques multipolaires chez le pigeon. Bien que Medina et Reiner (1994) aient confirmé que les corps cellulaires ChAT-ir observés autour du NIO présentaient une taille et une localisation qui correspondaient aux neurones ectopiques décrits par

Hayes et Webster (1981), ils ont noté que les cellules ChAT-ir du NIO, démontrant une très faible immunoréactivité, étaient retrouvées seulement chez quelques pigeons, particulièrement lorsque les cerveaux étaient moins bien fixés. Par ailleurs, aucune cellule ChAT-ir n'a été observée dans le NIO du poulet (Sorenson, Parkinson, Dahl, & Chiappinelli, 1989).

La présence d'une dense innervation cholinergique dans le NIO du pigeon (Medina & Reiner, 1994), des niveaux élevés de récepteurs nicotiniques sur les périkarya de la plupart des neurones et dans le neuropile du NIO du poulet (Britto, Keyser, Lindstrom, & Karten, 1992 ; Sorenson & Chiappinelli, 1992), ainsi que le caractère cholinociceptif des neurones du NIO proposé par Nickla et al. (1994) suggèrent donc la présence de projections cholinergiques provenant de structures afférentes au NIO. Cependant, peu d'informations sont disponibles concernant l'origine de ces projections cholinergiques, mais étant donné que le NIO reçoit principalement des projections du toit optique (Cowan & Powel, 1963 ; Mcgill & al., 1966a, b ; Crossland & Hugues, 1978 ; Uchiyama, 1989 ; Woodson & al., 1991 ; Miceli & al., 1993), une origine tectale ne peut être exclue. D'ailleurs, de nombreux neurones de la couche 9 et principalement de la couche 10 du tectum optique ont été démontrés comme étant ChAT-ir chez le pigeon (Medina & Reiner, 1994 ; Bagnoli & al., 1992) et le poulet (Sorenson & al., 1989). Cependant, il a été suggéré que ces dernières cellules des couches 9/10 du TO étaient à l'origine des projections

tectales sur le noyau isthmique parvocellulaire (Ipc) et/ou le noyau géniculé latéral ventral (GLv) (Hunt & Künzle, 1976 a, b ; Reiner & Karten, 1982 ; Hunt & Brecha, 1984 ; Woodson & al., 1991). Par ailleurs, des neurones ChAT-ir ont été décrits dans d'autres structures du tronc cérébral qui se projettent sur les cellules centrifuges visuelles : dans le ZpNIII du pigeon (appelé aussi l'aire tegmentale ventrale : Medina & Reiner, 1994), ainsi que dans le RPO du poulet (Sorenson & al., 1989) et du pigeon (Medina & Reiner, 1994). Enfin, une immunoréactivité pour le ChAT a été démontrée dans les motoneurones du N.VI, ainsi que dans un groupe de petits neurones situé ventrolatéralement entre la racine du n.VI et du complexe moteur facial (Medina & Reiner, 1994), ce dernier groupe pourrait correspondre à la région afférente au NIO appelée Zp-n.NVI (Miceli & al., 1997).

Immunoréactivité NOS

Différentes études ont porté sur la distribution du monoxyde d'azote (NO) dans le cerveau des oiseaux, en employant l'histochimie NADPH-diaphorase (poulet : Brüning, 1993 ; Montagnese & Csillag, 1996 ; Morgan, Miethke, & Li, 1994 ; pigeon : Meyer, Banuelos-Pineda, Montagnese, Ferres-Meyer, & Gonzales-Hernandez, 1994 ; caille : Panzica, Arévalo & al. 1994, 1996 ; perruche *Melopsittacus undulatus* : Cozzi, Massa, & Panzica, 1997), dont l'activité révèle la présence d'oxyde nitrique synthase (NOS) qui est l'enzyme de

synthèse du NO. Certains auteurs rapportent que la distribution du marquage de l'activité NADPH-d et de l'immunoréactivité pour le NOS est identique (Decker & Reuss, 1994 ; Hashikawa, Leggio, Hattori, & Yui, 1994 ; Valtschanoff, Weinberg, Kharazia, Nakane, & Schmidt, 1993 ; Dawson, Bredt, Fotuhi, Hwang, & Snyder, 1991), tandis que d'autres observent que ces deux techniques de marquage reflétant l'activité du NO n'identifient pas nécessairement les mêmes populations neuronales (Kharazia, Schmidt, & Weinberg, 1994 ; Spessert & Layes, 1994 ; Spessert, Wohlgemuth, Reuss, & Layes, 1994 ; Traub, Solodkin, Meller, & Gebhart, 1994 ; Vizzard, Erdman, Roppolo, Förstermann, & de Groat, 1994). En effet, certains auteurs ont suggéré que l'activité NADPH-d serait seulement positive dans les neurones synthétisant activement le NO, alors que la détection immunohistochimique révélerait tous les neurones contenant du NO (Rodrigo & al., 1994). Un autre avantage lié à l'utilisation de cette dernière technique se rapporte au fait que le NOS semble être moins influencé par les variations de la fixation tissulaire que la méthode d'histochimie NADPH-d (Gonzalez-Hernandez, Perez De La Cruz, & Mantolan-Sarmiento, 1996). Ces différentes données semblent donc justifier l'utilisation de l'immunohistochimie NOS dans l'étude présente.

Des recherches portant sur la distribution de l'activité NADPH-d dans le cerveau ont montré la présence de ce marqueur dans les cellules du NIO du poulet (Brüning, 1993 ; Montagnese & Csillag,

1996) et de la perruche (Cozzi & al., 1997), ce qui n'a pas été observé chez la caille (Panzica & al., 1994 ; Cozzi & al., 1997). De plus, il a été démontré que les éléments NADPH-d de la rétine dégénéraient après la destruction du NIO ou du tractus de projection isthmo-optique, impliquant ainsi que les terminaisons isthmo-optiques contiennent des niveaux élevés d'activité NADPH-d, suggérant qu'elles pourraient utiliser le NO comme messager chimique (Morgan & al., 1994). D'ailleurs, il a déjà été suggéré que chez les mammifères, le NO jouait un rôle important dans la régulation des fonctions visuelles, étant donné la mise en évidence de neurones NADPH-d à tous les niveaux du système visuel (Gonzalez-Hernandez, Conde-Sedin, & Meyer, 1992 ; Mitrofanis, 1992). Par ailleurs, différentes données suggèrent que le NO pourrait jouer un rôle important chez les oiseaux tant au niveau rétinien, que dans les centres visuels appartenant aux voies principales thalamofuges et tectofuges (Panzica & al., 1994 ; Cozzi & al., 1997), ainsi que centrifuges (Cozzi & al., 1997).

Les résultats de Brüning (1993), ayant rapporté la présence d'un neuropile intensément marqué dans le NIO, amènent à suggérer que des structures utilisant le NO se projettent au NIO. Certaines structures semblent donc plus susceptibles d'être à l'origine de ces projections utilisant le NO. Ainsi, de nombreux neurones marqués avec l'enzyme NADPH-d ont été observés dans les couches 9 (Brüning, 1993) et 10 du TO du poulet (Brüning, 1993) et du pigeon (Meyer & al., 1994). Cependant, Meyer et al. (1994) suggèrent que

ces dernières se projetteraient à l'Ipc. Par ailleurs, l'innervation NADPH-d du NIO pourrait aussi provenir des structures extra-tectales. D'ailleurs, des neurones marqués par l'histochimie NADPH-d ont été retrouvés dans les régions correspondantes au ZpNIII et à l'AVT chez le poulet (Brüning, 1993 ; Montagnese & Csillag ; 1996), la caille (Panzica & al., 1994, 1996) et la perruche (Cozzi & al., 1997). La PRF, incluant le Zp-n.NVI, est également un candidat possible puisque des cellules réagissant au NADPH-d ont été mises en évidence à travers la formation réticulée pontique de la caille (Panzica & al., 1994) et de la perruche (Cozzi & al., 1997).

Objectifs

Le but de la présente étude a été de déterminer les propriétés neurochimiques des cellules centrifuges, ainsi que des neurones afférents dans les couches 9/10 du TO, le ZpNIII, l'AVT et le Zp-n.NVI en employant la technique d'immunohistofluorescence et les anticorps dirigés contre le GABA, le ChAT et le NOS. Dans certaines expériences, cette technique a été combinée avec le marquage des cellules centrifuges après injection intraoculaire du traceur RITC et le marquage des cellules afférentes au SVC soit, 1) par transport rétrograde du traceur FG après son injection dans le NIO/EN ; soit 2) par transport transneuronal du traceur RITC suivant son injection intraoculaire.

Méthode

Douze pigeons anesthésiés avec une solution de Kétamine/Rompan (respectivement, 50/20 mg/kg), ont reçu une injection intraoculaire d'une solution de 0.4% wt./vol. (wv) d'acide kaïnique (KA) immédiatement suivie d'une seconde injection intraoculaire de 50 ul de 10% w/v du fluorochrome RITC (10%) contenant 2% de DMSO. Six autres pigeons ont reçu une injection unilatérale stéréotaxique (coordonnées, antérieur : 1.75mm, lateral :2.5mm, and profondeur : 8.5mm (Karten & Hodos, 1967)) de 0.1-0.2 ul d'une solution de 5% (w/v) du traceur Fluoro-Gold (FG) (5%) dans le NIO.

Après un temps de survie de 18-23 jours, pour les injections intraoculaires de RITC, et de 7-8 jours, pour les injections de FG dans le NIO, les pigeons anesthésiés ont été perfusés par voie intracardiaque, soit avec 0.9% de saline suivie de 4% de paraformaldéhyde et 0,2% de glutaraldéhyde dans un tampon phosphate 0,1M pH 7,3, et finalement avec le même fixatif contenant 30% de sucrose. Les cerveaux ont ensuite été extraits pour être préservés dans 30% de sucrose dans un tampon phosphate 0, 1M pH 7,3 à 4°C, pendant 2 jours, et ensuite sectionnés dans un plan frontal à une épaisseur de 20-40 µm à l'aide d'un microtome à congélation. Les coupes ont immédiatement été montées sur des lames gélatinées.

Pour la procédure de double marquage immunohistofluorescent, les coupes sont récupérées dans le PBS 0,02M pH 7,4 à 4° C, préincubées avec 3% de sérum normal de chèvre (NGS) et incubées une nuit dans l'anticorps GABA (1/1000, Incstar), 68 à 72 heures dans l'anticorps ChAT (1/1000, Johnson & Epstein, 1986) et NOS (1/800, Cedarlane). Les anticorps anti-lapin sont dilués avec du PBS 0,02M pH 7,4 et cette dernière solution est utilisée pour les lavages avant l'incubation dans un deuxième anticorps. Ce dernier conjugué au *Goat anti-rabbit* (GAR) FITC est utilisé pour les coupes provenant des pigeons ayant reçus une injection intraoculaire de RITC, et le GAR TRITC est utilisé pour les coupes provenant des cerveaux ayant reçu une injection de FG dans le NIO. Il est à noter, que lors du traitement des coupes, le Triton X-100 a été enlevé de la procédure afin de diminuer la diffusion du traceur RITC. De plus, pour chacune des différentes manipulations, des coupes contrôles ont été effectuées, afin d'éliminer la possibilité d'un marquage non-spécifique. Ces coupes ont été traitées dans les mêmes solutions d'incubation, mais sans l'anticorps.

Après le double marquage immunohistofluorescent, les coupes sont montées sur des lames gélatinées et examinées à l'aide d'un microscope à fluorescence Ploemopack Leitz adapté avec des systèmes de filtre-miroir N2 (550nm), I2 (470nm) et A (360nm) pour identifier respectivement le RITC, le FITC et le FG.

Le *ELF* ™*-AP immunohistochemestry kit* (Molecular Probes, Inc.) (ELF) a aussi été utilisé comme technique complémentaire. Cette technique a permis de vérifier si certains résultats trouvés avec le double marquage immunohistofluorescent étaient confirmés par cette autre technique. D'ailleurs, il a été démontré que ce type de marquage est net, éclatant et durable (Huang, You, Haugland. Paragas, Olson, & Haugland, 1993), ce qui indique un marquage précis.

Résultats

La distribution des cellules marquées suivant l'injection intraoculaire du traceur transneuronal RITC correspond aux descriptions de différentes études utilisant les mêmes technique de marquage (Miceli & al., 1993, 1997).). Le marquage des cellules tectales des couches 9/10, après une injection de FG directement dans le NIO, démontre une distribution très près de la précédente et semblable à ce qui a été rapporté dans différentes études employant d'autres traceurs rétrogrades injectés directement dans le NIO de la caille (Uchiyama & Watanabe, 1985) et du pigeon (Woodson & al.,1991).

La technique de double marquage (RITC/FITC) a permis de mettre en évidence la présence de neurones ChAT-ir dans la quasi-totalité des cellules centrifuges du NIO (fig. 1A-B), ce qui a également été démontré dans un grand nombre de cellules ChAT-ir de la région des EN (fig.1C-D). L'utilisation de la technique ELF, en simple marquage, a confirmé ces données au niveau du NIO (fig.1E), mais un moins grand nombre de cellules ChAT-ir ont alors été observées, ces dernières étant situées notamment dans les couches des cellules centrifuges périphériques du noyaux. Dans le NIO, la grande majorité des cellules qui forment les convolutions laminaires des neurones centrifuges sont NOS-ir (fig.1F), ainsi que dans la vaste région ectopique (fig.1G-H). Les seules cellules GABA-ir trouvées dans le

NIO présentent une petite taille et sont situées dans la zone neuropillaire du noyau. Une absence de cellules centrifuges GABAergiques est également remarquée dans la région ectopique.

Au niveau des cellules afférentes tectales se projetant aux cellules centrifuges, une immunoréactivité pour le ChAT en double marquage (RITC/FITC) est observée dans les couches 9/10 (fig.2A-B). Cependant, un nombre plus important de neurones uniquement ChAT-ir est présent dans les couches 9/10 du TO. Ces dernières couches du TO contiennent également des cellules NOS-ir doublement marquées avec le traceur transneuronal RITC (fig.2C-D). L'immunoréactivité pour le GABA est largement répandue dans les cellules du TO, incluant la couche 9 et particulièrement la couche 10. Au niveau des ces deux couches, aucune cellule GABA-ir n'a présenté un double-marquage suivant l'injection intraoculaire de FG (fig.2E-F). Ces résultats ont également été confirmés avec la technique ELF, après l'injection de FG dans le NIO. La présente étude démontre qu'un grand nombre de cellules situées dans les structures extra-tectales afférentes aux cellules centrifuges sont ChAT-ir et NOS-ir dans l'AVT et plus particulièrement dans le ZpNIII, dont plusieurs sont également marquées par le traceur transneuronal RITC (fig.3A-D). Enfin, une absence d'immunoréactivité pour le GABA est notée dans la région du ZpNIII et de l'AVT. Au niveau de la PRF, qui inclue le Zp-n.NVI, les seules cellules doublement marquées (RITC/FITC) sont NOS-ir (fig.3E-F).

Conclusion

Différentes études électrophysiologiques ont montré que les réponses visuelles enregistrées au niveau des cellules ganglionnaires sont facilitées par l'activation des fibres centrifuges (Galifret, Condé-Courtine, Repérant, & Serviere, 1971 : Miles, 1972). Cet effet facilitateur serait produit grâce à une action inhibitrice des fibres centrifuges sur les influences inhibitrices du champ réceptif des cellules ganglionnaires rétiniennes (Holden, 1978, 1982; Pearlman & Hughes, 1973). Un mécanisme possible pourrait impliquer l'inhibition par les fibres centrifuges, des cellules amacrines GABAergiques et glycinergiques qui, à leur tour, exerceraient des influences inhibitrices sur les cellules ganglionnaires (Frumkes, Miller, Slaughter, & Dacheux, 1981 ; Uchiyama, 1989). Cependant, la présente étude confirme que le GABA, principal neurotransmetteur inhibiteur dans le système nerveux central (Mugnaini & Oertel, 1985 ; Otterson & Storm-Mathisen, 1985), n'est pas utilisé par les fibres centrifuges et que les seuls éléments GABAergiques, dans le NIO, sont des interneurones (Miceli & al., 1995). Par ailleurs, la présente étude a démontré que l'innervation GABAergique du NIO ne provient pas des cellules afférentes aux cellules centrifuges qui sont situées dans les structures afférentes échantillonées. À l'inverse, la mise en évidence de cellules centrifuges ChAT-ir indique que l'influence centrifuge, sur les cellules amacrines de la rétine, est excitatrice. Par conséquent, l'ensemble de ces données indique que le

mécanisme d'influence centrifuge implique une désactivation des cellules centrifuges dans le NIO par l'intermédiaire, soit des interneurones intrinsèques, soit par les afférences inhibitrices GABAergiques extrinsèques. Inversement, une activation est aussi possible par l'intermédiaire, soit d'une afférence inhibitrice GABAergique extra- ou intra-nucléaire sur des interneurones intrinsèques, soit par une afférence extrinsèque excitatrice sur les cellules centrifuges.

Ces afférences cholinergiques allant aux cellules centrifuges seraient modulées par le NO, qui est possiblement colocalisé dans les mêmes neurones, étant donné que ces deux messagers chimiques présentent une distribution similaire au niveau de plusieurs des structures étudiées. De même, il a été démontré (Vincent, Satoh, Armstrong, & Fibiger, 1983 ; Pasqualotto & Vincent, 1991 ; Panzica & Garzino, 1994) et suggéré (Brüning, 1993 ; Panzica & al., 1994 ; Montagnese & Csillag, 1996) que le NO était colocalisé avec le ChAT dans différentes structures du cerveau, incluant le NIO (Brüning, 1993). Par ailleurs, il a été observé que chez les mammifères, des neurones NADPH-d sont présents à tous les niveaux du système visuel, suggérant que le NO joue un rôle important dans la régulation des fonctions visuelles (Gonzalez-Hernandez & al., 1992 ; Mitrofanis, 1992). De plus, différentes données ont suggéré que le NO pourrait jouer un rôle important au niveau rétinien chez les oiseaux, ainsi que dans les centres visuels appartenant aux voies principales

thalamofuges et tectofuges (Cozzi & al., 1997; Panzica & al., 1994), ainsi que centrifuges (Cozzi & al., 1997). Ainsi, la présente étude démontre que le NO joue un rôle important à tous les niveaux du SVC, tant au niveau tecto-NIO-rétine qui est considéré comme la voie principale spécifiquement visuelle et des autres systèmes non visuels spécifiques qui jouent un rôle dans la modulation sur le SVC. Il est intéressant de noter l'influence du NO au niveau de plusieurs composantes du SVC : (1) la boucle de rétroaction rapide rétino-tecto-NIO-rétinienne qui facilite le transfert de l'information visuelle dans des portions particulières du champ visuel ; (2) des systèmes de projections diffuses et non-spécifiques du tronc cérébral (ZpNIII/AVT) qui pourraient fournir des effets à plus long terme sur la mise en marche de l'activité des neurones centrifuges probablement reliée aux mécanismes tels que ceux modulant l'état général de vigilance ; (3) des afférences au SVC provenant des centres oculomoteurs du tronc cérébral (Zp-n.NVI/PRF) qui pourraient fournir une modulation de la sensibilité rétinienne en fonction du mouvement des yeux ou de la fixation de ces derniers (Miceli & al., 1997).

Références

Angaut, P., & Repérant, J. (1978). A light and electron microscopic study of the nucleus isthmo-opticus in the pigeon. Archives d'anatomie microscopique et de morphologie expérimentale, 67, 63-78.

Bagnoli, P., Fontanesi, G., Alesci, R., & Erichsen, J.T. (1992). Distribution of neuropeptide Y, substance P, and Choline acetyltransferase in the developing visual system of the pigeon and effects of unilateral retina removal. Journal of Comparative Neurology, 318, 392-414.

Britto, L.R.G., Keyser, K.T., Lindstrom, J.M., & Karten, H.J. (1992). Immunohistochemical localization nicotinic acetylcholine receptor subunits in the mesencephalon and diencephalon of the chick (*Gallus gallus*). Journal of Comparative Neurology, 317, 325-340.

Brüning, G. (1993). Localisation of NADPH-diaphorase in the brain of the chicken. Journal of Comparative Neurology, 334, 192-208.

Cowan, W.M. (1970). Centrifugal fibers to the avian retina. British Medical Bulletin, 26, 112-118.

Cowan, W.M. & Powel, T.P.S. (1963). Centrifugal fibres in the avian visual system. Proceedings of the Royal Society, 158, 232-252.

Cozzi, B., Massa, R., & Panzica, G.C. (1997). The NADPH-diaphorase-containing system in the brain of the budgerigar (*Melopsittacus undulatus*). Cell and Tissue Research, 287(1), 101-112

Crossland, W.J., & Hughes, C.P. (1978). Observations on the afferent and efferent connections of the avian isthmo-optic nucleus. Brain Research, 145, 239-256.

Crossland, W.J. (1979). Identification of tectal synaptic terminals in the avian isthmooptic nucleus. Dans A.M. Granda, & J.H. Maxwell (Éds), Neural Mechanisms of Behavior in the Pigeon (pp. 267-286). New York : Plenum Press.

Dawson, T.M., Bredt, D.S., Fotuhi, M., Hwang, P.M., & Snyder, S.H. (1991). Nitric oxide synthase and neuronal NADPH diaphorase are in brain and peripherical tissues. Proceeding National Academy Sciences USA., 88, 7797-7801.

Decker, K., & Reuss, S. (1994). Nitric oxide-synthesizing neurons in the hamster suprachiasmatic nucleus : a combined NOS- and NADPH-staining and retinohypthalamic tract tracing study. Brain Research, 666, 284-288.

Domenici, L., Waldvogel, H.J., Matute, C., & Streit, P. (1988). Distribution of GABA-like immunoreactive in the pigeon brain. Neuroscience, 71, 14-28.

Enna, S.J., & Gallager, J.P. (1983). Biochemical and electrophysiological characteristics of mammalian GABA receptors. In J.R. Smythies & R.J. Bradley (Éds), International Review of Neurobiology (Vol. 24) (pp. 181-212). New York.

Frumkes, T.E., Miller, R.F., Slaughter, M., & Dacheux, R.F. (1981). Physiological and pharmacological basis of GABA and glycine action on neurons of mudpuppy retina, III : Amacrine-mediate inhibitory influences on ganglion cell receptive-field organization : a model. Journal of Neuropsysiology, 45, 783-804.

Galifret, Y., Condé-Courtine, F., Repérant, J., & Serviere, J. (1971). Centrifugal control in the visual system of the pigeon. Vision Research, 3, 185-200.

Granda, R.H., & Crossland, W.J. (1989). GABA-like immunoreactivity of neurons in the chicken diencephalon and mesencephalon. Journal of Comparative Neurology, 287, 455-469.

Gonzalez-Hernandez, T., Conde-Sedin, M., & Meyer, G. (1992). Laminar distribution and morphology of NADPH-diaphorase containing neurons in the superior colliculus and underlying periaqueductal gray of the rat. Anatomy and Embryology, 189, 245-250.

Gonzalez-Hernandez, T., Perez De La Cruz, M.A., & Mantolan-Sarmiento, B. (1996). Histochemical detection of neurons that produce nitric oxide : Effect of different fixative parameters and immunoreactivity against non-neuronal NOS antisera. Journal of Histochemistry and Cytochemistry, 44 (12), 1399-1413.

Hahmann, U., & Güntürkün, O. (1992). Visual-discrimination deficits after lesions of the centrifugal visual system in pigeons (*Columba livia*). Visual Neuroscience, 9, 225-233.

Hashikawa, T., Leggio, M.G., Hattori, R., & Yui, Y. (1994). Nitric oxide synthase immunoreactivity colocalised with NADPH-diaphorase histochemistry in monkey cerebral cortex. Brain Research, 641, 341-349.

Hayes, B.P., & Webster, K.E. (1981). Neurons situated outside the isthmo-optic nucleus and projecting to the eye in adult birds. Neuroscience Letters, 26, 107-112.

Holden, A.L. (1990). Centrifugal pathways to the retina: which way does the "searchlight" point. Visual Neuroscience, 4, 493-495.

Holden, A.L. (1982). Electrophysiology of the avian retina. Progress in Retinal Research, 1, 79-196.

Holden, A.L. (1978). Centrifugal action on pigeon retinal ganglion cells. Journal of Physiology (London), 282, 8P.

Huang, Z., You, W., Haugland. R.P., Paragas, V.B., Olson, N. & Haugland, R.P. (1993). Journal of Histochemistry and Cytochemistry, 41, 313-317.

Hunt, S.P., & Brecha, H. (1984). The avian optic tectum : A synthesis of morphology and biochemistry. In H. Vanegas (Éds), Comparative Neurology of the Optic Tectum (pp.619-648). New York : Plenum Press.

Hunt, S.P., & Künzle, H. (1976a). Observations on the projections and intrinsic organization of the pigeon optic tectum : an autoradiographic study based on anterograde and retrograde, axonal and dendritic flow. Journal of Comparative Neurology, 170, 153-172.

Hunt, S.P., & Künzle, H. (1976b). Seletive uptake and transport of label within three identified neuronal systems after injection of 3H-GABA into the pigeon optic tectum : an autoradiographic and golgi study. Journal of Comparative Neurology, 170, 173-90.

Johnson, C.D., & Epstein, M.L. (1986). Monoclonal antibodies and polyvalent antiserum to chicken choline acetyltransferase. Journal of Neurochemistry., 46, 968-976.

Karten, H.J., & Hodos, W. (1967). A stereotaxic Atlas of the brain of the pigeon (*Columba livia*). Baltimore, Maryland : Johns Hopkins Press.

Kharazia, N.V., Schmidt, H.H.H.W., & Weinberg, R.J. (1994) Type I nitric oxide synthase fully accounts for NADPH-diaphorase in rat striatum, but not cortex. Neuroscience, 62, 983-987.

Mc Gill, J.I., Powel, T.P.S., & Cowan, W. M. (1966a). The retinal representation upon the optic tectum and isthmo-optic nucleus in the pigeon. Journal of Anatomy, 100, 5-33.

Mc Gill, J.I., Powel, T.P.S., & Cowan, W. M. (1996b). The organization of the projection of the centrifugal fibres to the retina in the pigeon. Journal of Anatomy, 100, 35-49.

Medina, L., & Reiner, A. (1994). Distribution of choline acetyltransferase immunoreactivity in the pigeon brain. Journal of Comparative Neurology, 342, 497-537.

Meyer, G., Banuelos-Pineda, J., Montagnese, C., Ferres-Meyer, G., & Gonzales-Hernandez, T. (1994). Laminar distribution and morphology of NADPH-diaphorase containing neurons in the optic tectum. Journal of Brain Research, 3, 445-452.

Miceli, D., Repérant, J., Bavikati, R., Rio, J.-P., & Volle, M. (1997). Brainstem afferents upon retinal projecting ishtmo-optic and ectopic neurons of the pigeon centrifugal visual system demonstrated by retrograde transneuronal transport of rhodamine ß-isothiocyanate. Visual Neuroscience, 14, 213-224.

Miceli, D., Repérant, J. Marchand, L., & Rio, J.-P. (1993). Retrograde transneuronal transport of the fluorescent dye Rhodamine β-isothiocyanate from the primary and centrifugal visual systems in the pigeon. Brain Research, 601, 289-298.

Miceli, D., Repérant, J., Rio, J.P., & Medina, M. (1995). GABA immunoreactivity in the nucleus isthmo-optic of the centrifugal

visual system in the pigeon: A light and electronmicroscopic study. Visual Neuroscience, 12, 425-441.

Miles, F.A. (1972). Centrifugal control of the avian retina.III. Effects of electrical stimulation of the isthmo-optic tract on the receptive field properties of retinal ganglion cells. Brain Research, 48, 115-129.

Mitrofanis, J. (1992). NADPH-diaphorase reactivity in the ventral and dorsal lateral geniculate nuclei of rats. Visual Neuroscience, 9, 211-216.

Montagnese, C.M., & Csillag, A. (1996). Comparative distribution of NADPH-diaphorase activity and tyrosine hydroxylase immunoreactivity in the diencephalon and mesencephalon of the domestic chicken (gallus domesticus). Anatomy and Embryology, 193, 427-439.

Morgan, I.G., Miethke, P., & Li, Z.K. (1994). Nitric oxide a transmitter of the centrifugal projection to the avian retina. Neuroscience Letters, 168, 5-7.

Mugnaini, E., & Oertel,W.H. (1985). An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD imunohistochemistry. In A. Björklund, & T. Hökfelt (Éds), Handbook of Chemical Neuroanatomy : GABA and Neuropeptides in the CNS. (Vol. IV), (pp. 437-609). Elsevier, Amsterdam.

Nickla, D.L., Gottlieb, M.D., Marin, G., Rojas, X., Britto, L.R.G., & Wallman, J. (1994). The retinal targets of centrifugal neurons and the retinal neurons projecting to the accessory optic tectum system. Visual Neuroscience, 11, 401-409.

O'Leary, D.D.M., & Cowan, W.M. (1982). Further studies on the development of the isthmo-optic nucleu with special reference

to the occurrence and fate of ectopic and ipsilaterally projecting neurons. Journal of Comparative Neurology, 212, 399-416.

Ottersen, O.P., & Storm-Mathisen, J. (1985). Neurons containing or accumulating transmitter amino acids. In A. Björklund, T. Hökfelt, & M.J. Kuhar (Éds), Handbook of Chemical Neuroanatomy : Classical Transmitters and Transmitter Receptor in the CNS, (Vol. III), (pp.141-245). Elsevier, Amsterdam.

Panzica, G.C., Arévalo, R., Sanchez, F., Alonso, J.R., Aste, N., Viglietti-Panzica, C., Aijon, J., & Vazquez, R. (1994). Topographical distribution of reduced nicotinamide adenine dinucleotide phosphate-diasphorase in the brain of the japanese quail. Journal of Comparative Neurology, 342, 97-114.

Panzica, G.C., & Garzino, A. (1994). The mesopontine NADPH-diaphorase system in the Japanese quail. Society for Neuroscience Abstracts, 20, 1560.

Panzica, G.C., Garzino, A., & Garcia Ojeda, E. (1996). Coexistence of NADPH-diaphorase and tyrosine hydroxylase in the mesencephalic catecholaminergic system of the Japanese quail. Journal of Chemical Neuroanatomy, 11, 37-47.

Pasqualotto, B.A., & Vincent, S.R. (1991). Galanin and NADPH-diaphorase coexistence in cholinergic neurons of the rat basal forebrain. Brain Research, 551, 78-86.

Pearlman, A.L., & Hughes, C.P. (1973). Functional role of efferents to the avian retina : effects of reversible cooling of the isthmo-optic nucleus. Journal of Comparative Neurology, 166, 123-132.

Reiner, A., & Karten, H.J. (1982) Laminar distribution of the cells of origin of the descending tectofugal pathway in the pigeon (*Columba livia*). Journal of Comparative Neurology, 204, 165-187.

Repérant, J., Miceli, D., Vesselkin, N.P., & Molotchnikoff, S. (1989). The centrifugal visual system of vertebrates: a century-old search reviewed. International Review of Cytology, 118, 115-171.

Rodrigo, J., Springall, D.R., Uttenthal, O., Bentura, M.L., Abadia-Molina, F., Riveros-Moreno, V., Martinez-Murillo, R., Polak, J.M., & Moncada, S. (1994). Localization of nitric oxide synthase in the adult rat brain. Philos. Trans. R. Soc. Lond. B. Biol. Sci., 345, 175-221.

Shortess, G.K., & Klose, E.F. (1977). Effects of lesions involving efferent fibers to the retina in pigeons (*Columba livia*). Physiology Behavior, 18, 409-414.

Somogyi, P. (1989). Synaptic organiztion of GABAergic neurons and $GABA_A$ receptors in the lateral geniculate nucleus and visual cortex. In D.K. Lam, & L.D. Gilbert (Éds). Neural Mechanisms of Visual Perception (pp.35-92). Texas : Portfolio Pub.

Sorenson, E.M., & Chiappinelli, V.A. (1992) Localization of 3H-nicotine I-kappa-bungarotoxin, and I-alpha- bungarotoxin binding to nicotinic sites in the chicken forebrain and midbrain. Journal of Comparative Neurology, 323, 1-12.

Sorenson, E.M., Parkinson, D., Dahl,. J.L., & Chiappinelli, V.A. (1989). Immunohistochemical localization of choline acetyltransferase in the chicken mesencephalon. Journal of Comparative Neurology, 281, 641-657.

Spessert, R., & Layes, E. (1994). Fixation conditions affect the intensity but not the pattern of NADPH-diaphorase staining as a marker for neuronal nitric oxide synthase in rat olfactory bulb. The Journal of Histochemistry and Cytochemistry, 42, 1309-1315.

Spessert, R., Wohlgemuth, C., Reuss, S., & Layes, E. (1994). NADPH-diaphorase activity of nitric oxide synthase in the olfactory bulb : co-factor specificity and characterization regarding the interelation to no formation. The Journal of Histochemistry and Cytochemistry, 42, 569-575.

Traub, R.J., Solodkin, A., Meller, S.T., & Gebhart, G.F. (1994). Spinal cord NADPH-diaphorase histochemical staining but not nitric oxide synthase immunoreactivity increases following carrageenan- produced hindpaw inflammation in the rat. Brain Research., 668, 204-210.

Uchiyama, H. (1989). Centrifugal pathways to the retina: influence of tectum. Visual Neuroscience, 3, 183-206.

Uchiyama, H., & Watanabe, M. (1985). Tectal neurons projecting to the isthmo-optic nucleus in the Japanese quail. Neuroscience Letters, 58, 381-385.

Valtschanoff, J.G., Weinberg, R.J., Kharazia, V.N., Nakane, M., & Schmidt, H.H.H.W. (1993). Neurons in rat hippocampus that synthesize nitric oxide. Journal of Comparative Neurology, 331, 111-121.

Veenman, C.L., & Reiner, A. (1994). The distribution of GABA-containing perikarya, fibres, and terminals in the forebrain and midbrain of pigeons, with particular reference to the basal ganglia and its projection targets. Journal of Comparative Neurology, 339, 209-250.

Vincent, S.R., Satoh, K., Armstrong, D.M., & Fibiger, H.C. (1983). NADPH-diaphorase : a selective histochemical marker for the cholinergic neurons of the pontine reticular formation. Neuroscience Letters, 43, 31-36.

Vizzard, M.A., Erdman, S.L., Roppolo, J.R., Förstermann, U., & de Groat, W.C. (1994). Differential localization of neuronal nitric oxide synthase immunoreactivity and NADPH-diaphorase activity in the cat spinal cord. Cell Tissue Research., 278, 299-309.

Ward, R., Repérant, J., & Miceli, D. (1991). The centrifugal visual system: what can comparative studies tell us about its evolution and possible function? In P. Bagnoli, & W. Hodos (Éds), The Changing Visual System (pp.61-76). New York: Plenum Press.

Weidner, C., Desroches, A.M., Repérant, J., Kirpitchnikova, E., & Miceli, D. (1989). Comparative study of the centrifugal visual system in the pigmented and glaucomatous albino quail. Biological Structures and Morphology, 2, 89-93.

Weidner, C., Repérant, J., Desroches, A.-M., Miceli, D., & Vesselkin, N.P. (1987). Nuclear origin of the centrifugal visual pathway in birds of prey. Brain Research, 436, 153-160.

Wolf-Oberhollenzer, F. (1987). A study of the centrifugal projections to the pigeon retina using two fluorescent markers. Neuroscience Letters, 73, 16-20.

Woodson, W., Reiner, A., Anderson, K., & Karten, H.J. (1991). Distribution, laminar location, and morphology of tectal neurons projecting to the isthmo-optic nucleus and the nucleus isthmi, pars parvocellularis in the pigeon (*Columbia Livia)* and chick (*Gallus domesticus*), a retrograde labelling study. Journal of Comparative Neurology, 305, 470-488.

Woodson, W., Shimizu, T., Wild, J.M., Schimke, J., Cox, K., & Karten, H.J. (1995). Centrifugal projections upon the retina : An anterograde tracing study in the pigeon (*Columba livia*). Journal of Comparative Neurology, 362, 489-509.

Partie II - Manuscrit en anglais

ChAT, NOS and GABA immunoreactivity in the nucleus isthmo-opticus and ectopic neurons of the pigeon centrifugal visual system and their afferent brain-stem projections

Christine Bertrand [1], Dom Miceli [1], Jacques Repérant [1,2] and Monique Médina [2]

[1] Laboratoire de Neuropsychologie Expérimentale et Comparée, Universté du Québec, Trois-Rivières, Canada and [2] INSERM U-106, Hôpital de la Salpétrière, Paris, France

For correspondence: Dr. Dom Miceli,
Département de psychologie,
Université du Québec,
C.P. 500,
Trois-Rivières, Québec,
G9A 5H7, Canada

Telephone NO: (819) 376-5085

Running tittle: ChAT, NOS and GABA immunoreactivity in the pigeon CVS.

Manuscript: 45 Pages; 4 Figures

ChAT, NOS and GABA immunoreactivity in the nucleus isthmo-opticus and ectopic neurons of the pigeon centrifugal visual system and their afferent brain-stem projections

Summary The immunohistofluorescent technique was used to investigate ChAT, NOS and GABA immunoreactivity (-ir) of neurons in the nucleus isthmo-opticus (NIO) and in ectopic neurons (EN) of the pigeon centrifugal visual system (CVS) as well as in their afferents stemming from cells in layers 9/10 of the optic tectum (OT), area ventralis-Tsai (AVT), zona peri-NIII (Zp.NIII) and in the pontine reticular formation (PRF) including the zona peri-n.NVI (Zp.n.NVI). This technique was combined with the retrograde fluorescent labelling of the centrifugal cells after intraocular injection of Rhodamine ß-isothiocyanate (RITC) and of their afferent cells via either 1) retrograde transport of Fluoro-gold (FG) injected into the NIO/EN or 2) retrograde transneuronal transport of RITC injected intraocularly. The double-labelling results showed that the majority of the centrifugal cells of the NIO and many EN were immunoreactive to ChAT and NOS, but not to GABA. Numerous tectal projection neurons in layers 9/10 were NOS-ir, a lesser number ChAT-ir and none GABA-ir. Extra-tectal afferent neurons in ZpNIII and AVT were ChAT-ir and NOS-ir, in the Zp.n.NVI/PRF region were NOS-ir and all were GABA-immunonegative. The results indicate an excitatory cholinergic influence of centrifugal NIO cells and the EN upon their retinal target neurons and of some brain-stem afferent systems upon the centrifugal neurons and that these pathways also utilise nitric oxide. Interactions between the different neurochemical and

inhibitory/excitatory components of the CVS and of the brain-stem visual and non visual afferent systems are discussed in relation to the possible role this system plays in modulating visual attention.

Key words: Centrifugal visual system; GABA; ChAT; NOS; Pigeon.

INTRODUCTION

A centrifugal visual system (CVS), defined by its afferent brain input to the retina, has been described in all vertebrate groups including man. Although numerous studies have been carried out on the CVS, its functional role has as yet not been clearly elucidated, which has given rise to rather diverse hypotheses (for reviews, see Repérant et al., 1989; Uchiyama, 1989; Ward et al., 1991). The avian CVS is particularly well suited for anatomical studies because of its large retinopetal projection from the mesencephalic tegmentum stemming from the highly developed and differentiated nucleus isthmo-opticus, and from the associated, rather dispersed ectopic neurons (EN) (Cowan, 1970; Hayes & Webster, 1981). For many years, the only well-established afferent input to the NIO was the major projection from the optic tectum (OT), originating in the layers 9/10, which constitutes a relay of the retino-tectal-NIO-retinal feedback loop, with the NIO as the key structure (McGill et al., 1966a, 1966b). For a long time, the data provided in the literature concerning other afferents to the NIO was fragmentary and often contradictory (see Repérant et al., 1989; Ward et al., 1991). However, more recently, through the use of a retrograde transneuronal transport technique using the fluorescent dye Rhodamine B-isothiocyanate : RITC (Miceli et al., 1993), it has been possible to identify several extra-tectal brain-stem structures which project upon the centrifugal visual neurons (area ventralis-Tsai: AVT, zona peri-NIII: Zp.NIII and the

pontine reticular formation: PRF including the zona peri-NVI: Zp.n.NVI, Miceli et al., 1997).

There is very little, if any, information available regarding the neurochemical properties of the CVS and its associated input systems. Veenman & Reiner (1994) reported that a few NIO cells were immunoreactive for glutamic acid decarboxylase, the synthetic enzyme of Gamma-aminobutyric acid (GABA). However, it has been demonstrated that the GABA-immunoreactive (-ir) cells are not centrifugal, but are instead interneurons situated within the neuropilar zone of the nucleus (Miceli et al., 1995). Moreover, numerous GABAergic terminals have been described in the NIO, as well as GABA-ir myelinated axons, suggesting that the GABAergic input upon centrifugal neurons is also extra nuclear in origin (Miceli et al., 1995).

Since almost half the axon terminals within the NIO are GABA-ir (Miceli et al. 1995), and since the NIO mainly receives projections from the optic tectum (Cowan & Powell, 1963; McGill et al., 1966a, b; Crossland & Hughes, 1978; Uchiyama, 1989; Woodson et al., 1991; Miceli et al., 1993), it is possible that the GABAergic input originates in tectal layers 9/10. Electron microscopic studies have also shown that the GABAergic terminals of the NIO exhibit synaptic profiles characterised by round-shaped vesicles (Miceli et al., 1995), and that this same terminal type is a characteristic feature of tecto-NIO fibers

(Angaut & Repérant, 1978; Crossland, 1979). Furthermore, Hunt and Künzle (1976b) observed that cells situated within the afferent layers 9/10 of the pigeon OT express GABA. The presence of GABAergic synaptic terminals within the NIO containing pleomorphic rather than rounded synaptic vesicles suggests that these might also be of extra-tectal origin.

Bagnoli et al. (1992) provided evidence of centrifugal neurons in the NIO and within the ectopic region which were immunoreactive for choline acetyltransferase (ChAT), the synthetic enzyme of acetylcholine. Medina & Reiner (1994) also suggested the presence of ChAT within the ectopic and NIO neurons, although these exhibited a very weak immunoreactivity for ChAT, and were found only in some pigeons where the brains were less fixed. In contrast, no ChAT-ir cells have been observed in the NIO of the chicken (Sorenson et al., 1989).

Various data indicate that the NIO also receives cholinergic input. A dense cholinergic innervation has been observed in the pigeon NIO (Medina & Reiner, 1994), as well as high levels of nicotinic receptors found upon the perikarya of most of the cells and within the neuropile of the NIO of the chicken (Britto et al., 1992; Sorenson & Chiappinelli, 1992). There is little information available regarding the origin of these cholinergic projections. Numerous ChAT-ir neurons have been described in layers 9/10 of the OT, although

mainly in layer 10, in the pigeon (Bagnoli et al., 1992; Medina & Reiner, 1994) and in the chicken (Sorenson et al., 1989). ChAT-ir neurons have also been reported within other brain-stem structures which project upon the visual centrifugal neurons: Zp.NIII of the pigeon (also referred to as the ventral tegmental area, Medina & Reiner, 1994), as well as in the reticularis pontinus oralis (RPO) of the chicken (Sorenson et al., 1989) and pigeon (Medina & Reiner, 1994). Lastly, ChAT immunoreactivity has been demonstrated within the motor neurons of the n.NVI, as well as within a population of small neurons situated ventrolaterally to the latter (Medina & Reiner, 1994), which appears to correspond to the region shown to provide input to the NIO, referred to as Zp.n.NVI (Miceli et al., 1993).

Different studies on the distribution of nitric oxide (NO) within the brain, using NADPH-d histochemistry, whose activity reveals nitric oxide synthase (NOS), the synthetic enzyme of NO, have demonstrated its presence within the NIO of the chicken (Brüning, 1993; Montagnese & Csillag, 1996) and the budgie *Melopsittacus undulatus* (Cozzi et al., 1997), but no such activity in the NIO was mentioned in a study in the quail (Panzica et al., 1994). Moreover, it has been shown that the isthmo-optic terminals in the chicken contain high levels of NADPH-d activity, suggesting that they might utilise NO as a chemical messenger (Morgan et al., 1994). Brüning (1993) noted the presence of an intensely labelled neuropile within

the NIO, which suggests that structures using NO project upon the NIO.

Some brain-stem structures seem to be better candidates for the origin of NO projections upon the centrifugal neurons. Numerous cells labelled with the NADPH-d enzyme have been observed in the chicken OT, including layer 10 (Brüning, 1993; Meyer et al., 1994). NADPH-d-ir neurons have also been described in the AVT of the chicken (Brüning, 1993; Montagnese & Csillag, 1996), quail (Panzica et al., 1994, 1996) and budgie (Cozzi et al., 1997).

In the present study, the immunohistofluorescent technique was used to investigate ChAT, NOS and GABA immunoreactivity of neurons in the NIO and in ectopic neurons of the pigeon centrifugal visual system (CVS) as well as in afferents upon the latter stemming from cells in layers 9/10 of the OT, AVT, Zp.NIII and in the PRF including Zp.n.NVI. This technique was combined with the retrograde axonal labelling of the centrifugal cells following intraocular injection of the fluorescent dye RITC and of their afferent cells via either 1) retrograde transport of the fluorescent tracer Fluoro-gold (FG) after its injection into the NIO/EN or 2) transneuronal transport of RITC following its intraocular injection.

METHODOLOGY

Twelve pigeons anesthetized with ketamine/Rompun (50/20 mg/kg respectively) received an intraocular injection of a 0.4% W/V solution of kainic acid (KA) immediately followed by a second intraocular injection of 50ul of 10% W/V of the fluorochrome RITC (10%) containing 2% DMSO. In these experiments, the eye injections were performed using a 1-ml capacity syringe (26G/2 diameter needle tip) and a Hamilton 50 ul syringe for RITC and KA respectively). In another set of experiments, six pigeons received a unilateral stereotaxic injection (anterior coordinates: 1.75 mm, lateral: 2.5 mm and depth: 8.5 mm (Karten & Hodos, 1967)) of a 0.1 to 0.2 ul solution of 5% (W/V) of the tracer Fluoro-Gold (FG) into the NIO using micropipettes having tip diameters of 50 - 65 um, which were adapted to 1.0 ul capacity Hamilton syringes.

Following survival times ranging from 18 to 23 days for the intraocular RITC injections and from 7 to 8 days for the FG injections into the NIO, the pigeons were perfused transcardially, with 0.9% saline followed by 4% paraformaldehyde/0.2% glutaraldehyde in a 0.1M phosphate buffer, pH 7.3, and finally with the same fixative containing 30% sucrose. The brains were removed and kept in 30% sucrose in a 0.1M phosphate buffer, pH 7.3, at 4°C for 2 days, and were subsequently sectioned in the frontal plane at a thickness of

20-40 um on a freezing microtome. The sections were immediately mounted onto gelatin-coated slides and stored at -20°C.

For the double labelling immunohistofluorescent procedure, the sections were recovered in 0.02M PBS, pH 7.4, at 4°C, preincubated with 3% normal goat serum (NGS) and incubated either overnight in a GABA antibody (1/1000, Incstar), 68 to 72 hours in antibodies for ChAT (1/1000, Johnson & Epstein, 1986) and NOS (1/800, Cedarlane). The anti-rabbit antibodies were diluted in 0.02M PBS, pH 7.4, and the latter solution was used for rinsing prior to incubation with the secondary antibody. The latter, Goat anti-rabbit (GAR) FITC (2 hours) conjugated antibody, was used for those sections from experiments where the pigeons had received an intraocular injection of RITC. A GAR-TRITC secondary antibody was used in those experiments where FG had been injected into the NIO. In these experiments using RITC as an axonal tracer, brain sections were processed without Triton X-100 in order to minimise the diffusion of the tracer out of the cell bodies.

Following double labelling immunohistofluorescence, the sections were mounted onto gelatin-coated slides and observed under a Leitz Ploemopack fluorescence microscope adapted with filter mirrors N2 (550 nm), I2 (470 nm) and A (360 nm) for identifying RITC, FITC and FG respectively.

The intraocular injection of RITC produced a reliable transneuronal labelling of afferent structures to the NIO. However, with the subsequent immunohistofluorescent labelling, the tracer was observed to diffuse within the different preparations, and the intensity of the somatic labelling in some cells was reduced, and in some cases disappeared altogether. In contrast, the direct retrograde labelling produced by the NIO injection of FG was far more resistant to diffusion during the immunohistofluorescent procedure. Unfortunately, an accurate injection into the NIO was difficult to perform, given the small size of the nucleus and its location deep within the brain beneath the cerebellum. Consequently, the precise identification of NIO afferent neurons was less precise due to the diffusion at the injection site of the FG tracer into neighbouring structures.

The *Elf*^{TM} *AP immunohistochemistry* procedure (ELF, Molecular Probes Inc.) was also used as a complementary method in the double labelling immumohistofluorescent procedure. With this technique, the fluorescent labelling was of higher intensity providing greater resolution and was more resistant to fading (Huang et al., 1993).

In all immunohistofluorescent experiments, control sections were also processed according to the same procedures described previously, except that the primary antibody was omitted. Under

these conditions, a complete absence of labelling was observed in all of the brain structures investigated.

RESULTS

Retrograde labeling following intraocular injections of RITC and NIO injections of FG

Following an intraocular injection of the RITC tracer, strongly labelled cells were found contralaterally in the NIO and the EN region. The RITC labelled cells in the NIO were round or slightly oval in shape whereas, in the EN region, they were more heterogeneous in shape, ranging from triangular to oval. Occasionally, some large multipolar cells were also labelled in the ectopic region.

After retrograde transneuronal transport of RITC from the eye, labelled cell bodies were found in the contralateral OT. Such cells were round to oval-shaped and formed a distinct narrow band of labelling at the layers 9/10 border region. Elsewhere, RITC neurons were observed contralaterally to the intraocular injection within the Zp.NIII. This region, generally composed of multipolar neurons, is situated laterally and medially to the oculomotor nerve where it exits at the base of the brain and dorsally extends to the ventral limits of the red nucleus. Lateral to the Zp.NIII, a second group of labelled neurons was observed within the AVT. They were of small

diameter, bipolar and topographically segregated from the labelled cells in Zp.NIII. Lastly, labelled RITC cells were observed widely distributed within the PRF and within a restricted dorsomedial region corresponding to Zp.n.NVI. The latter is mainly composed of large round-shaped cells, but also contains smaller triangular-shaped neurons. Among the various structures studied, this was the only population of neurons which was labelled bilaterally with RITC.

The intensity of the various afferent cells labelled transneuronally with RITC was observed to weaken after processing with FITC immunohistofluorescence, whereas the intensity of the first-order RITC labelling in the NIO and EN region remained relatively high. When double-labelling with the ELF technique, the RITC fluorescence in the NIO and ectopic neurons was maintained, while the transneuronal labelling in the afferent brain-stem neurons disappeared completely.

The FG tracer injections into the NIO labelled afferent cells within the layers 9/10 of the OT, Zp.NIII, AVT and Zp.n.NVI. The FG injection sites mainly involved the lateral portion of the NIO and the EN region, and dorsally, invaded portions of the ventrolateral cerebellum. Regardless of the fact that, at the NIO injection site, the FG tracer was found to diffuse into numerous neighbouring structures which resulted in very widespread somatic labelling within the brain, the distribution of FG labelled afferent neurons

within any given portion of layers 9/10 of the OT was always comparable to that observed after intraocular injection and transneuronal transport of RITC. Thus, the double-label studies using the FG axonal tracing method were only performed in the OT and the intensity of FG fluorescence was remarkably well maintained throughout the double-labelling procedure.

Immunoreactivity in the NIO and the EN

The results demonstrated the presence of ChAT-ir within the NIO neurons. With the double labelling procedure using the retrograde transneuronal transport of the RITC tracer (Fig. 1A) injected intraocularly, the quasi totality of the centrifugal NIO neurons also demonstrated ChAT (Fig. 1B) immunohistofluorescence (FITC). Many ChAT-ir cells were also observed within the EN region, some of which were centrifugal since they were double-labelled with RITC and FITC, respectively (Figs. 1C-D). Moreover, the ectopic region also contained single-labelled FITC (ChAT-ir) or RITC (centrifugal) neurons of similar morphology (Fig. 1D). In the NIO, the results using the single labelling ELF method confirmed the observations made with the double labelling (RITC-FITC) technique (Fig. 1E). However, with the ELF technique far fewer cells were labelled with the ChAT antibody and were localised mainly within the peripheral centrifugal cell layers of the nucleus.

A primary feature of cells observed to be immunoreactive for NOS was the great variability in the intensity of the labelling. Moderate labelling intensities were observed within the NIO and EN, in contrast to other brain structures such as the substantia grisia centralis (GCt), locus coeruleus (LoC) and the Zp.NIII, where the labelling was much more intense. Within the NIO, the NOS immunoreactivity was observed in the vast majority of the cells in the centrifugal cell laminae of the nucleus (Fig. 1F). NOS-ir neurons, labelled with FITC, were also identified within the ectopic region, some of which were centrifugal and double-labelled with RITC (Figs. 1G-H). Other smaller cells which were solely NOS-ir were found dispersed around these EN (Fig. 1H).

No GABA-ir centrifugal cells were found in the NIO and EN region. Nevertheless, some small GABA-ir cells which were not labelled with RITC were found within the NIO and located within the neuropilar zone of the nucleus. GABA-ir neurons were also observed within the ectopic region, but no double centrifugal cell labelling was detected.

Immunoreactivity of afferent cells upon the centrifugal cells

Optic Tectum

ChAT immunoreactivity was restricted to cell bodies within various layers of the OT, but was found mainly within layer 10 and to a

lesser degree layers 9 and 11. Some of the ChAT-ir cells in layers 9/10 were also labelled transneuronally with RITC (Figs. 2A-B). Most of the cells labelled in layers 9/10, were solely ChAT-ir and, in some cases, displayed a similar morphology to that of the NIO projecting neurons. Other smaller dispersed ChAT-ir neurons were found in regions immediately surrounding the RITC-positive cells.

The OT also contained a large number of NOS-ir neurons, and the most intensely labelled were localised in layers 13 and 14. Moderate and weak NOS immunoreactivity was found within cells dispersed respectively within layers 11/12 and 9/10. Some NOS-ir cells in layers 9/10 were double labelled with the RITC transneuronal tracer (Figs. 2C-D).

GABA-ir neurons were found to be distributed extensively throughout the different tectal layers and particularly within layers 2 - 4 and 10. Fewer GABA-ir cells were concentrated in the other layers, including layer 9. None of the GABA-ir cells in layers 9/10 were RITC double-labelled. The GABA-ir cells were round in shape, generally small in size, and distributed around the afferent tectal neurons (Figs. 2E-F). These same results were obtained in brain sections processed with the ELF technique following the injection of FG into the NIO. The FG labelled cells within layers 9/10 were often multipolar and of large diameter, and were surrounded by numerous smaller GABA-ir neurons.

Zp.NIII and AVT

ChAT-ir cells were identified within the region of the Zp.NIII and AVT. In Zp.NIII, the majority of the cells labelled with RITC were mainly multipolar and ChAT-ir double-labelled (Figs. 3A-B). In the adjacent AVT, far fewer cells were labelled transneuronally with RITC. They were small, oval or fusiform in shape and were double labelled with FITC (ChAT-ir). Within these two brain regions, the FITC-labelled ChAT-ir cells were more numerous than the RITC afferent neurons (Fig. 3B).

Several NOS-ir neurons were also observed within the Zp.NIII and in the AVT. Most of the multipolar neurons in the Zp.NIII labelled with the RITC tracer exhibited a strong NOS immunoreactivity. A small number of oval and fusiform-shaped cells labelled with RITC additionally displayed a weaker NOS immunoreactivity within the AVT (Figs. 3C-D). Lastly, GABA immunoreactivity was absent in the Zp.NIII and AVT neurons.

PRF (Zp.n.NVI)

Cells displaying a very intense ChAT immunoreactivity were observed within the PRF and in the vicinity of the Zp.n.NVI. However, these neuronal populations were topographically distinct

from that of the RITC afferent cells. None of the latter were double-labelled with the ChAT antibody.

Weak to moderate NOS immunoreactivity was observed in numerous cells afferent to the NIO (Figs. 3E-F). Such NOS-ir cells were generally round in shape and localised very close to the abducens nerve (NVI). In addition, some cells labelled weakly and exclusively with FITC (NOS-ir) were identified around neurons labelled with the RITC tracer. No GABAergic cells were found in the PRF region, including the Zp.n.NVI, which contained RITC-labeled afferent neurons.

DISCUSSION

Labelling of centrifugal cells within the NIO/EN and their afferents

Intensely labelled centrifugal neurons were found within both the NIO and the ectopic region following the intraocular injection of the RITC tracer. This is consistent with the results obtained in previous studies using different axonal tracers transported retrogradely following intraocular injections (Hayes & Webster, 1981; Wolf-Oberhollenzer, 1987; Weidner et al., 1987, 1989; Miceli et al., 1993, 1997). The transneuronal label was comparatively weaker and its distribution within layers 9/10 of the OT was similar to that

described in earlier studies using the same transneuronal labelling method (Miceli et al., 1993, 1997). Similarly, the tectal labelling in layers 9/10 obtained after FG injections directly into the NIO resembled that reported previously in studies using retrograde tracers injected directly into the NIO of the quail (Uchiyama & Watanabe, 1985) and pigeon (Woodson et al., 1991). As previously reported, extratectal afferents upon the centrifugal cells were demonstrated stemming from the Zp.NIII, the AVT and the PRF, including the Zp.n.NVI (Miceli et al. 1997).

ChAT immunoreactivity

The overall distribution of ChAT-ir cells within the NIO, the EN region and in some of the structures which project upon the centrifugal neurons is similar to that previously described in the brain of the pigeon (Bagnoli et al., 1992; Medina & Reiner, 1994) and chicken, and more specifically for the OT (Sorenson et al., 1989). The results of the present study demonstrated centrifugal ChAT-ir cells within the NIO and the ectopic region, especially when using the double labelling immunohistofluorescent technique. With this method, the distribution of ChAT cells was extensive throughout the NIO, whereas the ELF technique provided a higher level of differentiation, with ChAT-ir cells being observed mainly localised peripherally within the

nucleus. Consequently, it is possible that the double labelling immunohistofluorescent technique provides an overestimation of the number of centrifugal ChAT-ir neurons. The results of the present study partly confirm the results reported by Bagnoli et al. (1992), showing the presence of a restricted population of cholinergic cells within the medial pole of the NIO in the pigeon, as well as numerous ectopic ChAT-ir neurons. However, no ChAT immunoreactivity has been demonstrated in the NIO of the chicken (Sorenson et al., 1989). Medina & Reiner (1994) indicated that the ChAT-ir cell bodies observed around the NIO correspond in terms of size and location to the ectopic neurons described by Hayes & Webster (1981). However, the extremely weakly labelled ChAT-ir cells of the NIO were found only in some pigeons, and especially in those birds where the brains had been less well fixed (Medina & Reiner, 1994).

In addition to the differences observed in the NIO, a number of other distinctions between the ChAT immunoreactivity of other structures in the pigeon and chicken brain are worth noting. In various studies using the same anti-chicken antibody (Johnson & Epstein, 1986), ChAT-ir neurons were observed at the level of the thalamic visual nuclei in the pigeon (Güntürkun & Karten, 1991; Bagnoli et al., 1992; Medina & Reiner, 1994), but not in the chicken (Sorenson et al., 1989). These different observations suggest that the cholinergic nature of the NIO neurons should be considered with care. Beyond methodological differences associated with tissue

fixation, it is possible that similar (ChAT-like) antigenic sites exist specifically in the pigeon but not in the chicken (Medina & Reiner, 1994).

The present double-labelling results showed that some cells in layers 9/10 of the OT, afferent to the NIO, were also ChAT-ir. This is consistent with the high level of ChAT immunoreactivity found in numerous tectal cells in layer 10 in the pigeon (Bagnoli et al., 1992; Medina & Reiner, 1994) and the chicken (Sorenson et al., 1989), and in some layer 9 neurons in the pigeon OT (Medina & Reiner, 1994). These latter authors suggested that the tectal projections to the isthmic parvocellular nucleus (Ipc) and/or the ventral lateral geniculate (GLv) originate in these ChAT-ir cells (Hunt & Künzle, 1976a, b; Reiner & Karten, 1982; Hunt & Brecha, 1984; Woodson et al., 1991). Since our results indicate that some of the cells in layers 9/10 afferent to the NIO are cholinergic, the ChAT-ir cells also observed surrounding the afferent neurons, or the latter themselves by way of collaterals, may correspond to the ChAT-ir cells projecting to the Ipc and/or GLv.

Our data suggest that many of the NIO afferent cells located in the AVT, and particularly in the Zp-NIII, are also ChAT-ir. Previous immunohistochemical studies have shown the presence of ChAT-ir neurons in the ventral tegmental area in the pigeon (Medina & Reiner, 1994) that seems to correspond to the AVT and Zp-NIII.

High levels of nicotinic receptors were found on the perikarya of most of the neurons and in the neuropil of the chicken NIO (Britto et al., 1992; Sorenson & Chiappinelli, 1992). The dense cholinergic innervation previously shown in the pigeon NIO (Medina & Reiner, 1994) could originate both tectally and extra-tectally. The discovery of ChAT-ir cells afferent to the NIO suggests a multiple cholinergic influence on the centrifugal structure from layers 9/10 of the OT and from the Zp-NIII and the AVT.

The cells afferent to the centrifugal cells located in the Zp-n.NVI exhibited no ChAT immunoreactivity, although a population of highly intense ChAT-ir cells was found laterally to this region. These latter cells may correspond to the group of small ChAT-ir neurons described in the PRF by Medina & Reiner (1994).

NOS Immunoreactivity

The distribution of NOS immunoreactivity in the NIO and EN region, and in certain afferent structures, corresponds to the description obtained from various NADPH-d histochemical studies on NO distribution in the brains of different birds (chicken: Brüning, 1993; Morgan et al., 1994; Montagnese & Csillag, 1996; pigeon: Meyer et al., 1994; quail: Panzica et al., 1994, 1996; budgerigar *Melopsittacus undulatus*: Cozzi et al., 1997). The present study is the first to use

an NOS antibody to determine its immunoreactivity in the avian brain. Although some authors have described identical distributions for NADPH-d activity and NOS immunoreactivity (Dawson et al., 1991; Valtschanoff et al., 1993, Decker & Reuss, 1994; Hashikawa et al., 1994), others have observed that these two labelling techniques, reflecting NO activity, do not necessarily identify the same neuronal populations (Kharazia et al., 1994; Spessert & Layes, 1994; Spessert et al., 1994; Traub et al., 1994; Vizzard et al., 1994). It has been suggested that NADPH-d activity is positive only in neurons that actively synthetize NO, while immunohistochemical detection identifies all of the neurons containing NOS (Rodrigo et al., 1994). Another advantage to the latter approach is that NOS seems to be influenced less by variations in tissue fixation than the NADPH-d histochemical method (Gonzalez-Hernandez et al., 1996).

Our results revealed the presence of NOS-ir centrifugal neurons in the pigeon NIO. This is consistent with data obtained using the NADPH-d histochemical method in the chicken (Brüning, 1993; Montagnese & Csillag, 1996) and the budgerigar *Melopsittacus undulatus* (Cozzi et al., 1997). It has also been shown that the NADPH-d elements in the retina degenerate following destruction of the NIO or the isthmo-optic tract (Morgan et al., 1994). NADPH-d has not been reported in the quail NIO (Panzica et al., 1994). Our study has shown, for the first time, that some of the centrifugal cells in the EN region also exhibit NOS immunoreactivity.

The study has also revealed the presence of NOS-ir cells in the pigeon OT, including some in layers 9/10. A large number of afferent neurons in these layers also react positively to the NOS antibody. These NOS-ir afferent cells may correspond to the neurons labelled by the NADPH-d enzyme previously identified in layers 9 and 10 of the OT in the chicken (Brüning, 1993) and in the pigeon (Meyer et al., 1994). It has been suggested that these latter neurons may project onto the Ipc (Meyer et al., 1994), but our double-labelling results show instead that some cells in the same region project upon the NIO. Given that only a small percentage of the NOS-ir cells in layers 9/10 project onto the centrifugal cells, it is possible that these same afferent cells by way of collateral axonal branching, or the NOS-ir cells in the broader regions of layer 10 are the source of the tecto-Ipc projections. Nevertheless, most of the afferent neurons in layers 9/10 of the OT which project upon the centrifugal cells utilize NO as a chemical messenger.

Among the extra-tectal structures studied, NOS-ir afferent cells were found in the AVT, and particularly in the Zp-NIII, where a higher degree of somatic immunoreactivity was observed. These data are consistent with the previous description of neurons labelled with NADPH-d in the corresponding regions in the chicken (Brüning, 1993; Montagnese & Csillag, 1996), quail (Panzica et al., 1994, 1996) and budgerigar *Melopsittacus undulatus* (Cozzi et al., 1997). With

respect to the PRF, including the Zp-n-NVI, some NOS-ir cells were identified which project to the centrifugal neurons. These latter may correspond to the cells found to be immunoreactive to NADPH-d to varying degrees throughout the PRF in the quail (Panzica et al., 1994) and to a high degree in the same region in the budgerigar *Melopsittacus undulatus* (Cozzi et al., 1997). It is therefore possible that the different afferent structures sampled in the present study are the source of the NADPH-d innervation described in the NIO (Brüning, 1993).

GABA Immunoreactivity

The distribution of GABA-ergic cells in the NIO and EN region, and in the afferent structures sampled, is similar to that described in the brain of the pigeon (Domenici et al., 1988; Miceli et al., 1995) and chicken (Granda et al., 1989). The present study confirmed that none of the NIO centrifugal cells were GABAergic (Miceli et al., 1995), and the results showed no GABA immunoreactivity in the EN. GABA-ir interneurons were found only in the interpilar regions of the NIO and this is consistent with the results obtained in a previous light and electron microscopic study (Miceli et al., 1995).

Our results also showed that none of the tectal cells afferent to the centrifugal cells were GABA-ir. Although some GABA-ir cells were similar in diameter to the latter, most displayed a smaller

somata, and a proportionally larger nucleus surrounded by a narrow rim of cytoplasm, suggesting that they are in fact interneurons (Repérant et al., 1981). Various studies have suggested that GABA could be used by neurons in layer 10 of the OT projecting to the GLv (Hunt & Künzle, 1976a; Veenman & Reiner, 1994) and/or the tecto-Ipc (Hunt & Künzle, 1976b; Hunt et al., 1977; Veenman & Reiner, 1994). These tecto-Ipc and tecto-Glv neurons may correspond, at least in part, to the many GABA-ir neurons surrounding the cells afferent to the centrifugal cells.

Given that the terminals in the NIO, containing round synaptic vesicles, degenerate following lesions of the OT (Crossland, 1979), Miceli et al. (1995) suggested that the various categories of GABAergic endings with the same synaptic vesicle profiles might originate in the tectum. This has not been confirmed by the present results, since the afferent cells in the OT were GABA-immunonegative. The possibility of extra-tectal GABAergic projections has also been suggested, based on the fact that GABAergic terminals containing pleomorphic synaptic vesicles were also found in the NIO (Miceli et al., 1995). However, the results of the present study exclude the existence of GABAergic afferent projection cells in the Zp-NIII, AVT and PRF, including Zp-n-NVI. Other evidence for the presence of GABAergic afferents to the NIO is the finding of myelinated GABA-ir axons within the NIO (Miceli et al., 1995). Consequently, their origin may be related to the other afferents to

the centrifugal cells that were not sampled in the present study, i.e. the broader region of the mesencephalic reticular formation (Miceli et al. 1997). However, the afferents may also stem from cells which project, not directly upon the centrifugal neurons, but instead upon the GABAergic interneurons within the NIO. The transneuronal transport of RITC from the eye may be specifically transsynaptic and thus the second-order uptake of the tracer occurs solely through those terminals making contact with the centrifugal neurons (Miceli et al, 1993). Consequently, afferent neurons projecting to the GABAergic interneurons within the NIO may not have been labelled with the RITC transneuronal labelling method.

NO and its colocalization

The results of the present study concerning NOS and ChAT immunoreactivity in both centrifugal cells and their afferents indicate strong similarities in their topographical distribution and morphological characteristics. Descriptions of NADPH-d-ir cells in the brains of different birds (quail: Panzica et al., 1994, 1996; chicken: Brüning, 1993; Montagnese & Csillag, 1996; pigeon: Meyer et al., 1994; budgerigar *Melopsittacus undulatus*: Cozzi et al., 1997) and of ChAT-ir cells in the pigeon (Bagnoli et al., 1992; Medina & Reiner, 1994) and chicken (Sorenson et al, 1989) also suggest a correspondence between the two in some regions of the brain. Both NOS and ChAT have already been found in several regions of the

brains of mammals, including the mesopontine region (Vincent et al., 1983; Pasqualotto & Vincent, 1991), and in the quail LoC (Panzica & Garzino, 1994). Some investigators have suggested that acetylcholine is a potential candidate for colocalization with NADPH-d in the avian brain (Brüning, 1993; Panzica et al., 1994; Montagnese & Csillag, 1996). Brüning (1993) proposed that several nuclei in the avian brain-stem, including the NIO, contained both NADPH-d and ChAT. Our immunohistofluorescence results revealed that centrifugal cells in the NIO were ChAT-ir and that the vast majority of neurons located in the centrifugal cell layers were also NOS-ir. It is therefore highly probable that both NOS and ChAT are colocalized in such neurons. Moreover, based on the distribution and morphology of the ChAT-ir and NOS-ir cells, both of the neuroactive substances may be present in the centrifugal cells of the ectopic region and especially in the Zp-NIII, where most of the afferent cells expressed both immunoreactivities. Similarly, such a colocolization may exist in AVT and OT afferent neurons, but the smaller number of ChAT-ir and NOS-ir cells detected in these regions makes the correspondence more difficult to establish. In order to clearly demonstrate NOS and ChAT colocolization in cells within the different structures a double-labelling immunohistofluorescence study would have to be carried out using both antibodies simultaneously.

Immunohistochemical data recently obtained using electronic microscopy have shown that some centrifugal cells of the NIO in the

pigeon are glutamate-ir (Rio, 1996). Given that the vast majority of the centrifugal cells in the NIO are NOS-ir, it is possible that they colocalize NO and glutamate. Nitric oxide has been shown to reduce the sensitivity of AMPA and NMDA glutamate receptors (Crepel & Jaillard, 1990; Shibuki & Okada, 1991; Manzoni et al., 1992), and correspondingly, in the CVS, NO may alter the effects of glutamate release within the retina.

Nitric oxide seems to play an important role as a neurotransmitter or neuromoderator and intracellular messenger in various parts of the central and autonomic nervous system in vertebrates (Bredt & Snyder, 1992; Snyder, 1992; Vincent & Hope, 1992). It has been shown that NO may act either as a retrograde messenger, by modulating presynaptic activity following postsynaptic release (for review, see Garthwaite, 1991; Bredt & Snyder, 1992; Wiklund et al., 1993), or as a neurotransmitter following presynaptic release (Garthwaite, 1991). In mammals, NADPH-d neurons are present at all levels of the visual system, suggesting that NO plays an important role in regulating visual functions (Gonzalez-Hernandez et al., 1992; Mitrofanis, 1992). Moreover, various data suggest that NO may play an important role in the retina in birds, and in the visual centres of the thalamofugal, tectofugal (Panzica et al., 1994; Cozzi et al., 1997) and centrifugal (Cozzi et al., 1997) pathways. Our results demonstrating the presence of NO in the NIO and EN, as well as in the tectal and extra-

tectal afferent neurons suggest that it may have a significant influence on CVS function.

The functional role of the CVS

The CVS of grain-eating birds such as the pigeon, quail and chicken is particularly well-developed (10,000 to 12,000 retinopetal neurons). Based on preferential projections from the ventral tectum to the NIO and centrifugal projections to the ventral retina, Holden (1990) postulated that the CVS plays a role in behavioral attention during the search for food (searchlight hypothesis). Other studies have also lent support to the involvement of the avian CVS in feeding behavior (Shortess & Klose, 1977; Weidner et al., 1987; Hahmann & Güntürkün, 1992). Woodson et al. (1995) suggested that the CVS increased retinal stabilization of gaze, to improve the precision with which small objects are identified, and involves a centrifugal input upon displaced ganglion cells in the retina which project onto the accessory optic system (Nickla et al., 1994). Finally, it has also been suggested that the CVS selectively increases retinal sensitivity to novel or meaningful stimuli (food objects, detection of predators, etc.) within the visual field ("highlighting hypothesis": Uchiyama, 1989; Miceli et al., 1995).

In view of the different hypotheses, CVS function would appear to involve a dynamic process of selectively increasing the visual

attention with regard to either rather large (superior *vs* inferior) or smaller more punctate regions of the visual field. Electrophysiological studies have shown that the visual responses recorded in the ganglion cells are facilitated by the activation of centrifugal fibers (Galifret et al., 1971; Miles, 1972). The facilitating effect may be produced by the inhibiting action of the centrifugal fibers on the inhibitory influences of the receptive field of the retinal ganglion cells (Pearlman & Hughes, 1973; Holden, 1978, 1982). One mechanism may be that the centrifugal fibers exercise an inhibiting effect on the GABAergic and glycinergic amacrine cells, which in turn exercise an inhibitory effect on the ganglion cells (Frumkes et al., 1981; Uchiyama, 1989). However, the present study confirmed that GABA, the major inhibitory neurotransmitter in the central nervous system (Mugnaini & Oertel, 1985; Otterson & Storm-Mathisen, 1985), is not used by the centrifugal fibers, and that the only GABAergic elements in the NIO are the intrinsic interneurons (Miceli et al., 1995). Conversely, the identification of ChAT-ir centrifugal cells suggests that the centrifugal influence on the retinal amacrine cells is excitatory. In the mammalian visual thalamus, it has been shown that ACh produces an excitatory effect directly on geniculate cells (Pasik et al., 1990). A similar action by the centrifugal cells on the target retinal cells has also been suggested following the finding that centrifugal endings are aspartate-ir (Uchiyama, 1995) and glutamate-ir (Rio, 1996). Moreover, it has been shown that amacrine cells, which are the target of the convergent-type NIO endings, are

not inhibitory because they have never been observed to be GABA-ir (Nickla et al., 1994), but are probably excitatory since they have been found to be highly glutamate-ir and aspartate-ir (Uchiyama et al., 1995).

All these data, taken together, suggest that the centrifugal influence involves a deactivation of the centrifugal cells in the NIO either through intrinsic interneurons, or through extrinsic GABAergic inhibitory inputs. In contrast, activation is also possible via either an extra- or intra-nuclear GABAergic inhibitory input on the intrinsic interneurons, or by an excitatory extrinsic action on the centrifugal cells stemming from the brain-stem afferents which have been shown in the present study to be cholinergic. These cholinergic afferents to the NIO seem to be modulated by NO, which may be colocalized in the same neurons. Thus NO would appear to play a significant role at all levels of the CVS: (1) within the main tecto-NIO-retinal pathway for facilitating the transfer of visual information related to specific portions of the visual field; (2) within the diffuse and non-specific projection systems in the brain-stem (Zp-NIII/AVT) which may contribute to longer-term effects on centrifugal neuronal activity, possibly associated with mechanisms involved in regulating general states of arousal; (3) in afferent systems from the oculomotor centres of the brain-stem (Zp-n-NVI/PRF) which may modulate retinal sensitivity in accordance with eye movements or gaze (Miceli et al., 1997).

Acknowledgements

This work was supported by NSERC 0053 and FRSQ-INSERM collaborative research grants. The authors thank Dr. M.L. Epstein for graciously supplying the ChAT antibodies, Nadine Mayotte, Michel Volle and Michel Bélanger for their excellent technical assistance.

References

Angaut, P. & Repérant, J. (1978). Alight and electron microscopic study of the nucleus isthmo-opticus in the pigeon. Archives d'anatomie microscopique et de morphologie expérimentale, 67, 63-78.

Bagnoli, P., Fontanesi, G., Alesci, R. & Erichsen, J.T. (1992). Distribution of neuropeptide Y, substance P, and Choline acetyltransferase in the developing visual system of the pigeon and effects of unilateral retina removal. Journal of Comparative Neurology, 318, 392-414.

Bredt, D.S. & Snyder, S.H. (1992). Nitric oxide, a novel neuronal messenger. Neuron, 8, 3-11.

Britto, L.R.G., Keyser, K.T., Lindstrom, J.M. & Karten, H.J. (1992). Immunohistochemical localization nicotinic acetylcholine receptor

subunits in the mesencephalon and diencephalon of the chick (*Gallus gallus*). Journal of Comparative Neurology, 317, 325-340.

Brüning, G. (1993). Localization of NADPH-Diaphorase in the brain of the chicken. Journal of Comparative Neurology, 334, 192-208.

Cowan, W.M. (1970). Centrifugal fibers to the avian retina. British Medical Bulletin, 26, 112-118.

Cowan, W.M. & Powel, T.P.S. (1963). Centrifugal fibres in the avian visual system. Proceedings of the Royal Society, 158, 232-252.

Cozzi, B., Massa, R. & Panzica, G.C. (1997). The NADPH-diaphorase-containing system in the brain of the budgerigar (*Melopsittacus undulatus*). Cell and Tissue Research, 287, 101-112.

Crepel, F. & Jaillard, D. (1990). Protein kinases, nitric oxide and long-term depression of synapses in the cerebelum. Neuroreport, 1, 133-136.

Crossland, W.J. (1979). Identification of tectal synaptic terminals in the avian isthmooptic nucleus. In Neural Mechanisms of Behavior in the Pigeon, ed. Granda, A.M. & Maxwell, J.H., pp. 267-286. New York: Plenum Press.

Crossland, W.J. & Hughes, C.P. (1978). Observations on the afferent and efferent connections of the avian isthmo-optic nucleus. Brain Research, 145, 239-256.

Dawson, T.M., Bredt, D.S., Fotuhi, M., Hwang, P.M. & Snyder, S.H. (1991). Nitric oxide synthase and neuronal NADPH diaphorase are in brain and peripherical tissues. Proceedings National Academy Sciences USA., 88, 7797-7801.

Decker, K. & Reuss, S. (1994). Nitric oxide-synthesizing neurons in the hamster suprachiasmatic nucleus: a combined NOS- and NADPH-staining and retinohypthalamic tract tracing study. Brain Research, 666, 284-288.

Domenici, L., Waldvogel, H.J., Matute, C. & Streit, P. (1988). Distribution of GABA-like immunoreactive in the pigeon brain. Neuroscience, 71, 14-28.

Frumkes, T.E., Miller, R.F., Slaughter, M. & Dacheux, R.F. (1981). Physiological and pharmacological basis of GABA and glycine action on neurons of mudpuppy retina, III: Amacrine-mediate inhibitory influences on ganglion cell receptive-field organization: a model. Journal of Neuropysiology, 45, 783-804.

Galifret, Y., Condé-Courtine, F., Repérant, J. & Serviere, J. (1971). Centrifugal control in the visual system of the pigeon. Vision Research, 3, 185-200.

Garthwaite, J. (1991). Glutamate, nitric oxide and cell-cell signaling in the nervous system. Trends Neuroscience, 14, 60-67.

Granda, R.H. & Crossland, W.J. (1989). GABA-like immunoreactivity of neurons in the chicken diencephalon and mesencephalon. Journal of Comparative Neurology, 287,455-469.

Gonzalez-Hernandez, T., Conde-Sedin, M., & Meyer, G. (1992). Laminar distribution and morphology of NADPH-diaphorase containing neurons in the superior colliclus and underlying periaqueductal gray of the rat. Anatomy and Embryology, 189, 245-250.

Gonzalez-Hernandez, T., Perez De La Cruz, M.A. & Mantolan-Sarmiento, B. (1996). Histochemical detection of neurons that produce nitric oxide: Effect of different fixative parameters and immunoreactivity against non-neuronal NOS antisera. Journal of Histochemistry and Cytochemistry, 44 (12), 1399-1413.

Güntürkün, O. & Karten, H.J. (1991). An immunohistochemical analysis of the lateral geniculate complex in the pigeon (*Columba livia*). Journal of Comparative Neurology, 314, 721-749.

Hahmann, U. & Güntürkün, O. (1992). Visual-discrimination deficits after lesions of the centrifugal visual system in pigeons (*Columba livia*). Visual Neuroscience, 9, 225-233.

Hashikawa, T., Leggio, M.G., Hattori, R. & Yui, Y. (1994). Nitric oxide synthase immunoreactivity colocalised with NADPH-diaphorase histochemistry in monkey cerebral cortex. Brain Research, 641, 341-349.

Hayes, B.P. & Webster, K.E. (1981). Neurons situated outside the isthmo-optic nucleus and projecting to the eye in adult birds. Neuroscience Letters, 26, 107-112.

Holden, A.L. (1990). Centrifugal pathways to the retina: which way does the "searchlight" point. Visual Neuroscience, 4, 493-495.

Holden, A.L. (1982). Electrophysiology of the avian retina. Progress in Retinal Research, 1, 79-196.

Holden, A.L. (1978). Centrifugal action on pigeon retinal ganglion cells. Journal of Physiology (London), 282, 8P.

Huang, Z., You, W., Haugland. R.P., Paragas, V.B., Olson, N. & Haugland, R.P. (1993). Journal of Histochemistry and Cytochemistry, 41, 313-317.

Hunt, S.P., & Brecha, H. (1984). The avian optic tectum : A synthesis of morphology and biochemistry. In :Comparative Neurology of the Optic Tectum. ed. H. Vanegas., pp. 619-648. New York: Plenum Press.

Hunt, S.P. & Künzle, H. (1976a). Observations on the projections and intrinsic organization of the pigeon optic tectum: an autoradiographic study based on anterograde and retrograde, axonal and dendritic flow. Journal of Comparative Neurology, 170, 153-172.

Hunt, S.P. & Künzle, H. (1976b). Selective uptake and transport of label within three identified neuronal systems after injection of 3H-GABA into the pigeon optic tectum: an autoradiographic and golgi study. Journal of Comparative Neurology, 170, 173-190.

Hunt, S.P., Streit, P., Kunzle, H. & Cuénod, M. (1977). Characterization of the pigeon isthmo-tectal pathway by selective uptake and retrograde movement of radioactive compounds and by golgi-like horseradish peroxidase labeling, Brain Research, 129, 197-212.

Johnson, C.D. & Epstein, M.L. (1986). Monoclonal antibodies and polyvalent antiserum to chicken choline acetyltransferase. Journal of Neurochemistry, 46, 968-976.

Karten, H.J. & Hodos, W. (1967). A stereotaxic Atlas of the brain of the pigeon (*Columba livia*). Baltimore, Maryland: Johns Hopkins Press.

Kharazia, N.V., Schmidt, H.H.H.W. & Weinberg, R.J. (1994). Type I nitric oxide synthase fully accounts for NADPH-diaphorase in rat striatum, but not cortex. Neuroscience, 62, 983-987.

Manzoni, O, Prezeau, L., Martin, P., Deshager, S., Bockaert, J. & Fagni, L. (1992). Nitric oxide-induced blockade of NMDA receptors. Neuron, 8, 653-662.

Mc Gill, J.I., Powel, T.P.S. & Cowan, W. M. (1966a). The retinal representation upon the optic tectum and isthmo-optic nucleus in the pigeon. Journal of Anatomy, 100, 5-33.

Mc Gill, J.I., Powel, T.P.S. & Cowan, W. M. (1996b). The organization of the projection of the centrifugal fibres to the retina in the pigeon. Journal of Anatomy, 100, 35-49.

Medina, L. & Reiner, A. (1994). Distribution of choline acetyltransferase immunoreactivity in the pigeon brain. Journal of Comparative Neurology, 342, 497-537.

Meyer, G., Banuelos-Pineda, J., Montagnese, C., Ferres-Meyer, G. & Gonzales-Hernandez, T. (1994). Laminar distribution and morphology of NADPH-diaphorase containing neurons in the optic tectum. Journal of Brain Research, 35, 445-452.

Miceli, D., Repérant, J., Bavikati, R, Rio, J.-P. & Volle, M. (1997). Brainstem afferents upon retinal projecting ishtmo-optic and ectopic neurons of the pigeon centrifugal visual system demonstrated by retrograde transneuronal transport of rhodamine ß-isothiocyanate. Visual Neuroscience, 14, 213-224.

Miceli, D., Repérant, J. Marchand, L. & Rio, J.-P. (1993). Retrograde transneuronal transport of the fluorescent dye Rhodamine β-isothiocyanate from the primary and centrifugal visual systems in the pigeon. Brain Research, 601, 289-298.

Miceli, D., Repérant, J., Rio, J.P. & Medina, M. (1995). GABA immunoreactivity in the nucleus isthmo-optic of the centrifugal visual system in the pigeon: Alight and electronmicroscopic study. Visual Neuroscience, 12, 425-441.

Miles, F.A. (1972). Centrifugal control of the avian retina.III. Effects of electrical stimulation of the isthmo-optic tract on the receptive field properties of retinal ganglion cells. Brain Research, 48, 115-129.

Mitrofanis, J. (1992). NADPH-diaphorase reactivity in the ventral and dorsal lateral geniculate nuclei of rats. Visual Neuroscience, 9, 211-216.

Montagnese, C.M. & Csillag, A. (1996). Comparative distribution of NADPH-diaphorase activity and tyrosine hydroxylase immunoreactivity in the diencephalon and mesencephalon of the domestic chicken (gallus domesticus). Anatomy and Embryology, 193, 427-439.

Morgan, I.G., Miethke, P. & Li, Z.K. (1994). Nitric oxide a transmitter of the centrifugal projection to the avian retina. Neuroscience Letters, 168, 5-7.

Mugnaini, E. & Oertel,W.H. (1985). An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD imunohistochemistry. In Handbook of Chemical Neuroanatomy: GABA and Neuropeptides in the CNS. Vol. IV, ed. Björklund, A. & Hökfelt, T., pp. 437-609. Elsevier, Amsterdam.

Nickla, D.L., Gottlieb, M.D., Marin, G., Rojas, X., Britto, L.R.G. & Wallman, J. (1994). The retinal targets of centrifugal neurons and the retinal neurons projecting to the accessory optic tectum system. Visual Neuroscience, 11, 401-409.

Ottersen, O.P. & Storm-Mathisen, J. (1985). Neurons containing or accumulating transmitter amino acids. In Handbook of Chemical Neuroanatomy : Classical Transmitters and Transmitter Receptor in the CNS, Vol. III, ed. Björklund. A., Hökfelt, T. & Kuhar, M.J., pp.141-245. Elsevier, Amsterdam.

Panzica, G.C., Arévalo, R., Sanchez, F., Alonso, J.R., Aste, N., Viglietti-Panzica, C., Aijon, J. & Vazquez, R. (1994). Topographical distribution of reduced nicotinamide adenine dinucleotide phosphate-diasphorase in the brain of the japanese quail. Journal of Comparative Neurology, 342, 97-114.

Panzica, G.C. & Garzino, A. (1994). The mesopontine NADPH-diaphorase system in the Japanese quail. Society for Neuroscience Abstracts, 20, 1560.

Panzica, G.C., Garzino, A. & Garcia Ojeda, E. (1996). Coexistence of NADPH-diaphorase and tyrosine hydroxylase in the mesencephalic catecholaminergic system of the Japanese quail. Journal of Chemical Neuroanatomy, 11, 37-47.

Pasik, P., Monlinar-Rode, R. & Pasik, T. (1990). Chemically specified systems in the dorsal lateral geniculate nucleus of mammals. In Vision and the brain. ed. B. Cohen, & I. Bodis-Wollner, pp.43-83. New York : Raven Press Ltd.

Pasqualotto, B.A. & Vincent, S.R. (1991). Galanin and NADPH-diaphorase coexistence in cholinergic neurons of the rat basal forebrain. Brain Research, 551, 78-86.

Pearlman, A.L. & Hughes, C.P. (1973). Functional role of efferents to the avian retina: effects of reversible coolig of the isthmo-optic nucleus. Journal of Comparative Neurology, 166, 123-132.

Reiner, A., & Karten, H.J. (1982) Laminar distribution of the cells of origin of the descending tectofugal pathway in the pigeon (*Columba livia*). Journal of Comparative Neurology, 204, 165-187.

Repérant, J., Miceli, D., Vesselkin, N.P. & Molotchnikoff, S. (1989). The centrifugal visual system of vertebrates: a century-old search reviewed. International Review of Cytology, 118, 115-171.

Repérant, J., Peyrichoux, J. & Rio, J.-P. (1981). Fine structure of the superficial layers of the viper optic tectum: A Golgi and electron-microscopic study. Journal of Comparative Neurology, 199,393-417.

Rio, J-P. (1996). Organisation anatomo-fonctionnelle et évolution du système visuel centrifuge des vertébrés. Thèse de Doctorat es Sciences, M.N.H.N., Paris.

Rodrigo, J., Springall, D.R., Uttenthal, O., Bentura, M.L., Abadia-Molina, F., Riveros-Moreno, V., Martinez-Murillo, R., Polak, J.M. & Moncada, S. (1994). Localization of nitric oxide synthase in the adult rat brain. Philos. Trans. R. Soc. Lond. B. Biol. Sci., 345, 175-221.

Shibuki, K. & Okada, D. (1991). Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum. Nature London., 349, 326-328.

Shortess, G.K. & Klose, E.F. (1977). Effects of lesions involving efferent fibers to the retina in pigeons (*Columba livia*). Physiology Behavior, 18, 409-414.

Snyder, S.H. (1992). Nitric oxide: first in a new class of neurotransmitters. Science, 257, 494-496.

Sorenson, E.M. & Chiappinelli, V.A. (1992) Localization of 3H-nicotine I-kappa-bungarotoxin, and I-alpha- bungarotoxin binding to nicotinic sites in th chicken forebrain and midbrain. Journal of Comparative Neurology, 323, 1-12.

Sorenson, E.M., Parkinson, D., Dahl,. J.L. & Chiappinelli, V.A. (1989). Immunohistochemical localization of choline acetyltransferase in the chicken mesencephalon. Journal of Comparative Neurology, 281, 641-657.

Spessert, R. & Layes, E. (1994). Fixation conditions affect the intensity but not the pattern of NADPH-diaphorase staining as a marker for neuronal nitric oxide synthase in rat olfactory bulb. Journal of Histochemistry and Cytochemistry, 42, 1309-1315.

Spessert, R., Wohlgemuth, C., Reuss, S. & Layes, E. (1994). NADPH-diaphorase activity of nitric oxide synthase in the olfactory bulb: co-factor specificity and characterization regarding the interelation to no formation. Journal of Histochemistry and Cytochemistry, 42, 569-575.

Traub, R.J., Solodkin, A., Meller, S.T. & Gebhart, G.F. (1994). Spinal cord NADPH-diaphorase histochemical staining but not nitric oxide synthase immunoreactivity increases following carrageenan-produced hindpaw inflammation in the rat. Brain Research., 668, 204-210.

Uchiyama, H. (1989). Centrifugal pathways to the retina: influence of tectum. Visual Neuroscience, 3, 183-206.

Uchiyama, H., Ito, H. & Tauchi, M. (1995). Retinal neurons specific for centrifugal modulation of vision. NeuroReport, 6, 889-892.

Uchiyama, H. & Watanabe, M. (1985). Tectal neurons projecting to the isthmo-optic nucleus in the Japanese quail. Neuroscience Letters, 58, 381-385.

Valtschanoff, J.G., Weinberg, R.J., Kharazia, V.N., Nakane, M. & Schmidt, H.H.H.W. (1993). Neurons in rat hippocampus that synthesize nitric oxide. Journal of Comparative Neurology, 331, 111-121.

Veenman, C.L. & Reiner, A. (1994). The distribution of GABA-containing perikarya, fibres, and terminals in the forebrain and midbrain of pigeons, with particular reference to the basal ganglia and its projection targets. Journal of Comparative Neurology, 339, 209-250.

Vincent, S.R., & Hope, B.T., (1992). Neurons that say NO. Trends of Neuroscience., 15, 108-113.

Vincent, S.R., Satoh, K., Armstrong, D.M. & Fibiger, H.C. (1983). NADPH-diaphorase: a selective histochemical marker for the

cholinergic neurons of the pontine reticular formation. Neuroscience Letters, 43, 31-36.

Vizzard, M.A., Erdman, S.L., Roppolo, J.R., Förstermann, U. & de Groat, W.C. (1994). Differential localization of neuronal nitric oxide synthase immunoreactivity and NADPH-diaphorase activity in the cat spinal cord. Cell Tissue Research., 278, 299-309.

Ward, R., Repérant, J. & Miceli, D. (1991). The centrifugal visual system: what can comparative studies tell us about its evolution and possible function? In The Changing Visual System., ed. P.Bagnoli & W. Hodos, pp. 61-76. New York: Plenum Press.

Weidner, C., Desroches, A.M., Repérant, J., Kirpitchnikova, E. & Miceli, D. (1989). Comparative study of the centrifugal visual system in the pigmented and glaucomatous albino quail. Biological Structures and Morphology, 2, 89-93.

Weidner, C., Repérant, J., Desroches, A.-M., Miceli, D. & Vesselkin, N.P. (1987). Nuclear origin of the centrifugal visual pathway in birds of prey. Brain Research, 436, 153-160.

Wiklund, C.V., Olgart, C., Wiklund, N.P. & Gustafsson, L.E. (1993). Modulation of cholinergic and substance p-like neurotransmission by nitric oxide in the guinea-pig ileum. Br. J. Pharmacol, 110, 833-839.

Wolf-Oberhollenzer, F. (1987). A study of the centrifugal projections to the pigeon retina using two fluorescent markers. Neuroscience Letters, 73, 16-20.

Woodson, W., Reiner, A., Anderson, K. & Karten, H.J. (1991). Distribution, laminar location, and morphology of tectal neurons projecting to the isthmo-optic nucleus and the nucleus isthmi, pars parvocellularis in the pigeon (*Columbia Livia)* and chick (*Gallus domesticus*), a retrograde labelling study. Journal of Comparative Neurology, 305, 470-488.

Woodson, W., Shimizu, T., Wild, J.M., Schimke, J., Cox, K. & Karten, H.J. (1995). Centrifugal projections upon the retina: An anterograde tracing study in the pigeon (*Columba livia*). Journal of Comparative Neurology, 362, 489-509.

Annexe

LEGENDS

Figure 1. Double-labeling technique (A-D). A) Somatic retrograde labeling of NIO centrifugal neurons following the intraocular injection of RITC and visualized using the N2 filter system and in B) the same region observed with the I2 filter system demonstrating that numerous centrifugal cells are ChAT-ir (FITC labeled). X93. C-D) Similar double-labeling respectively of RITC centrifugal ectopic neurons (EN), some of which (indicated by arrows) are ChAT-ir (FITC labeled). X270. E) ELF technique: showing ChAT-ir cells in the NIO and in the underlying region of ectopic neurons as visualized using the A filter system. X83. F) Immunohistofluorescent technique showing the labeling (RITC) of NOS-ir neurons within the centrifugal cell layers of the NIO. X91. G-H) Double-labeling technique showing respectively a labeled EN after the intraocular injection of RITC and the same region showing the same cell (arrow) to be NOS-ir (FITC labeled). X216.

Figure 2. Optic tectum. Double-labeling techniques. A) Retrograde RITC labeling of afferent neurons at the layers 9/10 border region of the optic tectum (OT) following transneuronal transport of the tracer from the eye and in B) the same region showing that some of the cells (indicated by arrows are also ChAT-ir (FITC labeled). X365. C-D) Similar double-labeling of respectively RITC afferent tectal cells in layers 9/10 and that some (arrows) are also NOS-ir (FITC). X350. E)

Retrograde FG labeling of cell bodies in tectal layers 9/10 following an injection of the tracer into the NIO and in F) the same region demonstrating the distribution of numerous smaller GABA-ir (RITC labeled) cells non double-labeled with the FG cells in E. X220.

Figure 3. Double-labeling technique. A-B) Respectively, RITC retrograde transneuronal labeling of afferent neurons in the ZpNIII and the same region containing FITC labeled ChAT-ir cells, some of which appear double-labeled (arrows). X212. C-D) Similar double labeling of respectively AVT afferent neurons (RITC) and the latter also immunolabeled (arrows) with FITC (NOS-ir). X220. E-F) RITC afferent cells in the Zp-n.NVI and some FITC immunolabeled (NOS-ir) cells in the same region, some of which were double-labeled (arrows). X223.

Figure 4. Schematic representation of frontal sections through the pigeon brain showing the location of NOS-ir (open triangle), ChAT-ir (black diamond) and GABA-ir (asterisk) cells. The latter were observed in the NIO and EN as well as in afferent neurons in different brain-stem structures found to project upon the centrifugal neurons. The centrifugal neurons were ChAT-ir and NOS-ir, whereas GABA-ir cells corresponded to interneurons within the neuropilar region of the NIO. Abbreviations: CbI: Nucleus cerebrallis internus; CbM: Nucleus cerebrallis intermedius; CCV: Commissura cerebralis ventralis; CP: Commissura posterior; CT: Commissura tectalis; EM:

Nucleus ectomamillaris; GCt: Sustancia grisea centralis; Imc: Nucleus isthmi, pars magnocellularis; MLD: Nucleus mesencephalicus lateralis, pars dorsalis; MRF: Formatio reticularis medialis mesencephali; nVI: Nucleus nervi abducentis; nVII: Nucleus nervi facialis; PL: Nucleus pontis lateralis; PRF: Formatio reticularis pontis; R: Nuclei raphes; V: Ventriculus; VeL: Nucleus vestibularis lateralis; VLV: Nucleus ventralis lemnisci lateralis.

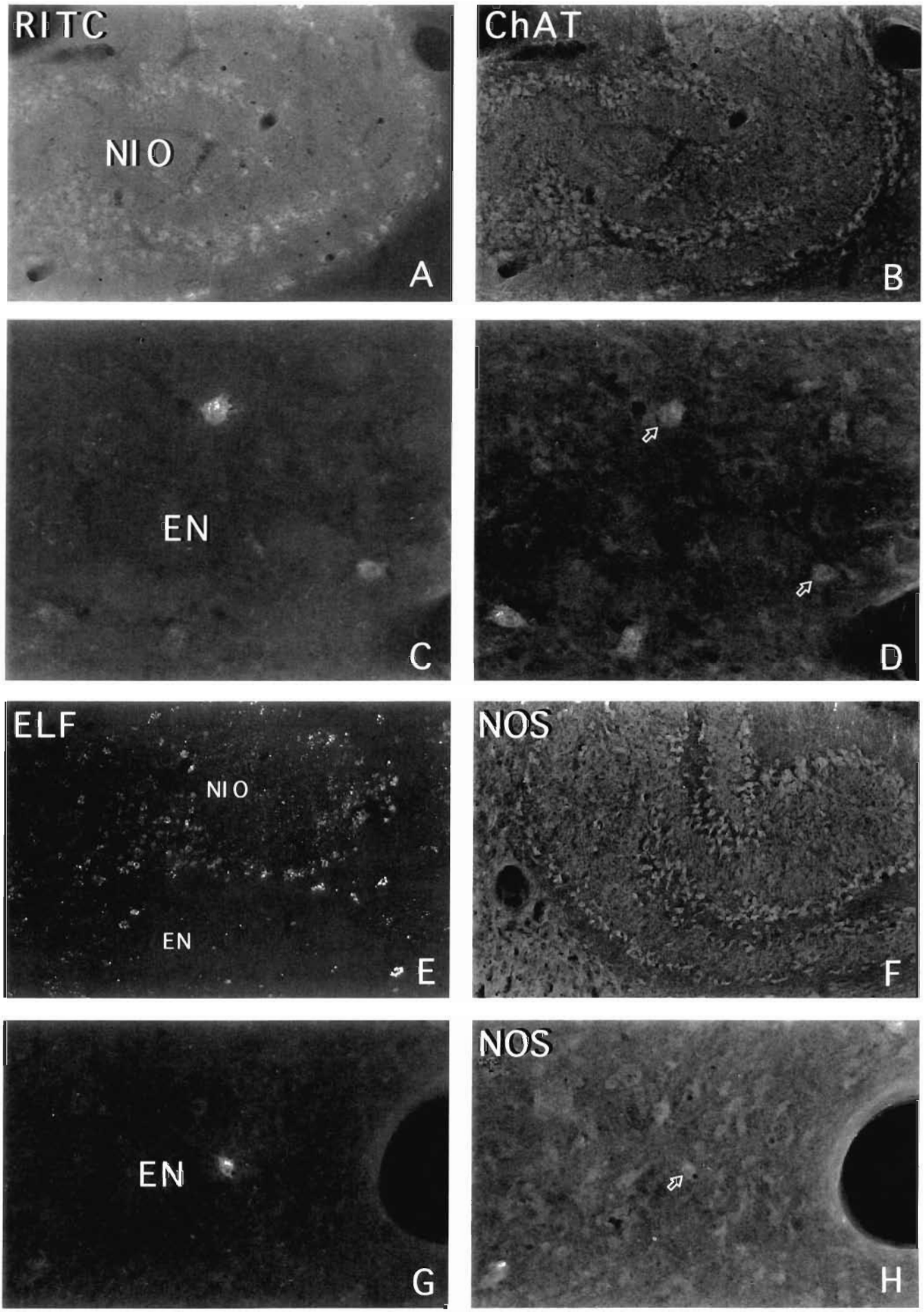

Figure 1

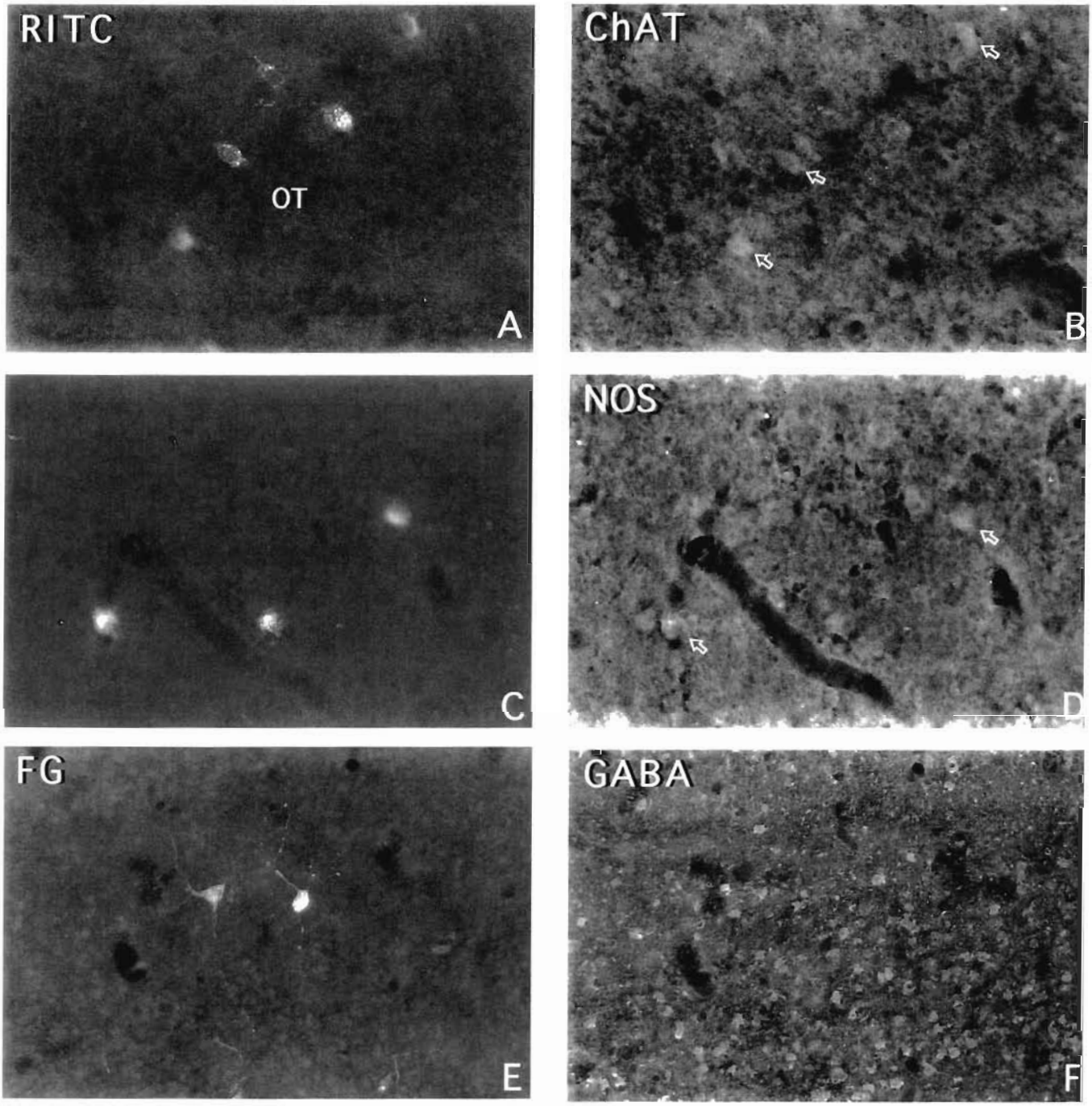

Figure 2

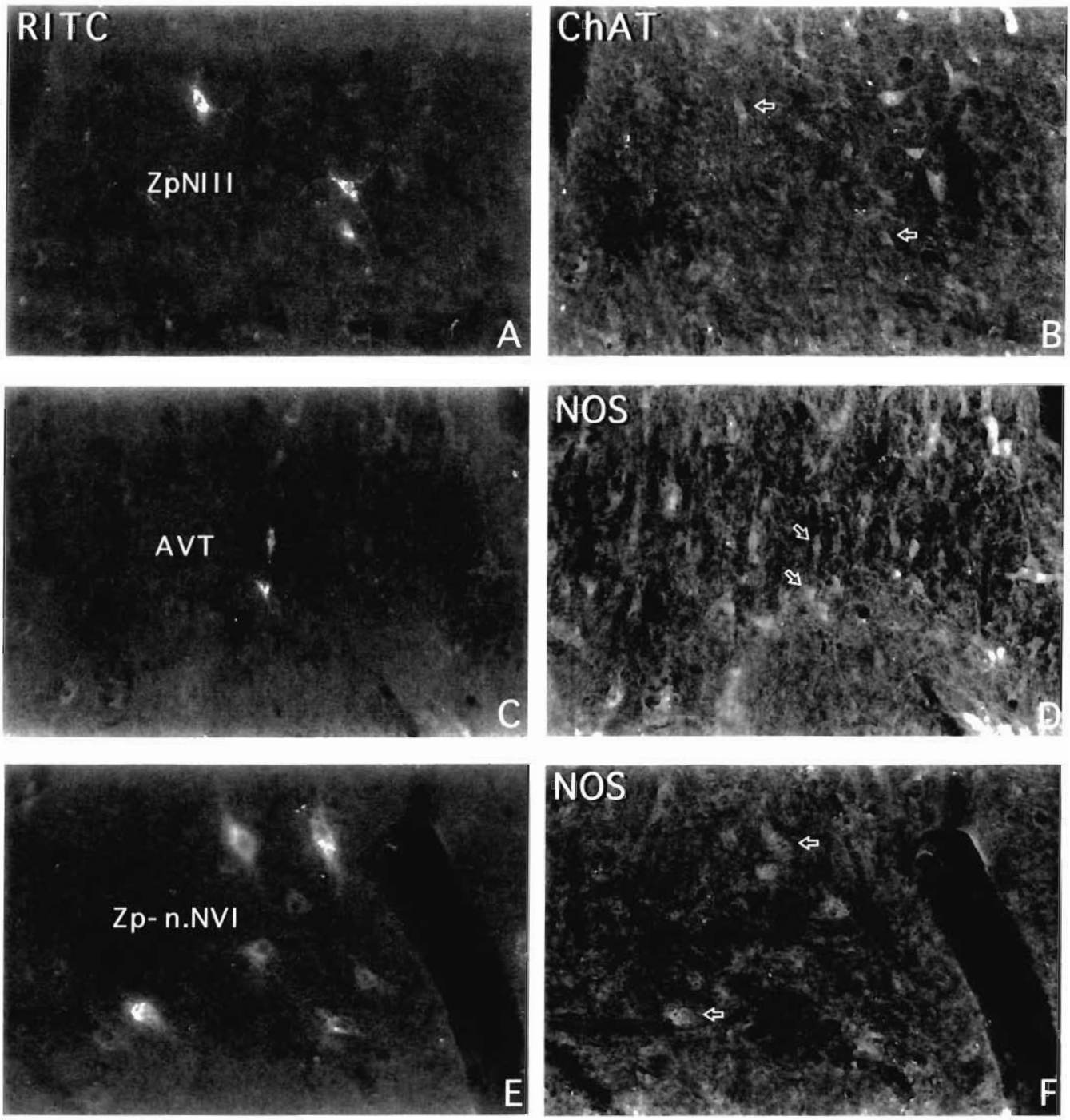

Figure 3

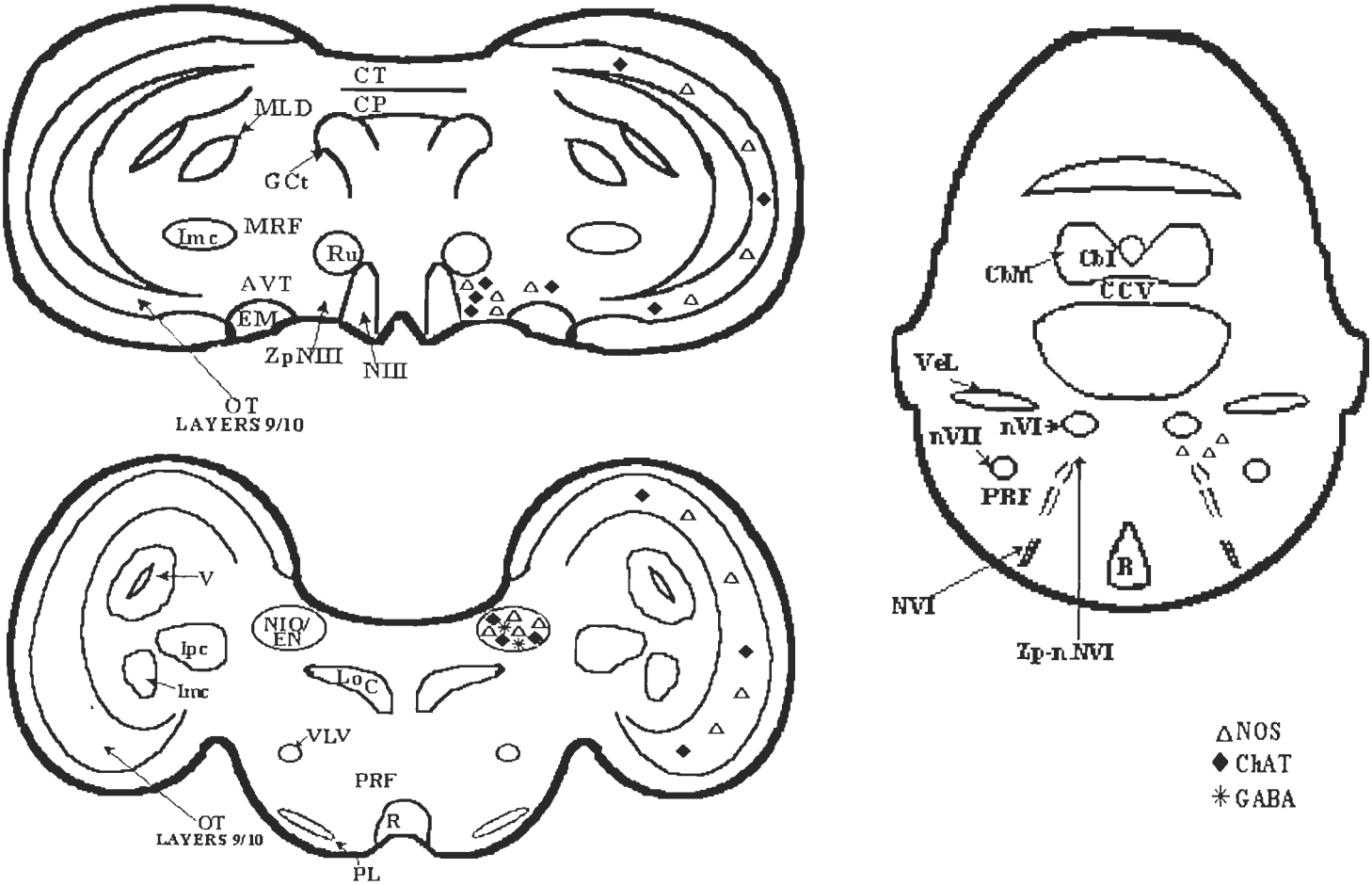
CT
CP
MLD
GCt
Imc
MRF
Ru
AVT
EM
ZpNIII
NIII
OT
LAYERS 9/10
V
NIO/
EN
Ipc
Imc
LoC
VLV
PRF
R
OT
LAYERS 9/10
PL
ChM
ChI
CCV
VeL
nVII
nVI
PRF
R
NVI
Zp-n.NVI
ΔNOS
◆ ChAT
✳ GABA

FIGURE 4