

# Dopaminergic innervation of the brain in pigeons. The presumed 'Prefrontal Cortex'

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**Abstract.** In the postero-dorso-lateral neostriatum (PDLNS) of the pigeon we found a high density of tyrosine hydroxylase and perineuronal nests of fibres labelled with anti-dopamine antibody. These data confirm our earlier indirect evidence that PDLNS receives a dense dopaminergic innervation and therefore can be compared with the mammalian prefrontal cortex.

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**Key words:** immunohistochemistry, species comparisons, avian neostriatum, tyrosine hydroxylase

## INTRODUCTION

The prefrontal cortex (PFC) in mammals is defined as the area innervated by the thalamic mediodorsal nucleus (MD) (Rose and Woolsey 1948, Divac and Öberg 1990). Considerable differences in the organization of the thalami in birds (Karten and Hodos 1967) and mammals (e.g. Paxinos and Watson 1986) make the commonly used topographic definition of the MD uncertain (Clark and Meyer 1950). Thus, the 'dorsomedial nucleus' of Karten and Hodos may not be the bird counterpart of the 'mediodorsal nucleus' in mammals. In consequence, the presence in birds of an equivalent of mammalian PFC was not even considered until a number of studies showed that in mammals this cortical area receives exceptionally dense dopaminergic innervation in comparison with other cortical areas (Divac et al. 1978 and references therein). This observation suggested that dopaminergic innervation could indicate in the bird brain the presence and localization of the tissue equivalent to the mammalian PFC.

Dopamine has been used previously to redefine parts of the bird brain. For example, in this way paleostriatum augmentatum was found to correspond to the mammalian neostriatum (Juorio and Vogt 1967, Baker-Cohen 1968). Thus, a formation in the pigeon brain that is particularly densely innervated by dopamine-containing fibres and is not to be confused with other formations with pronounced dopaminergic innervation such as neostriatum, amygdala, septum and the entorhinal cortex (Divac and Mogensen 1985), may be equivalent to the mammalian PFC. The results of several experiments concurred in suggesting that postero-dorsolateral neostriatum (PDLNS) is a candidate for being the pigeon equivalent of mammalian PFC: First, catecholamine histochemistry showed that PDLNS contains a dense network and pericellular baskets of catecholamine-containing fibres which responded as dopaminergic to pretreatment with reserpine and L-DOPA (Divac and Mogensen 1985). Essentially the same results have been recently obtained with anti-dopamine antibodies

(Waldmann and Güntürkün 1993). Secondly, biochemical measurements revealed a high concentration of non-precursor dopamine in PDLNS as compared to other regions equivalent to mammalian cerebral cortex (Divac et al. 1985). Thirdly, a functional similarity between PFC and PDLNS was found with the neurobehavioural approach (Mogensen and Divac 1982, 1993, Gagliardo et al., submitted). In addition to these positive identification signs, one could add negative: PDLNS has not been 'claimed' by any sensory or motor system. In conclusion, PDLNS may be equivalent to mammalian PFC or one of its divisions.

The most important element in this hypothesis is a denser dopaminergic innervation and pericellular baskets of dopamine-containing axons in PDLNS as compared to other cortical equivalents in the pigeon brain. Presently we have tried to verify this observation using immunohistochemistry for detection of tyrosine hydroxylase and dopamine.

## METHODS

### Dopamine immunohistochemistry

Six adult pigeons of unknown gender, weighing 400-520 g were used in this part of study. The birds were anaesthetized with Equithesin (Divac and Mogensen 1985) injected intramuscularly. The transcardial perfusion started with about 25 ml of phosphate buffered saline (PBS) pH 7.4 at room temperature followed by 300 ml of ice-cold 3.5% glutaraldehyde in 0.1M phosphate buffer, containing 1% sodium metabisulfite ( $\text{Na}_2\text{S}_2\text{O}_4$ ) with pH adjusted to 7.4. The brain was taken out of the skull, divided in two by a coronal or sagittal cut and post-fixed in the same fixative for 2 to 4 h. The caudal and lateral blocks were rinsed for at least 24 h in PBS and cut at 40  $\mu\text{m}$  on a vibratome either in coronal or sagittal plane. The sections were transferred to a cryoprotective solution (25% ethyleneglycol, 20% glycerol in 0.1 M phosphate buffer, final pH 7.48) and stored in this protectant at  $-20^\circ\text{C}$  for two to eight weeks. Prior to the immunohistochemical procedure the sections were first rinsed in 1% sodium

metabisulfite dissolved in 0.1 M phosphate buffer (PMBS) and then placed in 1% sodiumborohydride (NaBH<sub>4</sub>) in PMBS for 10 min. Following several rinses in PMBS the sections were preincubated with 10% normal horse serum for one hour and then with a mouse primary antibody against dopamine (Changaud et al. 1987) diluted 1:5,000 for 48 h at +4°C. This monoclonal antibody has been shown to be highly specific when used for the detection of dopamine in glutaraldehyde-fixed tissue (Changaud et al. 1987). After a thorough rinse with PMBS the sections were incubated with a biotinylated horse antimouse antibody diluted 1:200 (Vector) for 2 h at room temperature. Following rinses, sections were incubated for 2 additional hours with an avidin-peroxidase complex diluted to 1:100 (Vectastain ABC kit, Vector). Peroxidase localization was revealed by exposure of sections to 0.05% 3,3'-diaminobenzidine tetrahydrochloride and 0.03% hydrogen peroxyde. The rinsed sections were mounted on slides and finally exposed to 1% OsO<sub>4</sub> in order to enhance the visibility of the reaction product. Thereafter the sections were dried and coverslipped with DPX (Merck).

### Tyrosine hydroxylase (TH)

Twelve adult male pigeons (*Columba livia*, 1-2 years old, 450-550 g body weight, provided by S. Abdel'Al, Basle, Switzerland) were killed by decapitation, brains were immediately removed and rapidly frozen on dry ice. Sections 10 µm thick were cut with a microtome cryostat (Leitz, Zürich, Switzerland), mounted onto gelatin-coated slides and stored at -20°C until used. (TH)-like immunoreactivity (THLI) was detected as previously reported for substance P-like immunoreactivity (Dietl et al. 1985). The antibodies to TH were produced by one of us (Thibault et al. 1981). Tissue sections were brought to room temperature and fixed for 2 h in 4% paraformaldehyde in 0.05 M phosphate buffer saline (PBS). The following steps were performed at room temperature in a PBS 0.1M, pH 7.4. After fixation, tissues were washed twice for 10 min in PBS and preincubated for 30 min in PBS containing 1% bovine serum albumine (Serva, FRG) and

1% normal goat serum (Nordic Immunologicals, The Netherlands). Then, tissues were incubated for 30 min in the same preincubation buffer containing in addition 0.3% Triton X-100 (Serva, FRG) and an antibody against TH at a dilution of 1/5000. The incubation was continued at 4°C overnight and ended next day with a further 30 min period at room temperature. After that, tissues were rinsed two times for 15 min in PBS and incubated with a second antibody, a goat anti-rabbit radiolabelled with [<sup>125</sup>I]iodine at 300,000 cpm/ml (8.2 µCi/g; New England Nuclear, Dreieich, FRG). Finally, sections were washed 8 times for 15 min in PBS, dipped in cold distilled water, dried under a stream of air.

The specificity of immunohistochemical labelling was controlled either by omitting the specific anti-TH antibody in the first incubation or by preabsorption of the anti-TH antibody with 1 µg/ml of TH (Tassin et al. 1978, Dietl et al. 1985). Under these conditions no immunoreactive labelling was observed on the sections.

Autoradiograms were generated by apposing the labelled tissue sections to a tritium-sensitive Ultrafilms (LKB, Sweden). The exposures were 1 and 4 days for <sup>125</sup>I-ligands. Films were then developed in D-19 Kodak developer, washed and fixed. To permit a precise anatomical localization of the labelling, adjacent sections were stained with cresyl violet or for acetylcholinesterase reaction and the atlas of the pigeon brain by Karten and Hodos (1967) was employed. To estimate the density of the labelling, and increase the contrast, an image-analysis system (MCID, Imaging Res. Inc., Ontario, Canada) was used.

For microscopic examination and microphotography a Leitz microscope equipped with Orthomat was used.

## RESULTS

### Dopamine immunohistochemistry

In the brains prepared for dopamine immunohistochemistry, microscopic examination of the telencephalon revealed in PDLNS a dense network

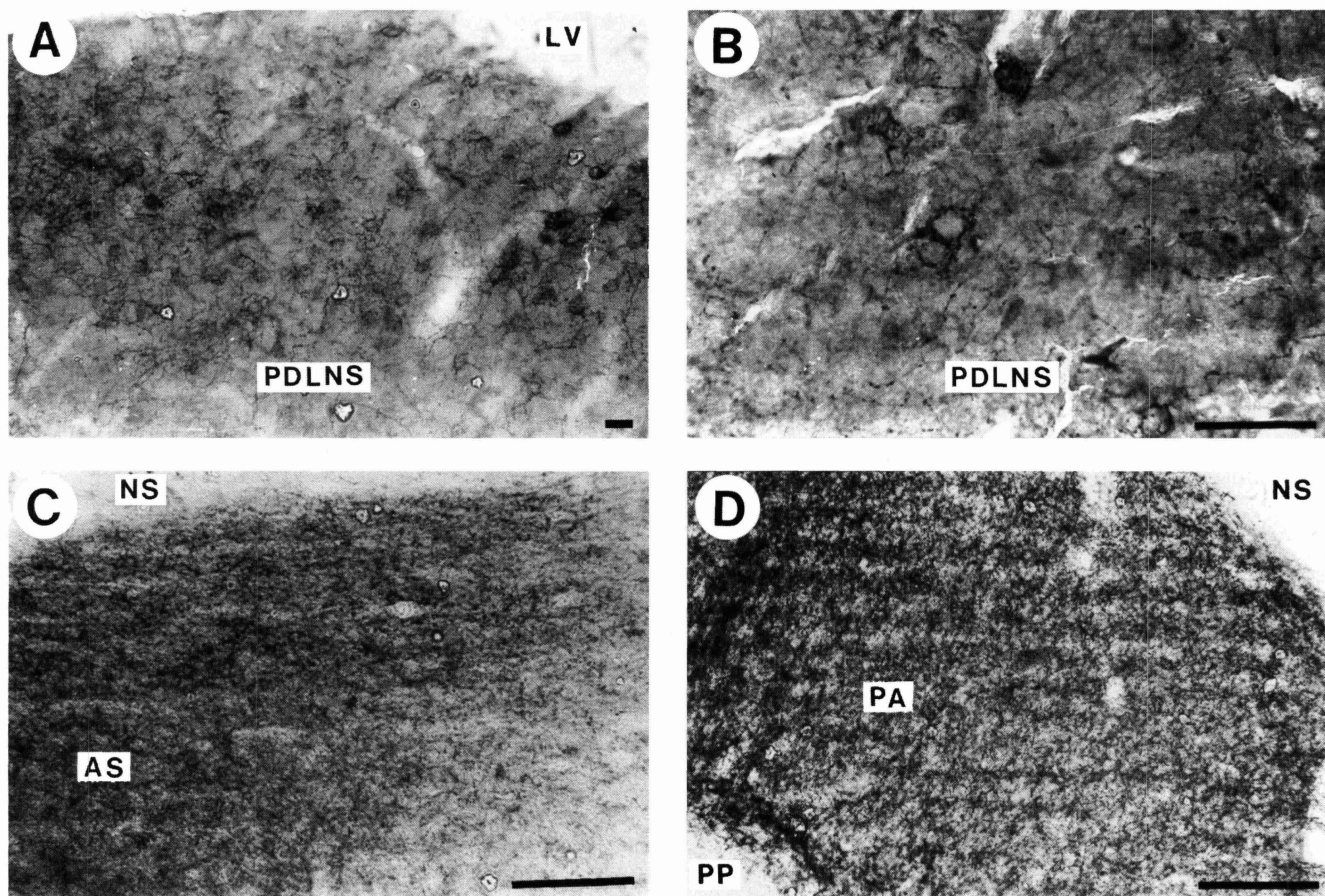


Fig. 1. Dopamine-like immunohistochemical staining in some structures of the pigeon brain. A and B, characteristic pericellular baskets of labelled fibres are seen in PDLNS at two different magnifications; C, the labelling is characteristically disperse in the archistriatum; D, the density of labelled fibres is largest in the paleostriatum augmentatum. Abbreviations: AS, archistriatum; LV, lateral ventricle; NS, neostriatum; PA, paleostriatum augmentatum; PDLNS, postero-dorso-lateral neostriatum; PP, paleostriatum primitivum. For topography consult Fig. 2 and Karten and Hodos (1967). Calibration: 25  $\mu$ m in A, 50  $\mu$ m in B, C and D.

of labelled fibres which made numerous pericellular baskets (Fig. 1A and B). In some brains the staining intensity of PDLNS was even macroscopically stronger than that of other dorsal telencephalic regions (Fig. 2A). The archistriatum and paleostriatum augmentatum were intensely stained, but the distribution of dopamine fibres differed from that in PDLNS (compare Fig. 1A and B with 1C and D).

### Tyrosine hydroxylase immunohistochemistry

This labelling in PDLNS was conspicuously stronger than in other dorsal telencephalic regions (Fig. 2 B and C).

## DISCUSSION

The present results show that among the regions in the pigeon brain that are comparable to the mammalian cerebral cortex (Karten and Hodos 1967, Karten 1969), PDLNS is the largest formation characterized by a dense dopaminergic innervation forming perineuronal nests. In this way PDLNS resembles the prefrontal cortex (PFC) in the rat (Emson and Koob 1978, Palkovits et al. 1979, Slopsoma et al. 1982, Tassin et al. 1987, Van Eden et al. 1987, Yoshida et al. 1988; for the review of the work prior to 1978 see Divac et al. 1978). Dopamine-containing fibres have been seen in some areas of the rat cortex outside the PFC (Berger et al.

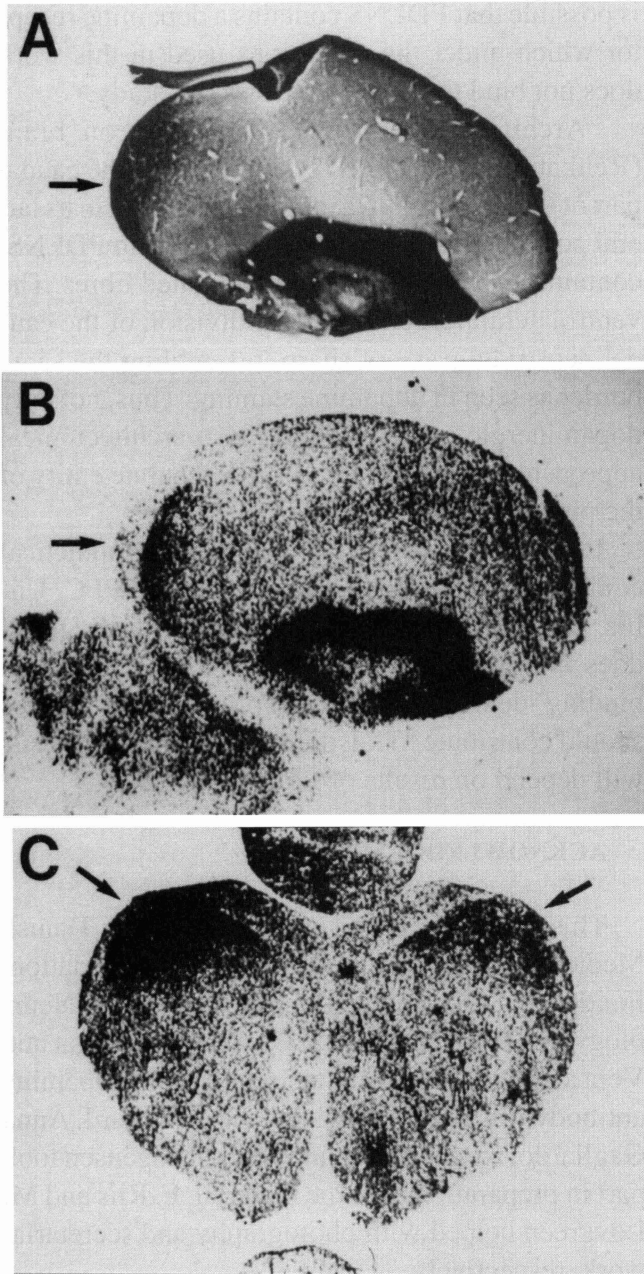


Fig. 2. Distribution of dopamine (A) and tyrosine hydroxylase (B and C) in parasagittal (A and B) and horizontal (C) sections of pigeon brains. The rostral direction is to the right in A and B, and down in C. Arrows point toward PDLNS. The asymmetric intensity of staining of TH is probably due to uneven pressure of the tissue against the film. Notice in A and B also the intensely labelled ring of the paleostriatum augmentatum, the bird equivalent of the mammalian neostriatum.

1985, Phillipson et al. 1987) but the density of innervation and the regional amount of dopamine are considerably smaller (references above). In pri-

mates, dense dopaminergic innervation has been found not only in the PFC but also all other cortical areas (review in Berger et al. 1991). This difference between primates and lower mammalian species suggests that dopaminergic innervation of the cerebral cortex or cortical equivalents may indicate PFC only if a steep gradient in this innervation is found. In primates, the amount of dopamine rather than density of dopamine-containing fibres (Brown and Goldman 1977, Björklund et al. 1978, Berger et al. 1991) may be used to indicate the localization of PFC.

Several studies employing different techniques have shown that PDLNS in pigeons is more densely innervated with dopaminergic fibres than any other cortical equivalent: catecholamine fluorescence with pharmacological elimination of the noradrenergic fluorescence (Divac and Mogensen 1985), immunohistochemistry of dopamine (Waldmann and Güntürkün 1993 and present data), immunohistochemistry of tyrosine hydroxylase (Shimizu and Karten 1990 and present data). The amount of dopamine, determined biochemically in tissue samples, was higher in PDLNS than in any other major part of the cortical equivalent (Divac et al. 1985).

Some pericellular nests of axons stained for tyrosine hydroxylase have been seen in the hyperstriatum accessorium by Shimizu and Karten (1990). There are several ways to interpret this observation. First, PFC may be represented by two spatially separated regions in the pigeon brain (see discussion in Divac and Mogensen 1985). Second, the hyperstriatum accessorium may correspond to the cingulate portion of the dopaminergic innervation of the mesial cortex in the rat (Lindvall et al. 1978). In the rat, ablations of the pregenual PFC induce a significantly stronger impairment in delayed alternation than lesions of the supragenual cortex (Larsen and Divac 1978). Similarly, selective lesions in the PDLNS but not in the hyperstriatum accessorium induced delayed alternation impairment (Mogensen and Divac 1993). Finally, the opinions vary about the usefulness of tyrosine hydroxylase as a marker of dopamine-containing axons. Hökfelt et al. (1976, 1984) and Lewis et al. (1988) find it acceptable, but Berger et al. (1985, 1991) have reser-



variations. The dopamine immunohistochemistry, however, stains pericellular baskets in hyperstriatum accessorium (O. Güntürkün, personal communication), removing the doubt about identity of the transmitter.

It is not likely that all parameters found in PFC of one species will be found in PFC of all other species. We already know about similarities and differences of PFC in different mammalian species (Divac and Öberg 1990). Some differences in PFC of the rat and PDLNS of the pigeon have been described especially in activity of glutamic acid decarboxylase (Bissoli et al. 1988). Such differences should be catalogued but cannot alone falsify the hypothesis about PFC and PDLNS equivalence. On the other hand, the values for the AChE in the PFC and PDLNS are among the highest in both species. The same work revealed a chemical difference between the rat and pigeon hippocampi: among the respective cortical samples the activity of AChE has the highest value in the rat hippocampus, but among the lowest in the pigeon. This difference cannot be sufficient to discard the equivalence of the hippocampi in mammals and birds.

In their studies on dopamine receptors in the pigeon brain, Palacios and collaborators (Dietl and Palacios 1988, Camps et al. 1990) consistently found low binding of sulpride in PDLNS. This indication of low density of D2 receptors in this formation is an agreement with the results on dopamine receptors in rats (Tassin et al. 1978, Charuchinda et al. 1987, Lidow et al. 1991, Wamsley et al. 1989). Binding of Schering 23390, a ligand to D1 receptors, however, was inconsistent in different specimens (Dietl and Palacios 1988). A dense dopaminergic innervation and absence of a similarly high density of dopamine receptors in PDLNS appears to be an instance of the mismatch of amounts of a transmitter and density of corresponding receptors emphasized by Herkenham (1987). It should be noticed, however, that an increasing number of dopamine receptor subtypes (D1 to D5) have been described in mammalian brain (Sibley and Monsma 1992). These subtypes, however, have not been characterized in brains of other vertebrates. It

is possible that PDLNS contains a dopamine receptor which under the conditions used in this work does not bind the ligands used in our study.

Architectonical study of the pigeon brain (Rehkämper et al. 1985) has revealed in the caudal part of the neostriatum a region (Ne16) that in its lateral portion, which closely corresponds to PDLNS, contains a dense network of myelinated fibres. The ventrolateral border of this subdivision of the caudal neostriatum is not sharp, resembling the same border as seen in dopamine staining. Thus, not only dopaminergic innervation but also architectonical approach singles out PDLNS as a separate entity of the pigeon brain.

In summary, our data support the hypothetical equivalence of PDLNS and mammalian PFC. Unlike the mammalian PFC, however, the PDLNS does not consistently display a comparable high binding density of D1 receptors. Whether this should contribute to falsification of our hypothesis, will depend on results of further work.

#### ACKNOWLEDGEMENTS

This research was supported by the Danish Medical Research Council, Hasselblad Foundation, Fonden af 1870, Foundation for Research in Neurology, Ib Henriksens Fond, Leo Nielsens Legat and Vera and Carl Johan Michaelsens Legat. Dopamine antibody was kindly provided by M. Geffard. Anna Gagliardo, José Regidor and Jesper Mogensen took part in preparation of some material. F. Riis and M. Løvgreen helped with photography and secretarial work, respectively.

#### REFERENCES

- Baker-Cohen K.F. (1968) Comparative enzyme histochemical observations on submammalian brains. Part I. Striatal structures in reptiles and birds. *Ergebn. Anat. EntwGesch.* 40: 1-41.
- Berger B., Gaspar P., Verney C. (1991) Dopaminergic innervation of the cerebral cortex: unexpected differences between rodents and primates. *Trends Neurosci.* 14: 21-27.
- Berger B., Verney C., Alvarez C., Vigny A., Helle K.B. (1985) New dopaminergic terminal fields in the motor, visual

- (area 18<sup>b</sup>) and retrosplenial cortex in the young and adult rat. Immunohistochemical and catecholamine histochemical analyses. *Neuroscience* 15: 983-998.
- Bissoli R., Battistini S., Guarnieri T., Contestabile A. (1988) Regional levels of neurotransmitter markers in the pigeon telencephalon: A comparison with possibly homologous areas of the rat telencephalon. *J. Neurochem.* 50: 1731-1737.
- Björklund A., Divac I., Lindvall O. (1978) Regional distribution of catecholamines in monkey cerebral cortex. Evidence for a dopaminergic innervation of the primate prefrontal cortex. *Neurosci. Lett.* 7: 115-119.
- Brown R.M., Goldman P.S. (1977) Catecholamines in neocortex of rhesus monkeys: Regional distribution and ontogenetic development. *Brain Res.* 124: 576-580.
- Camps M., Kelly P.H., Palacios J.M. (1990) Autoradiographic localization of dopamine D1 and D2 receptors in the brain of several mammalian species. *J. Neurol. Transm.* 80: 105-127.
- Chagnaud J.-L., Mons N., Tuffet S., Grandier-Vazeilles X., Geffard M. (1987) Monoclonal antibodies against glutaraldehyde-conjugated dopamine. *J. Neurochemistry* 49: 487-494.
- Charuchinda C., Supavilai P., Karobath M., Palacios J.M. (1987) Dopamine D2 receptors in the rat brain: Autoradiographic visualization using a high-affinity selective agonist ligand. *J. Neurosci.* 7: 1352-1360.
- Clark Le Gros W.E., Meyer M. (1950) Anatomical relationships between the cerebral cortex and the hypothalamus. *Br. Med. Bull.* 6 341-345.
- Dietl M., Arluison M., Mouchet P., Feuerstein C., Manier M., Thibault J. (1985) Immunocytochemical demonstration of catecholaminergic cell bodies in the spinal cord of the rat. *Histochemistry* 82: 385-390.
- Dietl M.M., Palacios J.M. (1988) Neurotransmitter receptors in the avian brain: 1. Dopamine receptors. *Brain Res.* 439: 354-359.
- Divac I., Björklund A., Lindvall O., Passingham R.E. (1978) Converging projections from the mediodorsal thalamic nucleus and mesencephalic dopaminergic neurons to the neocortex in three species. *J. Comp. Neurol.* 180: 59-72.
- Divac I., Braestrup C., Nielsen M. (1981) Spiroperidol, naloxone, diazepam and QNB binding in the monkey cerebral cortex. *Brain Res. Bull.* 7: 469-477.
- Divac I., Mogensen J. (1985) The prefrontal 'cortex' in the pigeon. Catecholamine histofluorescence. *Neuroscience* 15: 677-682.
- Divac I., Mogensen J., Björklund A. (1985) The prefrontal 'cortex' in the pigeon. Biochemical evidence. *Brain Res.* 439: 354-359.
- Divac I., Öberg R.G.E. (1990) Prefrontal cortex: the name and the thing. In: *Structure and development of the forebrain in lower vertebrates* (Eds. W. Schwerdtfeger and P. Gereroth) (Experimental Brain Research Series) Springer Verlag, Berlin, p. 213-220.
- Emson P.D., Koob G.F. (1978) The origin and distribution of dopamine-containing afferents to the rat frontal cortex. *Brain Res.* 142: 249-267.
- Herkenham M. (1987) Mismatches between neurotransmitter and receptor localizations in brain: observations and implications. *Neuroscience* 23: 1-38.
- Hökfelt T., Johansson O., Fuxe K., Goldstein M., Park D. (1976) Immunohistochemical studies on the localization and distribution of monoamine neurons systems in the rat brain. I. Tyrosine hydroxylase in the mes- and diencephalon. *Med. Biol.* 55: 427-453.
- Hökfelt T., Johansson O., Goldstein M. (1984) Central catecholamine neurons with special reference to adrenaline neurons. In: *Handbook of chemical anatomy* (Eds. A. Björklund and T. Hökfelt). Vol. 2. Classical transmitters in the CNS, Part 1. Elsevier, Amsterdam, p 157-276.
- Juorio A.V., Vogt M. (1967) Monoamines and their metabolites in the avian brain. *J. Physiol.* 189: 489-518.
- Karten H.J. (1969) The organization of the avian telencephalon and some speculations on the phylogeny of the amniote telencephalon. *Ann. N.Y. Acad. Sci.* 167: 164-180.
- Karten H.J., Hodos W. (1967) A stereotaxic atlas of the brain of pigeon (*Columba livia*). John Hopkins Press, Baltimore, 193 p.
- Lewis D.A., Foote S.L., Goldstein M., Morrison J.H. (1988) The dopaminergic innervation of monkey prefrontal cortex: a tyrosine hydroxylase immunohistochemical study. *Brain Res.* 449: 225-243.
- Lidow M.S., Goldman-Rakic P.S., Gallager D.W., Rakic P. (1991) Distribution of dopaminergic receptors in the primate cerebral cortex: quantitative autoradiographic analysis using [3H]raclopride, [3H]spiperone and [3H]SCH23390. *Neuroscience* 40: 657-671.
- Lindvall O., Björklund A., Divac I. (1978) Organization of catecholamine neurons projecting to the frontal cortex in the rat. *Brain Res.* 142: 1-24.
- Mogensen J., Divac I. (1982) The prefrontal 'cortex' in the pigeon. Behavioral evidence. *Brain Behav. Evol.* 21: 60-66.
- Mogensen J., Divac I. (1993). Behavioural effects of ablation of the pigeon-equivalent of the mammalian prefrontal cortex. *Behav. Brain Res.* 55: 101-107.
- Palkovits M., Zaborszky M., Brownstein M.J., Fekete M.I.K., Hernon J.P., Kanyicska B. (1979) Distribution of norepinephrine and dopamine in cerebral cortical areas of the rat. *Brain Res. Bull.* 4: 593-601.
- Paxinos G., Watson C. (1986) The rat brain in stereotaxic coordinates. 2nd edition, Academic, Sydney.
- Phillipson O.T., Kilpatrick I.C. and Jones M.W. (1987) Dopaminergic innervation of the primary visual cortex in the rat, and some correlations with human cortex. *Brain Res. Bull.* 18: 621-633.
- Rehkämper G., Zilles K., Schleicher A. (1985) A quantitative approach to cytoarchitectonics. X. The areal pattern of the neostriatum in the domestic pigeon, *Columba Livia* f.d. A

- cyto- and myeloarchitectonical study. *Anat. Embryol.* 171: 345-355.
- Rose J.E., Woolsey C.N. (1948) The orbitofrontal cortex and its connections with the mediodorsal nucleus in rabbit, sheep and cat. In: *The frontal lobes. Proc. Assoc. Res. Nerv. Ment. Dis.* p. 210-232.
- Shimizu T., Karten H.J. (1990) Immunohistochemical analysis of the visual Wulst of the pigeon (*Columba livia*). *J. Comp. Neurol.* 300: 346-369.
- Sibley D.R., Monsma F.J. Jr. (1992) Molecular biology of dopamine receptors. *TIPS* 13: 61-69.
- Slopsoema J.S., Van Der Gugten J., De Bruin J.P.C. (1982) Regional concentrations of noradrenaline and dopamine in the frontal cortex of the rat: dopaminergic innervation of the prefrontal subareas and lateralization of prefrontal dopamine. *Brain Res.* 250: 197-200.
- Tassin J.P., Bockaert J., Blanc G., Stinus L., Thierry A.M., Lavie S., Prémont J., Glowinski J. (1978) Topographical distribution of dopaminergic innervation and dopaminergic receptors of the anterior cerebral cortex of the rat. *Brain Res.* 154: 241-251.
- Thibault J., Vidal D., Gros F. (1981) *In vitro* translation of mRNA from rat pheochromocytoma tumors, characterization of tyrosine hydroxylase. *Biochem. Biophys. Res. Commun.* 99: 960-968.
- Van Eden C.G., Hvorneman E.M.D., Buijs R.M., Matthijssen M.A.H., Geffard M., Uylings H.B.M. (1987) Immunocytochemical localization of dopamine in the prefrontal cortex of the rat at the light and electron microscopical level. *Neuroscience* 22: 849-862.
- Waldmann C., Güntürkün O. (1993) The dopaminergic innervation of the pigeon caudolateral forebrain - Immunocytochemical evidence for a prefrontal cortex in birds. *Brain Res.* 600: 225-234.
- Wamsley J.R., Gehlert D.R., Filloux F.M., Dawson T.M. (1989) Comparison of the density and distribution of D1 and D2 dopamine receptors in the rat brain. *J. Chem. Neuroanat.* 2: 119-137.
- Yoshida M., Sakai M., Kani K., Nagatsu I., Tanaka M. (1988) The dopaminergic innervation as observed by immunohistochemistry using anti-dopamine serum in the rat cerebral cortex. *Experientia* 44: 700-702.

Received 20 January 1994, accepted 10 May 1994