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Autoradiographic localization of D1-like dopamine receptors in the forebrain of male and female Japanese quail and their relationship with immunoreactive tyrosine hydroxylase

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Abstract

The distribution of D1-like dopamine receptors was studied in the brain of male and female Japanese quail (*Coturnix japonica*) by means of quantitative autoradiography with ^{3}H-labelled D1 selective antagonist, SCH 23390, serving as a ligand. A specific, saturable, high affinity binding of this ligand was demonstrated. High densities of binding sites were detected in the lobus parolfactorius, olfactory tubercle, and paleostriatum augmentatum. Medium densities were observed in the entire neostriatum and in the external layers of the optic tectum. Similar levels of binding outlined the paleostriatum primitivum, the nucleus pretectalis and the nucleus intercollicularis. Low but significant levels of receptors were also present in the medial preoptic area at the level of the sexually dimorphic medial preoptic nucleus and throughout the infundibulum, as well as in the ectostriatum, medial and lateral septum, and nucleus accumbens. At the level of the medial septum, just dorsal to the anterior commissure, two circular areas of high receptor density corresponding to the nucleus of the septal commissure were also observed. No sex difference in receptor density could be detected in any of these areas. All areas containing high densities of D1 receptors also contained high densities of tyrosine hydroxylase (TH) fibers. However, certain areas characterized by a high density of TH-immunoreactive fibers did not contain appreciable densities of D1-like dopamine receptors. The distribution of this receptor and its relationship to TH-immunoreactivity is consistent with observations made in other vertebrates, suggesting that the dopaminergic system is evolutionarily highly conserved among amniote vertebrates.

Keywords: Dopamine receptors; Male sexual behavior; Quantitative autoradiography; Preoptic area; Birds

1. Introduction

The catecholamine, dopamine, has been implicated in the control of a variety of behaviors including male reproductive behavior, feeding behavior, and defensive responses (Blackburn et al., 1992; Meisel and Sachs, 1994). This role for dopamine has been most convincingly demonstrated in rodents, but also suggested in relation with some of these behaviors in birds (Balthazart and Ball, 1992; Absil et al., 1994). A full understanding of the behavioral implications of dopamine has been hindered by the relative lack of information concerning the neuroanatomical organization of the dopamine system in non-mammalian vertebrates. In birds, dopamine has been measured via fluorometric assays or high performance liquid chromatography (HPLC) in sub-regions of the brain in pigeons (*Columba livia*; Jurio and Vogt, 1967), quail (*Coturnix japonica*; Ottinger et al., 1986; Ottinger and Balthazart, 1987; Balthazart et al., 1992), and zebra finches (*Taeniopygia guttata*; Barclay et al., 1988, 1990). Its biosynthetic enzyme, tyrosine hydroxylase (TH), has been localized via immunohistochemical methods in the brain of Japanese quail (Bailhache and Balthazart, 1993), zebra finches (Bottjer, 1993) and parts of the brain of other avian species (Reiner et al., 1983; Knigge and Piekut, 1985; Kiss and Peczely, 1987; Anderson et al., 1991; Berk, 1991). Dopamine itself has been visualized with the use of an immunocytochemical procedure in various

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species (Steinbusch and Tilders, 1987), including chickens (Moons et al., 1994) and pigeons (Divac et al., 1994). Dopamine receptors have been investigated via autoradiography in the brain of pigeons (Richfield et al., 1987; Dietl and Palacios, 1988) and European starlings (*Sturnus vulgaris*; Casto and Ball, 1994).

Dopamine has been linked to male reproductive function in quail (Balthazart and Ball, 1992; Absil et al., 1994), and some aspects of its functions, such as variation in dopamine turnover (Balthazart et al, 1992) and the localization of TH (Bailhache and Balthazart, 1993), have already been studied in the quail brain. Receptors represent another component in the chain of actions by which dopamine exerts its effects. The anatomical localization of receptor types is an important step toward an understanding of how receptors might be causally involved in a particular function and for subsequent studies of the regulation of receptors. It is also useful to compare the distribution of receptor subtypes to other markers of dopamine action. Localizing receptor subtypes could be particularly important for the investigation of dopamine action in relation to male sexual behavior. Dopamine is known to stimulate male sexual behavior in rats, acting, in part, through a D1 receptor subtype (Blackburn et al. 1992; Meisel and Sachs, 1994). In particular, the infusion of the D1 selective antagonist, SCH 23390, into the preoptic area has been shown to significantly retard male copulatory behavior in rats (Hull et al., 1989). The preoptic region is important for the activation of male copulatory behaviour in other species including quail, where it has been shown that the action of testosterone in the preoptic region is necessary and sufficient for the activation of male copulatory behavior (Balthazart and Surlemont 1990a, 1990b; Balthazart and Ball, 1992).

In this paper, we use autoradiography procedures to localize 'D1-like' receptors in the brains of male and female quail. We selected the D1 antagonist SCH 23390 as the ligand for labeling this subtype because it is known to be effective in retarding male sexual behavior in several species (Meisel and Sachs, 1994). In order to understand the relationship of these binding sites to the dopaminergic system in quail, we also compare the distribution of SCH 23390 binding sites in the quail brain with the distribution of the biosynthetic enzyme, TH. The binding characteristics of SCH 23390 have been described in detail in mammals. We performed additional studies to confirm that this ligand binds to D1-like receptors in quail in a manner similar to what has been described in mammals.

2. Materials and methods

2.1. Subjects and tissue preparation

Male and female Japanese quail (*Coturnix japonica*) were obtained as juveniles from a local dealer in Belgium and kept in individual cages for a few weeks until they were sexually mature. In males, this is demonstrated by the presence of a large cloacal gland and, in females, by regular egg laying. During this time, food and water were available ad libitum and birds were exposed to a photoperiod of 16 h of light and 8 h of dark per day.

For autoradiographic studies, birds were killed by decapitation and their brains were quickly removed and frozen on powdered dry ice. Frozen brains were cut on a cryostat, and for 8 males and 8 females, coronal sections (16 μm) were collected throughout the entire telencephalon, diencephalon and mesencephalon. For one male, sections in the sagittal plane were collected. Adjacent sections were thaw-mounted on gelatin-coated slides (two per slide), dried at room temperature, and stored at −70°C until receptor binding assays were performed. The sampling protocol for brain sections from each bird used in the assay consisted of using four adjacent sections (two for determination of total binding, and two for determination of nonspecific binding; see below), then skipping six sections and repeating this sampling procedure.

An additional group of adult birds was used for describing the distribution of the dopamine synthesizing enzyme, TH. These birds were deeply anesthetized with Hypnodil (Janssen Pharmaceutica, Beerse, Belgium, 50 mg/kg body weight) and injected intravenously with 50 μl of heparin solution (Sigma H-7005, 20 mg/ml or 3340 units/ml). They were then perfused through the heart with a saline solution (9 g/l; 0.15 M NaCl), followed by 300–500 ml of fixative (paraformaldehyde 4% and 0.1% glutaraldehyde in 0.15 M phosphate buffer (PB), pH 7.2). Brains were immediately dissected out of the skull, postfixed one hour in the fixative solution without glutaraldehyde and placed overnight in a 20% sucrose solution in 0.1 M PB, pH 7.2. They were then frozen on powdered dry ice and stored in a freezer at −75°C until used.

2.2. Drugs

[^{3}H]SCH 23390 (SA 60.3 Ci/mmol) was obtained from New England Nuclear (Boston, MA). The following unlabeled ligands were used as cold competitors in the general autoradiography procedure and/or in the validations of receptor specificity: (+)butaclamol HCl (a general D1/D2 antagonist), (+)SCH 23390 HCl (a D1-specific antagonist), (−)sulpiride (a D2-specific antagonist), (−)apomorphine HCl (a general D1/D2 agonist), (+)SKF 38393 HCl (a D1-specific agonist), (−)propyl-norapomorphine HCl (−PNApomorphine, a D2-specific agonist). In every case, the inactive enantiomer was also used. Additional competition studies were also carried out with the endogenous ligand, dopamine HCl, and with the potent antagonist, *cis*-flupentixol. All unlabeled drugs were obtained from Research Biochemicals Incorporated (RBI, Natick, MA).

2.3. Quantitative receptor autoradiography

The assay was adapted from previously described procedures (Dawson et al., 1986; Casto and Ball, 1994). A 50 mM Tris-HCl (pH 7.4), 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ buffer was used in this assay. For the assay, slides were first fan-dried for 30 min. They were then preincubated in buffer at room temperature for 30 min. Next, slides were incubated at room temperature for 60 min in 2.0 nM [^{3}H]SCH 23390 (specific activity: 73.6 Ci/mmol, Dupont-New England Nuclear, Boston, MA) in buffer. This was followed by two 5 min washes in ice cold buffer and a final dip in ice cold distilled water before fan-drying. Nonspecific binding was estimated by incubating adjacent sections in a medium containing 2.0 nM [^{3}H]SCH 23390 and a cold competitor, (+)butaclamol HCl (2 μM concentration). The slides were exposed to tritium-sensitive Hyperfilm (Amersham Corps, Arlington Heights, IL) for 13 days. The film was developed for 5 min in Kodak D-19 developer, and fixed for 2 min with Kodak rapid fixer.

Digitized autoradiograms were analyzed with 'Image', an image analysis software package (version 1.41, Wayne Rasband, NIH, Bethesda, MD). All binding data were determined from film density in areas of interest (see below). Tritium standards (American Radiolabeled Chemicals Inc. St. Louis, MO) were co-exposed with the slides and used to convert optical density to fmol of tritiated ligand specifically bound per mg of protein. Mean protein content has been previously determined in quail and song birds (Ball et al., 1989) and is approximately 10% of tissue wet weight. Specific binding in areas of interest was defined as the density of binding in the absence of butaclamol (total binding) minus binding in the presence of butaclamol (nonspecific binding) in adjacent sections. Measurement of all structures was performed bilaterally in order to test for hemispheric differences in receptor density; however, repeated measures analyses of variance (ANOVA) revealed no significant hemispheric differences in [^{3}H]SCH 23390 binding; therefore, density measures from the right and left sides were averaged, yielding a mean receptor density value per region. The neuroanatomical nomenclature used in this report is based on previous studies of the quail and chicken brain (Baylé et al., 1974; Kuenzel and VanTienhoven, 1982; Kuenzel and Masson, 1988, Panzica et al., 1991).

2.4. Binding characteristics

The binding characteristics of [^{3}H]SCH 23390 have been thoroughly described in mammals. Similar binding characteristics seem to be present in birds based on one study of European starlings (Casto and Ball, 1994). Additional experiments were performed in order to confirm that [^{3}H]SCH 23390 binds with similar characteristics in quail. To minimize variance in receptor density between sections, 20 μm brain slices were sectioned from minced brain tissue prepared from the rostral forebrains of three male quail and formed in a mold with a diameter of 11 mm. The rostral forebrain was chosen because it contains structures in the basal ganglia that are known to receive an enriched dopaminergic innervation. Assays were carried out using the same procedures as in receptor autoradiography except that at the end of the assay, sections were wiped from the slides with glass fiber filters (Whatman International Ltd., Maidstone, England) and radioactivity bound per slice was determined by scintillation counting.

To determine the affinity (apparent dissociation constant, Kd) and saturability (maximum number of binding sites, B_{max}) of [^{3}H]SCH 23390 binding, brain-mash sections were incubated with a range (0.1–14.6 nM) of [^{3}H]SCH 23390 concentrations that bracketed the incubation concentration used in the receptor autoradiography (described above). Nonspecific binding was estimated at every other concentration by incubating sections with 2 μM butaclamol. Sections were wiped off slides with glass fiber filters and the bound radioactivity was quantified by scintillation counting.

Competition experiments were performed in order to evaluate the specificity of [^{3}H]SCH 23390 for D1 dopamine receptors. Two sections of brain mash were incubated with a 2 nM concentration of [^{3}H]SCH 23390 and one of two different concentrations (10^{-6} and 10^{-8} M) of several unlabeled dopamine receptor specific compounds or their inactive enantiomers (described above). In addition, five concentrations (10^{-5} to 10^{-9} M) of unlabeled dopamine were used to compete with a 2 nM concentration of [^{3}H]SCH 23390. Finally, the displacement of [^{3}H]SCH 23390 binding by the two most active antagonists ((+)butaclamol and SCH 23390) was statistically compared with the displacement produced by similar concentrations of another D1 antagonist, *cis*-flupentixol (five sections of brain mash in each condition). As in the saturation experiments, sections were wiped off slides and counted in a scintillation counter.

Because [^{3}H]SCH 23390 has been reported to bind to serotonin receptors (5-hydroxytryptamine, 5-HT), in particular the 5-HT_2 sub-type (see Dawson et al., 1986), another competition experiment was performed on film. The competitive 5-HT_2 receptor antagonist, ketanserin (200 nM concentration, RBI, Natick, MA), was added to a small subset of the slide mailers containing incubation medium and a subset containing incubation medium plus butaclamol. The autoradiograms of sections incubated with and without ketanserin were compared to determine the amount of nonspecific and total binding resulting from the ligand binding to 5-HT_2 receptors.

2.5. Tyrosine hydroxylase (TH) immunocytochemistry

Brains (8 males and 8 females) were cut with a

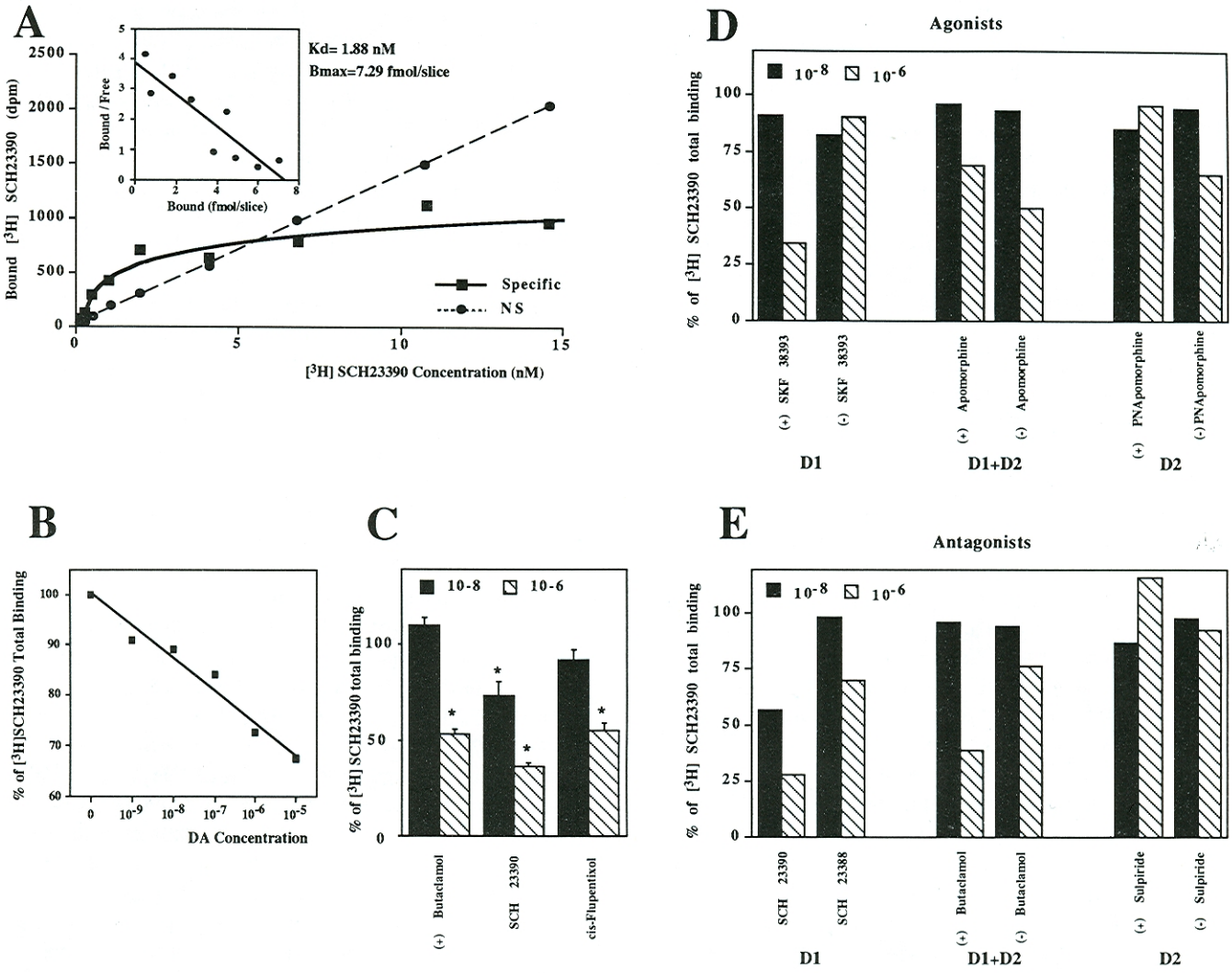

Fig. 1. Saturation and specificity analysis of the [^{3}H]SCH 23390 binding in the quail brain. A. Direct plot of the saturation binding for [^{3}H]SCH 23390 measured at equilibrium conditions (60 min at 23°C) with specific binding defined as the excess over non-specific binding derived from counting the concentrations of [^{3}H]SCH 23390 incubated in the presence of 2 μM of (+)butaclamol. The insert shows the Scatchard plot of the specific binding. Five brain mash sections were used at each concentration and counted in a scintillation counter in individual vials. Sections were counted twice, average disintegrations per minute (dpm) were calculated for each section, the dpms for the five sections from each concentration of ligand were then averaged and reported as the average total dpm or non-specific dpm for each concentration of the ligand. These dpm values were then transformed into fmols of [^{3}H]SCH 23390. B. Effect of five increasing doses of the endogenous ligand, dopamine, on the total binding of [^{3}H]SCH 23390. For each concentration of dopamine, 5 individually mounted brain-mash sections were incubated with a 2 nM concentration of [^{3}H]SCH 23390 in the presence of the specified dopamine concentration. C. Statistical comparison of the ability of a low (10^{-8}) and a high (10^{-6}) dose of three D1 antagonists to compete for [^{3}H]SCH 23390 binding sites. Five replicates of each condition were analyzed. The one-way ANOVA of these data indicated a significant effect of the drugs ($F_{6,28} = 27.272$, $P \leq 0.0001$); asterisks indicate significant inhibition compared to the total binding for $P = 0.05$ as determined by the Fisher protected least significant difference test. There was no significant difference between the effects of (+)butaclamol and *cis*-flupentixol at the 10^{-6} M concentration. D and E. Bar graphs illustrating the ability of a low (10^{-8}) and a high (10^{-6}) dose of a selection of dopaminergic agonists (C) or antagonists (D) to compete for binding sites with a 2 nM concentration of [^{3}H]SCH 23390. PNApomorphine = propylnorapomorphine. The drugs used in these competitions are either pure D1, mixed D1+D2 or pure D2 agonists and antagonists. The active and inactive enantiomers have also been compared in each case. Two sections were used for each concentration of each competitor and average values are reported in each case. Two sections of brain mash were also incubated with a 2 nM concentration of [^{3}H]SCH 23390 in the absence of any cold competitor. The mean dpm from these two sections served as the standard of 100% [^{3}H]SCH 23390 binding to which cold competitors could be compared.

cryostat in the coronal plane at 30 μm thickness starting at the rostral end. A standard peroxidase anti-peroxidase (PAP) immunocytochemical procedure using diaminobenzidine as the chromogen was then used to visualize TH on free floating sections. Bailhache and Balthazart (1993) contains a full description of the methods and results of this study. We focus here on the relationship between TH-immunoreactive structures and D1 receptors.

3. Results

3.1. Saturation and specificity analysis

Results of saturation and competition experiments suggest that [^{3}H]SCH 23390 binding to homogenized brain tissue was saturable, specific, and of high affinity (see Fig. 1). Scatchard analysis of the binding data indicated a K_d of 1.88 nM and a B_{max} of 7.29 fmol/slice (see Fig. 1A). Non-specific binding exceeded specific

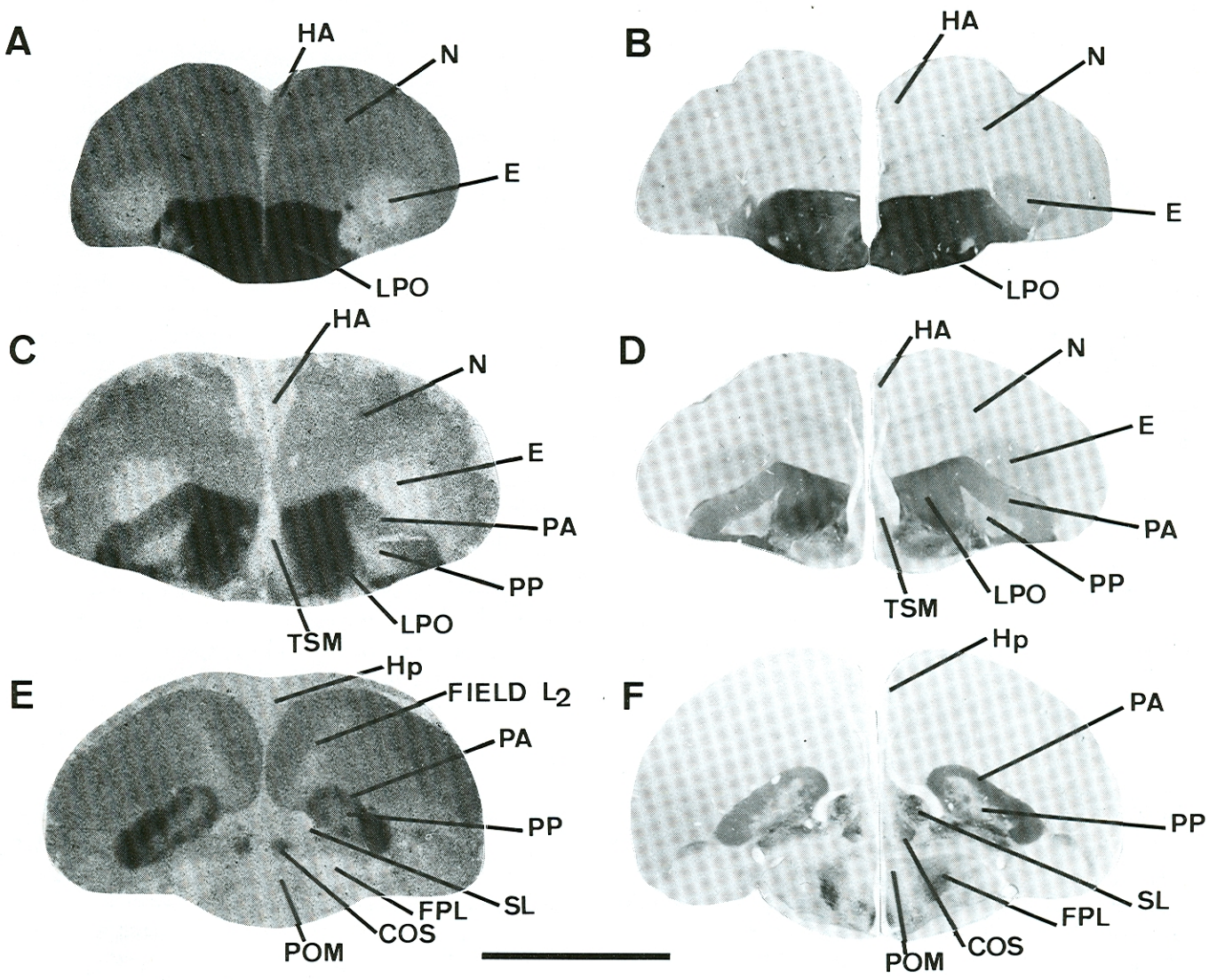

Fig. 2. Comparison of the distribution of [^{3}H]SCH 23390 binding (panels A, C and E) and of the immunoreactive tyrosine hydroxylase (panels B, D and F) at three levels of the quail brain. The pairs of panels A-B, C-D and E-F are organized in a rostral to caudal order. A high level of concordance was observed in the distribution of these two neurochemical markers (see for example the LPO, E, PA and PP). However, some discrepancies were present, namely in the lateral septum (SL) and in the lateral parts of the preoptic area. The panels illustrating the autoradiographic and immunocytochemical data were prepared by necessity with sections derived from different brains because autoradiography has to be performed on fresh frozen material while the immunocytochemical localization of TH can only be done reliably on perfused brains. In addition, the two sets of sections were treated by different procedures which resulted in differential swelling and distortion of the tissue. Therefore, the sections selected for each pair show slight differences in the topographical organization, although they have been selected to represent levels in the brain that are as similar as possible. Magnification bar = 5 mm.

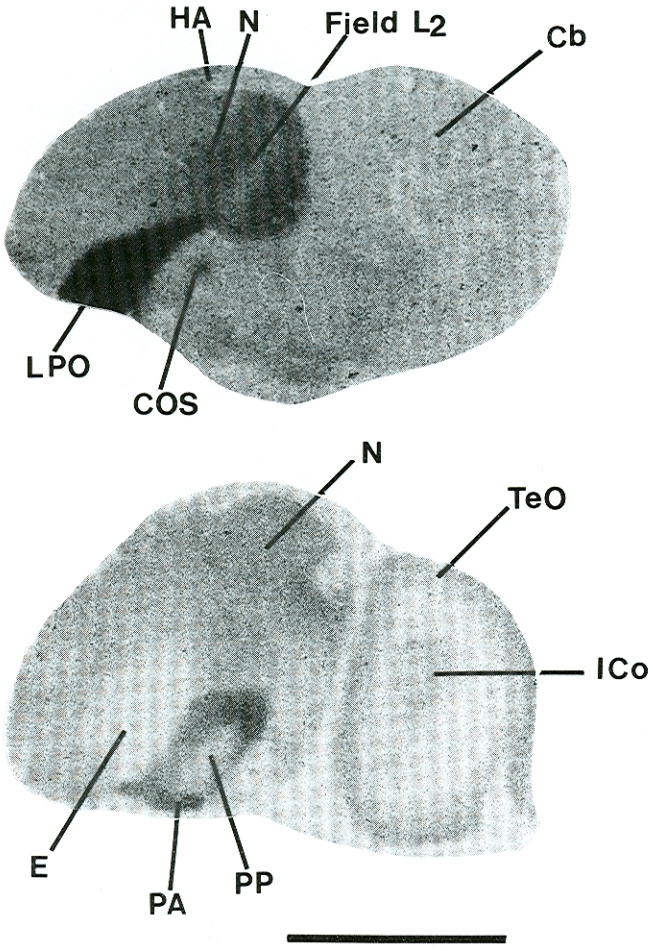

Fig. 3. Two autoradiograms in the sagittal plane illustrating the [^{3}H]SCH 23390 binding in the quail brain. Section A is more medial than section B. Magnification bar = 5mm.

binding in the higher range of ligand concentration (above 5nM). The binding of SCH 23390 was markedly displaced by dopamine (Fig. 1B). A comparison of the displacement of the ligand by a variety of selective and non-selective dopamine compounds revealed an order of potency characteristic of a D1-like receptor. The D1-specific compounds had a higher potency than non-selective compounds or D2-specific compounds. This was true for both agonists and antagonists. The inactive enantiomers had little or no effect in this study (Figs 1D and 1E). Very high and comparable displacements of [^{3}H]SCH 23390 binding were obtained in sections incubated in the presence of cold SCH 23390, (+)butaclamol or *cis*-flupentixol at the 10^{-6} M concentration (Fig. 1C). No additional decrease in binding was observed when ketanserin was added to the incubation medium.

3.2. Regional distribution of [^{3}H]SCH 23390 binding in the quail brain

Representative autoradiograms illustrating the [^{3}H]-SCH 23390 binding at different levels in the rostral to caudal axis of the quail forebrain are presented in Figs. 2 (left part, coronal sections) and 3 (sagittal sections).

D1 receptor binding was quantified in 8 males and 8 females as described above and the results of these analyses are summarized in Fig. 4.

The highest densities of receptors were observed in the rostral part of the telencephalon, specifically in the lobus parolfactorius, the paleostriatum augmentatum, and the olfactory tubercle that is ventral to these structures. Medium to high levels of binding were also present in the entire neostriatum and in the external layers of the optic tectum. Similar levels of binding outlined the paleostriatum augmentatum as well as the nucleus pretectalis and the nucleus intercollicularis. This latter nucleus was easily identified both in its medial and lateral part by a high density of D1 receptors relative to the surrounding mesencephalon. The nucleus mesencephalicus lateralis, pars dorsalis, an auditory nucleus, appeared as a hole almost devoid of binding sites in the middle of the nucleus intercollicularis. A subregion of the primary auditory projection, Field L, was also characterized by a very low level of receptor density in comparison to the surrounding medial caudal neostriatum and lateral caudal neostriatum. This area appears to correspond to sub-region L_2 in the nomenclature proposed by Bonke et al. (1979) and sub-region L_{2A} in the nomenclature of Fortune and Margoliash (1992).

Low but significant levels of receptors were also present in the medial preoptic area at the level of the sexually dimorphic medial preoptic nucleus and throughout the infundibulum as well as in the ectostriatum, in the medial and lateral septum, in the nucleus accumbens. At the level of the medial septum, just above the anterior commissure, two circular areas of high receptor density compared to the surroundings were also detected. They appeared to correspond to the nucleus of the septal commissure as defined by Baylé et al. (1974).

3.3. D1 receptor density in male and female quail

The entire set of quantitative data collected in 8 males and 8 females was first analyzed by a two way ANOVA with the sex of the birds being considered as an independent factor and the brain area considered as a repeated factor. This analysis indicated the absence of an overall sex difference in binding ($F_{1,10} = 0.0036$, $P = 0.9535$) but demonstrated a significant effect of the area considered ($F_{20,180} = 74.682$, $P < 0.0001$) and no interaction between the two factors ($F_{20,180} = 0.6330$, $P = 0.08842$). In this general analysis, data corresponding to a few subjects had to be ignored because, due to technical reasons (damaged sections), these could not be sampled for all brain areas considered.

A second series of analyses was therefore completed by Student's *t*-test to insure that no sex differences lim-

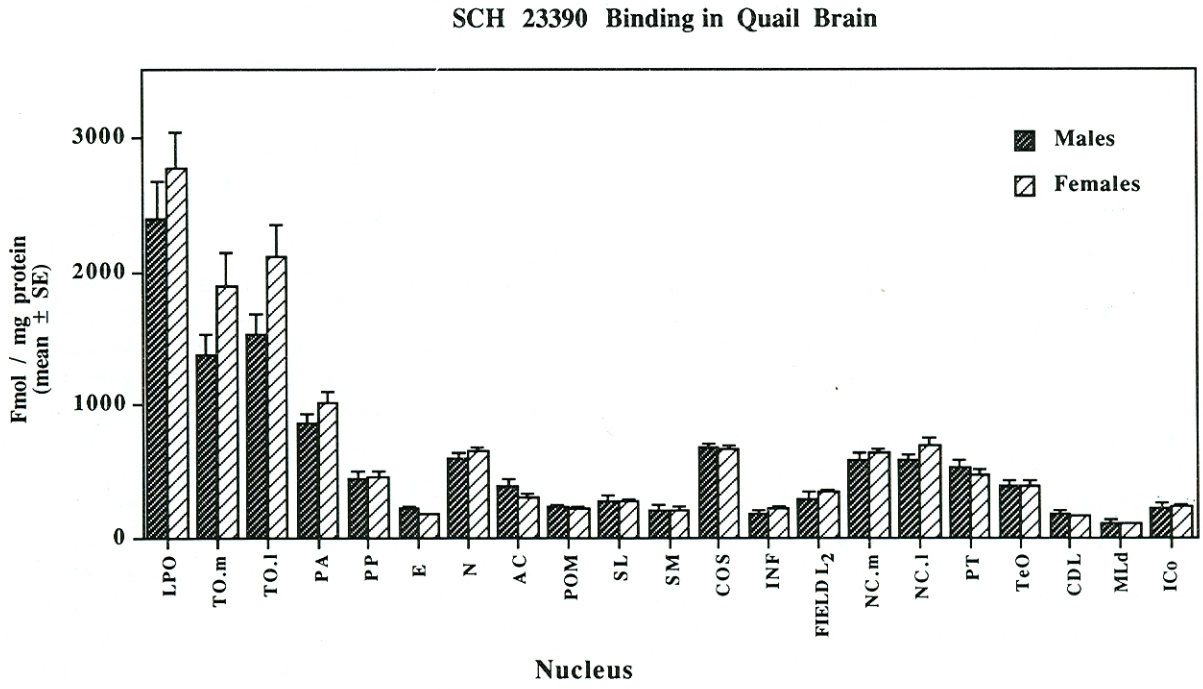

Fig. 4. Bar graph illustrating the specific binding of [^{3}H]SCH 23390 in various brain nuclei of male and female Japanese quail. No significant sex difference in receptor density was detected in any structure. Areas that were quantified are arranged in a rostral to caudal order.

ited to a single area went undetected. In agreement with the general analysis, the comparison of data for each brain region in males and females revealed no statistically significant differences in mean density of binding ($P > 0.05$ by Student's t-test).

3.4. *Comparison between [^{3}H]SCH 23390 binding and TH immunoreactivity in the quail brain*

We previously described the distribution of the dopamine synthesizing enzyme, TH, in the quail brain (Bailhache and Balthazart, 1993) and this information is compared here with the distribution of the D1 receptors. Representative coronal sections taken at similar levels in the quail brain and stained by immunocytochemistry for TH or labelled by autoradiography for the D1 receptor subtype are shown in Fig. 2 (right and left parts, respectively). In general, TH-immunoreactive fibers were present in brain areas that were shown here to contain high densities of D1 receptors. It is clear, however, that the reverse was not necessarily true and that in some areas that were densely innervated by TH-immunoreactive fibers, very low levels of D1 receptors were detected. In the rostral telencephalon, excellent correspondence between TH-immunoreactivity and D1 receptors was found at the level of the lobus parolfactorius, paleostriatum augmentatum, tuberculum olfactorium, paleostriatum primitivum and ectostriatum. These first three structures were very heavily labelled by [^{3}H]SCH 23390 binding and they also contained very high densities of TH-immunoreactive fibers. By contrast, the last two regions were almost devoid of binding and of immunoreactive material (see Fig. 2A-B, C-D). At the level of the anterior commissure, dense [^{3}H]SCH 23390 binding still defined the paleostriatum augmentatum which surrounds the paleostriatum primitivum. This structure exhibited a very low level of binding (Fig. 2E-F). This also matched well with the presence/absence of TH fibers. Interestingly, two dark areas of high D1 receptor density were observed above the anterior commissure at the level of the nucleus commissuralis septi and these areas were also clearly identified by a higher density of TH fibers compared to the surrounding area. In contrast, dense TH innervation was present in the lateral septum and in the lateral parts of the preoptic area, but very low levels of receptor were detected in these areas (see Fig. 2E-F). Finally at the mesencephalic level, structures such as the nucleus pretectalis and the nucleus intercollicularis could be outlined by both a higher level of D1 receptors and TH-immunoreactive fibers compared to surrounding areas. Other regions, however, contained very high densities of TH-immunoreactive fibers (e.g., the fasciculus prosencephali lateralis or the ventral preoptic area, Fig. 2E-F) but they were not clearly labelled by the [^{3}H]SCH 23390 binding.

4. Discussion

4.1. Characterization of the D1 dopamine receptor in the quail brain

The saturation analysis performed here demonstrated that the binding of [^{3}H]SCH 23390 in the quail brain has a high affinity in the nanomolar range and is saturable. The apparent dissociation constant that we observed in quail is in the same order of magnitude as values previously reported for rats (Richfield et al., 1987) and starlings (Casto and Ball, 1994). Accordingly, similar levels of maximal binding have been observed in these species and in the present work. Much lower receptor densities (approximately 10-fold based on a comparison of specific areas measured on the film autoradiograms) have, however, been reported in pigeons (Richfield et al., 1987; Dietl and Palacios, 1988). These data appear to reflect real species differences rather than experimental artefacts, because they have been confirmed within the same study utilizing identical methods (Richfield et al., 1987). The functional significance of such species differences is difficult to ascertain with the data available at present, but it can be noted that the brain dopamine levels appear to be roughly the same in pigeons (Jurio and Vogt, 1967) and doves (*Streptopelia risoria*; Lea et al., 1992) as they are in rats (e.g., Renner and Luine, 1984) or in other avian species including zebra finches (Barclay and Harding, 1988, 1990) and quail (Balthazart et al., 1992). With the possible exception of the pigeon, the relative levels of dopamine and D1 receptors appear to be similar across a diversity of vertebrate species.

The competition studies performed with various dopamine antagonists and agonists revealed a competitive order of potency characteristic of D1 receptors as previously described in pharmacological studies of mammalian species (Gingrich and Caron, 1993). Dopamine receptors have been divided pharmacologically into two major groups: the 'D1-like' receptors and the 'D2-like' receptors. In general, the 'D1-like' receptors stimulate adenylyl cyclase (AC) and show a high affinity for benzazepines, such as SCH 23390, and low affinity for butyrophenones (Gingrich and Caron, 1993). 'D2-like' receptors inhibit AC but stimulate K^+ channels and have a high affinity for butyrophenones and certain benzemides but a low affinity for benzazepines (Gingrich and Caron, 1993). Molecular cloning studies, based on mammalian species, suggest that there are two D1-like receptor genes and three D2-like genes (Gingrich and Caron, 1993). Recent work in non-mammalian vertebrates such as *Xenopus laevis* and domestic chickens (*Gallus domesticus*) indicate that there are more than two D1-like receptors in the vertebrate brain (Sugamore et al., 1994; Demchyshyn et al., 1994). These authors have named these receptors D1A, D1B, D1C and D1D. The chicken brain was reported to contain the D1A, D1B and D1D receptor sub-types. It is highly likely that these three forms of the D1-like receptors are present in the quail brain and that the ligand SCH 23390 is labeling at least a portion of all three types. However, until more is known about the exact binding properties of these three D1-like subtypes, we can not entirely dismiss the possibility that some might not have been identified with the present approach.

Dopamine receptor ligands, including SCH 23390, have been shown to exhibit significant affinity for what are now referred to as the 5-HT_{2A} and 5-HT_{2c} serotonin receptor sub-types (e.g. Dawson et al., 1986; McQuade et al., 1988; see Peroutka, 1994 and Sadou and Hen, 1994 for discussion of 5-HT receptor nomenclature). There was no evidence for such a serotoninergic component to the receptor densities observed in the present study because incubating the sections with high levels of ketanserin had no apparent effect. However, without more extensive competitions with a variety of serotoninergic compounds, we can not completely dismiss the possibility that a sub-set of the binding sites labeled by SCH 23390 in the quail brain represent 5-HT receptors. In particular, binding to the 5-HT_{2c} receptor sub-type should be investigated further given that ketanserin does not block these sites well and Palacios and Dietl (1988) have reported high densities of these receptors in the forebrain of pigeons.

4.2. Anatomical localization of D1 dopamine receptors in the quail brain

There have been three previous reports localizing D1 receptors with autoradiographic methods in parts of the avian brain. Two of these studies focused exclusively on the basal ganglia (Richfield et al., 1987; Casto and Ball, 1994). Neurochemical criteria, including the relative densities of receptor sub-types, are among several methods that can be profitably employed in attempts to reconstruct the evolution of the vertebrate brain and establish homologies among regions in different species (Brauth, 1990). Studies of the dopamine system in various vertebrate species have been used to infer homologies among the various structures that make up the basal ganglia (e.g., Parent, 1986; Camps et al., 1990). In this study, we find that D1 receptors are enriched in the lobus parolfactorius (LPO) and the paleostriatum augmentatum (PA), two areas that are thought to be homologous to the caudate-putamen (Parent, 1986; Reiner et al., 1984). The paleostriatum primitivum (PP), the avian homologue of the globus pallidus, contains a low density of receptors relative to these other structures. These findings are in good accord with other avian studies of the D1 receptor in birds (Richfield et al., 1987; Dietl and Palacios, 1988).

Work on a variety of mammals has found the highest levels of D1 receptors in the caudate-putamen complex

(e.g., Dawson et al., 1986; Richfield et al., 1987; Camps et al., 1990). We detected moderate and uniform densities of D1 receptors in telencephalic areas such as the neostriatum. An equivalent receptor density was also observed in other telencephalic areas such as the various parts of the hyperstriatum. Because of our inability to differentiate among the sub-divisions of the hyperstriatum on the basis of D1 receptor density, no specific measures are provided. It has been claimed that subdivisions of the neostriatum and the hyperstriatum are homologous to parts of the neocortex in mammals (e.g., Karten and Shimizu, 1989; Karten, 1991; Hartwig, 1993). Moderate densities of D1 receptors have been observed in the cortex of several mammalian species including rats, rhesus monkeys, and humans (Boyson et al., 1986; Dawson et al., 1986; Savasta et al., 1986; Cortés et al., 1989; Lidow et al., 1991). The presence of D1 receptors in the neostriatum and ventral hyperstriatum is consistent with the claim that these areas are homologous to the neocortex. Divac et al. (1994) have identified a dense plexus of immunoreactive dopamine in a sub-region of the pigeon neostriatum (the posterodorso-lateral neostriatum). They have argued that this is further evidence that this sub-region of the neostriatum is comparable to the mammalian prefrontal cortex. However, as noted by Karten (1991), many areas of the avian neostriatum and hyperstriatum lack a laminar organization characteristic of the mammalian neocortex. This laminar organization is apparent when examining the distribution of D1 receptors in the brains of monkeys and humans (e.g., Cortés et al., 1989; Lidow et al., 1990, 1991), but no such organization is apparent in the quail brain or in the autoradiograms illustrating the density of D1 receptors in quail.

Detectable but low levels of receptor density were measured in preoptic area and parts of the hypothalamus such as the infundibular complex. A similar pattern has also been described in humans and rats (Boyson et al., 1986; Dawson et al., 1986; Cortés et al., 1989). Other notable features of the binding pattern in quail include structures in the visual system such as the outer layers of the optic tectum and the nucleus pretectalis that contain moderate densities of receptors. This is especially striking in the case of pretectalis, where the boundaries of the nucleus are sharply defined by the high D1 receptor density. Moderate levels of D1 receptors have similarly been reported in the homologous structures of the mammalian brain. Within the telencephalon, there is also a striking absence of receptors in a region that corresponds to a sub-region of the primary auditory projection area, Field L (Fig. 2E). Dietl and Palacios (1988) found a similar lack of binding in pigeons, as is apparent in their Fig. 1, panel B. This sub-region appears to correspond to Field L_2 in the nomenclature of Bonke et al. (1979) based on work with guinea fowl (Numida meleagris) or L_{2A} based on the more recent nomenclature proposed by Fortune and Margoliash (1992) for this area of the brain in zebra finches. This sub-region receives its main input from the nucleus ovoidalis and projects to the ventral hyperstriatum and to other parts of the neostriatum (Bonke et al., 1979). In mammals, auditory areas have apparently not been highlighted by the pattern D1 receptor density.

In the mesencephalon, the nucleus intercollicularis/nucleus mesencephalicus lateralis, pars dorsalis complex exhibits a heterogeneous pattern of receptor density. This complex has recently been the subject of an extensive review with new recommendations concerning nomenclature based on a variety of neurochemical criteria (Puelles et al., 1994). It has been recommended that the entire complex be referred to as the avian torus semicircularis, a name widely used in the older literature. Based on this classification, the high D1 receptor density we observed corresponds most closely to the external subdivision of the toral nucleus, possibly extending into the shell part of the central nucleus and into the caudomedial shell nucleus of the toral nucleus (see Puelles et al., 1994 for a detailed description of these areas). In the previous, simpler classification, this would have been identified as the medial and lateral parts of the nucleus intercollicularis surrounding the nucleus mesencephalicus lateralis (MLd), which is an area of low D1 receptor density. This general heterogeneous pattern of high D1 receptor density is reminiscent of what we have reported previously for α_2 adrenergic receptors and muscarinic cholinergic receptors (Ball and Balthazart, 1990).

4.3. Relationship between the distribution of D1 dopamine receptors and immunoreactive TH in quail brain

A number of discrete dopamine cell groups have been identified in mammals and the major projections from these dopamine cells have been established (see Björklund and Lindvall, 1984 for review). In a recent study, we identified the TH-immunoreactive cell groups in the quail brain and tentatively proposed a classification taking into account the most probable homologies with the mammalian cell groups (Bailache and Balthazart, 1993). The areas of the quail brain containing TH-immunoreactive fibers were also detailed in that study. They overlap extensively with the brain regions that are shown here to contain D1 receptors. In the cases where we do not observe any overlap, it could be because of the sensitivity of the receptor labeling procedure or because the D2 receptor subtype is the predominant subtype in that region. In addition, immunoreactivity for TH does not only label dopamine structures; therefore, in some cases, noradrenaline fibers were also labeled. Despite the potential technical differences between these methods, the neuroanatomical correspondence between these two views of the dopamine

system is excellent, especially in the telencephalic areas (see Fig. 2). Discrepancies were more frequent in the diencephalon than in the telencephalon. For example, a substantial number of immunoreactive TH cells and fibers were observed throughout the hypothalamus, while the density of D1 receptors in the corresponding area was very low and certainly did not allow the identification of subregions within the hypothalamus as the TH immunocytochemistry does. A comparison of regional variations in receptor density must be done cautiously. A full interpretation of such variations requires information concerning the dynamics of the transmitter system; in many cases, a low receptor density may be biologically significant for synaptic transmission. Romero et al. (1990, 1991) have also claimed that certain TH immunoreactive neurons in the chick diencephalon do not actually synthesize dopamine because the final enzyme necessary for dopamine synthesis, dopa decarboxylase, is not present in these cells. Thus, the localized lack of correspondence between TH immunoreactivity and D1 receptor density observed in the quail hypothalamus will only be better understood when more data on the dopamine system in this species become available (e.g., distribution of D2 receptors, of dopamine itself, other measures of D1 receptor distribution).

4.4. Functional implications of the distribution of D1 dopamine receptors in the quail brain in relation to male sexual behavior

Previous experiments in quail have demonstrated that the dopamine turnover as measured by the α-methyl-*para*-tyrosine-induced depletion of the transmitter concentration is significantly higher in several areas of male brains as compared to female brains (Balthazart et al, 1992). Furthermore, this sexually differentiated dopamine turnover was still present when birds were placed in identical endocrine conditions, i.e., when subjects of both sexes were gonadectomized and eventually submitted to a replacement therapy with testosterone. This suggests that the sex difference in dopamine turnover does not result from a differential activation by steroids in adulthood but, rather, represents a sexually differentiated characteristic presumably organized by neonatal steroids. There is a large amount of evidence, derived primarily from rat studies, demonstrating that dopamine is implicated in the activation of male sexual behavior (e.g., Blackburn et al., 1992; Meisel and Sachs, 1994). This also seems to be the case in quail as shown by recent pharmacological studies (Absil et al., 1994). Since sexual behavior is sexually differentiated in quail and can only be activated by testosterone in males (Adkins, 1975; Balthazart et al., 1983), it is tempting to speculate that the sexually differentiated dopamine turnover could be an important part of the causal mechanisms controlling the behavioral sex difference (Balthazart and Ball, 1992).

The previously identified sex difference in dopamine turnover was present in the medial part of the preoptic area, a brain region where steroid action is necessary and sufficient to activate male reproductive behavior (Balthazart and Surlemont, 1990a; 1990b). Low, but nevertheless measurable, levels of D1 receptors are described here in the preoptic area, but we failed to detected a sex difference in this region or in any other area that was studied. However, dopamine turnover is different in males and females in this area so it is clear that there are sex differences in dopamine neurotransmission in the quail preoptic area. This neurotransmitter system could be involved in the sexually dimorphic activation of male sexual behavior as suggested by a number of studies in rats demonstrating that the injection of D1 agonists or antagonists in the preoptic region modulates the induction of sex behavior by steroids (Hull et al., 1986, 1992; Meisel and Sachs, 1994).

A discrete area of high D1 receptor density was also identified in the present study in the ventral part of the septal region at the level of the nucleus commissuralis septi (COS). As illustrated in Fig. 2F, these receptors are associated with a high density of TH-immunoreactive fibers. Our immunocytochemical work has identified aromatase-immunoreactive cells in this general area (Balthazart et al., 1990a, 1990b) and double-label studies have also shown that some of these aromatase-immunoreactive cells are entirely surrounded by TH-immunoreactive fibers and punctate structures; hence, dopamine regulation of aromatase activity is also suggested by this anatomical organization (Bailhache et al., 1991). The demonstration of relatively high concentrations of D1 receptors certainly reinforces this suggestion. It is probable that this brain area is homologous to a part of the ventral striatal region of the rat brain, a region that appears to play an important role in the control of 'motivated' behaviors and in particular of appetitive sexual behavior (Everitt et al., 1989; Everitt, 1990). It is clearly established that dopamine is implicated in the control of these motivated processes (Everitt, 1990; Blackburn et al., 1992). The conversion of testosterone to estrogens is catalyzed by the enzyme, aromatase. This enzyme has been previously implicated in the control of consummatory aspects of sexual behavior in quail (Balthazart and Foidart, 1993). Recent data indicate that appetitive aspects of this behavior are also estrogen-dependent (Balthazart et al., 1995). It is therefore likely that appetitive male sexual behavior is also controlled by the aromatization of testosterone in the brain. The discrete region of the septal area in which TH-immunoreactive fibers, aromatase-immunoreactive cells and D1 receptors co-exist may therefore represent

a key locus where dopamine interacts with steroid-driven processes to control motivational aspects of copulatory behavior. This hypothesis is currently under investigation in our laboratories.

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Appendix: abbreviations used in figures

AC	nucleus accumbens
Cb	cerebellum
CDL	area corticoidea dorsolateralis
COS	nucleus commissuralis septi
E	ectostriatum
Field L_2	auditory projection in neostriatum
FPL	fasciculus prosencephali lateralis
HA	hyperstriatum accessorium
Hp	hippocampus
ICo	nucleus intercollicularis
INF	infundibulum
LPO	lobus parolfactorius
MLd	nucleus mesencephalicus lateralis, pars dorsalis
NC.l	neostriatum caudale, pars lateralis
NC.m	neostriatum caudale, pars medialis
N	neostriatum
PA	paleostriatum augmentatum
POM	nucleus preopticus medialis
PP	paleostriatum primitivum
PT	nucleus pretectalis
SL	nucleus septalis lateralis
SM	nucleus septalis medialis
TeO	tectum opticum
TO.l	tuberculum olfactorium, pars lateralis
TO.m	tuberculum olfactorium, pars medialis
TSM	tractus septomesencephalicus

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