Characterization of the Dopaminergic System in the Brain of an African Cichlid Fish, *Astatotilapia burtoni*

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ABSTRACT

Catecholamines, such as dopamine, are evolutionarily ancient neurotransmitters that play an essential role in mediating behavior. In vertebrates, dopamine is central to the nigrostriatal motor and mesolimbic reward systems. Despite its importance, the distribution of the dopaminergic system has not been well studied in the teleost brain. The African cichlid fish *Astatotilapia burtoni* has become an important model system in social neuroscience and lends itself to uncovering how social decisions are implemented in the brain. To understand better where dopamine acts to regulate social behavior in this species, we have determined the distribution of putative dopaminergic cells and fibers (by tyrosine hydroxylase immunohistochemistry) and dopamine receptors (by in situ hybridization for the D_{1A} and D_2 dopamine receptor subtypes) throughout the forebrain and part of the mesencephalon of *A. burtoni*. Tyrosine hydroxylase immunoreactivity was evident in several regions of the fore- and midbrain, in support of putative homologies to tetrapods. Additionally, the D_{1A} and D_2 receptors were identified in brain regions known to modulate social behavior in other vertebrates, including the proposed teleost homologues of the mammalian amygdalar complex, hippocampus, striatum, preoptic area, anterior hypothalamus, ventromedial hypothalamus, and ventral tegmental area/substantia nigra pars compacta. Tyrosine hydroxylase-immunoreactive fibers as well as D_{1A} and D_2 receptor expression overlap almost completely in their distribution. These results significantly extend our understanding of the distribution of the dopaminergic system in the teleost brain and suggest a conserved role of dopamine in modulating behavior across vertebrates. J. Comp. Neurol. 519:75–92, 2011.

INDEXING TERMS: Indexing terms: dopamine receptor; D_{1A}; D_2; tyrosine hydroxylase; posterior tuberculum; hypothalamus; reward system; social behavior

Dopamine (DA) is an evolutionarily ancient catecholamine present in most eukaryotes, including plants and all animals (Wintle and Van Tol, 2001; Callier et al., 2003; Kulma and Szopa et al., 2007). In many animals, DA plays an essential role as a modulatory neurotransmitter in many behavioral and decision-making processes, such as pair bonding, aggression, sexual behavior, learning, and memory (Young and Wang, 2004; Kindt et al., 2007; Hull and Dominguez, 2007; Ryding et al., 2008; Dalley and Everitt, 2009; Krashes et al., 2009). Although the dopaminergic system has been well characterized in mammals, not much is known about how and where the dopaminergic system acts to modulate behavior in teleosts. Here, we characterize the distribution of tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of catecholamines (Levitt et al., 1965), and the D_{1A} and D_2 dopamine receptor subtypes in the brain of the African cichlid fish, *Astatotilapia burtoni*. This species has become an established model system for the study of social decision-making and neural plasticity (Hofmann, 2003; Robinson et al., 2008).

By using TH as a marker, the distribution of putative dopaminergic cells in the brain has been determined in several teleost species, including goldfish (Hornby et al., 1987), freshwater electric fish (*Apteronotus leptorhynchus*; Sas et al., 1990), and zebrafish (*Danio rerio*; Rink and Wullimann, 2001; Kaslin and Panula, 2001). The use of a DA-specific antiserum in the European eel (*Anguilla anguilla*; Roberts et al., 1989) and stickleback (Ekström et al., 1990) has provided support for the idea that most

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TH-immunoreactive cells in the telencephalon and diencephalon of teleost fish are indeed dopaminergic. Additionally, Ma (1994) established that there are no noradrenergic/adrenergic neurons rostral to the midbrain-hindbrain boundary in zebrafish by using immunohistochemistry for TH and dopamine-beta-hydroxylase (DBH), establishing that TH cells in the forebrain are all dopaminergic. Recently, it has become clear that all vertebrates with the exception of mammals have two paralogs encoding TH, *th1* and *th2* (Filippi et al., 2010; Yamamoto et al., 2010), opening up potentially exciting avenues of research into the function of the two paralogs in the brains of non-mammalian vertebrates. These studies have greatly increased our understanding of putative neural homologies between fish and tetrapod vertebrates. However, because TH is essential to the synthesis of all catecholamines (including norepinephrine and epinephrine), it is also crucial to determine the distribution of the sites of dopaminergic action that may play important roles in regulating behavior. Surprisingly, the neural distribution of the DA receptors has been described in only one teleost, the European eel, *Anguilla anguilla* (Kapsimali et al., 2000).

Dopamine receptors are organized into two major categories, D_1-like and D_2-like, based on pharmacology and sequence (for review see Callier et al., 2003). The D_1-like receptors are expressed mainly postsynaptically, whereas the D_2-like receptors can be found in both the pre- and the postsynaptic terminals and may act as autoreceptors (Callier et al., 2003). The D_{1A} receptor has been suggested to mediate most of the D_1 receptor-related dopamine effects in the vertebrate brain, as this subtype is most highly expressed in the brain and is abundant in regions known to mediate motivation and reward processing, such as the striatum and amygdala (Reiner and Northcutt, 1992; Braford, 1995; Kapsimali et al., 2000; Callier et al., 2003). Studying the neural distribution of a D_1-like receptor allows us to map the distribution of cells that are receptive to dopamine yet do not produce dopamine themselves, as would at least partially be the case when mapping the distribution of D_2-like receptors, insofar as they can function as autoreceptors (Callier et al., 2003) and could therefore be expressed in both cells producing dopamine and cells receptive to dopamine.

Remarkably little is known about the distribution of the dopaminergic system in species with plastic behavioral phenotypes, even though dopamine is involved in modulating behavior, and this information could give us a better understanding of which brain regions may be sites of modulation of neural and behavioral plasticity by DA. *A. burtoni* is an excellent model system with which to study the mechanisms by which DA modulates social behavior and neural plasticity. There is an extensive body of work on these fish, both in the wild and in laboratory settings, chronicling their complex yet easily quantified social behaviors (Fernald, 1977). Males display robust phenotypic plasticity in the form of two distinct phenotypes (Hofmann, 2003; Robinson et al., 2008): dominant males are colorful, are reproductively active, and defend territories where they court and spawn with females, whereas subordinate males display cryptic coloration, are

Abbreviations

AC	anterior commissure
An	anterior thalamic nucleus
aTn	anterior tuberal nucleus
Cn	central nucleus of the inferior lobe
CP	central posterior thalamic nucleus
CV	cerebellar valvula
D	dorsal (pallial) part of the telencephalon
Dc	central part of D
Dc-2	subdivision of Dc
Dd	dorsal part of D
DH	dorsal hypothalamus
Dl	lateral part of D
Dld	dorsal region of Dl
Dlg	granular region of Dl
Dlv	ventral region of Dl
Dlvv	ventral zone of Dlv
Dm	medial part of D
Dm-1,2,3	subdivisions of Dm
Dm2c	caudal part of Dm-2
Dn	diffuse nucleus of the inferior lobe
Dp	posterior part of D
Dx	unassigned part of D
E	entopeduncular nucleus
GR	corpus glomerulosum pars rotunda
H	habenula
HC	horizontal commissure
IL	inferior lobe
LHn	lateral hypothalamic nucleus
LPGn	lateral preglomerular nucleus
LR	lateral recess
LT	longitudinal torus
LZ	zona limitans of the diencephalon
MB	mammillary body
mPGn	medial preglomerular nucleus
nLT	nucleus of the lateral torus
nMLF	nucleus of the medial longitudinal fascicle
OB	olfactory bulb
OPT	optic tract
OT	optic tectum
P	pituitary
PAG	periaqueductal grey
PGCn	preglomerular commissural nucleus
PN	prethalamic nucleus
POA	preoptic area
PPd	dorsal periventricular pretectal nucleus
PPr	rostral periventricular pretectal nucleus
pTGN	preglomerular tertiary gustatory nucleus
pTn	posterior tuberal nucleus
PVO	paraventricular organ
ST	semicircular torus
TPp	periventricular nucleus of the posterior tuberculum
V	ventral (subpallial) division of the telencephalon
Vc	central part of V
Vd	dorsal nucleus of V
Vdc	caudal region of Vd
Vdr	rostral region of Vd
VH	ventral hypothalamus
Vi	intermediate part of V
Vl	lateral part of V
VM	ventromedial thalamic nucleus
Vp	postcommissural nucleus of V
vPPn	ventral portion of the periventricular pretectal nucleus
Vs	supracommissural nucleus of V
Vsl	lateral region of Vs
Vsm	medial region of Vs
vTn	ventral tuberal nucleus
Vv	ventral part of V

reproductively suppressed, and school with females. Importantly, individual males can adjust their behavioral tactics depending on the immediate social environment and frequently change from one phenotype to another (Hofmann et al., 1999). More recently, *A. burtoni* has also become a model system for the study of female mate choice decisions (Clement et al., 2005). Describing the dopaminergic system in both males and females of this species will be an important step toward elucidating the modulatory role of DA in social decision-making in both sexes.

Based on insights from many vertebrate classes, there are two neural networks that seem to regulate social behavior and/or encode the salience of (social) stimuli. The neural substrate of social behavior has been described by Newman (1999) as the "social behavior network" in mammals and has been expanded to reptiles, birds, and teleosts (Newman, 1999; Crews, 2003; Goodson, 2005). The core nodes of Newman's network are involved in multiple forms of social behavior, are reciprocally connected, and contain sex steroid hormone receptors. Many studies indicate that the neural network where evaluations of stimulus salience are made is the so-called reward system (including but not limited to the midbrain dopaminergic system; Deco and Rolls, 2005; Wickens et al., 2007). Although the brain regions involved in the social behavior network and the dopaminergic reward system have been well studied in mammals, homologizing these brain areas with structures in the teleost brain has been contentious (Nieuwenhuys et al., 1998; Northcutt, 2008). However, a consensus for at least partial homologies has been emerging from neurochemical, hodological, developmental, and stimulation/lesion studies that provide homology support for most of the relevant areas in the teleost brain (Rink and Wullimann, 2001, 2002; Portavella et al., 2002; Wullimann and Mueller, 2004; Northcutt, 2006, 2008; Bruce and Braford, 2009).

In the current study, we sought first to characterize the distribution of dopaminergic cell groups throughout the telencephalon, diencephalon, and some mesencephalic structures of the cichlid *A. burtoni*, a teleost fish with remarkable social plasticity, using an antiserum to TH. We also tested the hypothesis that the D_{1A} and D_2 receptors are widely distributed throughout fore- and midbrain of *A. burtoni* and predicted that DA receptor (D_{1A} and D_2) expression and TH-immunoreactive (TH-ir) fibers would be present in brain regions known to be important for the regulation of social behavior in teleost fish and other vertebrates.

MATERIALS AND METHODS

Animals

A. burtoni from a wild-caught stock population were kept in aquaria under conditions mimicking their natural environment: pH 8.0, 28°C water temperature, and 12 hour:12 hour light:dark cycle with 10-minute dusk and dawn periods (Fernald and Hirata, 1977b). Gravel substrate and terracotta shelters provide the substrate that facilitates the establishment and maintenance of territories necessary for reproduction (Fernald and Hirata, 1977a). Fish were fed every day with cichlid flakes (Arcata Pet Supplies). The animals chosen for this study were dominant and subordinate males that had been in their respective social states for at least 4 weeks and gravid females. All work was carried out in compliance with the Institutional Animal Care and Use Committee at The University of Texas at Austin.

Where possible, we followed the neuroanatomical nomenclature of Fernald and Shelton (1985) for the diencephalon and mesencephalon and Burmeister et al. (2009) for the telencephalon. However, for the prethalamic nucleus (PN), we adopted the nomenclature of Meader (1934), although this region has been identified as the anterior preglomerular nucleus (aPGn) by Fernald and Shelton (1985). This was done to avoid confusion with functionally different regions in percomorphs and cyprinids (Braford and Northcutt, 1983; Yamamoto and Ito, 2005, 2008; Northcutt, 2006). For the corpus glomerulosum pars rotunda (GR), we followed the nomenclature of Brickner (1929), although this area has been identified as the glomerular nucleus (Gn) by Fernald and Shelton (1985). For the central nucleus of the inferior lobe, we followed the nomenclature of Ahrens and Wullimann (2002) and Yang et al. (2007). The nomenclature for the periaqueductal gray was used according to Forlano et al. (2001). Parvocellular, magnocellular, and gigantocellular portions of the preoptic area were identified as described by Braford and Northcutt (1983) based on cell size and location.

Cloning of A. burtoni dopamine D_{1A} and D_2 receptor cDNA

To obtain the *A. burtoni* D_{1A} sequence, we designed degenerate primers using CODEHOP (http://blocks.fhcrc.org/codehop.html) based on the zebrafish D_{1A} sequence (GenBank accession No. FJ208849) and homologous sequences from stickleback, medaka, and pufferfish obtained by blasting the zebrafish sequence against the genomes of these three species (http://genome.ucsc.edu/). The *A. burtoni* D_{1A} receptor was isolated in a nested PCR approach: Using whole-brain cDNA as template, we first performed a PCR with two outside primers with an annealing temperature of 56°C and 30 rounds of amplification (see Table 1 for primer details). This initial PCR product was then used as template in a nested PCR with two inside primers with an annealing temperature of 56°C and 30 rounds of amplification. This nested PCR

resulted in a 376-base-pair fragment of the *A. burtoni* D_{1A} receptor, which we cloned into a pCRII-TOPO vector (Invitrogen, Carlsbad, CA). The partial mRNA sequence has been submitted to GenBank (accession No. GU322020).

To obtain the *A. burtoni* D_2 sequence, we designed primers to the dopamine receptor D_2 of the Nile tilapia, *Oreochromis niloticus* (Genbank accession No. AY673985). Using whole-brain cDNA as template, we performed a touchdown PCR that began with an annealing temperature of 70°C and decreased the annealing temperature by 1°C per cycle for 20 cycles. We then continued the PCR for 20 more cycles at 50°C annealing temperature. This touchdown approach yielded a 482-base-pair product, which we cloned into a pCRII-TOPO vector (Invitrogen). This partial mRNA sequence has been submitted to GenBank (accession No. HM008640).

TABLE 1.
Oligonucleotide Primers Used for Cloning Dopamine D_{1A} and D_2 Receptors

Primer name	Primer sequence
D_{1A} outer forward primer	5′-TGTGGGTGGCCTTCGAYATHATGTG
D_{1A} nested forward primer	5′- CGTGCAGCTGAACTGGCAYAARGC
D_{1A} outer reverse primer	5′- GGCCCAGCCGAACCANACRAA
D_{1A} nested reverse primer	5′- CCAGCAGCACACGAACACNCCCATDAT
D_2 forward primer	5′- AGAGCAGAAGCACCCCTACA
D_2 reverse primer	5′- TGGCGATCACACAGAGAGAC

Dopamine receptor phylogenetic analysis

Based on the partial mRNA sequence described above, we determined the *A. burtoni* D_{1A} and D_2 receptor amino acid sequences. To assess whether our putative DA receptor sequences indeed encode D_{1A} and D_2, we compared them with the D_{1A} and D_2 protein sequences of multiple species as well as a human adrenergic receptor as an outgroup (Genbank accession Nos. and species information: *H. sapiens* D_{1A}: NP_000785.1; *H. sapiens* D_2: AAB26274.1; *R. norvegicus* D_{1A}: NP_036678; *R. norvegicus* D_2: NP_036679.1; *X. laevis* D_{1A}: AAA50828; *X. laevis* D_2: NP_001095212.1; *A. anguilla* D_{1A1}: AAC60067.1; *A. anguilla* D_{1A2}: AAC60069.1; *A. anguilla* D_{2A}: ABH06893.1; *A. anguilla* D_{2B}: ABH06894.1; *G. gallus* D_1: NP_001138320.1; *G. gallus* D_2: NP_001106761.1; *H. sapiens* beta 1 adrenergic receptor: AAD53697.1). Using the Mega 4 freeware package (http://www.megasoftware.net/m_con_select.html), we aligned the sequences with ClustalW and generated a bootstrapped nearest neighbor-joining gene tree (Fig. 1).

In situ hybridization

Dominant (n = 4) and subordinate (n = 4) males and gravid females (n = 4) were sacrificed and their brains rapidly dissected, frozen in O.C.T. compound (Tissue-Tek), and stored at −80°C. Brains were then cryosectioned at

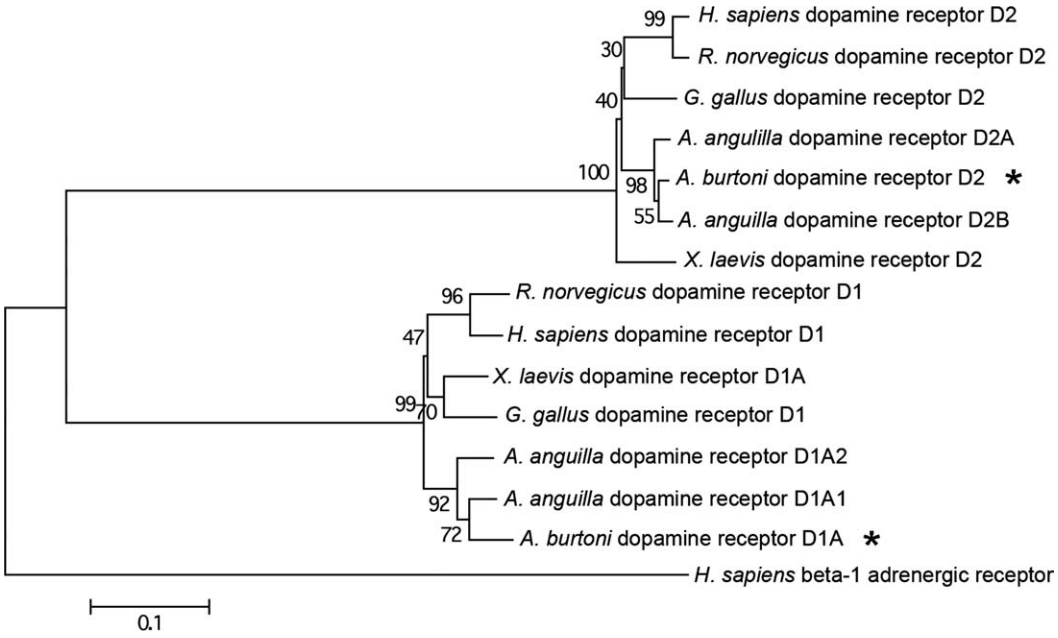

Figure 1. Comparison of *A. burtoni* D_{1A} and D_2 receptors with other vertebrates. A neighbor-joining tree shows that the *A. burtoni* D_{1A} sequence is most closely related to *A. anguilla* D_{1A} receptors and is distinct from the *A. burtoni* D_2 receptor, which clusters with the *A. anguilla* D_2 subtypes.

20 μm into four series and thaw-mounted onto Super-Frost Plus slides (Erie Scientific Co., Portsmouth, NH) that were stored at −80°C for 4–6 weeks until processing for in situ hybridization (ISH). Sections were fixed in cold 4% paraformaldehyde (pH 7.2) for 10 minutes and washed in 0.01 M (1×) phosphate-buffered saline (PBS). The sections were then incubated in 0.1 M triethanolamine (TEA; pH 8.0) for 10 minutes, followed by 15 minutes in freshly prepared TEA/0.25% acetic anhydride, rinsed in 2× SSC, dehydrated in increasing ethanol series, air dried, and stored at −80°C. Riboprobes were reverse-transcribed in the presence of fluorescein-labeled UTP (Roche, Indianapolis, IN) using a T7/SP6 Maxiscript in vitro transcription kit (Ambion, Austin, TX) to produce antisense or sense probes that were fluorescein labeled. The template used to make the D_{1A} receptor probe was 376 base pairs in length (see above), and the template to make the D_2 receptor probe was 482 base pairs in length. As a result of the method of probe synthesis, each probe contains 69 base pairs from the site of RNA synthesis (either the T7 or SP6 promoter) to the start of the gene insert. Therefore, the D_{1A} probe was 445 base pairs in length and the D_2 receptor probe was 551 base pairs in length. One series of sections was used for the D_{1A} ISH and another series of the same brains was used for the D_2 receptor ISH. Slides were then warmed to room temperature, air dried, and preequilibrated in hybridization buffer (50% formamide, 5× SSC, 5× Denhardt's solution, 125 mg/ml Baker's yeast tRNA, 250 mg/ml denatured herring sperm DNA) for 2 hours at 65°C. Sections were then incubated in riboprobe overnight at the same temperature. Experimental slides were exposed to antisense fluorescein-labeled probe, whereas control slides were incubated with sense fluorescein-labeled probe. Additional control slides were treated with RNase before hybridization with antisense probe.

After RNase A treatment at 37°C for 15 minutes, sections were washed in a decreasing series of SSC and equilibrated in 150 mM NaCl/100 mM Tris (pH 7.5) at room temperature before incubation in 1:1,000 antifluorescein-alkaline phosphatase Fab fragments (Roche) in 0.5% Tween 20/PBS for 2 hours at room temperature. Sections were then washed in 100 mM Tris (pH 7.5). Chromogenic product was formed using BM Purple (Roche) at room temperature until the desired darkness was achieved and was terminated simultaneously for all slides. Slides were then washed, dehydrated in an ethanol series ending in xylene, and coverslipped with Permount (Fisher Scientific, Itasca, IL).

Immunohistochemistry

Dominant (n = 4) and subordinate (n = 4) males and gravid females (n = 2) were sacrificed and their brains rapidly dissected and fixed in 4% paraformaldehyde in 1× PBS (pH 7.4) at 4°C overnight. Brains were then washed in 1× PBS and cryoprotected in 30% sucrose in 1× PBS overnight at 4°C before embedding in O.C.T. and storing at −80°C. Brains were then cryosectioned at 20 μm into six series and thaw-mounted onto Super-Frost Plus slides (Erie Scientific Co.) that were stored at −80°C until processing for immunohistochemistry (IHC).

Sections were removed from −80°C and air dried before being fixed in chilled 4% paraformaldehyde in 1× PBS, pH 7.4, for 10 minutes. Sections were then rinsed in PBS and incubated in 3% hydrogen peroxide in PBS for 20 minutes. After washing in PBS, antigen retrieval was performed by incubating in boiling citrate buffer (10 mM citric acid, 0.05% Tween 20, pH 6.0). After 2 minutes, the boiling citrate buffer was replaced twice and incubated for 5 minutes each, followed by a PBS wash. After blocking for 1 hour in blocking solution (5% normal goat serum and 0.3% Triton X-100 in PBS), sections were incubated in 1:500 primary antibody (rabbit anti-TH; Millipore, Berdford, MA; catalog No. AB152) in PBS with 2% normal goat serum and 0.3% Triton X at room temperature overnight. This commercial polyclonal antibody was raised against denatured TH from rat pheochromocytoma. Sections were then rinsed, incubated for 2 hours in a biotinylated goat anti-rabbit secondary antibody (Vector, Burlingame, CA), and rinsed again. After treatment with the ABC peroxidase staining kit (Vector) according to the manufacturer's instructions, immunoreactivity was visualized by using 3,3′-diaminobenzidine (DAB) substrate (Vector). Sections were then counterstained with cresyl violet, dehydrated, and coverslipped with Permount (Fisher Scientific). For control sections, all procedures were the same, except that primary antibody was omitted.

Western blot characterization of TH antibody

To determine whether the antibody would bind specifically to the cichlid antigen, we extracted protein from whole brain using a Mammalian Cell Lysis kit (Sigma, St. Louis, MO) according to the manufacturer's instructions. Whole-brain protein extract was run on an SDS-PAGE gel in replicate, in which one half of the gel was used for downstream Western blotting and the other half exposed to Coomassie stain to verify protein presence. Whole-brain extract on the gel was transferred onto a nitrocellulose membrane overnight. The membrane was then blocked in 5% dry milk in wash buffer [0.5% Triton X-100, 0.1% Tween-20 in 1× Tris-buffered saline (TBS)], incubated in primary antibody (1:2,000 TH in 1× TBS and 2% NaN_3) for 1 hour, washed five times for 3 minutes each in wash buffer, and then incubated in goat anti-rabbit HRP-conjugated antibody (Southern Biotech, Birmingham, AL) in blocking solution for 30 minutes. After washing five

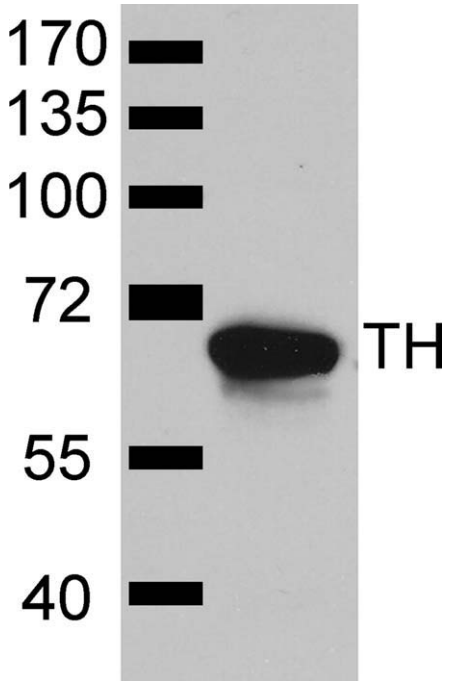

Figure 2. Confirmation of tyrosine hydroxylase antibody specificity. Western blot was used to confirm specificity of the TH antiserum against *A. burtoni* whole-brain extract. Ladder units are in kilodaltons.

times for 3 minutes each with wash buffer, the membrane was exposed to HRP substrate (Immobilon Western, Millipore) and exposed to film for 2 minutes. By using the TH antibody, a prominent band was visualized putatively representing TH1 at the predicted size of 64 kDa (Fig. 2) and a weaker band putatively representing TH2. This antiserum is the same as that of Yamamoto et al. (2010), who established that this polyclonal antibody recognizes both isoforms of TH (th1 and th2), although it appears to have somewhat lower affinity for th2.

Photomicroscopy

Brightfield optics were used to visualize ISH and IHC staining throughout the brain at low (×5) and high (×20) magnification. Photographs were taken with a digital camera (AxioCam MRc; Zeiss) attached to a Zeiss AxioImager.A1 AX10 microscope using the AxioVision (Zeiss) image acquisition and processing software. Images were compiled and brightness and contrast enhanced in Adobe Photoshop CS3.

RESULTS

Dopamine receptor sequences

We have cloned a single mRNA transcript from each of the *A. burtoni* D_{1A} and D_2 receptors and confirmed the identity of these sequences by constructing a gene tree with DA receptors of other vertebrates (Fig. 1). The European eel is reported to have two D_{1A} isoforms (D_{1A1} and D_{1A2}) and two D_2 isoforms (D_{2A} and D_{2B}) from two different genes (Cardinaud et al., 1997). Similarly, genome sequence databases indicate that two other teleosts, the three-spined stickleback, *Gasterosteus aculeatus*, as well as the medaka, *Oryzias latipes*, also have two copies for each of these receptor genes. Thus, *A. burtoni* most likely has another isoform of both receptors as well. However, for the purposes of this study, we chose to describe the distribution of two receptors from different families rather than isoforms within a family, as we have found that teleost-specific isoforms of receptors tend to have nearly identical distribution patterns in *A. burtoni* (Munchrath and Hofmann, 2010), although this does not necessarily imply that the same is true for other receptors.

Distribution of the dopaminergic system

Here we describe the dopaminergic system in the brain of *A. burtoni* by characterizing the distribution of TH staining by immunohistochemistry and D_{1A} and D_2 mRNA expression by in situ hybridization. We present distribution maps along with representative photomicrographs of brain regions for TH protein, D_{1A} mRNA, and D_2 mRNA at each level of the brain. For each representative section, the left map shows the nomenclature displayed on the left side, and TH immunoreactivity is represented by dots on the right side. The antibody used to detect TH binds to both TH1 and TH2. The right brain map shows D_{1A} mRNA on the left and D_2 mRNA on the right represented by dots. The distributions shown here are representative of gravid females and both dominant and subordinate males, because there were no qualitative differences (in total presence or absence) between sexes or phenotypes.

Telencephalon

Robust staining for TH was seen in both cell bodies and fibers throughout the telencephalon of *A. burtoni*. Control slides showed no staining of cell bodies or fibers. D_{1A} and D_2 mRNAs are widely distributed throughout the telencephalon, and D_2 mRNA seems to be more widespread than D_{1A} mRNA. Control slides, including both slides exposed to sense probe and slides pretreated with RNase A and incubated with antisense probe, showed no staining.

We found strong TH immunoreactivity in discrete areas of the dorsal and ventral telencephalon (Figs. 3, 4). TH-ir cell bodies and fibers are present in the olfactory bulb (OB; Fig. 3A1), where cell bodies are in the granule layer and fibers are contained in the granule and mitral cell layers. The granule cell layer of the olfactory bulb also contains D_{1A} and D_2 receptor expression. The TH-ir fibers appear to extend out of the OB into the dorsal telencephalon (D) along the boundary zone between the dorsal region

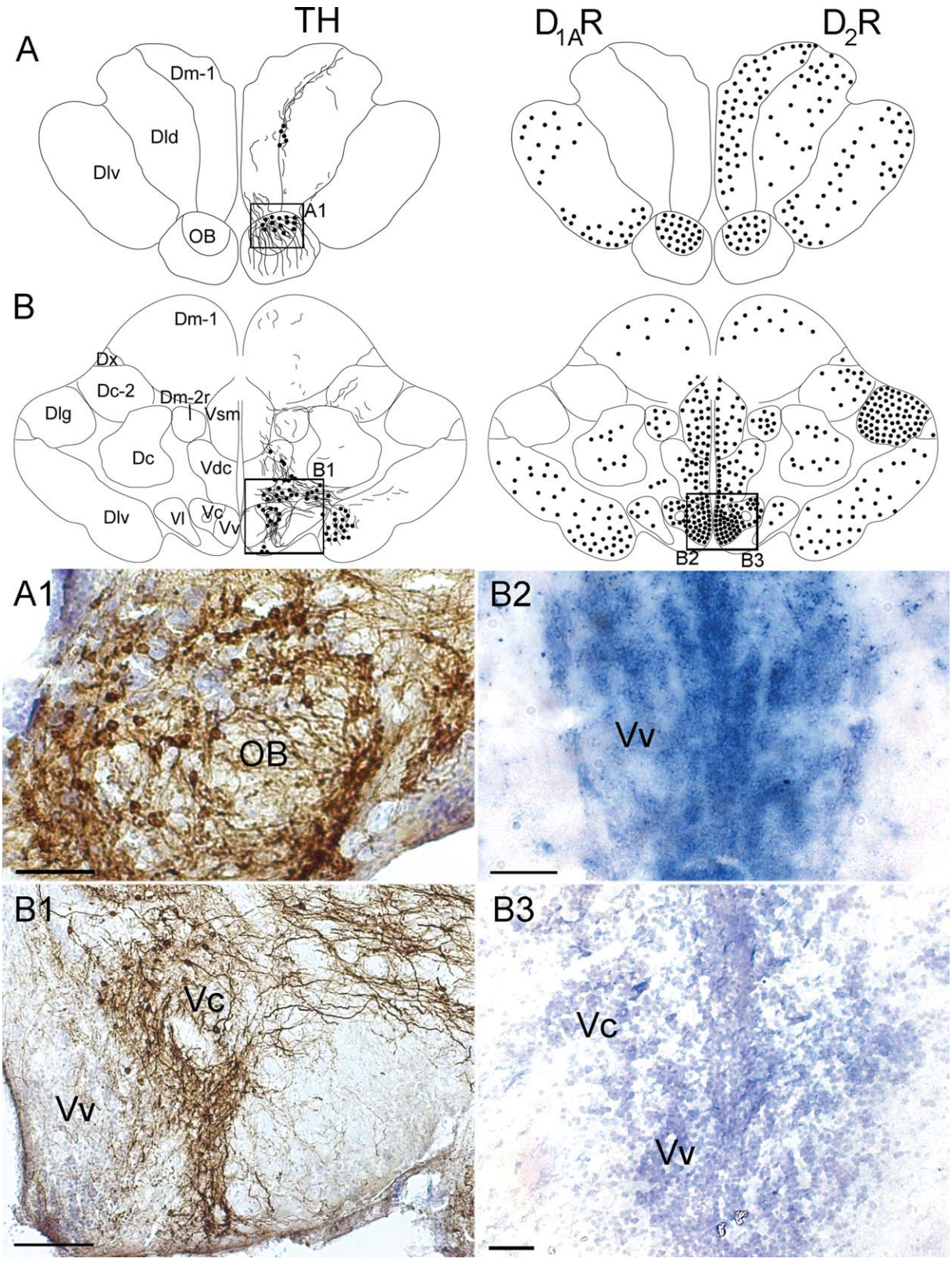

Figure 3. **A–B3:** Distribution of the dopaminergic system in the rostral telencephalon of *A. burtoni*. Representative sections of the telencephalon are presented as the first image in each panel with nomenclature labeled on the left portion and TH-ir cell bodies shown as dots and fibers shown as lines on the right side of the brain. The second panel presents the distribution of D_{1A} mRNA in the left part and D_2 mRNA on the right portion of the representative section, both represented by dots. Representative micrographs are in the bottom panels and show TH immunoreactivity in the olfactory bulb (OB; A1) and in the ventral and central parts of the ventral telencephalon (Vv and Vc, respectively, B1) The micrographs in the second column shows D_{1A} mRNA in Vv (B2) and D_2 mRNA in Vv (B3). Scale bars = 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

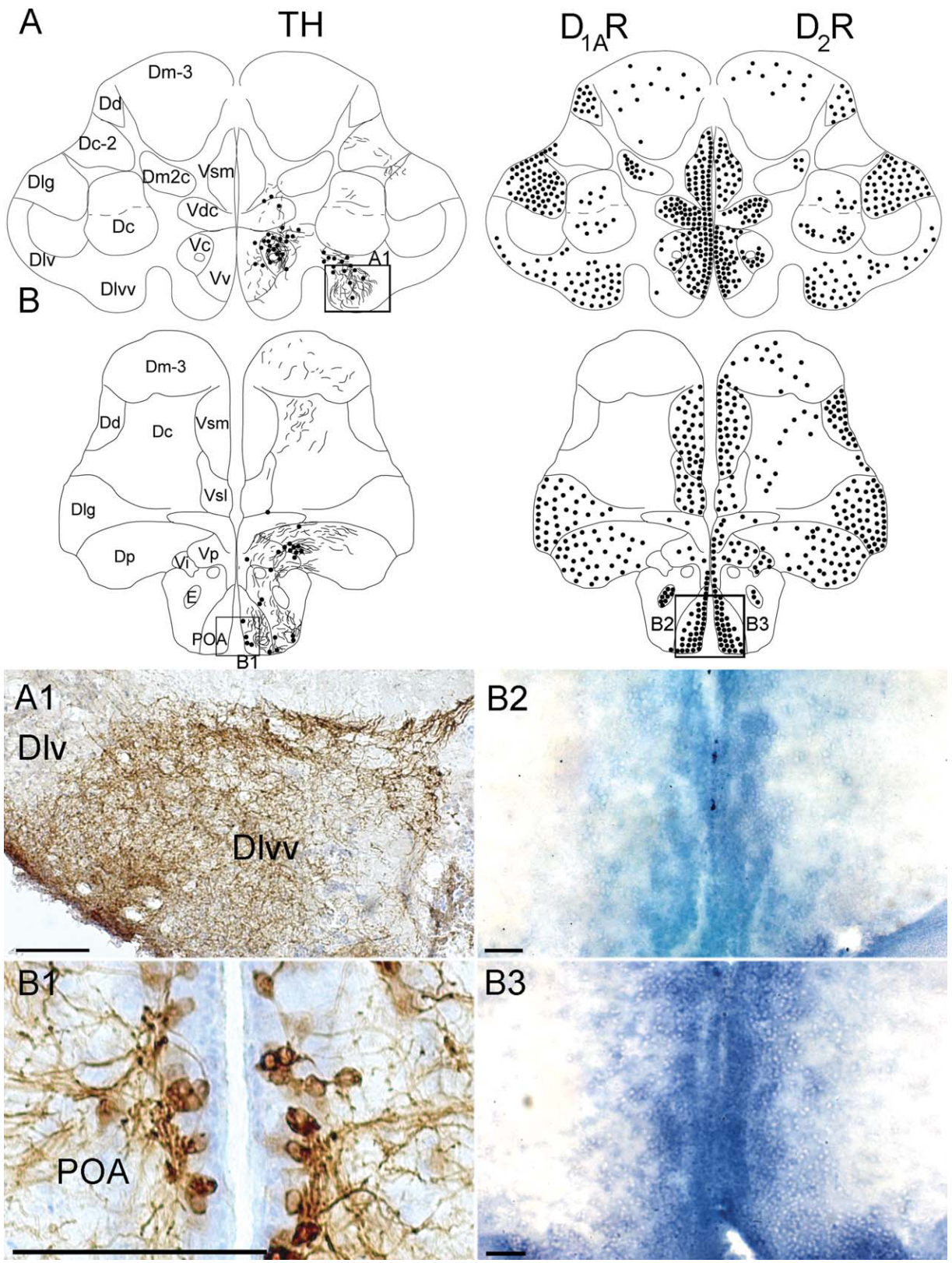

Figure 4. **A–B3:** Distribution of dopaminergic system in the caudal telencephalon of *A. burtoni*. Representative sections of the telencephalon are presented as the first image in each panel with nomenclature labeled in the left portion and TH-ir cell bodies shown as dots and fibers shown as lines on the right side of the brain. The second panel presents the distribution of D_{1A} mRNA on the left part and D_2 mRNA on the right portion of the representative section, both represented by dots. Representative micrographs are in the bottom panels and show TH-ir cell bodies in the ventral portion of the lateral part of the dorsal telencephalon (Dlv; A1) and the POA (B1). The micrographs in the second column shows D_{1A} and D_2 expression within the POA (B2 and B3, respectively). Scale bars = 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of the lateral part of D (Dld) and the medial part of the D (Dm; Fig. 3A). We find D_2 expression within this zone between Dld and Dm, but no D_{1A} receptor expression. Dorsal to the olfactory bulb, D_{1A} receptor expression is found within the ventral region of the lateral part of D (Dlv). The D_2 receptor mRNA is found within the Dlv as well as the dorsal region of Dl and the medial part of D (Dm-1). More caudally, there are TH-ir fibers in the central part of D (Dc), and these fibers extend into discrete subregions of Dlv (Fig. 3B). Dl is heterogeneous for TH immunoreactivity in that the granular region of Dl (Dlg) shows sparse TH signal, whereas the ventral regions of Dl (Dlv, Dlvv) display an abundance of TH immunoreactivity, mostly in fibers (Figs. 3B, 4A). Both D_{1A} and D_2 are expressed within Dm, Dc, Dlv, Dlg, and the dorsal part of D (Dd). However, we found only D_2 expression within Dc-2, but no D_{1A} mRNA. D_{1A} mRNA is present only in the rostral region of Dd, whereas D_2 is present throughout the rostral-caudal axis of this part.

In the ventral telencephalon (V), there are many TH-ir cell bodies in the central part of V (Vc) that appear to wrap around the medial olfactory tract (Fig. 3B). This bundle of TH-ir fibers appears to extend into the ventral part of V (Vv). There are also cell bodies and fibers between Vc and the caudal region of the dorsal part of V (Vdc). D_{1A} and D_2 mRNAs are abundant in Vv, Vc, Vdc, and the medial region of the supracommissural part of V (Vsm; Figs. 3B, 4A).

The preoptic area is rich in TH immunoreactivity (Fig. 4B); strong signal was found in all three teleost POA cell types: gigantocellular, magnocellular, and parvocellular. The caudal regions of the POA contain many TH-ir cell bodies that appear to send projections out of the POA and into the posterior part of D (Dp; Fig. 4B). D_{1A} and D_2 expression is also abundant in the POA (Fig. 4B2,3) and Dp, which overlaps well with the distribution of TH-ir fibers. Dorsal to the POA, TH-ir fibers are present within the intermediate part of V (Vi) and the postcommissural part of V (Vp). D_{1A} and D_2 mRNAs are found within Vp, but only D_2 is present within Vi. Finally, both D_{1A} and D_2 mRNAs are found in the entopeduncular nucleus (E).

Diencephalon and some mesencephalic structures

TH-ir fibers are more widespread in the diencephalon, whereas TH-ir cell bodies are more restricted to discrete brain regions. The distributions of the D_{1A} and D_2 expression in the diencephalon are also widespread and more similar to each other than the distribution patterns of mRNA found in the telencephalon. The distributions of the D_{1A} and D_2 mRNA overlap well with TH-ir fibers.

In the rostral diencephalon, there is an abundance of TH-ir fibers extending throughout the rostral-ventral hypothalamic regions (Fig. 5A). There are TH-ir cell bodies and fibers within the ventromedial thalamic nucleus (VM; Fig. 5A1) and in the rostral regions of the periventricular pretectal nucleus (PPr, Fig. 5B1). In this transition between the telencephalon and diencephalon, both D_{1A} and D_2 mRNAs are present within optic tectum (OT), VM, PPr, habenula (H), and ventral tuberal nucleus (vTn). However, only D_2 is present within the prethalamic nucleus (PN).

In the rostral hypothalamic regions, there are TH-ir cell bodies in the anterior tuberal nucleus (aTn) and the dorsal hypothalamus (DH) and many TH-ir fibers that spread into the ventral hypothalamus (VH) and LHn. Both D_{1A} and D_2 mRNAs are present in the anterior tuberal nucleus (aTn), dorsal hypothalamus (DH), and ventral hypothalamus (VH). Both the D_{1A} and the D_2 receptor mRNAs are present in the lateral hypothalamic nucleus (LHn), although D_{1A} mRNA is present only in the caudal portion of this region. Dorsal to these hypothalamic nuclei, there are TH-ir cell bodies in the zona limitans of the diencephalon (LZ), although this region does not contain D_{1A} or D_2 receptor mRNAs. Farther dorsally, the ventromedial and anterior thalamic nucleus (VM and An, respectively) contains both D_{1A} and D_2 receptor mRNAs but no TH-ir cells and fibers. Finally, D_{1A} and D_2 mRNAs are present within the preglomerular tertiary gustatory nucleus (pTGN) and the inferior lobe (IL), where there are also some TH-ir fibers.

The densest cluster of TH-ir cell bodies appears in the periventricular nucleus of the posterior tuberculum (TPp; Fig. 6). D_{1A} and D_2 mRNAs are also present within the TPp (Fig. 6A2,3). Ventral to the TPp, the paraventricular organ (PVO) contains both TH-ir cell bodies and some D_{1A} and D_2 mRNA. Dorsal to the TPp, the central posterior thalamic nucleus (CP) contains both D_{1A} and D_2 mRNA but no TH immunoreactivity. Farther dorsally, the dorsal periventricular pretectal nucleus (PPd) contains TH-ir cell bodies as well as D_{1A} and D_2 expression. More caudally, some mesencephalic regions emerge, including the torus semicircularis (ST; Fig. 6B), which shows both D_{1A} and D_2 receptor expression. Medial to the semicircular torus, the ventral portion of the periventricular pretectal nucleus (vPPn) contains both TH-ir fibers and D_{1A} and D_2 mRNA. Throughout the diencephalon, the prominently situated corpus glomerulosum pars rotunda (GR) contains both D_{1A} and D_2 expression as well as TH-ir fibers and cell bodies in the small cells of this region. The central nucleus of the inferior lobe (Cn) contains D_2 receptor expression in the rostral portion; D_{1A} mRNA or TH-ir fibers were not detectable in this region. The periaqueductal gray (Fig. 7A) contains TH-ir cell bodies and fibers as well as D_{1A} and D_2 mRNA expression. More caudally, the nucleus of the medial longitudinal fascicle (nMLF) contains TH-ir fibers as well as D_{1A} and D_2 mRNA. Ventral to the

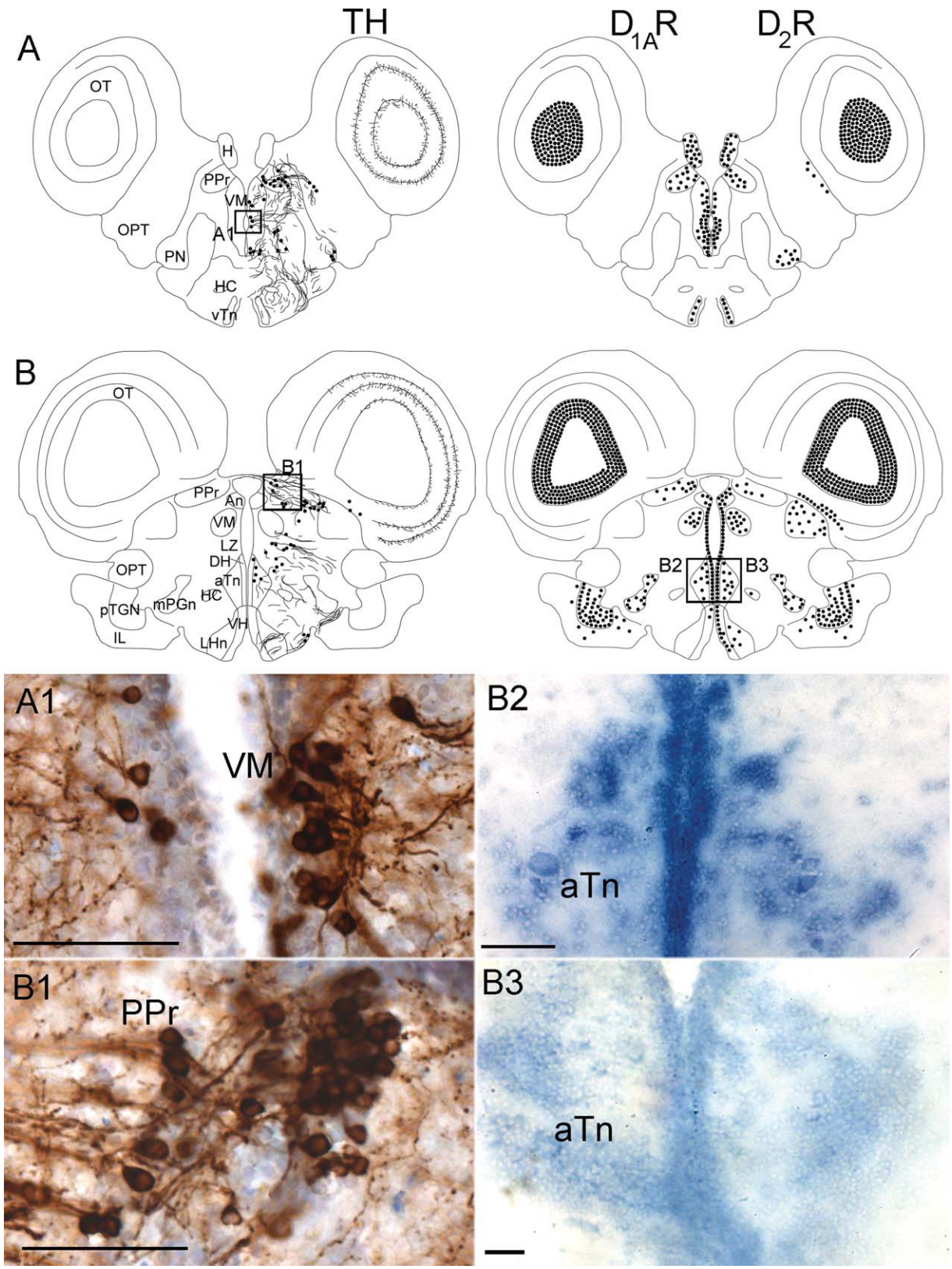

Figure 5. **A–B3:** Distribution of the dopaminergic system in the rostral diencephalon of *A. burtoni*. Representative sections of the rostral diencephalon are presented as the first image in each panel with nomenclature labeled in the left portion and TH-ir cell bodies shown as dots and fibers shown as lines on the right side of the brain. The second panel presents the distribution of D_{1A} mRNA on the left part and D_2 mRNA on the right portion of the representative section, both represented by dots. Representative micrographs are in the bottom panels and show TH-ir cells in the ventromedial thalamic nucleus (VM; A1) and the rostral periventricular pretectal nucleus (PPr; B1). The micrographs in the second column shows D_{1A} and D_2 mRNA in the anterior tuberal nucleus (aTn; B2 and B3, respectively). Scale bars = 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

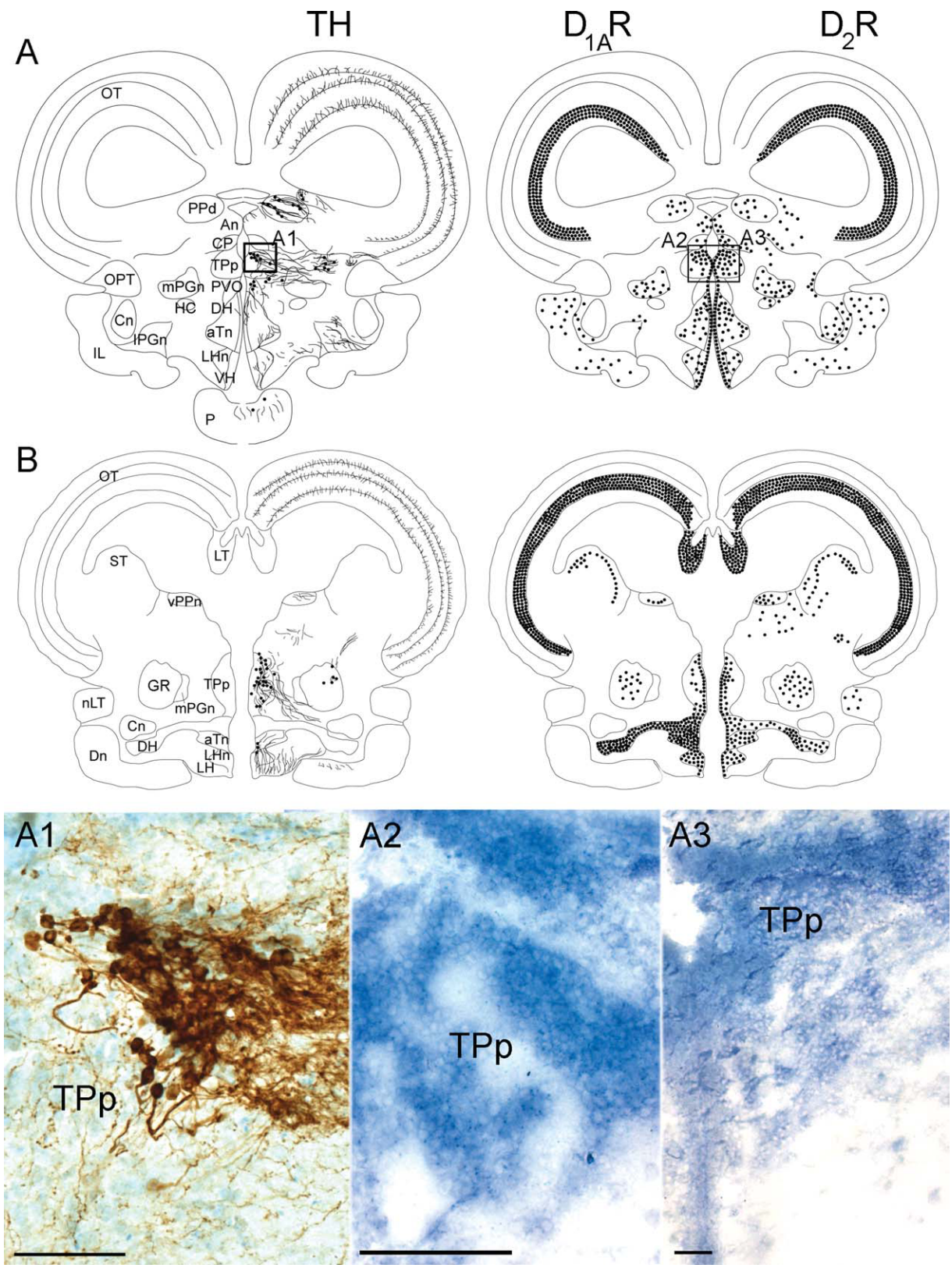

Figure 6. **A,B:** Distribution of the dopaminergic system in the diencephalon of *A. burtoni*. Representative sections of the diencephalon and some mesencephalic regions are presented as the first image in each panel with nomenclature labeled in the left portion and TH-ir cell bodies shown as dots and fibers shown as lines on the right side of the brain. The second panel presents the distribution of D_{1A} mRNA in the left part and D_2 mRNA in the right portion of the representative section, both represented by dots. Representative micrographs are in the bottom panels and show TH-cell bodies, D_{1A} mRNA, and D_2 mRNA in the periventricular nucleus of the posterior tuberculum (TPp; A1, A2, and A3, respectively). Scale bars = 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

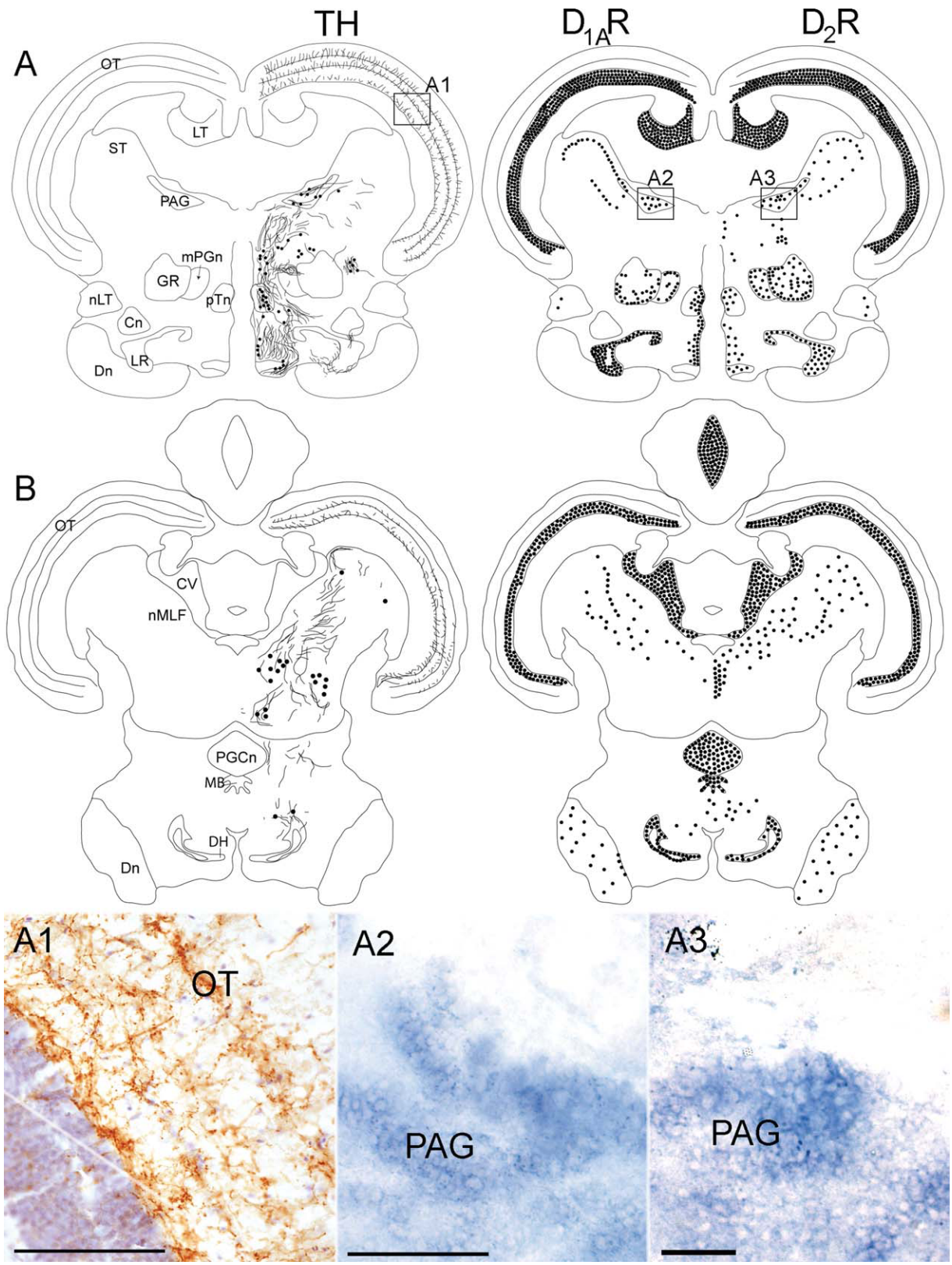

Figure 7. **A,B:** Distribution of the dopaminergic system in the caudal diencephalon of *A. burtoni*. Representative sections of the caudal diencephalon and mesencephalon are presented as the first image in each panel with nomenclature labeled in the left portion and TH-ir cell bodies shown as dots and fibers shown as lines on the right side of the brain. The second panel presents the distribution of D_{1A} mRNA in the left part and D_2 mRNA in the right portion of the representative section, both represented by dots. Representative micrographs are in the bottom panels and show TH-ir fibers in the optic tectum (OT; A1), D_{1A} mRNA, and D_2 mRNA in the periaqueductal gray (PAG; A2, A3, respectively). Scale bars = 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

nMLF are the preglomerular commissural nucleus (PGCn) and the mammillary body (MB), both of which contain D_{1A} and D_2 receptor expression, but TH immunoreactivity is absent. Finally, we observed characteristic TH-ir fiber staining in layers of the optic tectum (OT). D_{1A} and D_2 mRNA is abundant within the OT as well. Ventral to the optic tectum, the longitudinal torus (LT) and, more caudally, the cerebellar valvula (CV) are also rich in D_{1A} and D_2 expression.

DISCUSSION

Our results are consistent with an important role of the dopaminergic system in the modulation of neural circuits in *A. burtoni* and teleosts in general. We have shown that there are only a few cell groups that can synthesize DA as detected by TH immunoreactivity, whereas TH-ir fibers and D_{1A} and D_2 receptor mRNAs are widely distributed throughout the telencephalon and diencephalon and some mesencephalic structures. Furthermore, we found D_{1A} mRNA in brain regions that are thought to process stimulus salience and/or to modulate social behaviors in amniotes. We also found excellent correspondence between TH-ir fiber patterns and the distribution of D_{1A} and D_2 mRNA, although there are some brain regions with dopamine receptor expression but lacking TH-ir fibers, including Dd, Vsm, and entopeduncular nucleus (E) in the telencephalon, and the preglomerular tertiary gustatory nucleus (pTGN), preglomerular commissural nucleus (PGCn) torus longitudinalis, cerebellar granular layers, and lateral torus in the diencephalon and mesencephalon. This discrepancy may be due to a lack of sensitivity in fiber staining, or TH2 fibers may innervate this region and are thus less likely to be detected with the antibody used here (see also Yamamoto et al., 2010). Finally, there are no qualitative (complete presence or absence) differences in D_{1A} expression or TH immunoreactivity between females and dominant and subordinate males. This result suggests that any variation between or within sexes likely is due to quantitative differences in expression rather than qualitative differences. Indeed, there were differences in in situ hybridization detection intensity between males and females, which are indicative of quantitative variation, although a more robust method of measurement is needed to quantify these potential differences.

Comparison of the cichlid dopamine system with that of other teleosts

In the following, we compare our findings to the presence or absence of D_{1A} and D_2 receptor mRNAs and cells or fibers immunoreactive for TH or DA in a variety of teleosts.

Tyrosine hydroxylase

TH cell bodies are in a few distinct nuclei in the forebrain of *A. burtoni*, whereas TH-ir fibers are widely distributed throughout the *A. burtoni* telencephalon and diencephalon. The antibody used in this study recognizes both isoforms of TH (th1 and th2), although it has a lower affinity for th2 (Yamamoto et al., 2010). In zebrafish, the th1 is most widely distributed, and most telencephalic and diencephalic regions express either th1 or both th1 and th2, with very few regions expressing th2 exclusively, such as some hypothalamic regions such as the dorsal PVO intermediate nucleus (Yamamoto et al., 2010). Although the antibody used here recognizes both TH1 and TH2, the distribution in *A. burtoni* is strikingly similar to that of TH1 in zebrafish, suggesting that there is variation in *th1* and *th2* expression distribution in the teleost lineage. More studies are needed to gain insights into how much variation exists across teleosts and the role that this may play in species differences in behavior. The wide distribution pattern in *A. burtoni* of TH-ir fibers and the relatively restricted distribution of the TH-ir cell bodies is consistent with previously published results in a freshwater electric fish (*Apteronotus leptorhynchus*; Sas et al., 1990) and zebrafish (*Danio rerio*; Rink and Wullimann, 2001; Yamamoto et al., 2010) and also patterns of DA immunoreactivity in the European eel (*Anguilla anguilla*; Roberts et al., 1989). The distribution pattern of TH-ir cells in *A. burtoni*, zebrafish, and *A. leptorhynchus* is very similar to that of DA-ir cells in *A. anguilla* and distinctly different from DBH-ir cells in *A. leptorhynchus*. Since DA is a precursor in norepinephrine synthesis, it is possible that TH-ir cells may synthesize norepinephrine rather than dopamine. However, when we compare *A. leptorhynchus* staining patterns of TH with those of DBH (a marker for noradrenergic cells), our analysis suggests that the patterns of TH staining are dopaminergic rather than noradrenergic.

When comparing DA-ir cells in *A. anguilla* and TH immunohistochemistry in *A. burtoni*, zebrafish, and *A. leptorhynchus*, regions that consistently contain TH-ir cell bodies are the olfactory bulb, central and intermediate regions of the ventral telencephalon (Vc and Vi), POA, rostral periventricular pretectal nucleus (PPr), periventricular nucleus of the posterior tuberculum (TPp), ventromedial thalamic nucleus (VM), and hypothalamic regions. Brain areas that consistently lack TH or DA immunoreactivity in these species are the medial and dorsal regions of the dorsal telencephalon (Dm and Dd), dorsal subregion of the lateral region of D (Dld), optic tectum, central posterior nucleus, anterior tuberal nucleus, and semicircular torus.

Regions that differ between teleost species in the distribution of TH- or DA-ir cell bodies are numerous in the V and D regions of the telencephalon as well as regions of the diencephalon. We focus here on particular regions where *A. burtoni* TH-ir cell distribution differs from that of *A. anguilla* dopamine-ir cells. *A. burtoni*, zebrafish, and

A. leptorhynchus have TH-ir cells within the intermediate nucleus of V (Vi) and the ventromedial nucleus (VM), but *A. anguilla* does not have dopamine-ir neurons in these regions. Similarly, the pituitary of *A. burtoni* has TH-ir neurons, although no dopamine-ir neurons were found in the pituitary of *A. anguilla*. Within the central subregion of the dorsal region of V (Vdc), *A. burtoni* and zebrafish have TH-ir cells, whereas *A. leptorhynchus* does not, and *A. anguilla* shows no DA immunoreactivity. The medial subregion of the supracommissural region of V (Vsm) did not have TH-ir cells in *A. burtoni*, nor are they present in *A. leptorhynchus*. However, zebrafish have TH immunoreactivity in this region and *A. anguilla* shows dopamine immunoreactivity here. Interestingly, *A. anguilla* has DA-ir cells within the central region of D (Dc), but no TH-ir cells are seen within this region in *A. burtoni*, zebrafish, or *A. leptorhynchus*. The postcommissural nucleus of V (Vp) in *A. anguilla* contains DA-ir cells, and *A. burtoni* have TH-ir cells, but zebrafish and *A. leptorhynchus* lack TH-positive cells. Additionally, there is a distinct cell population in the ventral subregion of the ventral region of Dl (Dlvv) in *A. burtoni* that is absent in *A. anguilla*, *A. leptorhynchus*, and zebrafish. Finally, we observed TH immunoreactivity in the posterior subregion D (Dp) and the ventral subregion of the lateral region of D (Dlv) of *A. burtoni*, but these cells were not labeled in *A. leptorhynchus*, *A. anguilla*, or zebrafish. Additional studies will be needed to determine whether the regions labeled by TH immunoreactivity in *A. burtoni* (and other species) that differ from DA staining in *A. anguilla* are due to species differences, labeling of cells that are noradrenergic, technical variation, nomenclatural differences, or unresolved questions about homologies.

D_{1A} and D_2 receptors

Our phylogenetic analysis confirmed that we have cloned D_{1A} and D_2 mRNA transcripts from *A. burtoni*. We have found the D_{1A} and D_2 receptor mRNA to be widely distributed throughout the telencephalon and diencephalon of *A. burtoni*, and their distribution pattern corresponds well with that of TH-ir fibers.

The distribution of D_{1A} mRNA has been described in only one other teleost, the European eel *A. anguilla* (Kapsimali et al., 2000), a relatively minor group of teleosts. The distributions of D_{1A} receptor mRNA in *A. burtoni* and *A. anguilla* are remarkably similar, with only a few exceptions in the telencephalon and diencephalon. The concordance of these distribution patterns suggests that the formation of the dopaminergic system is highly conserved among diverse lineages of teleosts. Regions of the telencephalon that contain D_{1A} expression in the telencephalon of *A. burtoni* but not *A. anguilla* include the dorsal and lateral subdivisions of D (Dd, Dlv, and Dlvv), the postcommissural nucleus of V (Vp), and the semicircular torus (ST). A region of the diencephalon that differs in D_{1A} expression between *A. burtoni* and *A. anguilla* is the periventricular nucleus of the posterior tuberculum (TPp), which shows D_{1A} expression in *A. burtoni* but not *A. anguilla*. Additionally, the rostral periventricular pretectal nucleus (PPr) and ventromedial nucleus (VM) express D_{1A} in *A. burtoni*, but only the D_{1A1} receptor in *A. anguilla* is expressed in these regions. Finally, the optic tectum in *A. burtoni* contains D_{1A} expression, but only the D_{1A2} receptor is expressed here in *A. anguilla*.

The distribution of D_2 mRNA has been described in the European eel *A. anguilla* (Pasqualini et al., 2009) and the rainbow trout *O. mykiss* (Vacher et al., 2003), although the coverages of the telencephalon and diencephalon were not described in as much detail as presented here, making direct comparisons difficult. The most striking differences in D_2 mRNA distributions between *A. burtoni* and rainbow trout or European eel are in the telencephalon. Neither *A. anguilla* nor trout is reported to have D_2 mRNA in Dl or Dm. This is surprising, insofar as we report D_2 mRNA in both of these regions in *A. burtoni*, which also contain TH-ir fibers, especially in Dlv. The discrepancies could be due to species differences or differences in technique sensitivity.

The cichlid dopamine system in comparison with the tetrapod vertebrates

Although the structure of the teleost brain has long been a source of uncertainty for neuroanatomists, the realization that the teleost brain develops via eversion of the neural tube, rather than inversion as in tetrapods and other nonteleost fish, has precipitated much progress (Wullimann and Mueller, 2004; Yamamoto et al., 2007). Neurochemical, hodological, developmental, and lesion studies have been crucial in assigning various brain regions to putative mammalian homologies (Rink and Wullimann, 2001, 2002; Portavella et al., 2002; Wullimann and Mueller, 2004; Northcutt, 2006, 2008; Bruce and Braford, 2009; Nieuwenhuys, 2009). A tentative consensus has emerged from these studies, which is fundamental for reconstructing the evolution of neural circuits that are crucial in the evaluation of rewarding stimuli (the mesolimbic dopaminergic system; Wise, 2002) and the regulation of social behavior (Newman's social behavior network; Newman, 1999). In this section we discuss the teleost brain in reference to putative partial mammalian homologies, although these putative partial homologies should still be considered debatable.

In amniotes, the mesolimbic dopaminergic system consists of the ventral tegmental area (VTA) projecting to many forebrain nuclei in what has been described as the reward system and is important for reinforcing learned

behavior (Young and Wang, 2004). Regions that receive input from this dopaminergic system include the basolateral amygdala, hippocampus, nucleus accumbens, ventral pallidum, striatum, bed nucleus of the stria terminalis (BNST), and lateral septum. Most of these brain nuclei contain DA receptors in reptiles (Smeets et al., 2001, 2003), birds (Schnabel et al., 1997; Durstewitz et al., 1998; Sun and Reiner, 2000; Absil et al., 2001), and mammals (Savasta et al., 1986; Camps et al., 1990; Weiner et al., 1991; Mansour et al., 1991; Jansson et al., 1999; Hurd et al., 2001). However, there is an exception; the D_{1A} receptor is not present (or at least has not been reported) within the avian VTA. To our knowledge, the neuroanatomical distribution of DA receptors has not yet been mapped throughout an entire amphibian brain, although the D_2 receptor has been described in the anuran auditory midbrain (Endepols et al., 2000).

The putative homologies to these forebrain nuclei are somewhat contentious, but can tentatively be summarized as follows: the medial part of the dorsal telencephalon (Dm) is a putative partial homologue of the mammalian lateral amygdala (Portavella et al., 2004; Northcutt, 2006); the lateral region of the dorsal telencephalon (Dl) is a putative homologue to the mammalian hippocampus (Portavella et al., 2004; Northcutt, 2006); the ventral region of the ventral telencephalon (Vv) is a putative (partial) homologue of the mammalian nucleus accumbens (Northcutt, 1995; Braford, 2009); the dorsal and central parts of the ventral telencephalon (Vc/Vd) are a putative homologue of the vertebrate striatum (Wullimann and Rink, 2002); the postcommissural nucleus of the ventral telencephalon (Vp) is a putative homologue of the basal amygdala (Nieuwenhuys and Meek, 1990); and the posterior tuberculum (TPp) has been suggested to be at least functionally equivalent (Rink and Wullimann, 2001), if not homologous (Luo et al., 2008), to the mammalian VTA/substantia nigra pars compacta (see below). Our results show that TH-ir fibers are present and that the D_{1A} and D_2 receptors are expressed within all of these brain regions in *A. burtoni*, providing neurochemical evidence in support of these suggested homologies in the mesolimbic reward system.

Given its important role in regulating mammalian behavior, much attention has been given to finding the anamniote homologue to the mammalian VTA (Rink and Wullimann, 2001; Luo et al., 2008). In mammals, the functional connection between the VTA and the nucleus accumbens is widely considered the core of the dopaminergic reward system (Spanagel and Weiss, 1999). Neurochemical, developmental, and hodological evidence points to the posterior tuberculum as the putative VTA homologue. Rink and Wullimann (2001) found that the posterior tuberculum is the teleostean dopaminergic system ascending to the striatum, similarly to the case in mammals (Fallon and Moore, 1978), and suggested that this region in teleosts might be functionally equivalent and possibly homologous to the mammalian VTA/substantia nigra pars compacta. More recently, neurochemical evidence coupled with morpholino knockout studies in developing zebrafish, targeting the transcription factor Nr4a2, which is essential for both development and terminal differentiation of ventral mesencephalic DA neurons in mammals (Saucedo-Cardenas et al., 1998), has provided support for the notion that the posterior tuberculum is in fact homologous to the VTA (Zetterström et al., 1997; Le et al., 1999; Luo et al., 2008). It is unclear at this point whether the posterior tuberculum represents the mammalian substantia nigra, VTA, or both, insofar as it is possible that the separation of midbrain dopaminergic cell populations into the distinct substantia nigra and VTA happened after the anamniote-amniote transition. Once neurochemical markers become available that differentiate the substantia nigra from the VTA, many of these questions can be answered.

The social behavior network that was originally described for mammals (Newman, 1999) has now been expanded to other vertebrate classes (Crews, 2003; Goodson, 2005). The brain regions in this network are mostly hypothalamic and, by definition, mediate social behavior and express steroid hormone receptors (Newman, 1999). The nodes of this network include the preoptic area (POA), anterior hypothalamus, ventromedial hypothalamus, medial amygdala (MeAMY) and BNST, periaqueductal gray, and lateral septum (LS). These regions contain DA receptors in every vertebrate class studied, including reptiles (Smeets et al., 2001, 2003), birds (Schnabel et al., 1997; Durstewitz et al., 1998; Sun et al., 2000; Absil et al., 2001), and mammals (Savasta et al., 1986; Camps et al., 1990; Weiner et al., 1991; Mansour et al., 1991; Jansson et al., 1999; Hurd et al., 2001). The only exception seems to be in the avian ventromedial hypothalamus, which does not contain (or has not been reported to express) D_1 receptors. Notably, the POA also contains dopamine-producing cells in all vertebrates (Smeets and Reiner, 1994), and the role of DA in modulating behavior in the POA is well established in mammals (Miller and Lonstein, 2005; Hull and Dominguez, 2006). The putative homologs of these brain regions in teleosts are much less contentious than those of the mesolimbic reward system discussed above and can be summarized as follows: the supracommissural region of the ventral telencephalon (Vs) is the homologue of the medial amygdala/bed nucleus of the stria terminalis (Northcutt, 1995); the ventral and lateral nuclei of the ventral telencephalon (Vv and Vl) are homologous to the lateral septum (Wullimann and Mueller, 2004); the POA is

the homologue of the mammalian medial preoptic area (Moore and Lowry, 1998); the ventral tuberal region is the homologue of the anterior hypothalamus (Kittelberger et al., 2006); the anterior tuberal region is homologous to the ventromedial hypothalamus (Forlano et al., 2005); and the PAG is the mammalian periaqueductal gray (Forlano et al., 2001). We have shown here that the D_{1A} and D_2 receptors are expressed in each of these brain regions in *A. burtoni*, suggesting that DA plays an important role in modulating social behaviors in teleosts as well as other vertebrates.

Finally, the results presented here confirm previous reports that the number of dopaminergic cell groups in fore- and midbrain differs drastically between teleosts and mammals (for review see Smeets and Reiner, 1994). This general pattern suggests that the number of dopaminergic cell groups in the hypothalamus decreased in the vertebrate lineage leading to mammals, whereas the opposite is true for the midbrain (Parent et al., 1984). It is possible that, in the course of evolution, the number of dopaminergic cell groups changed independently in fore- and midbrain; alternatively, the dopaminergic cells in the forebrain of anamniotes may have begun to migrate to the midbrain during amniote brain development. More studies of the developmental origin of dopaminergic cell groups in each vertebrate class will be needed to address this possibility.

CONCLUSIONS

We have demonstrated that the D_{1A} and D_2 receptors are expressed in the brain of the cichlid fish, *A. burtoni*, in regions that regulate social behavior across amniotes. We have also found distinct patterns of cell groups positive for TH immunoreactivity as well as a high degree of correspondence between the patterns of D_{1A} and D_2 receptor expression overlap with TH fiber staining. This pattern suggests that DA may play an important role in the regulation of complex social behavior, behavioral plasticity, and evaluation of stimulus salience in *A. burtoni*. Future work will use pharmacological manipulations and quantitative histochemistry to dissect further the role DA may play in regulating social behavior either on its own or in concert with steroid hormones and/or neuropeptide pathways.

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