

SEROTONIN 5-HT_{1A} RECEPTOR BINDING SITES IN THE BRAIN OF THE PIGEON (*COLUMBA LIVIA*)

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Abstract—Present knowledge about the serotonergic system in birdbrains is very limited, although the pigeon was used as an animal model in various studies focused on the behavioral effects of serotonergic transmission. In the mammalian brain the 5-HT_{1A} receptor is the most widespread serotonin receptor type, and is involved in various functions. Less is known about the distribution of 5-HT_{1A} receptors in the avian species. Therefore, we analyzed serotonin 5-HT_{1A} receptor binding sites in the pigeon brain using quantitative *in vitro* receptor autoradiography with the selective radioligand [³H]-8-hydroxy-2-(di-n-propylamino)tetralin ([³H]-8-OH-DPAT). The receptor is differentially distributed throughout the pigeon brain. High levels of 5-HT_{1A} receptors are found in the nucleus pretectalis (PT). Moderate densities were detected in the tectum, as well as in the telencephalic nidopallium and hyperpallium. Very low levels were found in the hippocampal formation, the amygdaloid complex, the basal ganglia, and several thalamic nuclei. Furthermore, local variations in 5-HT_{1A} receptor densities support the concept of further subdivisions of the entopallium. The regional distribution patterns of 5-HT_{1A} receptors mostly display a similar distribution as found in homologue brain structures of mammals. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: 5-HT_{1A} receptor, [³H]-8-OH-DPAT, avian, nucleus pretectalis, entopallium, MVL.

Serotonin (5-HT) is a modulatory neurotransmitter that is involved in a variety of physiological and behavioral functions. In mammals, dysfunction of the serotonergic system has been linked to various diseases such as depression, schizophrenia, Alzheimer's disease, and eating disorders (Müller et al., 2007; Michelsen et al., 2008; Remington, 2008; Terry et al., 2008; Akimova et al., 2009; Polter and

Li, 2010). In addition, growing evidence found in many species indicates that 5-HT modulates learning and memory (Jacobs and Azmitia, 1992; Winsauer et al., 1996; Meneses, 1999; Clarke et al., 2004; Meneses and Perez-Garcia, 2007; Müller et al., 2007; Bert et al., 2008; Gasbarri et al., 2008; González-Burgos and Feria-Velasco, 2008; Sitaraman et al., 2008; Sambeth et al., 2009; Bari et al., 2010).

Serotonin binds to multiple receptors (Hoyer et al., 2002; Green, 2006), which are widely distributed throughout the brain (Chalmers and Watson, 1991; Baumgarten and Grozdanovic, 1995; Barnes and Sharp, 1999; Riad et al., 2000). One of the most prominent is the 5-HT_{1A} receptor, which was first cloned and described by Fargin et al. (1988). The 5-HT_{1A} receptor belongs to the G protein coupled receptor superfamily and binds to a Gi/o protein (Innis and Aghajanian, 1987; Polter and Li, 2010). It displays a high affinity for 5-HT and occurs both pre- and postsynaptically (Hall et al., 1985; van Wijngaarden et al., 1990; Riad et al., 2000). As somatodendritic autoreceptors, 5-HT_{1A} receptors modulate the activity of 5-HT neurons, whereas they modify neuronal activity in terminal areas as postsynaptic receptors (Müller et al., 2007).

The distribution of 5-HT_{1A} receptors in the brain has been investigated by several methods in rodents, non-human primates, and humans. Thereby, a high correlation between receptor binding with [³H]-8-hydroxy-2-(di-n-propylamino)tetralin ([³H]-8-OH-DPAT) and 5-HT_{1A} mRNA densities has been shown (Chalmers and Watson, 1991; Pompeiano et al., 1992). High 5-HT_{1A} receptor densities were detected in the dorsal and median raphe nuclei and in areas of the limbic system such as the hippocampus, the lateral septum, the amygdala, as well as the entorhinal and cingulate cortices (Glaser et al., 1985; Zilles et al., 1985, 2000; Palacios et al., 1990; Yilmazer-Hanke et al., 2003). Moderate binding was detected in the olfactory bulb, the thalamus, hypothalamus, and several brain stem nuclei as well as neocortical areas. Low levels, or no binding, were reported in the basal ganglia and cerebellum (Gozlan et al., 1983; Marcinkiewicz et al., 1984; Zilles et al., 1985, 2000; Hall et al., 1997; Vergé et al., 1986; Albert et al., 1990; Palacios et al., 1990; Pompeiano et al., 1992; Kawaja, 1995; Kia et al., 1996a,b; Farde et al., 1997; Raurich et al., 1999; Hume et al., 2001; Maeda et al., 2001; Geyer et al., 2005; Palchoudhuri and Flügge, 2005; Eickhoff et al., 2007; Topic et al., 2007). 5-HT_{1A} receptors play a role in executive functions, anxiety related behavior, learning and reinforcement, feeding behavior, and locomotor activity (Zilles et al., 2000; Yilmazer-Hanke et al., 2003; Müller

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Abbreviations: Al, arcopallium intermedium; APH, area parahippocampalis; Ee, entopallium externum; Eie, entopallium internum pars externale; Eii, entopallium internum pars internale; Gld, lateral geniculate nucleus; HA, hyperpallium apicale; HD, hyperpallium densocellulare; HI, hyperpallium intercalatum; IP, nucleus interpeduncularis; MVL, mesopallium ventrolaterale; NCL, nidopallium caudolaterale; NIL, nidopallium intermedium laterale; PT, nucleus pretectalis; Rt, nucleus rotundus; TnA, nucleus taenia amygdalae; TPO, temporo-parieto-occipitalis; [³H]-8-OH-DPAT, [³H]-8-hydroxy-2-(di-n-propylamino)tetralin.

et al., 2007; Sumiyoshi et al., 2007; Topic et al., 2007; Borg, 2008; Perez-Garcia and Meneses, 2009).

In pigeons, immunohistochemical studies revealed serotonin fibers and terminals to be broadly distributed throughout the brain. They were particularly prominent in several structures of the telencephalon (arcopallium pars dorsalis, nucleus taeniae, area parahippocampalis, septum), diencephalon (nuclei preopticus medianus, magnocellularis, nucleus geniculatus lateralis pars ventralis, nucleus triangularis, nucleus pretectalis), mesencephalon-rhombencephalon (superficial layers of the optic tectum, nucleus of the basal optic root, nucleus isthmo-opticus), and in most of the cranial nerve nuclei (Krebs et al., 1991; Challet et al., 1996). To date, detailed information about the distribution of 5-HT receptors in the avian brain is very sparse. One study described binding sites for [³H]-8-OH-DPAT in the basal ganglia (Dietl and Palacios, 1988), and a second study used [³H]-5-HT binding in the telencephalon of pigeons, which is non-selective for the different receptor types (Waeber et al., 1989). In addition, the role of 5-HT_{1A} receptor signaling in behavioral outcome and cognitive functions is less investigated in the avian brain compared with mammals. Only a few studies suggest a role for this receptor type in ingestive behavior, circadian rhythm, sleep (Tejada et al., 2011; Fuchs et al., 2006; Garau et al., 2006; Da Silva et al., 2007; Campanella et al., 2009; Dos Santos et al., 2009), and impulsive reactions (Wolff and Leander, 2000). It was demonstrated that 5-HT modulates executive function during working memory in pigeons (Karakuyu et al., 2007) and possibly plays a role in visual attention switching (Miceli et al., 1999, 2002) and ingestive behavior (Güntürkün et al., 1989). Hence, comprehensive information about the regional distribution of 5-HT_{1A} receptor densities is needed to constitute a relevant fundament for behavioral and pharmacological studies in birds. Furthermore, since the avian and mammalian pallia are partly homologous but differ in their morphological organization (Jarvis et al., 2005), 5-HT_{1A} receptor densities could be relevant to compare homologue and analogue structures in birds and mammals. Therefore, we analyzed the distributions of the 5-HT_{1A} receptor with the selective radioligand [³H]-8-OH-DPAT in the pigeon's CNS.

EXPERIMENTAL PROCEDURES

We examined a total of six pigeons (*Columba livia*) of unknown sex. Animals were decapitated and the brains removed from the skull, frozen immediately in isopentane at −40 °C and stored at −70 °C. Serial coronal 10 μm sections were cut with a cryostat microtome (2800 Frigocut E, Reichert-Jung, Vienna, Austria). Sections were thaw-mounted on gelatinized slides and freeze-dried before use for receptor autoradiography or histological staining for the visualization of cell bodies (Merker, 1983).

RECEPTOR AUTORADIOGRAPHY

Binding sites for serotonergic 5-HT_{1A} receptors were labeled with [³H]-8-OH-DPAT (Arvidsson et al., 1981; Hjorth and Carlsson, 1982) according to a previously published standardized protocol (Zilles et al., 2002a,b), which consists of three steps: (1) A preincubation step of 30 min at

room temperature in buffer (170 mM Tris–HCl buffer with 4 mM CaCl₂ and 0.01% ascorbic acid, pH 7.6) removed endogenous ligand from the tissue. (2) During the main incubation step, binding sites were labeled with 1 nM [³H]-8-OH-DPAT in buffer for 60 min at room temperature either in the presence of 1 μM 5-hydroxy tryptamine as a displacer (non-specific binding), or without the displacer (total binding). Specific binding is the difference between total and non-specific binding. Since non-specific binding sites amounted to less than 10% of total binding sites, total binding was considered equivalent to specific binding. (3) A final rinsing step of 5 min at 4 °C in buffer eliminated unbound radioactive ligand from the sections.

Sections were air-dried overnight and subsequently coexposed for 8 weeks against a tritium-sensitive film (Hyperfilm, Amersham, Braunschweig, Germany) with plastic [³H]-standards (Microscales, Amersham) of known concentrations of radioactivity. Adjacent sections were stained with a Nissl staining for cytoarchitectonic analysis.

IMAGE ANALYSIS

Autoradiographs were digitized (Schleicher et al., 2005; Zilles et al., 2002a) by means of a KS-400 image analyzing system (Kontron, Germany) connected to a CCD camera (Sony, Tokyo) equipped with an S-Orthoplanar 60-mm macro lens (Zeiss, Germany). The images were stored with a resolution of 512×512 pixels and 8-bit gray value. Images of coexposed microscales were used to compute a calibration curve by nonlinear, least squares fitting, which defined the relationship between gray values in the autoradiographs and concentrations of radioactivity. This enabled the pixel-wise conversion of the gray values of an autoradiograph into the corresponding concentrations of radioactivity. These concentrations of binding sites occupied by the ligand under incubation conditions are transformed into receptor binding site densities at saturation conditions by means of the equation: $(K_D + L)/A_S \times L$, where K_D is the equilibrium dissociation constant of ligand-binding kinetics, L is the incubation concentration of ligand, and A_S the specific activity of the ligand.

ANATOMICAL IDENTIFICATION

The borders of the structures as defined by the atlas of Karten and Hodos (1967) were microscopically identified in the sections processed for the visualization of cell bodies and traced on prints of the digitized autoradiographs. The mean gray values in anatomically identified brain regions (one to five sections per animal and region) are transformed into binding site concentrations (fmol/mg protein). The 5-HT_{1A} receptor densities measured in numerous anatomical structures are summarized in Table 1.

STATISTICAL ANALYSIS

To investigate the binding site density differences between the subdivisions in the entopallium a Friedman ANOVA was conducted. For post hoc analysis, pair-wise comparisons were run with Wilcoxon rank test. All analysis was

Table 1. [³H]8-OH-DPAT binding in the pigeon brain

Brain area	Binding density			
	fmol/mg protein	±SD	Relative density compared with MBV (%)	
Hyperpallium accesorium (HA)	494	115	41	++
Hyperpallium densocellulare (HD)	378	71	31	++
Hyperpallium intercalatum (HI)	688	146	57	+++
Mesopallium (M)	300	49	25	++
Mesopallium dorsale (MD)	313	59	26	++
Mesopallium ventrale (MV)	286	58	24	+
Nucleus MVL	425	50	35	++
Nidopallium (N)	587	90	49	++
Nidopallium caudolaterale (NCL)	374	67	31	++
Nidopallium intermedium laterale (NIL)	629	86	52	+++
Entopallial belt (Ep)	338	70	28	++
Entopallium externum (Ee)	208	54	17	+
Entopallium internum pars externale (Eie)	99	33	8	+
Entopallium internum pars internale (Eii)	n.d.	n.d.	n.d.	n.d.
Nucleus commissuralis septi (CoS)	269	116	22	+
Nucleus septalis lateralis (SL)	378	54	31	++
Nucleus septalis medialis (SM)	408	96	34	++
Nucleus diagonalis Brocae (NDB)	342	63	28	++
Nucleus basorostralis pallii (Bas)	121	15	10	+
Arcopallium anterius (AA)	175	13	14	+
Arcopallium intermedium (AI)	229	28	19	+
Arcopallium dorsale (AD)	194	21	16	+
Arcopallium mediale (AM)	n.d.	n.d.	n.d.	n.d.
Nucleus posterioris amygdalopallii (PoA)	175	20	14	+
Hippocampus (Hp)	136	12	11	+
Area parahippocampalis (APH)	156	25	13	+
Area corticoidea dorsolateralis (CDL)	236	101	20	+
Area temporo-parieto-occipitalis (TPO)	252	115	21	+
Field L2 (L2)	296	75	24	+
Medial striatum (MSt)	136	14	11	+
Lateral striatum (LSt)	116	13	10	+
Globus pallidus (GP)	94	14	8	+
Nucleus intrapeduncularis (INP)	120	12	10	+
Olfactory tubercle (Otu)	194	74	16	+
Bed nucleus of the stria terminalis, (BST)	164	24	14	+
Nucleus taeniae amygdalae (TnA)	133	15	11	+
Ventral pallidum (VP)	134	6	11	+
Nucleus dorsolateralis anterior thalami, pars lateralis dorsolateralis (DLLdl)	164	100	14	+
Nucleus dorsolateralis anterior thalami, pars lateralis dorsomedialis (DLLdm)	127	37	10	+
Nucleus dorsolateralis anterior thalami, pars lateralis ventrolateralis (DLLvl)	160	95	13	+
Nucleus dorsolateralis anterior thalami, pars lateralis ventromedialis (DLLvm)	126	71	10	+
Nucleus rotundus (Rt)	59	27	5	+
Nucleus subrotundus (SRt)	n.d.	n.d.	n.d.	n.d.
Nucleus ovoidalis (Ov)	76	3	6	+
Nucleus superficialis parvocellularis (SPC)	120	16	10	+
Nucleus triangularis (T)	71	11	6	+
Nucleus pretectalis (PT)	1210	160	100	MBV++++
Nucleus spiriformis medialis (SpM)	n.d.	n.d.	n.d.	n.d.
Nucleus spiriformis lateralis (SpL)	n.d.	n.d.	n.d.	n.d.
Nucleus principalis precommissuralis (PPC)	259	50	21	+
Nucleus geniculatus lateralis. Pars ventralis (Glv)	370	72	31	++
Nucleus subpretectalis/nucleus interstitio-pretecto-subpretectalis (Sp/IPS)	40	3	3	+
Tectum opticum lamina 1	132	22	11	+
Tectum opticum laminae 2–4	597	104	49	++
Tectum opticum lamina 5	620	116	51	+++
Tectum opticum laminae 6–7	545	87	45	++
Tectum opticum laminae 8–13	200	28	17	+

Table 1. Continued

Brain area	Binding density			
	fmol/mg protein	±SD	Relative density compared with MBV (%)	
Tectum opticum lamina 14	66	10	5	+
Nucleus interpeduncularis (IP)	550	138	45	++
Nucleus intercollicularis (ICo)	408	116	34	++
Molecular layer	23	6	2	+
Purkinje+granular cell layer	33	12	3	+

[3H]8-OH-DPAT binding values are shown in fmol/mg protein. Data is presented as mean±SD. The percentage binding values for each structure and the qualitative classification are compared with the structure with the maximal binding (MBV). +++++, very high; +++, high; ++, moderate; +, low; n.d., non detectable.

performed using Statistica 9.0 (StatSoft, Europe GmbH, Hamburg, Germany).

RESULTS

Quantitative receptor autoradiography

Telencephalon. Autoradiographic analysis revealed a widespread, but heterogeneous distribution of 5-HT_{1A} receptors in the pigeon's telencephalon (Table 1). In the following, we will provide a detailed account of our findings.

Pallial structures. High 5-HT_{1A} receptor densities were seen in the hyperpallium intercalatum (HI), one of the pseudolayers of the avian Wulst. In contrast, the most dorsal layer of the Wulst, the hyperpallium apicale (HA) and the hyperpallium densocellulare (HD) showed moderate densities (Fig. 1A–D; Table 1). The second area with a high 5-HT_{1A} receptor concentration was the nidopallium. Therein, the nidopallium intermedium laterale (NIL) showed the highest binding values. The area temporo-parieto-occipitalis (TPO) could be easily discriminated from the nidopallium because TPO showed only low densities. In contrast to the nidopallium, the overall labeling in the entopallium was relatively low with the notable exception of its belt subregion, which showed comparable densities to those of the nidopallium caudolaterale (NCL) (Fig. 1B–H; Table 1). Different subdivisions of the entopallium were visible (Fig. 2). A comparison of binding site densities between the entopallial belt (Ep), the entopallium externum (Ee), and the entopallium internum pars externale (Eie) using a Friedman ANOVA showed a significant overall effect [chi square ($n=6$, $df=2$)=12, $P<0.01$]. Binding site densities decreased from Ep to Ee to Eie (all $P<0.05$; Wilcoxon). 5-HT_{1A} receptors were not detectable in the entopallium internum pars internale (Eii). Within the mesopallium, the mesopallium ventrolaterale (MVL) showed a high binding site density (Fig. 1A–C; Table 1). All septal nuclei showed moderate binding site densities (Fig. 1D–F; Table 1). Only low 5-HT_{1A} receptor concentrations were detected in the arcopallium, with lowest values in the nucleus taenia amygdalae (TnA) (Fig. 1G–L; Table 1). Additionally, low densities of 5-HT_{1A} receptors were found in the dorsolateral corticoid area (CDL), area parahippocampalis (APH) and hippocampus (Hp) (Fig. 1D–L; Table 1).

Subpallial structures. The basal ganglia showed relatively low 5-HT_{1A} receptor concentrations when compared with those of the Wulst or the nidopallium. Densities were similar in all subpallial areas, with highest values in the olfactory tubercle (Otu) and the bed nucleus of the stria terminalis (BST), and with lowest concentrations in the globus pallidus (GP) (Fig. 1A–H; Table 1).

Diencephalon. In the thalamic nuclei overall densities were low. Highest 5-HT_{1A} receptor densities were found in the nucleus geniculatus lateralis, pars ventralis (Glv), and lowest in the nucleus subrotundus (SRT). All parts of the nucleus dorsolateralis anterior thalami, pars lateralis (DLL) were labeled, with higher densities in the lateral regions than in the medial ones. Low receptor densities were also detected in the nucleus rotundus (Rt) and in the nucleus ovoidalis (Ov) (Fig. 1H–L, Table 1).

The highest 5-HT_{1A} receptor densities in the pigeon's brain were detected in the nucleus pretectalis (PT). They were two-fold higher than those in the visual Wulst or the nidopallium, and almost 12-fold higher when compared with other diencephalic nuclei.

The nucleus principalis precommissuralis (PPC) contained moderate densities, whereas binding densities in the nucleus subpretectalis/nucleus interstitio-pretectosubpretectalis (Sp/IPS) were close to zero (Fig. 1H–L; Table 1).

Mesencephalon-rhombencephalon. The nucleus interpeduncularis (IP) showed quite high 5-HT_{1A} receptor densities. The nucleus intercollicularis (ICo) was moderately labeled. In the optic tectum a stepwise increase of 5-HT_{1A} receptor densities was found from layer 1 to layer 5, with a peak in layer 5 and a stepwise decrease until layer 14, which presented the lowest densities in this structure. For comparison, 5-HT_{1A} receptor densities in layer 5 of the optic tectum were comparable with those measured in the Wulst or the nidopallium (Fig. 1I–L, Table 1).

Cerebellum. 5-HT_{1A} receptors occurred at very low densities in the cerebellar cortex with higher concentration in the Purkinje cell layer and in the granule cell layer than in the molecular layer (Table 1).

Fig. 1. Color-coded autoradiographs of [^3H]8-OH-DPAT binding in the pigeon's CNS. For each sectioning level in a series of coronal sections the color-coded autoradiograph is shown (A–L). Images were arranged in rostro-caudal sequence (left, middle, and right column). Color coding indicates density of 5-HT $_{1A}$ receptor binding sites in fmol/mg protein. Note that color-coding of each image is optimized to the overall density. The maximum binding level is not included in the color graphs but is shown in the table (Table 1). Abbreviations used are defined in the autoradiography binding data table (Table 1). For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

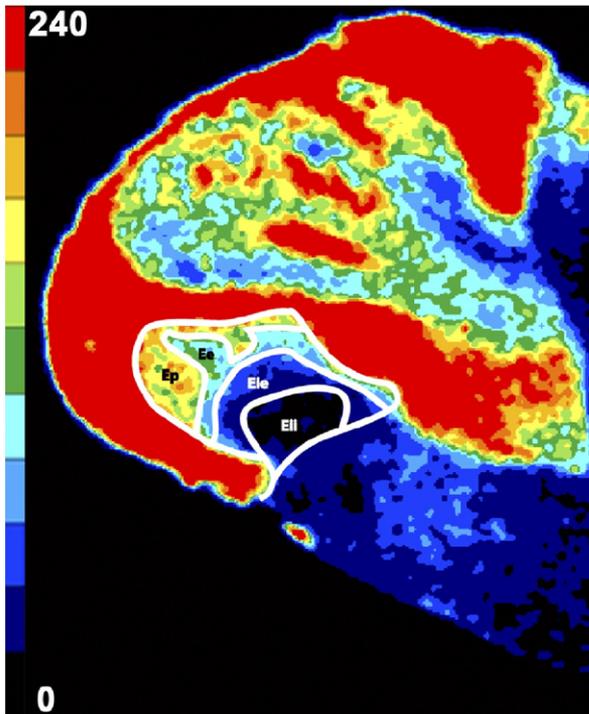


Fig. 2. Color-coded autoradiograph showing the heterogeneous 5-HT_{1A} receptor distribution in the pigeon's entopallium. Contrast enhancement is optimized to the density of 5-HT_{1A} receptors in the entopallium. The 5-HT_{1A} receptors showed a lamina-type allocation in the entopallium. Densities increased from the ventromedial area to the belt region. Ep, Entopallial belt; Ee, Entopallium externum; Ele, Entopallium internum pars externale; Eii, Entopallium internum pars internale. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

DISCUSSION

5-HT_{1A} receptors were widely and heterogeneously distributed throughout the pigeon's brain. The highest density was found in the PT. This is in line with the dense innervation of the avian PT by serotonergic fibers (Cozzi et al., 1991; Challet et al., 1996; Metzger et al., 2002). Since PT is the source of visual and nonvisual input to the superficial layers of the optic tectum, it may modulate the inhibitory control of retinotectal transmission (Gamlin and Cohen, 1988; Gamlin et al., 1996) through 5-HT_{1A} receptors. Lesions of PT as well as of the nucleus spiriformis lateralis (SpL) result in impairment of behavior involving tracking and pecking moving targets (Bugbee, 1979). Further, PT has reciprocal connections with the subpretectal nucleus SP and therefore, PT may control attention shift from one eye to the other (Theiss et al., 2003). The PT receives further input from the basal ganglia that underlines its function in visuomotor processing (Reiner et al., 1982). Like in birds, a prominent innervation of the area pretectalis with serotonergic fibers was reported for rats, turtles, and fish (Ueda et al., 1983; Lüth and Seidel, 1987; Cuadrado et al., 1993).

In the optic tectum 5-HT_{1A} receptors showed a laminar specific distribution. The major retino-recipient layer 5 displays the highest density of 5-HT_{1A} receptors in the optic

tectum, and is also densely innervated by serotonergic fibers (Metzger et al., 2006). Layer 5b of the optic tectum, which is a major retino-recipient layer, receives further input from PT (Gamlin et al., 1996). Since the optic tectum and PT showed a high density of 5-HT_{1A} receptors, the receptors may play a substantial role of 5-HT_{1A} receptors in controlling the output of these regions.

A nucleus in the brain stem, the IP, also showed a quite high density of 5-HT_{1A} receptors. This finding matches with the presence of a high density of 5-HT fibers and terminals in IP (Challet et al., 1996), and is in line with other autoradiography studies in human and rats that also showed high binding sites for 5-HT receptors in IP (Palacios et al., 1983; Kaulen et al., 1986). In birds IP has been implicated in appetitive and consummatory male sexual behavior (Dermont et al., 1999). Further, a lesion study in rats showed that IP is enclosed in a network of controlling avoidance behavior (Hammer and Klingberg, 1990). Rats with IP lesions were hypoactive and showed diminished exploratory behavior. Because behavioral drug tests in mammals have shown that 5-HT_{1A} receptors are also involved in appetitive and avoidance behavior, the same may be true for the avian species. Indeed, it was detected in ringdoves that systemic injections of 8-OH-DPAT increased locomotor activity (Tejada et al., 2011).

In the thalamic nuclei 5-HT_{1A} receptors are found to occur at moderate densities. The relatively highest density was detected in the ventral part of the lateral geniculate nucleus (Glv), which receives a strong serotonergic innervation, though to a lesser extent than PT (Cozzi et al., 1991; Challet et al., 1996). Furthermore, the Glv receives direct input from the retina and afferents from the visual Wulst, has reciprocal connections with the optic tectum, and projects to the pretectal nuclei (Guiloff et al., 1987; Güntürkün and Karten, 1991). Lesions of the Glv had shown that this area is involved in visuomotor function (Guiloff et al., 1987). The dorsal part of the lateral geniculate nucleus (Gld), contains relatively low 5-HT_{1A} receptor densities. The Gld is also a retinorecipient optic center in the thalamus, projects to the Wulst, and is part of one of the two ascending visual pathways in birds, the thalamofugal pathway (Karten et al., 1973). In addition, the Rt, which is the thalamic in- and output structure of the second visual pathway, the tectofugal pathway (Rogers and Deng, 1999; Hellmann and Güntürkün, 2001; Schmidt and Bischof, 2001; Folta et al., 2004), also contained very low 5-HT_{1A} receptor densities.

The overall 5-HT_{1A} receptor density in the pigeon Wulst was high. This implicates that 5-HT_{1A} receptors are critically involved in the function of the avian Wulst, which is in part comparable with the function of primary visual, somatosensory and motor cortices in mammals (Keary et al., 2010; Ng et al., 2010; Reiner et al., 2005; Iwaniuk and Wylie, 2006). The avian Wulst is also a part of the thalamofugal pathway in birds (Hodos et al., 1973; Karten et al., 1973; Shimizu and Bowers, 1999). It was suggested that the processing of visual information in the thalamofugal tract is associated with the performance of more complex visual tasks that include a more detailed analysis of infor-

mation, like during migration behavior (Budzynski et al., 2002). The thalamofugal pathway corresponds to the mammalian geniculostriate pathway (Shimizu and Karten, 1990). The Gld, the thalamic relay station of the thalamofugal projection receives input from the central area of the pigeon's retina, and thus, from the lateral visual field (Remy and Güntürkün, 1991). Consequently, lesions of the thalamofugal system affect discrimination tasks in the lateral but not in the frontal field of view (Güntürkün and Hahmann, 1999; Budzynski and Bingman, 2004). The highest 5-HT_{1A} receptor density was detected in the HI, which is one of the pseudolayers of the avian Wulst. Pseudolayers are nuclear structures that do not display the laminar organization of the mammalian cerebral cortex where columns have an orthogonal position to laminae (Medina and Reiner, 2000; Butler et al., 2005). HI receives visual input from the Gld and is also part of the thalamofugal system (Güntürkün and Hahmann, 1999). In addition, HI is the output layer that gives rise to projections to the dorsocaudal telencephalon like the area parahippocampalis and the area corticoidea dorsolateralis (Shimizu et al., 1995). In most avian species the Wulst contains three further pseudolayers (Medina and Reiner, 2000). The intermediate layer is a thin band of granule cells, the interstitial part of the hyperpallium apicale (IHA), which is a major recipient for sensory thalamic input (Watanabe et al., 1983; Wild, 1987; Shimizu et al., 1995). The HD receives only visual thalamic input and mainly projects to subpallial and pallial parts. The most superficial layer, the HA, is the main output layer and projects to the striatum, the thalamus and the brainstem, as well as to other pallial structures (Reiner and Karten, 1982; Veenman et al., 1995; Shimizu et al., 1995; Medina and Reiner, 2000). In addition, HA receives afferents from all other layers of the Wulst (Shimizu et al., 1995). 5-HT_{1A} receptor had lower densities in HA and HD than in HI. HI/HD showed comparable concentrations of 5-HT_{1A} receptors to those measured in layers II–III of human V1 (Eickhoff et al., 2007). Taken together, 5-HT_{1A} receptors can play a crucial role in controlling Gld output to the Wulst and hence to higher associative structures.

The nidopallium was also enriched in 5-HT_{1A} receptors, with higher amounts in the NIL than in its medial parts. In addition, the associative (Güntürkün, 2005) forebrain structure NCL was also densely labeled, and could be subdivided into a medial and a lateral part (Herold et al., 2011). The dense receptor labeling is in contrast to the few serotonergic terminals within NCL (Challet et al., 1996). However, our findings are in accordance with the results of Karakuyu et al. (2007), who examined serotonin efflux during a working memory paradigm in pigeons. They observed serotonin release in the NCL, but not in the striatum during working memory tasks. Since the serotonin release was independent of a short term memory component, the authors concluded that serotonin within NCL could control executive functions like attention switching without being involved in the process of memorization of stimulus information. Because of the relatively high 5-HT_{1A} receptor densities measured in the NCL, future studies should con-

firm a specific role of this receptors type for executive functions. For example, in rats, 5-HT_{1A} receptor modulations in the mPFC have been shown to be very important for optimal attention functioning (Carli et al., 2006). 5-HT_{1A} receptor densities in the nidopallium are comparable with those found in frontal areas of humans and monkeys (Herold et al., 2011; Goldman-Rakic et al., 1990) but are different from the findings in rats (Herold et al., 2011; Pazos and Palacios, 1985).

In birds the former archistriatum has been subdivided into a somatosensory arcopallium and a complex of structures that are comparable with the mammalian amygdaloid complex (Reiner et al., 2004; Saint-Dizier et al., 2009). In birds, the amygdaloid complex includes the nucleus posterior amygdalopallialis (PoA), the TnA, and the area subpallialis amygdalae (SpA), and has been linked to visceral and limbic functions because of its connections with the hypothalamus and caudal brain stem nuclei. A dense 5-HT innervation was found for limbic structures like the TnA, the parahippocampal area (APH), hippocampus (HP), and area septalis in pigeons, chicken, and quails (Yamada et al., 1985; Cozzi et al., 1991; Challet et al., 1996). Recently it was shown that injections of the 5-HT_{1/2/7} receptor antagonist Metergoline and the 5-HT_{1B/1D} agonist GR46611 into TnA induced hypophagic responses, whereas the same treatment in the arcopallium intermedium (AI) resulted in a selective increase in water intake (Campanella et al., 2009). These effects seemed to be regionally specific because Metergoline and GR46611 injections into the arcopallium mediale (AM) failed to affect those behaviors. In line with this, we found only low 5-HT_{1A} densities in TnA and AI, supporting the view that transmission through 5-HT_{1A} receptors in those regions plays no, or only a minor role in ingestive behavior (Campanella et al., 2009). However, our findings are in contrast to those of the mammalian amygdaloid nuclei, which contain high or intermediate 5-HT_{1A} receptor concentrations (Hall et al., 1997; Yilmazer-Hanke et al., 2003; Palchaudhuri and Flügge, 2005; Perez-Garcia and Meneses, 2008). Further, a strong serotonergic innervation of the hippocampus and the parahippocampal area has been reported, however the 5-HT_{1A} receptor density observed in the present study is low. This is not a contradictory finding, since 5-HT binds to a variety of further 5-HT receptors. Moreover, the boundaries of the avian hippocampal formation are still not completely determined. Krebs et al. (1991) detected dense 5-HT termination fields in the dorsomedial hippocampus. In our data, we could identify this field in the hippocampus by the highest concentration of 5-HT_{1A} receptors at the dorsomedial hippocampus, and a further field at the caudal and ventromedial site of the hippocampus. Future studies are clearly needed to clarify the exact boundaries of different areas in the hippocampal formation of the avian species. The low overall density of 5-HT_{1A} receptors in the avian hippocampus is in contrast to that in rats and other mammals (Topic et al., 2007; Pazos and Palacios, 1985; Zilles et al., 1985, 2000; Wree et al., 1987; Zilles, 1989; Kraemer et al., 1995; Aznavour et al., 2009).

Thus, low 5-HT_{1A} receptor densities in the pigeon's hippocampal formation may demonstrate an avian-specific situation and implicate that this receptor type might have a different role in the avian hippocampal formation than in mammals. In contrast, all septal nuclei showed dense labeling that is in line with a strong serotonergic innervation in pigeons (Challet et al., 1996) and published 5-HT_{1A} receptor densities for cats, rats, non-human primates, and humans (Aznavour et al., 2009; Aznavour and Zimmer, 2007; Khawaja, 1995; Pazos and Palacios, 1985).

Within the telencephalon, the entopallium is the end station of the tectofugal pathway (Hodos and Karten, 1970; Manns et al., 2007; Valencia-Alfonso et al., 2009). Because of the projections and connectivity of the entopallium, this structure was compared with the human extrastriate cortex (Veenman et al., 1995). Only a few serotonergic terminals were found in the entopallium of pigeons (Challet et al., 1996). In line with this, labeling of 5-HT_{1A} receptors in the entopallium was low, except for the entopallial belt (perientopallium; Ep), which showed comparable densities to those of the NCL. The Ep serves as an intermediary between the core components of the entopallium and the subsequent projections to the nidopallium and the arcopallium, although the Ep is also reached by a minute thalamic projection (Krütfeldt and Wild, 2004, 2005). Some authors compare the neurons of the Ep to layers II and III neurons of the mammalian neocortex (Shimizu and Karten, 1990; Shimizu et al., 1995; Veenman et al., 1995). Herein, our results support this suggestion, because layers II and III of the human extrastriate cortex also contain the highest numbers of 5-HT_{1A} receptors when compared with the other neocortical layers (Zilles et al., 2004; Eickhoff et al., 2007). The input region of the entopallium is the entopallial core, which can be further subdivided into an entopallium externum (Ee) and internum (Ei) (Hellmann et al., 1995; Krütfeldt and Wild, 2005). Our results in principle support such a subdivision. We found a heterogeneous 5-HT_{1A} receptor distribution within the entopallial core, with higher densities in the Ee than in the Eie, and no labeling in the Eii. This could imply that these subdivisions have functional implications, which have to be analyzed in further studies determining the role of the entopallium within the tectofugal pathway. To date, it is not clear whether neurons of the core components are comparable with layer IV neurons in the neocortex of mammals, or whether they are a mixture composed of layer IV and V neurons (Krütfeldt and Wild, 2005). Our results show a low concentration for 5-HT_{1A} receptors in Eie and Eii. This was also found in layers IV and V of the human extrastriate cortex (Eickhoff et al., 2007). In line with the finding of a laminar and columnar organization in the avian auditory cortex (Wang et al., 2010), our results support the idea that the entopallium may have a similar laminar-type organization (Wild and Krütfeldt, 2010).

In pigeons, the medial entopallium has strong reciprocal connections to an area of the ventrolateral mesopallium dorsal to the entopallium (Krütfeldt and Wild, 2005). This area is distinct in Nissl-stained sections and was described in the former hyperstriatum ventrale as hyperstriatum ven-

trale ventrolaterale (HVvl) (Husband and Shimizu, 1999). This area may be compared with the nucleus MVL in the zebra finch (Krütfeldt and Wild, 2004), and could be also observed in sparrows, canaries, and chicken (Huber and Crosby, 1929; Stokes et al., 1974; Alpár and Tömböl, 2000). In our study, this area showed a dense labeling for 5-HT_{1A} receptors that differed clearly from the rest of the mesopallium confirming a nuclear structure. Therefore, our findings support the idea of Krütfeldt and Wild (2005) that this area is comparable with the nucleus MVL in the zebra finch.

In the basal ganglia low 5-HT_{1A} receptor densities were detected. These findings are in line with one of the few studies that determined the distributions of serotonin 5-HT_{1A} receptors in the avian brain (Dietl and Palacios, 1988). Low densities were also found in the basal ganglia of mammals (Dietl and Palacios, 1988; Palomero-Gallagher et al., 2009). Thus, this result underlines the conservation of receptor distribution patterns in the basal ganglia of different species over a long span of separate evolution.

CONCLUSION

In conclusion, 5-HT_{1A} receptors were prominent in regions that process visual information and higher cognitive functions. In contrast to mammals, low binding sites were detected in limbic structures such as the hippocampus or the amygdala. Future functional studies should address these differences and similarities between the serotonergic systems in avian and mammalian brains. We detected comparable densities of 5-HT_{1A} receptors in pallial structures that have been compared with different layers of specific structures in the mammalian neocortex. Herein, our results support the idea of a nucleus to lamina homology between avian and mammalian brain structures. However, it seems to be necessary to delineate some structures more precisely because as in case of the entopallium it is indicated that the entopallium itself has a laminar-type organization.

Acknowledgments—Supported by a grant from the BMBF through the Bernstein Focus Group “Varying Tunes” to OG.

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(Accepted 26 October 2011)
(Available online 03 November 2011)