

Research Reports

Quantitative Autoradiographic Mapping of Serotonin Receptors in the Rat Brain.

I. Serotonin-1 Receptors

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The distribution of serotonin-1 (5-HT_1) receptors in the rat brain was studied by light microscopic quantitative autoradiography. Receptors were labeled with [^3H]serotonin ($5\text{-}[^3\text{H}]\text{HT}$), 8-hydroxy-2-[N -dipropylamino- ^3H]tetralin ($8\text{-OH-}[^3\text{H}]\text{DPAT}$), [^3H]LSD and [^3H]mesulergine, and the densities quantified by microdensitometry with the aid of a computer-assisted image-analysis system. Competition experiments for $5\text{-}[^3\text{H}]\text{HT}$ binding by several serotonin-1 agonists led to the identification of brain areas enriched in each one of the three subtypes of 5-HT_1 recognition sites already described (5-HT_{1A} , 5-HT_{1B} , 5-HT_{1C}). The existence of these 'selective' areas allowed a detailed pharmacological characterization of these sites to be made in a more precise manner than has been attained in membrane-binding studies. While $5\text{-}[^3\text{H}]\text{HT}$ labeled with nanomolar affinity all the 5-HT_1 subtypes, the other ^3H -labeled ligands labeled selectively 5-HT_{1A} ($8\text{-OH-}[^3\text{H}]\text{DPAT}$), 5-HT_{1C} ([^3H]mesulergine) and both of them ([^3H]LSD). Very high concentrations of 5-HT_1 receptors were localized in the choroid plexus, lateroseptal nucleus, globus pallidus and ventral pallidum, dentate gyrus, dorsal subiculum, olivary pretectal nucleus, substantia nigra, reticular and external layer of the entorhinal cortex. The different fields of the hippocampus ($\text{CA}_1\text{--CA}_4$), some nuclei of the amygdaloid complex, the hypothalamic nuclei and the dorsal raphe, among others, also presented high concentrations of sites. Areas containing intermediate densities of 5-HT_1 receptors included the claustrum, olfactory tubercle, accumbens, central grey and lateral cerebellar nucleus. The nucleus caudate-putamen and the cortex, at the different levels studied, presented receptor densities ranging from intermediate to low. Finally, in other brain areas — pons, medulla, spinal cord — only low or very low concentrations of 5-HT_1 receptors were found. From the areas strongly enriched in 5-HT_1 sites, dentate gyrus and septal nucleus contained 5-HT_{1A} sites, while globus pallidus, dorsal subiculum, substantia nigra and olivary pretectal nucleus were enriched in 5-HT_{1B} . The sites in the choroid plexus, which presented the highest density of receptors in the rat brain, were of the 5-HT_{1C} subtype. The distribution of 5-HT_1 receptors reported here is discussed in correlation with the distribution of serotonergic neurons and fibers, the related anatomical pathways and the effects which appear to be mediated by these sites.

INTRODUCTION

Pharmacological, electrophysiological and biochemical findings have revealed the existence of multiple brain serotonin (5-HT) recognition sites^{1,90}. On the basis of the results obtained in binding studies in rat brain, Peroutka and Snyder¹⁰⁹ classified these sites as 5-HT_1 , labeled by $5\text{-}[^3\text{H}]\text{HT}$ with high affinity and 5-HT_2 , which are recognized with high affinity by [^3H]spiperone. [^3H]LSD labels both receptor sites.

The possibility of the existence of different subtypes of 5-HT_1 -binding sites has received considera-

ble support in the recent years. Some serotonergic agonists, such as the piperidyl indole RU 24 969 ($5\text{-methoxy-3-[1,2,3,6-tetrahydro-4-pyridinyl]1H-indole}$)⁴⁰ and the 8-OH-DPAT ($8\text{-hydroxy-2-[}n\text{-dipropylamino]tetralin}$)⁵⁵ present Hill coefficients lower than 1 when displacing $5\text{-}[^3\text{H}]\text{HT}$ binding in the rat brain cortex^{54,57,81}. In a similar way, the neuroleptic spiperone and some β -blockers also inhibit $5\text{-}[^3\text{H}]\text{HT}$ binding in a biphasic manner (refs. 80, 86, 105, and Engel et al., in preparation). These results suggested the presence of two subtypes of 5-HT_1 sites, 5-HT_{1A} and 5-HT_{1B} , corresponding respectively to the high-

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and the low-affinity sites of spiperone- and 8-OH-DPAT-competition curves¹⁰⁵. Recently, we have characterized¹⁰³ a new type of 5-[³H]HT-binding site, mainly localized in the mammalian choroid plexus and which is not recognized with high affinity by the 5-HT_{1A} or 5-HT_{1B} 'selective' compounds described above, namely RU 24 969, 8-OH-DPAT, spiperone and β -blockers, but it is labeled by nanomolar concentrations of [³H]LSD as well as by the serotonin-2 ligand [³H]mesulergine²². We have named this site 5-HT_{1C}¹⁰³.

Considerable efforts are being made in attempts to establish functional correlates for 5-HT₁ sites. A link of 5-HT₁ sites to a 5-HT-sensitive adenylate cyclase has been the subject of much discussion (refs. 17, 42, 88, 107; see ref. 106 for a review). In a similar way, contradictory results have been reported on the proposed mediation of the dog basilar artery contraction by 5-HT₁ sites^{85,108}.

A good correlation has been found between the 5-HT₁ affinities of several drugs and their effects in models of presynaptic 5-HT receptors^{38,48,73}, although some discrepancies between the activities of the drugs in the 'presynaptic' test and in the binding assay have arisen (see ref. 44 for a discussion). It has been suggested that these discrepancies might reflect the existence of the different subtypes of 5-HT₁ recognition sites. It has also been reported that the '5-HT syndrome', a behavioral syndrome consisting of several complex motor reflexes, is mediated by 5-HT₁ sites⁶⁹. In addition to 5-HT itself and some 5-HT precursors, this syndrome is also produced by 8-OH-DPAT¹²⁷ and blocked by some β -blockers^{53,127}. Furthermore, both 5-HT agonists RU 24 969 and 8-OH-DPAT appear to produce circling behavior in rats with striatal lesions^{15,57}. The participation of the different subtypes of 5-HT₁ receptors in these effects is not clear at the present time (see Discussion).

The knowledge of the anatomical distribution of these 5-HT₁ sites in the brain constitutes an important requirement in order to better characterize these sites. It would allow (1) the detailed pharmacological characterization of the different subtypes of 5-HT₁ receptors, by analyzing the binding of 5-[³H]HT in areas enriched in one subtype, and (2) the correlation of receptor densities in different brain areas with the physiological activities proposed to be mediated by 5-HT₁ sites, clarifying their functional signifi-

cance. In this regard, quantitative autoradiographic techniques provide, besides a high level of anatomical resolution, the adequate estimation of receptor densities and affinities^{26,95,101}.

Autoradiographic visualization of 5-HT₁ sites in the central nervous system has been carried out using 5-[³H]HT^{14,138} and [³H]LSD⁷⁵ as ligands. Attempts to analyze separately the distribution of 5-HT_{1A} and 5-HT_{1B} sites have been recently reported using 5-[³H]HT³⁴ and 8-OH-[³H]DPAT⁷². We have demonstrated the presence of 5-HT_{1C} sites in the rat choroid plexus²⁵. We report here a detailed mapping of the serotonin-1 receptors in the rat brain, using 5-[³H]HT, [³H]LSD, 8-OH-[³H]DPAT and [³H]mesulergine as ligands, and analyzing the distribution of the three subtypes throughout the different brain areas.

MATERIALS AND METHODS

Rats (male, Wistar, 180–250 g) were sacrificed by intracardial perfusion with phosphate-buffered saline containing 0.1% formalin, after pentobarbital anesthesia (50 mg/kg). The brains were removed and frozen in liquid nitrogen. 10 μ m thick tissue sections were cut using a microtome-cryostat (Leitz 1720, from Leitz, Wetzlar, F.R.G.) mounted onto gelatin-coated microscope slides and stored at –20 °C until used.

Incubation conditions for each ³H-labeled ligand, including buffer solution, concentration of ligand, incubation time and exposure time are summarized in Table I. Blanks were generated by coincubation with 1 μ M LSD in the case of 5-[³H]HT and with 100 nM 5-HT for the other ³H-labeled ligands. In competition studies, consecutive tissue sections were incubated in the presence of increasing concentrations of displacers.

The binding of the different ³H-labeled ligands used, 5-HT, LSD, 8-OH-DPAT and mesulergine, to slide mounted brain rat sections has been already kinetically characterized, the saturation parameters and incubation procedures being defined^{14,72,74,75,102,138}. The conditions defined in these studies were used in the present work, with slight modifications.

After the washing period, tissues were dried with cold air and autoradiograms were generated by ap-

TABLE I

Incubation conditions used for the labeling of 5-HT₁ receptors

Ligand	[Ligand] (nM)	Preincubation protocol	Buffer	Incubation		Washing protocol	Exposure time
				temperature	time		
5-[³ H]HT	2	30 min at room temperature, in incubation buffer	0.17 M Tris-HCl-4 mM CaCl ₂ -0.01% ascorbic acid (pH 7.6)	room temp.	60 min	1 × 5 min (4 °C)	60 days
8-OH-[³ H]-DPAT	2	same as above	same as above	room temp.	60 min	2 × 5 min (4 °C)	60 days
[³ H]LSD	5	5 min at room temperature, in incubation buffer (-pargyline)	0.17 M Tris-HCl-4 mM CaCl ₂ -0.01% ascorbic acid-0.15 M NaCl-10 μM pargyline (pH 7.6)	room temp.	60 min	2 × 10 min (4 °C)	100 days
[³ H]-Mesulergine	5	15 min at room temperature, in incubation buffer	0.17 M Tris-HCl (pH 7.7)	room temp.	2 h	2 × 10 min (4 °C)	30 days

posing the labeled tissue to [³H]Ultrofilm (LKB, Sweden) as described by Unnerstall et al.¹³⁰. Brain grey and white matter standards, containing known amounts of a non-volatile ³H-labeled compound were exposed to the film along with the labeled tissue sections⁹⁵. Films were analyzed using a computerized image-analysis system which provides values of receptor densities expressed in fmol/mg protein⁹⁵. Four to six bilateral measurements were made per nucleus and the level described in each animal and the mean value was calculated. Values of 'specific binding' for each rat were obtained by subtracting the non-specific binding from the total value. The mean value from equivalent areas in different animals was calculated. Results reported in this study are from at least two separate experiments for each ³H-labeled ligand, involving tissues from 3 animals per experiment.

Percent occupancy of receptor sites by the different ³H-labeled ligands was estimated according to the equation:

$$\% \text{ occupancy} = 100 L / (L + K_d (1 + I/K_i))$$

where L = concentration of radioactive ligand; K_d =

equilibrium dissociation constant; I = concentration of unlabeled drug, and K_i = inhibition constant of unlabeled drug.

Brain nuclei were identified and named using the rat brain atlas of Paxinos and Watson¹⁰⁰. Cortical areas were named according to Krieg^{60,61}.

5-[³H]HT (26.8 Ci/mmol) and [³H]LSD (69.3 Ci/mmol) were obtained from New England Nuclear, Dreieich, F.R.G. [³H]Mesulergine (85 Ci/mmol) was synthesized and labeled by Dr. R. Voges of the Biopharmaceutical Department, Sandoz Ltd. (Basle, Switzerland). 8-OH-[³H]DPAT (105 Ci/mmol) was obtained from CEA, Saclay (France). Other substances were either purchased or kindly provided by the original manufacturers.

RESULTS

Pharmacological characterization of 5-HT₁ binding to some brain areas

The pharmacology of the binding of the 5-HT₁ ³H-labeled ligands was analyzed by quantitative autoradiography in several brain areas showing relevant densities of autoradiographic grains. The competi-

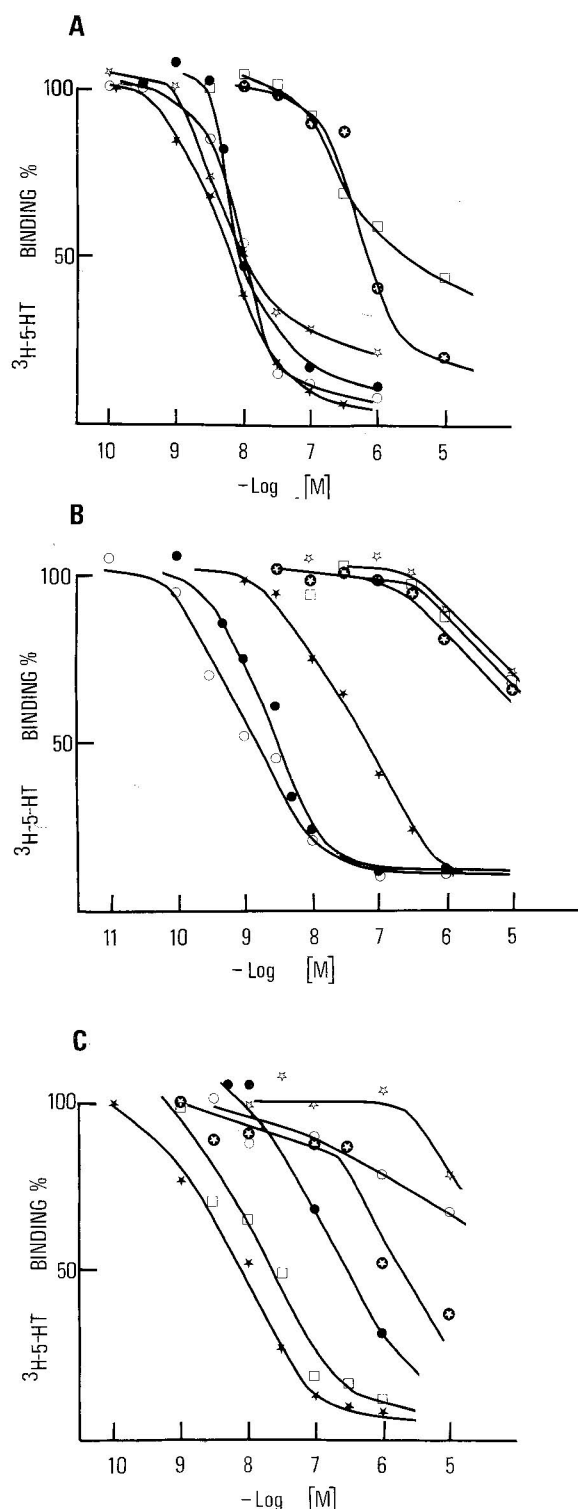


Fig. 1. Competition curves for $5\text{-}[^3\text{H}]\text{HT}$ binding to the dentate gyrus of the hippocampus (A), dorsal subiculum (B) and choroid plexus (C), measured by quantitative autoradiography. Curves correspond to (-)21 009 (○), RU-24 969 (●), 8-OH-DPAT (☆), LSD (★), mesulergine (□) and spiperone (⊙).

tion profile for $5\text{-}[^3\text{H}]\text{HT}$ binding of several drugs including 'selective' 5-HT_1 agonists and serotonergic antagonists, was studied. The analysis of the competition curves, some of them shown in Fig. 1, as well as the K_i values obtained for the different drugs (Table II) revealed the presence of three types of $5\text{-}[^3\text{H}]\text{HT}$ -binding sites, with different pharmacological properties. $5\text{-}[^3\text{H}]\text{HT}$ binding to the dentate gyrus of the hippocampus was inhibited with nanomolar affinity by 8-OH-DPAT, LSD, RU 24 969 and the β -blocker (-)21 009 (4[ter-butyl-amino-2-hydroxypropoxy]indol-2-carbonic acid isopropylester), while the other compounds used showed low to very low affinity for these binding sites (Figs. 1A, 2-5, 7 and 8, and Table II). In contrast, $5\text{-}[^3\text{H}]\text{HT}$ binding to the dorsal subiculum was displaced with nanomolar affinity by RU 24 969 and (-) 21 009. While LSD showed an intermediate affinity for these sites, the other drugs tested inhibited $5\text{-}[^3\text{H}]\text{HT}$ binding to this area with low or very low affinity (Figs. 1B, 2, 3, 5 and 8, and Table II). Finally, in the choroid plexus, only LSD, mesulergine and mianserin recognized the binding of $5\text{-}[^3\text{H}]\text{HT}$ with high affinity (Figs. 1C, 3-5, 7-9, and Table II).

According to the definition of the different subtypes of 5-HT_1 -recognition sites already mentioned, and to the affinities presented by the 'subtype-selective' ligands, $5\text{-}[^3\text{H}]\text{HT}$ sites in the dentate gyrus correspond to the 5-HT_{1A} subtype, while those in the dorsal subiculum exhibit the characteristics of 5-HT_{1B} sites. The pharmacology of $5\text{-}[^3\text{H}]\text{HT}$ binding to the choroid plexus is closely comparable to that presented by 5-HT_{1C} sites, as defined in membrane-binding studies (Table III).

In addition to these three anatomical areas, other rat brain nuclei presented a similar enrichment in one or the other subtype of 5-HT_1 sites. Table II summarizes the K_i values of different compounds for $5\text{-}[^3\text{H}]\text{HT}$ binding in the areas already described, as well as in others. A close correlation exists between K_i values for the different drugs in the dorsal subiculum, globus pallidus and substantia nigra (Table II). On the other hand, K_i values found in dentate gyrus, stratum oriens of the CA_1 field of the hippocampus and lateroseptal nucleus are also very similar (Table II). This pharmacological characterization demonstrates the existence of the three subtypes of 5-HT_1 -recognition sites in different nuclei of the rat brain. In

TABLE II

Affinities (K_i)¹ of several drugs for 5-[³H]HT binding to rat brain areas measured by quantitative autoradiography

Drug	Brain area						
	Dorsal subiculum	Globus pallidus	Substantia nigra (reticular)	Lateral septal nucleus (dorsal)	Dentate gyrus	CA ₁ field hippocampus	Choroid plexus
LSD	29.9	25.2	44.9	ND	3.6	7.2	4.1
RU 24 969	1.5	0.6	3.5	9.0	5.4	10.2	130.2
8-OH-DPAT	>3000	>3000	>5000	19.2	4.8	11.4	>4000
(-) 21 009	0.6	1.7	0.9	6.0	7.2	48.1	>4000
Mesulergine	>5000	>3000	ND	ND	>1000	>3000	11.8
Spiperone	>3000	>3000	>3000	ND	359.3	209.6	710.1
Ketanserin	1796.4	>1000	>1000	ND	598.8	>1000	354.0
Mianserin	143.7	ND ²	ND	ND	269.5	ND	13.6
Cinanserin	>3000	>5000	ND	>5000	>2000	ND	2000
(±) Propranolol	149.7	173.7	ND	ND	257.5	509.0	>5000
Pirenperone	>5000	>5000	ND	>2000	>5000	ND	1000

¹ nM.² Not determined.

contrast to these areas, where a predominant presence of one subtype was found, in many other brain nuclei, the existence of several subtypes of 5-[³H]HT-binding sites was demonstrated. An example of these areas is the neocortex. In the lamina IV of the frontoparietal somatosensory cortex, biphasic competition curves were obtained with several drugs (not shown).

A similar pharmacological characterization was carried out in the same areas for the other ³H-labeled ligands. While [³H]mesulergine labeled sites in the choroid plexus with the characteristics of 5-HT_{1C}-binding sites, binding of 8-OH-[³H]DPAT to the dentate gyrus and to the lateroseptal nucleus displayed the properties of 5-HT_{1A} sites. Finally, [³H]LSD labeled with high affinity, areas enriched in both 5-

HT_{1A} and 5-HT_{1C} (not shown).

It must be pointed out that, in addition to 5-HT_{1A} and 5-HT_{1C} [³H]LSD labeled 5-HT₂ and dopaminergic receptors, as it has been already reported. Similarly, [³H]mesulergine, aside from labeling 5-HT_{1C} sites, also labeled 5-HT₂ receptors. All these binding sites were not displaced by 100 nM 5-HT, concentration used to define non-specific binding.

Distribution of 5-HT₁ receptors in rat brain

In the following, the detailed distribution of 5-HT₁ sites in the rat brain is analyzed. 5-[³H]HT binding was measured in all the areas studied. In some selected nuclei, [³H]LSD and [³H]mesulergine binding were also quantified. In addition, binding of 8-OH-[³H]DPAT was qualitatively evaluated in several structures.

In order to determine the presence of the different subtypes of 5-HT₁ sites in each brain area, we combined the maximal densities obtained using the ³H-labeled ligands, with data from displacement experiments. For that, the inhibition of 5-[³H]HT binding induced by 10 nM (-) 21 009 and 10 nM 8-OH-DPAT was quantified in each area. The percent inhibition produced by these concentrations of both compounds in some areas previously defined as 'selectively' enriched in 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites were analyzed and, from these data, the interval for the inhibition of 5-[³H]HT binding in the three populations was calculated (Table IV). Percent inhibition values in areas presenting more than one subtype of 5-HT₁ site

TABLE III

Comparison of the affinities of several drugs for 5-[³H]HT binding in rat choroid plexus (autoradiographic studies) and in pig choroid plexus (binding assays)

Drug	Binding ¹ K_i (nM)	Autoradiography K_i (nM)
LSD	4.8	4.1
RU 24 969	165.1	130.2
8-OH-DPAT	>5000	>4000
(-) 21 009	2120.6	>4000
Mesulergine	5.2	11.8
Spiperone	>2000	710.1
Ketanserin	155.0	354.0
Mianserin	12.6	13.6
Cinanserin	1676.2	2000

¹ Taken from ref. 103.

TABLE IV

Inhibition of 5-[³H]HT binding by (-) 21 009 and 8-OH-DPAT in different brain areas

Area	% 5-[³ H]HT binding in the presence of 10 nM (-) 21 009	% 5-[³ H]HT binding in the presence of 10 nM 8-OH-DPAT
<i>5-HT_{1A} sites</i>		
Dentate gyrus	71.1	16.3
CA ₁ (oriens)	71.9	32.3
Lateral septal nucleus (dorsal)	79.6	42.7
Mean ± S.E.M.	74.2 ± 2.7	30.4 ± 7.8
(95% confidence limits)	(68.8–79.6)	(14.8–46.4)
<i>5-HT_{1B} sites</i>		
Dorsal subiculum	26.3	100.0
Globus pallidus	36.3	100.0
Substantia nigra (reticular)	29.5	77.4
Mean ± S.E.M.	30.7 ± 3.0	92.5 ± 7.7
(95% confidence limits)	(24.7–36.7)	(77.1–100)
<i>5-HT_{1C} sites</i>		
Choroid plexus (lateral ventricle)	90.3	88.5
Choroid plexus (third ventricle)	91.0	100.0
Choroid plexus (fourth ventricle)	76.1	94.7
Mean ± S.E.M.	85.8 ± 4.9	94.4 ± 3.4
(95% confidence limits)	(76.0–95.6)	(87.6–100)

in a given proportion, could be also obtained. In this way, the presence of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites in each brain area was predicted.

We have arbitrarily designated areas with 'very high' density of 5-HT₁ sites, as those presenting max-

imal densities of binding above 2700 fmol/mg protein, when labeled with 5-[³H]HT. High densities were between 2100 and 2700 fmol/mg protein. Intermediate levels of sites were between 1200 and 2100 fmol/mg protein. Low density areas were between 400 and 1200 fmol/mg protein. Areas presenting densities below 400 were considered as 'very low' areas. The values of these densities for the individual brain areas are listed in Table V. Values correspond to maximal densities of receptors, obtained from the percent occupancies, which were calculated as previously described. The distribution of these receptors is illustrated in the photographic atlas presented in Figs. 2–10. The reader is referred to the table and figures for further details.

Olfactory system. The external plexiform layer of the olfactory bulb, anterior olfactory nucleus, olfactory tubercle and piriform cortex (area 51b) presented intermediate concentrations of 5-HT₁ sites, while in the internal granular layer of the bulb, a somewhat lower density of sites was found. The piriform cortex, olfactory tubercle and anterior olfactory nucleus appeared to contain the three subtypes of 5-HT₁ receptors. In contrast, the external plexiform layer was enriched in 5-HT_{1A} sites and the internal granular layer contained predominantly 5-HT_{1B} sites (Table V and Figs. 3–6 and 7A–C).

Amygdala. The different nuclei of the amygdaloid complex presented an important concentration of autoradiographic grains. In the posteromedial and anterior cortical nuclei, high densities of 5-HT₁ receptors were found. The other nuclei showed intermediate concentration of sites. All these amygdaloid nuclei were mainly enriched in 5-HT_{1B}-recognition sites (Table V and Figs. 4, 5 and 7D–F).

Septal region. The lateroseptal nucleus presented very high concentrations of 5-HT₁ receptors, the majority being of the 5-HT_{1A} class. The bed nucleus of the stria terminalis contained an intermediate density of specific binding (Table V and Figs. 3, 6D–F and 7A–C).

Basal ganglia. In the basal ganglia, very high concentrations of 5-HT₁ sites were present in the globus pallidus and ventral pallidum. While the entopeduncular nucleus presented a high density of autoradiographic grains, the nucleus accumbens and the caudate-putamen only contained intermediate concentrations of specific binding. Most of the binding found

TABLE V

Maximal densities of 5-HT₁ receptors in the rat brain

Area	Specific binding (fmol/mg protein)			³ H]LSD (mean ± S.E.M.)	³ H]Mesulergine (mean ± S.E.M.)	8-OH[³ H]- DPAT (qualitative)
	5-[³ H]HT					
	Mean ± S.E.M.	% binding in the presence of 10 nM (-)-21009	% binding in the presence of 10 nM 8-OH-DPAT			
<i>Olfactory system</i>						
External plexiform layer of the olfactory bulb	1286.8 ± 175.8	85.7	57.6	—	—	—
Internal granular layer of the olfactory bulb	1034.1 ± 49.5	59.4	81.2	—	—	—
Anterior olfactory nucleus	1757.8 ± 180.2	65.9	49.1	—	204.2 ± 31.8	—
Olfactory tubercle	1787.3 ± 210.9	58.9	78.3	607.4 ± 143.6	180.3 ± 12.1	—
Primary olfactory cortex	1647.3 ± 43.9	69.1	86.9	559.1 ± 116.6	—	—
<i>Amygdala</i>						
Lateral amygdaloid nucleus	2075.6 ± 286.3	33.5	90.0	808.6 ± 51.7	178.7 ± 34.0	—
Basomedial amygdaloid nucleus	1420.9 ± 139.0	41.9	100.0	—	—	—
Central amygdaloid nucleus	1361.4 ± 212.6	56.9	100.0	—	—	—
Medial amygdaloid nucleus	1627.3 ± 214.8	42.5	83.3	—	—	—
Anterior cortical amygdaloid nucleus	2230.9 ± 362.2	45.8	67.0	—	—	—
Posteromedial cortical amygdaloid nucleus	2439.0 ± 487.3	35.0	68.4	602.3 ± 145.8	—	—
<i>Septal region</i>						
Lateral septal nucleus, dorsal part	3047.3 ± 289.5	79.6	42.7	—	—	high
Lateral septal nucleus, intermediate part	2802.4 ± 412.4	69.0	53.7	—	—	high
Lateral septal nucleus, ventral part	2988.0 ± 199.7	74.9	46.7	2012.1 ± 327.3	—	—
Bed nucleus of the stria terminalis	1971.7 ± 118.7	52.1	74.8	—	—	—
<i>Basal ganglia</i>						
Accumbens nucleus	1309.2 ± 9.0	52.7	97.2	—	—	—
Globus pallidus	3431.9 ± 180.4	36.3	100.0	102.3 ± 52.1	—	very low
Ventral pallidum	3614.8 ± 400.2	28.5	100.0	91.5 ± 8.7	—	—
Entopeduncular nucleus	2266.0 ± 195.1	15.7	100.0	—	—	—
Caudate-putamen, head	1178.2 ± 114.6	42.4	96.2	—	—	—
Caudate-putamen, body	1230.9 ± 2.9	44.4	97.0	—	—	low
Caudate-putamen, tail	1081.7 ± 47.3	61.1	97.6	—	—	—
<i>Hippocampus</i>						
Dentate gyrus	3401.9 ± 168.7	71.1	16.3	2903.1 ± 145.0	—	very high
CA ₁ field, oriens layer	2082.4 ± 80.4	71.9	32.3	—	—	high
CA ₁ field, stratum radiatum	1788.2 ± 386.3	64.7	41.1	—	—	—

TABLE V continued

Maximal densities of 5-HT₁ receptors in the rat brain

Area	Specific binding (fmol/mg protein)					
	5-[³ H]HT			[³ H]LSD (mean ± S.E.M.)	[³ H]Mesulergine (mean ± S.E.M.)	8-OH[³ H]- DPAT (qualitative)
	Mean ± S.E.M.	% binding in the presence of 10 nM (-)-21009	% binding in the presence of 10 nM 8-OH-DPAT			
CA ₁ field, lacunosum molecular layer	2501.2 ± 283.4	65.1	28.6	2112.1 ± 451.6	166.9 ± 54.3	—
CA ₂ field, oriens layer	1030.9 ± 61.7	73.0	42.1	—	—	—
CA ₂ field, pyramidal layer	1341.9 ± 130.9	70.1	41.5	—	—	—
CA ₃ field, oriens layer	1860.7 ± 8.3	60.8	41.2	1428.1 ± 168.4	—	high
CA ₃ field, pyramidal layer	773.1 ± 55.6	84.4	59.8	1243.6 ± 101.1	—	—
CA ₄ field	2615.6 ± 475.1	73.7	21.7	—	—	—
Dorsal subiculum	2841.7 ± 46.0	26.3	100.0	108.2 ± 35.6	—	low
<i>Hypothalamus</i>						
Ventromedial hypothalamic nucleus	2667.5 ± 234.1	43.2	69.3	454.8 ± 20.3	—	low
Lateral hypothalamic area	1363.6 ± 144.6	41.3	90.6	—	—	—
Dorsomedial hypothalamic nucleus	1977.0 ± 247.3	47.3	86.3	—	—	—
Posterior hypothalamic nucleus	1355.6 ± 61.9	36.6	80.1	—	—	—
Medial mammillary nucleus	1792.9 ± 204.8	48.6	89.2	—	69.3 ± 12.1	—
<i>Thalamus</i>						
Lateral habenular nucleus	855.1 ± 111.9	56.7	63.8	—	—	—
Anterior pretectal area	996.5 ± 11.4	33.6	53.6	—	—	—
Olivary pretectal nucleus	2761.4 ± 331.2	32.7	93.2	84.1 ± 13.2	—	—
Central medial thalamic nucleus	1707.3 ± 236.0	67.4	84.7	912.1 ± 101.8	133.9 ± 42.7	—
Laterodorsal thalamic nucleus	1193.6 ± 87.0	48.6	95.2	—	—	—
Ventrolateral thalamic nucleus	349.2 ± 32.9	42.0	87.9	—	—	—
Ventroposterior thalamic nucleus, lateral part	194.3 ± 31.7	50.3	86.8	—	—	—
Parafascicular nucleus	961.9 ± 151.9	61.6	89.3	—	—	—
Reuniens thalamic nucleus	1529.5 ± 132.1	38.3	100.0	—	—	—
Reticular thalamic nucleus	181.2 ± 30.9	48.5	89.5	—	—	—
Dorsal lateral geniculate nucleus	1074.1 ± 146.8	24.9	100.0	—	—	—

TABLE V continued

Maximal densities of 5-HT₁ receptors in the rat brain

Area	Specific binding (fmol/mg protein)			[³ H]LSD (mean ± S.E.M.)	[³ H]Mesulergine (mean ± S.E.M.)	8-OH[³ H]- DPAT (qualitative)
	5-[³ H]HT					
	Mean ± S.E.M.	% binding in the presence of 10 nM (-)-21009	% binding in the presence of 10 nM 8-OH-DPAT			
Medial geniculate nucleus	595.8 ± 88.3	47.4	63.2	—	—	—
<i>Clastrum, neo and cingulate cortex</i>						
Clastrum	1491.4 ± 178.5	67.5	75.2	1028.5 ± 195.9	185.2 ± 19.8	low
Frontal cortex (Area 10)						
lamina I	1309.0 ± 69.5	69.6	92.3	483.9 ± 18.3	—	—
lamina II	1149.7 ± 38.3	59.3	91.9	—	—	—
laminae III–IV	1681.9 ± 66.0	69.7	79.1	803.4 ± 166.6	102.3 ± 35.8	—
lamina V	1715.3 ± 159.2	62.8	72.7	—	—	—
lamina VI	1573.1 ± 48.7	57.3	73.2	—	—	—
Frontoparietal motor cortex (Area 2)						
lamina I	798.7 ± 115.8	65.4	90.5	321.8 ± 74.7	—	low
laminae II–III	1002.6 ± 166.8	60.8	93.0	—	—	very low
lamina IV	1554.6 ± 208.3	69.0	76.7	—	—	intermediate
lamina V	1744.6 ± 110.7	61.0	71.4	907.4 ± 270.1	—	intermediate
lamina VI	1316.3 ± 90.0	53.3	61.1	718.4 ± 200	—	—
Frontoparietal somatosensory cortex (Area 2a)						
lamina I	1053.1 ± 67.5	57.1	98.2	—	—	—
laminae II–III	917.3 ± 146.3	58.9	94.8	—	—	—
lamina IV	1461.4 ± 184.3	54.7	73.7	634.1 ± 112.8	84.2 ± 7.9	—
lamina V	1532.9 ± 80.7	70.1	60.4	—	—	—
lamina VI	1243.1 ± 128.5	58.6	57.5	—	—	—
Striate cortex (Area 18)						
lamina I	600.4 ± 89.7	66.0	67.2	—	—	low
laminae II–III	688.2 ± 195.8	56.2	72.1	—	—	—
lamina IV	782.4 ± 172.9	65.3	50.4	952.8 ± 104.5	51.3 ± 9.8	intermediate
lamina V	715.8 ± 109.0	72.8	59.5	1071.8 ± 148.8	—	—
lamina VI	686.8 ± 153.4	67.0	59.5	—	—	—
Anterior cingulate cortex (Area 24)						
lamina I	1860.4 ± 31.9	28.9	72.2	—	—	—
laminae II–III	1633.1 ± 203.4	32.1	59.9	—	—	—
lamina IV	2333.4 ± 332.6	44.5	49.8	—	—	—
lamina V	2109.2 ± 50.2	35.3	68.4	—	—	—
Retrosplenial cortex (Area 29b)						
lamina I	1100.9 ± 136.3	40.9	75.8	—	—	—
laminae II–III	798.5 ± 65.8	51.3	88.7	—	—	—
lamina IV	740.2 ± 89.0	52.9	77.7	—	—	—
lamina V	624.1 ± 100.9	54.5	77.3	—	—	—
lamina VI	572.4 ± 64.6	59.1	72.3	—	—	—
<i>Entorhinal cortex</i>						
Principalis						
external layer	2844.6 ± 140.2	69.8	49.5	—	—	high
Intermediate layer	1213.9 ± 40.7	28.2	63.9	—	—	—
Principalis						
internal layer	2499.0 ± 359.2	60.3	48.1	—	—	—
<i>Cerebellum</i>						
Stratum moleculare	265.3 ± 48.7	32.7	72.8	—	—	—
Stratum granulosum	374.6 ± 5.8	52.8	29.7	—	—	—

TABLE V *continued**Maximal densities of 5-HT₁ receptors in the rat brain*

Area	Specific binding (fmol/mg protein)			$[^3\text{H}]\text{LSD}$ (mean $\pm$ S.E.M.)	$[^3\text{H}]\text{Mesulergine}$ (mean $\pm$ S.E.M.)	8-OH $[^3\text{H}]$ - DPAT (qualitative)
	5- $[^3\text{H}]\text{HT}$					
	Mean $\pm$ S.E.M.	% binding in the presence of 10 nM (-)-21009	% binding in the presence of 10 nM 8-OH-DPAT			
Lateral nucleus	1283.9 $\pm$ 352.9	21.0	89.1	45.6 $\pm$ 11.8	—	very low
Infracerebellar nucleus	456.0 $\pm$ 83.6	37.1	94.1	—	—	—
Intermediate nucleus	543.4 $\pm$ 36.0	18.3	89.6	—	—	—
Midbrain						
Superficial grey layer of the superior colliculus	1604.3 $\pm$ 97.5	32.8	76.5	—	—	very low
Optic nerve layer of the superior colliculus	928.0 $\pm$ 67.5	32.9	81.4	—	—	—
Principal oculomotor nucleus	1266.5 $\pm$ 131.4	33.6	54.6	—	—	—
Central grey (periaqueductal)	1456.0 $\pm$ 21.4	39.6	81.1	142.1 $\pm$ 23.4	—	—
Dorsal raphé nucleus	2348.7 $\pm$ 317.3	60.1	43.6	2207.4 $\pm$ 121.5	—	high
Median raphé nucleus	1150.0 $\pm$ 266.0	49.8	50.8	—	—	—
Substantia nigra, reticular	2742.4 $\pm$ 135.8	29.5	77.4	243.1 $\pm$ 37.8	—	low
Substantia nigra, compacta	819.7 $\pm$ 28.0	28.3	100.0	—	—	—
Ventral tegmental area	823.6 $\pm$ 400.7	31.5	62.4	—	—	—
Interpeduncular nucleus	1140.4 $\pm$ 297.5	39.4	51.8	—	—	—
Pons						
Nucleus of the spinal tract of the trige- minal nerve, oral part	707.0 $\pm$ 90.4	48.3	53.4	—	—	—
Facial nucleus	773.9 $\pm$ 51.8	33.1	53.1	—	—	—
Pontine nuclei	419.7 $\pm$ 54.3	21.8	71.0	—	—	—
Nucleus of the solitary tract	1184.6 $\pm$ 106.8	50.4	59.3	—	71.6 $\pm$ 9.8	—
Raphé obscurus nucleus	761.4 $\pm$ 155.8	48.2	89.6	—	—	—
Medulla oblongata						
Ambiguous nucleus	745.8 $\pm$ 77.8	21.8	72.2	—	—	—
Gracile nucleus	860.9 $\pm$ 79.5	18.4	57.2	—	—	—
Cuneate nucleus	616.0 $\pm$ 85.8	23.0	51.0	—	—	—
Inferior olive	793.7 $\pm$ 158.0	44.4	77.5	—	—	—
Hypoglossal nucleus	710.0 $\pm$ 3.6	26.4	95.4	—	—	—
Nucleus of the spinal tract of the trigeminal nerve, caudal part	836.3 $\pm$ 123.8	39.4	52.3	—	—	—
Nucleus of the spinal tract of the trigeminal nerve, gelatinosus subnucleus	1322.1 $\pm$ 193.9	59.3	43.1	—	—	—
Medial vestibular nucleus	791.7 $\pm$ 41.9	35.7	69.0	—	—	—

TABLE V continued

Maximal densities of 5-HT₁ receptors in the rat brain

Area	Specific binding (fmol/mg protein)			³ H]LSD (mean ± S.E.M.)	³ H]Mesulergine (mean ± S.E.M.)	8-OH[³ H]- DPAT (qualitative)
	5-[³ H]HT					
	Mean ± S.E.M.	% binding in the presence of 10 nM (-)-21009	% binding in the presence of 10 nM 8-OH-DPAT			
<i>Spinal cord</i>						
Layer 1 of Rexed	1011.2 ± 169.8	89.6	42.4	—	91.4 ± 21.6	—
Layer 4 of Rexed	1099.1 ± 31.2	73.0	62.8	—	—	—
Layer 9 of Rexed	303.8 ± 27.9	43.8	68.9	—	—	—
Layer 10 of Rexed	458.5 ± 63.2	54.3	78.4	—	—	—
<i>Choroid plexus</i>						
Lateral ventricle	3496.0 ± 530.2	90.3	88.5	3787.9 ± 391	1563.7 ± 82.1	very low
Third ventricle	3381.7 ± 402.5	91.0	100.0	2751.4 ± 23.2	—	—
Fourth ventricle	4265.3 ± 279.5	76.1	94.7	3381.0 ± 293.7	1444.5 ± 308.7	very low

in the basal ganglia corresponded to the 5-HT_{1B} class (Table V and Figs. 3–5, 6D–F and 7).

Hippocampus. The hippocampal formation was very enriched in 5-HT₁ receptors. The dentate gyrus and the dorsal subiculum contained very high concentrations of sites. In the different fields of the hippocampus (CA₁–CA₄), high to intermediate densities of specific binding were found, depending on the layer analyzed. While binding to the dorsal subiculum showed the pharmacological characteristics of 5-HT_{1B} sites, the other hippocampal areas mostly contained 5-HT_{1A} sites (Table V and Figs. 2–5, 7D–F and 8).

Hypothalamus. High concentrations of 5-HT₁ receptors were found in the ventromedial hypothalamic nucleus. Other hypothalamic nuclei presented intermediate densities of specific binding. Most of the binding to these structures exhibited characteristics of the 5-HT_{1B} class (Table V and Figs. 3–5 and 7D–F).

Thalamus. Marked differences were observed in the density of 5-HT₁ receptors throughout the thalamus. Some nuclei, such as the ventrolateral and the reticular nuclei presented very low densities of specific binding. Low concentrations were found in the laterodorsal nucleus and the dorsal lateral geniculate nucleus. The central medial and the olivary pretectal nuclei were rich in 5-HT₁ sites. 5-HT₁ receptors in the thalamus presented the pharmacological profile of 5-HT_{1B} sites, although some 5-HT_{1C} binding appeared to exist in the central medial nucleus (Table V and Figs. 3–5 and 7D–F).

Clastrum, neo and cingulate cortex. The clau-

trum presented an intermediate density of 5-HT₁ receptors. The three subtypes of 5-HT₁ sites appeared to be present in this structure (Table V and Figs. 3, 5 and 6).

In the neocortex, intermediate concentrations of specific binding were found in the different layers of the frontal (area 10) and frontoparietal cortex (areas 2 and 2a), while the striate cortex (area 18) presented only low concentrations of sites. It can be said that the internal laminae (IV–VI) were richer in 5-HT₁ binding than the external ones (I–III). In the frontal cortex, most of the binding to all the laminae was of the 5-HT_{1B} class, although 5-HT_{1A} and 5-HT_{1C} sites were also present. In contrast, in the other regions studied, 5-HT_{1B} sites were predominant in the external laminae, but both 5-HT_{1A} and 5-HT_{1B} sites appeared to coexist in laminae IV–VI. As indicated above, the presence of low densities of 5-HT_{1C} sites at these levels, cannot be excluded.

Regarding the cingulate cortex, in the anterior cingulate cortex (area 24) intermediate to high concentrations of specific binding were found, the internal layers being the most enriched. A predominant but not exclusive presence of 5-HT_{1B} sites was detected. Finally, the retrosplenial cortex (area 29b) presented low densities of 5-HT₁ receptors in all the layers (Table V and Figs. 2–8).

Entorhinal cortex. The external layer of the entorhinal cortex presented a very high concentration of 5-HT₁ receptors. In the internal layer, a high density of autoradiographic grains was found, while only intermediate concentrations were present in the intermediate layer. Both 5-HT_{1A} and 5-HT_{1B} sites ap-

Figs. 2–10. Atlas of autoradiographic localization of 5-[³H]HT-binding sites in the rat brain. Dark regions represent areas with high receptor densities. Anatomical levels shown in these figures correspond to the plates 9, 12, 15, 20, 26, 30, 36, 42, 50, 55, 60, 65 and 66 of the rat brain atlas from Paxinos and Watson¹⁰⁰. Abbreviations used are listed separately in the text. Autoradiograms shown correspond to the total binding of 5-[³H]HT (A, D) and that in the presence of 10 nM (–)21 009 (B, E) and 100 nM 8-OH-DPAT (C, F). See text for further details.

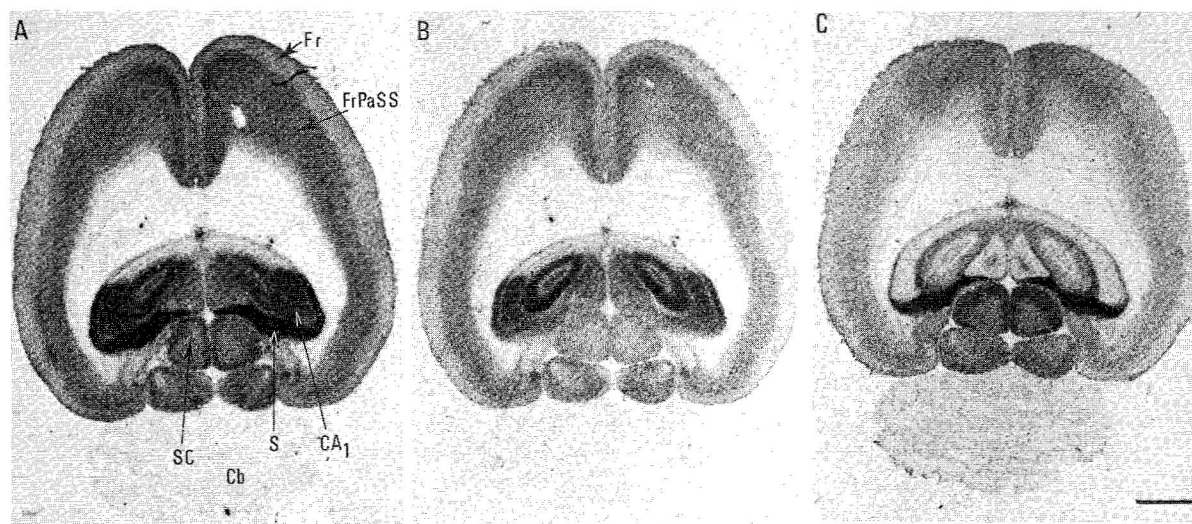


Fig. 2. 5-[³H]HT-binding sites in a dorsal horizontal section. Note the high concentrations of binding in the dorsal subiculum (S) and other regions of the hippocampus, such as the CA₁ field (CA₁). The superior colliculus (SC) and the internal layers of the neocortex also present important densities of 5-HT₁ receptors. Binding to the dorsal subiculum and the superior colliculus is inhibited by (–)21 009 (B) and not by 8-OH-DPAT (C), corresponding to 5-HT_{1B} sites. The opposite pattern is seen in the other areas of the hippocampus (5-HT_{1A} sites). Bar = 0.2 cm.

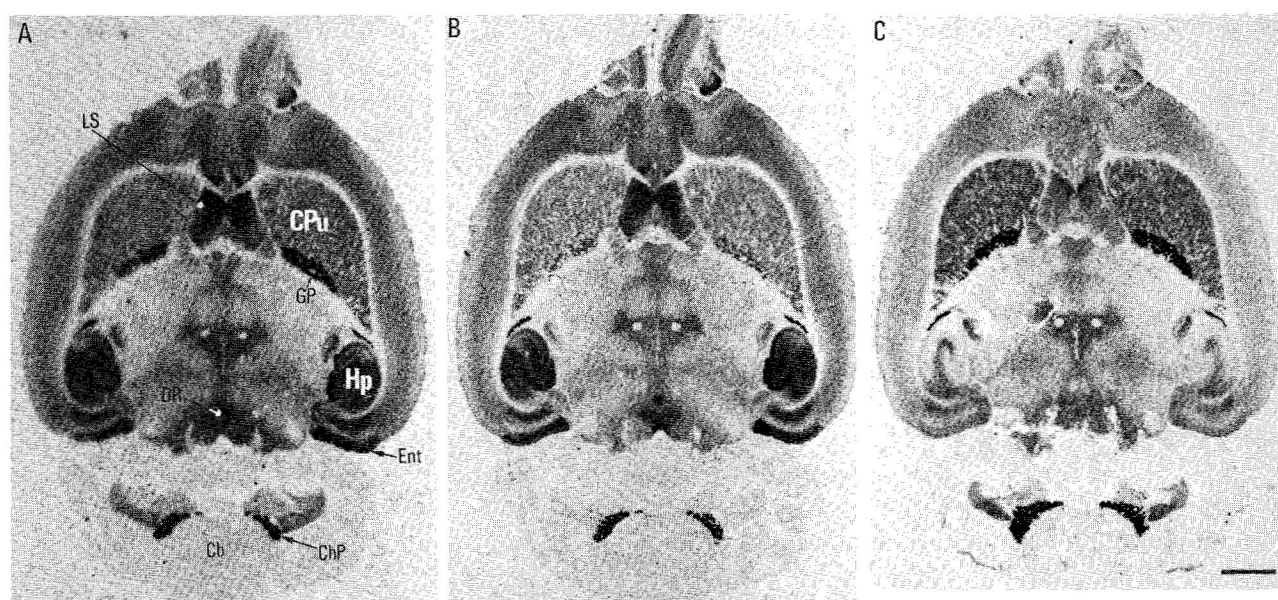


Fig. 3. 5-[³H]HT-binding sites in a horizontal section more ventral than those in Fig. 2. Very high concentrations of receptors are present in choroid plexus (ChP), lateral septal nucleus (LS), globus pallidus (GP), hippocampus (Hp) and entorhinal cortex (Ent). The caudate-putamen nucleus (CPu), some thalamic nuclei, the dorsal raphe nucleus (DR) and the deep cerebellar nuclei are also labeled by 5-[³H]HT. Sites where the binding is displaced by (–)21 009 (5-HT_{1B} sites) include the globus pallidus, caudate-putamen and cerebellar nuclei (B). Areas where 8-OH-DPAT shows high affinity for 5-[³H]HT binding (5-HT_{1A} sites) are the lateral septal nucleus, the hippocampus and the entorhinal cortex (C). 5-[³H]HT binding to the choroid plexus is not inhibited by these drugs (5-HT_{1C} sites). Bar = 0.2 cm.

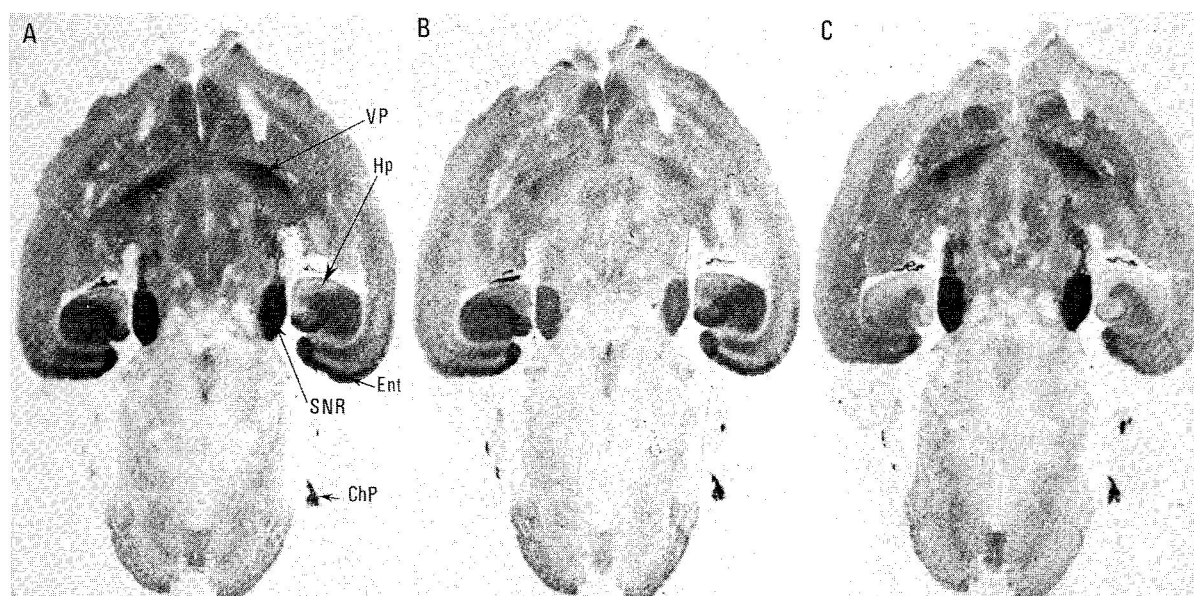


Fig. 4. 5-[^3H]HT-binding sites in a ventral horizontal section. Note the high affinity of (-)-21 009 (B) for the binding to the ventral pallidum (VP) and substantia nigra (SNR), while 8-OH-DPAT (C) inhibits 5-[^3H]HT binding to the hippocampus (Hp). Bar = 0.2 cm.

peared to exist in these areas, although 5-HT_{1A} sites were predominant in the internal and external layers (Table V and Figs. 3, 4 and 8D–F).

Cerebellum. While the density of receptors in the different layers of the cerebellum was very low, the deep nuclei presented detectable amounts of specific binding ranging from intermediate (lateral nucleus) to low densities (infracerebellar and intermediate nuclei). Binding to these nuclei presented the pharmacological characteristics of 5-HT_{1B} sites (Table V and Figs. 2, 3, 5 and 9).

Midbrain. A heterogeneous distribution of 5-HT₁ receptors was found in the midbrain, the richest areas being the substantia nigra (reticular), the dorsal raphe and the superficial grey layer of the superior colliculus. In contrast, other structures, such as the optic layer of the superior colliculus or the ventral tegmental area presented only low concentrations of specific binding. In most of the areas, 5-HT_{1B} sites were predominant, although the other 5-HT₁ subtypes were also present. An exception was the dorsal raphe nucleus, which appeared to be richer in 5-HT_{1A} sites (Table V and Figs. 2–5 and 8).

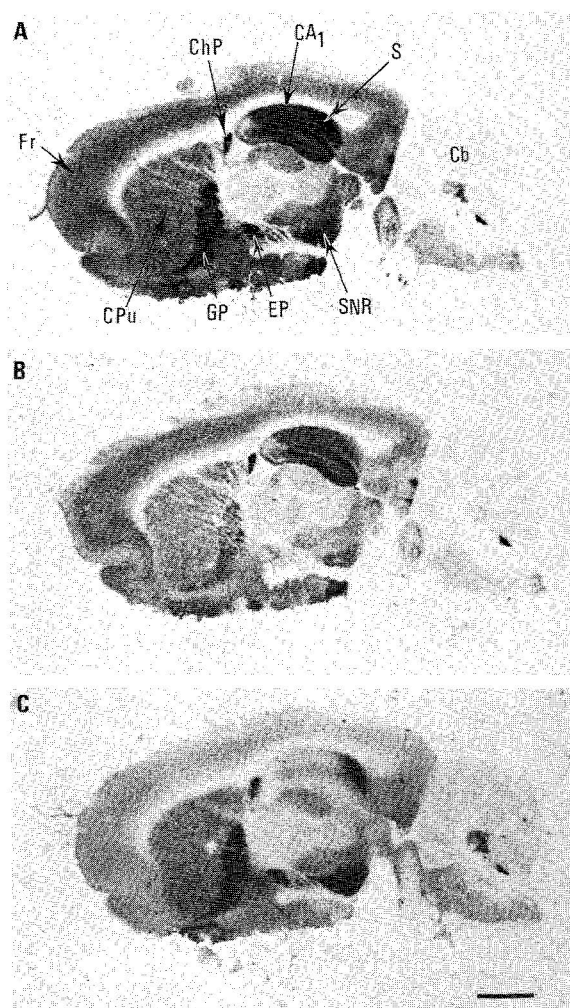


Fig. 5. Sagittal sections showing 5-[^3H]HT binding. Areas highly labeled include CA₁ field of the hippocampus (CA₁) (5-HT_{1A} sites), dorsal subiculum (S), globus pallidus (GP), substantia nigra reticular (SNR) (5-HT_{1B} sites) and choroid plexus (ChP) (5-HT_{1C} sites). Bar = 0.2 cm.

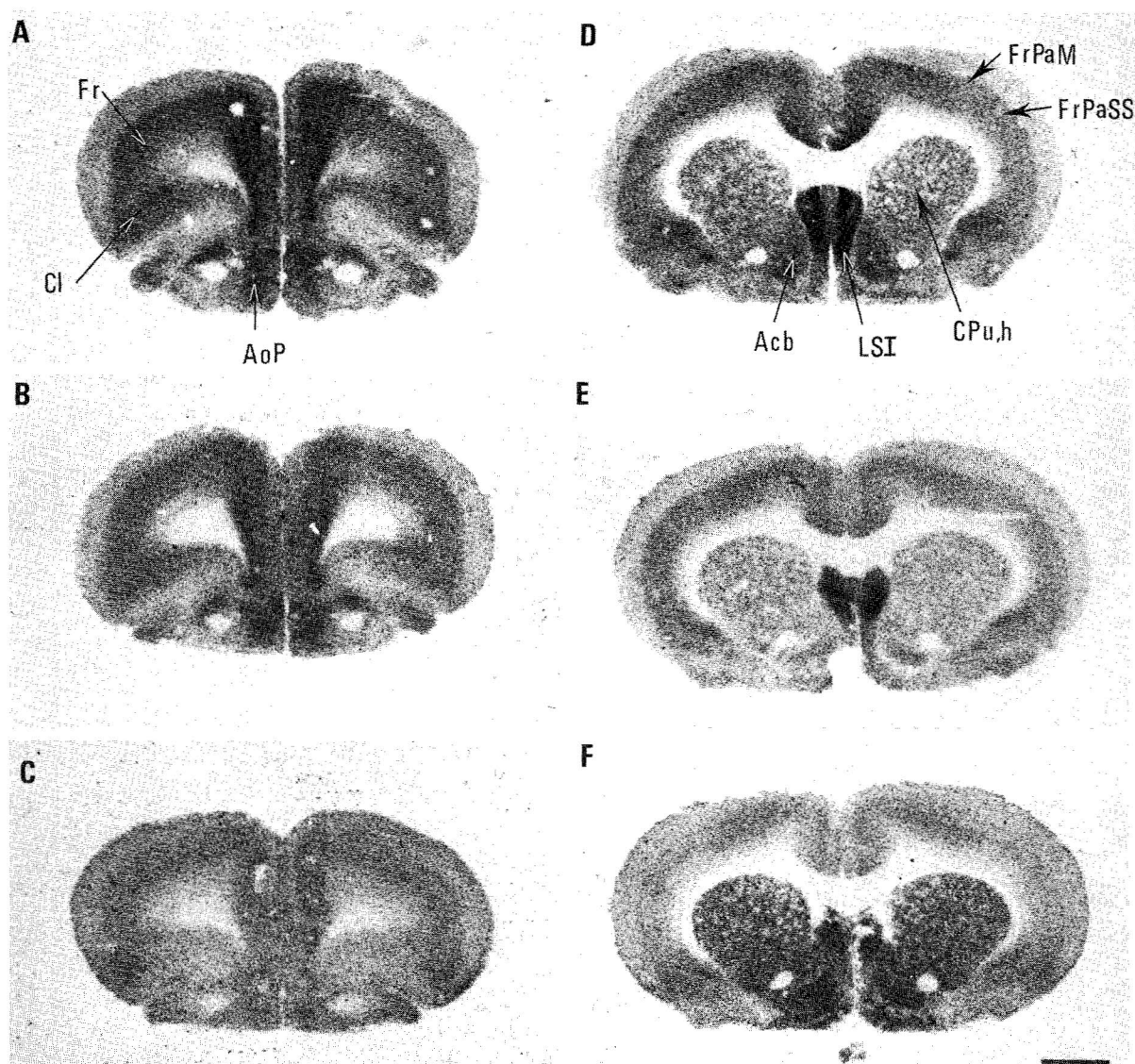


Fig. 6. 5-[^3H]HT binding in coronal sections through the frontal cortex (A–C) and the caudate-putamen (D–F). Note that 5-[^3H]HT binding to caudate-putamen (CPu,h) and accumbens (Acb) is inhibited with high affinity by (–)21 009 (E), showing that it is of the 5-HT_{1B} subtype. In contrast, binding to the lateral septal nucleus (LSI) is displaced by 8-OH-DPAT. Binding to the different areas of the neocortex is partially affected by both drugs. Bar = 0.2 cm.

Pons. Only low concentrations of 5-HT₁ receptors were found in the pons, although the nucleus of the solitary tract contained a higher density of autoradiographic grains. As in midbrain, 5-HT_{1B} sites appeared to be predominant (Table V and Figs. 4, 5 and 9).

Medulla oblongata. The different nuclei of the medulla presented low concentrations of 5-HT₁ receptors, except the gelatinous subnucleus of the nucleus of the spinal tract of the trigeminal nerve, where an

intermediate density of specific binding was found. The presence of 5-HT_{1A} and 5-HT_{1B} sites was apparent in these areas, although in most of them, 5-HT_{1B} was predominant (Table V and Figs. 4, 5 and 9).

Spinal cord. In the cervical part of the spinal cord, laminae of the dorsal horn presented intermediate densities of sites, which had the characteristics of 5-HT_{1A}-recognition sites. In contrast, in the ventral horn, the concentration of specific binding was low or very low, and of the 5-HT_{1B} class. The presence of

some 5-HT_{1C} binding in the dorsal horn was also found (Table V and Fig. 10).

Choroid plexus. The choroid plexuses presented the highest concentrations of 5-HT₁ receptors, having the characteristics of 5-HT_{1C} sites (Table V and Figs. 3–5, 7–9).

DISCUSSION

The results presented in this paper provide a quan-

titative mapping of the 5-HT₁ receptors in the rat brain. Furthermore, they demonstrate the presence of three different populations of 5-HT₁-binding sites, supplying a detailed pharmacological characterization of these sites, as well as their anatomical distribution throughout the different areas and nuclei of the brain.

Since several compounds inhibit 5-[³H]HT binding to the cortex in a biphasic manner, two subtypes of 5-HT₁ sites, 5-HT_{1A} and 5-HT_{1B} have been proposed

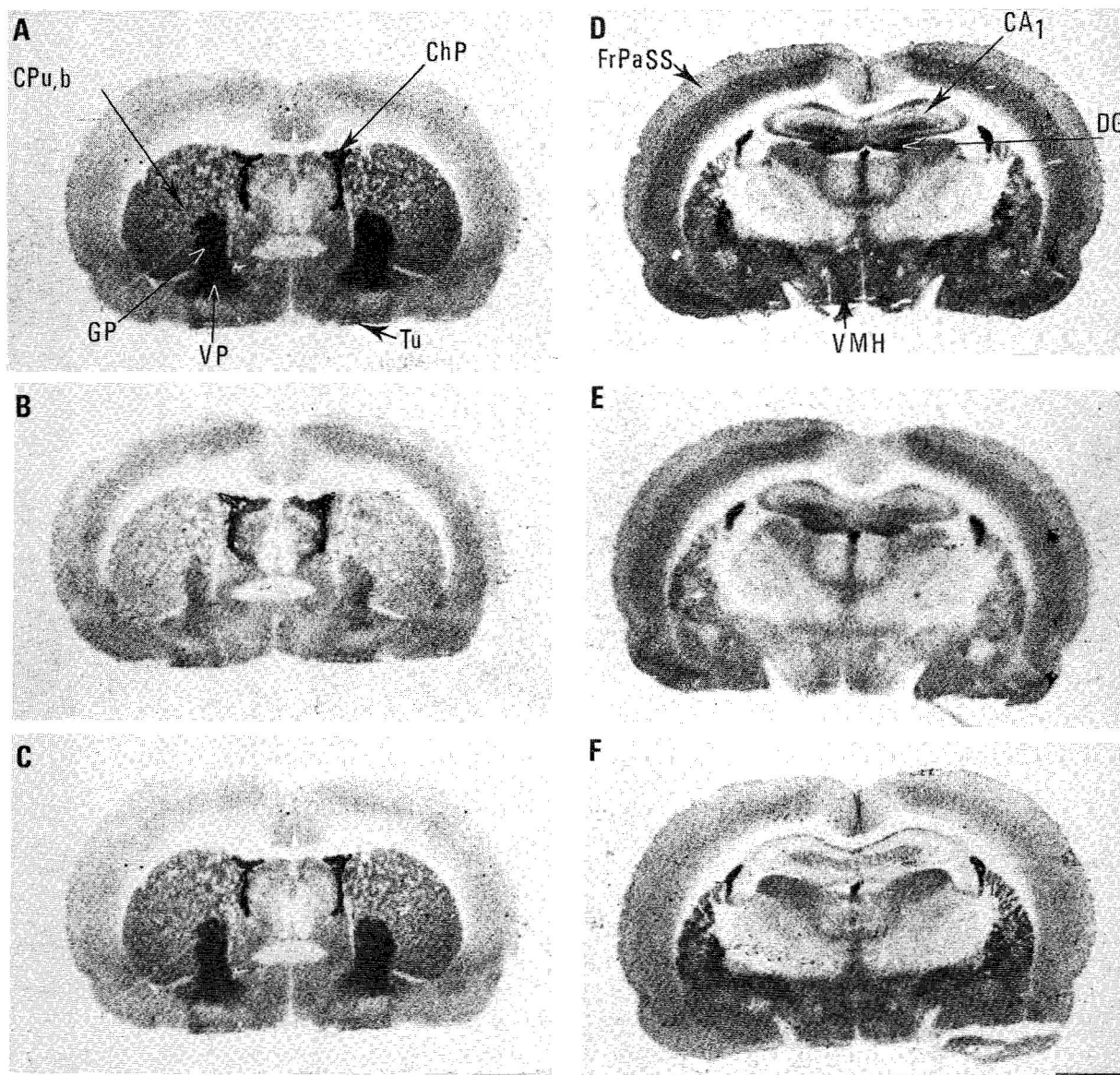


Fig. 7. 5-[³H]HT binding in coronal sections through the globus pallidus (A–C) and the anterior thalamus (D–F). Structures where 5-HT_{1A} sites are present include lateral septal nucleus, dentate gyrus (DG) and CA₁ field of the hippocampus (CA₁). Areas enriched in 5-HT_{1B} are globus pallidus (GP), ventral pallidum (VP), caudate-putamen (CPu,b) and hypothalamus (VMH). The binding to the choroid plexuses (ChP) is of the 5-HT_{1C} class. Bar = 0.2 cm.

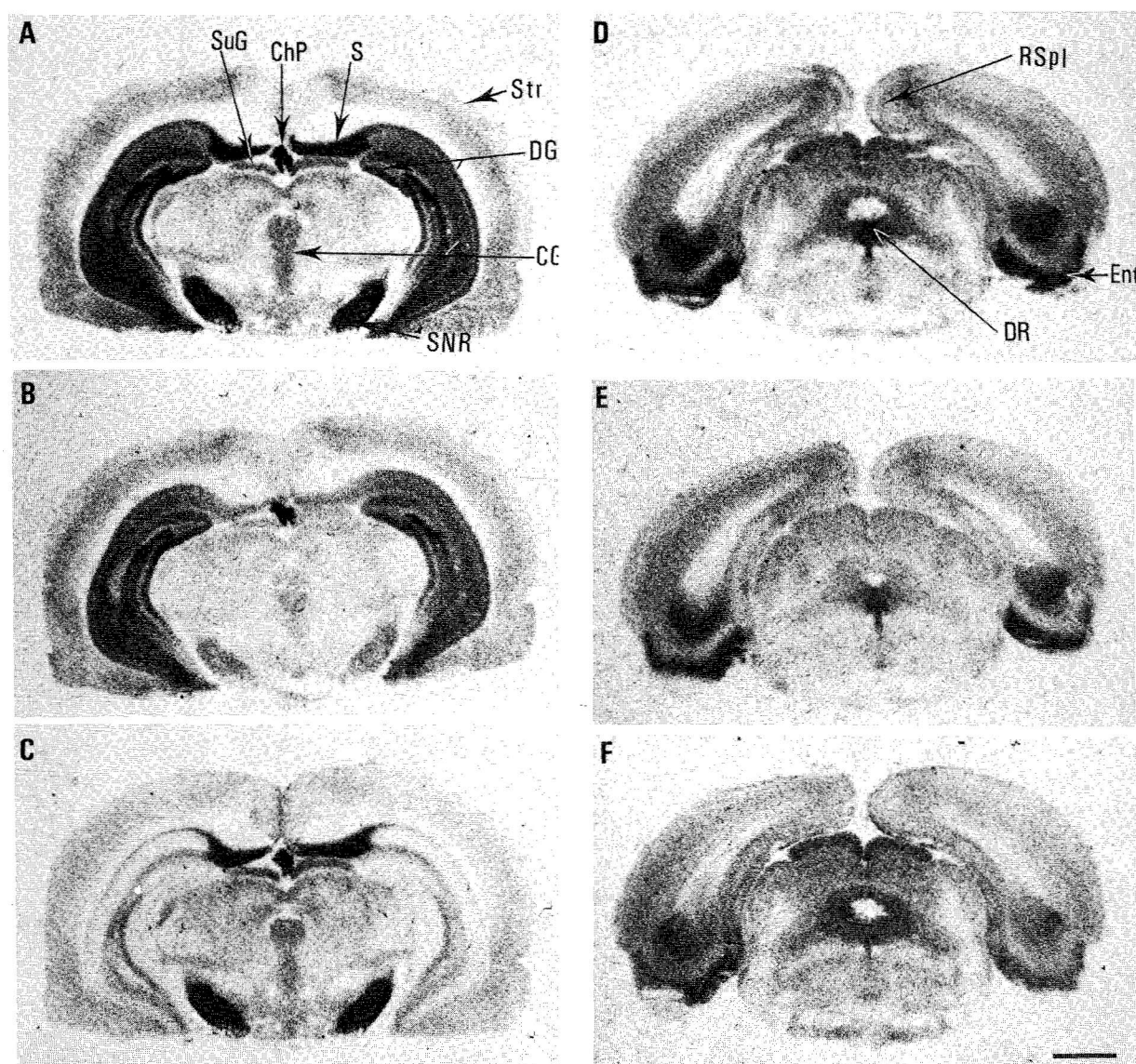


Fig. 8. 5-[³H]HT binding in coronal sections through the upper (A–C) and the lower midbrain (D–F). Autoradiograms show the intense labeling of the dorsal subiculum (S), substantia nigra reticular (SNR) and superficial grey layer of the superior colliculus (SuG), among others, having the characteristics of 5-HT_{1B} sites. On the other hand, the dentate gyrus (DG) and other areas of the hippocampus present binding of the 5-HT_{1A} class. Bar = 0.2 cm.

(see Introduction). Recently, we have reported the presence of a third subtype of 5-HT₁ site: 5-HT_{1C}, in pig choroid plexus membranes¹⁰³. By combining quantitative autoradiography with binding-competition experiments, we have (1) identified brain areas enriched in only one of these 5-HT₁ receptor subtypes, and (2) carried out the pharmacological characterization of these sites by analyzing the affinities of several drugs for the 5-HT₁ receptors located in these areas. This pharmacological approach would

have been difficult to attain by membrane-binding techniques, because of the difficulty in dissecting by hand numerous small brain areas.

In this way, we have identified areas enriched in 5-HT_{1A} sites: dentate gyrus, several fields of the hippocampus, lateroseptal nucleus; finding that 8-OH-DPAT, LSD, RU-24 969 and the β -blocker (–) 21 009 (ref. 39) recognized these sites with very high affinity. In contrast to other results published¹⁰⁵, spiperone showed only low affinity for the above-mentioned

sites, although clearly higher than those presented for the other 5-HT₁ sites (Table II). Areas presenting only 5-HT_{1B} sites included globus pallidus, substantia nigra reticular part and dorsal subiculum, among others. While 8-OH-DPAT showed low affinity for these sites, RU-24 969 and (-) 21 009 recognized them with very high affinity. In the case of the β -blocker, the affinity for these 5-HT_{1B} sites was higher than the one shown for 5-HT_{1A} sites. It appears clear from these data that 8-OH-DPAT and (-) 21 009 are the com-

pounds showing a better discriminatory capacity to differentiate both subtypes (Table II). Finally, 5-HT_{1C} sites were found in a very high concentration in the choroid plexuses, and their pharmacology was very similar to that reported¹⁰³ in membrane-binding studies (Tables II and III).

The analysis of the areas labeled with the different ³H-labeled ligands was in agreement with the data from competition experiments. 8-OH-[³H]DPAT labeled with high affinity those brain areas which pre-

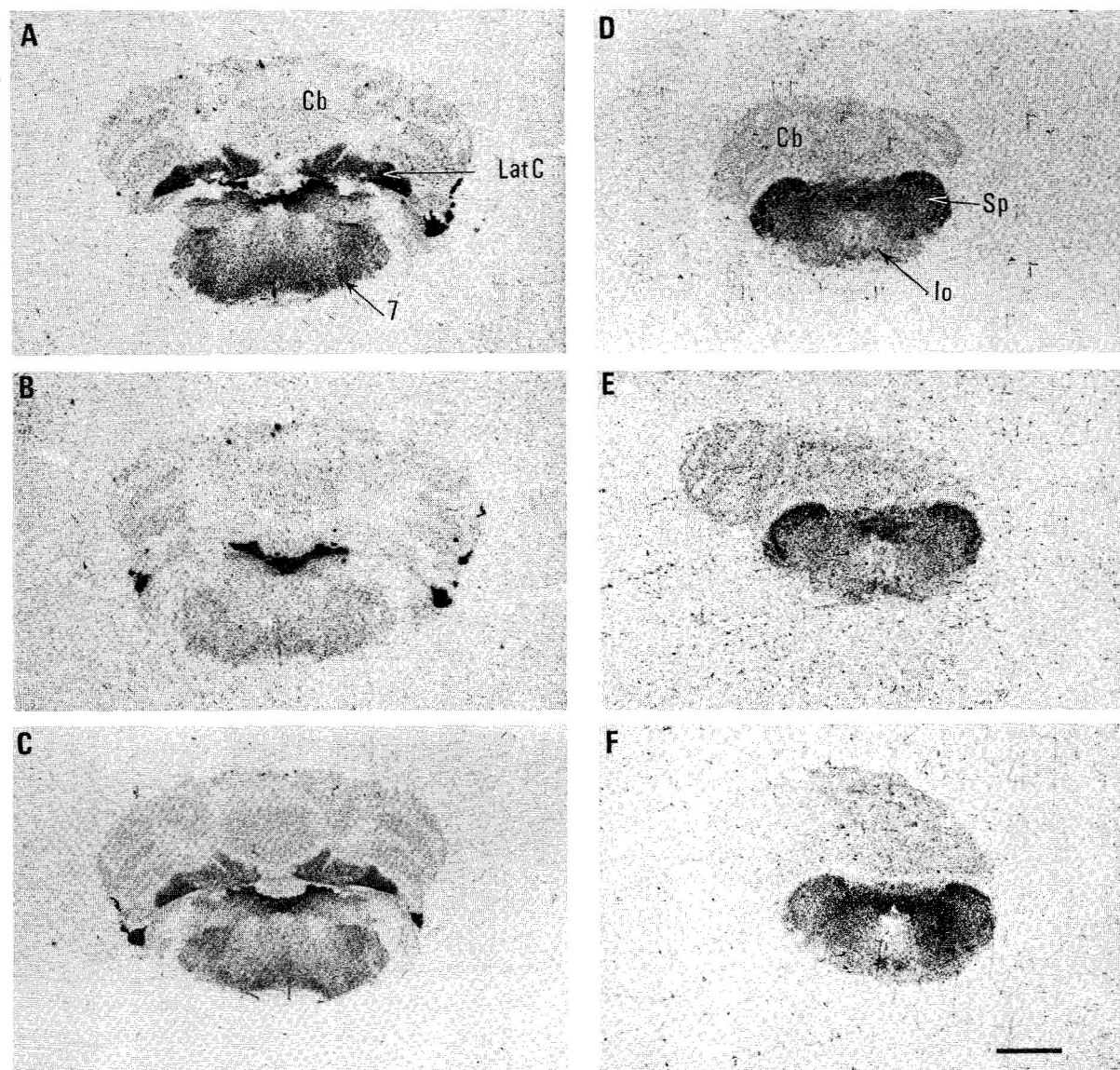


Fig. 9. 5-[³H]HT binding in coronal sections through the pons (A-C) and medulla oblongata (D-F). Note the labeling of the lateral cerebellar nucleus (LatC), which is inhibited by (-)21 009 (B), and that on the choroid plexus, which is not affected by (-)21 009 (B) or 8-OH-DPAT (C). Bar = 0.2 cm.

sented the characteristics of 5-HT_{1A} sites when 5-[³H]HT was used as a ligand. $B_{\max}$ values of [³H]LSD binding were similar to those obtained with 5-[³H]HT in the areas where no 5-HT_{1B} sites were present, but clearly lower in those enriched in this subtype. Binding of [³H]mesulergine only reached high concentrations in the choroid plexus, an area extremely enriched in 5-HT_{1C} receptor sites. The difference between the maximal densities found in this structure with 5-[³H]HT and [³H]mesulergine could be due to the different labeling conditions used for both ligands (Table V). A multiplicity of serotonergic recognition sites has also been proposed on the basis of data obtained from electrophysiological studies¹. However, further work is needed in order to clarify the possible relationship between the different 'S' receptors

proposed by Aghajanian¹ and the 5-HT sites defined with radiolabeled ligands¹¹⁵.

It must be pointed out that, in addition to 5-HT₁ receptors, [³H]LSD and [³H]mesulergine labeled 5-HT₂ receptors in the rat brain, as has been already described^{22,102,109}. [³H]LSD also labeled dopaminergic receptors¹⁹.

The distribution of 5-HT₁ sites reported here correlate well with the results obtained in membrane-binding assays performed in hand-dissected rat brain regions using 5-[³H]HT and [³H]LSD^{16,110}. Similar distribution of 5-HT₁ receptors has been found in other mammalian species, including guinea-pig and human^{29,110}. Our results are also in very good agreement with those reported in previous autoradiographic studies, performed using 5-[³H]HT, [³H]LSD and 8-OH-[³H]DPAT, both in the pattern of general distribution of 5-HT₁ receptors^{14,75,138} and in the distribution of 5-HT_{1A} and 5-HT_{1B} subtypes^{34,72}. In our study, we confirm and extend these results, providing a quantitative analysis, and defining the pharmacological profile of the three 5-HT₁-recognition sites.

5-HT₁ receptors presented a very heterogeneous distribution in the rat brain. Choroid plexus, basal ganglia and hippocampus were very rich in these sites. The neocortex and the hypothalamus also showed high concentrations of receptors. In contrast to this, in brainstem and spinal cord, the density of 5-HT₁ receptors was low, although some mesencephalic structures, i.e., substantia nigra, still presented high concentrations of specific binding (Figs. 2–5).

Regarding the functional significance of 5-HT₁ receptors, some behavioral responses have been proposed to be linked to these sites. While many of the 5-HT motor effects are mediated by 5-HT₂ receptors^{23,67,107,135}, in other models, 5-HT₁ sites seem to be involved. The 5-HT syndrome, a complex behavioral syndrome, has been proposed to be mediated by 5-HT₁ receptors⁶⁹. This syndrome is produced by 5-HT itself, as well as by some serotonergic precursors and consists of several motor activities, including forepaw treading, Straub's tail, hindlimb abduction and head weaving, among others^{50,58}. Although no full agreement exists on this point⁹³, the 5-HT₁ mediation of this behavior is further confirmed by the fact that 8-OH-DPAT induces the syndrome¹²⁷ and some β -blockers antagonize it^{53,127}. A second motor

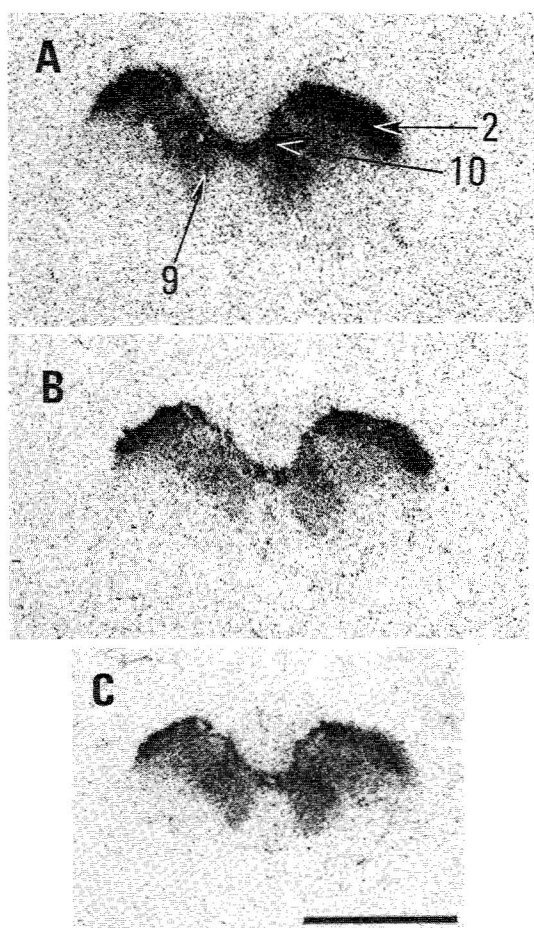


Fig. 10. 5-[³H]HT binding in a coronal section through the cervical spinal cord. 5-HT₁ sites on the dorsal horn (2) appear to be of the 5-HT_{1A} class, while those on the ventral horn (9) could be considered as 5-HT_{1B} sites. Bar = 0.2 cm.

effect which appears to be related to 5-HT₁ activation is the circling behavior in striatum-lesioned rats. Both agonists RU-24 969 and 8-OH-DPAT produce this behavior^{15,57}. Finally, it has been reported that the indole 5-HT agonists, such as RU-24 969, originate myoclonic jerking in guinea-pigs⁷⁰ and hyperlocomotion in mice and rats^{52,128}. A good correlation has been shown between the potencies of these compounds displacing 5-[³H]HT binding in the brainstem and those inducing myoclonus⁷⁰. However, caution is required in analyzing the motor effects produced by 5-HT agonists, because of the interactions which have been demonstrated between 5-HT and dopaminergic receptors in this regard^{36,52}. Although most of these 5-HT₁ behavioral effects have been claimed to be brainstem or spinally mediated^{59,70}, recent results have demonstrated that at least some components of the 5-HT syndrome are not dependent on these lower structures³⁵. Our autoradiographic data reveal the presence of 5-HT₁ receptors in many structures known to be involved in motor activity. The spinal cord, substantia nigra, cerebellar nuclei, basal ganglia, entopeduncular nucleus and motor cortex present important concentrations of receptors, mainly of the 5-HT_{1B} subtype. The claustrum, a structure also connected to the motor cortex²⁸ is also rich in 5-HT₁ sites. These results support a predominant role of 5-HT_{1B}-recognition sites in these effects. However, an involvement of 5-HT_{1A} sites is possible, since they are also present in these structures.

There is also considerable evidence for an inhibitory role of central 5-HT systems on male rat sexual behavior^{3,117}. In contrast, administration of 8-OH-DPAT causes marked stimulatory changes in this sexual behavior, reducing number of intromissions preceding ejaculation and shortening the ejaculatory latency⁴. Furthermore, this sexual facilitatory effect of 8-OH-DPAT is not blocked by the administration of several serotonergic antagonists^{2,4}. These results complicate the analysis of the relationship between 5-HT₁ receptors and sexual behavior, suggesting a possible presynaptic mediation, as will be discussed later. Nevertheless, the presence of high concentrations of 5-HT_{1A} receptors in the different areas of the hippocampus, as well as in other structures of the limbic system, including the septum, which is strongly connected to hippocampal areas^{5,71}, could indicate a role of these sites in the mediation of this behavior.

A role of 5-HT₁ receptors in the control of visual activity can be suggested, according to the distribution of these sites in some brain areas. The olivary pretectal nucleus and the superficial grey layer of the superior colliculus, both connected with the visual pathway^{120,134} present high concentrations of 5-HT₁ receptors. Other structures related to the visual system, such as the claustrum⁶⁵, the striate cortex and lateral geniculate nucleus^{47,119,126} also contain important receptor densities. Most of these areas are mainly enriched in 5-HT_{1B}-recognition sites.

5-HT agonists have been reported to induce stimulation of rat prolactin secretion (see ref. 77). Although this effect appears to be mediated by 5-HT₂ receptors⁷⁶, a role of 5-HT₁ sites cannot be excluded⁴¹. The important presence of 5-HT_{1B}-recognition sites in different nuclei of the hypothalamus, an area which has been related to these effects⁷⁷, could suggest an involvement of 5-HT_{1B} sites in this endocrine response.

The central 5-HT systems are known to be involved in the regulation of nociceptive activity, mainly through a raphé-spinal projection interconnected with the endogenous opioid pathways (see ref. 10 for a review), although brainstem serotonergic connections are also relevant¹². It has been also shown that this serotonergic role varies depending on the methodological approach used^{12,13,121,136}. Furthermore, it has been recently suggested that spinal 5-HT receptors related to nociceptive control are of the 5-HT₁ class^{121,139}. Our autoradiographic data support this hypothesis, showing the presence of 5-HT₁ receptors in the raphé nuclei and spinal cord, as well as in some other brainstem structures. Regarding the spinal cord, most of the receptors present in the dorsal horn are of the 5-HT_{1A} subtype, suggesting a role of these sites in this system.

Some vegetative functions, such as the control of blood pressure and respiration are known to be influenced by central 5-HT activity^{8,63}. Although our data reveal the presence of 5-HT₁ receptors in several brainstem nuclei which play an important role in these functions, such as the nucleus of the solitary tract, further work is needed to clarify the 5-HT involvement in these effects.

In the last few years, the role of 5-HT receptors in some neurological diseases has been the matter of increasing research. A decrease in the number of 5-HT

receptors in the cortex of patients suffering from senile dementia of the Alzheimer type has been reported^{18,30,113}. Although the decrease in 5-HT₂ receptors appears to be greater, 5-HT₁ receptors are also affected, mainly in those patients presenting a later stage of the disease^{18,30}. A role of diminished number of 5-HT₁ sites in the impaired cognitive activity characteristic of this disease is therefore possible. Related to this, the involvement of serotonergic transmission in learning and memory processes, as has been shown in animals^{7,92} and in humans¹³², must be taken into consideration.

A role of central 5-HT activity in the mechanisms of schizophrenia has also been investigated. While an increase in the striatal concentration of 5-HT in brains from schizophrenic patients has been reported³¹, a clear decrease of [³H]LSD binding to frontal cortical membranes has also been found¹¹. These data do not allow any statement on the possible role of 5-HT₁ receptors in this disease. In a similar way, while considerable evidence has accumulated on the relationship between antidepressant treatments and number of cortical 5-HT₂ receptors, no clear effect has been shown in the case of 5-HT₁ sites (refs. 82, 123; see ref. 6 for a review). Finally, the involvement of 5-HT mechanisms in the pathogenesis of migraine has been proposed^{20,112} and the prophylactic effect of the 5-HT antagonist methysergide in some types of migraine is well-known (see ref. 64). Whether 5-HT₁ sites are involved in this process is unknown.

One of the relevant findings of this work is the characterization of some β -blockers as drugs with high affinity and selectivity for the 5-HT_{1B}-recognition site. This fact confirms and extends previous reports showing the ability of β -blockers to displace 5-[³H]HT binding in a stereospecific way^{80,86}. It has also been established that some β -blockers antagonize several *in vivo* and *in vitro* effects induced by serotonergic agonists (refs. 27, 51; see ref. 133 for a review). On the other hand, β -adrenergic agonists have been reported to increase some 5-HT behavioral effects^{91,94}. Taken together, these data suggest an interaction of β -adrenergic drugs with central 5-HT₁ receptors. This interaction could explain some of the neurological effects induced by β -blockers in humans, such as their therapeutic activity in schizophrenic states^{124,137} and in migraine^{43,131}.

The localization of 5-HT₁ receptors described here correlates well with the distribution of serotonergic terminals. Most of the areas presenting important concentrations of receptors have been found to contain nerve-endings from the serotonergic neurons, mainly located in the raphe nuclei^{9,24,99,122}. The hippocampus, the septal nuclei, the neocortex, the basal ganglia, the different nuclei of the hypothalamus, the olfactory system, or the substantia nigra are structures where the presence of 5-HT, 5-HT fibers and biochemical markers have been reported^{24,32,37,45,46,56,68,99,116,122}. The choroid plexuses have also been shown to contain serotonergic innervation^{21,122}. However, some areas very enriched in 5-HT₁ sites do not present a high level of serotonergic innervation. This is the case for the dorsal subiculum or the entopeduncular nucleus^{24,122}. The existence of discrepancies between the distribution of fibers and receptors has been found with many neurotransmitters^{96,98} and several explanations have been proposed (see ref. 62). Furthermore, it should be kept in mind that the brain contains also 5-HT₂ receptors, whose distribution is different from that of 5-HT₁ sites^{75,102} and this could explain some apparent discrepancies.

Our results on the distribution and pharmacology of 5-HT_{1C} sites confirm our previous reports on this subtype^{25,103}, showing that the pharmacological profile obtained by quantitative autoradiography is identical to that obtained in membrane-binding assays¹⁰³. The choroid plexuses are especially enriched in these 5-HT_{1C} sites, presenting the highest density of 5-HT₁ receptors in the rat brain. Very high concentrations of 5-HT₁ binding in the choroid plexus have also been found in other mammalian species, such as pig¹⁰³ and human⁹⁷. The choroid plexuses present an important 5-HT innervation, the fibers arising from the raphe nuclei and ending on the blood vessels that supply the plexuses^{83,87}. In addition, there are 5-HT fibers on the supraependymal surface of the ventricular system¹¹⁴. Beside the choroid plexus, 5-HT_{1C} sites are also present in other brain areas, as has already been reported in pig brain¹⁰⁴. The possible physiological significance of 5-HT_{1C} sites is not known at the present time. Since they are mainly localized in the choroid plexuses, they may play a role in the regulation of the production of cerebrospinal fluid, a function strongly linked to these plexuses³³.

An important question concerns the cellular localization of 5-HT₁ receptors. Using several presynaptic models, such as the inhibition of 5-HT release from brain synaptosomes or the regulation of noradrenaline release in the sympathetic endings of canine saphenous vein, several authors have found a correlation between the activity of several agonists and antagonists in these tests and their affinity for 5-[³H]HT binding^{38,43,73}, although certain discrepancies have been reported for some drugs^{48,84}. Engel et al. have shown a good correlation between a subpopulation of 5-HT₁ receptors and 'presynaptic' activity³⁸. 8-OH-DPAT produces some physiological effects which appear to be related to 5-HT autoreceptors^{2,125}. In addition, this compound has been shown to stimulate presynaptic 5-HT autoreceptors in the central nervous system, but only at micromolar concentrations^{49,54}. This fact, and other biochemical data suggest a link of 5-HT autoreceptors to 5-HT_{1B} sites^{78,79}. Another approach to study the cellular localization of 5-HT₁ receptors has been the analysis of the effects of different types of lesions on 5-[³H]HT binding. In this regard, it has been reported that chemical lesioning of serotonergic neurons with 5,7-dihydroxytryptamine increased the amount of 5-[³H]HT binding in the hippocampus, where 5-HT_{1A} sites are predominant⁸⁹, indicating a supersensitivity of these receptors, similar to that observed in behavioral studies¹²⁹. In contrast, electrolytic lesions of the raphe nuclei did not modify 5-HT₁ binding in the cortex¹⁶

and the destruction of the 5-HT fibers of the fornix-fimbria and cingulum bundle produced no change in 5-[³H]HT binding to the hippocampus¹¹¹. Although the clarification of this point is beyond the scope of the present work, we have recently found (Pazos et al., in preparation) that electrolytic lesions of the septum induce a decrease of 5-HT_{1A} and 5-HT_{1B} sites in different areas of the hippocampus, measured by quantitative autoradiography. Since the septo-hippocampal projection is one of the main central cholinergic pathways⁷¹, these results suggest the presence of 5-HT₁ receptors on cholinergic fibers. This finding is not surprising, because an interaction between serotonergic and cholinergic neurons in the central nervous system has been demonstrated¹¹⁸.

In conclusion, sites with the pharmacological properties of 5-HT₁-binding sites are heterogeneously distributed in the rat brain and three subtypes of 5-HT₁ sites can be identified with different pharmacological profiles and anatomical distribution. The presence of these receptors in different brain areas correlates well with the proposed role of 5-HT₁ sites in several central functions. Although there are some discrepancies on this point⁶⁶, these data seem to provide good evidence to consider 5-HT₁ sites as receptors.

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ABBREVIATIONS USED IN FIGURES

2	layer 2 of Rexed	Fr	frontal cortex
7	facial nucleus	FrPaM	frontoparietal cortex, motor area
9	layer 9 of Rexed	FrPaSS	frontoparietal cortex, somatosensory area
10	layer 10 of Rexed	Hp	hippocampus
AoP	anterior olfactory nucleus, posterior part	lo	inferior olive
CA ₁	field CA ₁ of the hippocampus	LatC	lateral cerebellar nucleus
Cb	cerebellum	LS	lateral septal nucleus
CG	central (periaqueductal) grey	LSI	lateral septal nucleus, intermediate part
ChP	choroid plexus	RSpl	retrosplenial cortex
Cl	claustrum	S	dorsal subiculum
CPu	caudate-putamen	SC	superior colliculus
CPu,b	caudate-putamen, body	SNR	substantia nigra, reticular part
CPu,h	caudate-putamen, head	Sp	nucleus of the spinal tract of the trigeminal nerve, caudal part
DG	dentate gyrus	Str	striate cortex
DR	dorsal raphe nuclei	SuG	superficial grey layer of the superior colliculus
Ent	entorhinal cortex	Tu	olfactory tubercle
EP	entopeduncular nucleus	VMH	ventromedial hypothalamic nucleus
		VP	ventral pallidum

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