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Quantitative Autoradiographic Mapping of Serotonin Receptors in the Rat Brain. II. Serotonin-2 Receptors

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The distribution of serotonin-2 (5-HT$_2$) receptors in the rat brain was studied by light microscopic quantitative autoradiography. Receptors were labeled with four ligands: [^{3}H]ketanserin, [^{3}H]mesulergine, [^{3}H]LSD and [^{3}H]spiperone, which are reported to show high affinity for 5-HT$_2$ receptors. Co-incubation with increasing concentrations of several well-known 5-HT$_2$-selective drugs, such as pirenperone, cinanserin and ketanserin, resulted in an inhibition of the binding of the four ^{3}H-labeled ligands to the same areas. However, all of them recognized, in addition to 5-HT$_2$ sites, other populations of binding sites. Receptor densities were quantified by microdensitometry with the aid of a computer-assisted image-analysis system. Our results reveal a heterogenous distribution of 5-HT$_2$ receptor densities in the rat brain. Very high concentrations were localized in the claustrum, olfactory tubercle and layer IV of the neocortex. The anterior olfactory nucleus, piriform cortex and layer I of neocortex were also rich in 5-HT$_2$ receptors. Intermediate concentrations of receptors were found in caudate putamen, nucleus accumbens, layer V of neocortex, ventral dentate gyrus and mammillary bodies. Areas containing only low concentrations of receptors included the thalamus, hippocampus, brainstem, medulla, cerebellum and spinal cord. The specificity of the different ligands used is discussed in terms of the other populations of sites recognized by them. The distribution of 5-HT$_2$ receptors here reported is discussed in correlation with (a) the known distribution of serotoninergic terminals, (b) the specific anatomical systems and (c) the central effects reported to be mediated by 5-HT$_2$-selective drugs.

INTRODUCTION

The heterogeneity of brain serotonin (5-HT)-recognition sites has been supported by many biochemical and pharmacological data[1,43]. Peroutka and Snyder[55] classified 5-HT receptor sites into 5-HT$_1$, labeled by 5-[^{3}H]HT with nanomolar affinity, and 5-HT$_2$, labeled with high affinity by [^{3}H]spiperone. More recently, it has been suggested that 5-HT$_1$ receptors are heterogeneous, and three subclasses of these sites have been proposed[13,39,53].

Regarding 5-HT$_2$ sites, there appears to be a good correlation between the potencies of many drugs in the competition for the serotonin-2 binding sites and those showed in several serotonin-mediated effects, such as the 5-hydroxytryptophan (5-HTP)-induced head twitches in several mammalian species, the tryptamine-induced clonic seizures in rats, and the 5-HT-induced contraction of basilar artery in dogs, among others[11,23,34,36,41,54,70]. These results suggest that 5-HT$_2$-binding sites are, in fact, functional receptors.

Several ligands have been proposed for the study of these receptors. In addition to [^{3}H]spiperone and [^{3}H]LSD, described as 5-HT$_2$ ligands in the initial report of Peroutka and Snyder[55], other ^{3}H-labeled compounds have been developed in the search for more specific ligands for these sites. [^{3}H]Mianserin[57] and [^{3}H]metitepine[42] have been assayed. [^{3}H]Ketanserin, developed by Leysen et al.[34] shows high-affinity binding to 5-HT$_2$ sites from brain tissues in several species[34,64]. Closse[10] has reported the high-affinity binding of the ergot derivative [^{3}H]mesulergine, to 5-HT$_2$ receptors in rat brain cortex. [^{125}I]LSD also shows high affinity for these sites[20].

Light microscopic autoradiography is useful to localize receptors, because of the level of anatomical resolution provided, as well as its high sensitivity. When quantitative autoradiography is used it is possible to obtain a precise estimation of receptor densi-

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ties and drug affinities[14,44].

In the case of 5-HT_2 sites, quantification of their distribution in the central nervous system could be correlated to the physiological responses mentioned above. Autoradiographic visualization of these sites has been carried out in very limited way using [^{3}H]LSD and [^{3}H]spiperone[38,45,71]. We report a more detailed mapping of the serotonin-2 receptors in the rat brain, using [^{3}H]ketanserin, [^{3}H]mesulergine, [^{3}H]LSD and [^{3}H]spiperone as ligands.

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). After removing the brains, 10 μm thick sections were prepared as described in the preceding paper[52].

Incubation conditions for each ^{3}H-labeled ligand, including buffer solution, concentration of ligand, incubation time and compounds used to generate blanks, are summarized in Table I. In competition studies, consecutive tissue sections were incubated in the presence of increasing concentrations of displacers.

The binding of [^{3}H]spiperone and [^{3}H]LSD to slide-mounted brain rat sections has been already kinetically and pharmacologically characterized[38,45]. The conditions defined in these studies were used in the present work. In order to determine an optimal washing time, allowing the best specific to non-specific binding ratio, sections were transferred, at the end of incubation with ^{3}H-labeled ligands, to ice-cold buffer for different periods of time. Then, tissues were wiped from the slides with a glass fiber filter and radioactivity was determined by liquid-scintillation counting. Washing curves generated for [^{3}H]ketanserin and [^{3}H]mesulergine showed that the highest specific to non-specific ratio was obtained after two 10-min consecutive washes. Accordingly, this washing protocol was used in all the experiments. Association of both ligands to binding sites was studied in a similar way and 2 h incubation time was selected for routine experiments.

The saturation kinetics of [^{3}H]ketanserin and [^{3}H]mesulergine were also examined. The binding of both ligands was saturable and of high affinity with apparent K_d of 1.0 nM ([^{3}H]ketanserin) and 1.5 nM ([^{3}H]mesulergine). These results are in good agreement with those obtained using fresh tissue homogenates[10,34].

For autoradiography, tissues were incubated in the conditions described above (Table I). At the end of

TABLE I

Incubation conditions used for the labeling of 5-HT_2 receptors

Ligand	*[Ligand] (nM)*	*Preincubation protocol*	*Buffer*	*Incubation*		*Washing protocol*	*Blank generation*
				Temperature	*Time*		
[^{3}H]Ketanserin	2	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)	10^{-6} M methysergide
[^{3}H]Mesulergine	5	same as above	0.17 M Tris-HCl (pH 7.7)	room temp.	2 h	2 × 10 min (4 °C)	3×10^{-7} M ketanserin
[^{3}H]LSD	5	5 min at room temperature, in incubation buffer (–pargyline)	0.17 M Tris-HCl–4 mM $CaCl_2$–0.15 M NaCl–0.01% ascorbic acid–10 mM pargyline (pH 7.6)	room temp.	60 min	2 × 10 min (4 °C)	10^{-6} M cinanserin
[^{3}H]Spiperone	0.4	none	0.17 M Tris-HCl–1 mM $MgCl_2$–2 mM $CaCl_2$–5 mM KCl–120 mM NaCl (pH 7.7)	room temp.	30 min	2 × 5 min (4 °C)	3×10^{-7} M ketanserin

the washing period, tissues were dried with a stream of cold air and autoradiograms were generated by apposing the labeled tissue to [^{3}H]Ultrofilm (LKB, Sweden) as described by Unnerstall et al.[68]. Brain grey and white matter standards, containing known amounts of a non-volatile ^{3}H-labeled compound were exposed to the film along with the labeled tissue sections[44]. Exposure period was 30 days for [^{3}H]ketanserin and [^{3}H]mesulergine, 100 days for [^{3}H]LSD and 90 days for [^{3}H]spiperone. Films were analyzed using a computerized image-analysis system which provides values of receptor densities expressed in fmol/mg protein[44]. Measurements were made as described in the accompanying paper[52]. Results reported in this study are from, at least, two separate experiments for each ^{3}H-labeled ligand, involving tissues from 3 animals per experiment.

Percent occupancy of receptor sites by the different ^{3}H-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 Watson[50]. Cortical areas were named according to Krieg[28,29].

Slides consecutive to those used in the autoradiographic experiments were stained for acetylcholinesterase (AChE) activity[26].

[^{3}H]Ketanserin (64.6 Ci/mmol), [^{3}H]LSD (69.3 Ci/mmol), and [^{3}H]spiperone (22 Ci/mmol) were obtained from New England Nuclear Dreieich, F.R.G. [^{3}H]Mesulergine (85 Ci/mmol) was synthesized and labeled by Dr. R. Voges of the Biopharmaceutical Department, Sandoz Ltd. (Basle, Switzerland). Other chemicals and drugs were of commercial origin or kindly provided by the original manufacturers.

RESULTS

Pharmacological characterization of the binding of the different ^{3}H-labeled ligands to some brain areas

The pharmacology of the binding of the ^{3}H-labeled ligands was studied by quantitative autoradiography in selected brain areas showing high densities of binding sites. Binding to areas labeled by the four ligands, such as the lamina IV of the neocortex, was inhibited with nanomolar affinity by pirenperone, cinanserin, spiperone and other 5-HT$_2$ ligands, while required micromolar concentrations of 5-HT and other 5-HT$_1$ ligands to be completely blocked. This is illustrated in Fig. 1, and some competition curves for [^{3}H]mesulergine (A) and [^{3}H]ketanserin (B) binding in the frontoparietal motor cortex, are shown in Fig. 2. It is noteworthy to mention that K_i values obtained with several drugs in this competition experiments on [^{3}H]ketanserin and [^{3}H]mesulergine binding correlate well with those reported in membrane-binding experiments (Table II).

In contrast, binding of these ligand to other brain areas did not present the pharmacological characteristics of serotonin-2 receptors. [^{3}H]Ketanserin labeled with high affinity the caudate-putamen and the dorsal raphé, as well as other structures, but the binding to these areas was not inhibited by serotoninergic drugs (Figs. 1B and 3). For example, displaceable [^{3}H]ketanserin binding to the caudate-putamen represented only around 40% of the total binding (Fig. 3), in contrast to an 80% in other areas (Fig. 2B). Binding of [^{3}H]mesulergine to the choroid plexus was inhibited with high affinity by 5-HT, but not by ketanserin or spiperone (Figs. 1A and 4), indicating that this is a 5-HT$_1$ site (5-HT$_{1C}$), as will be discussed later. Regarding the other ^{3}H-labeled ligands, our results confirmed the labeling of 5-HT$_1$ and dopaminergic receptors by [^{3}H]LSD (Fig. 1C) and that of the dopaminergic as well as spirodecanone sites by [^{3}H]spiperone (Fig. 1D) (see below).

Because of the lack of complete specificity of the different ^{3}H-labeled ligands, we defined 5-HT$_2$ sites as those labeled by the four ^{3}H-labeled ligands and whose binding was: (1) inhibited by pirenperone, cinanserin, spiperone and ketanserin; (2) non-displaceable by nanomolar concentrations of serotonin and (3) non-displaceable with high affinity by sulpiride or haloperidol. When these criteria were considered, the distribution of 5-HT$_2$ sites obtained with the four ^{3}H-labeled ligands was similar.

Distribution of 5-HT$_2$ receptors in rat brain

The distribution throughout the brain of 5-HT$_2$ receptors presenting these characteristics was hetero-

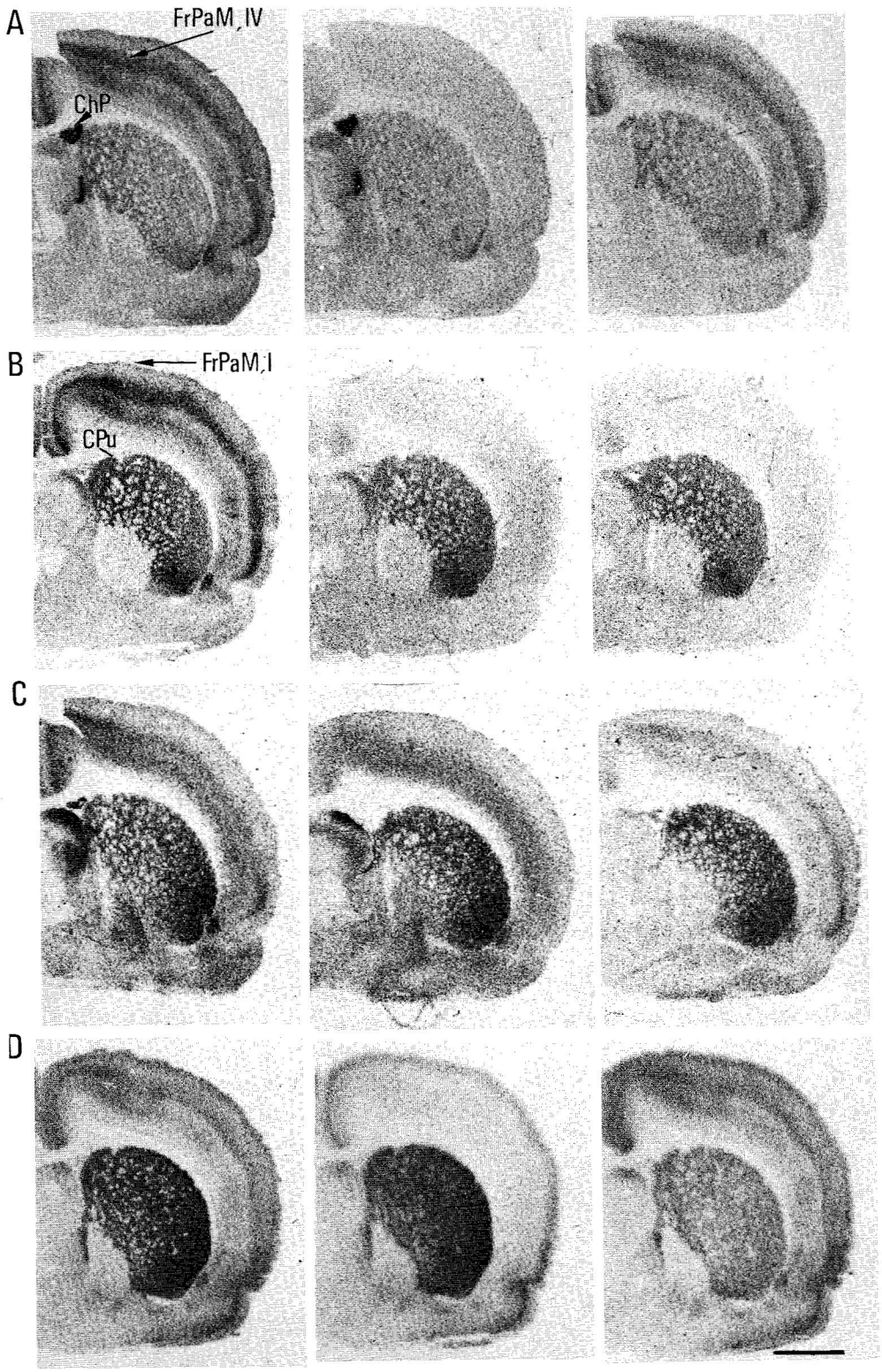


Fig. 1. Specificity of the different ^{3}H-labeled ligands used to label 5-HT$_2$ receptors. Autoradiographic localization of the binding sites in the rat brain. Coronal sections at the level of the nucleus caudate-putamen. Dark regions represent areas with high receptor densities. Abbreviations used are listed separately in the text. A illustrates the total binding of [^{3}H]mesulergine (left), and that in the presence of 3×10^{-7} M cinanserin (middle) and 3×10^{-7} M 5-HT (right). In B, is shown the total binding of [^{3}H]ketanserin (left) and the binding when slides are coincubated with 10^{-7} M methysergide (middle) and 10^{-7} M spiperone (right). C shows the structures labeled by [^{3}H]LSD alone (left) and in the presence of 3×10^{-7} M cinanserin (middle) and 10^{-7} M 5-HT (right). D illustrates the total binding of [^{3}H]spiperone (left), and that obtained by coincubation with 10^{-7} M ketanserin (middle) and 10^{-6} M sulpiride (left). For details and further explanation, see text. Bar = 0.2 cm.

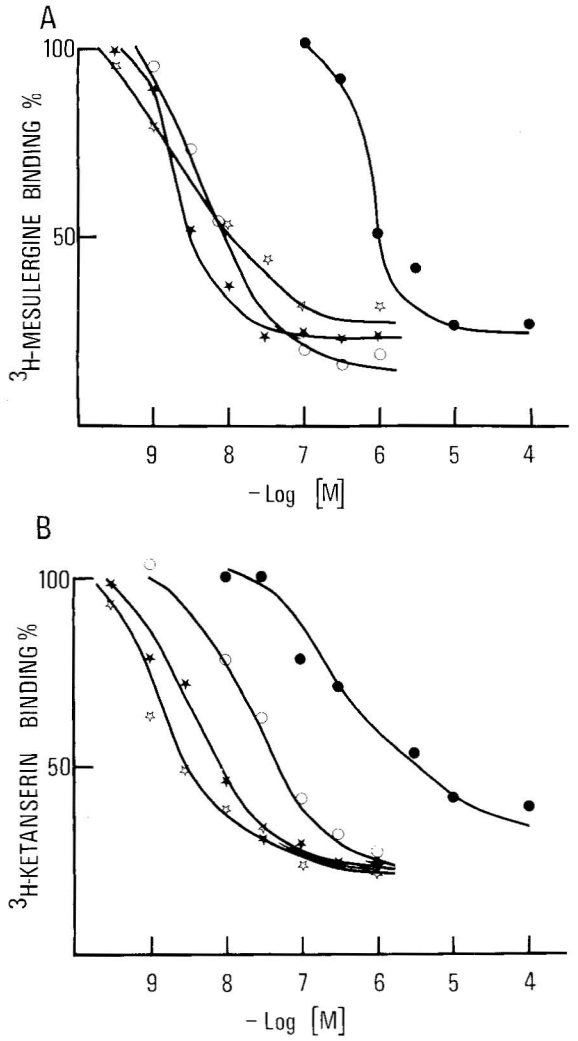


Fig. 2. Competition curves for [^{3}H]mesulergine (A) and [^{3}H]ketanserin (B) binding to the lamina IV of the rat frontoparietal cortex, measured by quantitative autoradiography. Curves correspond to cinanserin (○), 5-HT (●), spiperone (☆) and pirenperone (★).

geneous. In the following, this distribution is analyzed in detail. In all the areas studied, [^{3}H]ketanserin and [^{3}H]mesulergine binding was quantified. [^{3}H]LSD and [^{3}H]spiperone binding were also measured in some selected areas. Although the absolute values of maximal densities varied depending on the ligands (Table III), the rank order of densities in the different nuclei was fully comparable for the four compounds. A very good correlation between the maximal densities of sites using [^{3}H]mesulergine and [^{3}H]ketanserin was obtained (correlation coefficient $= 0.87$; $P < 0.001$).

TABLE II

Comparison of the affinities of several drugs for [^{3}H]ketanserin- and [^{3}H]mesulergine-binding sites in rat frontal cortical membranes (binding assays) and in the lamina IV of the frontoparietal cortex (autoradiographic studies)

Drug	*[^{3}H]Ketanserin*		*[^{3}H]Mesulergine*	
	Binding K_i (nM)	*Autoradiography K_i (nM)*	*Binding K_i (nM)*	*Autoradiography K_i(nM)*
Spiperone	1.9[a]	0.5	1.0[c]	1.0
Mianserin	7.1[b]	11.3	4.6[c]	5.5
Methysergide	3.0[a]	1.3	1.9[c]	0.9
Pirenperone	1.3[b]	1.7	0.5[b]	0.8
Mesulergine	5.1[b]	15.1	1.6[c]	3.7
Cinanserin	4.1[b]	8.3	7.9[c]	1.8
5-HT	3.8[b,H]	32.6[H]	296.0[c]	214.0
	339.2[b,L]	1509.2[L]		
Propranolol	2881.2[b]	3000	2661.0[b]	3000
8-OH-DPAT	3000[d]	3000	–	–

[a] Taken from ref. 34.
[b] Taken from ref. 51.
[c] Taken from ref. 10.
[d] Taken from ref. 51. Data in pig cortex.
[H] High-affinity site.
[L] Low-affinity site.

We have arbitrarily designated areas with 'very high' density of sites, those with a maximal density higher than the 65% of that found in the most en-

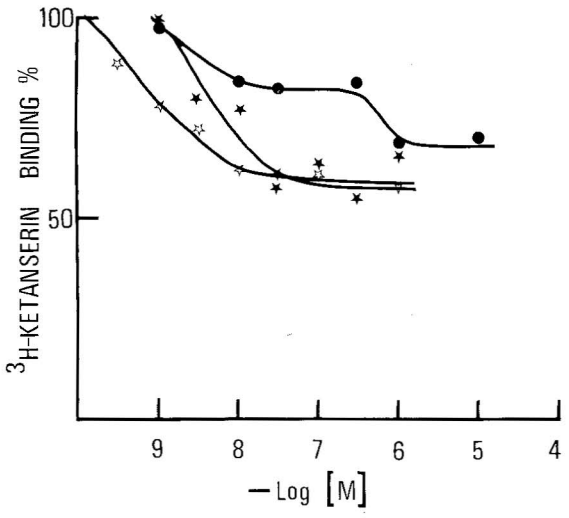


Fig. 3. Competition curves for [^{3}H]ketanserin binding to the rat caudate-putamen, measured by quantitative autoradiography. For symbols, see Fig. 2.

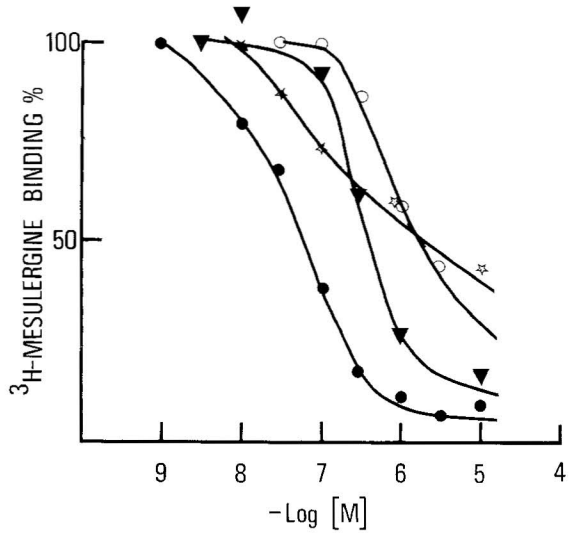


Fig. 4. Competition curves of ketanserin (▼) and other drugs (see Fig. 2 for symbols) for [^{3}H]mesulergine binding to the rat choroid plexus.

riched area for each ligand. 'High' densities were between 50 and 65%. 'Intermediate' levels of binding sites were between 30 and 50% of the highest value found. Finally, 'low' and 'very low' densities of sites were between 10 and 30%, and below 10%, respectively. The values of these densities for the individual brain areas are listed in Table III. Values correspond to maximal densities of receptors, obtained from the percentage occupancies, which were calculated as previously described (see Materials and Methods). The distribution of these receptors is illustrated in the photographic atlas presented in Figs. 1 and 5–9. The reader is referred to the table and figures for further details.

Olfactory system. The external plexiform layer of the olfactory bulb presented a low density of 5-HT_2 sites, while very low concentrations of sites were found in the other layers of this structure, such as the internal granular layer. Very high levels of specific binding were present in the different layers of the olfactory tubercle, with all the ^{3}H-labeled ligands, except for [^{3}H]LSD. The anterior olfactory nucleus, specially in the posterior part, as well as the piriform cortex (area 51b) and the insulae Callejae presented a high to intermediate density of autoradiographic grains (Figs. 6, 7, 8A,B and 9A,B).

Amygdala. Low levels of specific binding were present over the amygdaloid complex, although the lateral and the anterior cortical nuclei were somewhat more enriched in binding sites than the other nuclei. Most of [^{3}H]mesulergine binding to the lateral amygdaloid nucleus was inhibited by 5-HT, but not by spiperone or cinanserine. (Figs. 8C and 9C).

Basal ganglia. In the basal ganglia, marked differences in the 5-HT_2 receptor densities were observed. While intermediate levels of binding sites were found in the nucleus accumbens and in the body and tail of the caudate-putamen nucleus, low to very low concentrations of receptors were present in the head of the caudate-putamen, as well as in the globus pallidus. It is noteworthy to mention that the caudate-putamen, as well as the accumbens, presented the highest level of [^{3}H]ketanserin binding but, as has already been mentioned, most of this binding was not inhibited by any serotoninergic drug. In the same way, high concentrations of dopaminergic binding were found in this nucleus, when [^{3}H]LSD or spiperone were used as ligands (Figs. 1, 6, 7, 8B,C and 9B,C).

Septal region. Very low densities of autoradiographic grains were present over the septal nuclei, as well as in the bed nucleus of the stria terminalis (Figs. 1, 6, 8B and 9B).

Hippocampus. In the hippocampal formation, the concentrations of 5-HT_2 receptors were low or very low in all the areas, except in the ventral dentate gyrus, which presented an intermediate density of binding sites. However, a high density of [^{3}H]LSD binding was present in many structures of the hippocampus, but it had the characteristics of 5-HT_1 receptors, being inhibited by nanomolar concentrations of serotonin, but not by pirenperone or ketanserin. Other non-5-HT_2-binding sites were labeled in the hippocampus by [^{3}H]mesulergine (lacunosum moleculare layer) (5-HT_{1C} sites) and [^{3}H]spiperone (CA_1 field) (spirodecanone sites) (Figs. 5–7, 8C,E and 9C,E).

Hypothalamus. The medial and posterior part of the medial mammillary nucleus presented an intermediate to high concentration of autoradiographic grains, while the different nuclei of the hypothalamus showed low to very low levels of specific binding (Figs. 7, 8C,D and 9C,D).

Thalamus. Low densities of 5-HT_2 receptors were found in the thalamus, except in a small area, at the posterior level, probably corresponding to the parafascicular nucleus, where intermediate concentra-

TABLE III

Maximal densities of 5-HT_2 receptors in different areas of the rat brain

Values are mean ± S.E.M.

Area	*Specific binding (fmol/mg protein)*			
	[^{3}H]mesulergine	*[^{3}H]ketanserin*	*[^{3}H]LSD*	*[^{3}H]spiperone*
Olfactory system				
External plexiform layer of the olfactory bulb	132.2 ± 12.6	107.2 ± 11.2	197.3 ± 42.3	114.1 ± 48.2
Internal granular layer of the olfactory bulb	64.1 ± 29.3	3.9 ± 2.4	–	–
Anterior olfactory nucleus, posterior part	222.9 ± 22.4	281.1 ± 56.3	351.6 ± 10.9	458.8 ± 5.9
Olfactory tubercle	373.1 ± 54.8	378.9 ± 73.8	213.2 ± 48.5	766.0 ± 88.1
Primary olfactory cortex (area 51b)	279.6 ± 18.7	205.7 ± 31.2	327.5 ± 80.6	663.2 ± 99.5
Septal region				
Lateral septal nucleus	28.1 ± 12.3	46.7 ± 3.7	–	–
Bed nucleus of the stria terminalis	12.3 ± 6.2	32.2 ± 5.1	–	–
Basal ganglia				
Accumbens nucleus	164.3 ± 7.6	139.8 ± 20	93.0 ± 30.7	306.7 ± 77.3
Globus pallidus	32.5 ± 2.8	10.6 ± 1.8	–	–
Caudate-putamen, head	72.1 ± 9.8	0.0	45.7 ± 22.9	196.4 ± 8.1
Caudate-putamen, body	174.9 ± 22.0	124.1 ± 38.3	231.8 ± 26.0	–
Caudate-putamen, tail	184.9 ± 33.1	193.2 ± 42.6	172.9 ± 23.3	210.3 ± 7.8
Amygdala				
Lateral amygdaloid nucleus	97.6 ± 10.1	84.4 ± 15.0	–	264.8 ± 18.0
Basomedial amygdaloid nucleus	60.8 ± 19.6	64.9 ± 28.6	–	–
Medial amygdaloid nucleus	60.9 ± 5.9	75.0 ± 14.8	–	–
Anterior cortical amygdaloid nucleus	80.4 ± 15.6	101.0 ± 17.2	–	–
Hippocampus				
Dentate gyrus, dorsal part	16.2 ± 3.7	19.2 ± 12.7	–	–
Dentate gyrus, ventral part	196.9 ± 30.6	102.8 ± 19.4	–	–
Lacunosum molecular layer of the CA_1 field	19.3 ± 10.4	8.1 ± 2.5	–	–
Oriens layer of the CA_1 field	35.8 ± 5.9	19.4 ± 0.1	12.9 ± 3.2	–
Stratum radiatum of the CA_1 field	23.8 ± 5.4	28.6 ± 14.7	–	–
Oriens layer of the CA_3 field	11.1 ± 8.1	32.4 ± 20.6	–	–
Pyramidal cell layer of the CA_3 field	40.4 ± 8.8	25.0 ± 2.9	39.9 ± 20.0	–
Dorsal subiculum	25.7 ± 3.0	13.0 ± 9.6	–	–
CA_4 field	140.5 ± 21.3	102.1 ± 21.4	–	–
Hypothalamus				
Ventromedial hypothalamic nucleus	50.8 ± 8.8	68.5 ± 5.0	31.8 ± 15.9	127.6 ± 14.7
Lateral hypothalamic area	58.5 ± 9.7	65.9 ± 17.2	–	–
Medial mammillary nucleus, medial part	214.7 ± 11.0	227.6 ± 63.4	450.8 ± 25.6	402.2 ± 1.1
Thalamus				
Lateral habenular nucleus	87.5 ± 1.6	58.4 ± 41.6	63.6 ± 43.4	–
Anterior pretectal area	57.4 ± 17.1	18.5 ± 11.2	84.5 ± 17.8	–
Central medial thalamic nucleus	24.9 ± 6.3	40.8 ± 21.4	41.5 ± 20.8	–
Laterodorsal thalamic nucleus	50.1 ± 12.3	56.3 ± 16.3	67.4 ± 7.4	35.1 ± 21.0
Ventrolateral thalamic nucleus	33.4 ± 10.7	44.7 ± 31.8	20.5 ± 10.3	–
Parafascicular nucleus	167.2 ± 9.1	145.5 ± 3.2	–	–
Dorsal lateral geniculate nucleus	37.0 ± 10.7	11.5 ± 8.3	–	–
Medial geniculate nucleus	39.4 ± 7.6	2.1 ± 1.8	–	–
Claustrum, neo- and cingulate cortex				
Claustrum	497.7 ± 51.0	598.4 ± 80.5	516.7 ± 74.0	1020.1 ± 112.3

TABLE III *continued*

Area	*Specific binding (fmol/mg protein)*			
	[³H]mesulergine	*[³H]ketanserin*	*[³H]LSD*	*[³H]spiperone*
Frontal cortex (Area 10)				
lamina I	305.5 ± 14.6	299.2 ± 8.1	292.6 ± 3.3	–
lamina II	248.4 ± 16.8	132.2 ± 19.6	110.9 ± 43.4	–
lamina IV	594.8 ± 17.7	603.3 ± 30.4	340.3 ± 29.8	909.6 ± 48.2
lamina V	243.8 ± 40.1	236.8 ± 38.1	77.9 ± 46.1	524.4 ± 34.9
lamina VI	127.2 ± 24.8	98.5 ± 16.8	87.9 ± 38.8	134.1 ± 36.9
Frontoparietal motor cortex (Area 2)				
lamina I	255.7 ± 23.8	117.1 ± 13.4	136.1 ± 31.4	–
lamina II	173.1 ± 23.6	165.7 ± 21.2	53.5 ± 38.8	–
lamina IV	562.7 ± 77	382.1 ± 18.9	237.6 ± 15.9	964.6 ± 85.7
lamina Va	255.6 ± 28.5	180.3 ± 60.9	220.2 ± 60.1	576.4 ± 21.6
lamina Vb	341.6 ± 18.7	257.6 ± 32.4	–	–
lamina VI	101.2 ± 16.8	61.5 ± 24.9	66.7 ± 39.9	–
Frontoparietal somatosensory cortex (Area 2a)				
lamina I	176.3 ± 22.7	202.4 ± 22.2	33.7 ± 15.8	–
lamina II	145.7 ± 22.4	174.6 ± 31.3	–	–
lamina IV	339.1 ± 43.2	319.5 ± 31.6	253.9 ± 49.6	571.7 ± 42.4
lamina V	110.7 ± 14.4	163.6 ± 31.1	10.1 ± 4.8	–
lamina VI	82.2 ± 9.4	100.2 ± 11.6	–	–
Striate cortex (Area 18)				
lamina I	–	60.5 ± 5.5	–	–
lamina II	63.6 ± 20.7	0.7 ± 0.3	–	–
lamina IV	250.5 ± 9.1	77.4 ± 9.3	243.4 ± 114	545.0 ± 184.2
lamina V	202.3 ± 19.0	162.3 ± 12.9	–	–
lamina VI	49.9 ± 9.5	20.7 ± 2.4	67.4 ± 42.6	–
Anterior cingulate cortex (Area 24)				
lamina I	276.4 ± 38.0	137.2 ± 12.4	362.8 ± 69.1	–
lamina II	156.6 ± 15.0	104.6 ± 4.2	28.7 ± 12.5	–
lamina IV	473.7 ± 22.6	410.6 ± 26.2	432.2 ± 46.5	842.8 ± 71.5
lamina V	188.4 ± 45.6	129.9 ± 30.1	234.9 ± 108.5	–
lamina VI	108.0 ± 10.3	20.0 ± 14.3	–	–
Retrosplenial cortex (Area 29b)				
lamina I	15.5 ± 6.1	4.2 ± 3.2	–	–
lamina II	1.5 ± 0.3	8.8 ± 5.2	–	–
lamina IV	3.9 ± 2.1	6.5 ± 0.8	–	–
lamina V	20.1 ± 0.2	7.8 ± 5.2	–	–
lamina VI	13.7 ± 5.8	22.4 ± 10.1	–	–
Entorhinal cortex				
Principalis internal layer	252.7 ± 39.3	201.3 ± 28.7	–	–
Cerebellum				
Stratum moleculare of the cerebellum	0.0	43.6 ± 13.2	–	–
Stratum granulosum of the cerebellum	0.0	23.3 ± 8.5	–	–
Midbrain				
Superficial grey layer of the superior colliculus	45.3 ± 8.5	60.0 ± 3.9	–	–
Optic nerve layer of the superior colliculus	35.5 ± 8.6	16.4 ± 11.3	–	–
Principal oculomotor nucleus	7.3 ± 6.5	7.2 ± 6.1	7.4 ± 6.1	–
Acessory oculomotor nucleus	20.7 ± 1.3	32.9 ± 18.1	–	–
Central grey (periaqueductal)	9.4 ± 7.1	28.6 ± 8.1	–	–
Dorsal raphé nucleus	31.7 ± 0.7	23.1 ± 19.2	–	77.6 ± 15.6
Median raphé (superior central) nucleus	10.1 ± 4.9	12.0 ± 6.1	25.6 ± 21.2	17.0 ± 1.4

TABLE III *continued*

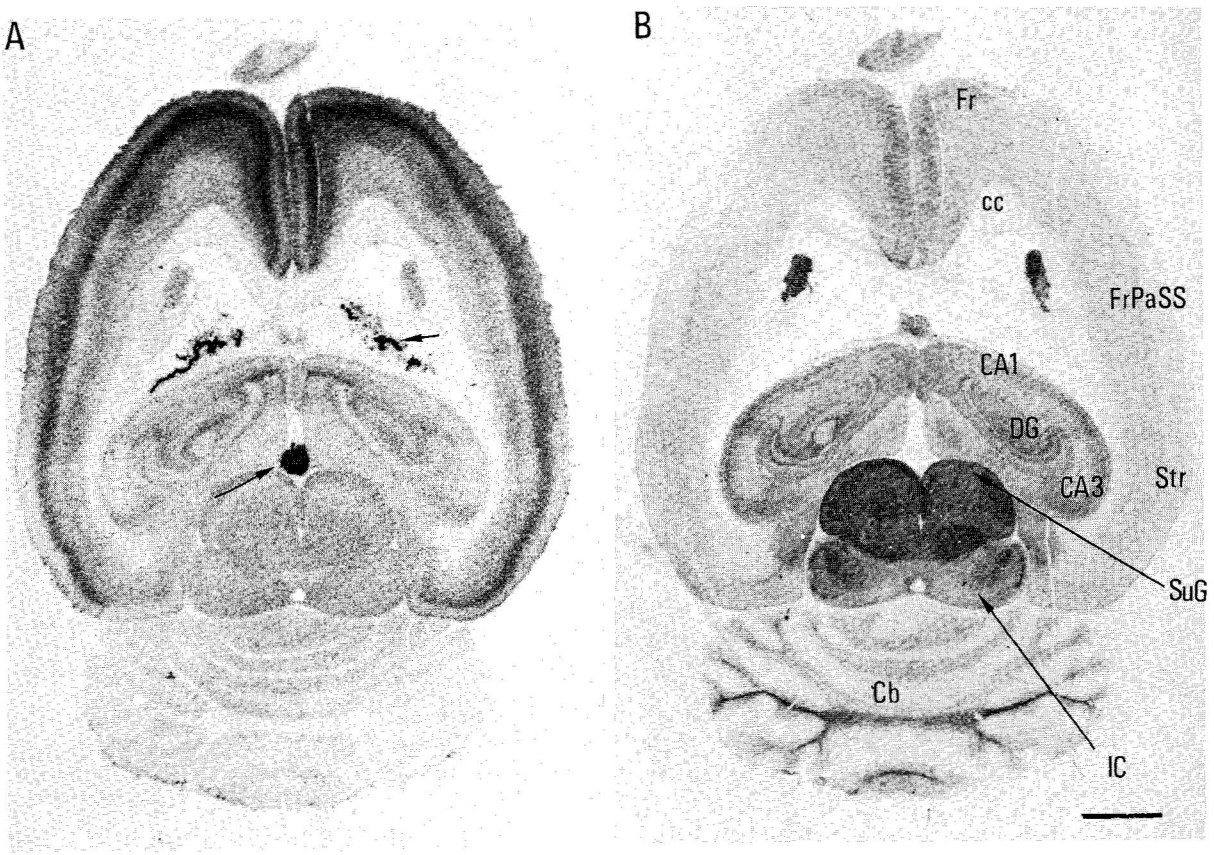
Area	*Specific binding (fmol/mg protein)*			
	[³H]mesulergine	*[³H]ketanserin*	*[³H]LSD*	*[³H]spiperone*
Substantia nigra, reticular part	54.8 ± 13.4	29.4 ± 11.7	–	–
Substantia nigra, compact part	38.0 ± 25.1	37.4 ± 25.2	–	–
Ventral tegmental area	15.6 ± 3.8	10.7 ± 5.6	–	–
Interpeduncular nucleus	21.7 ± 1.9	7.6 ± 5.1	–	–
Pons				
Cuneiform nucleus	0.0	18.5 ± 11.7	–	–
Dorsal cochlear nucleus	28.5 ± 10.4	11.1 ± 6.2	–	–
Raphé magnus nucleus	46.7 ± 12.2	14.6 ± 10.1	–	–
Nucleus of the spinal tract of the trigeminal nerve, oral part	40.1 ± 5.2	24.7 ± 11.2	–	–
Facial nucleus	136.4 ± 31.7	45.1 ± 10.6	67.8 ± 12.4	100.9 ± 28.4
Pontine nuclei	–	5.7 ± 6.1	–	–
Pontine reticular nucleus, oral part	20.1 ± 5.9	25.2 ± 18.7	–	–
Gigantocellular reticular nucleus	30.2 ± 6.8	15.9 ± 2.3	–	–
Nucleus of the solitary tract	42.5 ± 14.7	69.3 ± 11.5	–	82.1 ± 6.6
Medulla oblongata				
Prepositus hypoglossal nucleus	71.8 ± 8.8	65.9 ± 7.2	–	66.9 ± 17.7
Medial vestibular nucleus	44.9 ± 4.8	42.6 ± 10.7	–	–
Hypoglossal nucleus	116.6 ± 23.1	27.3 ± 21.4	–	120.2 ± 16.1
Ambiguus nucleus	50.1 ± 0.1	23.3 ± 17.4	36.8 ± 25.1	–
Gracile nucleus	50.5 ± 10.3	87.8 ± 14.0	–	–
Cuneate nucleus	47.1 ± 7.9	35.3 ± 13.8	–	–
Inferior olive	145.5 ± 15.3	17.4 ± 11.2	1.2 ± 0.3	246.3 ± 42.1
Nucleus of the spinal tract of the trigeminal nerve, caudal part	34.8 ± 6.5	20.2 ± 15.5	–	–
Spinal cord				
Layer 2 of Rexed	0.0	6.2 ± 5.1	–	–
Layer 7 of Rexed	0.0	6.4 ± 2.9	–	–
Choroid plexus				
Lateral ventricle	44.0 ± 24.2	42.3 ± 13.7	–	–
Third ventricle	81.3 ± 8.0	0.0	50.0 ± 41.0	0.5 ± 0.1
Fourth ventricle	73.0 ± 51.1	–	33.4 ± 26.5	–

tions of binding sites were found. Areas such as the dorsal lateral geniculate nucleus, medial geniculate nucleus, ventrolateral and laterodorsal nuclei showed a very low concentration of autoradiographic grains. The central medial nucleus and the anterior pretectal area, as well as the lateral habenula presented low densities of specific binding. The binding of [³H]mesulergine to the central medial nucleus displayed the pharmacological characteristics of a 5-HT_1 site (Figs. 6, 7, 8C,D).

Claustrum. Very high concentrations of 5-HT_2 receptors were found in the claustrum with the different ³H-labeled ligands used (Figs. 6, 8A,B and 9A,B).

Neo- and cingulate cortex. A very high concentration of 5-HT_2 receptors was found in the cortex. These sites were present in all the areas and laminae of the neocortex. The highest density of binding sites was localized to a continuous band which included lamina IV and extended to part of lamina III, depending upon the cortical area. In the following, this band has been named lamina IV for the sake of simplicity.

In the neocortex, a decreasing rostrocaudal gradient of 5-HT_2 sites was observed in this band. While very high densities were found in the frontal (area 10) and in the frontoparietal motor cortex (area 2), only high concentrations of sites were present in the frontoparietal sensory (area 2a) and striate cortex (area 18). High to intermediate grain concentrations were present over the lamina I. Lamina V contained varying concentrations of sites in different cortical areas.



Figs. 5–9. Atlas of autoradiographic localization of [^{3}H]mesulergine- and [^{3}H]ketanserin-binding sites in the rat brain. Anatomical levels shown in these figures correspond to the plates 9, 13, 19, 24, 26, 30, 37, 48, 61 and 65 of the rat brain atlas from Paxinos and Watson[50]. See text for further details.

Fig. 5. [^{3}H]Mesulergine-binding sites in a dorsal horizontal section (A) and consecutive section incubated for acetylcholinesterase activity (B). Note the high concentrations of binding in lamina I and IV at the different levels of the neocortex and the low densities of sites in other structures, including hippocampus and colliculi. Arrows indicate the intensely labeled choroid plexuses, where the binding is not of the 5-HT$_2$ class. Bar = 0.2 cm.

The highest densities in this lamina were observed in frontoparietal motor cortex. Contrasting with that, the density of 5-HT$_2$ sites in this lamina in the auditory cortex was very low. Intermediate to low densities were found in lamina II, while low to very low were presented in lamina VI (Figs. 1, 5–7, 8A–F and 9A–F).

It should be pointed out that [^{3}H]spiperone labeled with high affinity a non-5-HT$_2$ area in the lamina I, previously described as spirodecanone site. In a similar way, [^{3}H]LSD labeled in the deep laminae of the neocortex, an important population of 5-HT$_1$ receptors (Fig. 1C,D).

In the cingulate cortex, the anterior cingular cortex (area 24) presented a pattern of specific binding similar to that described in the neocortex, the highest concentrations of receptors being found in lamina IV and I. In contrast in the retrosplenial cortex (area 29b), the binding was very low in all the laminae (Figs. 1, 5–7, 8A–F and 9A–F).

Entorhinal cortex. High to intermediate densities of sites were found in the entorhinal cortex (area 28a), the highest concentration of specific binding being found in the principalis internal layer (Figs. 6, 8E,F and 9E,F).

Cerebellum. Specific binding in the different areas, as well as in the deep cerebellar nuclei was very low (Figs. 5–7, 8G and 9G).

Midbrain. The density of 5-HT$_2$-binding sites was low or very low in all the nuclei of the midbrain.

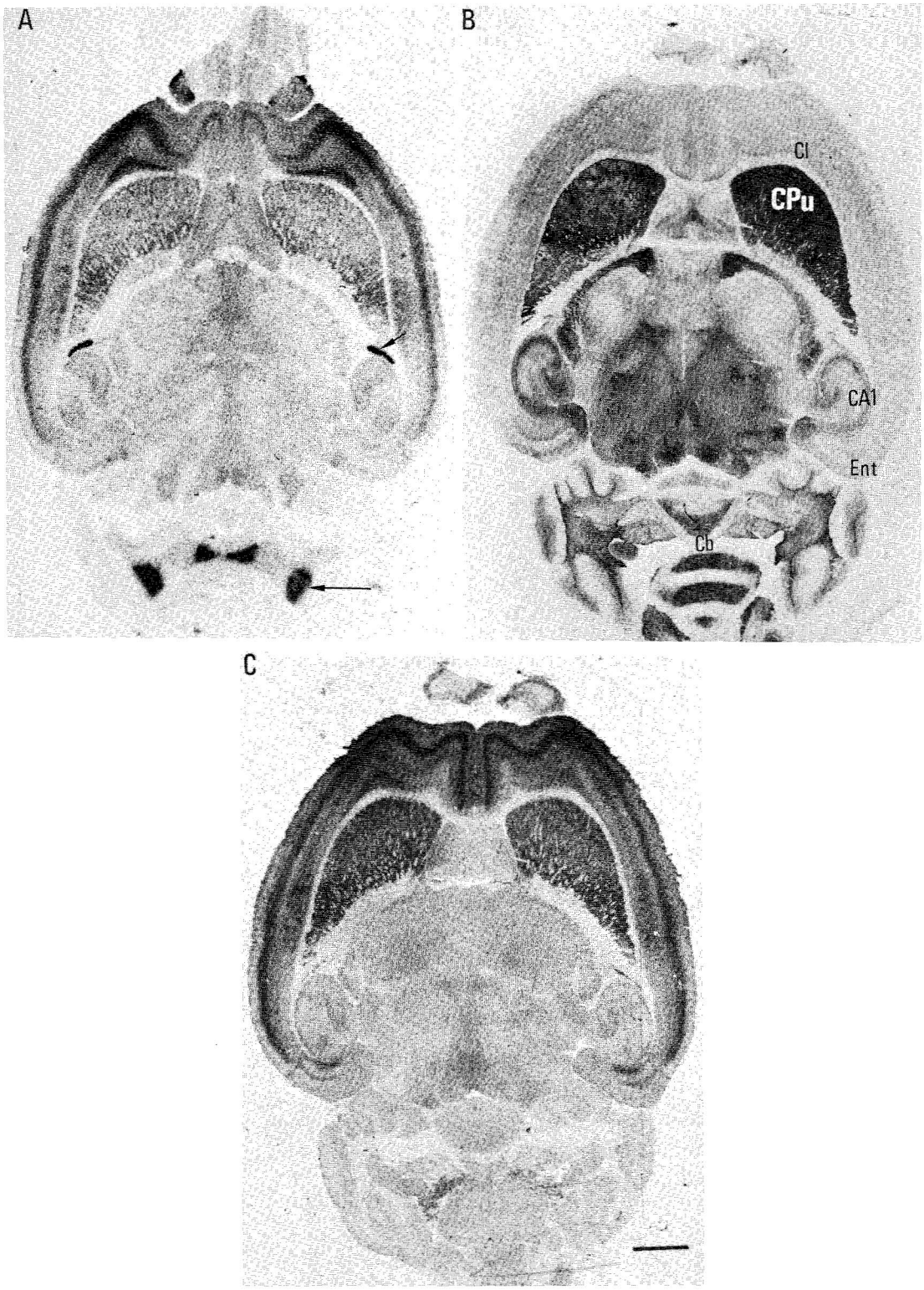


Fig. 6. [^{3}H]Mesulergine (A) and [^{3}H]ketanserin (C) binding sites in a medial horizontal section, and consecutive section incubated for acetylcholinesterase activity (B). Both ligands label with high affinity the neocortex and the claustrum (Cl). Note the high concentrations of [^{3}H]mesulergine sites in the choroid plexuses (arrows) and those of [^{3}H]ketanserin binding in the nucleus caudate-putamen (CPu) which do not represent 5-HT_2 receptors. Bar = 0.2 cm.

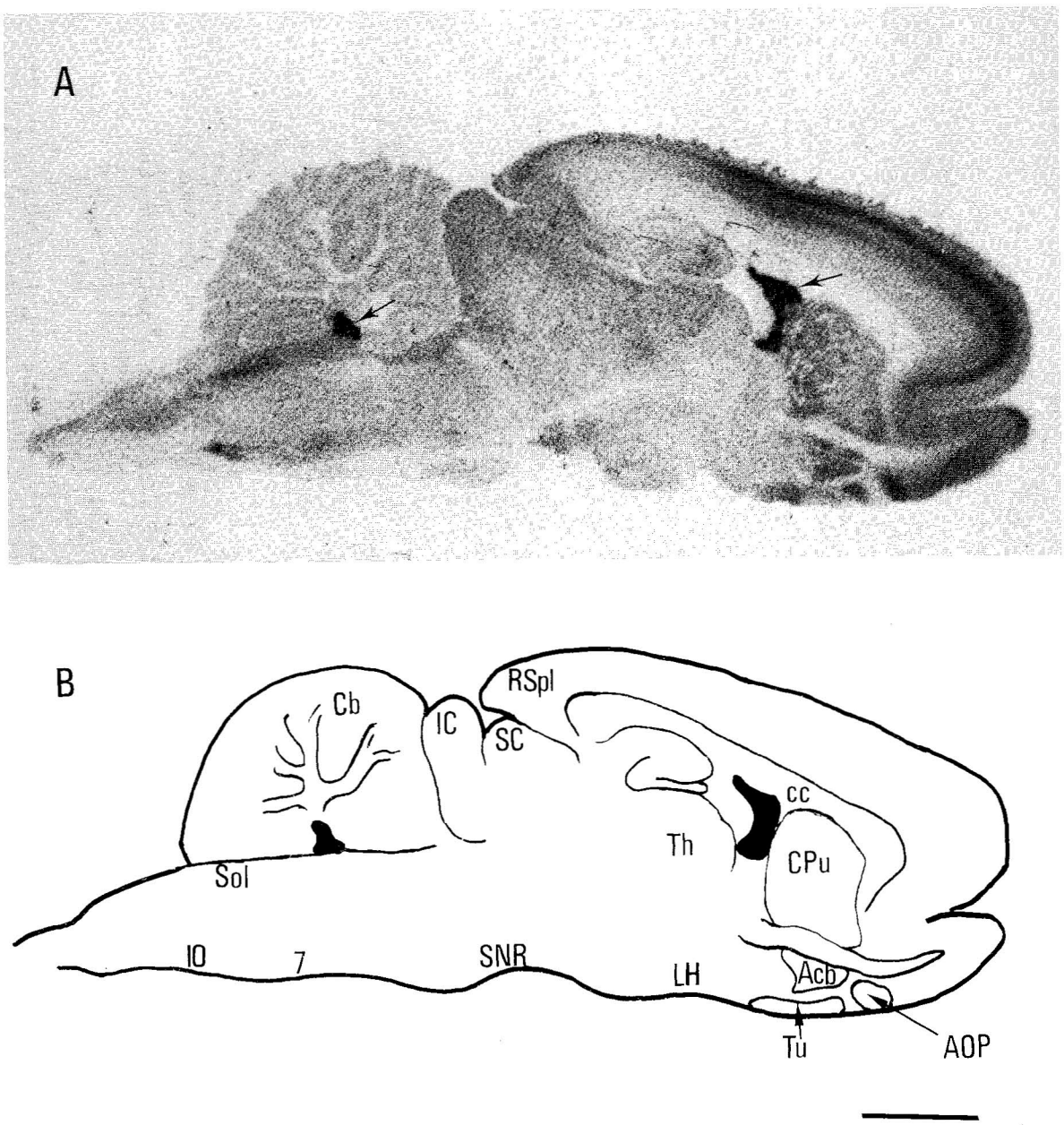


Fig. 7. Sagittal level showing [^{3}H]mesulergine binding (A) and corresponding schematic drawing (B). Highest concentration of binding sites are present in lamina IV of the neocortex — except at the retrosplenial level (RSpl) — choroid plexuses (arrows), olfactory tubercle (Tu) and anterior olfactory nucleus (AOP). Bar = 0.2 cm.

However, very high concentrations of [^{3}H]ketanserin binding were found in the dorsal raphé nuclei, substantia nigra compacta and ventral tegmental area, but they were not inhibited by any serotoninergic drug. [^{3}H]LSD also labeled with high affinity some nuclei in the midbrain, with the characteristics of 5-HT_1 sites (Figs. 5–7, 8E,F and 9E,F).

Pons. Specific binding in the different areas of the pons ranged from low to very low levels, although the facial nucleus was somewhat richer in 5-HT_2 receptors. A significant part of the binding sites labeled by [^{3}H]mesulergine in the facial nucleus and in the nu-

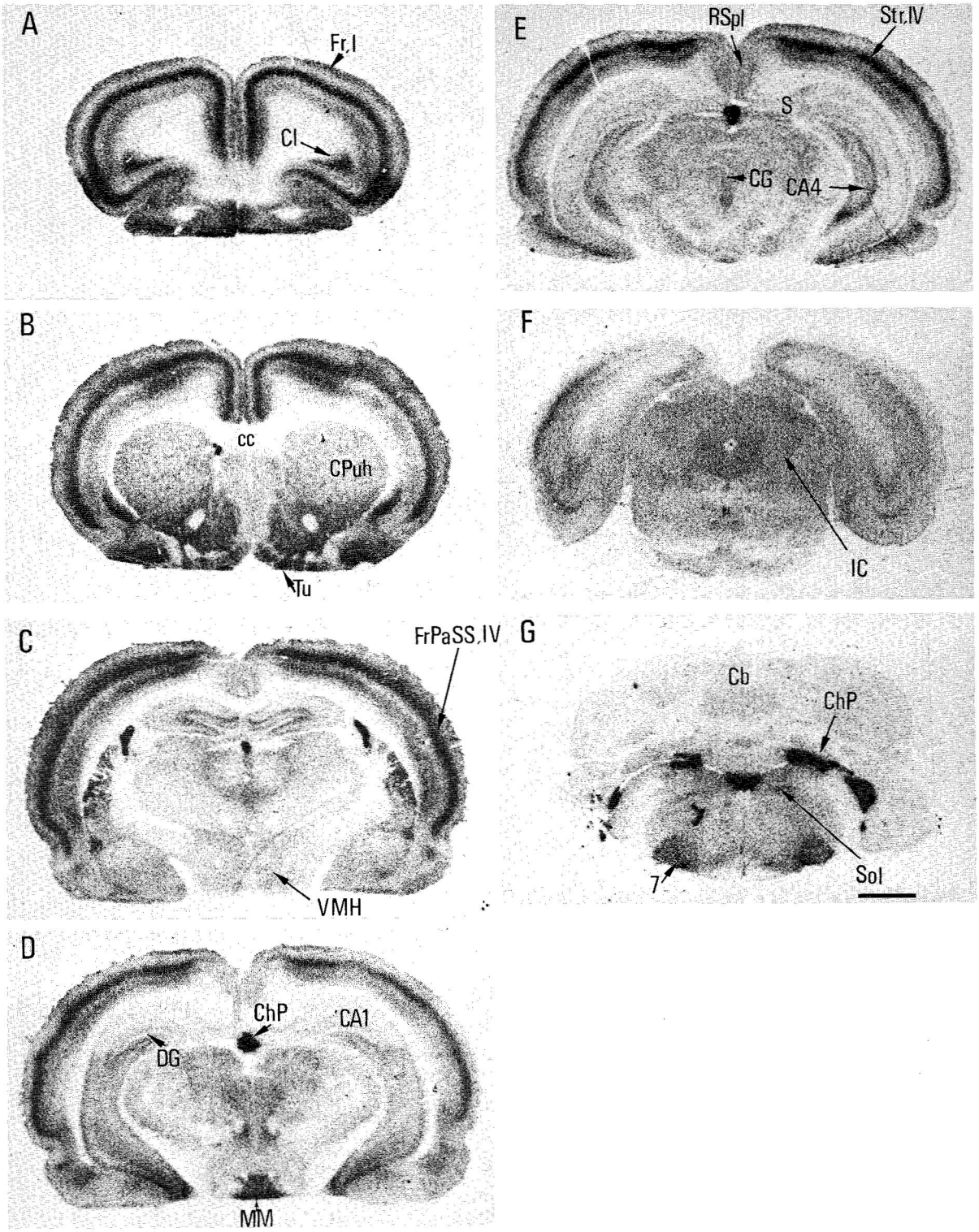


Fig. 8. Localization of [^{3}H]mesulergine-binding sites at different levels. Coronal sections through the frontal cortex (A), the nucleus caudate-putamen (B), the anterior thalamus (C), the posterior thalamus (D), the upper midbrain (E), the lower midbrain (F) and the pons (G). The highest receptor concentrations are present in the lamina IV of the neocortex (FrPaSS, IV), claustrum (Cl) and olfactory tubercle (Tu). Note also the presence of high to intermediate concentrations of specific binding in the layers I and V of the neocortex at some levels, anterior olfactory nucleus, caudate-putamen (CPuh), accumbens, mammillary bodies (MM), ventral dentate gyrus (DG) and facial nucleus (7). The intense labeling of the choroid plexuses (ChP) does not correspond to 5-HT_2 receptors. Bar = 0.2 cm.

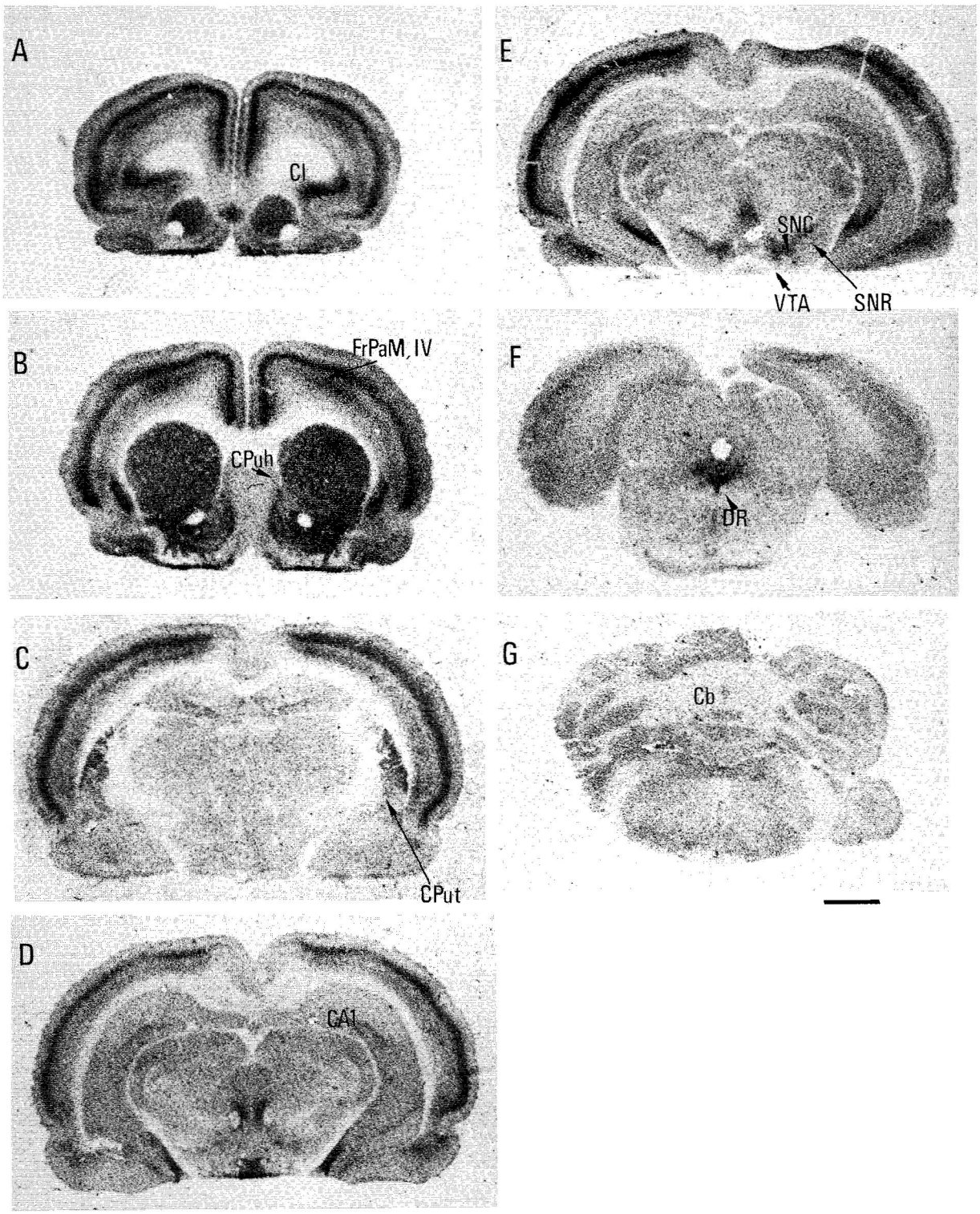


Fig. 9. Localization of [^{3}H]ketanserin-binding sites at the levels shown in Fig. 8. The autoradiograms demonstrate the presence of high or intermediate concentrations of receptors in the same structures labeled by [^{3}H]mesulergine (see Fig. 8). Note the very high concentrations of sites in areas such as the caudate-putamen (CPuh), substantia nigra, pars compacta (SNC) and dorsal raphé (DR), where most of the binding is not displaced by serotoninergic ligands. Bar = 0.2 cm.

cleus of the solitary tract was inhibited by 5-HT, but not by serotonin-2 drugs (Figs. 7, 8F,G and 9F,G).

Medulla oblongata. Most of nuclei in this part of the brainstem presented very low concentrations of autoradiographic grains. An exception was the inferior olive which showed low to intermediate densities of 5-HT_2 receptors when [^{3}H]mesulergine or [^{3}H]spiperone were used as ligands. However, no significant density of binding was seen with [^{3}H]ketanserin and [^{3}H]LSD in this area. As in the other structures studied, [^{3}H]LSD labeled with high affinity other nuclei, displaying the pharmacological properties of 5-HT_1 sites (Fig. 7).

Spinal cord. Very low levels of 5-HT_2-binding sites were found throughout the gray matter in the spinal cord (not shown).

Choroid plexus, Although [^{3}H]mesulergine and [^{3}H]LSD labeled with very high affinity the choroid plexuses at the different levels studied, this binding was not inhibited by serotonin-2 ligands, but by nanomolar concentrations of serotonin, as has been previously described (Figs. 1A–C, 5–7 and 8C–G).

DISCUSSION

The results presented above provide a detailed quantitative description of the distribution of 5-HT_2 receptors in rat brain, using four ^{3}H-labeled ligands. Previous studies dealing with this issue were carried out using non-selective 5-HT_2 compounds such as [^{3}H]LSD[38,71] or [^{3}H]spiperone[45] and in these studies no quantification was attempted. Several findings of the present study could be underlined. First, the kinetic and pharmacological properties of the binding of the new ^{3}H-labeled ligands mesulergine and ketanserin are closely comparable to those reported for these ligands in membrane-binding assays. Thus, the binding of these ligands to mounted tissue sections was saturable with K_d values similar to those obtained in homogenate preparations[10,34]. Similar characteristics have been previously documented for [^{3}H]LSD[38,71] and [^{3}H]spiperone[45]. More important, by combining a quantitative autoradiographic approach with classical competition experiments, it was possible to analyze the pharmacological characteristics of the binding of these ligands to microscopic brain areas. In many regions, we found K_i values for several drugs which are identical to those reported in previous homogenate studies (Table II). This finding supports an identity of the sites visualized by autoradiography with the proposed 5-HT_2 receptors.

The four ^{3}H-labeled ligands used labeled the same populations of sites in the rat brain, when their binding was studied under the adequate conditions to visualize 5-HT_2 receptors (see above). The rank order of maximal receptor densities in the different areas and nuclei of the brain showed a very good correlation between the different compounds, although the absolute values of the densities varied depending on the ^{3}H-labeled ligand (Table III).

An important finding in our study was that none of the compounds used to label 5-HT_2 receptors were completely selective for these sites. [^{3}H]LSD labeled in many brain areas 5-HT_1 sites, as well as dopamine receptors (Fig. 1C), as has been described[8,38,55]. In the same way, the reported binding of [^{3}H]spiperone to dopamine receptors and to 'spirodecanone'-binding sites[25,45] was also seen (Fig. 1D). Surprisingly, the reputed 5-HT_2-selective ligands [^{3}H]mesulergine and [^{3}H]ketanserin also labeled sites which did not have the characteristics of 5-HT_2 receptors. The highest concentration of [^{3}H]mesulergine binding in the rat brain was found in the choroid plexus, and this binding was inhibited by serotonin, but not by spiperone or ketanserin (Fig. 1A). We have demonstrated that these sites correspond to the 5-HT_{1C} subtype[13]. Other nuclei containing low concentrations of these sites were also labeled by [^{3}H]mesulergine (Fig. 8C). Finally, it was observed that in some areas containing very high concentrations of [^{3}H]ketanserin-binding sites, mainly the caudate-putamen and the dorsal raphé, most of the binding was not inhibited by any serotoninergic drug (Figs. 1B and 9). Therefore, these sites should be considered as 'ketanserin chemical recognition sites' rather than serotoninergic receptors. Recently, these nuclei have been reported as the most enriched brain areas in 5-HT_2 sites, in a study where no pharmacological assessment was carried out[65]. These findings emphasize that a pharmacological characterization of the autoradiographic binding sites is always required, before any statement on specific receptor labeling can be made.

The distribution of 5-HT_2 receptors found in this study agrees very well with preliminary autoradiographic data[38,45,71] as well as with the results obtained in biochemical assays performed in membranes from

hand-dissected rat brain regions and using LSD, spiperone or ketanserin as ^{3}H-labeled ligands[7,31,34,58]. A similar distribution of these receptors has been found in the brain of other mammalian species, such as guinea-pig, bovine[58] and human[64], although the pharmacological profile of these receptors in the human brain seems to be different[51].

It can be stated that 5-HT_2 receptor density was much higher in telencephalic areas than in meso-or metencephalic areas (Fig. 7). The neocortex and the claustrum, as well as several components of the olfactory system were very enriched in these sites. Some structures related to the limbic system — anterior cingular cortex and mammillary nuclei — and basal ganglia also presented high concentrations of receptors. In contrast, the density of sites in the other parts of the brain was low or very low (Figs. 5–7).

The widespread presence and the high density of 5-HT_2 sites in the different layers of the cortex, supports a role of these receptors in the regulation of many brain functions. It must be pointed out that most of the central effects so far related to the 5-HT_2 receptors are motor behavioral syndromes with different degrees of complexity, such as the 5-HTP-induced head twitches in several mammalian species, the wet-dog shake behavior or the tryptamine-induced clonic seizures in rat[11,23,34,36,41,54,70]. Although Bedard and Pycock[4] suggested that these effects could be hind-brain and spinally mediated, it remains unclear whether the behavioral model used in this case, reflex wet-dog shakes after lesions at the posterior commissura, is fully comparable to those previously described[70]. Moreover, it has been recently reported that not all the serotonin-mediated behavioral syndromes are related to 5-HT_2 receptor activation[36]. It has been proposed that the typical 5-HT_2 behavioral models require the activity of rostral brain structures[36,70]. The high concentrations of 5-HT_2 receptors present in the neocortex (including the frontoparietal motor area), some structures of the basal ganglia, as well as the claustrum, which has connections with 'motor' cortex, among others[15,61] support the influence of these areas on those motor behavioral effects. Furthermore, the presence of receptors in some olfactory and limbic structures could indicate a role of 5-HT_2 sites in these systems. A physiological significance of these receptors in the control of the visual activity could be also suggested, because of the high concentration found in the visual cortex (area 17) and in the claustrum, an area strongly connected with the visual pathway[32,63].

Regarding the possible role of 5-HT_2 receptors in some clinical situations, considerable evidence has accumulated on the relationship between antidepressant treatments and these receptors. It has been reported that the chronic administration of antidepressant drugs decreases the number of 5-HT_2 receptors in the rat brain[6,16,40,56,60,67]. Another antidepressant therapy, the chronic electroconvulsive shock, has been reported to induce the opposite effect, increasing the number of sites[22,27,69] (see ref. 2 for a recent discussion).

Recently, decreases in the number of cortical 5-HT_2 receptors have been described in membranes from patients suffering senile dementia type Alzheimer[59]. Although it is too early to speculate on the pathological meaning of this finding, it is tempting to suggest that cortical 5-HT_2 receptors could play a role in cognitive processes.

An important question is how the localization of these 5-HT_2 receptors correlates with the distribution of serotoninergic terminals. It should be kept in mind that besides 5-HT_2 sites, the brain contains also other 5-HT-recognition sites, such as the 5-HT_1, whose distribution is different from that of 5-HT_2 sites[5,13,18,37,38,71]. A detailed quantitative mapping of 5-HT_1 sites is reported in the accompanying paper[52]. It is apparent that most of the areas presenting high concentrations of 5-HT_2 receptors contain nerve terminals arising from the different serotoninergic neurons, mainly located within the raphé nuclei[3,9,17,65]. The caudate-putamen nucleus, the different layers of the neocortex, the olfactory tubercle or the medial mammillary bodies are areas where the presence of serotonergic fibers and markers is well-documented[9,12,19,21,24,35,49,62,66]. However, the density of 5-HT terminals in the claustrum is rather low[66] and does not correlate with the very high concentration of 5-HT_2 receptors found in this area. The existence of discrepancies between the distribution of fibers and receptors has been reported for other many neurotransmitters, such as noradrenaline, histamine or γ-aminobutyric acid (GABA)[46–48] and several explanations have been proposed for this fact. The lack of physiological function of some of the binding sites, the influence on the density of receptors by factors

unrelated to innervation or the nature of the cells containing the receptors, among others, are examples of these explanations. None of these hypotheses completely accounts for the differences observed in several systems[30]. On the other hand, many brain areas which are extremely enriched in serotoninergic terminals presented low or very low densities of 5-HT_2 receptors. As mentioned above, the presence of 5-HT_1 receptors in these areas[5,13,18,37,38,52,71] could explain the lack of 5-HT_2 receptors.

The question of the cellular localization of 5-HT_2 sites is beyond the resolution limits of the technique used in this work. More or less specific lesions combined with membrane-binding assays have been used by others in attempt to determine the nature of the cells bearing 5-HT_2 receptors in brain. The results of these studies[7,33] suggest that 5-HT_2 receptors in the rat cortex are postsynaptic, not related to noradrenergic or dopaminergic systems and probably located in some populations of interneurons. Further work is necessary to clarify this problem. In any case, our technique does not provide sufficient resolution to clarify this issue.

In conclusion, our results indicate that sites with the pharmacological properties of 5-HT_2 receptors have been localized at the light microscopic level and found to be highly heterogeneously distributed in the rat brain. The mapping of these receptors supports the involvement of these receptors particularly in cortical functions and provides a detailed information on the localization of these sites throughout the CNS.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

7	facial nucleus
Acb	accumbens nucleus
AOP	anterior olfactory nucleus, posterior part
CA_1	field CA_1 of the hippocampus
CA_3	field CA_3 of the hippocampus
CA_4	field CA_4 of the hippocampus
Cb	cerebellum
cc	corpus callosum
CG	central (periaqueductal) grey
ChP	choroid plexus
Cl	claustrum
CPu	caudate-putamen
CPuh	caudate-putamen, head
CPut	caudate-putamen, tail
DG	dentate gyrus
DR	dorsal raphé nuclei
Ent	entorhinal cortex
Fr	frontal cortex
Fr, I	lamina I of the frontal cortex
FrPaM, I	lamina I of the frontoparietal cortex, motor area
FrPaM, IV	lamina IV of the frontoparietal cortex, motor area
FrPaSS	frontoparietal cortex, somatosensory area
FrPaSS, IV	lamina IV of the frontoparietal cortex, somatosensory area
IC	inferior colliculus
IO	inferior olive
LH	lateral hypothalamic area
MM	medial mammillary nucleus, medial part
RSpl	retrosplenial cortex
S	dorsal subiculum
SC	superior colliculus
SNC	substantia nigra, compact part
SNR	substantia nigra, reticular part
Sol	nucleus of the solitary tract
Str	striate cortex
Str, IV	lamina IV of the striate cortex
SuG	superficial grey layer of the superior colliculus
Th	thalamus
Tu	olfactory tubercle
VMH	ventromedial hypothalamic nucleus
VTA	ventral tegmental area

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