Quantitative Autoradiography of Multiple 5-HT₁ Receptor Subtypes in the Brain of Control or 5,7-Dihydroxytryptamine-Treated Rats

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The distribution of the 2 main types (A and B) of 5-HT₁ binding sites in the rat brain was studied by light-microscopic quantitative autoradiography. The 5-HT₁ₐ sites were identified using ³H-8-hydroxy-2-(N-dipropylamino)tetralin (³H-8-OH-DPAT) or ³H-5-HT as the ligand. In the latter case, it was shown that ³H-5-HT binding to 5-HT₁ₐ sites corresponded to that displaceable by 0.1 μM 8-OH-DPAT or 1 μM spiperone. The "non-5-HT₁ₐ" sites labeled by ³H-5-HT in the presence of 0.1 μM 8-OH-DPAT corresponded mainly to 5-HT₁ᵦ sites. 5-HT₁ₐ binding was notably high in limbic regions (dentate gyrus, CA₁ and CA₃ hippocampal regions, lateral septum, frontal cortex), whereas 5-HT₁ᵦ binding was particularly concentrated in extrapyramidal areas (caudate nucleus, globus pallidus, substantia nigra). Except in the latter regions, where only one class of 5-HT₁ sites was found, both 5-HT₁ₐ and 5-HT₁ᵦ sites existed in all areas examined. The selective degeneration of serotoninergic neurons produced by an intracerebral injection of 5,7-dihydroxytryptamine was associated only with a significant loss of 5-HT₁ₐ binding to the dorsal raphe nucleus (−60%) and of 5-HT₁ᵦ binding to the substantia nigra (−37%). These results are discussed in relation to the possible identity of 5-HT₁ₐ and/or 5-HT₁ᵦ sites with the presynaptic 5-HT autoreceptors controlling nerve impulse flow and neurotransmitter release in serotoninergic neurons.

Pharmacological, biochemical and electrophysiological studies have clearly demonstrated the presence of multiple recognition sites for 5-HT in the CNS (Aghajanian, 1981; Hamon et al., 1984a; Nelson et al., 1980; Peroutka and Snyder, 1979). Ligand binding studies first suggested that 2 types of 5-HT receptors could be discriminated (Peroutka and Snyder, 1979): the 5-HT₁ class, which exhibits a high affinity for ³H-5-HT and 5-HT agonists; and the 5-HT₂ class which has a higher affinity for ³H-spiperone and other 5-HT antagonists, notably ³H-ketanserin (Leysen et al., 1982).

More recently, evidence has accumulated in support of the heterogeneity of 5-HT₁ recognition sites. Thus, Pedigo et al. (1981) postulated the existence of 5-HT₁ₐ and 5-HT₁ᵦ subtypes as a possible explanation of the biphasic displacement by spiperone of ³H-5-HT specifically bound to rat cortical membranes. Support for this hypothesis appeared with the discovery that a new 5-HT agonist, 8-hydroxy-2-(di-N-propylamino)tetralin

(8-OH-DPAT), is, in fact, a selective ligand of 5-HT₁ₐ binding sites (Hall et al., 1985; Hamon et al., 1984b; Middlemiss and Fozard, 1983). Subsequent developments in the synthesis of radioactive ligands demonstrated that the situation is probably even more complex, since the use of a new 5-HT antagonist, ³H-mesulergine, allowed Pazos et al. (1984b) to characterize a third subtype of 5-HT₁ binding sites, called 5-HT₁c, that is particularly abundant in the choroid plexus.

Although numerous positive correlations have been observed between the pharmacological profiles of these various 5-HT₁ binding sites and the pharmacological characteristics of the 5-HT receptors mediating specific behaviors and biological responses (Blackburn et al., 1984; Engel et al., 1983, 1984; Lucki et al., 1984; Middlemiss, 1984; Shenker et al., 1985; Tricklebank et al., 1985), the problem is still far from being solved, and all investigators do not agree about the possible identity of 5-HT₁ binding sites with true 5-HT receptors (Leysen, 1984).

Since the presence of a given receptor in particular brain areas can be of great informative value [for instance, the presence of opiate receptors *within* and *outside* the brain regions involved in the transmission and control of nociceptive messages (see Wamsley et al., 1982), we have studied the detailed distribution of the 5-HT₁ₐ and 5-HT₁ᵦ subtypes in the rat brain, in an attempt to determine whether those binding sites were located, as expected, in the brain regions involved in the various 5-HT-elicited behaviors and biological responses. For this purpose we used the quantitative autoradiographic technique (Rainbow et al., 1982) applied to brain sections incubated with ³H-8-OH-DPAT or ³H-5-HT under conditions ensuring the selective labeling of 5-HT₁ₐ or 5-HT₁ᵦ sites. Furthermore, since data in the literature support that the presynaptic 5-HT autoreceptor controlling 5-HT release from serotoninergic terminals has the same pharmacological properties as the 5-HT₁ᵦ type (Engel et al., 1983; Middlemiss, 1984), we investigated whether some 5-HT₁ᵦ sites (and/or 5-HT₁ₐ sites) disappeared or were at least significantly reduced following selective degeneration of serotoninergic innervation by 5,7-dihydroxytryptamine (5,7-HT) intracerebral administration.

Materials and methods

Chemicals

Labeled compounds

³H-5-hydroxytryptamine creatinine sulfate (³H-5-HT, 12.5–13.8 Ci/mmol) was purchased from Amersham International plc (U.K.) and was purified just before use (Hamon et al., 1974). ³H-8-hydroxy-2-(di-N-propylamino)tetralin (³H-8-OH-DPAT, 95–125 Ci/mmol) was supplied by C.E.A. (France).

Other compounds.

5-Hydroxytryptamine creatinine sulfate (5-HT) was from Merck, pargyline from Sigma, fluoxetine from Eli Lilly and Co., lysergic acid di-

ethylamide (LSD) from Sandoz, guanosine 5'-triphosphate (GTP) from Boehringer-Mannheim, spiperone from Janssen Pharmaceutica, 5,7-dihydroxytryptamine (5,7-HT) from Regis, and reserpine and desmethylimipramine from Ciba-Geigy. Unlabeled 8-OH-DPAT was a gift from Prof. L. Pichat (C.E.A., France).

Animals and treatments

Reserpine

Male Wistar rats weighing 250–300 g were treated with reserpine (10 mg/kg, i.p.) 24 hr before being sacrificed. Control of the extensive 5-HT depletion due to the treatment was made by HPLC coupled to electrochemical detection of the indoleamine high-performance liquid chromatography in the striatum, hippocampus, and cerebral cortex, according to the procedure described in detail elsewhere (Hall et al., 1985).

5, 7-HT lesion

Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame. Thirty minutes after an i.p. injection of desmethylimipramine (25 mg/kg, i.p.), 5,7-HT (8 μg in 4 μl of isotonic saline containing 0.1% ascorbic acid) was slowly infused into the right serotoninergic bundle coming out the anterior raphe nuclei, as described by Nelson et al. (1978). After a 18 d recovery, rats were used for quantitative autoradiography (see below) or for biochemical control of the lesion: measurements of 5-HT levels (Hall et al., 1985) and tryptophan hydroxylase activity (see Hamon et al., 1981).

Autoradiography

Rats were anesthetized with chloral hydrate and then sacrificed by an intracardial perfusion with 500 ml of 0.15 M Sörensen buffer, pH 7.4, before removing the brain from the skull. This perfusion procedure allowed a marked decrease in the nonspecific binding of ³H-8-OH-DPAT or ³H-5-HT to brain sections, particularly onto the meninges (not shown). The brains were then frozen at −30°C using isopentane cooled by liquid nitrogen. Coronal sections (16 μm) were cut at −15°C in a cryostat, thaw-mounted onto gelatin-coated glass slides, and stored at −20°C until use (within less than 2 weeks).

The sections were labeled in vitro according to the procedure of Rainbow et al. (1982). Tissues were preincubated at 20°C for 30 min in 0.17 M Tris-HCl buffer, pH 7.6, and then incubated for 60 min in the same buffer supplemented with 0.01% ascorbic acid, 10 μM pargyline, 10 μM fluoxetine, and generally 2 nM of the appropriate tritiated ligand. Blanks were generated by adding 10 μM 5-HT to the incubation medium. In experiments designed to characterize the 5-HT₁ subsites, 0.1 μM 8-OH-DPAT, 1 μM spiroperidol, or 0.1 μM LSD was added to the incubation medium.

After incubation, washing (2 × 5 min in ice-cold buffer and quick dipping in distilled water), and drying with cold air, slices were put in tight contact with a sheet of tritium-sensitive film (³H-Ultrofilm, LKB, Sweden) and exposed in the dark at 4°C for 3 months (³H-5-HT) or 2 months (³H-8-OH-DPAT). Alternatively, sections were scraped off the slides and bound tritium was measured by liquid scintillation counting. This procedure has been used notably for the determination of the apparent K_d of ³H-8-OH-DPAT binding. In this case, brain sections were incubated with 7 different concentrations of the labeled ligand ranging between 0.20 and 8.5 nM.

Autoradiograms were developed in Kodak Microdol (10 min at 20°C), and their quantitative analysis was performed with a Quantimet 720 densitometer (Cambridge, U.K.). Results are expressed in optical density (O.D.) units. As already pointed out (Rainbow et al., 1984), the nonlinear response of LKB Ultrofilm to increasing amounts of radioactivity is often a limitation to the use of O.D. measurements. However, in the present case, control experiments carried out with ³H-standards showed that, in studies using ³H-5-HT, the measured O.D. was proportional to bound ³H:
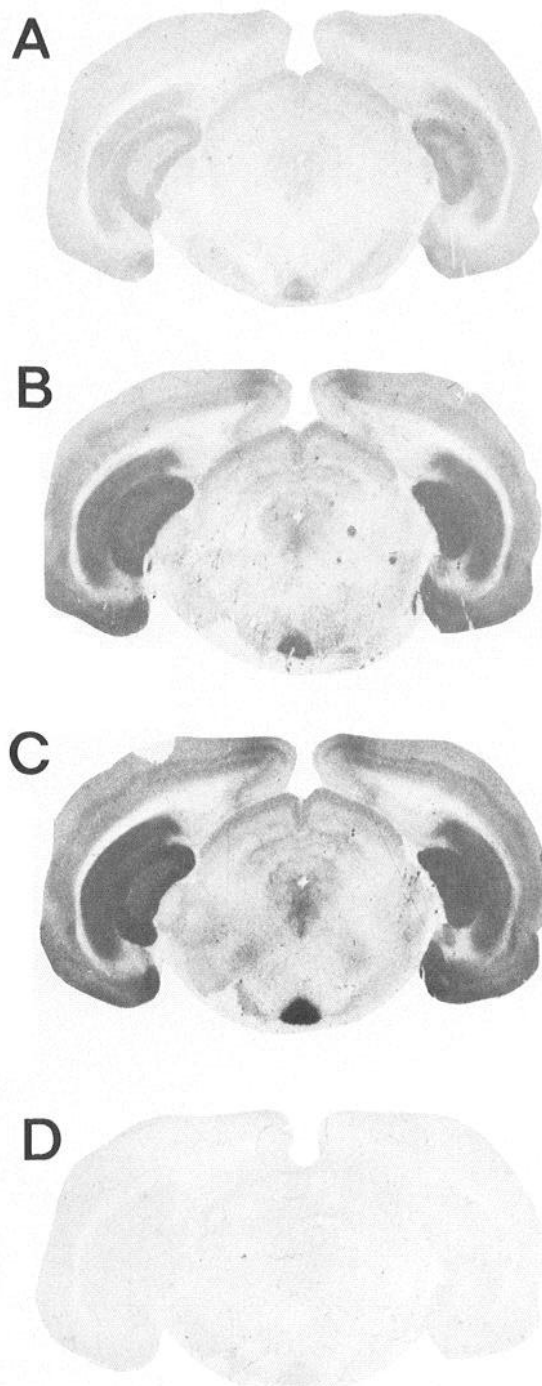
$$O.D. = 0.052 \times B^*$$

where B^* is bound ³H, expressed in nCi/mg protein.

Results

Characteristics of ³H-8-OH-DPAT binding to brain sections

The direct counting of ³H-8-OH-DPAT specifically bound to brain sections at the hippocampal level allowed the determi-

Figure 1. Effects of GTP or Mn²⁺ on ³H-8-OH-DPAT binding to rat brain sections. Coronal sections were taken at A = −6.3 mm from the bregma, according to the stereotaxic atlas of Paxinos and Watson (1982). *A,* ³H-8-OH-DPAT binding in the presence of 0.5 mM GTP. O.D. measurements indicated an average 72% reduction compared to autoradiogram *B. B,* Autoradiogram of ³H-8-OH-DPAT bound under standard assay conditions (see Materials and Methods). *C,* ³H-8-OH-DPAT binding in the presence of 2 mM MnCl₂. Compared to autoradiogram *B,* the mean O.D. increase due to Mn²⁺ reaches +27% in all the structures exhibiting specific ³H-8-OH-DPAT binding (hippocampus, dorsal raphe nucleus, interpeduncular nucleus, etc.). *D,* Nonspecific ³H-8-OH-DPAT binding in the presence of 10 μM 5-HT.
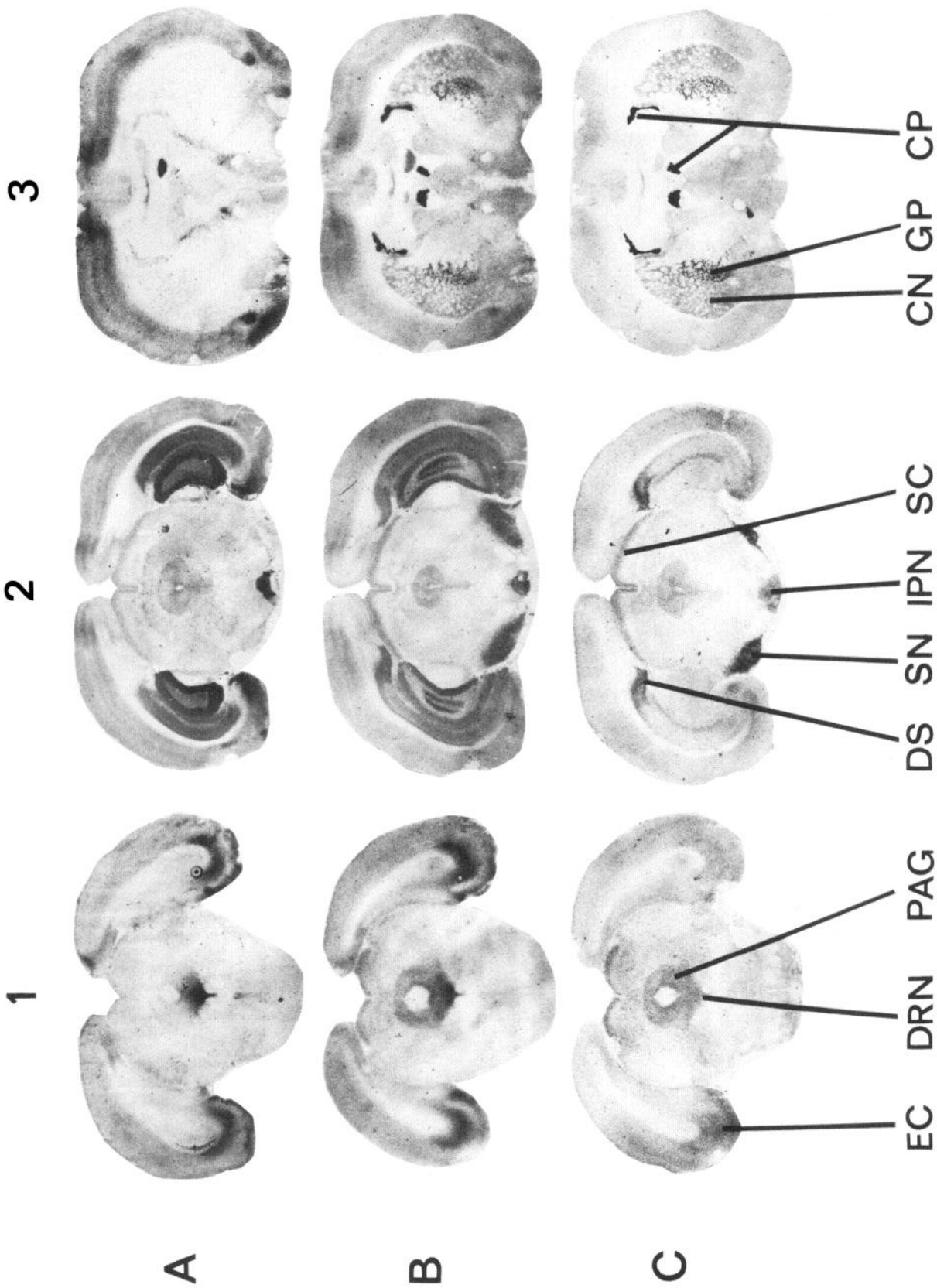
Figure 2. Autoradiographic evidence of 5-HT$_{1A}$ and 5-HT$_{1B}$ sites in the rat brain. Adjacent coronal sections taken at A = −7.6 mm (*1*), −5.8 mm (*2*), −1.3 mm (*3*) from the bregma (Paxinos and Watson, 1982) were incubated with 2 nM ^{3}H-8-OH-DPAT (*A*), 2 nM ^{3}H-5-HT (*B*), or 2 nM ^{3}H-5-HT plus 0.1 μM 8-OH-DPAT (*C*). Autoradiograms were obtained following an exposure of 2 (*A*) or 3 (*B, C*) months. Abbreviations: *CN*, caudate nucleus; *CP*, choroid plexus; *DRN*, dorsal raphe nucleus; *DS*, dorsal subiculum; *EC*, entorhinal cortex; *GP*, globus pallidus; *IPN*, interpeduncular nucleus; *PAG*, periaqueductal gray; *SN*, substantia nigra.

Table 1. Regional distribution of 5-HT$_{1A}$ binding sites in the rat brain

	Specific ^{3}H-binding to 5-HT$_{1A}$ sites (O.D.)		
Brain structure	^{3}H-8-OH-DPAT (5-HT, 10 μM)	^{3}H-5-HT (8-OH-DPAT, 0.1 μM)	^{3}H-5-HT (spiperone, 1 μM)
Dentate gyrus	0.293 ± 0.030	0.094 ± 0.012	0.092 ± 0.009
Lateral septum	0.286 ± 0.035	0.073 ± 0.011	0.069 ± 0.012
Dorsal raphe nucleus	0.261 ± 0.024	0.061 ± 0.009	0.064 ± 0.008
Entorhinal cortex	0.254 ± 0.028	0.057 ± 0.006	0.055 ± 0.008
Interpeduncular nucleus	0.226 ± 0.016	0.047 ± 0.004	0.044 ± 0.007
Hippocampal CA$_1$	0.209 ± 0.021	0.051 ± 0.006	0.054 ± 0.006
Central amygdala	0.157 ± 0.016	0.029 ± 0.004	0.032 ± 0.004
Ventral hypothalamus	0.130 ± 0.016	0.026 ± 0.004	0.034 ± 0.004
Dorsal subiculum	0.126 ± 0.014	0.013 ± 0.003	0.014 ± 0.003
Frontal cortex	0.124 ± 0.014	0.035 ± 0.005	0.036 ± 0.005
Hippocampal CA$_3$	0.112 ± 0.017	0.028 ± 0.004	0.028 ± 0.005
Superior colliculus	0.076 ± 0.005	0.015 ± 0.002	0.020 ± 0.004
Stria terminalis	0.070 ± 0.009	0.009 ± 0.003	0.009 ± 0.002
Parietal cortex	0.064 ± 0.009	0.012 ± 0.003	0.016 ± 0.003
Periaqueductal gray	0.057 ± 0.011	0.013 ± 0.005	0.021 ± 0.005
Dorsal hypothalamus	0.055 ± 0.014	0.011 ± 0.003	0.014 ± 0.003
Caudate-putamen	nd	nd	nd
Globus pallidus	nd	nd	nd
Substantia nigra	nd	nd	nd
Choroid plexus	nd	nd	nd

$$r_4 = 0.961 \qquad r_2 = 0.993$$
$$r_3 = 0.958$$

Measurements of optical density (O.D.) were made on autoradiographic films from tissue sections incubated with ^{3}H-8-OH-DPAT (2 nm) or ^{3}H-5-HT (2 nm). The ^{3}H-ligand binding (expressed as O.D. units) to 5-HT$_{1A}$ sites corresponded to total ^{3}H-8-OH-DPAT binding minus that persisting with 10 μM 5-HT (left-hand column) or to total ^{3}H-5-HT binding minus that found when 0.1 μM 8-OH-DPAT (middle column) or 1 μM spiperone (right-hand column) was included in the incubation mixture. Each value is the mean O.D. ± SEM of 24 measurements made in 4 rats for each assay condition.

r_1, Coefficient of the correlation between ^{3}H-8-OH-DPAT binding and the ^{3}H-5-HT binding component selectively inhibited by 0.1 μM 8-OH-DPAT.

r_2, Correlation coefficient between the O.D. due to ^{3}H-5-HT bound to 8-OH-DPAT (0.1 μM)-sensitive sites and the O.D. due to ^{3}H-5-HT bound to spiperone (1 μM)-sensitive sites.

r_3, Correlation coefficient between ^{3}H-8-OH-DPAT binding and the ^{3}H-5-HT binding component selectively inhibited by 1 μM spiperone.

nd, Not detectable.

nation of an apparent K_d of 1.5 nM, similar to that found previously using hippocampal membranes, $K_d = 2$–4 nM (Gozlan et al., 1983; Hall et al., 1985). As illustrated in Figure 1, GTP (0.5 mM) markedly reduced, whereas Mn^{2+} (2 mM MnCl$_2$) enhanced ^{3}H-8-OH-DPAT specific binding to most brain regions. In contrast, the nonspecific binding that persisted when 10 μM 5-HT was included in the incubation medium was unaffected by GTP or Mn^{2+}. Indeed, the nonspecific binding was extremely low under all assay conditions (Fig. 1).

Regional distribution of ^{3}H-8-OH-DPAT specific binding in rat brain: comparison with regional distribution of 8-OH-DPAT-sensitive ^{3}H-5-HT binding

Previous studies by our group (Marcinkiewicz et al., 1984) have shown that the localization of ^{3}H-8-OH-DPAT binding sites is not superimposed over that of ^{3}H-5-HT binding sites, notably in the substantia nigra, dorsal subiculum, choroid plexus, and striatum. Confirmation of these findings is shown in Figure 2. Thus, an intense labeling of these structures was obtained using ^{3}H-5-HT (Fig. 2B) but not ^{3}H-8-OH-DPAT (Fig. 2A) as the ligand.

Since ^{3}H-5-HT bound to all 5-HT$_1$ sites, whereas ^{3}H-8-OH-DPAT bound solely to 5-HT$_{1A}$ sites (Hamon et al., 1984b; Mid-

dlemiss and Fozard, 1983), attempts were made to dissociate ^{3}H-5-HT binding to 5-HT$_{1A}$ sites from that to "non-5-HT$_{1A}$" sites with an appropriate concentration of "cold" 8-OH-DPAT. As shown in Figure 2C, the addition of 0.1 μM 8-OH-DPAT to the incubation mixture resulted in a marked reduction of ^{3}H-5-HT binding in those regions exhibiting ^{3}H-8-OH-DPAT binding capacity such as the entorhinal cortex, dorsal raphe nucleus, and dentate gyrus. In contrast, ^{3}H-5-HT binding remained unaffected in the substantia nigra, dorsal subiculum, choroid plexus, and striatum (Fig. 2C). Since 0.1 μM 8-OH-DPAT has been shown to saturate 5-HT$_{1A}$ sites completely without altering ^{3}H-5-HT binding to 5-HT$_{1B}$ sites (Hamon et al., 1984b), the data illustrated in Figure 2 indicate that 5-HT$_{1A}$ sites could be identified either directly with ^{3}H-8-OH-DPAT as the ligand or indirectly as those where 0.1 μM 8-OH-DPAT inhibited ^{3}H-5-HT binding. Conversely, "non-5-HT$_{1A}$" sites could be identified as those labeled by ^{3}H-5-HT *in the presence* of 0.1 μM 8-OH-DPAT. Although these "non-5-HT$_{1A}$" sites probably correspond to both 5-HT$_{1B}$ and 5-HT$_{1C}$ sites (Pazos et al., 1984b), they are presently referred to as being mainly 5-HT$_{1B}$ sites, since recent extensive autoradiographic studies by Pazos and Palacios (1985) have shown that the density of 5-HT$_{1C}$ sites is low (usually less than 10%) compared to that of other 5-HT$_1$ sites in any region (except the choroid plexus) of the rat brain.

Table 2. Regional distribution of 5-HT$_{1B}$ binding sites in the rat brain

Brain structure	Specific ^{3}H-5-HT binding to 5-HT$_{1B}$ sites (O.D.)	
	^{3}H-5-HT + 0.1 μM 8-OH-DPAT	^{3}H-5-HT + 1 μM spiperone
Substantia nigra	0.132 ± 0.012	0.106 ± 0.017
Choroid plexus[a]	0.102 ± 0.018	0.082 ± 0.012
Dorsal subiculum	0.090 ± 0.019	0.082 ± 0.018
Globus pallidus	0.083 ± 0.014	0.069 ± 0.014
Entorhinal cortex	0.066 ± 0.003	0.068 ± 0.016
Interpeduncular nucleus	0.065 ± 0.011	0.067 ± 0.015
Periaqueductal gray	0.065 ± 0.009	0.054 ± 0.013
Superior colliculus	0.061 ± 0.012	0.056 ± 0.014
Dorsal raphe nucleus	0.060 ± 0.009	0.057 ± 0.010
Lateral septum	0.058 ± 0.010	0.055 ± 0.009
Ventral hypothalamus	0.057 ± 0.004	0.049 ± 0.011
Dorsal hypothalamus	0.054 ± 0.005	0.045 ± 0.010
Stria terminalis	0.052 ± 0.004	0.043 ± 0.009
Central amygdala	0.044 ± 0.006	0.037 ± 0.009
Caudate-putamen	0.041 ± 0.009	0.035 ± 0.009
Dentate gyrus	0.031 ± 0.005	0.033 ± 0.008
Parietal cortex	0.030 ± 0.006	0.026 ± 0.009
Frontal cortex	0.029 ± 0.005	0.028 ± 0.006
Hippocampal CA1	0.025 ± 0.003	0.019 ± 0.002
Hippocampal CA3	0.018 ± 0.004	0.018 ± 0.005

———— $r = 0.979$ ————

Measurements of optical density (O.D.) were made on autoradiographic films from tissue sections incubated with 2 nM ^{3}H-5-HT plus 0.1 μM 8-OH-DPAT or 1 μM spiperone. Each value is the mean O.D. ± SEM of 24 measurements in 4 rats for each assay condition.

r, Coefficient of the correlation between ^{3}H-5-HT binding in the presence of 0.1 μM 8-OH-DPAT and that in the presence of 1 μM spiperone.

[a] Although the choroid plexus is included in this series, the characteristics of ^{3}H-5-HT binding to this structure did not correspond exactly to those expected from authentic 5-HT$_{1B}$ sites (see Table 3).

Figure 3. Respective proportions of 5-HT$_{1A}$ and 5-HT$_{1B}$ binding sites in various brain areas. For each region examined, ^{3}H-5-HT binding to 5-HT$_{1A}$ (*left*) or 5-HT$_{1B}$ (*right*) sites is expressed as the percentage of total (specific) ^{3}H-5-HT binding (to 5-HT$_1$ sites). Each bar is the mean of 12–24 separate determinations. Variations were less than 5%. Abbreviations as in Figure 2.

Respective distributions of 5-HT$_{1A}$ and 5-HT$_{1B}$ sites in rat brain
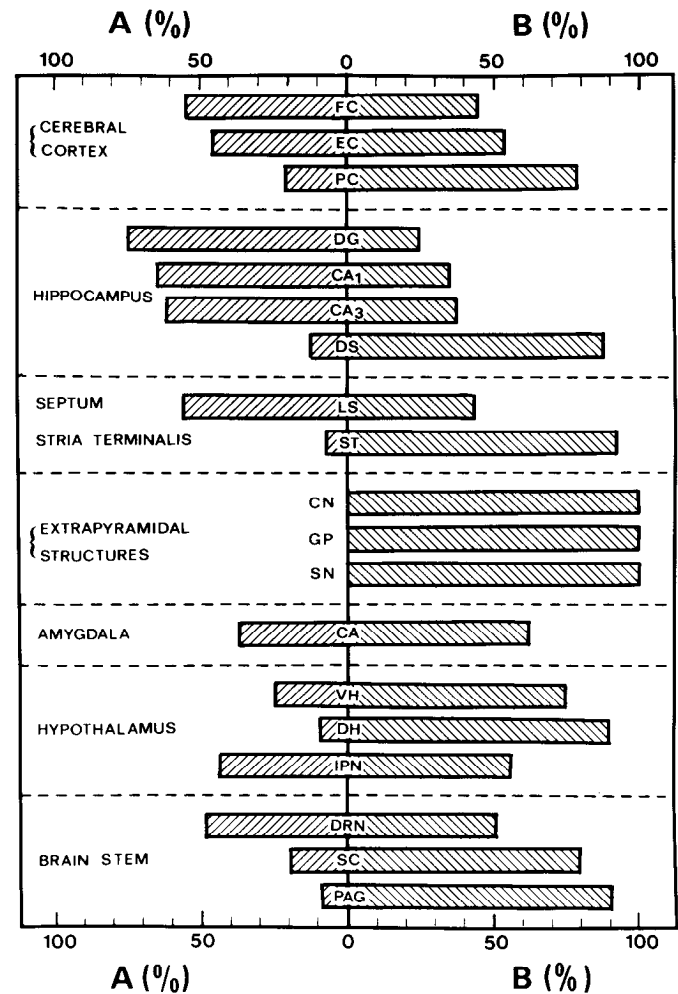
5-HT$_{1A}$ sites

Since 1 μM spiperone allows complete occupancy of 5-HT$_{1A}$ sites without interacting with 5-HT$_{1B}$ sites (Emerit et al., 1985; Middlemiss and Fozard, 1983; Pedigo et al., 1981), ^{3}H-5-HT binding susceptible to inhibition by 1 μM spiperone was also studied and compared with both ^{3}H-5-HT binding sensitive to 0.1 μM 8-OH-DPAT and ^{3}H-8-OH-DPAT binding in various brain regions.

As shown in Table 1, the regional distributions of specific 5-HT$_{1A}$ sites identified by these 3 methods were highly correlated ($r \geq 0.95$). 5-HT$_{1A}$ binding sites were particularly abundant in the hippocampus (dentate gyrus > CA$_1$ >> CA$_3$), lateral septum, dorsal raphe nucleus, interpeduncular nucleus, and entorhinal cortex. No 5-HT$_{1A}$ binding sites could be detected in the substantia nigra, globus pallidus, caudate-putamen, and choroid plexus, and a low to moderate density of 5-HT$_{1A}$ binding sites was found in the other structures examined (Table 1).

5-HT$_{1B}$ sites

As indicated in Table 2, the regional distributions of specific sites labeled by ^{3}H-5-HT in the presence of 0.1 μM 8-OH-DPAT or 1 μM spiperone were very similar ($r = 0.98$). Since these sites corresponded mainly (if not exclusively, see above) to 5-HT$_{1B}$

sites, it can be inferred that the highest density of 5-HT$_{1B}$ binding sites was found in the substantia nigra, followed by the dorsal subiculum and globus pallidus. Very low densities of 5-HT$_{1B}$ sites were detected in the hippocampus (gyrus dentatus > CA$_1$ ≥ CA$_3$) and frontal and parietal cortex, and a fairly constant moderate density of 5-HT$_{1B}$ sites was found in the other brain areas examined.

Respective proportions of 5-HT$_{1A}$ and 5-HT$_{1B}$ sites in various brain regions

Of the 19 regions examined, only 3 exhibited an apparently homogeneous population of 5-HT$_1$ sites, namely, the caudate-putamen, globus pallidus, and substantia nigra, which contained exclusively a "non-5-HT$_{1A}$" receptor type, probably the 5-HT$_{1B}$ type (Fig. 3). A higher proportion of 5-HT$_{1B}$ sites compared to 5-HT$_{1A}$ sites occurred in the parietal cortex, dorsal subiculum, stria terminalis, central amygdala, hypothalamic areas, superior colliculi, and periaqueductal gray. In contrast, hippocampal areas (dentate gyrus, CA$_1$, CA$_3$) contained more 5-HT$_{1A}$ sites than 5-HT$_{1B}$ sites, and other regions (cerebral cortex, lateral septum, interpeduncular nucleus, dorsal raphe nucleus) had approximately similar proportions of the 2 binding sites (Fig. 3).

Table 3. Effects of LSD on specific ³H-5-HT binding to brain regions enriched in 5-HT$_{1A}$ sites or 5-HT$_{1B}$ sites and to the choroid plexus

Structures	Specific ³H-5-HT binding (O.D. units)	
	−	+0.1 μM LSD (%)
Brain regions enriched in 5-HT$_{1A}$ sites		
Lateral septum	0.130 ± 0.009	0.019 ± 0.006 (15)
Dentate gyrus	0.124 ± 0.008	0.008 ± 0.003 (6)
Dorsal raphe nucleus	0.118 ± 0.012	0.013 ± 0.002 (11)
Brain regions enriched in 5-HT$_{1B}$ sites		
Substantia nigra	0.128 ± 0.009	0.056 ± 0.008 (44)
Dorsal subiculum	0.102 ± 0.006	0.056 ± 0.006 (55)
Globus pallidus	0.089 ± 0.009	0.047 ± 0.004 (53)
Choroid plexus	0.104 ± 0.014	0.016 ± 0.005 (15)

Adjacent coronal sections were incubated with 2 nM ³H-5-HT in the absence or presence of 0.1 μM LSD. Autoradiograms were analyzed by microdensitometry, and the results are the means ± SEM of O.D. measurements in 6–10 sections from 2–4 rats. Background (incubations in the presence of 10 μM 5-HT) was subtracted from all values.

Figures in parentheses are the percentages of specifically bound ³H-5-HT (in O.D. units) that persisted in the presence of 0.1 μM LSD compared to respective controls.

The case of the choroid plexus

Since ³H-8-OH-DPAT did not bind to the choroid plexus (Table 1), whereas ³H-5-HT gave an intense labeling that was reduced neither by 0.1 μM 8-OH-DPAT nor by 1 μM spiperone (Table 2), it may be that 5-HT₁ sites in the choroid plexus belonged to the 5-HT$_{1B}$ type. However, experiments with "cold" LSD (0.1 μM) as the displacing agent suggested that the situation was probably more complex.

Using ³H-8-OH-DPAT as the ligand, almost no binding (<10% of control) persisted in any brain area examined when 0.1 μM LSD was added to the incubation medium (not shown). In contrast, the inhibitory effect of LSD on ³H-5-HT binding exhibited marked regional differences. Almost complete inhibition was observed in areas containing mostly 5-HT$_{1A}$ sites (Table 3) whereas approximately 50% inhibition took place in brain regions enriched in 5-HT$_{1B}$ sites (Table 3). Surprisingly, 0.1 μM LSD markedly inhibited (−85%) ³H-5-HT binding to specific sites in the choroid plexus (Table 3), where, however, neither 8-OH-DPAT nor spiperone bound, as expected for 5-HT$_{1B}$ sites.

Effects of reserpine treatment or 5,7-HT-induced lesion on ³H-8-OH-DPAT and ³H-5-HT specific binding to 5-HT$_{1A}$ and 5-HT$_{1B}$ sites in various brain regions

Twenty-four hours after the i.p. administration of reserpine (10 mg/kg), 5-HT levels in the cerebral cortex, hippocampus, and striatum were less than 5% of those found in control rats (0.38 ± 0.04, 0.45 ± 0.03, and 0.43 ± 0.04 μg/g, respectively; n = 6 in each case). However, O.D. measurements on autoradiographic films from tissue sections incubated with 2 nM ³H-8-OH-DPAT or ³H-5-HT did not reveal any significant difference between control and reserpine-treated rats in the 20 areas examined (data not shown).

The intracerebral injection of 5,7-HT (8 μg) resulted in a 80–90% reduction in tryptophan hydroxylase activity and 5-HT levels in the hippocampus and cerebral cortex on the injected (right) side. A marked decrease (−70 to −80%) in these 2 serotoninergic markers was also noted in the contralateral hippocampus and cerebral cortex. Tryptophan hydroxylase activity and 5-HT levels were also diminished in both striata (right, −72%; left, −60%) and in the anterior raphe area (−52%) of 5,7-HT-lesioned rats. Autoradiograms obtained after incubation of brain slices with either ³H-5-HT alone (for all 5-HT₁

Table 4. ³H-5-HT and ³H-8-OH-DPAT binding to the substantia nigra and dorsal raphe nucleus of control or 5,7-HT-treated rats

Structures	Specific ³H-ligand binding (O.D. units)		
	³H-5-HT		
	−	+0.1 μM 8-OH-DPAT	³H-8-OH-DPAT
Dorsal raphe nucleus			
Control	0.120 ± 0.014	0.061 ± 0.007	0.267 ± 0.032
5,7-HT	0.064[a] ± 0.010	0.054 ± 0.010	0.106[a] ± 0.013
	(−47%)	(−11%)	(−60%)
Substantia nigra			
Control	0.118 ± 0.010	0.132 ± 0.012	−
5,7-HT	0.091[a] ± 0.010	0.083[a] ± 0.014	−
	(−23%)	(−37%)	

Optical densities were measured on autoradiographic films from tissue sections incubated with 2 nM ³H-5-HT or 2 nM ³H-8-OH-DPAT for the selective labeling of all 5-HT₁ sites (with ³H-5-HT alone, left-hand column), "non-5-HT$_{1A}$" sites, i.e., mainly 5-HT$_{1B}$ sites (with ³H-5-HT plus 0.1 μM 8-OH-DPAT, middle column), or 5-HT$_{1A}$ sites (with ³H-8-OH-DPAT, right-hand column). Results are the means ± SEM of O.D. measurements in 12–24 sections from 4 rats in each group. Background was subtracted from all values.

Figures in parentheses are the percentage reductions due to 5,7-HT treatment.

[a] p < 0.05 compared to the respective control values (Student's t test).

sites), ³H-5-HT in the presence of 0.1 μM 8-OH-DPAT (for "non-5-HT$_{1A}$" sites), or ³H-8-OH-DPAT (for 5-HT$_{1A}$ sites) revealed that the 5,7-HT lesion affected none of these binding sites in most areas studied. However, 2 exceptions were noted: the dorsal raphe nucleus and the substantia nigra (Table 4). A marked decrease in ³H-8-OH-DPAT and ³H-5-HT binding was noted in the dorsal raphe nucleus of 5,7-HT-treated rats (Fig. 4, Table 4). However, when ³H-5-HT binding assays were performed in the presence of 0.1 μM 8-OH-DPAT, no significant difference could be detected between control and lesioned animals (Fig. 4, Table 4). Similarly, ³H-5-HT binding in the presence of 1 μM spiperone remained unaltered in 5,7-HT-treated rats compared to control animals (not shown).

The second exception concerned the substantia nigra, where a significant reduction of ³H-5-HT binding (to "non-5-HT$_{1A}$," i.e., to 5-HT$_{1B}$ sites) was found bilaterally in 5,7-HT-lesioned rats (Table 4).

Discussion

Numerous investigations have shown that quantitative autoradiography is a reliable technique with which to study the binding of labeled ligands to specific recognition sites in tissues, especially in the gray matter regions within the CNS (Pazos and Palacios, 1985; Pazos et al., 1984a; Rainbow et al., 1982, 1984). The present study further confirms this conclusion, since application of the autoradiographic technique to examination of the properties of ³H-8-OH-DPAT binding to 5-HT$_{1A}$ sites in brain gave the same results as those previously found using brain membranes. In particular, we observed that these 5-HT$_{1A}$ binding sites have a K_d of 1.5 nM and are modulated in opposite directions by GTP and Mn²⁺, in agreement with recent observations by Hall et al. (1985). However, binding studies with membranes allowed the detection of another class of specific sites for ³H-8-OH-DPAT, particularly in the striatum (Gozlan et al., 1983; Hall et al., 1985), where we failed to find any specific binding of this ligand using the present autoradiographic approach. Indeed, striatal sites have a lower affinity (K_d ~ 10 nM) than 5-HT$_{1A}$ sites for ³H-8-OH-DPAT, and the dissociation of this ligand is much faster from the striatal sites (Hall et al., 1985). Therefore, the present incubation conditions were not appropriate for the specific labeling of striatal sites by ³H-8-OH-
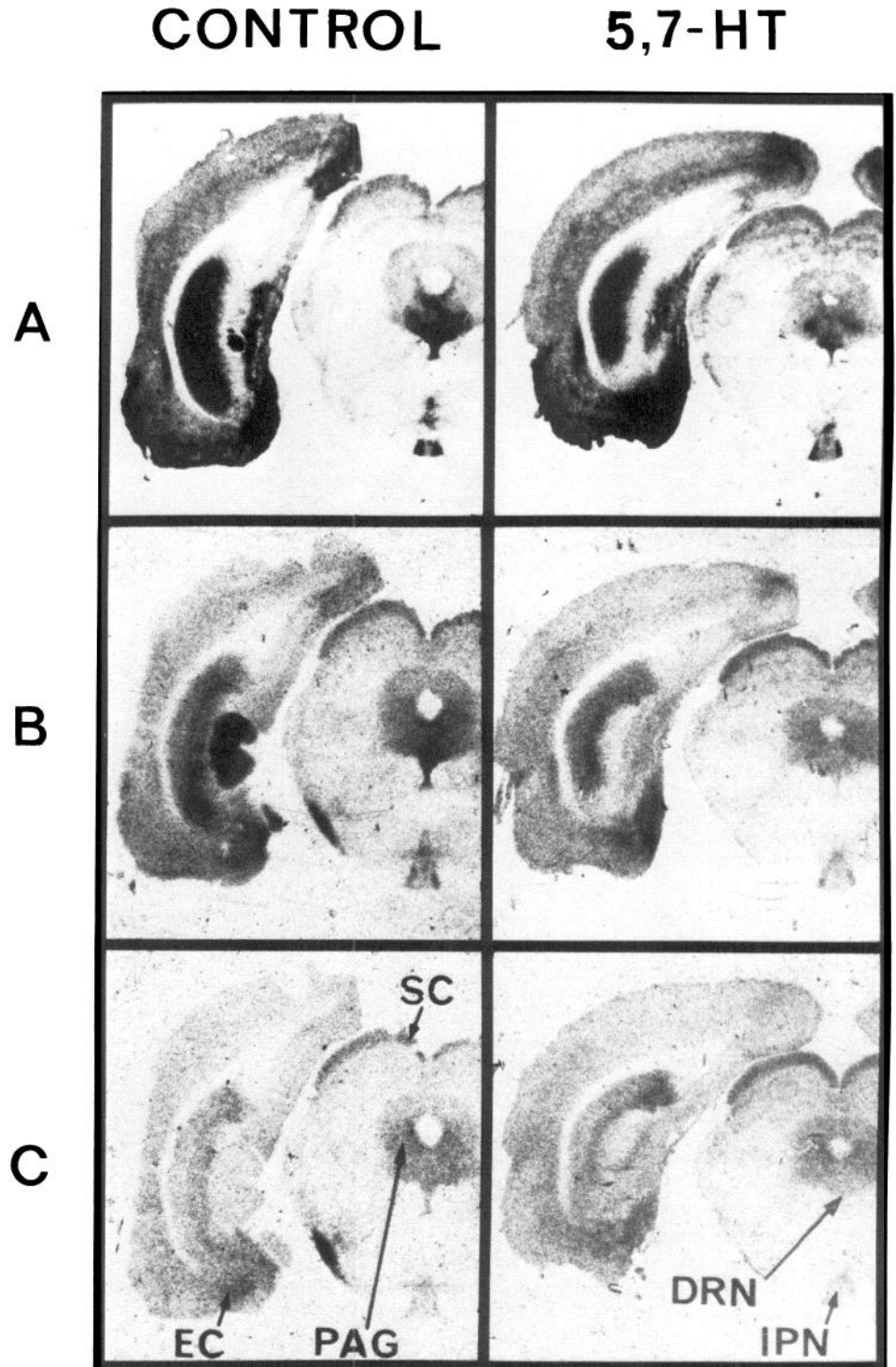
CONTROL   5,7-HT

Figure 4. Autoradiograms of ³H-8-OH-DPAT and ³H-5-HT binding to the dorsal raphe nucleus of a control or a 5,7-HT-treated rat. Coronal sections were taken at A = −7.0 mm from the bregma (Paxinos and Watson, 1982). *A,* ³H-8-OH-DPAT binding. *B,* ³H-5-HT binding. *C,* ³H-5-HT binding in the presence of 0.1 μM 8-OH-DPAT. Abbreviations as in Figure 2. Similar results were obtained in 4 rats in each group.

DPAT. Attempts to reveal ³H-8-OH-DPAT striatal sites using higher concentrations of the labeled ligand (8–12 nM) and less extensive washing procedure were largely unsuccessful due to the high level of nonspecific binding found on autoradiographic films under such conditions (unpublished observations).

Detailed microdensitometric analysis of the brain regions, where bound ³H-5-HT could be displaced by 0.1 μM 8-OH-DPAT or 1 μM spiperone, indicated that they superimposed exactly with those selectively labeled by nanomolar concentrations of ³H-8-OH-DPAT. Since previous studies using brain membranes (Hall et al., 1985; Hamon et al., 1984b; Middlemiss and Fozard,

1983) demonstrated that the ³H-5-HT binding component that can be displaced by 0.1 μM 8-OH-DPAT corresponds in fact to the 5-HT$_{1A}$ sites previously defined by Nelson et al. (1980), the present observations confirm that ³H-8-OH-DPAT can be used directly for the selective labeling of these sites in brain sections (see Marcinkiewicz et al., 1984; Pazos and Palacios, 1985; Vergé et al., 1985). Conversely, ³H-5-HT binding, which persisted in the presence of 0.1 μM 8-OH-DPAT, involved the second main class of 5-HT$_1$ sites, i.e., the 5-HT$_{1B}$ type (Nelson et al., 1980). Some contribution of 5-HT$_{1C}$ sites was also probable under this condition, but the density of 5-HT$_{1C}$ sites was so low compared

to that of 5-HT$_{1B}$ sites in most brain regions (Pazos and Palacios, 1985) that these "non-5-HT$_{1A}$" sites in fact corresponded (almost exclusively) to 5-HT$_{1B}$ sites. In agreement with this conclusion, examination of the displacement by 0.1 μM LSD of ^{3}H-5-HT specifically bound to "non-5-HT$_{1A}$" sites revealed that, except in the choroid plexus where 5-HT$_{1C}$ sites exist (Pazos and Palacios, 1985), LSD action in all brain areas considered resembled that found in the globus pallidus (Table 3 and data not shown), where 5-HT$_{1B}$ sites predominate (Pazos and Palacios, 1985).

Owing to the selective labeling of 5-HT$_{1A}$ sites by ^{3}H-8-OH-DPAT and of 5-HT$_{1B}$ sites by ^{3}H-5-HT in the presence of 0.1 μM 8-OH-DPAT (or 1 μM spiperone), the respective distributions of these 2 binding classes could be examined in detail. In general, 5-HT$_{1A}$ sites are less widely distributed than the 5-HT$_{1B}$ sites, and only a few areas contain more 5-HT$_{1A}$ than 5-HT$_{1B}$ sites: hippocampus, lateral septum, frontal cortex; however, none of these can be considered as a "pure" region with regard to the 5-HT$_{1A}$ binding class. In contrast, several areas, notably the substantia nigra, globus pallidus, and caudate-putamen, contain only "non-5-HT$_{1A}$", i.e., 5-HT$_{1B}$ sites. The choroid plexus exhibited no ^{3}H-8-OH-DPAT binding but was intensely labeled by ^{3}H-5-HT (in the absence or presence of 0.1 μM 8-OH-DPAT), as expected for a structure containing only 5-HT$_{1B}$ sites. However, further observations indicated that the properties of 5-HT$_1$ sites in the choroid plexus are distinct from those of 5-HT$_{1B}$ sites in brain tissues. Thus, using LSD (0.1 μM) as a displacing drug, we noted that ^{3}H-5-HT binding was almost completely inhibited in regions containing mostly 5-HT$_{1A}$ sites but only partially in those enriched in 5-HT$_{1B}$ sites. Therefore, LSD can be considered as a ligand acting preferentially at 5-HT$_{1A}$ sites. Surprisingly, LSD (0.1 μM) also suppressed ^{3}H-5-HT binding to choroid plexus, in contrast to that expected from the existence of 5-HT$_{1B}$ sites in this structure. Accordingly, the 5-HT$_1$ sites labeled by ^{3}H-5-HT in the choroid plexus probably correspond neither to 5-HT$_{1A}$ nor to 5-HT$_{1B}$ sites. These results support the recent findings of Pazos et al., who proposed calling these binding sites 5-HT$_{1C}$ (Pazos et al., 1984b; Pazos and Palacios, 1985).

The preferential location of 5-HT$_{1A}$ binding sites in limbic regions (hippocampus, septum, etc.) might suggest that the selective 5-HT$_{1A}$ agonist 8-OH-DPAT would influence behaviors depending on the "limbic brain" (Gray, 1982). Indeed, anxiolytic (-like) (Engel et al., 1984) and antidepressant (-like) (Curzon et al., 1985) effects of 8-OH-DPAT have been described in rats. Furthermore, new anxiolytic drugs such as buspirone and TVX Q 7821 (isapirone) have recently been shown to act in the nanomolar range on 5-HT$_{1A}$ binding sites in the hippocampus (Glaser and Traber, 1983; Glaser et al., 1985; Gozlan et al., 1983). It is therefore highly probable that 5-HT$_{1A}$ binding sites in limbic regions participate in the regulation of behaviors such as those associated to affective experience and emotion (see Trimble and Zarifian, 1984).

In contrast, 5-HT$_{1B}$ sites are particularly concentrated in the extrapyramidal areas (caudate-putamen, globus pallidus, etc.) involved in the control of motor function (Dray, 1979). Interestingly, a marked hyperlocomotion is usually observed following the administration of RU 24969 (Hunt and Oberlander, 1981), a 5-HT agonist possibly acting preferentially on 5-HT$_{1B}$ sites (Doods et al., 1985; Sills et al., 1984). However, 5-HT$_{1B}$ sites also exist outside extrapyramidal areas (see Fig. 3), and probably participate in the control of other central functions in addition to motor function. Similarly, 5-HT$_{1A}$ sites are found not only in limbic areas, but also in extralimbic regions. Therefore, it would be an obvious oversimplification to consider that 5-HT$_{1A}$ sites are exclusively involved in the control of specific behaviors associated with emotion and affect.

A well-known function of some 5-HT receptors, namely those located presynaptically on serotoninergic cell bodies and/or den-

drites (Aghajanian, 1981; De Montigny et al., 1984) and terminals (Göthert, 1980), consists of triggering feedback mechanisms that exert a negative influence on the nerve impulse flow and the neurotransmitter release process in these neurons. Recently, Engel et al. (1983) and Middlemiss (1984) proposed that the presynaptic autoreceptors located on serotoninergic terminals may correspond to 5-HT$_{1B}$ sites, and direct demonstration of this hypothesis has been attempted in the present study by examining the fate of 5-HT$_{1B}$ (and 5-HT$_{1A}$) sites following the selective degeneration of serotoninergic neurons in 5,7-HT-treated rats. However, the only region where ^{3}H-5-HT binding to 5-HT$_{1B}$ sites was significantly reduced following the lesion was the substantia nigra. This change could not be ascribed to some artifactual alteration due to the disappearance of endogenous 5-HT in this structure, since the marked 5-HT depletion following reserpine treatment was not associated with any modification of ^{3}H-8-OH-DPAT or ^{3}H-5-HT binding to 5-HT$_{1A}$ and 5-HT$_{1B}$ sites in all regions examined. Therefore, the loss of 5-HT$_{1B}$ sites in the substantia nigra was probably the consequence of the local degeneration of serotoninergic terminals. Whether such sites correspond to the presynaptic autoreceptors (Engel et al., 1983; Middlemiss, 1984) is, however, far from being proved, as no loss of 5-HT$_{1B}$ sites occurred in such other regions as the striatum, hypothalamus, and cerebral cortex, where presynaptic autoreceptors controlling 5-HT release have been found (see Hamon et al., 1984a, b). It is therefore more likely that 5-HT autoreceptors located on presynaptic serotoninergic terminals are neither 5-HT$_{1A}$ nor 5-HT$_{1B}$ sites but correspond to other sites with distinct pharmacological properties (see Gozlan et al., 1983). The situation was much clearer at the level of serotoninergic cell bodies and/or dendrites within the dorsal raphe nucleus, since a marked reduction of ^{3}H-8-OH-DPAT or ^{3}H-5-HT binding to 5-HT$_{1A}$ sites was observed following intracerebral administration of 5,7-HT. Since this treatment induced a parallel local decrease in tryptophan hydroxylase activity, it can be postulated that the 5-HT$_{1A}$ binding sites that disappeared were associated with tryptophan hydroxylase-containing neurons, i.e., corresponded to the presynaptic 5-HT autoreceptors located on serotoninergic cell bodies and/or dendrites. Interestingly, in addition to 5-HT itself, which is released locally, very probably from 5-HT cell bodies and dendrites (Bourgoin et al., 1981; Héry et al., 1986), 5-HT$_{1A}$ agonists are especially active on these presynaptic autoreceptors and trigger a marked reduction of the nerve impulse flow within serotoninergic raphe neurons (De Montigny et al., 1984; Eison et al., 1984; Sprouse and Aghajanian, 1985). Therefore, these reported pharmacological observations, as well as the present anatomical evidence, support the contention that the 5-HT autoreceptors located on cell bodies and/or dendrites of serotoninergic raphe neurons are of the 5-HT$_{1A}$ type.

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