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Autoradiographic visualisation of [^{3}H]5-carboxamidotryptamine binding sites in the guinea pig and rat brain

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Abstract

We have investigated the distribution of [^{3}H]5-carboxamidotryptamine ([^{3}H]5-CT) binding sites by in vitro autoradiography on sections of guinea-pig and rat brain. In saturation studies, the ligand recognised a saturable, homogeneous population of binding sites with an affinity ranging from 0.19–0.45 nM depending on the region. The labelling pattern was heterogeneous, and the displacement pattern with different competing drugs selective for different 5-HT receptor subtypes was complex. [^{3}H]5-CT appeared to label 5-HT$_{1B}$/5-HT$_{1D}$ sites in the substantia nigra, globus pallidus and caudate/putamen, as the binding in these regions was displaced by the 5-HT$_{1B/1D}$ receptor selective agents sumatriptan, CP-122,288 and GR-127,935. In the hippocampus and lateral septum, the very dense [^{3}H]5-CT binding was displaced with high affinity by the 5-HT$_{1A}$ receptor selective agonist 8-hydroxy-dipropylaminotetralin ((±)-8-OH-DPAT), dihydroergotamine and 5-HT. In contrast the affinity of the 5-HT$_1$ receptor antagonists spiperone and methiothepine was much lower than their previously published potency at 5-HT$_{1A}$ receptors. The affinity of agonists, taken together with the fact that the distribution of these [^{3}H]5-CT sites overlaps that of [^{3}H]8-OH-DPAT binding sites in serial sections, suggest that these sites correspond to 5-HT$_{1A}$ receptors. Their atypical properties deserve further investigations. While [^{3}H]5-CT binding at 5-HT$_{1B/1D}$ sites and these atypical 5-HT$_{1A}$ sites was inhibited by the GTP analogue 5′-β,γ-imidotriphosphate, [^{3}H]5-CT binding in the superficial cortical layers and in midline thalamic nuclei was insensitive to this agent. It was however displaced by low concentrations of spiperone, clozapine and methiothepine, but not by sumatriptan, CP-122,288, GR-127,935 or dihydroergotamine. This binding profile is similar to that of 5-HT$_7$ receptors, while the spatial distribution of these sites matches the known distribution of 5-HT$_7$ messenger RNA. We did not find evidence of [^{3}H]5-CT labelling to 5-HT$_5$ receptors, in spite of their reported high affinity for this ligand. It is concluded that [^{3}H]5-CT, in the presence of selective blockers, can be used to investigate the properties of 5-HT$_{1A}$, 5-HT$_{1B/1D}$ and 5-HT$_7$ receptors in the rodent brain, although further studies are required to explain the atypical features of [^{3}H]5-CT binding in 5-HT$_{1A}$ receptors containing regions.

Keywords: 5-HT$_{1D}$ receptor; 5-HT$_{1A}$ receptor; 5-HT$_7$ receptor

1. Introduction

The first evidence that the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) exerts its various physiological actions by activating multiple subtypes of receptors was deduced from functional studies (Gaddum and Picarelli, 1957). Two decades later, the introduction of radioligand binding techniques to study the pharmacological profile receptors resulted in the detection of different binding sites for 5-HT in the central nervous system (Peroutka and Snyder, 1979). A first classification was established in an attempt to reconcile functional data with the results of binding studies (Bradley et al., 1986). This classification recognised three different classes of receptors, termed 5-HT$_1$, 5-HT$_2$ and 5-HT$_3$. One of the criteria used to identify 5-HT$_1$ receptors was that the agonist 5-carboxamidotryptamine (5-CT) should be more or equally potent than the endogenous transmitter 5-HT in functional or binding experiments. During the last 6 years, the introduction of molecular biology techniques has resulted in the cloning and sequencing of 14 different subtypes (Branchek, 1993). While some of them were

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already known from previous studies, about one-half is still lacking an established functional correlate. A modified classification has recently been agreed upon, which takes into account the signal transduction systems used by the receptors, as well as the sequence homologies existing between them (Hoyer et al., 1994). With the exception of 5-HT_3 receptors, which form an ion channel, all the known subtypes belong to the superfamily of receptors coupled to GTP-binding proteins. The 5-HT_1 class (5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1B/1D\beta}$, 5-HT_{1E}, 5-HT_{1F}) is linked to adenylate cyclase inhibition. 5-HT_2 receptors (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}) increase phosphatidylinositide turnover. 5-HT_4, 5-HT_6 and 5-HT_7 receptors stimulate adenylate cyclase. The second messenger system used by 5-HT_{5A} and 5-HT_{5B} is unknown.

The advantage of radioligand binding studies is their ability to define a detailed pharmacological profile for a receptor subtype. In vitro autoradiography combines these techniques with the possibility to visualise the regional distribution of binding sites with a specific pharmacological profile. This feature has been instrumental in the characterisation of novel 5-HT receptor subtypes (Pazos et al., 1984,1985). Ligand binding autoradiography can also be used to determine which brain structures contain the binding sites for the corresponding recently cloned genes. In particular, the brain distribution of 5-HT_7 receptors has not been reported. These sites have been labelled in transfected cells using [^{3}H]5-HT (Bard et al., 1993; Ruat et al., 1993; Tsou et al., 1994), [^{125}I]LSD (Lovenberg et al., 1993), [^{3}H]LSD and [^{3}H]spiperone (Meyerhof et al., 1993), and they also display a nanomolar affinity for 5-CT. While being more selective than [^{3}H]5-HT and [^{125}I]LSD, which have a nanomolar affinity for most 5-HT receptor subtypes, 5-CT also recognises with high affinity 5-HT_{1A}, 5-$HT_{1B/1D\beta}$ and 5-$HT_{1D\alpha}$ binding sites in the brain. In addition, 5-CT is a potent agonist at several vascular 5-HT_1-like receptors which do not fully conform to the presently recognised subtypes and might thus represent new subtypes (see Hoyer et al., 1994). Finally, 5-CT has been reported to be extremely potent at putative prejunctional receptors inhibiting neuropeptide release from trigemino-vascular terminals (Buzzi et al., 1991), suggesting the existence of a new receptor subtype (Lee and Moskowitz, 1993).

Radiolabeled 5-CT can thus be expected to label multiple populations of sites (see Mahle et al., 1991), including presently uncharacterized receptors. The higher potency of 5-CT in functional models (see above) and its higher affinity for some binding sites (including 5-HT_{1A}, 5-$HT_{1B/1D}$ and 5-HT_7) suggest that [^{3}H]5-CT might be a better radioligand than [^{3}H]5-HT. The low affinity of 5-CT for 5-HT_{2C} and 5-HT_6 receptors also indicates that the binding profile of [^{3}H]5-CT might be less complex (see Hoyer et al., 1994). In the present study, we have used tritiated 5-carboxamidotryptamine ([^{3}H]5-CT) in autoradiographic studies in order to determine the distribution of its binding sites in the guinea-pig and rat brain. In order to characterise the binding profile of [^{3}H]5-CT binding sites in different brain areas, we have also performed a series of competition studies using drugs showing some selectivity for the expected populations of [^{3}H]5-CT binding sites: (±)8-hydroxydipropylaminotetralin (8-OH-DPAT; 5-HT_{1A}), sumatriptan, CP-122,288 (5-methyl-aminosulfonylmethyl-3-(*N*-methylpyrrolidin-2*R*-yl-methyl)-1*H*-indole), GR-127,935 (2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazine-1-yl)-phenyl]-amide) (5-$HT_{1B/1D}$), dihydroergotamine (5-HT_{1A}, 5-$HT_{1B/1D}$), spiperone (5-HT_{1A}, 5-HT_7), clozapine, (+)-butaclamol (5-HT_7), methiothepin (non-selective 5-HT_1 antagonist).

2. Materials and methods

2.1. Materials

[^{3}H]5-CT was obtained from NEN (Boston, MA, USA) at a specific activity of 22.8 Ci/mmol, [^{3}H]8-OH-DPAT was obtained from Amersham (Arlington Heights, IL, USA) at a specific activity of 205 Ci/mmol. GR-127,935 and sumatriptan were provided by Glaxo. CP-122,288 was obtained from Pfizer. 5′-β,γ-imidotriphosphate was purchased from Sigma (St. Louis, MO, USA). All other drugs were obtained from RBI (Natick, MA, USA).

2.2. Autoradiography

Adult male rats (Sprague-Dawley, 200–250 g) and adult guinea-pigs (Hartley, 250–350 g) were sedated with inhaled chloroform and decapitated. The whole brain and upper cervical spinal cord was dissected and frozen over crushed dry ice. Frozen brains were sectioned (10 μm) using a cryostat-microtome (Leitz 1720, Leica, Deerfield, IL, USA). The sections were thaw-mounted onto gelatinised glass slides and stored at −25°C. [^{3}H]5-CT and [^{3}H]8-OH-DPAT binding sites were labelled as previously described for [^{3}H]5-HT (Waeber et al., 1989). Except in saturation studies, the concentration of the radioligands was 1 nM. In saturation studies, [^{3}H]5-CT was used at 0.08, 0.19, 0.36, 0.67, 1.33, 2.84 and 6.5 nM. Exposure times were respectively 3 weeks and 6 weeks for [^{3}H]8-OH-DPAT and [^{3}H]5-CT. Competition studies were performed by adding increasing concentrations of the displacers to the incubation medium of consecutive sections. Non-specific binding was defined by the addition of 10 μM 5-HT. The optical density of the autoradiograms over selected brain regions was measured using a comput-

erised image analysis system (M4, Imaging Research, St Catherines, Ontario, Canada). The system converted the optical density values to 'nCi/mg of tissue equivalent' after calibration with images of Amersham (Arlington Heights, IL, USA) tritiated polymer standards. The values were then converted to 'fmol bound ligand/mg of protein' assuming a uniform protein concentration of 0.1 mg/mg of tissue.

2.3. Data analysis

Data points from autoradiographic measurements were fitted by non-linear regression using Grafit (Erithacus Software, Staines, UK). In displacement experiments where a biphasic pattern was suspected, the improvement of fit using a biphasic model was assessed by calculating the F statistics:

$$F = \frac{(SS_1 - SS_2)/(df_1 - df_2)}{SS_2/df_2}$$

where the subscript 1 refers to the simplest model, SS_i being the sum of squares of the respective residuals and df_i the respective degrees of freedom.

3. Results

3.1. Saturation studies

Typical saturation curves of [^{3}H]5-CT binding sites in guinea pig hippocampus (CA1 region), substantia nigra and paraventricular thalamic nucleus are shown in Fig. 1. Also shown is the level of non-specific binding, which was significantly above film background only at the highest ligand concentration. The parameters (B_{max} and pK_D values) of the saturation curves obtained in different regions of the guinea pig brain are listed in Table 1. [^{3}H]5-CT apparently recognised a homogeneous population of binding sites in all regions examined. The affinity of the radioligand was very similar across regions (K_D = 0.19–0.45 nM). A very high B_{max} value was observed in the CA1 region of the hippocampus, high densities were found in the substantia nigra and superficial layer of the superior colliculus.

Table 1
Saturation study with [^{3}H]5-CT in different regions of the guinea-pig brain

Region	$K_D \pm$S.E.M. (nM)	B_{max} (fmol/mg protein)
Caudate/putamen	0.26±0.02	607± 14
Globus pallidus	0.33±0.03	1541± 33
Laterodorsal septum	0.23±0.02	1660± 35
Hippocampus (CA1)	0.41±0.06	4900±195
Cortex (superficial layers)	0.36±0.04	529± 16
Cortex (internal layers)	0.19±0.02	807± 20
Paraventricular thalamus	0.38±0.06	1078± 48
Substantia nigra	0.45±0.03	2400± 49
Superior colliculus	0.32±0.02	2220± 39

Parameters have been obtained by computerised curve-fitting to the density values read of the autoradiograms over the respective regions. Fitting was performed for each animal ($n \geq 6$) and the mean K_D and B_{max} values (±S.E.M.) are presented here.

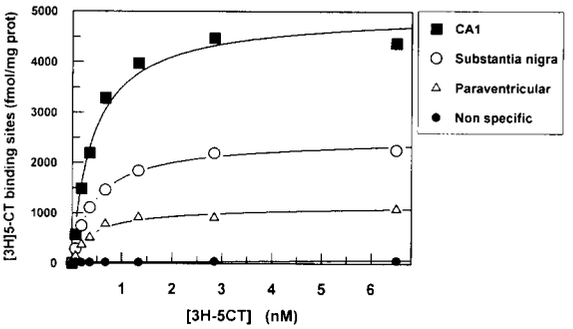


Fig. 1. [^{3}H]5-CT saturation curves obtained in three different regions of the guinea-pig brain, containing mostly 5-HT$_{1A}$ receptors (CA1 subfield of hippocampus), 5-HT$_{1D}$ receptors (substantia nigra) and 5-HT$_7$ receptors (paraventricular thalamic nucleus) (see Discussion). Non-specific binding is not significantly different from film background, except at the highest radioligand concentration. See Table 1 for affinities and B_{max} values.

3.2. Drug binding profile of [^{3}H]5-CT binding sites

Due to the non-specificity of [^{3}H]5-CT, the affinity of most displacing drugs was tested in the presence of blockers of either the 5-HT$_{1A}$ (100 nM (±)-8-OH-DPAT) or 5-HT$_{1B/1D}$ subtypes (20 μM sumatriptan), in order to restrict the complexity of the curves. Even under these conditions, some displacers competed for [^{3}H]5-CT binding sites with a biphasic profile. The affinity values at one or both binding sites are listed in Tables 2–9. For biphasic displacements, the proportion of the high affinity site in percent of the specific binding (in the presence of the blocker listed) are also given.

Methiothepin recognised a single population of [^{3}H]5-CT binding sites (in the presence of 100 nM (±)-8-OH-DPAT) in most regions of the guinea-pig brain (pK_D = 7.41–7.97). In addition to these sites, a lower affinity component was also present in the septum, hippocampus and trigeminal nucleus (pK_D = 5.13–5.93). In rat brain, both sites were observed in all regions, except in the paraventricular thalamic nucleus and central grey (where only the low affinity site was found) and in the trigeminal nucleus (containing only high affinity sites).

Sumatriptan and its conformationally restricted derivative *CP-122,288* displayed a subnanomolar affinity for [^{3}H]5-CT sites in all guinea-pig brain regions (in the presence of 100 nM (±)-8-OH-DPAT). A lower

Table 2
Affinity of methiothepin for [^{3}H]5-CT binding sites in different regions of the guinea-pig and rat brain

Region	Guinea-pig		Rat	
	$pK_D \pm$ S.E.M. (% of sites)	$pK_D \pm$ S.E.M.	$pK_D \pm$ S.E.M. (% of sites)	$pK_D \pm$ S.E.M.
Caudate/putamen	7.76 ± 0.09		7.46 ± 0.13 (24 ± 2)	5.86 ± 0.04
Globus pallidus	ND		7.15 ± 0.07 (64 ± 3)	5.61 ± 0.11
Laterodorsal septum	7.53 ± 0.28 (65 ± 11)	5.43 ± 0.34	7.20 ± 0.20 (36 ± 6)	5.52 ± 0.12
Hippocampus (CA1)	7.80 ± 0.45 (17 ± 6)	5.13 ± 0.13	7.44 ± 0.50 (17 ± 6)	5.18 ± 0.13
Cortex (superficial layers)	7.72 ± 0.08 (91 ± 3)	5.72 ± 0.16		
Cortex (internal layers)*	7.70 ± 0.28		8.22 ± 0.60 (25 ± 16)	5.51 ± 0.29
Paraventricular thalamus	7.41 ± 0.06		Not observed#	5.65 ± 0.11
Substantia nigra	7.80 ± 0.01		7.28 ± 0.42 (54 ± 26)	5.67 ± 0.42
Superior colliculus	7.49 ± 0.17		6.88 ± 0.05 (36 ± 1)	5.17 ± 0.03
Central grey	7.57 ± 0.11		Not observed#	5.92 ± 0.09
Trigeminal nucleus	7.97 ± 0.07 (60 ± 2)	5.93 ± 0.10	7.28 ± 0.05	

Methiothepine competition curves were established in the presence of 100 nM (±)-8-OH-DPAT to block 5-HT$_{1A}$ sites. *All layers in the rat, as no clear-cut laminar pattern of [^{3}H]5-CT labelling was observed in this species. #No site with an affinity ranging from 1 to 100 nM was observed in these regions.

Tables 2–9: For the displacement studies of [^{3}H]5-CT binding in different regions of the guinea-pig and rat brain using different cold competing drugs, parameters have been obtained by computerised curve-fitting to the density values read over the autoradiograms over the respective regions. Fitting was performed for each animal ($n \geq 3$) and the mean pK_D values (±S.E.M.) are presented. When the fit was significantly better using a biphasic model, pK_D values for the high and low affinity site are listed, as well as the proportion of high affinity sites.

Table 3
Affinities of sumatriptan and its conformationally restricted derivative CP-122,288 for [^{3}H]5-CT binding sites in different regions of the guinea-pig brain

Region	Sumatriptan		CP-122,288
	$pK_D \pm$ S.E.M. (% of sites)	$pK_D \pm$ S.E.M.	$pK_D \pm$ S.E.M. (% of sites)
Caudate/putamen	7.45 ± 0.24		7.57 ± 0.17
Globus pallidus	7.35 ± 0.25		7.53 ± 0.30
Laterodorsal septum	7.70 ± 0.11 (43 ± 1)	5.38 ± 0.10	6.83 ± 0.29
Hippocampus (CA1)	7.41 ± 0.46 (14 ± 2)	4.83 ± 0.29	6.44 ± 0.29
Cortex (superficial layers)	7.54 ± 0.25 (47 ± 2)	5.91 ± 0.20	7.13 ± 0.38
Cortex (internal layers)	7.84 ± 0.62 (68 ± 10)	5.41 ± 0.90	7.24 ± 0.33
Paraventricular thalamus	7.33 ± 0.74 (46 ± 20)	6.26 ± 0.66	7.13 ± 0.31
Substantia nigra	7.42 ± 0.32		7.62 ± 0.26
Superior colliculus	7.49 ± 0.31 (78 ± 4)	5.60 ± 0.65	7.43 ± 0.20
Central grey	7.38 ± 0.33		7.47 ± 0.18

CP-122,288 was tested at a maximal concentration of 200 nM, precluding the detection of a low affinity site. Sumatriptan and CP-122,288 competition curves were established in the presence of 100 nM (±)-8-OH-DPAT to block 5-HT$_{1A}$ sites.

Table 4
Affinity of the selective 5-HT$_{1D}$ receptor antagonist GR-127,935 for [^{3}H]5-CT binding sites in different regions of the guinea-pig and rat brain

Region	Guinea-pig		Rat	
	$pK_D \pm$ S.E.M. (% of sites)	$pK_D \pm$ S.E.M.	$pK_D \pm$ S.E.M. (% of sites)	$pK_D \pm$ S.E.M.
Caudate/putamen	8.74 ± 0.15 (92 ± 2)	5.52 ± 0.71	8.58 ± 0.40	
Laterodorsal septum	8.53 ± 0.81 (47 ± 11)	5.50 ± 0.68	8.85 ± 0.22 (74 ± 2)	< 5
Hippocampus (CA1)	Not observed#	5.69 ± 0.36	7.96 ± 0.61 (40 ± 5)	< 5
Cortex (superficial layers)	8.11 ± 0.71 (31 ± 5)	5.35 ± 0.52		
Cortex (internal layers)*	8.54 ± 0.15 (49 ± 1)	5.34 ± 0.17	8.87 ± 0.36 (75 ± 4)	< 5
Paraventricular thalamus	8.70 ± 0.60 (44 ± 6)	5.29 ± 0.62	8.10 ± 0.53 (55 ± 5)	< 5
Substantia nigra	8.59 ± 0.19		8.76 ± 0.16	
Superior colliculus	8.65 ± 0.25 (81 ± 3)	5.63 ± 0.59	8.65 ± 0.23 (82 ± 2)	< 5
Central grey	8.61 ± 0.21 (89 ± 2)	5.56 ± 0.70	8.55 ± 0.20 (90 ± 2)	< 5
Trigeminal nucleus	8.82 ± 0.37 (60 ± 4)	5.38 ± 0.34	8.72 ± 0.31 (66 ± 3)	5.34 ± 0.54

GR-127,935 competition curves were established in the presence of 100 nM (±)-8-OH-DPAT to block 5-HT$_{1A}$ sites. *All layers in the rat. #No site with an affinity ranging from 1 to 100 nM was observed in these regions.

Table 5
Affinity of the non-selective 5-HT_1 receptor agonist dihydroergotamine for [^{3}H]5-CT binding sites in different regions of the guinea-pig and rat brain

Region	Guinea-pig		Rat	
	$pK_D \pm$ S.E.M. (% of sites)	$pK_D \pm$ S.E.M.	$pK_D \pm$ S.E.M. (% of sites)	$pK_D \pm$ S.E.M.
Caudate/putamen	9.46 ± 0.31		9.19 ± 0.25	
Globus pallidus	9.58 ± 0.26		9.17 ± 0.07	
Laterodorsal septum	8.56 ± 0.32		8.84 ± 0.22	
Hippocampus (CA1)	8.38 ± 0.30		8.52 ± 0.19	
Cortex (superficial layers)	9.56 ± 0.32 (30 ± 9)	7.18 ± 0.48		
Cortex (internal layers)*	8.57 ± 0.44		8.65 ± 0.20	
Paraventricular thalamus	9.55 ± 0.32 (30 ± 11)	7.36 ± 0.49	8.59 ± 0.24 (61 ± 7)	6.93 ± 0.53
Substantia nigra	9.55 ± 0.21		9.14 ± 0.27	
Superior colliculus	8.93 ± 0.38		8.77 ± 0.29	
Central grey	9.98 ± 0.04 (70 ± 1)	8.25 ± 0.07	8.78 ± 0.28	

Dihydroergotamine competition curves were established in the absence of other blocking drugs. *All layers in the rat.

affinity component was also observed with sumatriptan, except in the caudate/putamen, globus pallidus, substantia nigra and central grey. CP-122,288 was tested at lower concentrations (see Discussion) and a low affinity site could not be observed with this ligand.

GR-127,935 curves (in the presence of 100 nM (±)-8-OH-DPAT) displayed a biphasic pattern in most guinea-pig and rat brain areas, with a high affinity component (pK_D = 8.38–9.98) and a much lower affinity component (pK_D 5.29–5.69 in guinea-pig, < 5 in rat). Homogeneous populations of sites were only observed in the caudate/putamen and substantia nigra, as well as in the hippocampus, where only low affinity sites were detected.

Dihydroergotamine seemed to show 2 classes of high affinity sites in the guinea-pig. The caudate/putamen, globus pallidus, superficial cortex, paraventricular thalamus, substantia nigra contained very high affinity sites (pK_D = 9.46–9.98), while the other regions contained a lower affinity population (pK_D = 8.38–8.93). The central grey appeared to contain both populations of sites. A similar dichotomy was observed in rat brain regions. In the superficial cortical layers and in the paraventricular thalamus, a component with a still lower affinity was also found.

Spiperone (in the presence of 20 μM sumatriptan) was a low affinity displacer in all guinea-pig and rat brain areas (pK_D 4.98–5.77). However, a high affinity component (pK_D = 7.52–8.15) was also observed in the superficial cortical layers, the paraventricular thalamus, the trigeminal nucleus, as well as in the hippocampus, but only in the rat.

(±)-8-OH-DPAT recognised a high affinity site in all brain regions (in the presence of 20 μM sumatriptan), except the rat caudate/putamen and substantia nigra. A low, and in general minor, affinity component was also observed in some areas.

Clozapine (in the presence of 100 nM (±)-8-OH-DPAT) was a low affinity displacer in all guinea-pig and rat brain areas (pK_D 4.93-5.91). However, a high affinity component (pK_D = 6.63–7.47) was also observed in the superficial cortical layers (only in guinea-pig) and in the paraventricular thalamus.

(+)-Butaclamol displayed a single population with intermediate affinity for [^{3}H]5-CT binding sites (in the presence of 100 nM (±)-8-OH-DPAT) in all guinea-pig

Table 6
Affinity of the non-selective 5-HT receptor antagonist spiperone for [^{3}H]5-CT binding sites in different regions of the guinea-pig and rat brain

Region	Guinea-pig		Rat	
	$pK_D \pm$ S.E.M. (% of sites)	$pK_D \pm$ S.E.M.	$pK_D \pm$ S.E.M. (% of sites)	$pK_D \pm$ S.E.M.
Caudate/putamen	5.02 ± 0.48		< 5	
Laterodorsal septum	5.63 ± 0.28		5.77 ± 0.27	
Hippocampus (CA1)	5.62 ± 0.19		7.55 ± 0.22 (17 ± 1)	5.66 ± 0.05
Dentate gyrus	5.73 ± 0.35		5.75 ± 0.22	
Cortex (superficial layers)	7.52 ± 0.44 (45 ± 5)	5.54 ± 0.36		
Cortex (internal layers)*	5.73 ± 0.35		7.64 ± 0.21 (27 ± 1)	5.52 ± 0.09
Paraventricular thalamus	7.08 ± 0.30 (80 ± 5)	4.98 ± 0.59	7.65 ± 0.49 (57 ± 8)	5.74 ± 0.53
Substantia nigra	< 5		< 5	
Superior colliculus	5.34 ± 0.32		5.42 ± 0.07	
Trigeminal nucleus	8.15 ± 0.63 (26 ± 6)	5.65 ± 0.32	ND	

Spiperone competition curves were established in the presence of 20 μM sumatriptan to block 5-HT_{1D} sites. *All layers in the rat.

Table 7
Affinity of the selective 5-HT_{1A} receptor agonist (±)-8-OH-DPAT for [^{3}H]5-CT binding sites in different regions of the guinea-pig and rat brain

Region	Guinea-pig		Rat	
	pK_D ± S.E.M. (% of sites)	pK_D ± S.E.M.	pK_D ± S.E.M. (% of sites)	pK_D ± S.E.M.
Caudate/putamen	8.28 ± 0.58 (58 ± 8)	< 5	5.20 ± 0.36	
Laterodorsal septum	8.35 ± 0.45 (86 ± 9)	5.86 ± 1.31	8.11 ± 0.33	
Hippocampus (CA1)	8.41 ± 0.16		8.30 ± 0.11	
Dentate gyrus	8.32 ± 0.26		8.28 ± 0.11	
Cortex (superficial layers)	8.25 ± 0.39 (73 ± 6)	5.70 ± 0.61		
Cortex (internal layers)*	8.45 ± 0.28 (91 ± 4)	5.22 ± 0.95	8.19 ± 0.11 (87 ± 1)	5.16 ± 0.47
Paraventricular thalamus	8.03 ± 0.52 (78 ± 10)	5.67 ± 0.81	8.73 ± 0.67 (39 ± 10)	6.66 ± 0.46
Substantia nigra	8.29 ± 0.66 (38 ± 5)	< 5	< 4	
Superior colliculus	8.27 ± 0.30 (87 ± 5)	5.30 ± 0.77	8.20 ± 0.25 (78 ± 3)	< 5
Trigeminal nucleus	8.21 ± 0.28		ND	

(±)-8-OH-DPAT competition curves were established in the presence of 20 μM sumatriptan to block 5-HT_{1D} sites. *All layers in the rat.

brain regions, except the hippocampus. The competition pattern was in general more complex in the rat, where high affinity components were observed in the globus pallidus, hippocampus and paraventricular thalamus. Low affinity components (pK_D = 5.16–5.67) were also found in some regions.

3.3. Guanosine nucleotide sensitivity of [^{3}H]5-CT binding sites

The effects of increasing concentrations of the GTP analogue 5′-β,γ-imidotriphosphate on [^{3}H]5-CT binding in different guinea-pig brain areas are shown in Table 10. The pEC_{50} of this compound in inhibiting [^{3}H]5-CT binding was below 5.0 in hippocampus, dentate gyrus and laterodorsal septum. pEC_{50} values were significantly higher in the other regions, except in the superficial cortical layers and the paraventricular thalamus, where 5′-β,γ-imidotriphosphate had no detectable effect on [^{3}H]5-CT binding. This compound was ineffective on at least one-third of the sites in all areas.

We also observed that in some individual rats and guinea-pig, 5′-β,γ-imidotriphosphate was virtually inefficient in all brain regions. The maximum effect of the GTP analogue was subsequently found to be inversely proportional to the age of the sections. A similar effect has previously been described for hippocampal 5-HT_{1A} receptors and shown to be accounted for by oxidation of the receptor (Emerit et al., 1991). We have thus included in our analysis only the sections cut less than one month before the incubation.

3.4. Regional distribution of [^{3}H]5-CT binding sites

The concentration of [^{3}H]5-CT binding sites in different regions of the guinea-pig brain, in the absence of blockers, in the presence of 100 nM (±)-8-OH-DPAT or of 20 μM sumatriptan (to block the major predicted targets of [^{3}H]5-CT, namely 5-HT_{1A} and 5-HT_{1D}), is shown in Fig. 2. The distribution of [^{3}H]5-CT binding sites in different guinea-pig brain areas is shown in Figs. 3–5, the rat brain distribution is shown in Fig. 6. These figures display the labelling pattern obtained in the presence of different blocking drugs. When possible, the concentration of the latter has been chosen at the plateau of biphasic displacement curves, in order to discriminate better between the

Table 8
Affinity of clozapine for [^{3}H]5-CT binding sites in different regions of the guinea-pig and rat brain

Region	Guinea-pig		Rat	
	pK_D ± S.E.M. (% of sites)	pK_D ± S.E.M.	pK_D ± S.E.M. (% of sites)	pK_D ± S.E.M.
Caudate/putamen	5.71 ± 0.11		5.47 ± 0.23	
Globus pallidus	5.89 ± 0.19		5.46 ± 0.30	
Laterodorsal septum	5.69 ± 0.13		5.62 ± 0.15	
Hippocampus (CA1)	5.29 ± 0.22		5.38 ± 0.15	
Cortex (superficial layers)	6.63 ± 0.32 (80 ± 10)	4.93 ± 0.89		
Cortex (internal layers)*	5.72 ± 0.12		5.36 ± 0.26	
Paraventricular thalamus	7.30 ± 0.25 (23 ± 2)	5.84 ± 0.08	7.47 ± 0.32 (16 ± 1)	5.41 ± 0.08
Substantia nigra	5.91 ± 0.17		5.33 ± 0.21	
Superior colliculus	5.69 ± 0.16		5.50 ± 0.16	
Trigeminal nucleus	5.71 ± 0.15		5.61 ± 0.26	

Clozapine competition curves were established in the presence of 100 nM (±)-8-OH-DPAT to block 5-HT_{1A} sites. *All layers in the rat.

Table 9
Affinity of (+)-butaclamol for [^{3}H]5-CT binding sites in different regions of the guinea-pig and rat brain

Region	Guinea-pig	Rat	
	$pK_D \pm$ S.E.M.	$pK_D \pm$ S.E.M. (% of sites)	$pK_D \pm$ S.E.M.
Caudate/putamen	6.65 ± 0.11	6.65 ± 0.11 (n_H = 0.63 ± 0.1)♦	
Globus pallidus	6.79 ± 0.14	7.14 ± 0.27 (40 ± 3)	5.67 ± 0.08
Laterodorsal septum	6.36 ± 0.42	6.79 ± 0.28 (37 ± 5)	5.45 ± 0.19
Hippocampus (CA1)	5.15 ± 0.19	7.72 ± 0.10 (15 ± 1)	5.21 ± 0.08
Cortex (superficial layers)	6.72 ± 0.29		
Cortex (internal layers)*	6.59 ± 0.39	6.58 ± 0.17 (49 ± 3)	5.23 ± 0.22
Paraventricular thalamus	6.54 ± 0.15	7.26 ± 0.60 (36 ± 8)	5.16 ± 0.49
Substantia nigra	6.83 ± 0.06	5.67 ± 0.05	
Superior colliculus	6.45 ± 0.22	5.61 ± 0.20	
Trigeminal nucleus	6.53 ± 0.24	6.17 ± 0.33	

(+)-Butaclamol competition curves were established in the presence of 100 nM (±)-8-OH-DPAT to block 5-HT$_{1A}$ sites. *All layers in the rat. ♦ Even though the data were best fitted with a Hill slope (n_H) significantly smaller than 1, the fit did not converge to a meaningful solution using a biphasic model.

different labelled sites. In guinea-pigs, without blocking drug, [^{3}H]5-CT binding sites are highly concentrated in the anterior olfactory nucleus, lateral septum, globus pallidus, dorsal and ventral hippocampus, superior colliculus, dorsal raphe, substantia nigra and entorhinal cortex (Fig. 3A, C, E and G, I, K). The distribution is very similar in rats (Fig. 6), which in addition display a dense labelling in the olfactory tubercle. Fig. 3 (B, D, F and H, J, L) illustrates the distribution of binding sites in the presence of 100 nM (±)-8-OH-DPAT (a 5-HT$_{1A}$ agonist). Binding is displaced in the anterior olfactory nucleus, lateral septum, hippocampus, raphe and entorhinal cortex.

The effect of other 5-HT$_{1A}$ drugs is shown in Fig. 4, at the level of the lateral septum and of the paraventricular thalamus. 5-HT$_{1B/1D}$ binding is eliminated by 20 μM sumatriptan (C and C′; the loss of 5-HT$_{1B/1D}$ binding can be observed in the caudate/putamen, globus pallidus and hypothalamus). In addition to 20 μM sumatriptan, the incubation medium was supplemented with 100 nM (±)-8-OH-DPAT (B, B′) and 1 μM spiperone (D, D′). While (±)-8-OH-DPAT efficiently displaces [^{3}H]5-CT in the septum, hippocampus and internal cortical layers, it does not affect labelling in the external cortical layers and midline thalamic nuclei. Conversely, binding in the latter areas is eliminated by spiperone, which does not affect binding in the former regions.

Table 10
Effect of the GTP analogue 5′-β,γ-imidotriphosphate on [^{3}H]5-CT binding in different regions of the guinea-pig brain

Region	$pEC_{50} \pm$ S.E.M. (M)	Maximal inhibition (% of total)
Caudate/putamen	5.42 ± 0.12	69 ± 5
Globus pallidus	5.75 ± 0.11	71 ± 4
Laterodorsal septum	4.48 ± 0.19	42 ± 6
Dentate gyrus	3.94 ± 0.36	51 ± 6
Hippocampus (CA1)	4.75 ± 0.25	53 ± 9
Cortex (superficial layers)	No effect	
Paraventricular thalamus	No effect	
Substantia nigra	5.69 ± 0.11	67 ± 4
Superior colliculus	5.28 ± 0.19	42 ± 5

Fig. 5 illustrates further the properties of [^{3}H]5-CT binding sites in guinea-pig at the level of the paraventricular thalamus. In the presence of 100 nM (±)-8-OH-DPAT (Fig. 5A), the 5-HT$_{1D}$ antagonist GR-127,935 (100 nM; Fig. 5B) inhibits binding in the caudate and hypothalamus (and other areas containing 5-HT$_{1D}$ sites, not shown), but not in the external cortical layers and midline thalamus. Labelling in the latter areas is also resistant to 100 nM dihydroergotamine (Fig. 5C; without 8-OH-DPAT), 1 μM sumatriptan (Fig. 5E), but not to 1 μM methiothepin (Fig. 5D).

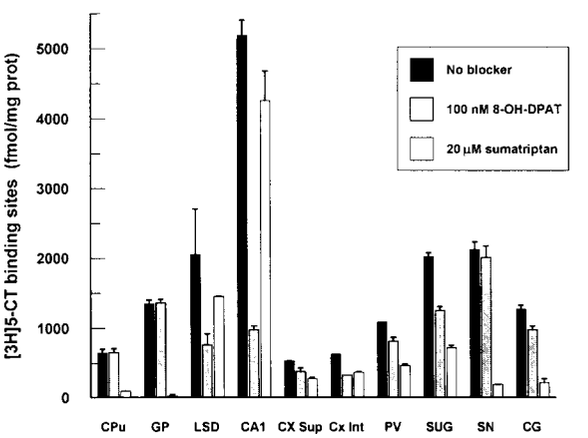


Fig. 2. Densities of [^{3}H]5-CT binding sites in different regions of the guinea-pig brain under different labelling conditions. 100 nM (±)-8-OH-DPAT and 20 μM sumatriptan block [^{3}H]5-CT binding to the majority of 5-HT$_{1A}$ and 5-HT$_{1D}$ receptors, respectively. Thus, while the globus pallidus (GP), caudate/putamen (CPu), substantia nigra (SN) and central grey (CG) contain almost exclusively 5-HT$_{1D}$ receptors, the dorsolateral septum (LSD) and hippocampus (CA1) contain a majority of 5-HT$_{1A}$ receptors. The superficial cortical layers (Cx Sup), internal cortical layers (Cx Int), paraventricular thalamic nucleus (PV) and superior colliculus (SUG) contain mixed populations of receptors.

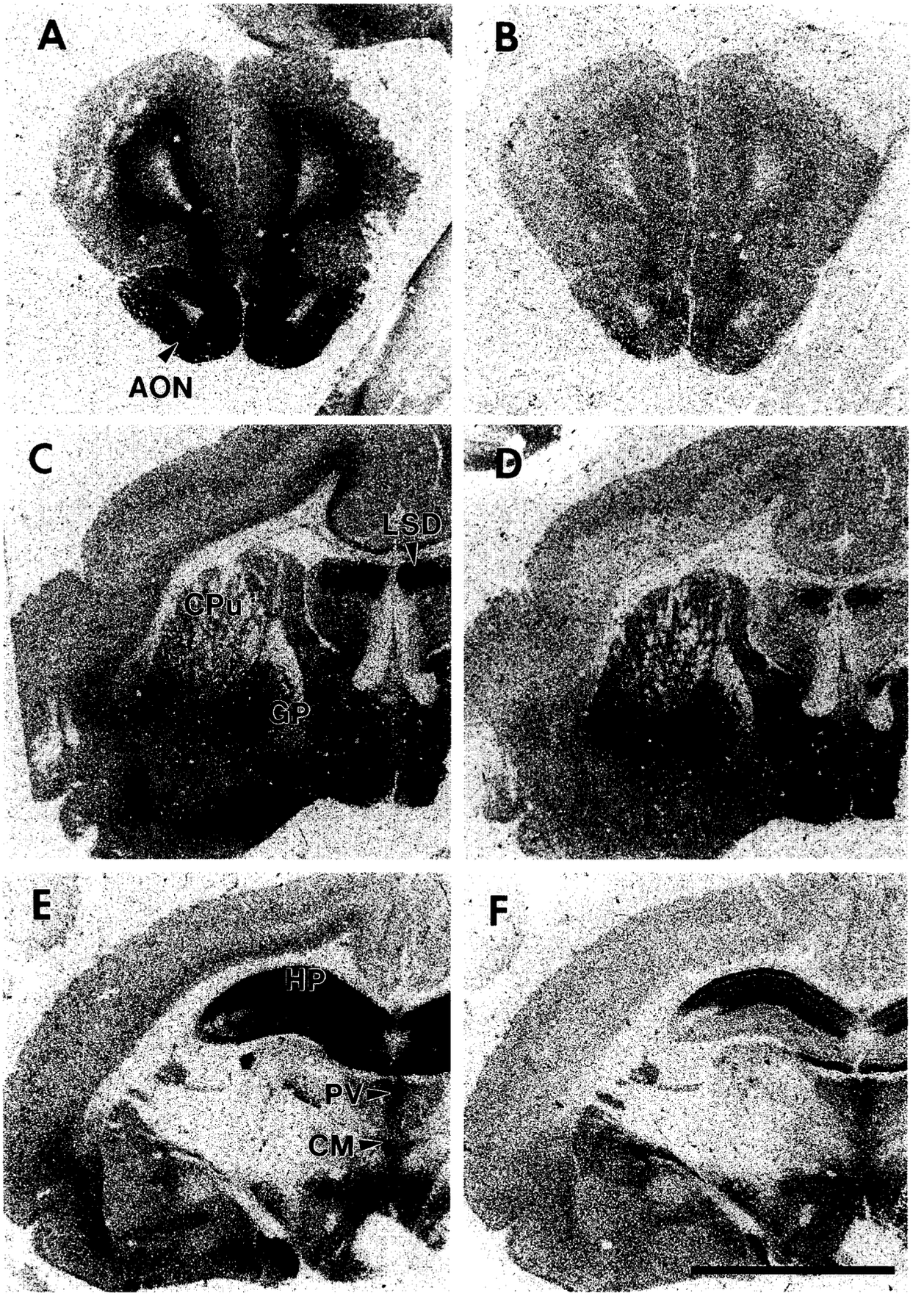


Fig. 3. Caption on page 40.

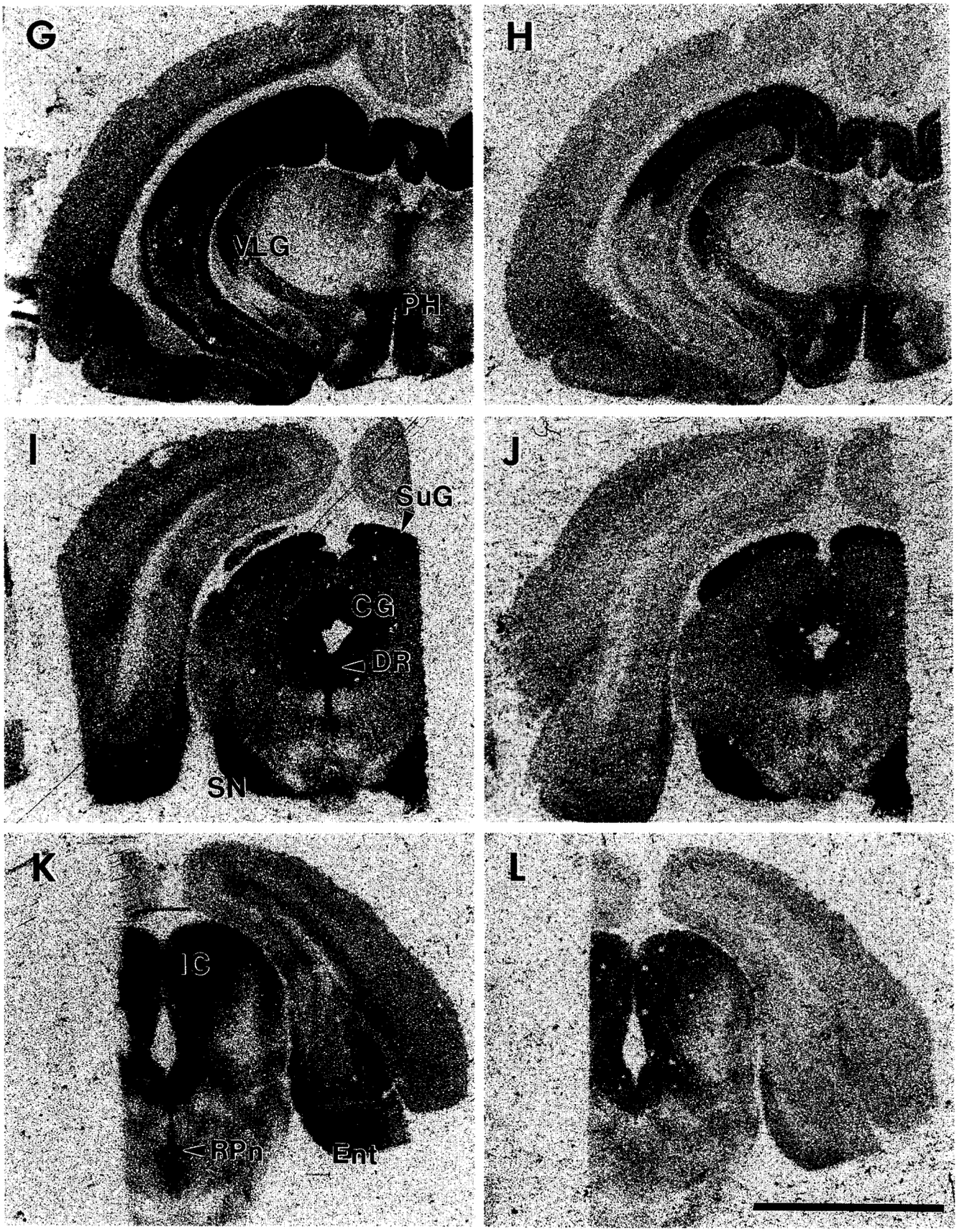


Fig. 3 (continued).

This concentration is sufficient to reduce binding to background levels in all areas except the hippocampus, which still shows a dense labelling. The pattern of this labelling corresponds to that of [^{3}H]8-OH-DPAT, which specifically recognises 5-HT$_{1A}$ sites (Fig. 5F). Note the absence of such sites in the external cortical layers and midline thalamus.

In the rat brain, after complete blockade of 5-HT$_{1D}$ sites (by 1 μM GR-127,935) and in the presence of 100 nM 8-OH-DPAT (blocking all sites in the raphe),

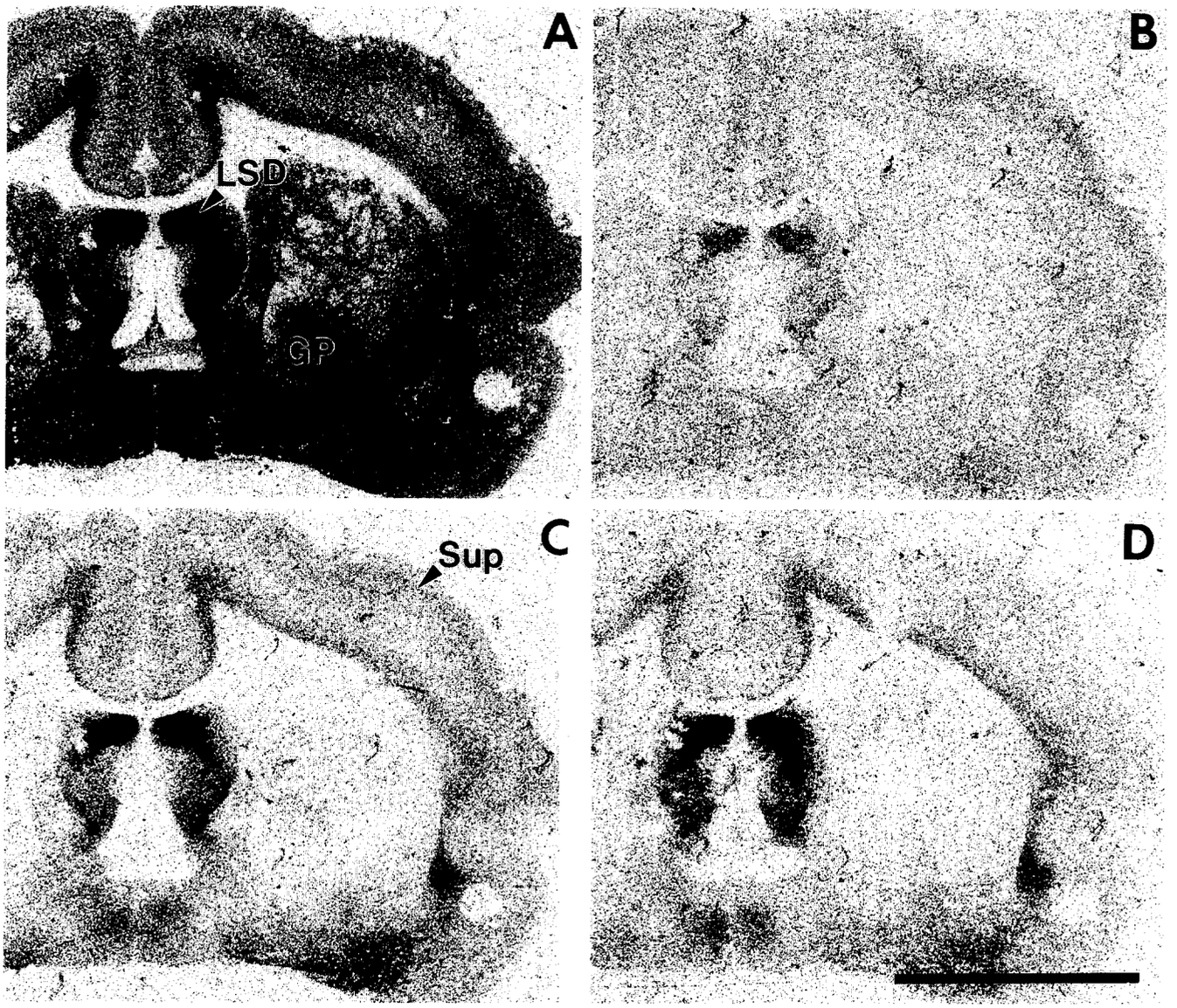


Fig. 4. Effects of 100 nM 8-OH-DPAT or 1 μM spiperone on non-5-HT$_{1D}$ [^{3}H]5-CT binding sites in the guinea-pig brain, at the levels of the striatum (A–D) and the hippocampus (A′–D′). Total [^{3}H]5-CT binding is shown in A, A′; binding remaining in the presence of 20 μM sumatriptan is shown in C, C′: binding to 5-HT$_{1D}$ sites is virtually eliminated under those conditions (see globus pallidus, GP). Sumatriptan is also present in B, B′, D, D′. 100 nM 8-OH-DPAT prevents [^{3}H]5-CT binding to the laterodorsal septum, hippocampus and internal cortical layers (B, B′) but does not affect binding in the superficial cortical layers (Sup), paraventricular (PV) and rhomboid (Rh) thalamic nuclei. D and D′ show the opposite effect of 1 μM spiperone. Scale bars: 5 mm.

Fig. 3. Autoradiographic distribution of [^{3}H]5-CT binding sites in coronal sections of guinea-pig brain. A, C, E and G, I, K show total binding; B, D, F and H, J, L show binding remaining in the presence of 100 nM of the 5-HT$_{1A}$ agonist (±)-8-OH-DPAT. Under the latter conditions, most of the binding disappears in the anterior olfactory nucleus (AON), laterodorsal septum (LSD), hippocampus (HP), dorsal raphe nucleus (DR), pontine raphe nucleus (RPn) and entorhinal cortex (Ent). The globus pallidus (GP), superficial grey layer of the superior colliculus (SuG) and substantia nigra (SN) remain densely labelled. Other abbreviations are: paraventricular thalamic nucleus (PV), central medial thalamic nucleus (CM), ventrolateral geniculate nucleus (VLG), posterior hypothalamic area (PH) and inferior colliculus (IC). Scale bar: 5 mm.

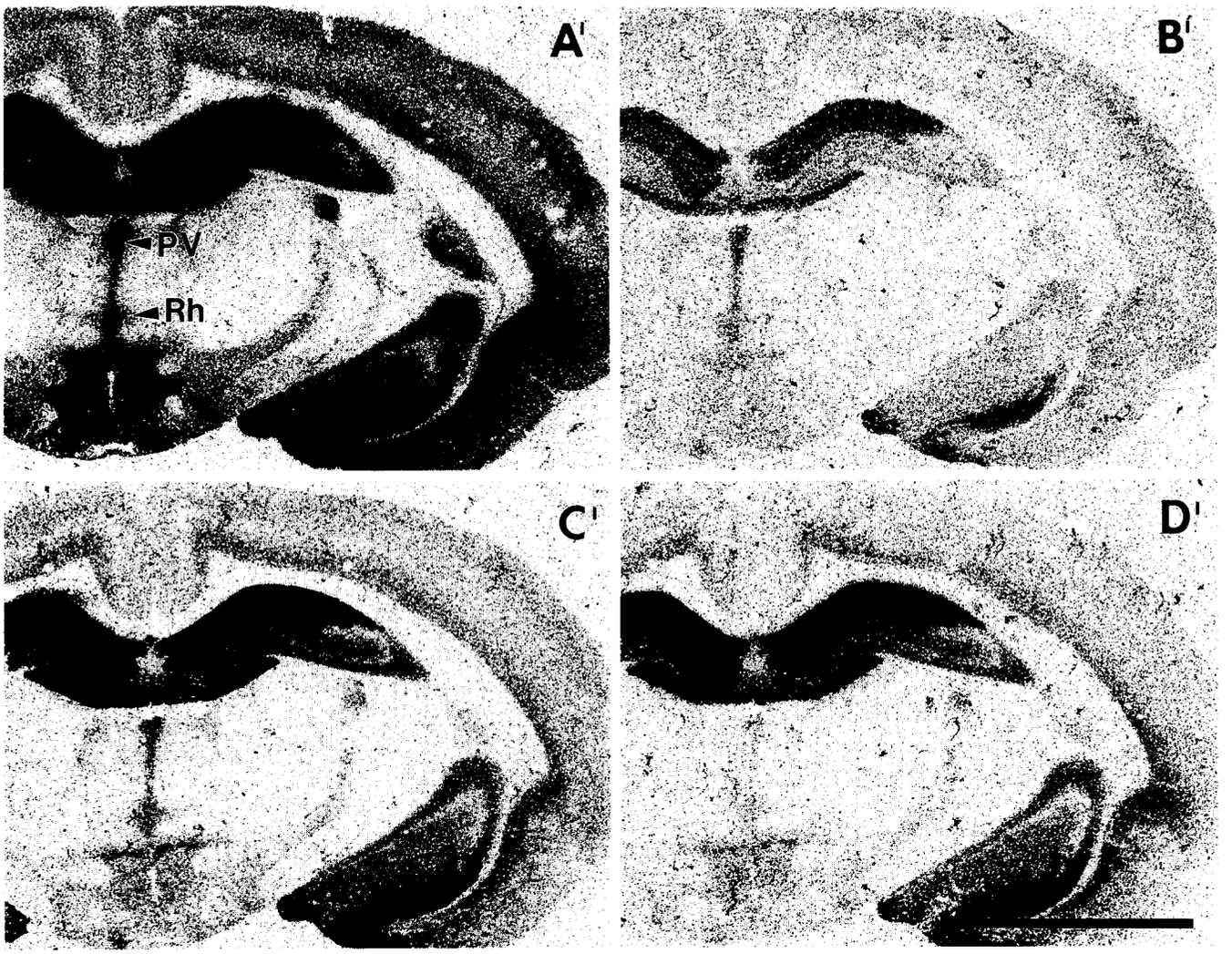


Fig. 4 (continued).

[^{3}H]5-CT labelling can still be observed in the cortex (without a clear laminar pattern), septum, hippocampus, midline and centrolateral thalamic nuclei, superior colliculus and interpeduncular nucleus.

4. Discussion

The main finding of the present study is the characterisation of the pharmacological properties of [^{3}H]5-CT binding sites in different regions of guinea-pig and rat brain. As expected in view of its non-specificity and previous studies (Mahle et al., 1991), this radioligand labels multiple subtypes of receptors. Its affinity is very high and does not significantly vary across regions. Binding is saturable and appears to be monophasic in the concentration range examined.

In order to minimally mask potential novel recognition sites, we have tried to study [^{3}H]5-CT binding properties either in the absence of blocking, or with the lowest possible concentration of a single drug (100 nM (±)-8-OH-DPAT for 5-HT$_{1A}$ receptors, 20 μM sumatriptan for 5-HT$_{1B/1D}$ receptors). Under these conditions, some regions contain homogeneous populations of a receptor subtype. It is then possible to characterise [^{3}H]5-CT binding at this site which provides affinity values which can then be used as reference in regions containing a mixture of subtypes. Thus, the substantia nigra and globus pallidus consistently yielded monophasic competition patterns, in agreement with the known predominance of 5-HT$_{1B}$ (rat) or 5-HT$_{1D}$ (guinea-pig) receptors in these areas.

Affinity values of dihydroergotamine, sumatriptan and methiothepin were in agreement with previous studies (Nowak et al., 1993), although, at variance with this study, methiothepin recognised only one population of sites in the guinea-pig substantia nigra. [^{3}H]5-CT has been shown to bind to multiple sites in guinea-pig frontal cortex membranes (Mahle et al., 1991). The authors suggested that they could be accounted for by

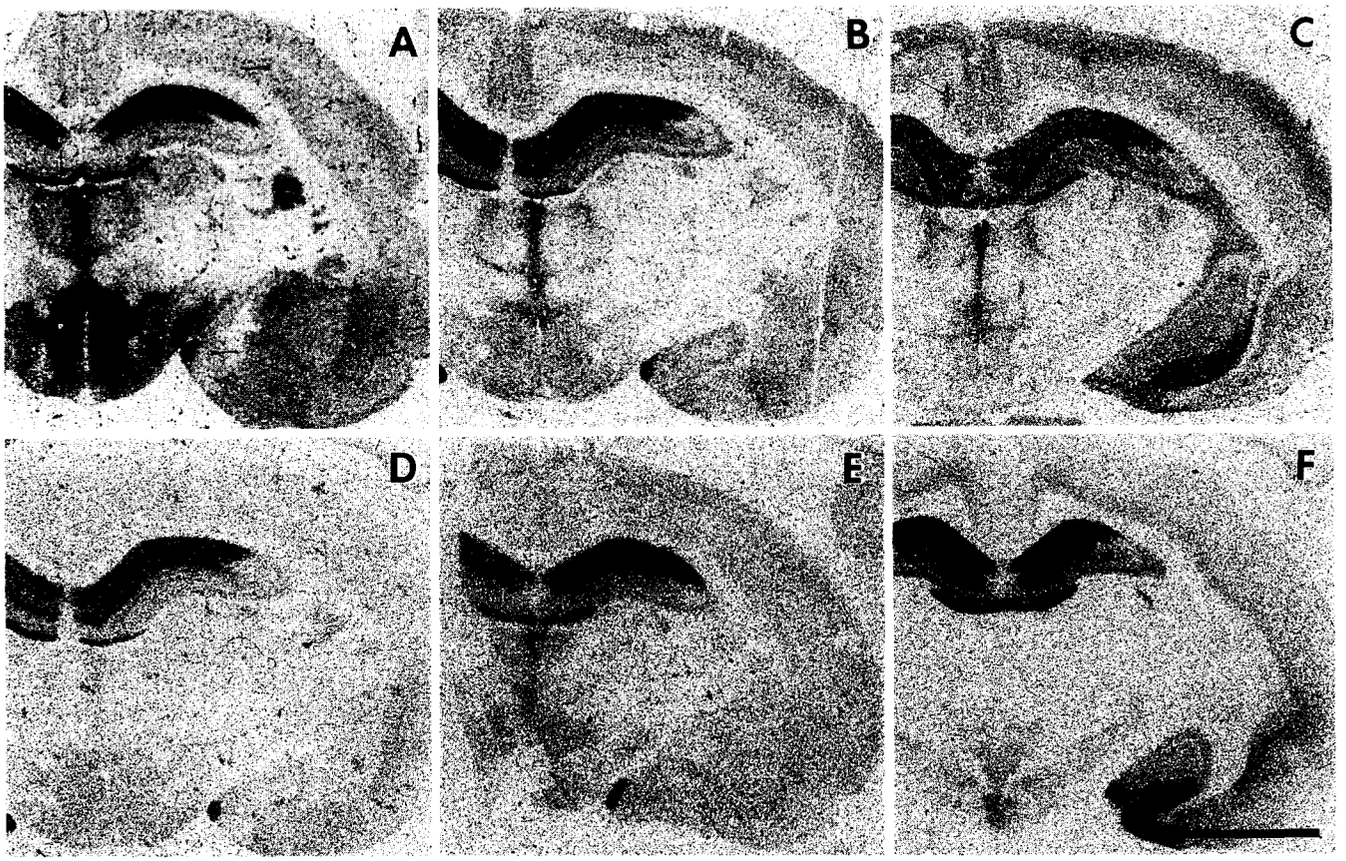
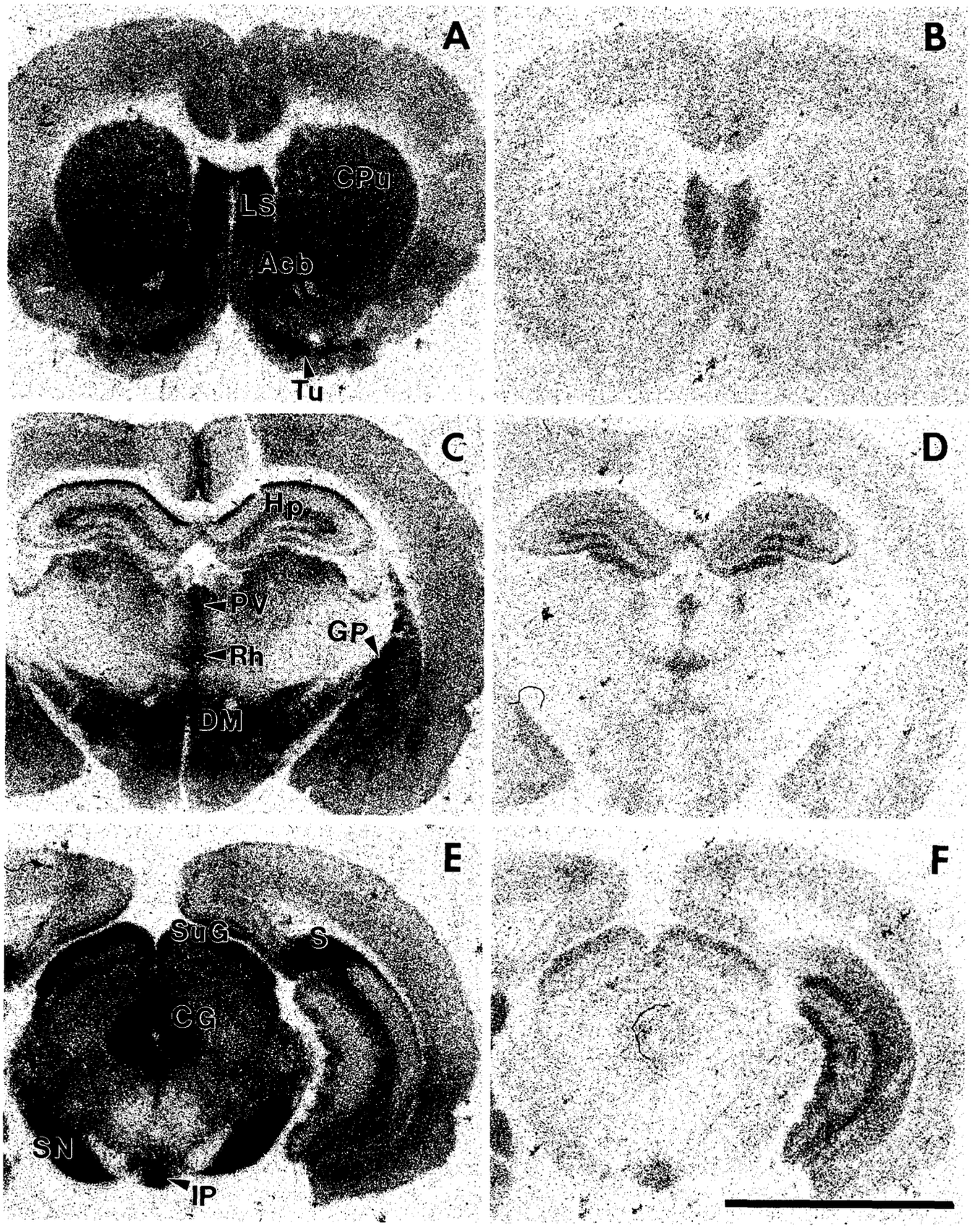


Fig. 5. Further characterisation of [^{3}H]5-CT binding sites in the hippocampus, superficial cortical layers and midline thalamic nuclei in the guinea-pig brain. 100 nM 8-OH-DPAT is present in A, B and E (total binding at the same level is displayed in Fig. 3 and Fig. 4'). The 5-HT_{1D} antagonist GR-127,935 (100 nM, B) and the 5-HT_{1D} agonist sumatriptan (1 μM, E) completely eliminate binding in the globus pallidus or substantia nigra (not shown here), leaving thalamic and cortical sites mostly unaffected. A nearly identical image is obtained with 100 nM dihydroergotamine (in the absence of 8-OH-DPAT, C), as this drug possesses high affinity for both 5-HT_{1A} and 5-HT_{1D} receptors. Please note that the prominent [^{3}H]5-CT binding in the superficial cortical layers stands out only when both 5-HT_{1A} and 5-HT_{1D} sites are blocked (either by dihydroergotamine alone or by the combination of 8-OH-DPAT and GR-127,935), probably because of the different layer distributions of the 5-HT_{1A}, 5-HT_{1D} and 5-HT_7 receptors labelled under those conditions. GR-127,935 is a better blocker than sumatriptan, probably because it discriminates better between 5-HT_{1D} and 5-HT_7 receptors. 1 μM methiothepine eliminates binding in all brain regions, except the lateral septum (not shown here) and hippocampal formation (D). The pattern of labelling remaining under these conditions is very similar to that obtained by the specific labelling of 5-HT_{1A} receptors using 1 nM [^{3}H]8-OH-DPAT (F). Scale bar: 5 mm.

two 5-HT_{1D}-like receptors. In view of the present results and the recent cloning data, it is likely that these sites actually correspond to 5-HT_{1D} and 5-HT_7 receptors (see below).

[^{3}H]5-CT did not label 5-HT_{1A} binding sites with the expected pharmacological properties in the hippocampus. The affinity of hippocampal [^{3}H]5-CT binding sites for spiperone (5.62) and methiothepin (5.13 for the majority of the sites) is much lower than the previously published affinities of these drugs for 5-HT_{1A} sites (7.2 and 7.1) (Hoyer, 1989). However, all hippocampal [^{3}H]5-CT labelling is displaced by (±)-8-OH-DPAT and dihydroergotamine with affinities (8.41 and 8.38) slightly lower but comparable to published 5-HT_{1A} values (8.7 and 8.9) (Hoyer, 1989). Although 5-HT was not used in a full competition experiment, the fact that 10 μM 5-HT (non-specific binding) reduced binding to background level indicates that the affinity of 5-HT is lower than 100 nM. Thus hippocampal [^{3}H]5-CT binding sites seem to show a low affinity for antagonists (spiperone and methiothepin), while agonists (5-HT, (±)-8-OH-DPAT, dihydroergotamine

Fig. 6. Distribution of [^{3}H]5-CT binding sites in the rat brain, in the absence of displacer (A, C, E) and in the presence of 100 nM 8-OH-DPAT and GR 127935 (B, D, F), to block 5-HT_{1A} and 5-HT_{1D} receptors. Under these conditions, sites remain labelled in the paraventricular and rhomboid thalamic nuclei. Binding remaining in the other regions might be due to the incomplete blockade of 5-HT_{1A} receptors. Abbreviations are as in Fig. 3, and accumbens nucleus (Acb), olfactory tubercle (Tu), dorsomedial hypothalamic nucleus (DM), subiculum (S) and interpeduncular nucleus (IP). Scale bar: 5 mm.


A
CPu
LS
Acb
Tu
B
C
Hp
PV
GP
Rh
DM
D
E
SuG
S
CG
SN
IP
F

(Hoyer et al., 1989)) seem to display the expected affinity. Similar binding sites were observed in the internal cortical layers and the lateral septum, with a distribution identical with that of [^{3}H]8-OH-DPAT. This observation, taken together with the affinity of 5-HT$_{1A}$ agonists, indicate that these sites are accounted for by 'atypical' 5-HT$_{1A}$ sites, rather than a novel receptor subtype. Interestingly, they seem to be sensitive, at least partly, to the GTP analogue 5'-β,γ-imidotriphosphate. This site might be related to the 'independent' [^{3}H]8-OH-DPAT binding sites described by Nénonéné et al. (1994) in cortical and hippocampal membranes, although the latter exhibited a low affinity for the agonists (±)-8-OH-DPAT and 5-HT and was insensitive to guanine nucleotides. Alternatively, [^{3}H]5-CT might recognize an additional binding site on the 5-HT$_{1A}$ receptor, from which it could be displaced only by agonists, by an allosteric modification not induced by antagonists. Further studies are required to confirm the distinct behaviors of 5-HT$_{1A}$ agonists and antagonists and to establish the molecular basis of the atypical properties of these [^{3}H]5-CT binding sites.

Beside 5-HT$_{1B}$/5-HT$_{1D}$ and 5-HT$_{1A}$ receptors, [^{3}H]5-CT appears to label another population of sites. In the guinea-pig, [^{3}H]5-CT labelling remaining in the presence of 100 nM (±)-8-OH-DPAT and 20 μM sumatriptan can be observed in the superficial cortical layers, the paraventricular, intermediodorsal, centromedial and central lateral thalamic nuclei and in the hippocampus. The nature of the sites in the latter region cannot be identified with certainty, for the reason mentioned above, but they probably correspond to 5-HT$_{1A}$ receptors. The labelling pattern is similar in the presence of 100 nM dihydroergotamine (blocking simultaneously 5-HT$_{1A}$ and 5-HT$_{1B/1D}$ sites) or in the presence of both (±)-8-OH-DPAT and the 5-HT$_{1B/1D}$ antagonist GR-127,935 (100 nM). GR-127,935 is a more convenient blocker than sumatriptan, as it has a higher selectivity and affinity for 5-HT$_{1B/1D}$ sites. Replacing (±)-8-OH-DPAT by the 5-HT$_{1A}$ antagonist spiperone is not favourable, both because this agent does not recognise 5-HT$_{1A}$ sites in this system (see above) and because it seems to possess a high affinity for the cortical and thalamic sites.

The localisation and the pharmacological profile of these sites indicate that they may correspond to the recently cloned 5-HT$_7$ receptors (Ruat et al., 1993; Tsou et al., 1994). In transfected cell lines, 5-HT$_7$ binding sites are characterised by a relatively high affinity for atypical neuroleptics such as clozapine and (+)-butaclamol (Ruat et al., 1993). Indeed, the superficial cortex and the midline thalamic nuclei contain a binding component with a higher affinity for clozapine than the other brain regions. (+)-Butaclamol does not appear to discriminate between 5-HT$_{1D}$ and 5-HT$_7$ receptors in the guinea-pig. The pharmacological profile and the distribution of these [^{3}H]5-CT binding sites are similar to those reported by Jakeman et al. (1994), who described the presence of 5-HT$_7$ receptors in the guinea-pig. However, it should be mentioned that the poor selectivity of the drugs used in the present study does not allow to exclude the possibility that sites other than 5-HT$_7$ are also labelled by [^{3}H]5-CT. Further pharmacological studies will be necessary to confirm the identity of these sites. Drugs classically considered as 5-HT$_{2C}$ antagonists (mesulergine, lisuride, mianserine, cyproheptadine or ritanserine, but not the 5-HT$_{2A}$ antagonist ketanserin) have been shown to have a relatively high affinity for 5-HT$_7$ receptors; in the absence of really specific agents, these drugs might help to better characterize [^{3}H]5-CT binding sites.

The superficial cortical layers and the midline thalamic nuclei are the only regions where the GTP analogue 5'-β,γ-imidotriphosphate does not inhibit [^{3}H]5-CT binding. This might appear surprising, as 5-HT$_7$ receptors have been shown to be coupled to GTP-binding proteins. However, Szele and Pritchett (1993) have shown recently that agonist binding to 5-HT$_{2A}$ receptors expressed in transfected human embryonic kidney cells was not sensitive to GTP, although binding did activate the second messenger system. 5-HT$_7$ might also have an enhanced sensitivity to oxidation. This process has been shown to inhibit the effect of GTP on hippocampal 5-HT$_{1A}$ receptors (Emerit et al., 1991) and was indeed observed in the present study using [^{3}H]5-CT (see Section 3.3).

In contrast to what is observed in guinea-pig, (+)-butaclamol shows complex competition profile in rats, high affinity sites being observed in the thalamus, cortex, hippocampus and globus pallidus. The nature of the sites in the latter three regions is not clear, as the affinity of clozapine is compatible with the presence of 5-HT$_7$ sites only in rat thalamus. The distribution of non-5-HT$_{1A}$/non-5-HT$_{1B}$ [^{3}H]5-CT binding sites in the rat cortex is not concentrated in the external layers, as it is the case in the guinea-pig. Ruat et al. (1993) describe the presence of 5-HT$_7$ messenger RNA in retrosplenial cortex, hippocampus, medial amygdaloid nucleus, paraventricular thalamus, superior colliculus and raphe nuclei. With the exception of the latter region, non-5-HT$_{1A}$/non-5-HT$_{1B}$ [^{3}H]5-CT binding sites are present in all the regions described, suggesting that these sites might actually correspond to 5-HT$_7$ receptors. It should however be mentioned that the labelled sites observed under those conditions in the septum, hippocampus, superior colliculus, and interpeduncular nucleus could also be accounted for by incompletely blocked 5-HT$_{1A}$ receptors.

Another class of 5-HT receptors, comprising 5-HT$_{5A}$ and 5-HT$_{5B}$ receptors, has been described in the mouse and rat (Erlander et al., 1992; Matthes et al., 1993; Wisden et al., 1993). It has been labelled with [^{3}H]5-CT

in transfected cells and displays a low affinity for sumatriptan (Wisden et al., 1993). In situ hybridisation experiments have shown that the 5-HT_{5A} messenger RNA is expressed in cerebral cortex, hippocampus, habenula, olfactory bulb and cerebellum. 5-HT_{5B} messenger RNA is expressed predominantly in the habenula and in the CA1 field of the hippocampus. 5-HT_{5A} and 5-HT_{5B} receptor genes have been detected in humans, although the latter is a pseudogene (Graihle et al., 1994). It is nevertheless likely that these receptors exist in guinea-pig. Some of the [^{3}H]5-CT binding sites detected in the present study might thus be accounted for by 5-HT_5 receptors. However, 5-HT_{5B} displays a high affinity for dihydroergotamine and is not recognised by spiperone (Wisden et al., 1993); it is thus unlikely that [^{3}H]5-CT binding sites in the cortex and midline thalamic nuclei are accounted for by 5-HT_{5B} receptors. 5-HT_6 receptors have also been shown to have a high affinity for clozapine and other antipsychotic drugs (Monsma et al., 1993). However, considering their low affinity for 5-CT, it is unlikely that they were detected here.

In conclusion, we report here on the visualisation of [^{3}H]5-CT binding sites in the guinea-pig and rat brain. This radioligand labels 5-$HT_{1B/1D}$ receptors, and probably also 5-HT_{1A} and 5-HT_7 receptors. The latter sites are concentrated in the external cortical layers (in guinea-pig) and in the midline thalamic nuclei. It is worth mentioning that in the rat [^{3}H]5-HT-labelled sites in these thalamic nuclei are not displaced by 5-HT_{1A} or 5-HT_{1B} drugs (Pazos and Palacios, 1985), and probably correspond to 5-HT_7 receptors. While the advantage of [^{3}H]5-CT over [^{3}H]5-HT is its low affinity for 5-HT_{1E}, 5-HT_{1F} and 5-HT_{2C} receptors, it still remains a relatively non-selective tool, especially in the absence of selective blockers with high affinity for 5-HT_{1A} and 5-HT_5 receptors. Only a 5-HT_7-selective ligand will allow to study the spatial distribution of 5-HT_7 receptors, especially in areas where they represent a small proportion of total 5-HT receptors.

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