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A receptor autoradiographic and *in situ* hybridization analysis of the distribution of the 5-ht$_7$ receptor in rat brain

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1 Receptor autoradiography and *in situ* hybridization histochemistry have been used to delineate the distribution of the 5-ht$_7$ receptor and its mRNA in rat brain. Receptor autoradiographic studies were performed using [^{3}H]-5-carboxamidotryptamine (5-CT) as the radioligand. The binding characteristics of the masking compounds were determined in Cos-7 cells transfected with a panel of 5-HT receptor subtype cDNAs, including the rat 5-ht$_7$ cDNA. *In situ* hybridization studies were carried out with ^{35}S-labelled oligonucleotide probes to the rat 5-ht$_7$ mRNA.

2 Specific binding of [^{3}H]-5-CT was observed in many areas of the rat brain. Following co-incubation with 1 μM ergotamine, this binding was completely eliminated. After addition of the masking ligands, [^{3}H]-5-CT binding remained in layers 1-3 of cortex, septum, globus pallidus, thalamus, hypothalamus, centromedial amygdala, substantia nigra, periaquaductal gray, and superior colliculus. Addition of the antagonist, methiothepin, to the incubation regimen eliminated most of the remaining [^{3}H]-5-CT binding in the brain, with the exception of the globus pallidus and substantia nigra.

3 The 5-ht$_7$ mRNA was discretely localized in rat brain. The most intense hybridization signals were observed over the thalamus, the anterior hippocampal rudiment, and over the CA3 region of the hippocampus. Other regions containing hybridization signals included the septum, the hypothalamus, the centromedial amygdala and the periaquaductal gray. The regions exhibiting a modest receptor binding signal after methiothepin incubation, the globus pallidus and the substantia nigra, contained no 5-ht$_7$ hybridization signals, suggesting a non-5-ht$_7$ subtype in these two related structures.

4 The distribution of the 5-ht$_7$ receptor and its mRNA is suggestive of multiple roles for this novel 5-HT receptor, within several brain systems. The limbic system (centromedial amygdala, anterior hippocampal rudiment, hypothalamus) is particularly well-represented, indicating a potential role for the 5-ht$_7$ receptor in affective processes.

Keywords: 5-Hydroxytryptamine; receptor; autoradiography; *in situ* hybridization; limbic system

Introduction

The search for new 5-hydroxytryptamine (5-HT) receptor subtypes has yielded impressive results in recent years. The initial division of the 5-HT receptors into the 5-HT$_1$ and 5-HT$_2$ classes has now expanded to seven structurally and pharmacologically distinct classes (Hoyer *et al.*, 1994; Boess & Martin, 1994). The most recent addition to the list is the 5-ht$_7$ receptor, the cloning of which has been reported for man (Bard *et al.*, 1993), rat (Lovenberg *et al.*, 1993; Meyerhof *et al.*, 1993; Ruat *et al.*, 1993; Shen *et al.*, 1993), mouse (Plassat *et al.*, 1993), guinea-pig (Tsou *et al.*, 1994), and *Xenopus* (Nelson *et al.*, 1995). This 5-HT receptor has been shown to be positively coupled to adenylate cyclase activation and to possess a unique pharmacological profile (Bard *et al.*, 1993; Lovenberg *et al.*, 1993; Meyerhof *et al.*, 1993; Ruat *et al.*, 1993; Shen *et al.*, 1993). By *in situ* hybridization histochemistry, 5-ht$_7$ mRNA has been shown to be discretely localized in rat and guinea-pig brain and is most abundant in the thalamus, hippocampus, and hypothalamus (Lovenberg *et al.*, 1993; To *et al.*, 1995). Ligand binding studies have suggested a role for the 5-ht$_7$ receptor in circadian rhythms (Lovenberg *et al.*, 1993; Kawahara *et al.*, 1994), and in the efficacy of antipsychotic and antidepressant compounds (Roth *et al.*, 1994; Sleight *et al.*, 1995).

One particularly useful aspect of 5-ht$_7$ pharmacology is its high affinity for 5-carboxyamidotryptamine (5-CT). The other 5-HT receptors for which this is true are the 5-HT$_{1A}$, 5-HT$_{1B/1D}$, and 5-ht$_{5A/5B}$ receptors (Voigt *et al.*, 1991; Hamblin *et al.*, 1992; Peroutka, 1992; Adham *et al.*, 1993; Bruinvels *et al.*, 1993; Matthes *et al.*, 1993). As such, it is possible to employ the tritiated ligand [^{3}H]-5-CT, in the presence of subtype-selective displacers, to determine the localization of the 5-ht$_7$ receptor. At present, it is unknown if the autoradiographic distribution of the rat 5-ht$_7$ receptor matches that of its mRNA. We have thus compared the distributions obtained using radioligland binding and *in situ* hybridization histochemistry, and have determined that the localization of the 5-ht$_7$ receptor and its mRNA are similar in many, but not all, respects. A preliminary account of these results has been presented previously (Branchek *et al.*, 1994).

Methods

Radioligand binding studies

Membranes from Cos-7 cells transiently expressing the rat 5-ht$_7$ receptor gene were prepared as previously described (Branchek *et al.*, 1990) and [^{3}H]-5-CT binding was conducted according to the [^{3}H]-5-HT binding protocol outlined by Zgombick *et al.* (1991). [^{3}H]-5-CT saturation experiments were conducted to determine the equilibrium dissociation constant (K_d) of this radioligand for the rat 5-ht$_7$ receptor subtype which was used in subsequent autoradiographic studies. Saturation studies were performed with eight concentrations of [^{3}H]-5-CT, ranging from 50 pM to 7 nM. Competition studies were performed with 0.5 nM [^{3}H]-5-CT to determine the affinity of reference compounds acting at 5-HT receptors which were used as masking agents in the 5-ht$_7$ receptor autoradiographic studies. IC$_{50}$ values were converted to K_i values using the Cheng &

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Prusoff equation (1973). Nonspecific binding was defined by 10 μM unlabelled 5-HT. Membranes were incubated for 30 min at 37°C and the reaction terminated by vacuum filtration. Membrane protein concentrations were determined by the method of Bradford (1976) with bovine serum albumin used as standard.

Tissues

Male Sprague-Dawley rats (200–225 g) were purchased from Charles River (Wilmington, MA, U.S.A.). Animals were narcotized with CO_2 and decapitated, after which the brains were immediately removed and frozen in isopentane on dry ice and stored at −80°C until used. For receptor autoradiography, coronal sections through the rat brain were cut at 20 μm on a Hacker-Bright cryostat, thaw-mounted onto gelatin-coated slides, and stored at −20°C until used. For *in situ* hybridization, coronal sections were cut at 11 μm on a cryostat, thaw-mounted on to poly-L-lysine coated slides, and stored at −80°C until used. In addition, cell pellets from transiently transfected Cos-7 cells were frozen, sectioned at 11 μm, and thaw mounted on to poly-L-lysine coated slides for use in the receptor autoradiographic and *in situ* hybridization studies.

Receptor autoradiography

In autoradiographic studies, the agonist [^{3}H]-5-CT was used to label the 5-ht$_7$ as well as other binding sites in a variety of transfected cells and rat brain slices. The concentration of [^{3}H]-5-CT (0.5 nM) used to label the 5-ht$_7$ binding site could also result in the potential labelling of five additional 5-HT receptor subtypes: 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D\alpha}$, 5-ht$_{5A}$, and 5-ht$_{5B}$. Therefore, a number of masking ligands were included to block (or limit) the [^{3}H]-5-CT labelling of most non-5-ht$_7$ sites. Based on the results obtained in the radioligand studies described above, and on previously published data, the following incubation paradigm was devised (see Table 1).

Stock solutions (10^{-3} M) of all reagents were prepared on the day of the experiment. (−)-Pindolol and methiothepin were dissolved in methanol and added to the incubation buffer to give a final concentration of 160 nM and 5–100 nM, respectively. PAPP was dissolved in 100% ethanol, diluted 1:10 in H_2O then added for a final concentration of 30 nM. Ergotamine was dissolved and diluted 1:10 in H_2O then added to a final concentration of 1 μM.

Tissue sections were preincubated in Coplin jars for 15 min at room temperature in 40 ml of a buffer consisting of 50 mM Tris-HCl, 0.5 mM EDTA, 10 mM $MgSO_4$, 10 μM pargyline and 0.1% L-ascorbate. Sections were then incubated in the same buffer containing the radioligand, [^{3}H]-5-CT, at a final concentration 0.5 nM. Total binding was defined as the binding of the ligand in the absence of any drugs/displacers. Nonspecific binding was generated by the addition of ergotamine (1 μM). 5-ht$_7$ selective binding was determined in the following manner. Radioligand binding to 5-HT$_{1A}$ and 5-HT$_{1D\alpha}$ receptors was blocked with the addition of PAPP (30 nM) to the incubation buffer, while binding to the 5-HT$_{1B}$ receptor was blocked by the addition of (−)-pindolol at 160 nM or 1.6 μM. The removal of [^{3}H]-5-CT binding to the 5-ht$_7$ receptor was assessed by the addition of methiothepin at 10 or 100 nM. As 5-CT binds with high affinity to the 5-ht$_{5A}$ receptor (Matthes *et al.*, 1993), and methiothepin binds with moderate affinity to the 5-ht$_{5A}$ and 5-ht$_{5B}$ receptors, these conditions also enabled us to evaluate the binding of the radioligand to the 5-ht$_5$ receptors. In addition to the rat brain tissue sections the incubation conditions described above were also carried out on cryostat sections of pelleted Cos-7 cells which had been transfected with a panel of 5-HT receptor cDNAs.

After incubation, the sections were washed in ice cold (4°C) buffer twice for 5 min then quickly dipped in cold distilled water to remove any salts. The sections were dried in a stream of cool air, and exposed to Hyperfilm-^{3}H (Amersham) for six weeks. Films were developed with Kodak GBX developer and fixer.

In situ *hybridization*

Antisense and sense oligonucleotide probes to nucleotides 1341–1385 in the carboxy tail region of the rat 5-ht$_7$ mRNA (Lovenberg *et al.*, 1993; J. Bard, unpublished observations) were synthesized on a Cyclone Plus DNA synthesizer (Milligen/Biosearch) and gel-purified. The sequence of the sense probe JM71 was 5′ TCA ACC GGA AGC TCT CTG CTG CAG GCA TGC ATG AAG CCC TGA AAC 3′, while that for the antisense probe JM72 was 5′ GTT TCA GGG CTT CAT GCA TGC CTG CAG CAG AGA GCT TCC GGT TGA 3′. The probes were 3′-end labelled with [^{35}S]-dATP (1200 Ci mmol^{-1}, New England Nuclear, Boston, MA, U.S.A.) to a specific activity of 10^9 d.p.m. μg^{-1} using terminal deoxynucleotidyl transferase (Pharmacia; Milwaukee, WI, U.S.A.). The radiolabelled probes were purified on Biospin 6 chromatography columns (Bio-Rad; Richmond, CA), and diluted in hybridization buffer to a concentration of 1.5×10^4 c.p.m. μl^{-1}. The hybridization buffer was composed of 50% formamide, 4 × sodium citrate buffer (1 × SSC = 0.15 M NaCl and 0.015 M sodium citrate), 1 × Denhardt's solution (0.2% polyvinylpyrrolidine, 0.2% Ficoll, 0.2% bovine serum albumin), 50 mM dithiothreitol, 0.5 mg ml^{-1} salmon sperm DNA,

Table 1 Incubation paradigm used in the receptor autoradiographic studies

Incubation conditions	*Potential 5-HT subtypes visualized*	*Cell lines tested*	*Cell lines with signal*
Total binding (0.5 nM [^{3}H]-5-CT)	1A, 1B, 1Dα, 1Dβ 5A, 5B, 7	1A, 1B, 1Dα, 1Dβ 5A, 7	7>1A=1B>5A>1Dα
Ergotamine 1 μM (nonspecific)	None	1A, 1B, 1Dα, 1Dβ 5A, 7	None
PAPP 30 nM + (−)-pindolol 160 nM	5A, 5B, 7	1A, 1B, 1Dα, 1Dβ 5A, 7	7>>1A=1B=5A
PAPP 30 nM + (−)-pindolol 1.6 μM	5A, 5B, 7	1A, 1B, 1Dα, 1Dβ 5A, 7	7>>5A
PAPP 30 nM + (−)-pindolol 1.6 μM + Methiothepin 10 nM	5A, 5B>7	1A, 1B, 1Dα, 1Dβ 5A, 7	7>5A
PAPP 30 nM + (−)-pindolol 1.6 μM + Methiothepin 100 nM	5A, 5B	1A, 1B, 1Dα, 1Dβ 5A, 7	7>5A

Abbreviations: 5-CT, 5-carboxamidotryptamine; PAPP, *p*-Aminophenethyl-*m*-trifluoromethylphenyl piperazine

0.5 mg ml^{-1} yeast tRNA, and 10% dextran sulphate. Tissues were fixed in 4% paraformaldehyde, treated with 5 mM dithiothreitol, acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, delipidated with chloroform, and dehydrated in graded ethanols.

One hundred μl of the radiolabelled probe was applied to each section, which was then covered with a Parafilm coverslip. Hybridization was carried out overnight in humid chambers at 42°C. The following day the sections were washed in two changes of 2 x SSC for 1 h at room temperature, in 0.1 x SSC for 30 min at 50–60°C, and finally in 0.1 x SSC for 30 min at room temperature. Tissues were dehydrated in graded ethanols and apposed to Kodak XAR-5 film for 3 days to 6 weeks at −20°C, then dipped in Kodak NTB3 autoradiography emulsion diluted 1:1 with 0.2% glycerol water. After exposure at 4°C for 2 to 8 weeks, the slides were developed in Kodak D-19 developer, fixed, and counterstained with haematoxylin and eosin.

Probe specificity was established by comparing the hybridization signal obtained in Cos-7 cells transiently transfected with the rat 5-ht$_7$ receptor cDNA to that obtained in non-transfected Cos-7 cells. Both antisense and sense probes were employed according to the procedure described above.

Materials

Compounds were obtained from the following companies: [^{3}H]-5-CT (5-carboxamidotryptamine,[1,2-^{3}H]) (27.9 Ci mmol^{-1}) was obtained from New England Nuclear. 5-Hydroxytryptamine (5-HT), ergotamine tartrate and (−)-pindolol were purchased from Sigma (Saint Louis, MO, U.S.A.). Methiothepin maleate was purchased from BioMol (Plymouth Meeting, PA, U.S.A.), and PAPP (*p*-aminophenethyl-*m*-trifluoromethylphenyl piperazine) and unlabelled 5-CT were supplied by RBI (Natick, MA, U.S.A.).

Results

Radioligand binding in Cos-7 cells

High affinity, saturable [^{3}H]-5-CT binding was observed to membranes harvested from Cos-7 cells transiently expressing the rat 5-ht$_7$ receptor (Figure 1). The equilibrium dissociation constant (K_d) of [^{3}H]-5-CT from saturation studies was 0.28 ± 0.08 nM with a maximal site density (B_{max}) of 49 ± 9 pmol mg^{-1} protein. Specific binding was greater than 98% of total binding at the K_d value. The affinity constants (K_i values) of reference compounds for the rat 5-ht$_7$ receptor were determined in competition studies using [^{3}H]-5-CT. Specific binding was totally displaced in a monophasic manner by this collection of structurally diverse ligands (data not shown). The rank order of compounds to compete for [^{3}H]-5-CT-labelled rat 5-ht$_7$ receptors was consistent with previously published pharmacological values for this recombinant subtype (Ruat *et al.*, 1993; Shen *et al.*, 1993). The K_i values of these reference compounds for the rat 5-ht$_7$ subtype are summarized in Table 2.

Receptor autoradiography

Transfected cells Following the use of the incubation paradigm described above, the autoradiographic signals obtained with [^{3}H]-5-CT in the transfected cell lines followed the expected pattern. Under conditions of total binding, the Cos-7 cells transfected with either human 5-HT$_{1A}$, rat 5-HT$_{1B}$, mouse 5-ht$_{5A}$, or rat 5-ht$_7$ cDNA gave robust autoradiographic signals, all of which were eliminated by co-incubation with 1 μM ergotamine. Cells transfected with the guinea-pig 5-HT$_{1D\alpha}$ receptor exhibited a much lower level of total binding. Cells expressing the 5-ht$_7$ receptor gave the most intense signal, which was unaffected by incubation with 30 nM PAPP and either 160 nM or 1.6 μM (−)-pindolol. In contrast, the binding of [^{3}H]-5-CT to cells expressing the 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D\alpha}$, or 5-ht$_{5A}$ was markedly reduced under these conditions. In-

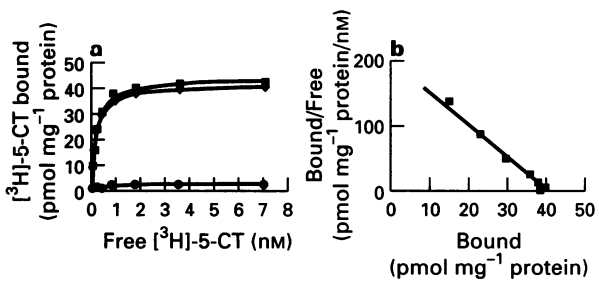

Figure 1 (a) Representative graph of the equilibrium dissocation constant (K_d) of [^{3}H]-5-CT for the recombinant rat 5-ht$_7$ receptor. Membranes were harvested from Cos-7 cells transiently expressing the rat 5-ht$_7$ receptor and incubated with eight concentrations of [^{3}H]-5-CT (50 pM-7 nM). The specific binding curve (◆) was generated by subtracting the nonspecific binding (●), obtained in the presence of 10 μM unlabelled 5-HT for 30 min at 37°C, from the total binding (■). Each data point represents the mean of triplicate determinations. K_d and B_{max} values were determined by nonlinear regression analysis and these values (■) are illustrated in the form of a representative Scatchard plot (b).

Table 2 Affinity (K_i in nM) of ligands used competing for a variety of radioligand binding sites

Receptor	*5-ht$_7$*		*5-HT$_{1A}$*		*5-ht$_{1B}$*	*5-ht$_{1D\alpha}$*	*5-ht$_{5A}$*	*5-ht$_{5B}$*
Compound	clone	tissue[a]	clone	tissue	tissue[f]	tissue[f]	clone[g]	clone[h]
5-CT	0.48	NA	0.85[b]	NA	0.74	0.74	1.4	1.3
5-HT	1.7	1.45	0.51[c]	0.54[c]	NA	NA	42	7.8
Ergotamine	45	5.24	NA	NA	NA	NA	27	31
PAPP	152	NA	NA	2.9[de]	371	9.5	NA	NA
(−)-Pindolol	3260	NA	37[b]	2.38[ge]	6.6	467	4010	>1000
Methiothepin	5.0	NA	79[b]	19.6[e]	NA	NA	92	44

The values shown for the 5-ht$_7$ receptor clone were generated as part of the present study, for the displacement of [^{3}H]-5-CT binding sites.

References for the K_i values cited above and radioligand used: [a]Sleight *et al.* (1995), rat hypothalamus, [^{3}H]-5-CT; [b]Boddeke *et al.* (1992), human clone, [^{3}H]-8-OH-DPAT; [c]Fujiwara *et al.* (1993), rat clone and rat hippocampus, [^{3}H]-5-HT; [d]Ransom *et al.* (1986), rat cortex, [^{3}H]-5-HT; [e]Emerit *et al.* (1991), rat hippocampus, [^{3}H]-8-OH-DPAT; [f]Bruinvels *et al.* (1993), rat brain, [^{125}I]-GTI; [g]Hen, personal communication, mouse clone [^{3}H]-5-CT; [h]Wisden *et al.* (1993), rat clone, [^{3}H]-5-CT. NA, not available; PAPP, *p*-Aminophenethyl-*m*-trifluoromethylphenyl piperazine.

cubation of the 5-ht$_7$-transfected cells with 100 nM methiothepin resulted in a significant loss of autoradiographic signal. These results indicate that the [^{3}H]-5-CT binding obtained in rat brain tissue sections, under identical masking conditions, is largely the result of binding to 5-ht$_7$ receptors.

Rat brain The total binding observed with [^{3}H]-5-CT was found in many areas of the rat brain. Regions exhibiting the highest density of signal were the septum, hippocampus (Figure 2a), deep layers of cortex, hypothalamus, globus pallidus, substantia nigra, and the dorsal horn of the cervical spinal cord. Other areas with significant [^{3}H]-5-CT binding included the thalamus, the amygdala, the dorsal raphe and raphe magnus, the periaquaductal gray, the superior colliculus, and the nucleus of the solitary tract. Incubation with 1 μM ergotamine completely displaced [^{3}H]-5-CT binding from rat brain sections (Figure 2b). Following incubation with the radioligand containing 30 nM PAPP and 160 nM (−)-pindolol, much of the [^{3}H]-5-CT binding in the brain was eliminated (Figure 2c). The binding which remained is what we have considered to be the combined binding to the 5-ht$_7$, as well as to the 5-ht$_{5A}$, and 5-ht$_{5B}$ receptor subtypes. As the affinity of 5-CT for the 5-ht$_{5A}$ and 5-ht$_{5B}$ receptors is 3 fold lower than that for the 5-ht$_7$ receptor, and as the radioligand was used at the K_D for 5-ht$_7$, we believe this to represent mainly the binding of [^{3}H]-5-CT to the 5-ht$_7$ receptor. Addition of either 10 nM or 100 nM methiothepin to the incubation paradigm (including PAPP and (−)-pindolol) eliminated the binding of [^{3}H]-5-CT to most of the brain (Figure 2d). The two areas in which some residual binding was detected were the globus pallidus and the substantia nigra, pars reticulata. Increasing the concentration of (−)-pindolol from 160 nM to 1.6 μM had no effect on the density of [^{3}H]-5-CT binding in rat brain sections.

The left side of the schematic diagrams in Figure 3 (a–k) and Tables 3–5 show the distribution of the autoradiographic signal remaining after incubation of tissue sections with 0.5 nM [^{3}H]-5-CT, 30 nM PAPP, and 160 nM (−)-pindolol. Telencephalic regions containing the most intense autoradiographic signals were the lateral and medial septum, and the medial nucleus of the amygdala. Moderate labelling was observed in the superficial layers of cortex, globus pallidus, substantia innominata, and posterior ventral regions of the hippocampus proper and subiculum. Weak labelling was seen in the cingulate, retrosplenial and piriform cortices, and in the taenia tecta, the entire hippocampus, and amygdalohippocampal region.

In the thalamus, the strongest signals were observed in the anterior ventral nucleus, paraventricular nucleus, centromedial nucleus, rhomboid nucleus, and intermediodorsal nucleus (Figures 2 and 3). Moderate labelling was seen in the anterodorsal, anteromedial, laterodorsal, reuniens, and paracentral thalamic nuclei. The mediodorsal nucleus, centrolateral, lateral geniculate, and medial geniculate nuclei were weakly labelled with [^{3}H]-5-CT. In the hypothalamus, the medial preoptic

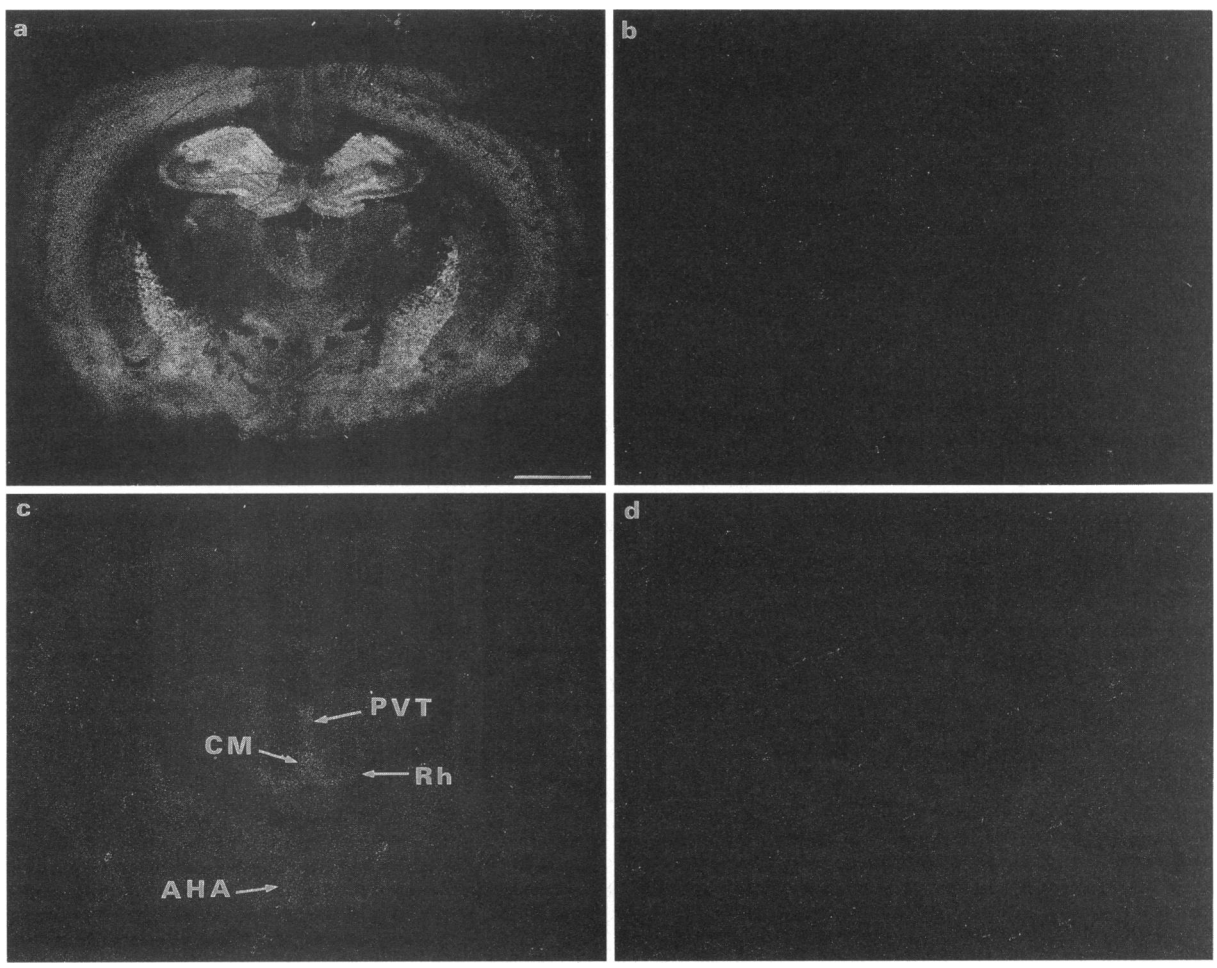

Figure 2 Autoradiographic localization of [^{3}H]-5-CT binding seen in coronal rat brain sections under various incubation conditions. These are reversal photomicrographs of the original Amersham ^{3}H Hyperfilms apposed to the tissue sections. (a) Total binding obtained with 0.5 nM [^{3}H]-5-CT. Strong signals are observed in many regions. (b) Nonspecific binding observed in the presence of 1 μM ergotamine. (c) [^{3}H]-5-CT binding observed after incubation with 30 nM PAPP and 160 nM (−)-pindolol. The most prominent binding which remains is in midline thalamic nuclei (PVT, Rh, CM). (d) An identical section to that in (c), with the addition of 10 nM methiothepin. Little binding remains under these conditions. Marker bar in (a) = 2 mm and applies to all panels.

Table 3 Summary table of 5-ht$_7$ receptor and mRNA localization in rat telencephalon

Region	*Abbreviation*	*[^{3}H]-5-CT binding*[a]	*Hybridization signal*[b]	*Figure 3 reference*
Cortex				
layers 1–3	Cx	+ +	+	a–h
piriform	Pir	+	+ +	a–e
cingulate	ACA	+	+ +	c
retrosplenial	RSP	+	+	e,f
Septal area				
lateral septum	LS	+ + +	+	b
medial septum	MS	+ + +	+	b
diagonal band n.	HDB	+	+	b
Hippocampus				
tenia tecta	TT	+	+ +	a
indusium griseum	IG	+	+ +	a
CA3	CA3	+	+ + +	d–g
parasubiculum	PaS	+	+ +	h
Amygdala				
substantia innominata	SI	+ +	–	c
medial n., dorsal	Me	+ + +	+	e
medial n., posterial ventral	MePV	+	+ +	e
amygdalohippocampal area	AHi	+	+ +	f
Basal ganglia				
globus pallidus	GP	+ +	–	c,d

[a]Qualitative evaluation by two observers of [^{3}H]-5-CT binding density on films, with masks included. Density was graded as low (+), moderate (+ +), or intense (+ + +).
[b]Qualitative evaluation by two observers of hybridization intensity as low (+), moderate (+ +) or intense (+ + +).

Table 4 Summary table of 5-ht$_7$ receptor and mRNA localization in rat diencephalon

Region	*Abbreviation*	*[^{3}H]-5-CT binding*[a]	*Hybridization signal*[b]	*Figure 3 reference*
Thalamus				
anterior ventral n.	AV	+ + +	+ + +	c
anterior dorsal n.	AD	+ +	+ + +	c,d
anterior medial n.	AM	+ +	+ + +	c
paraventricular n.	PVT	+ + +	+ + +	c–e
paratenial n.	PT	+	+	c
centromedial n.	CM	+ + +	+ +	d,e
paracentral n.	PC	+ +	+ +	d
rhomboid n.	Rh	+ + +	+ + +	d
reuniens n.	Re	+ +	+	d
mediodorsal n.	MD	+	+	d
intermediodorsal n.	IMD	+ + +	+ +	e
ventral nuclei	VL	–	+	d
centrolateral n.	CL	+	+	e
lateral dorsal n.	LD	+	+	e
dorsal lateral geniculate n.	DLG	+	+	f
ventral lateral geniculate n.	VLG	+	+	f
medial geniculate n.	MG	+	+	g
Hypothalamus				
medial preoptic nucleus	MPO	+ +	+ +	c
anterior hypothalamic area	AHA	+ +	+	d
paraventricular n.	PVH	+	–	d
dorsomedial n.	DMH	+	+	e
ventromedial n.	VMH	+	+	e
arcuate n.	Arc	+	+	e
medial mammillary n.	MM, ML	+ +	+ +	f
supramammillary n.	SuM	+ +	+ + +	f

[a]Qualitative evaluation by two observers of [^{3}H]-5-CT binding density on films, with masks included. Density was graded as low (+), moderate (+ +), or intense (+ + +).
[b]Qualitative evaluation by two observers of hybridization intensity as low (+), moderate (+ +) or intense (+ + +).

nucleus and the anterior hypothalamic area were moderately labelled with [^{3}H]-5-CT, as were the medial mammillary and supramammillary nuclei. The remainder of the hypothalamic nuclei were lightly labelled.

In the midbrain, moderate signals were observed in the superficial gray of the superior colliculus, and the substantia nigra, pars reticulata. Incubation with [^{3}H]-5-CT and the masks also resulted in weak labelling in the periaquaductal

Table 5 Summary table of 5-HT$_7$ receptor and mRNA localization in rat midbrain, pons, medulla and spinal cord

Region	*Abbreviation*	*[^{3}H]-5-CT binding*[a]	*Hybridization signal*[b]	*Figure 3 reference*
Midbrain, pons, medulla				
superior colliculus	SC	+ +	+	g
dorsal raphe	DR	+	–	g
substantia nigra	SNr	+ +	–	g
periaquaductal gray	PAG	+	+	g,h
pontine n.	Pn	+	+ +	h
dorsal tegmental n.	DTg	+	+ +	i
n. solitarius	NST	+	+	j
Spinal cord				
dorsal horn, lamina 1 and 2	DH, I,II	+	–	k

[a]Qualitative evaluation by two observers of [^{3}H]-5-CT binding density on films, with masks included. Density was graded as low (+), moderate (+ +), or intense (+ + +).
[b]Qualitative evaluation by two observers of hybridization intensity as low (+), moderate (+ +) or intense (+ + +).

gray, and in the dorsal raphe. In the pons and medulla, weak autoradiographic signals were seen in the pontine central gray, dorsal tegmental nucleus, and nucleus of the solitary tract, while in the spinal cord a moderate level of signal remained in the dorsal horn at all spine levels.

In situ *hybridization*

Controls In Cos-7 cells transiently transfected with the rat 5-ht$_7$ cDNA, hybridization with the radiolabelled antisense probes resulted in a striking signal which was visible in approximately 50% of the cells (Figure 4a). In contrast, no signal was detected after incubation of the transfected cells with radiolabelled sense probe (Figure 4b), nor did the antisense probe give any signal when incubated with mock-transfected Cos-7 cells (data not shown). In the brain sections, hybridization with the antisense probe resulted in moderately intense signals in some regions (Figure 4c). The only area labelled after incubation with the sense probe was the dentate gyrus of the hippocampus (Figure 4d).

Rat brain localization Cells exhibiting hybridization signal for 5-ht$_7$ mRNA were found in a number of rat brain regions (Figure 3a′–k′ and Tables 3–5). In all areas, the localization appeared to be restricted to neurones, although no concerted effort was made to visualize glial cells. In telencephalic regions, the most intense labelling was observed over neurones in the CA3 region of the hippocampus (Figure 4c), which was visible at all rostrocaudal levels. Moderate hybridization signals were seen over neurones in piriform cortex, in the granular layer of cingulate and retrosplenial cortices, and in the induseum griseum, taenia tecta, and parasubiculum. The posterior ventral region of the medial amygdaloid nucleus also contained a number of moderately labelled neurones, as did the amygdalohippocampal transition area. Weakly labelled neurones were observed in layer 2 of the dorsal neocortex (sensorimotor region), in the CA1 and CA2 regions of the hippocampus, in the lateral and medial septum, horizontal nucleus of the diagonal band, and in the dorsal region of the medial amygdaloid nucleus.

In the thalamus, most nuclei contained neurones with some level of hybridization signal (Figures 3 and 4c). The most prominent signals were observed over the paraventricular thalamic nucleus, and over the anterodorsal, anteroventral, anteromedial and rhomboid nuclei. Moderate hybridization signals were observed over neurones in the centromedial, intermediodorsal, and paracentral nuclei. Most of the remaining thalamic nuclei, including the paratenial, rhomboid, ventrolateral, lateral dorsal, and mediodorsal nuclei, the nucleus reuniens, and dorsal, ventral lateral, and medial geniculate nuclei contained scattered neurones with low numbers of silver grains over them.

In the hypothalamus, the most intense hybridization signals were localized to the medial preoptic nucleus (Figure 4c), and to the supramammillary and medial mammillary nuclei. Relatively weak labelling was seen over the anterior hypothalamic area, the lateral hypothalamus, the arcuate nucleus, the dorsomedial nucleus, and the ventral lateral subdivision of the ventromedial nucleus. One noteworthy aspect of the hypothalamic localization of the 5-ht$_7$ mRNA was the absence of signal in the suprachiasmatic nucleus (SCN). There were, however, weakly labelled neurones in the immediate vicinity of the dorsolateral aspect of the SCN, in the anterior hypothalamic area.

Few areas in the mesencephalon, pons, and medulla contained hybridization signals indicative of 5-ht$_7$ mRNA. Both the pontine nuclei and the dorsal tegmental nucleus contained neurones which were moderately labelled, while weak hybridization signals were present over the lateral portion of the periaquaductal gray, over the superficial gray layer of the superior colliculus, and over the nucleus of the solitary tract.

Discussion

The present study has defined the distribution of the rat 5-ht$_7$ receptor by both receptor autoradiographic and *in situ* hybridization techniques. Our *in situ* hybridization observations on mRNA localization confirm and extend those of previous investigators (Lovenberg *et al.*, 1993; Meyerhof *et al.*, 1993; Ruat *et al.*, 1993; Shen *et al* 1993; Tsou *et al.*, 1994; To *et al.*, 1995). Taken together with the receptor autoradiographic studies obtained with [^{3}H]-5-CT, our results indicate that distributions of the 5-ht$_7$ receptor and mRNA overlap in many regions. The regions exhibiting the strongest concordance between receptor and mRNA expression include superficial layers of neocortex, the lateral septum, the hypothalamus, some nuclei of the dorsal and midline thalamus, and parts of the hippocampal formation. The globus pallidus, substantia innominata, and substantia nigra exhibited [^{3}H]-5-CT binding but no 5-ht$_7$ mRNA. In two of these nuclei, the globus pallidus and substantia nigra, appreciable [^{3}H]-5-CT binding remained after incubation with methiothepin, suggesting that these binding sites may not represent 5-ht$_7$ receptors. This conclusion is supported by the observation that none of the major regions projecting into the globus pallidus and substantia nigra (e.g. the caudate-putamen) contain 5-ht$_7$ mRNA.

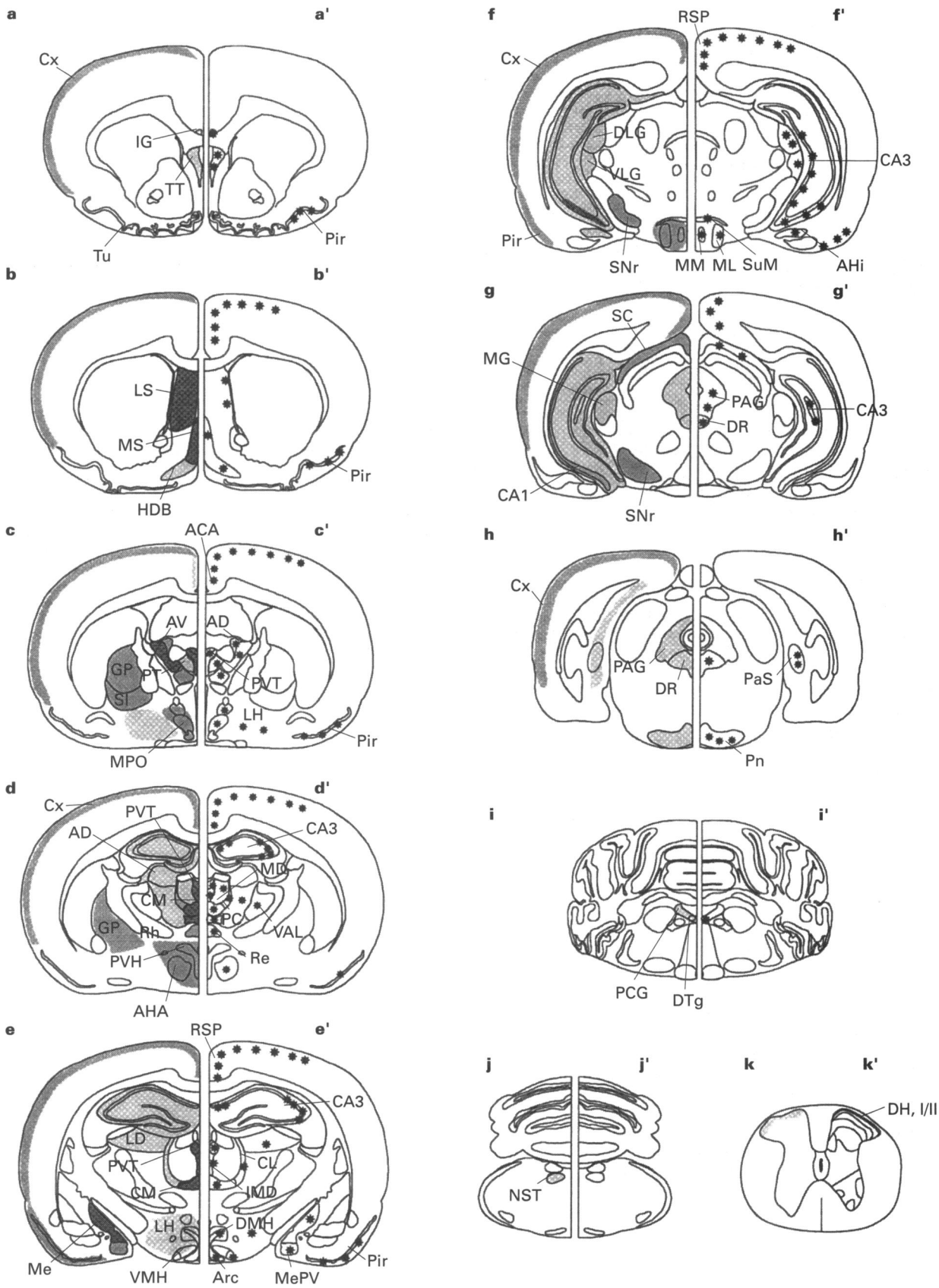

Figure 3 Schematic diagrams showing the distribution of the putative 5-ht$_7$ receptor and the 5-ht$_7$ mRNA, in a rostrocaudal series of coronal sections through the rat brain. The left hand side (a–k) of each drawing illustrates the distribution of [^{3}H]-5-CT binding after incubation with 30 nM PAPP and 160 nM (−)-pindolol. The graded shading of each nucleus is a qualitative indication of the relative intensity of the autoradiographic signal observed on films. The right hand side (a′–k′) of each diagram shows the distribution of 5-ht$_7$ mRNA determined from emulsion-coated sections of *in situ* hybridization material. Asterisks indicate the distribution of labelled cells. Abbreviations are given in Tables 3-5.

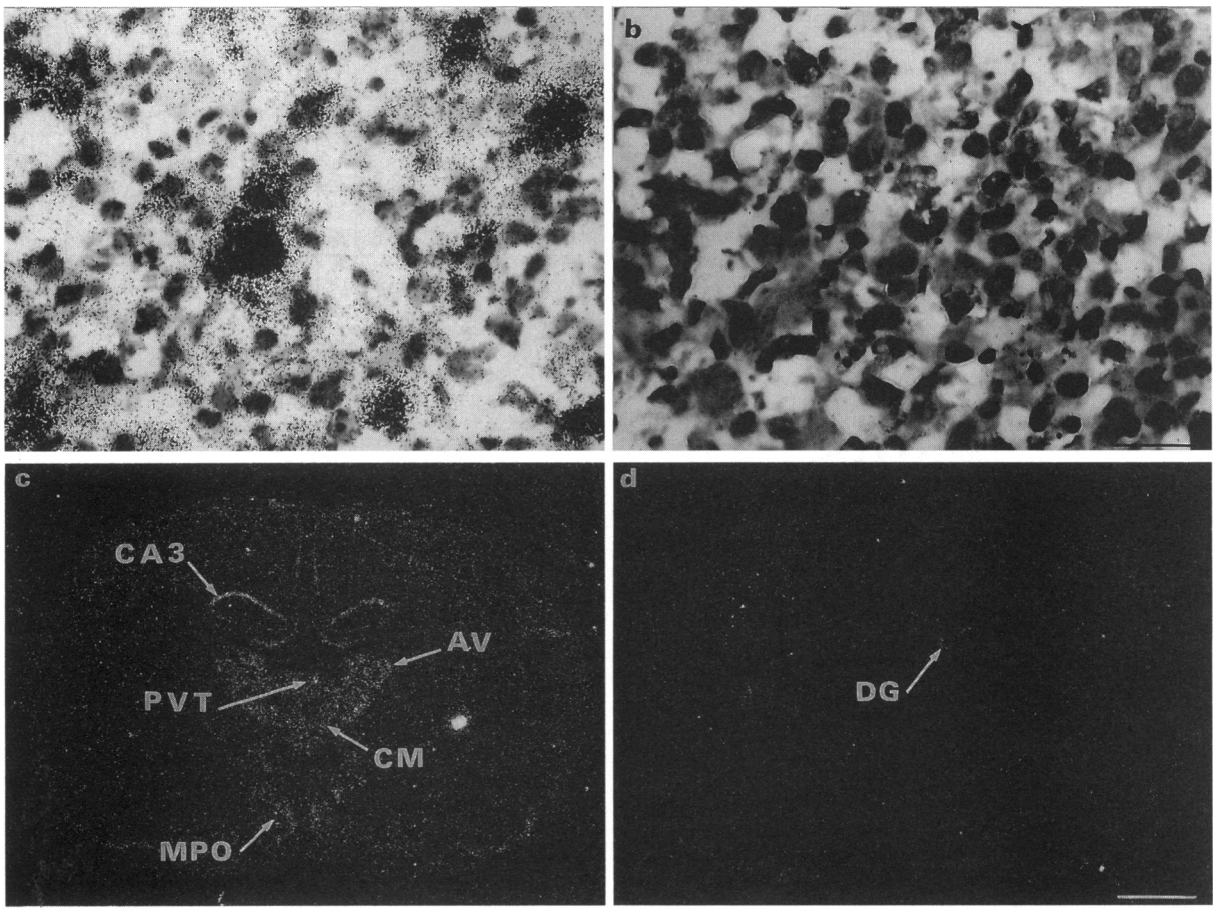

Figure 4 Photomicrographs showing the hybridization signals obtained with ^{35}S-labelled oligonucleotide probes to the rat 5-ht$_7$ receptor mRNA. (a) Antisense probe hybridization in transiently transfected Cos-7 cells expressing the 5-ht$_7$ receptor. Dense accumulations of silver grains are present over cells expressing very high amounts of the mRNA. (b) No signal is observed after incubation of the transfected cells with the radiolabelled sense probe. Marker bar = 50 μm and applies to (a) and (b). (c) Hybridization signal obtained in a coronal section of rat brain after incubation with the labelled antisense probe. At this level, the most intense signals are seen in the thalamus, hippocampus (CA3), and in the medial preoptic area of the hypothalamus. (d) A matching section to that in (c), showing the lack of hybridization signal in all brain regions except the dentate gyrus of the hippocampus (DG), after incubation with the sense probe. Marker bar = 2 mm and applies to (c) and (d).

Comparison with published results and 5-HT subtype exclusion

Several recent reports have described similar results in guinea-pig and rat brain (To *et al.*, 1995; Waeber & Moskowitz, 1995). Using [^{3}H]-5-CT and oligonucleotide probes selective for the guinea-pig 5-ht$_7$, To *et al.* (1995) report substantially the same localization of the 5-ht$_7$ receptor/mRNA as we have seen in the rat. Thus, in the guinea-pig as in the rat, the thalamus contains the highest density of 5-ht$_7$ receptor and mRNA, the cortical labelling is mainly restricted to superficial layers, and the midbrain/hindbrain contains little or no signal of either type. The only apparent discrepancy appears to be in the amygdala, where To *et al.* (1995) report localization of both 5-ht$_7$ receptor and mRNA in the basolateral and basomedial nuclei. We do not see either receptor or mRNA in these areas; however, this may reflect an issue of abundance rather than the absolute lack of 5-ht$_7$ in these regions. Interestingly, To *et al.* (1995) also appear to have residual binding in the guinea-pig globus pallidus and substantia nigra which is not accompanied by 5-ht$_7$ mRNA.

Waeber & Moskowitz (1995) have carried out similar autoradiographic studies with [^{3}H]-5-CT, albeit using different blockers. In their study of guinea-pig and rat brain, they used 100 nM 8-OH-DPAT to block 5-HT$_{1A}$ receptors, and 100 nM GR 127935 to block the 5-HT$_{1D}$ receptors. Under these conditions, the [^{3}H]-5-CT binding in rat brain is very similar to that obtained in the present study. Thus, binding sites remain in the lateral septum, thalamus, medial amygdala, hippocampus, and hypothalamus. We specifically avoided using 8-OH-DPAT in our studies due to the reported affinity of this compound for the 5-ht$_7$ receptor (Ruat *et al.*, 1993; Shen *et al.*, 1993), and thus the 5-ht$_7$ population may be underrepresented in the study of Waeber & Moskowitz (1995). However, the main difference between the current study and that of Waeber & Moskowitz (1995) is the interpretation of the [^{3}H]-5-CT binding which remains after blockade of the 5-HT$_{1A/1B/1D}$ receptors. Our interpretation (and see below) is that, with the exception of the globus pallidus and substantia nigra, the remainder of the binding is due to the 5-ht$_7$ receptor. Waeber & Moskowitz (1995) suggest instead that the [^{3}H]-5-CT binding after blockade, in all regions except the thalamus, may be due to binding to other 5-HT receptors, mainly the 5-HT$_{1A}$ receptor. In their study, there was little binding which remained in the globus pallidus and substantia nigra, suggesting that they achieved better blockade of 5-HT$_{1D}$ receptors than in the present study. In any case, it is clear that better pharmacological tools are needed to establish the exact location of the 5-ht$_7$ receptor in brain.

In addition to the 5-ht$_7$ receptor, a number of other rat 5-HT receptor subtypes have been reported to bind 5-CT with high affinity (for reviews see Hoyer *et al.*, 1994; Saudou & Hen, 1994). These include 5-HT$_{1A}$ (Bodekke *et al.*, 1992), 5-HT$_{1B}$ (Voigt *et al.*, 1991; Hamblin *et al.*, 1992; Adham *et al.*, 1993;

Bruinvels *et al.*, 1993), 5-HT$_{1D\alpha}$ (Hamblin *et al.*, 1992; Bach *et al.*, 1993); and 5-ht$_{5A/5B}$ receptors (Erlander *et al.*, 1993; Wisden *et al.*, 1993). The total binding of [^{3}H]-5-CT obtained in the receptor autoradiographic studies is consistent with the labelling of a large population of 5-HT receptors, and this population would appear to consist mainly of 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D\alpha}$ receptors. Based on previously published observations, the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors should be removed by the concentrations of PAPP (Ransom *et al.*, 1986) and (−)-pindolol (Bruinvels *et al.*, 1993), respectively, used in the present study. This would appear to be the case, as increasing the concentration of (−)-pindolol from 160 nM to 1.6 μM has no further effect on the density of [^{3}H]-5-CT binding in the rat brain. The 5-HT$_{1D\alpha}$ receptor should also be removed by PAPP (Bruinvels *et al.*, 1993). The binding which remains in the globus pallidus and substantia nigra after methiothepin treatment may reflect an incomplete blockade of the 5-HT$_{1D\alpha}$ receptor; however the cloned 5-HT$_{1D\alpha}$ receptor reportedly has a higher affinity for methiothepin (Hamblin *et al.*, 1992) than the 5-ht$_7$ receptor. Other observations indicating that the 5-ht$_7$ receptor population in rat brain is represented by the [^{3}H]-5-CT binding which remains after incubation with (−)-pindolol and PAPP include; (1) the receptor binding and 5-ht$_7$ mRNA distribution are closely correlated; (2) in groups of Cos-7 cells transfected with genes for individual 5-HT receptors, only cells transfected with the 5-ht$_7$ receptor exhibited autoradiographic signal with [^{3}H]-5-CT following use of the masking paradigm; and (3) as in the rat brain, use of the antagonist methiothepin eliminated much of the [^{3}H]-5-CT binding in the 5-ht$_7$-transfected cells.

Given the inevitable caveats associated with receptors with high B_{max}, the receptors which should remain are the 5-ht$_7$ and 5-ht$_{5A/5B}$ receptors. As incubation with methiothepin eliminates virtually all of the remaining [^{3}H]-5-CT binding, and the 5-ht$_7$ receptor has a higher affinity for both [^{3}H]-5-CT and methiothepin than do the 5-ht$_{5A/5B}$ receptors, we have attributed the remaining binding mostly to occupancy of the 5-ht$_7$ receptor. While no selective ligands exist for the 5-ht$_{5A/5B}$ receptors, *in situ* hybridization studies indicate that the distribution of the 5-ht$_{5A}$ and 5-ht$_{5B}$ mRNAs are more widespread than the distribution of [^{3}H]-5-CT would suggest (Erlander *et al.*, 1993; Matthes *et al.*, 1993; Wisden *et al.*, 1993).

Functional considerations

The 5-ht$_7$ receptor has been demonstrated to be positively coupled to adenylate cyclase (Bard *et al.*, 1993; Lovenberg *et al.*, 1993; Ruat *et al.*, 1993; Shen *et al.*, 1993; Tsou *et al.*, 1994), and it has been suggested that it is involved in physiological processes previously attributed to other 5-HT receptors. For example, 5-HT has been suggested as being involved in some aspect of circadian rhythm regulation (Morin *et al.*, 1990; Inouye & Shibata, 1994), based in large part on the 5-hydroxytryptaminergic innervation of the suprachiasmatic nucleus (SCN), which is the endogenous circadian pacemaker in mammalian brain (Moore, 1983). However, the issue of which receptor subtype(s) are involved in the phase-shifts of behaviour and SCN neuronal activity seen with 5-HT receptor agonists like 8-OH DPAT (Prosser *et al.*, 1990; Medanic & Gillette, 1992), has been largely unresolved. It has been suggested that a postsynaptic 5-HT$_{1A}$ receptor in the SCN is involved (Inouye & Shibata, 1994); however, there is little evidence for the localization of this subtype in the SCN (Roca *et al.*, 1993). Recent studies indicate that the pharmacological profile of 5-HT-related phase shifts correlates well with the pharmacological profile of the 5-ht$_7$ receptor (Lovenberg *et al.*, 1993; Kawahara *et al.*, 1994). However, neither the 5-ht$_7$ receptor nor its mRNA has been localized to the SCN (Lovenberg *et al.*, 1993; present study), so a firm conclusion concerning the involvement of the 5-ht$_7$ receptor in circadian rhythms is not yet possible. The localization of 5-ht$_7$ mRNA to the anterior hypothalamic area, immediately lateral and dorsal to the SCN, suggests the possibility that (1) the receptor could be localized to neurones whose dendritic fields extend into the SCN, and/or (2) that these anterior hypothalamic neurones have axonal projections into the SCN. Similar observations have been made for the guinea-pig SCN (To *et al.*, 1995).

Several reports have suggested that the 5-ht$_7$ receptor is involved in the effect of antipsychotic and/or antidepressant treatment (Roth *et al.*, 1994; Sleight *et al.*, 1995). The localization of this receptor and its mRNA in the septum, hypothalamus and amygdala further suggest a role for the 5-ht$_7$ receptor in so-called limbic processes. Chronic antidepressant treatment with the 5-HT uptake blocker, fluoxetine, significantly reduces the binding of [^{3}H]-5-HT to the 5-ht$_7$ receptor in the hypothalamus (Sleight *et al.*, 1995), suggesting a potential role of the 5-ht$_7$ receptor in depression. It will be interesting to ascertain if this is a specific effect related to a 5-HT receptor, or a non-specific side effect of chronic antidepressant treatment. Typical and atypical antipsychotics such as pimozide and clozapine, respectively, exhibit a high affinity for the 5-ht$_7$ receptor, suggesting that the therapeutic actions of these drugs may be exerted partially via this receptor (Roth *et al.*, 1994). It is important to keep in mind that both pimozide and clozapine have even higher affinities for other 5-HT (5-HT$_{2A}$), and non-5-HT (dopamine D$_2$ and α_1-adrenoceptor) receptors. A clearer picture of the involvement of the 5-ht$_7$ receptor in these processes awaits further study, either by pharmacological means or by well-designed and implemented antisense experiments.

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References

ADHAM, N., ELLERBROOK, B., HARTIG, P., WEINSHANK, R.L. & BRANCHEK, T. (1993). Receptor reserve masks partial agonist activity of drugs in a cloned rat 5-hydroxytryptamine$_{1B}$ receptor expression system. *Mol. Pharmacol.*, **43**, 427–433.

BACH, A.W.J., UNGER, L., SPRENGEL, R., MENGOD, G., PALACIOS, J., SEEBURG, P.H. & VOIGT, M.M. (1993). Structure functional expression and spatial distribution of a cloned cDNA encoding a rat 5-HT1D-like receptor. *J. Recept. Res.*, **13**, 479–502.

BARD, J.A., ZGOMBICK, J., ADHAM, N., VAYSSE, P., BRANCHEK, T.A. & WEINSHANK, R.L. (1993). Cloning of a novel human serotonin receptor (5-HT$_7$) positively linked to adenylate cyclase. *J. Biol. Chem.*, **268**, 23422–23426.

BODDEKE, H.W.G.M., FARGIN, A., RAYMOND, J.R., SCHOEFFTER, P. & HOYER, D. (1992). Agonist/antagonist interactions with cloned human 5-HT$_{1A}$ receptors: Variations in intrinsic activity studied in transfected HeLa cells. *Naunyn Schmied. Arch. Pharmacol.*, **345**, 257–263.

BOESS, F.G. & MARTIN, I.L. (1994). Molecular biology of 5-HT receptors. *Neuropharmacology*, **33**, 275–317.

BRADFORD, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 23422–23426.

BRANCHEK, T.A., ADHAM, N., MACCHI, M.J., KAO, H.T. & HARTIG, P.R. (1990). [3H]DOB (4-bromo-2,5-dimethoxy-phenylpropylamine) and [^{3}H]ketanserin label two affinity states of the cloned 5-hydroxytryptamine$_2$ receptor. *Mol. Pharmacol.*, **38**, 604–609.

BRANCHEK, T.A., GUSTAFSON, E.L., DURKIN, M.M, BARD, J.A. & WEINSHANK, R.L. (1994). Autoradiographic localization of 5-HT7 and its mRNA in rat CNS by radioligand binding and *in situ* hybridization histochemistry. *Br. J. Pharmacol.*, **112**, 100P.

BRUINVELS, A.T., PALACIOS, J.M. & HOYER, D. (1993). Autoradiographic characterisation and localisation of 5-HT$_{1D}$ compared to 5-HT$_{1B}$ binding sites in rat brain. *Naunyn Schmied. Arch. Pharmacol.*, **347**, 569–582.

CHENG, Y.C. & PRUSOFF, W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.*, **22**, 3099–3108.

EMERIT, M.B., MIQUEL, M.C., GOZLAN, H. & HAMON, M. (1991). The GTP-insensitive component of high-affinity [^{3}H]8-hydroxy-2-(di-n-propylamino)tetralin binding in the rat hippocampus corresponds to an oxidized state of the 5-hydroxytryptamine$_{1A}$ receptor. *J. Neurochem.*, **56**, 1705–1716.

ERLANDER, M.G., LOVENBERG, T.W., BARON, B.M., DE LECEA, L., DANIELSON, P.E., RACKE, M., SLONE, A.L., SIEGEL, B.W., FOYE, P.E., CANNON, K., BURNS J.E. & SUTCLIFFE, J.G. (1993). Two members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat brain. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 3452–3456.

FUJIWARA, Y., TOMITA, H., HIKIJI, M., KASHIHARA, K., OTSUKI, S., OHNUKI, T., HAMAGISHI, Y., OKI, T., SORA, I., ROESKE, W.R & YAMAMURA, H.I. (1993). Characterization of a cloned rat serotonin 5-HT1A receptor expressed in the HeLa cell line. *Life Sci.*, **52**, 949–958.

HAMBLIN, M.W., MCGUFFIN, R.W., METCALF, M.A., DORSA, D.M. & MERCHANT, K.M. (1992). Distinct 5-HT$_{1B}$ and 5-HT$_{1D}$ serotonin receptors in rat: structural and pharmacological characterization of the two cloned receptors. *Mol. Cell. Neurosci.*, **3**, 578–587.

HOYER, D., CLARKE, D.E., FOZARD, J.R., HARTIG, P.R., MARTIN, G.R., MYLECHARANE, E.J., SAXENA, P.R. & HUMPHREY, P.P.A. (1994). VII. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.*, **46**, 157–204.

INOUYE, S.-I.T. & SHIBATA, S. (1994). Neurochemical organization of circadian rhythm in the suprachiasmatic nucleus. *Neurosci. Res.*, **20**, 109–130.

KAWAHARA, F., SAITO, H. & KATSUKI, H. (1994). Inhibition by 5-HT$_7$ receptor stimulation of GABA$_A$ receptor-activated current in cultured rat suprachiasmatic neurones. *J. Physiol.*, **478**, 67–73.

LOVENBERG, T.W., BARON, B.M., DE LECEA, L., MILLER, J.D., PROSSER, R.A., REA., M.A., FOYE, P.E., RACKE, M., SLONE, A.L., SIEGEL, B.W., DANIELSON, P.E., SUTCLIFFE, J.G. & ERLANDER, M.G. (1993). A novel adenylyl cyclase-activating serotonin receptor (5-HT$_7$) implicated in the regulation of mammalian circadian rhythms. *Neuron*, **11**, 449–458.

MATTHES, H., BOSCHERT, U., AMLAIKY, N., GRAILHE, R., PLASSAT, J.L., MUSCATELLI, F., MATTEL, M.G. & HEN, R. (1993). Mouse 5-hydroxytryptamine$_{5A}$ and 5-hydroxytryptamine$_{5B}$ receptors define a new family of serotonin receptors: Cloning, functional expression, and chromosomal localization. *Mol. Pharmacol.*, **43**, 313–319.

MEDANIC, M. & GILLETTE, M.U. (1992). Serotonin regulates the phase of the rat suprachiasmatic circadian pacemaker in vitro only during the subjective day. *J. Physiol.*, **450**, 629–642.

MEYERHOF, W., OBERMUELLER, F., FEHR, S. & RICHTER, D. (1993). A novel rat serotonin receptor: Primary structure, pharmacology, and expression pattern in distinct brain regions. *DNA Cell. Biol.*, **12**, 401–409.

MOORE, R.Y. (1983). Organization and function of a central nervous system circadian oscillator. The suprachiasmatic hypothalamic nucleus. *Fed. Proc.*, **42**, 2783–2789.

MORIN, L.P., MICHELS, K.M., SMALE, L. & MOORE, R.Y. (1990). Serotonin regulation of circadian rhythmicity. *Ann. N.Y. Acad. Sci.*, **600**, 418–426.

NELSON, C.S., CONE, R.D., ROBBINS, L.S., ALLEN, C.N. & ADELMAN, J.P. (1995). Cloning and expression of a 5HT7 receptor from Xenopus laevis. *Receptors Channels*, **3**, 61–70.

PEROUTKA, S.J. (1992). Phylogenetic tree analysis of G protein-coupled 5-HT receptors: Implications for receptor nomenclature. *Neuropharmacology*, **31**, 609–613.

PLASSAT, J.-L., AMLAIKY, N. & HEN, R. (1993). Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase. *Mol. Pharmacol.*, **44**, 229–236.

PROSSER, R.A., MILLER, J.D. & HELLER, H.C. (1990). A serotonin agonist phase-shifts the circadian clock in the suprachiasmatic nuclei in vitro. *Brain Res.*, **534**, 336–339.

RANSOM, R.W., ASARCH, K.B. & SHIH, J.C. (1986). [^{3}H]1-[2-(4-aminophenyl)ethyl]-4-(3-trifluoromethylphenyl)piperazine: a selective radioligand for 5-HT$_{1A}$ receptors in rat brain. *J. Neurochem.*, **46**, 68–75.

ROCA, A.L., WEAVER, D.R. & REPPERT, S.M. (1993). Serotonin receptor gene expression in the rat suprachiasmatic nuclei. *Brain Res.*, **608**, 159–165.

ROTH, B.L., CRAIGO, S.C., CHOUDHARY, M.S., ULUER, A., MONSMA, JR. F.J., SHEN, Y., MELTZER, H.Y. & SIBLEY, D.R. (1994). Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J. Pharmacol. Exp. Ther.*, **268**, 1403–1410.

RUAT, M., TRAIFFORT, E., LEURS, R., TARDIVEL-LACOMBE, J., DIAZ, J., ARRANG, J.-M. & SCHWARTZ, J.-C. (1993). Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT$_7$) activating cAMP formation. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 8547–8551.

SAUDOU, F. & HEN, R. (1994). 5-hydroxytryptamine receptor subtypes in vertebrates and invertebrates. *Neurochem. Int.*, **25**, 503–532.

SHEN, Y., MONSMA, JR. F.J., METCALF, M.A., JOSE, P.A., HAMBLIN, M.W. & SIBLEY, D.R. (1993). Molecular cloning and expression of a 5-hydroxytryptamine$_7$ serotonin receptor subtype. *J. Biol. Chem.*, **268**, 18200–18204.

SLEIGHT, A.J., CAROLO, C., PETIT, N., ZWINGELSTEIN, C. & BOURSON, A. (1995). Identification of 5-hydroxytryptamine$_7$ receptor binding sites in rat hypothalamus: Sensitivity to chronic antidepressant treatment. *Mol. Pharmacol.*, **47**, 99–103.

TO, Z.P., BONHAUS, D.W., EGLEN, R.M. & JAKEMAN, L.B. (1995). Characterization and distribution of putative 5-ht$_7$ receptors in guinea-pig brain. *Br. J. Pharmacol.*, **115**, 107–116.

TSOU, A., KOSAKA, A., BACH, C., ZUPPAN, P., YEE, C., TOM, L., ALVAREZ, R., RAMSEY, S., BONHAUS, D.W., STEFANICH, E., JAKEMAN, L., EGLEN, R.M. & CHAN, H.W. (1994). Cloning and expression of a 5-hydroxytryptamine$_7$ receptor positively coupled to adenylyl cyclase. *J. Neurochem.*, **63**, 456–464.

VOIGT, M.M., LAURIE, D.J., SEEBURG, P.H. & BACH, A. (1991). Molecular cloning and characterization of a rat brain cDNA encoding a 5-hydroxytryptamine$_{1B}$ receptor. *EMBO J.*, **10**, 4017–4023.

WAEBER, C. & MOSKOWITZ, M. (1995). Autoradiographic visualisation of [^{3}H]5-carboxyamidotryptamine binding sites in the guinea pig and rat brain. *Eur. J. Pharmacol.*, **283**, 31–46.

WISDEN, W., PARKER, E.M., MAHLE, C.D., GRISEL, D.A., NOWAK, H.P., YOCCA, F.D., FELDER, C.C., SEEBURG, P.H. & VOIGT, M.M. (1993). Cloning and characterization of the rat 5-HT$_{5B}$ receptor: Evidence that the 5-HT$_{5B}$ receptor couples to a G protein in mammalian cell membranes. *FEBS Lett.*, **333**, 25–31.

ZGOMBICK, J.M., WEINSHANK, R.L., MACCHI, M., SCHECHTER, L.E., BRANCHEK, T.A. & HARTIG, P.R. (1991). Expression and pharmacological characterization of a canine 5-hydroxytryptamine$_{1D}$ receptor subtype. *Mol. Pharmacol.*, **40**, 1036–1042.

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