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Common receptors for hallucinogens in rat brain: a comparative autoradiographic study using [^{125}I]LSD and [^{125}I]DOI, a new psychotomimetic radioligand

Dennis J. McKenna[1], Adil J. Nazarali[1], Andrew J. Hoffman[2], David E. Nichols[2], C.A. Mathis[3] and Juan M. Saavedra[1]

[1]*Unit on Preclinical Neuropharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892 (U.S.A.),* [2]*Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, U.S.A. and* [3]*Lawrence Berkeley Laboratory, University of California, Berkeley, CA 92704 (U.S.A.)*

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The S and R enantiomers of the psychotomimetic $5HT_2$ agonist DOI (2,5-dimethoxy-4-iodophenylisopropylamine) were labeled with ^{125}I at high-specific activity. The regional distribution of binding sites for each of the enantiomers was investigated using in vitro quantitative autoradiography and compared to the regional distribution of [^{125}I]LSD in the rat brain. Saturable, specific binding of the radioligands was determined in cortical membrane homogenates. All radioligands exhibited specific binding in localized regions throughout the rat brain. Binding of [^{125}I]DOI enantiomers was completely displaced (>90%) by 1 μM of the corresponding unlabeled enantiomer; [^{125}I]LSD was completely displaced by 1 μM LSD. The choroid plexus showed the highest-density binding. Other regions showing high-density binding included the frontoparietal cortex (motor and somatosensory areas), anterior cingulate gyrus, lateral olfactory tubercle, nucleus accumbens, caudate nuclei, claustrum, nucleus of the lateral olfactory tract, dentate gyrus, mamillary nuclei, and motor trigeminal nuclei. In most regions, [^{125}I]S-DOI, the less active enantiomer, exhibited 25–40% of the amount of total binding as [^{125}I]R-DOI. In some regions, [^{125}I]R-DOI and [^{125}I]LSD had similar binding densities; in others, marked differences were apparent. The regional distribution of specific [^{125}I]R-DOI binding sites correlated with the distribution of $5HT_2$ receptors reported in previous studies. DOI and its analogs may have potential clinical applications for in vivo localization of $5HT_2$-receptors using positron emission tomography (PET) and similar techniques.

INTRODUCTION

Radioligand binding studies have demonstrated the existence of distinct central serotonin receptor subtypes. At least 5 receptor subtypes have been identified to date, including $5HT_{1a}$, $5HT_{1b}$, $5HT_{1c}$, $5HT_{1d}$, and $5HT_2$[36,39]. The existence of $5HT_3$-receptors, and of putative subyptes of $5HT_2$-receptors has also been postulated[42,50].

A number of studies have demonstrated the probable involvement of $5HT_2$-receptors in mediating the actions of hallucinogenic drugs such as LSD and the ring-substituted phenylisopropylamines[15]. These include radioligand competition studies[16], animal behavioral studies[4], and electrophysiological investigations[40]. In particular, Titeler and co-workers have recently characterized the halogenated psychotomimetic [^{3}H](±)DOB ((+,–)4-bromo-2,5-dimethoxyphenylisopropylamine) as a $5HT_2$-selective agonist radioligand[48,49]. These investigators have postulated that [^{3}H](±)DOB labels a guanyl nucleotide sensitive high-affinity 'state' of $5HT_2$-receptors[26], although this interpretation has been disputed[50].

Our own approach to the characterization of putative 'hallucinogen' receptors has emphasized the use of quantitative autoradiography to localize and characterize binding sites for hallucinogens in specific anatomical regions of rat brain. We have recently

Correspondence: D.J. McKenna, Department of Neurology, Neurological Research Laboratory, Stanford University Medical Center, 701 Welch Rd., Bldg. B (lower level), Palo Alto, CA 94304, U.S.A.

reported on the use of an iodine-containing psychotomimetic, R-(–)-2,5-dimethoxy-4-iodophenylisopropylamine (R-DOI), labeled with ^{125}I ([^{125}I]DOI), for the autoradiographic localization of high-affinity binding sites[30]. Like its brominated analog DOB, DOI shows a high selectivity for $5HT_2$-receptors[16], and its discriminative stimulus properties are consistent with those of a $5HT_2$-agonist[17]. Other investigators have used ^{125}I-labeled LSD ([^{125}I]LSD, IOL) as a $5HT_2$-selective ligand in both autoradiographic and membrane binding studies[1,13,18,20,33].

[^{125}I]LSD and [^{125}I]DOI are not identical in their receptor selectivity, however. We have shown using autoradiography that [^{125}I]R-DOI labels a subset of the receptors that are labeled by [^{125}I]LSD, by demonstrating that unlabeled hallucinogenic phenylisopropylamines such as DOB displace [^{125}I]LSD in specific regions, while unlabeled LSD displaces [^{125}I]R-DOI from virtually all binding sites in the rat forebrain[31]. Other investigators have shown that [^{125}I]LSD possesses a significant binding component to dopamine D_2-receptors[13,19,33] and to $5HT_{1c}$-receptors[22].

A further disadvantage in the use of [^{125}I]LSD to characterize hallucinogen receptors is the fact that halogenation of LSD apparently converts a potent hallucinogen into a potent hallucinogen antagonist. The brominated derivative, 2-bromo-LSD (BOL) has been shown to block the psychotomimetic activity of LSD[14], and iodination of the LSD molecule in this position may similarly disrupt the activity. In the case of phenylisopropylamine psychotomimetics such as DOB or DOI, however, the halogen substituent is an intrinsic part of the structure, and in fact the *presence* of a halogen or alkyl substituent in the 4-position results in a marked increase in psychotomimetic potency compared to the unsubstituted derivative 2,5-dimethoxyphenylisopropylamine[8].

[^{125}I]DOI thus represents a new class of relatively $5HT_2$-selective psychotomimetic radioligands that can be applied to the anatomical localization of $5HT_2$- and/or 'hallucinogen receptors'. In view of this fact, we felt it worthwhile to map and quantify the distribution of its specific binding sites in the rat brain, and to compare them with those of the only other radio-iodine labeled hallucinogen analog that is currently available, [^{125}I]LSD. We report here the results of our comparative mapping study.

MATERIALS AND METHODS

Synthesis of DOI enantiomers and radiosynthesis of [^{125}I]R-DOI and [^{125}I]S-DOI

The enantiomers of (2,5-dimethoxyphenyl)-2-aminopropane were prepared in 3 steps, from 1-(2,5-dimethoxyphenyl)-2-propanone using the asymmetric synthesis of Nichols et al.[35], in about 90% overall yield. The enantiomeric amines were individually treated with trifluoroacetic anhydride in benzene at reflux to afford the respective trifluoroacetamides at about 90% yield. Aromatic iodination of these amides was accomplished with ICl in acetic acid according to the method of Braun et al.[7] in 80–85% yield. Deprotection of these amides was performed in 2-propanol with aqueous KOH at room temperature thus producing the enantiomers of 2,5-dimethoxy-4-iodophenylisopropylamine in over 90% yield.

The radiosynthesis of the ^{125}I-labeled R- and S-enantiomers of 2,5-dimethoxy-4-iodophenylisopropylamine (DOI) followed the general labeling procedures given by Coenen[12] for the radiosynthesis of ^{77}Br-labeled 4-bromo-2,5-dimethoxyphenylisopropylamine with some modification. Complete synthetic procedures for the enantiomers of DOI and their ^{125}I-radiolabels appear elsewhere[28]. Briefly, the trifluoroacetyl derivative of 2,5-dimethoxyphenylisopropylamine (DMA) in trifluoroacetic acid was reacted with 5 mCi of [^{125}I]iodide in 0.1 M NaOH in the presence of dichloramine-T (DCT). The solution was stirred at room temperature for 2 h and the progress of the reaction observed by high-performance liquid chromatography (HPLC). Typical radiochemical yields at 2 h were 60–70%. The trifluoroacetic acid was removed with a stream of nitrogen gas and the residue taken up in isopropyl alcohol; 2 M KOH was added and the mixture was heated for 1 h at 50 °C. The solvent was evaporated, the residue taken up in water and extracted several times with dichloromethane; the organic phase was separated and the solvent evaporated. The residue was taken up in methanol and purified on a Waters Bondapack C18 column with a methanol/water (40/60) eluent. The overall yields of the [^{125}I]DOI products based on the amount of [^{125}I]sodium iodide used were 15–20%. The radiochemical purity of the product was greater than 99.5%. Quantitation of the UV mass peak that coincided with the radioactive peak of DOI led to es-

timates of the specific activity of the products as 1200 Ci/mmol for the S-isomer and 1700 Ci/mmol for the R-isomer.

Animals and tissue preparation

Experiments utilized adult male Sprague–Dawley rats (250 g b. wt.) which were maintained under standard laboratory conditions with lights on from 06.00 to 18.00 h for at least 1 week prior to sacrifice. Animals were killed by decapitation between 09.00 and 11.00 h and their brains were immediately removed and frozen in isopentane at −30 °C. Within 48 h tissue sections (16 μm) were cut in a cryostat at −17 °C and mounted on gelatin-coated glass slides and placed under vacuum at 4 °C overnight prior to incubation.

Histology

To aid in identification of brain areas and microscopic examinations, histological specimens were prepared from duplicate sections and stained for acetylcholinesterase after the method of Karnovsky[25]. Stained sections are shown in the fourth row in Figs. 1–2, and in Fig. 3.

Autoradiography

For autoradiography, slides were preincubated for 30 min at room temperature in 50 mM Tris buffer (pH 7.4) containing 0.1% ascorbate, 0.1% BSA and 4 mM $CaCl_2$. Following preincubation, slides containing adjacent brain sections were transferred to fresh buffer containing 500 pM of one of the following radioligands: [^{125}I]LSD (Amersham, Arlington Heights, IL), [^{125}I]R-DOI, or [^{125}I]S-DOI. After correction for decay the specific activity was as follows: [^{125}I]LSD, 796 Ci/mmol, [^{125}I]R-DOI, 963.3 Ci/mmol, [^{125}I]S-DOI, 741 Ci/mmol. Unlabeled LSD (obtained from National Institute on Drug Abuse) was used to define non-specific binding for [^{125}I]LSD, while unlabeled R- and S-enantiomers of DOI were used to define non-specific binding for [^{125}I]R-DOI and [^{125}I]S-DOI, respectively. Non-specific binding was defined with adjacent sections incubated with 500 pM of each radioligand in the presence of 1 μM unlabeled drug. Under these conditions non-specific binding was less than 10% of total binding for all 3 ligands.

Slides were incubated for 60 min at room temperature in the presence of radioligand, then washed 3 times (10 min) in ice-cold buffer, pH 7.4. Finally slides were dipped in ice-cold distilled water (1 min) and dried under a cold air stream. Slides were exposed to ^{3}H-sensitive Ultrofilm (LKB Industries, Rockville, MD) in X-ray cassettes at room temperature for 96 h. ^{125}I brain paste standards, cut to 16 μm thickness, were included in the cassettes and exposed simultaneously. Films were developed in undiluted Kodak D-19 developer at 4 °C for 4 min.

Film optical densities were quantified by computerized densitometry; the optical densities of the tissue sections were related to the optical densities of the ^{125}I standards, by comparison with standard curves generated by processing sets of brain-paste standards containing known amounts of radioactivity per mg protein, corrected for ^{125}I decay (ln o.d. vs ln dpm/mg protein). Complete details of the quantification procedures are described elsewhere[23].

Saturation studies

For saturation studies, frozen frontoparietal cortices from male Sprague–Dawley rats were homogenized in 50 mM Tris buffer (pH 7.4) containing 0.1% ascorbate, 4 mM $CaCl_2$ (Polytron setting, 8; 2 × 15 s). BSA was not included in the buffer used in binding studies with membrane homogenates. The homogenate was centrifuged (35,000 *g*, 20 min), the supernatant removed and the pellet resuspended in buffer. The centrifugation step was repeated once and the protein content of the final membrane suspension was determined using the method of Bradford[6]. The membrane suspension was diluted with buffer for 1 mg protein/ml. Binding assays were conducted in 96-well microtitre plates (Falcon 3040; Beckton, Dickinson & Co., Oxnard, CA) in 0.2 ml final volume (37 °C, 60 min incubation). Incubations consisted of 50 μg protein, and 0.02–30 nM concentrations of radioligand (3 replicates/concentration). Non-specific binding was defined at each concentration with 1 μM unlabeled drug. In an additional experiment, non-specific binding was defined by incubation with 1 μM of the $5HT_2$-selective antagonist ritanserin. Following incubation for 60 min, reactions were terminated by transferring reaction aliquots to a microsample filtration manifold (Schleicher and Schuell, Keene, NH) with an 8-tip multi-channel pipette, and suction filtering through Schleicher and Schuell No. 30 glass fiber filters, presoaked in 0.1%

TABLE I

Saturation data for R- and S-enantiomers of [^{125}I]DOI and [^{125}I]LSD

Parameters refer to high- (K_{d_H}, B_{max_H}) and Low- (K_{d_L}, B_{max_L}) affinity binding components of each radioligand as determined by the EBDA program[29]. Figures given are mean ± S.E.M. of 3 separate experiments. See Figs. 5–7 for representative saturation isotherms and Scatchard plots.

	K_{d_H} (nM)	B_{max_H} (fmol/mg protein)	K_{d_L} (nM)	B_{max_L} (fmol/mg protein)
[^{125}I]R-DOI	1.16 ± 0.02	92 ± 10	29 ± 7	536 ± 164
[^{125}I]S-DOI	2.1 ± 0.21	67 ± 19	18 ± 4	245 ± 60
[^{125}I]LSD	2.26 ± 0.47	1187 ± 349	5.46 ± 1.1	2224 ± 724

polyethylenimine. Filters were washed with an additional 1.0 ml ice-cold buffer, and counted in a gamma counter to determine concentration of bound ligand. Results of the saturation experiments were analyzed on an IBM PC using the computer programs EBDA and LIGAND[29].

RESULTS

The computer program EBDA[29] was used to produce Scatchard transformations of the binding data (Figs. 5–7, lower panels). The Scatchard plots were biphasic for all 3 radioligands, indicative of the prob-

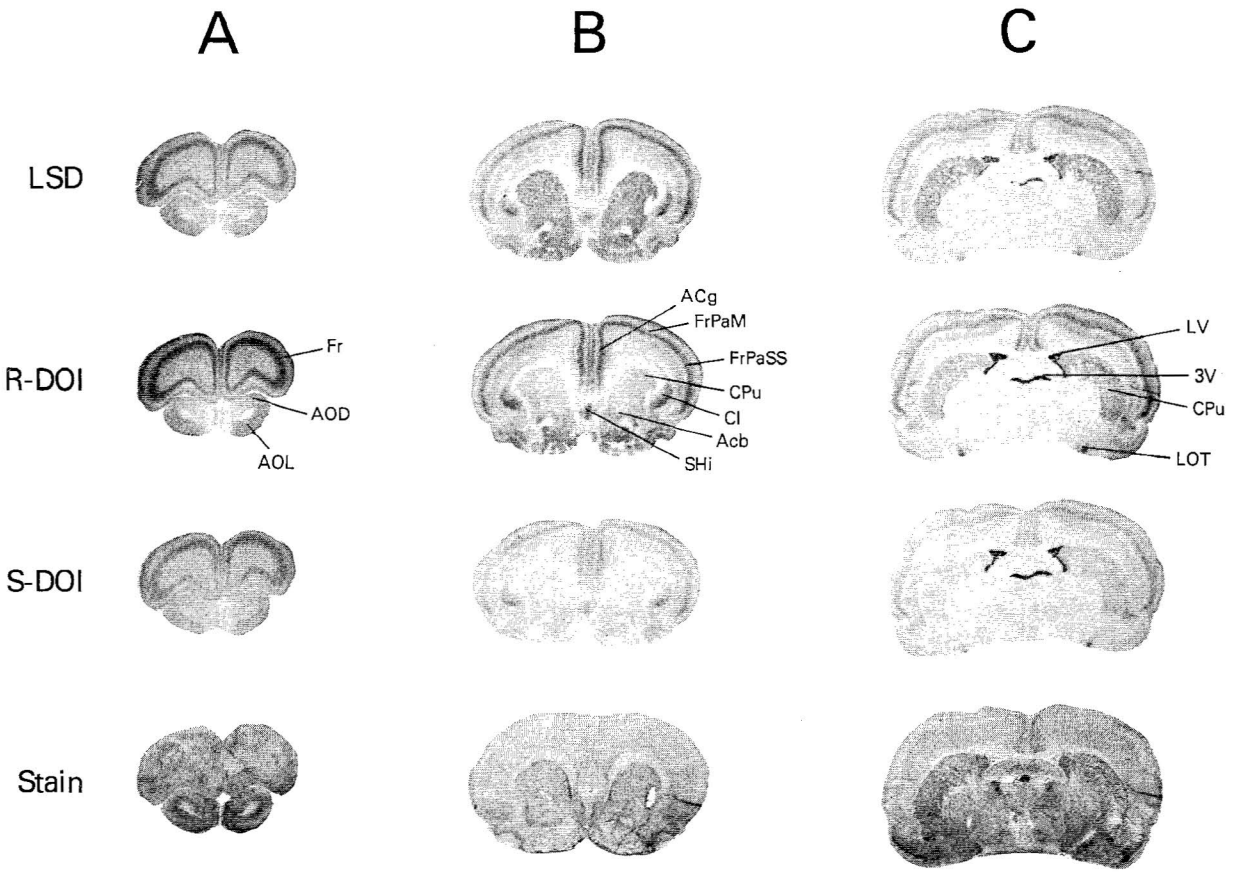

Fig. 1. Binding of [^{125}I]LSD, [^{125}I]S-DOI, and [^{125}I]R-DOI in adjacent coronal sections of rat brain. Radioligands used are shown in rows labeled at left; sections arranged in columns A–C; approximate stereotaxic coordinates based on Paxinos and Watson[37]. A: interaural, 13.2 mm. B: interaural, 10.7 mm. C: interaural, 7.7 mm. Bottom row shows adjacent sections stained for acetylcholinesterase[25]. Labeled areas in second row are defined in Table II.

able presence of more than one binding site. Apparent K_d and B_{max} values for the high- and low-affinity components of the Scatchard curves (Table I) were calculated via linear regression on segments of the curves using the EBDA program. Values given in Table I are mean ± S.E.M. of 3 independent experiments.

Results of representative saturation experiments in cortical membrane homogenates are shown in Figs. 5–7. The saturation isotherms (Figs. 5–7, upper panels) indicate that [^{125}I]LSD showed low levels of non-specific binding (>10%) even at 25 nM (Fig. 7A), while the [^{125}I]DOI enantiomers showed comparatively high levels of non-specific binding at all concentrations measured. Non-specific binding of [^{125}I]R-DOI exceeded specific binding at concentrations above 15 nM, while for [^{125}I]S-DOI, non-specific binding was greater than specific binding at concentrations above 3 nM. At 500 pM [^{125}I]R-DOI and [^{125}I]S-DOI showed similar levels of non-specific binding (25 and 29% of total binding, respectively) so this concentration was selected for the autoradiographic study; at this concentration non-specific binding for [^{125}I]LSD was 2.4% of total binding. Comparable levels of non-specific binding were observed when 1 μM ritanserin was used to define non-specific binding (21% of total binding at 500 nM).

In an additional experiment, we displaced 0.15 nM [^{125}I]R-DOI with unlabelled R-DOI (0–10^{-6} M) in the presence and absence of 10^{-5} M GppNHp. IC_{50} values were 0.26 ± 0.02 nM in the absence of GppNHp, and 0.32 ± 0.24 nM in the presence of GppNHp.

Specific binding sites for [^{125}I]LSD and both enan-

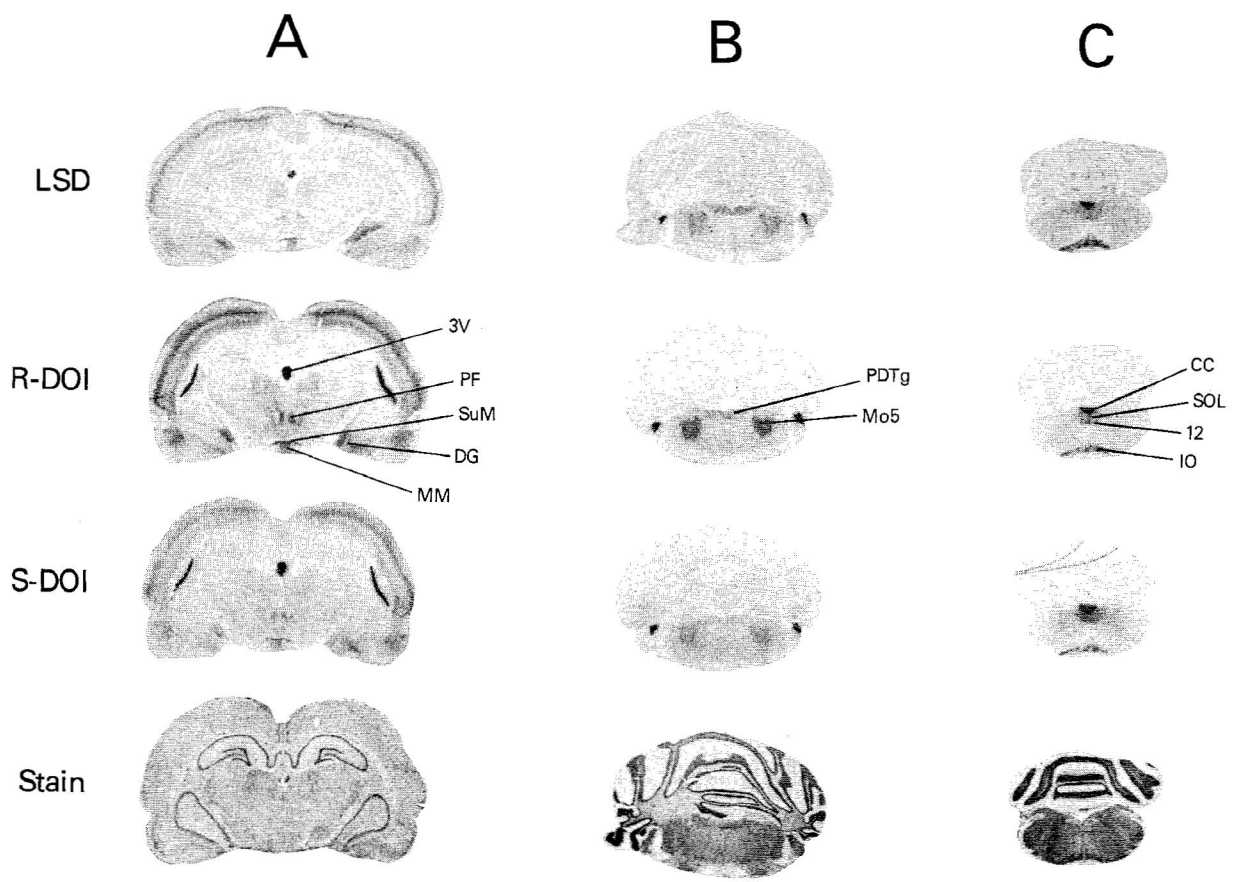

Fig. 2. Binding of [^{125}I]LSD, [^{125}I]S-DOI, and [^{125}I]R-DOI in adjacent coronal sections of rat brain. Radioligands used are shown in rows labeled at left; sections arranged in columns A–C; approximate stereotaxic coordinates based on Paxinos and Watson[37]. A: interaural, 4.7 mm. B: interaural, −1.8 mm. C: interaural, −4.8 mm. Bottom row shows adjacent sections stained for acetylcholinesterase[25]. Labeled areas in second row are defined in Table II.

tiomers of [^{125}I]DOI were detected in localized regions throughout the rat forebrain (Figs. 1–4, and Table II). In most regions, [^{125}I]S-DOI exhibited 25–40% of the amount of total binding as [^{125}I]R-DOI. The highest levels of total binding for all 3 compounds were detected in the choroid plexus (desig-

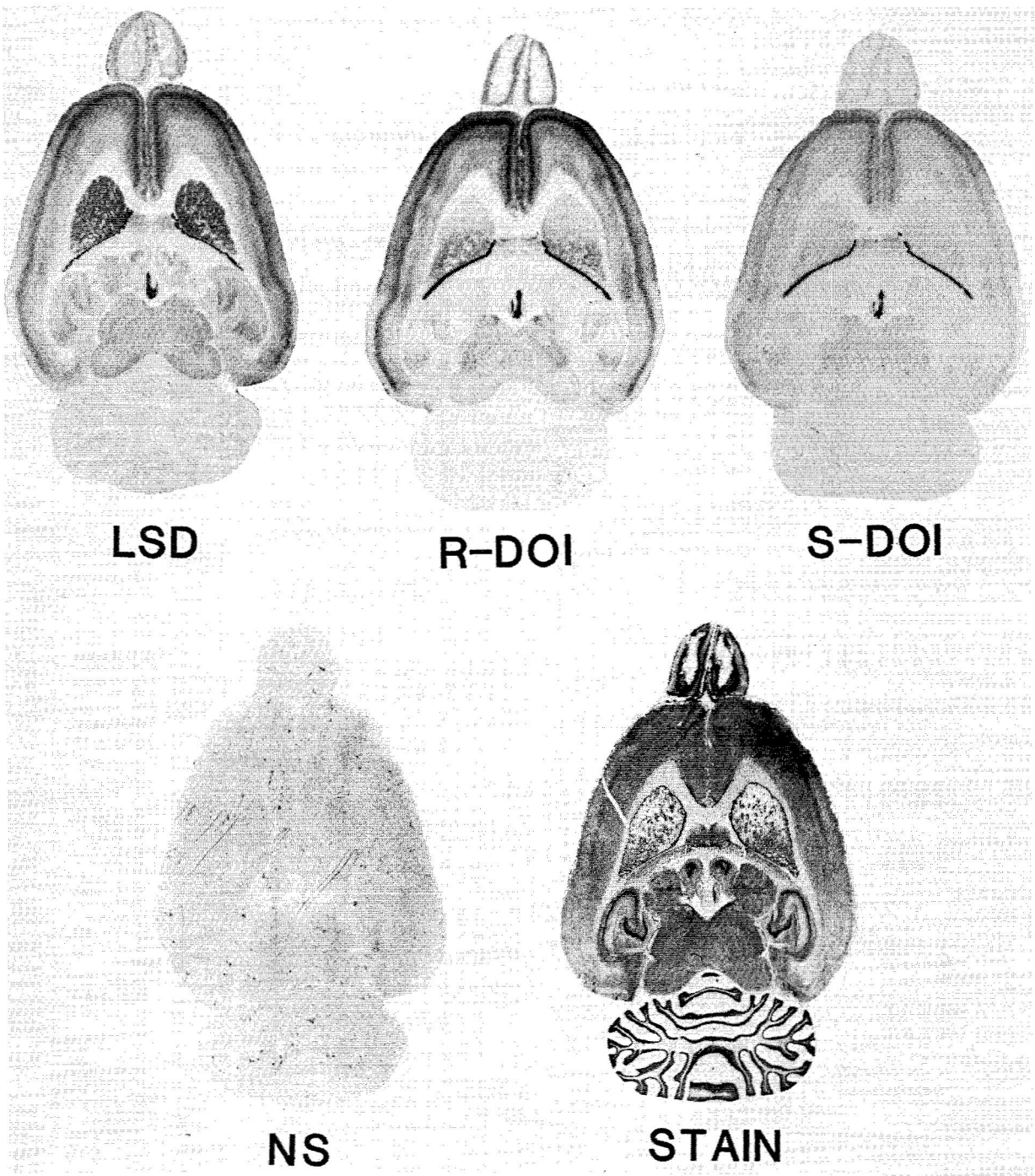

Fig. 3. Binding of [^{125}I]LSD, [^{125}I]S-DOI, and [^{125}I]R-DOI in adjacent horizontal sections of rat brain. Sections shown approximate stereotaxic coordinates in Plate 63 in Paxinos and Watson[37]. (Interaural: 5.9 mm; bregma: 4.1 mm). Section labeled 'stain' shows adjacent section stained for acetylcholinesterase[25]. Labeled areas are defined in Table II. NS, non-specific binding.

nated as the third and lateral ventricles and central canal in Table II and Figs. 1–3) and in these areas, the reduced binding of [^{125}I]S-DOI compared to [^{125}I]R-DOI was less apparent. Total binding for [^{125}I]LSD exceeded that of [^{125}I]R-DOI in the anterior cingulate cortex, accumbens nuclei, dentate gyrus, and caudate putamen (Table II, Figs. 1–3). [^{125}I]R-DOI and [^{125}I]LSD showed comparable levels of total binding in the motor and somatosensory areas of the frontoparietal cortex, septal hippocampal nuclei, parafasicular nuclei, hypoglossal nuclei, nuclei of the solitary tract, and inferior olive: in the other regions measured, total binding of [^{125}I]R-DOI exceeded that of [^{125}I]LSD (Table II, Figs. 1–3).

DISCUSSION

The apparent K_d and B_{max} values determined in our experiments with the DOI enantiomers are in close agreement with those reported previously by Johnson et al.[24]. Apparent K_d and B_{max} values for [^{125}I]LSD have been determined by various investigators[13,18], and are comparable to the values reported here.

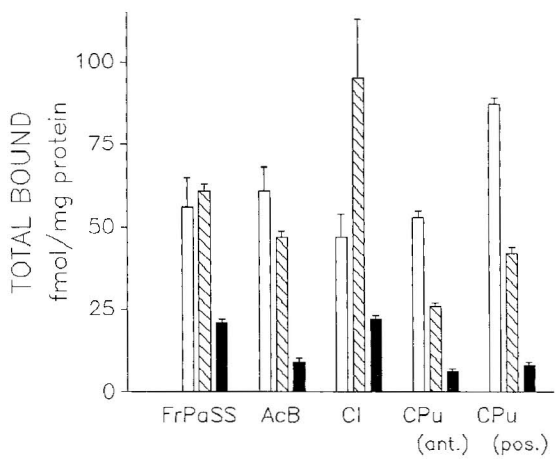

Fig. 4. Relative binding of [^{125}I]LSD (□), [^{125}I]S-DOI (■) and [^{125}I]R-DOI (▨) in selected regions of rat forebrain, measured by quantitative autoradiography. Concentrations of all radioligands was 500 pM. Error bars are standard errors, calculated from measurements of adjacent sections from 3 different animals. Abbreviations on abscissa are defined according to Paxinos and Watson[37]. FrPaSS, frontoparietal cortex, somatosensory area; AcB, accumbens nucleus; Cl, claustrum; CPu (ant.), caudate putamen, anterior portion; CPu (pos.), caudate putamen, posterior (peripallidal) portion.

The B_{max} of both enantiomers was similar, but the K_d of the S-isomer was approximately 2-fold higher than the R-isomer. This is consistent with the known lesser hallucinogenic potency of the S-isomer[2]. [^{125}I]LSD labeled the high-affinity site with a K_d approximately 2-fold higher than [^{125}I]R-DOI, and had a B_{max} more than 10-fold greater than that of either DOI enantiomer. The comparatively high density of the high-affinity binding of [^{125}I]LSD may represent antagonist binding to the 5HT$_2$-receptors, while the low-density binding of the DOI enantiomers is characteristic of agonist binding.

The significance of the low-affinity binding of the [^{125}I]DOI enantiomers and [^{125}I]LSD is not clear. The K_d of the low-affinity site labeled by [^{125}I]LSD is 3–6 times lower than the K_d of the low-affinity site labeled by the [^{125}I]DOI isomers, indicating that these sites are not identical. [^{125}I]LSD has been shown to label D$_2$-receptors as well as 5HT$_2$-receptors, and the K_d for the low-affinity binding determined in our experiments is in the same range as the K_d reported by Hartig and co-workers[19] for the D$_2$-binding component (9.1 nM) of [^{125}I]LSD in bovine caudate membranes. These investigators reported that the high-affinity component of [^{125}I]LSD binding represents 5HT$_2$-binding, while the low-affinity binding represents D$_2$-binding and our results support this interpretation. The localization of D$_2$-receptors in the rat cortex using quantitative autoradiography has been recently reported[5].

Analysis of our saturation data using the curve-fitting program LIGAND[29] indicated that the low-affinity binding of the [^{125}I]DOI enantiomers could be modeled either as a second site, or treated as a form of non-specific binding not displaced by 1 μM cold DOI (data not shown). Lyon et al.[26], reported that [^{3}H](+,–)DOB labels a guanyl nucleotide-sensitive low-density, high-affinity 'state' of cortical 5HT$_2$-receptors. We also found that the enantiomers of [^{125}I]DOI label a low density, high-affinity site, but in a preliminary experiment we could detect no reduction in high-affinity binding of [^{125}I]R-DOI in the presence of 10^{-5} M GppNHp. The low-affinity site labeled by [^{125}I]DOI may correspond to the antagonist 'state' of the 5HT$_2$-receptor; alternatively, it may represent a form of non-specific binding, or it may represent binding to a different receptor or receptor subtype. Clarification of these alternatives will re-

quire further investigations and are beyond the scope of the present study.

In the autoradiographic study, all 3 radioligands show high-density binding in the same areas in adjacent brain sections, with some notable exceptions. One of these is the much lower binding density of [^{125}I]S-DOI compared to [^{125}I]R-DOI; this is obvious in Figs. 1–3 and the quantitative comparison (Fig. 4, Table II) shows that the apparent differences are not artifacts since the relationship is preserved after correction for the different specific activity of the radioligands. The lower binding density of [^{125}I]S-DOI is consistent with its lower affinity for the receptors as demonstrated in the membrane saturation studies. The saturation curves (Figs. 5 and 6, upper panels) show that at the concentration used for autoradiography (0.5 nM), the concentration of specifically bound [^{125}I]R-DOI is nearly twice that of [^{125}I]S-DOI at the same concentration (4.38×10^{-3} M and 2.4×10^{-3} nM, respectively). This fact accounts for the ob-

TABLE II

Binding densities of LSD and DOI in different areas of the rat brain

*Figure**	*Area***	*Total binding (fmol/mg protein)****		
		LSD	*R-DOI*	*S-DOI*
1A	Frontal cortex (Fr)	61 ± 7	177 ± 4	57 ± 6
	Anterior olfactory nucleus, dorsal (AOD)	14 ± 2	78 ± 1	17 ± 3
	Anterior olfactory nucleus, lateral (AOL)	24 ± 1	118 ± 3	25 ± 4
1B	Frontoparietal cortex, motor area (FrPaM)	57 ± 6	58 ± 1	25 ± 4
	Frontoparietal cortex, somatosensory area (FrPaSS)	56 ± 9	61 ± 2	21 ± 1
	Anterior cingulate cortex (ACg)	91 ± 9	63 ± 0	22 ± 2
	Accumbens nucleus (Acb)	61 ± 7	47 ± 2	9 ± 0
	Claustrum (Cl)	47 ± 7	95 ± 18	22 ± 1
	Caudate putamen (CPu)	44 ± 2	33 ± 6	5 ± 1
	Septal hippocampal nucleus (SHi)	44 ± 9	55 ± 6	20 ± 2
1C	Frontoparietal cortex, motor area (FrPaM)	45 ± 6	84 ± 8	22 ± 1
	Frontoparietal cortex, somatosensory area (FrPaSS)	34 ± 9	86 ± 5	22 ± 2
	Anterior cingulate cortex (ACg)	50 ± 5	63 ± 0	22 ± 3
	Nucleus lateral olfactory tract (LOT)	19 ± 3	85 ± 5	25 ± 2
	Third ventricle (3V)	125 ± 4	143 ± 10	121 ± 12
	Lateral ventricle (LV)	126 ± 5	146 ± 10	116 ± 2
	Caudate putamen (CPu)	46 ± 13	64 ± 7	24 ± 4
2A	Third ventricle (3V)	225 ± 44	137 ± 12	169 ± 25
	Dentate gyrus (DG)	84 ± 8	18 ± 6	29 ± 8
	Parafascicular nucleus (PF)	58 ± 12	59 ± 9	36 ± 4
	Supramammillary nucleus (SuM)	17 ± 2	59 ± 11	18 ± 3
	Medial mammillary nucleus (MM)	52 ± 12	85 ± 1	32 ± 11
2B	Motor trigeminal nucleus (Mo5)	24 ± 2	74 ± 3	29 ± 5
	Posterodorsal tegmental area (PDTg)	19 ± 4	44 ± 2	13 ± 2
2C	Hypoglossal nucleus (12)	52 ± 7	59 ± 4	48 ± 5
	Central canal (CC)	147 ± 16	203 ± 13	147 ± 16
	Nucleus solitary tract (SOL)	20 ± 3	25 ± 5	21 ± 5
	Inferior olive (IO)	36 ± 11	42 ± 4	14 ± 5
3	Anterior caudate putamen (CPu)	53 ± 2	26 ± 1	6 ± 1
	Posterior caudate putamen (CPu)	87 ± 2	42 ± 2	8 ± 1
	Lateral ventricle (LV)	124 ± 9	152 ± 2	89 ± 5
	Third ventricle (3V)	227 ± 26	159 ± 11	99 ± 2
	Frontal cortex (Fr)	115 ± 3	104 ± 2	43 ± 2
	Frontoparietal cortex, Somatosensory area (FrPaSS)	57 ± 8	58 ± 1	15 ± 1

* Numbers refer to Figs. 1–3; letters refer to columns A–C in Figs. 1 and 2.

** Nomenclature and abbreviations for brain areas are after Paxinos and Watson[37]. Stereotaxic coordinates of brain areas listed in Table II are given in captions to Figs. 1–3. Abbreviations refer to labelled areas in Figs. 1–3.

*** Determined by computerized densitometry by comparison with ^{125}I standards[23]. Standard errors are based on measurements of adjacent sections from 3 animals measured independently. Concentration of the radioligands was 500 pM. See Table I for summary of K_d and B_{max} values for each radioligand as measured in membrane homogenates.

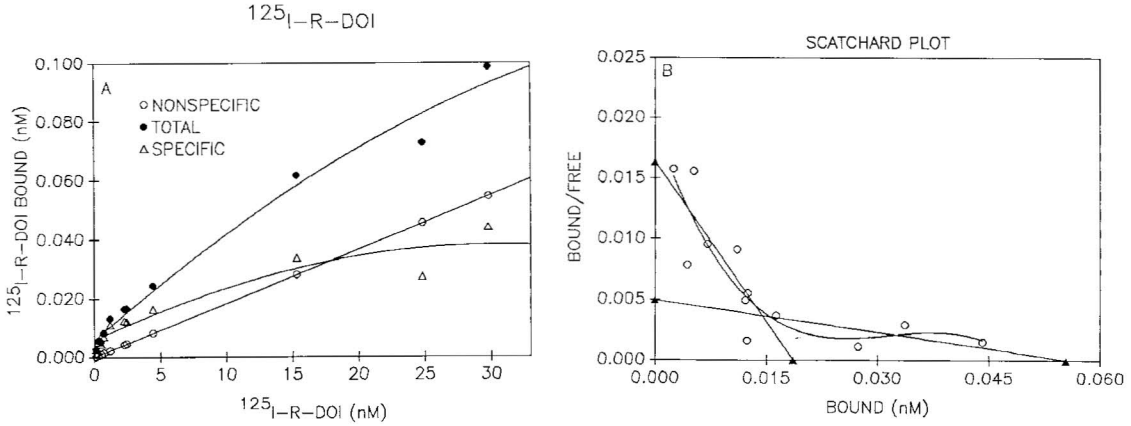

Fig. 5. Saturation isotherm (A) and Scatchard plot (B) for [^{125}I]R-DOI in rat cortex membranes. Data shown represent a typical experiment; each point plotted is the mean of triplicate determinations. The solid diagonal lines in (B) represent the high- and low-affinity binding components of the Scatchard plot, determined using the computer program EBDA[29]. The curved line represents the third-order regression coefficient.

served differences in binding density between the R- and S-enantiomers at the concentrations used.

Interestingly, the highest binding density for all 3 radioligands was detected in the choroid plexus, a region known to contain a high density of $5HT_{1c}$-receptors. Hoyer and co-workers[22] have already shown that [^{125}I]LSD labels $5HT_{1c}$-receptors in the choroid plexus with high affinity, and Lyon and co-workers[27] have shown that DOI and its congeners exhibit a similar binding profile at both $5HT_{1c}$- and $5HT_2$-receptors. The present study provides the first autoradiographic evidence that the enantiomers of [^{125}I]DOI bind to $5HT_{1c}$-receptors in the choroid plexus. Hartig[21] has commented on the apparent homologies between the $5HT_{1c}$-receptor and the $5HT_2$-receptor.

Preliminary autoradiographic studies in our laboratory with racemic [^{125}I]DOI showed much higher levels of non-specific binding than with either enantiomer (data not shown). In membrane homogenates, the S-enantiomer of [^{125}I]DOI had higher levels of non-specific binding (Fig. 6); this observation, combined with the fact that the less active S-enantiomer could compete with the R-enantiomer for specific binding, constitutes a strong argument for the use of enantiomerically pure radioligands in regional localization studies of this type. Nichols et al.[35], have

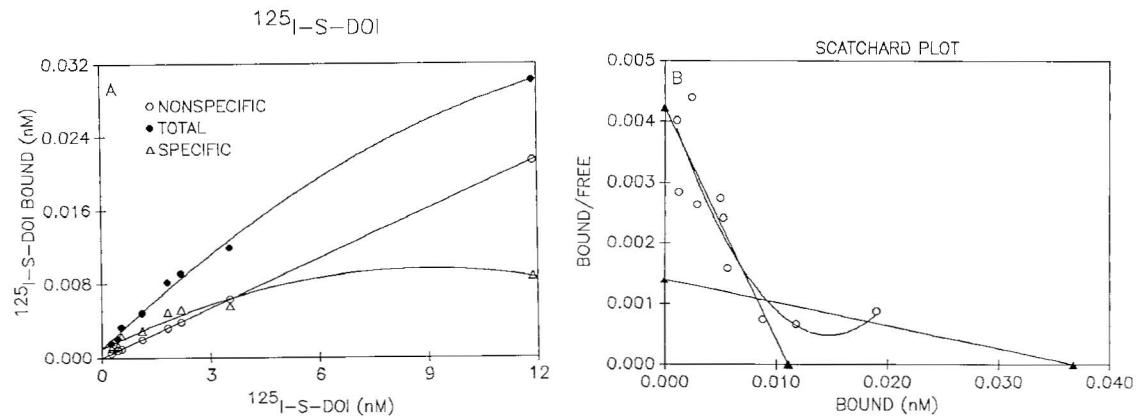

Fig. 6. Saturation isotherm (A) and Scatchard plot (B) for [^{125}I]S-DOI in rat cortex membranes. Data shown represent a typical experiment; each point plotted is the mean of triplicate determinations. The solid diagonal lines in (B) represent the high- and low-affinity binding components of the Scatchard plot, determined using the computer program EBDA[29]. The curved line represents the third-order regression coefficient.

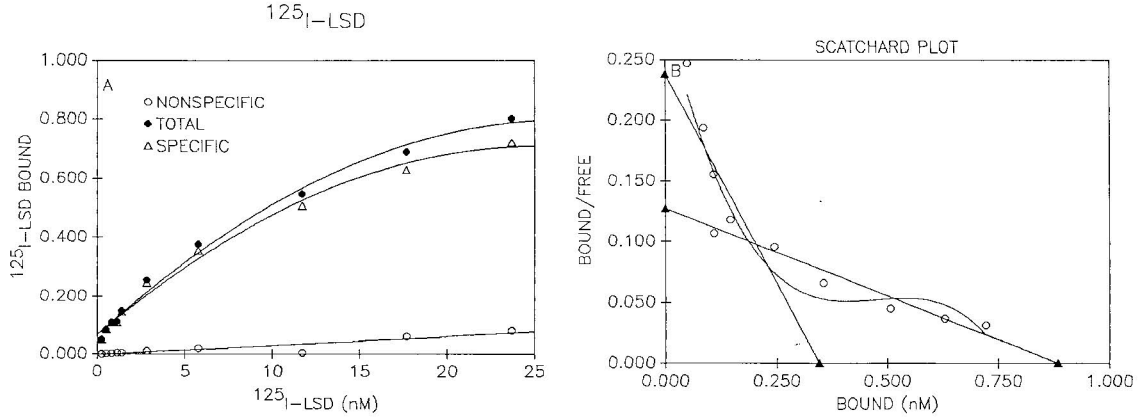

Fig. 7. Saturation isotherm (A) and Scatchard plot (B) for [^{125}I]LSD in rat cortex membranes. Data shown represent a typical experiment; each point plotted is the mean of triplicate determinations. The solid diagonal lines in (B) represent the high- and low-affinity binding components of the Scatchard plot, determined using the computer program EBDA[29]. The curved line represents the third-order regression coefficient.

described procedures for synthesizing pure enantiomers of DOM and its analogs.

With the exception of the high-density binding in the choroid plexus, the distribution of the binding for all 3 radioligands is in good agreement with the localization of $5HT_2$-receptors reported by Pazos et al.[38], using [^{3}H]ketanserin and [^{3}H]LSD.

It is interesting to note that in Pazos et al.[38], the claustrum contained the highest density of $5HT_2$-receptors of all brain regions. In our study one of the highest binding densities for [^{125}I]R-DOI was also localized in the claustrum. [^{125}I]LSD also had high-binding densities in this region (Table II, Figs. 1 and 4). The high-density localization of [^{125}I]R-DOI in the claustrum suggests that this structure may play an important and hitherto unrecognized role in mediating the effects of hallucinogens such as DOI and LSD. Numerous reciprocal connections between the claustrum and the frontal, auditory, motor, somatosensory, and visual cortices have been defined[9,12,34,47], and the existence of visual and somatosensory maps in the claustrum has also been demonstrated[11,32]. There is also evidence that the claustrum is involved in a multisensory link between the neocortex and the limbic system (hippocampus, dentate gyrus)[51]. Psychotomimetics exert characteristic effects on motor, auditory, somatosensory, affective, cognitive, and visual processes, all of which have been functionally linked to the claustrum. Our finding that both LSD and DOI bind with high density in the claustrum indicates that this structure should receive more attention as a potential anatomical site of action for hallucinogens and other $5HT_2$-agonists.

The clear differences between [^{125}I]LSD and [^{125}I]R-DOI binding in the caudate nucleus (Figs. 1B,C,3 and 4, Table II) is another indication of the high $5HT_2$ selectivity of [^{125}I]R-DOI. It can be seen from Fig. 1B and C and from the quantitative data in Table II and Fig. 4 that the binding of [^{125}I]R-DOI in the caudal (peripallidal) region of the caudate-putamen is almost twice that of the rostral portion; this difference is even more apparent in the horizontal sections shown in Fig. 3. The binding of [^{125}I]LSD in the caudate, however, is more or less homogenous throughout (Figs. 3 and 4, Table II). Altar and coworkers[1] utilized [^{125}I]LSD autoradiography to demonstrate the preferential localization of $5HT_2$-receptors in the caudal region of the caudate-putamen by showing that 50 nM of the $5HT_2$ selective drug ketanserin displaced 74% of the binding in the caudal area, but only 30% from the rostral caudate. Our results show that [^{125}I]R-DOI clearly delineates a region of higher binding density in the caudal part of the caudate-putamen without the use of any displacing drugs (Fig. 3). Considered together with the known high selectivity of DOI for $5HT_2$-receptors, these observations suggest that [^{125}I]R-DOI may become the ligand of choice for the in vitro localization of $5HT_2$-receptors.

It should also be noted that the work reported here

is not without clinical implications. DOI, DOB and related phenylisopropylamines are highly selective $5HT_2$-agonists which also happen to be potent psychotomimetics; thus, suitably labeled derivatives could be developed for in vivo PET imaging of $5HT_2$-receptors in humans. Such investigations could provide insights into the mechanisms of action of psychotomimetic drugs in humans. In addition, alterations in $5HT_2$-receptors have been implicated in treatments with antipsychotic drugs[3], antidepressants[43], and senile dementia of the Alzheimer's type[41]. Thus, the development of suitably selective $5HT_2$-radioligands for in vivo imaging studies could provide clinicians with a powerful tool for investigating the role of $5HT_2$-receptors in these disorders. Recent investigations have focused on [^{11}C]brom-LSD as a $5HT_2$-selective ligand for human PET studies[53]. DOI and some of its congeners have been labeled with ^{123}I (refs. 46, 52), ^{131}I (ref. 45), or ^{77}Br (ref. 44) for use in brain and whole-body imaging and brain–blood flow studies. These utilize relatively low-resolution imaging techniques compared to PET, however. ^{122}I and ^{75}Br are two positron-emitting isotopes that have been suggested[46] as potential labels for DOI and DOB, respectively. Since the $5HT_2$-selectivity of DOI and its congeners has now been demonstrated by receptor and autoradiographic studies, the use of appropriately labeled derivatives for in vivo PET studies in animals and humans would seem to be the next logical step.

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