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Abstract

The detailed distribution of histamine H_3 receptor mRNAs in rat brain was analyzed by *in situ* hybridization using a ^{33}P-labelled riboprobe and was combined for the first time with the detailed autoradiographic distribution of the receptor determined in the same animals with [^{125}I]iodoproxyfan, a selective radioligand. The signals generated on adjacent brain sections by each probe were quantified and/or rated and were compared in order to identify neuronal populations expressing the receptor. In addition, the cellular localization of the transcripts within various brain structures was analyzed in sections dipped in a photographic emulsion.

In the cerebral cortex, the strong mRNA expression in intermediate and deep layers indicates the presence of H_3 receptors on several types of neurons. The binding is dense except in layer V, suggesting that H_3 receptors are located on granule cells and apical dendrites of pyramidal cells. In addition to their localization on monoaminergic afferents, the dense binding in layer IV and strong mRNA expression in thalamic nuclei suggest the presence of heteroreceptors on thalamocortical projections. In the hippocampus, the strong mRNA expression but low binding in pyramidal layers of the CA1 and ventral CA3 fields suggest that H_3 receptors are abundant on efferent projections of pyramidal cells. In the dentate gyrus, some binding sites in the molecular layer may correspond to H_3 receptors synthesized in granule cells and coexpressed with H_1 and H_2 receptors in their dendrites. In the basal ganglia, H_3 receptors are highly expressed in the striatal complex and olfactory tubercles but not in islands of Calleja. Some of the striatal binding sites may correspond to presynaptic receptors present on afferents. The mRNAs in cortical layer V may encode for heteroreceptors on corticostriatal neurons. The presence of mRNAs in the substantia nigra pars compacta suggests that H_3 receptors are located upon nigrostriatal afferents. However, the absence of any signal in the ventral tegmental area indicates that some but not all dopaminergic neurons express H_3 receptors. In addition, the homogeneous mRNA expression within the caudate putamen and nucleus accumbens suggests that many striatal H_3 receptors are present on medium-sized, spiny projection neurons of both the direct and indirect movement pathways. In agreement, a dense binding, but low mRNA expression, is observed in external and internal pallidum and in substantia nigra pars reticulata. In the amygdala, the dense binding and mRNA expression indicate the presence of receptors on both afferents and projections. In the thalamus, the binding in some association nuclei may correspond to receptors present on neurons emanating from the deep cortical layers that strongly express the mRNAs, as well as receptors on the visual systems. However, the low binding and high mRNA expression in most nuclei indicate that many receptors are present upon thalamic projections. In the hypothalamus, the mRNA expression parallels the density of binding sites and is the highest in the tuberomammillary nucleus. Further investigation is needed to know if the dense binding and mRNA expression observed in other nuclei such as the paraventricular, ventromedial and medial tuberal nuclei correspond to pre- and/or postsynaptic receptors. mRNAs are also observed in several areas projecting to the tuberomammillary nucleus, such as the ventrolateral preoptic nucleus. In the lower brainstem, the high mRNA expression and very low binding in the locus coeruleus and raphe nuclei indicate that presynaptic rather than somatodendritic receptors regulate noradrenaline and serotonin release, respectively. A similar pattern in vestibular nuclei suggests that receptors located on projections account for the anti-vertigo properties of H_3 receptor antagonists. In the cerebellum, binding is hardly

detectable but a strong mRNA expression is found in most, if not all, Purkinje cells as well as in several central cerebellar nuclei, suggesting the presence of H_3 receptors on efferent projections.

The present study reports the first detailed quantification and/or rating of H_3 receptor mRNAs in the brain. The comparison, performed in the same animals, with the distribution of the H_3 receptor protein provides evidence for the presence of H_3 receptors on many neuronal perikarya, dendrites and projections. Although some localizations, mainly as auto- or heteroreceptors, are consistent with previous functional studies, the physiological role, if any, of most of these presynaptic or postsynaptic receptors remains to be established.

Keywords

histamine
receptor autoradiography
in situ hybridization
H_3 receptor mRNAs
[^{125}I]iodoproxyfan

Abbreviations

EDTA, ethylene diaminetetra-acetate
H_3R, H_3 receptor
TM, tuberomammillary nucleus

The histamine H_3 receptor (H_3R) was initially characterized as an autoreceptor controlling histamine synthesis and release (Arrang et al., 1983, 1987b). Thereafter, it was shown to inhibit presynaptically the release of other monoamines in brain and peripheral tissues as well as of neuropeptides from unmyelinated C fibers. In neurons, the H_3R mediates presynaptic inhibition of release of histamine, noradrenaline, serotonin, dopamine, glutamate, GABA and tachykinins (Schlicker et al., 1994; Hill et al., 1997; Brown et al., 2001; Schwartz and Arrang, 2002), presumably by inhibiting calcium channels (Arrang et al., 1985; Takeshita et al., 1998; Brown and Haas, 1999). The inhibition mediated by H_3autoreceptors expressed in the tuberomammillary nucleus (TM) constitutes a major regulatory mechanism of the activity and functions of histaminergic neurons (Schwartz and Arrang, 2002). However, the physiological role of the H_3Rs present on other neuronal populations remains largely unknown.

The modulation of agonist binding by guanylnucleotides (Arrang et al., 1998) and the sensitivity of various H_3R-mediated responses to pertussis toxin (Clark and Hill, 1996; Takeshita et al., 1998) suggested that the H_3R was a G_i/G_o protein-coupled heptahelical receptor. This proposal was confirmed by the recent cloning of H_3R cDNAs from human (Lovenberg et al., 1999), guinea-pig (Tardivel-Lacombe et al., 2000) and rat (Morisset et al., 2000; Lovenberg et al., 2000; Drutel et al., 2001).

Consistent data have been reported with respect to the autoradiographic localization of H_3R binding sites in rat brain using various radiolabelled agonists, i.e. [^{3}H](R)α-methylhistamine (Arrang et al., 1987a; Yanai et al., 1992; Ryu et al., 1995) and [^{3}H]N$^\alpha$-methylhistamine (Cumming et al., 1991, 1994; Cumming and Gjedde, 1994), or various radiolabelled antagonists, i.e. [^{3}H]*S*-methylthioperamide (Yanai et al., 1994) and [^{125}I]iodophenpropit (Jansen et al., 1994, 2000). Among these various radioligands, only [^{3}H](R)α-methylhistamine was used for a detailed mapping of rat brain (Pollard et al., 1993). However, being an agonist, its binding may be dependent on various factors including G proteins. In a preliminary study, we showed that the radiolabelled antagonist [^{125}I]iodoproxyfan was a reliable probe for autoradiography. Being iodinated, this radioligand displayed a much higher specific activity and therefore allowed a much more sensitive detection of the H_3R than that obtained with [^{3}H](R)α-methylhistamine, even after a short exposure time (Ligneau et al., 1994).

With the cloning of the H_3R, a preliminary and non-rated analysis of the gene transcripts was performed by *in situ* hybridization (Lovenberg et al., 1999). Following this cloning, evidence was obtained for the existence of H_3R isoforms (Tardivel-Lacombe et al., 2000; Morisset et al., 2000) which revealed a differential expression in various brain regions (Drutel et al., 2001; Morisset et al., 2001). In the present study, *in situ* hybridization was used for a detailed mapping of H_3R mRNAs and was combined for the first time with the autoradiographic mapping of the receptor performed in the same animals with [^{125}I]iodoproxyfan. Moreover, the densities of the signals obtained in both approaches on adjacent brain sections were rated and the complementary information provided by each method was taken into account to further identify neuronal populations expressing the receptor in brain. The rat was selected because most of the previous studies that established the localizations, signalling mechanisms and detailed pharmacology of the H_3R were performed in this species.

Experimental procedures

Tissue preparation

All experiments performed in the present study conformed to the National Institutes of Health guidelines (décret no. 2001-464, May 29, 2001, from the French Ministry of Agriculture). Male Wistar rats (Iffa-Credo, L'Arbresle, France) were killed by decapitation, their brain was removed rapidly, immediately frozen (−40°C) by immersion in monochlorodifluoromethane and stored at −70°C. Brain sections (10 μm) were prepared on a cryostat and thaw-mounted onto Superfrost slides. For *in situ* hybridization, sections adjacent to those prepared for incubation with [^{125}I]iodoproxyfan, were immediately fixed for 40 min at 4°C in 4% paraformaldehyde made up in 0.1 M phosphate-buffered saline, pH 7.4, and 0.1% diethylpyrocarbonate water. Sections were rinsed three times (5 min each) in 0.1 M phosphate-buffered saline, pH 7.4, dehydrated through graded ethanol and dried under a stream of cold air. All the sections were stored at −70°C until use.

Receptor binding autoradiography

Autoradiographic localization of [^{125}I]iodoproxyfan binding sites was determined essentially as described (Ligneau et al., 1994). In brief, sections were preincubated for 15 min in phosphate buffer in the presence of 0.1% bovine serum albumin and 1 μM S132 (a 1-substituted imidazole derivative displaying a very low affinity at H_3Rs and used to decrease non-specific labelling). They were then incubated for 1 h at 4°C in the same buffer with 15 pM [^{125}I]iodoproxyfan. Non-specific binding was determined in the presence of 1 μM (R)α-methylhistamine. After incubations, the sections were extensively washed, dried and apposed to a β_{max} Hyperfilm (Amersham, UK) for 5 days at 4°C.

In situ hybridization histochemistry

Sections were incubated at 37°C for 10 min with proteinase K (1 μg/ml), acetylated for 10 min (in 0.1 M triethanolamine, pH 8 and 0.25% acetic anhydride) at room temperature and dehydrated in graded ethanol up to 100%. Hybridization was performed overnight at 55°C in the presence of 4×10^6 d.p.m. of ^{33}P-labelled antisense or sense probes in hybridization buffer (50% formamide, 10% dextran sulfate, 2×standard saline citrate (SSC), 1×Denhardt's solution, 50 mM Tris–HCl buffer, 0.1% NaPPi, 0.1 mg/ml yeast tRNA, 0.1 mg/ml salmon sperm DNA, 1 mM EDTA). Subsequently, the sections were rinsed with 2×SSC for 5 min and incubated for 40 min at 37°C with RNAse A (200 μg/ml). The sections were then extensively washed in SSC, dehydrated in graded ethanol, dried and exposed for 1 week to a β_{max} Hyperfilm (Amersham) or dipped in liquid photographic LM1 emulsion (Amersham) for 2 weeks. Dipped sections were observed with a photomicroscope (Axiophot Zeiss, Carl Zeiss, Germany) after counterstaining of cell nuclei with Mayer's hemalun.

For the hybridization probe, a partial coding sequence of the rat H_3R was amplified from striatum cDNAs using primers 1 and 2 based on the sequence of the third transmembrane domain and the third intracellular loop of the human H_3R, respectively (Lovenberg et al., 1999) (primer 1: 5′-AGTCGGATCCAGCTACGACCGCTTCCTGTC-3′ and primer 2: 5′-AGTCAAGCTTGGAGCCCCTCTTGAGTGAGC-3′). The amplified fragment was sequenced and corresponded to nucleotides 636–1243 of the rat H_3R sequence. It was previously shown to hybridize to the various H_3R mRNA isoforms expressed in the brain or peripheral tissues (Morisset et al., 2001; Héron et al., 2001). After subcloning into pGEM-4Z (Promega, Charbonnières, France), ^{33}P-labelled antisense and sense strand RNA probes were prepared by *in vitro* transcription using a riboprobe kit (Promega).

Analysis of H_3 receptor binding sites and mRNAs

Labelled structures were identified on adjacent Nissl-stained sections and are indicated in abbreviated form (see *Abbreviations used in the figures*) according to the atlas of Paxinos and Watson (1998). The densities of [^{125}I]iodoproxyfan binding sites and the level of H_3R mRNA expression in these structures were arbitrarily rated from 0 to 4+ (double-blind evaluation by four observers). In addition, sections from four separate animals were also digitized using a camera and analyzed using Starwise/Autorad 210 program (Imstar, Paris, France), and the mean optical density of pixels was determined within the main brain regions.

Radiochemicals and drugs

[^{125}I]iodoproxyfan (specific activity of 2000 Ci/mmol at activity reference date) was prepared as described (Krause et al., 1997). S132 (3-cyclohexyl-*N*-[3-(1H-imidazol-1-yl)propyl]propanic amide) was synthesized by Prof. W. Schunack (Freie Universität Berlin, Germany). (R)α-methylhistamine was from Bioprojet (Paris, France).

Results

The densities of [^{125}I]iodoproxyfan binding sites and H_3R mRNAs in the various structures were quantified and/or arbitrarily rated and the results are given in Fig. 1 and Tables 1–5.

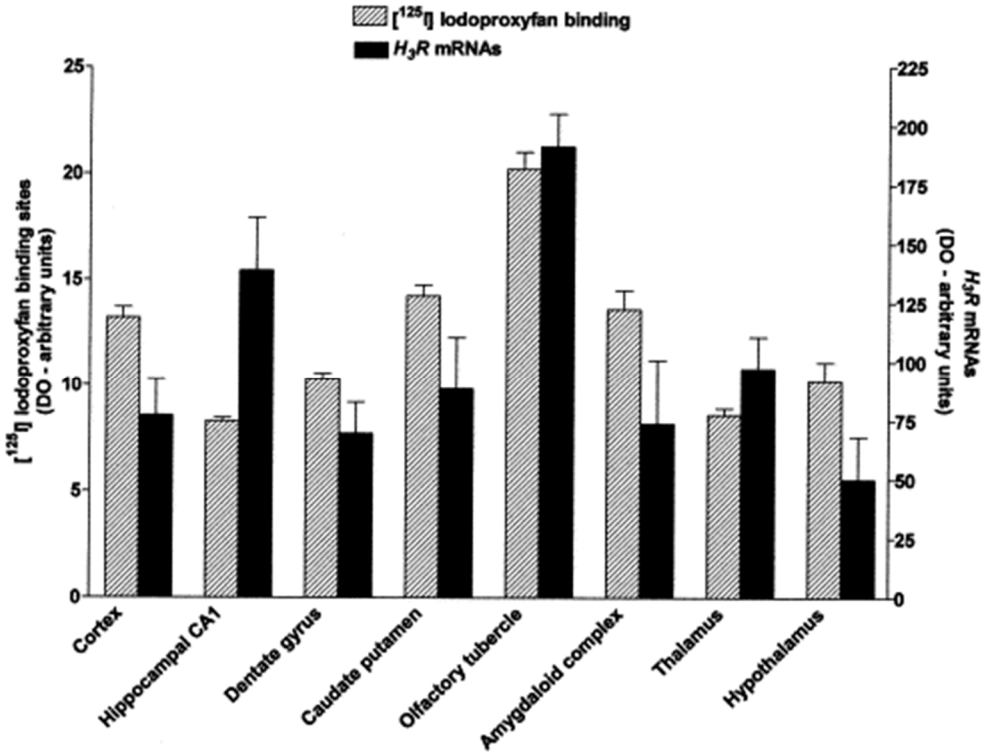

Fig. 1. Distribution of H_3R ([^{125}I]iodoproxyfan) binding sites and *H_3R* mRNAs in rat brain. The autoradiographic signals were measured as the mean optical density (OD) of pixels obtained with an image analyzer in the whole brain region, including all layers and/or nuclei. Results are means±S.E.M. of the values from four animals (4–10 sections per animal).

Table 1. Distribution of [^{125}I]iodoproxyfan binding sites and H_3R mRNAs in the rat cerebral cortex and hippocampus

Brain structures	[^{125}I]iodoproxyfan binding sites	H_3R mRNAs
I. Neocortex	Cortical layers	Cortical layers
Primary motor cortex (M1)	I,II,III,V: 4+, VI: 3+	I,II,III: 1–2+, V: 4+, VI: 2+
Secondary motor cortex (M2)	I,II: 2–3+, III: 4+, V: 3+, VI: 2+	I,II: 1–2+, III: 4+, V: 3+, VI: 2+
Somatosensory cortex (S1, S2)	I,II,III,IV: 3–4+, V: 1–2+, VI: 2–3+	I,II,III,IV: 1–2+, V: 4+, VI: 3+
Auditory cortex (Au1)	I,II,III,IV: 4+, V: 2+, VI: 3+	I,II,III: 1–2+, IV,V: 4+, VI: 3+
Visual cortex (V1, V2)	I,II,III,IV: 3–4+, V: 2+, VI: 3–4+	I,II,III,IV: 1–2+, V: 4+, VI: 2+
II. Allo- and periallocortices		
Prefrontal:		
Cingulate cortex (Cg1, Cg2)	3–4+	4+ (I,II: 2+)
Agranular insular cortex (AI)	3–4+	4+
Orbitofrontal cortex (LO, MO, VLO, VO)	3–4+	4+
Retrosplenial cortex (RSA, RSGa,b)	1–2+	3+ (I,II: 1–2+)
Perirhinal cortex (PRh)	3–4+	3–4+
Piriform cortex (Pir)	2+	4+
III. Hippocampic formation		
Ammon's Horn		
Stratum oriens (so)	0	0
Pyramidal cell layer	CA1: 1+, CA2/CA3: 0	CA1: 4+, CA2: 1+, $CA3_{ventral}$: 3+
Stratum lucidum (sl) of CA3	0–1+	0
Stratum radiatum (sr)	0	0
Stratum lacunosum moleculare (s l-m)	2+ (CA1)	0–1+ (CA1)
Dentate gyrus		
Granular layer (GrDG)	0	2+
Molecular layer (ml)	2+	0
Polymorph layer (PoDG)	0	1+
Subiculum (S)	2+ (molecular layer)	3+ (pyramidal cell layer)
Entorhinal cortex (Ent)	3–4+	I: 4+, II,III,IV: 2+, V,VI: 4+
Tenia tecta (TT)	3–4+	4+

Table 2. Distribution of [^{125}I]iodoproxyfan binding sites and H_3R mRNAs in the rat olfactory system, basal forebrain and amygdaloid complex

Brain structures	[^{125}I]iodoproxyfan binding sites	H_3R mRNAs
I. Olfactory system		
Anterior olfactory nucleus (AOD, AOL, AOM, AOP, AOV)	4+	3+
II. Basal forebrain		
Septal areas		
Lateral septal nucleus (LSD, LSI)	1+	2+
Medial septal nucleus (MS)	0–1+	2+
Nucleus of the diagonal band (HDB)	1+	0–1+
Basal ganglia		
Nucleus accumbens (AcbC, AcbSh)	4+	3+
Caudate putamen (CPu)	3–4+	3+
Olfactory tubercle (Tu)	4+	4+
Globus pallidus ext. (Gpe)	3–4+	0–1+
Globus pallidus int. (Gpi)	3–4+	0–1+
Islands of Calleja (ICj, ICjM)	0–1+	0
Substantia innominata (SI)	1–2+	0–1+
Subthalamic nucleus (STh)	1+	2+
III. Amygdaloid complex		
Medial and central nuclei (Me, Ce)	2–3+	2+
Lateral and basolateral nuclei (La, BL)	2–3+	3–4+
Bed nucleus of the stria terminalis (BST)	1+	2–3+

Table 3. Distribution of [^{125}I]iodoproxyfan binding sites and H_3R mRNAs in the rat thalamus, habenula and zona incerta

Brain structures	[^{125}I]iodoproxyfan binding sites	H_3R mRNAs
Thalamic sensory nuclei		
Dorsolateral geniculate nucleus (DLG)	1+	2–3+
Ventral posterolateral nucleus (VPL)	0–1+	3+
Ventral posteromedial nucleus (VPM)	0–1+	3+
Posterior nuclear group (PO)	1–2+	3+
Medial geniculate nucleus, dorsal part (MGD)	1–2+	3+
Medial geniculate nucleus, ventral part (MGV)	1–2+	3+
Medial geniculate nucleus, medial part (MGM)	0–1+	1+
Thalamic motor nuclei		
Ventrolateral nucleus (VL)	0–1+	3+
Ventromedial nucleus (VM)	0–1+	3–4+
Thalamic association nuclei		
Mediodorsal nucleus (MD, MDC, MDL, MDM)	1–2+	3+
Anterodorsal nucleus (AD)	0–1+	3+
Anteroventral nucleus (AV)	0–1+	3+
Anteromedial nucleus (AM)	1–2+	3–4+
Lateral posterior nucleus (LP)	2–3+	2–3+
Laterodorsal nucleus (LD)	2–3+	3–4+
Thalamic midline and intralaminar nuclei		
Paraventricular nucleus (PV, PVA, PVP)	3+	3–4+
Paratenial nucleus (PT)	1+	2–3+
Intermediodorsal nucleus (IMD)	2–3+	3+
Central nucleus (CL)	0–1+	3+
Central medial nucleus (CM)	0–1+	3–4+
Paracentral nucleus (PC)	0–1+	3+
Parafascicular nucleus (PF)	0–1+	1–2+
Reuniens nucleus (Re)	2–3+	3+
Rhomboid nucleus (Rh)	2–3+	3+
Thalamic reticular nucleus (Rt)	0–1+	2–3+
Habenular nucleus (Hb)	0	1+
Zona incerta (ZI)	0–1+	1–2+

Table 4. Distribution of [^{125}I]iodoproxyfan binding sites and H_3R mRNAs in the rat hypothalamus

Brain structures	[^{125}I]iodoproxyfan binding sites	H_3R mRNAs
Preoptic region		
Periventricular zone		
Periventricular nucleus (Pe)	1+	1+
Vascular organ of the lamina terminalis (VOLT)	1+	1+
Median preoptic nucleus (MnPO)	1+	1+
Anteroventral periventricular nucleus (AVPe)	1+	1+
Medial zone		
Medial preoptic area (MPA)	2+	2+
Medial preoptic nucleus (MPO)	2+	3–4+
Anterodorsal preoptic nucleus (ADP)	1+	1+
Parastrial nucleus (PS)	1+	1+
Lateral zone		
Lateral preoptic nucleus (LPO)	1+	2+
Magnocellular preoptic nucleus (MCPO)	0–1+	2+
Ventrolateral preoptic nucleus (VLPO)	1+	1+
Anterior region		
Periventricular zone		
Suprachiasmatic nucleus (SCh)	1+	1+
Paraventricular nucleus (PaAP)	2+	3+
Medial zone		
Anterior hypothalamic area (AH)	1+	1+
Anterior area, anterior part (AHA)	2+	3+
Anterior area, central part (AHC)	1+	1–2+
Anterior area, posterior part (AHP)	1+	1–2+
Lateral zone		
Lateral hypothalamic area (LH)	1+	1–2+
Supraoptic nucleus (SO)	1–2+	1–2+
Tuberal region		
Periventricular zone		
Arcuate nucleus (Arc)	1–2+	1+
Medial zone		
Tuber cinereum area (TC)	1+	1–2+
Ventromedial nucleus (VMH)	2–3+	3–4+
Dorsomedial nucleus (DM)	1–2+	2+

Lateral zone		
Medial tuberal nucleus (Mtu)	2–3+	2+
Terete nucleus (Te)	2–3+	3–4+
Magnocellular nucleus (MCLH)	1+	1+
Mammillary region		
Medial zone		
Posterior hypothalamic area (PH)	2+	2+
Premammillary nucleus (PMD, PMV)	1–2+	1+
Medial mammillary nucleus (MM)	2+	0–1+
Lateral mammillary nucleus (LM)	0–1+	1+
Supramammillary nucleus (SuM)	1–2+	2–3+
Lateral zone		
TM	4+	4+

Table 5. Distribution of [^{125}I]iodoproxyfan binding sites and H_3R mRNAs in the rat caudal areas

Brain structures	[^{125}I]iodoproxyfan binding sites	H_3R mRNAs
Substantia nigra, pars compacta (SNC)	1+	1+
Substantia nigra, pars reticulata (SNR)	4+	1+
Ventral tegmental area (VTA)	0–1+	0
Inferior colliculus (CIC, ECIC)	1+	1–2+
Superior colliculus (SC)	1+	1–2+
Nucleus of the optic tract (OT)	0–1+	1–2+
Raphe nuclei		
Dorsal raphe nucleus (DRD, DRV)	0–1+	2+
Median raphe nucleus (MnR)	0–1+	1+
Locus coeruleus (LC)	0–1+	3+
Nucleus of the solitary tract (Sol)	0–1+	2+
Pontine nuclei (Pn)	2+	2+
Cerebellar cortex		
Granular layer (GL)	0–1+	0–1+
Molecular layer (ML)	0–1+	0
Purkinje cell layer (P)	0–1+	3+
Dorsal cochlear nucleus (DC)	0–1+	2+
Inferior olive (IO)	0–1+	1–2+
Vestibular nuclei (Lve, MVeMC, MVePC, SpVe, SuVe, VeCb)	0–1+	2–3+
Facial nucleus (7)	0–1+	2+
Periaqueductal gray (PAG)	2+	2+

Autoradiograms from sagittal or frontal sections generated with [^{125}I]iodoproxyfan or with a selective antisense riboprobe are shown in Figs. 2–6. Figures 7–9 display the cellular localization of H_3R mRNAs in sections from various brain areas.

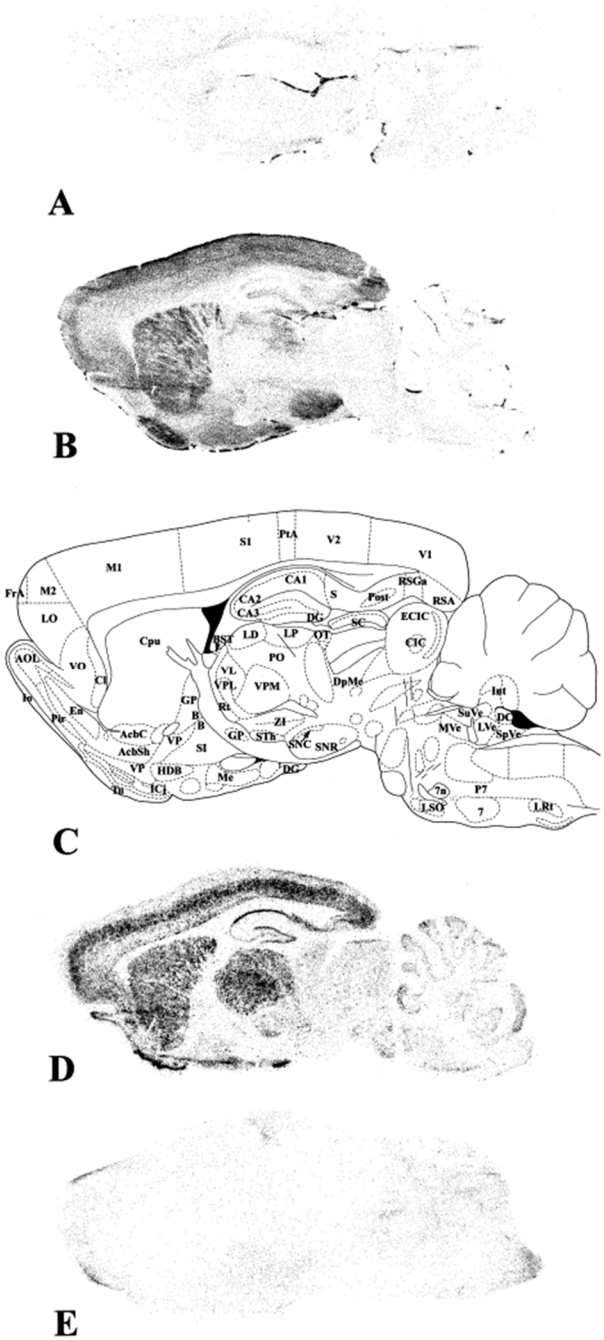

Fig. 2. Distribution of H_3R ([^{125}I]iodoproxyfan) binding sites and *H_3R* gene transcripts in rat brain sagittal sections. Sections were incubated with 15 pM [^{125}I]iodoproxyfan alone (total binding, B) or in the presence of 1 μM (R)α-methylhistamine (non-specific binding, A). The laterality of the sections is 2.4 mm (Paxinos and Watson, 1998) (C). Sections from the same level of separate animals were hybridized with ^{33}P-labelled antisense (D) or sense (E) riboprobes.

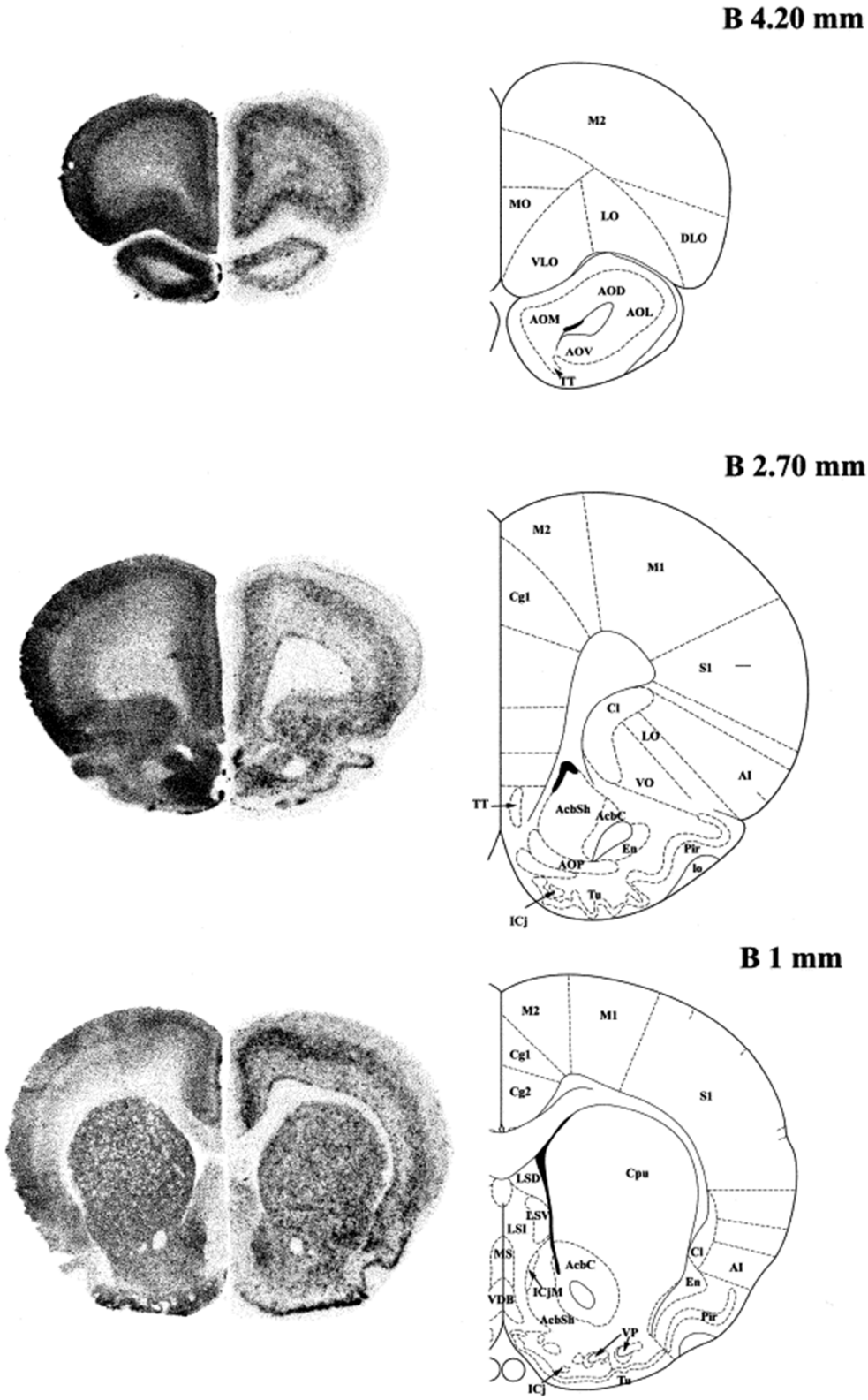

Fig. 3. Autoradiographic distribution of [^{125}I]iodoproxyfan binding sites and *H_3R* gene transcripts on serial frontal sections of the rat brain. H_3R binding sites (left) and mRNAs (right) were visualized on sections using [^{125}I]iodoproxyfan alone or a ^{33}P-labelled antisense riboprobe, respectively. The brain structures were identified at the corresponding anteriorities indicated by the bregma distance in mm (reproduced with permission from Paxinos and Watson, Academic Press, 1998).

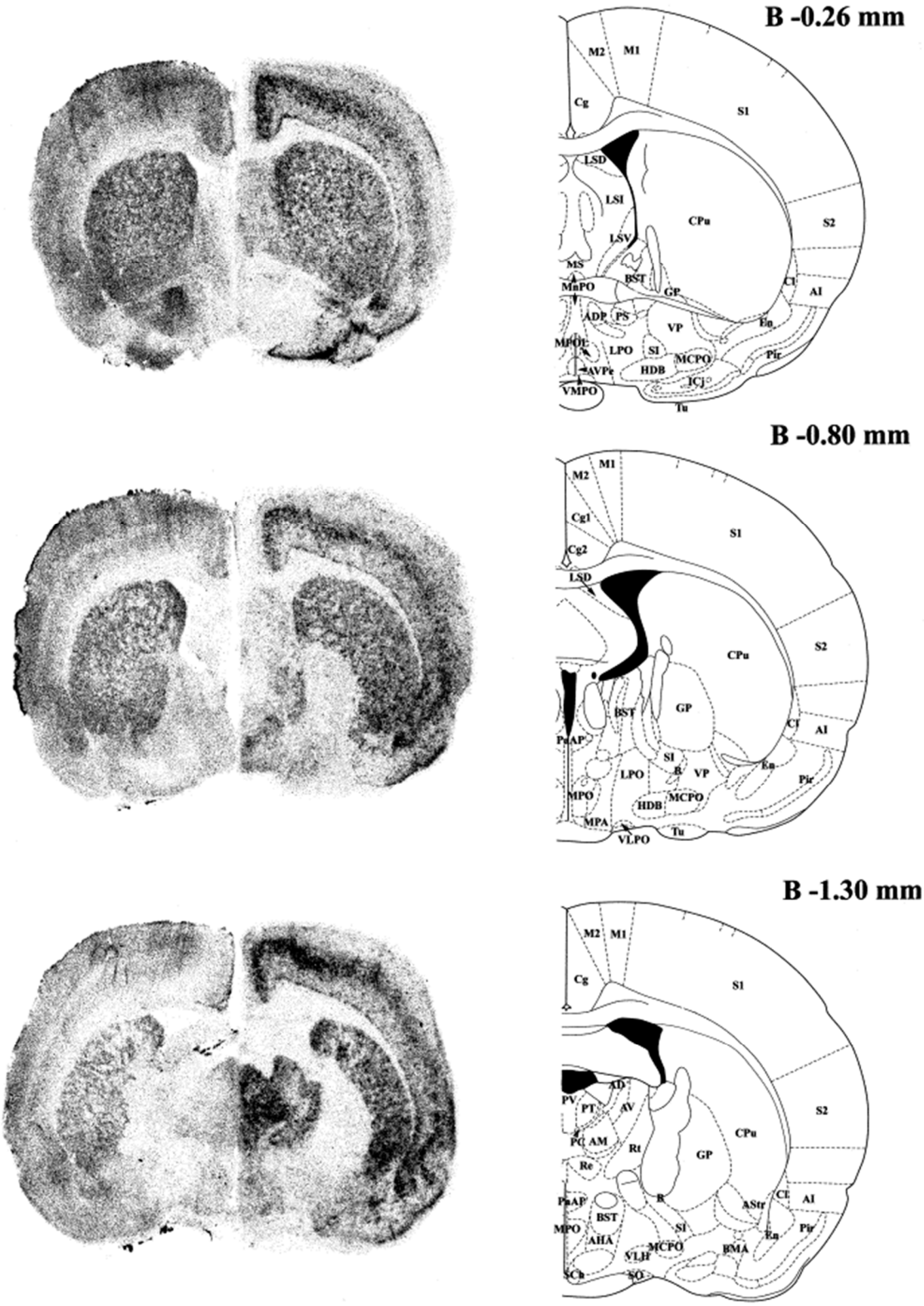

Fig. 4. Autoradiographic distribution of [^{125}I]iodoproxyfan binding sites and *H_3R* gene transcripts on serial frontal sections of the rat brain. H_3R binding sites (left) and mRNAs (right) were visualized on sections using [^{125}I]iodoproxyfan alone or a ^{33}P-labelled antisense riboprobe, respectively. The brain structures were identified at the corresponding anteriorities indicated by the bregma distance in mm (reproduced with permission from Paxinos and Watson, Academic Press, 1998).

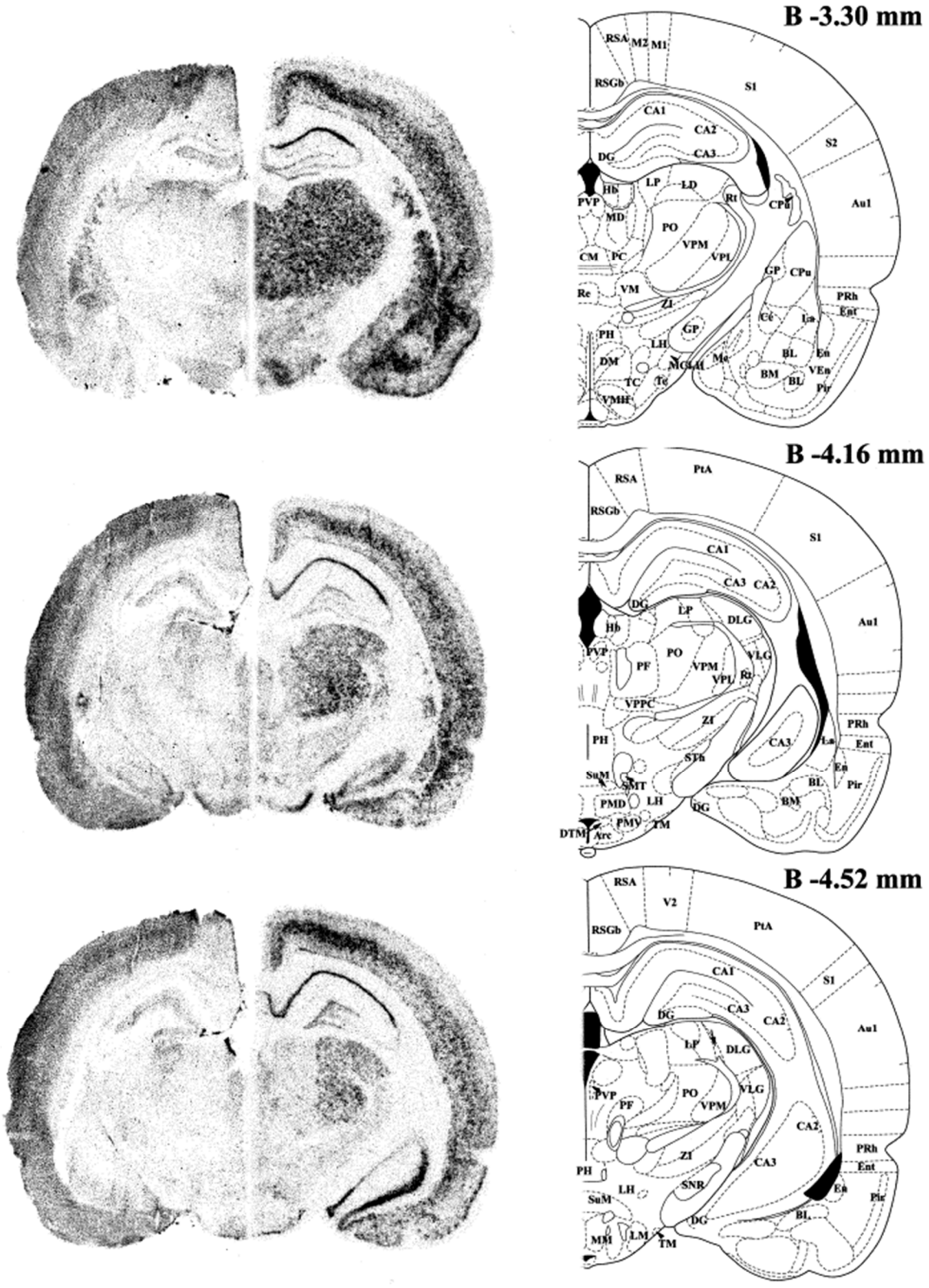

Fig. 5. Autoradiographic distribution of [^{125}I]iodoproxyfan binding sites and *H_3R* gene transcripts on serial frontal sections of the rat brain. H_3R binding sites (left) and mRNAs (right) were visualized on sections using [^{125}I]iodoproxyfan alone or a ^{33}P-labelled antisense riboprobe, respectively. The brain structures were identified at the corresponding anteriorities indicated by the bregma distance in mm (reproduced with permission from Paxinos and Watson, Academic Press, 1998).

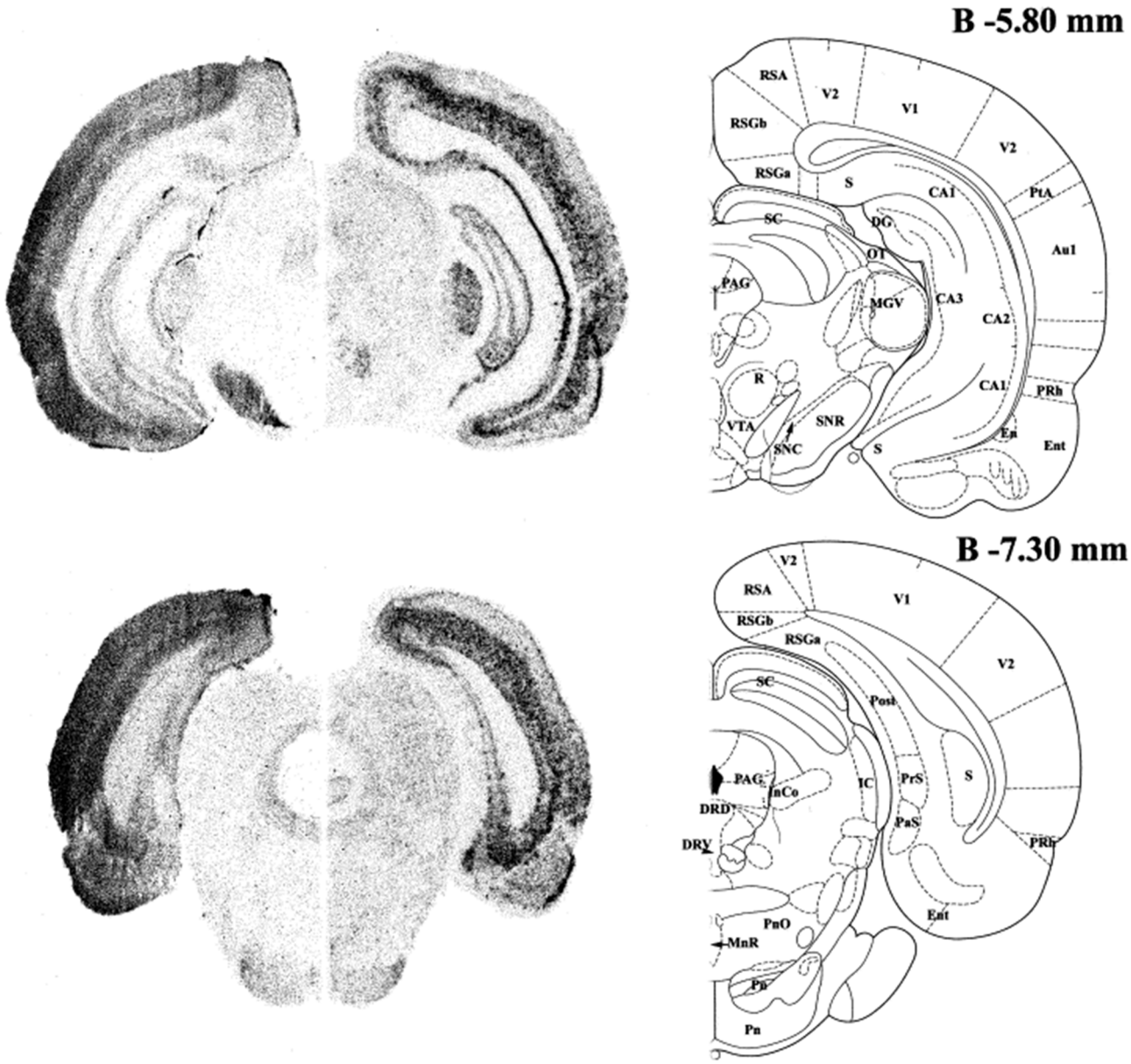

Fig. 6. Autoradiographic distribution of [^{125}I]iodoproxyfan binding sites and *H_3R* gene transcripts on serial frontal sections of the rat brain. H_3R binding sites (left) and mRNAs (right) were visualized on sections using [^{125}I]iodoproxyfan alone or a ^{33}P-labelled antisense riboprobe, respectively. The brain structures were identified at the corresponding anteriorities indicated by the bregma distance in mm (reproduced with permission from Paxinos and Watson, Academic Press, 1998).

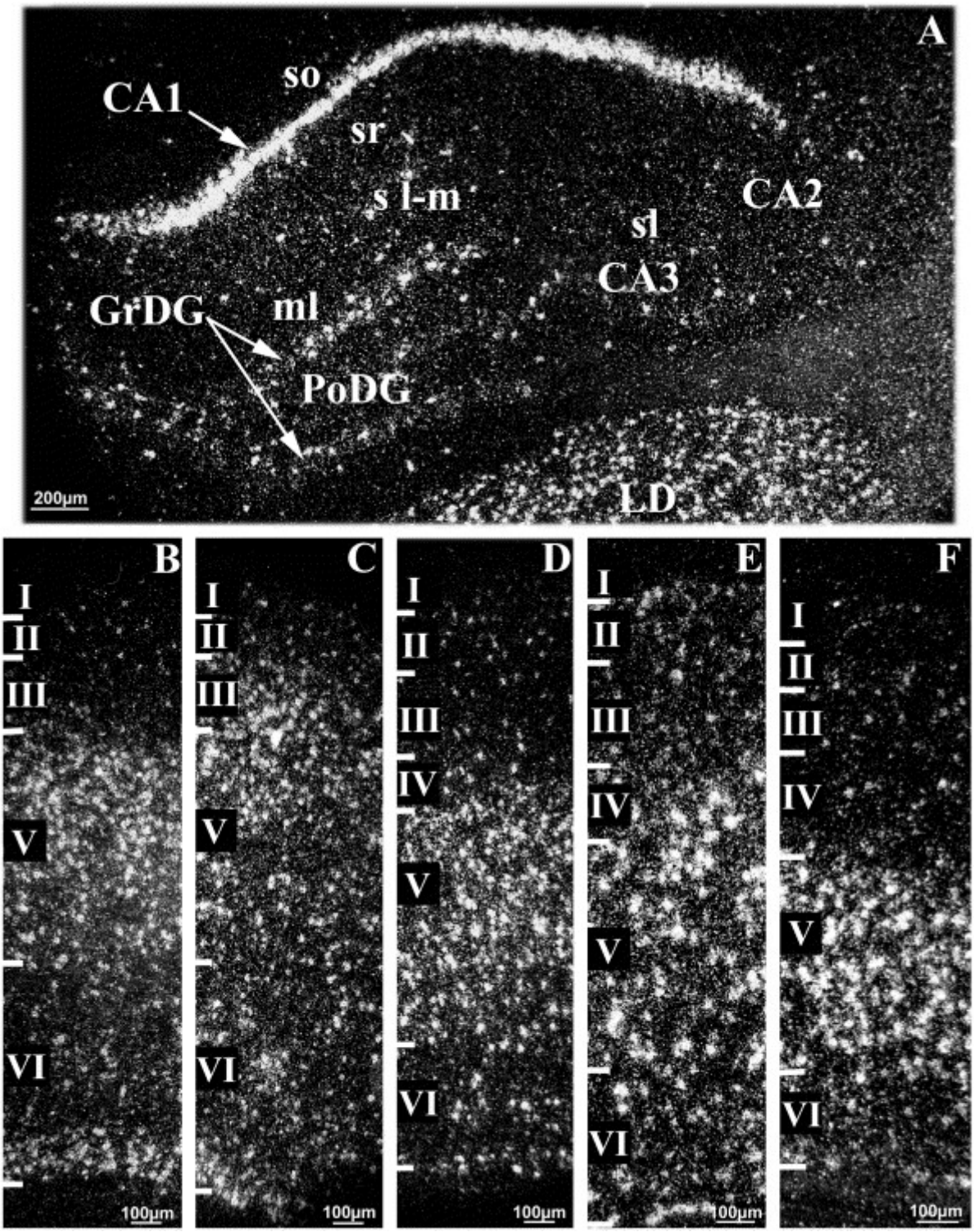

Fig. 7. Cellular localization of *H_3R* gene transcripts within the hippocampus and cerebral cortex as revealed by *in situ* hybridization using a photographic emulsion (dark field photomicrographs). (A) localization of *H_3R* mRNAs in the hippocampus. The arrows indicate the pyramidal cell layer of the CA1 field and the granule cell layer of the dentate gyrus (GrDG). (B–F) Localization of *H_3R* mRNAs in the cerebral cortex (B, primary motor cortex; C, secondary motor cortex; D, somatosensory cortex; E, auditory cortex; F, visual cortex). The cortical layers, identified on a Nissl-stained adjacent section, are indicated on the left-hand side.

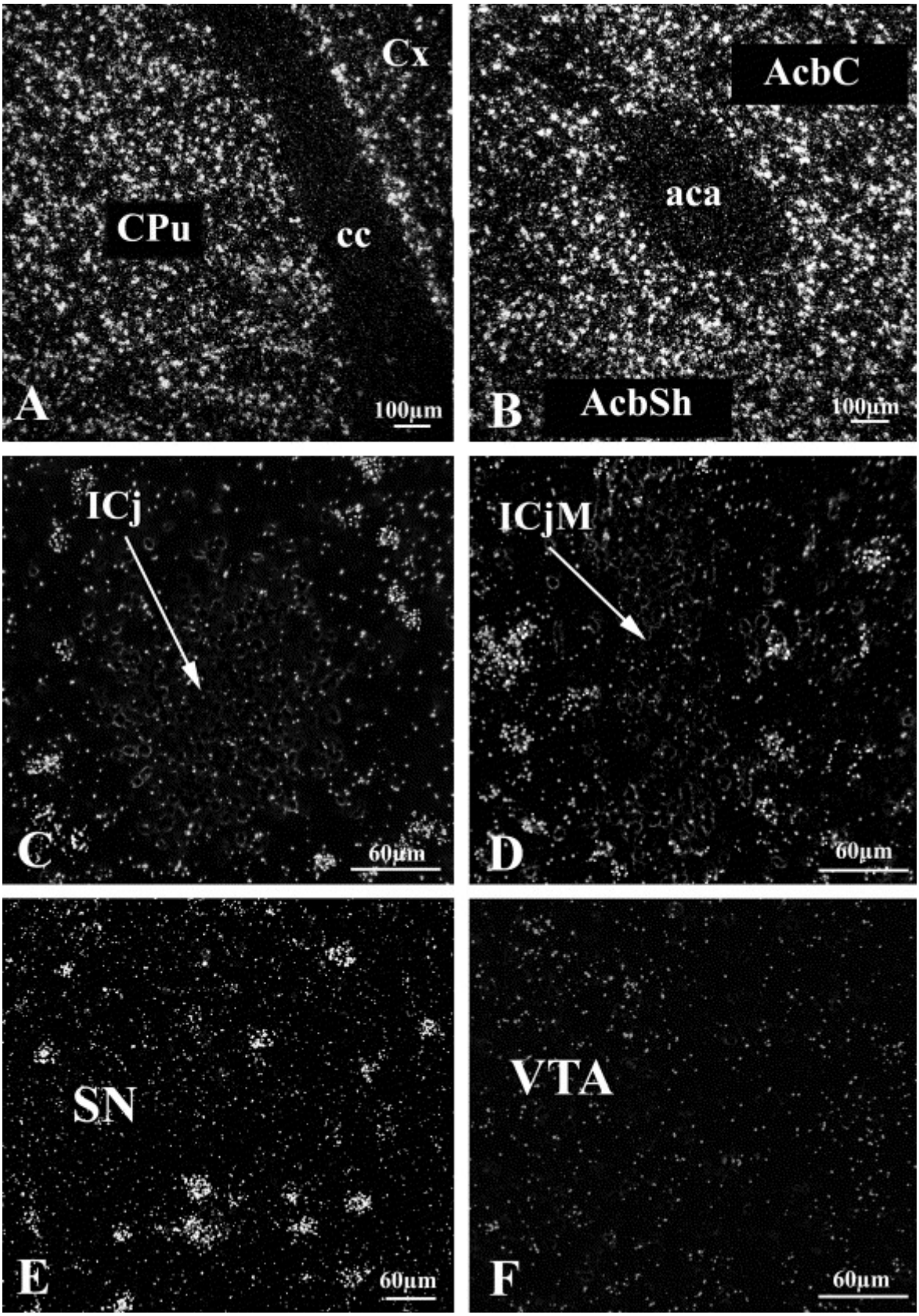

Fig. 8. Cellular localization of *H_3R* gene transcripts in the basal ganglia. Localization of *H_3R* mRNAs within the caudate putamen (A), nucleus accumbens (B), islands of Calleja (C, D), substantia nigra pars compacta (E) and ventral tegmental area (F), as revealed by *in situ* hybridization using a photographic emulsion (dark field photomicrographs).

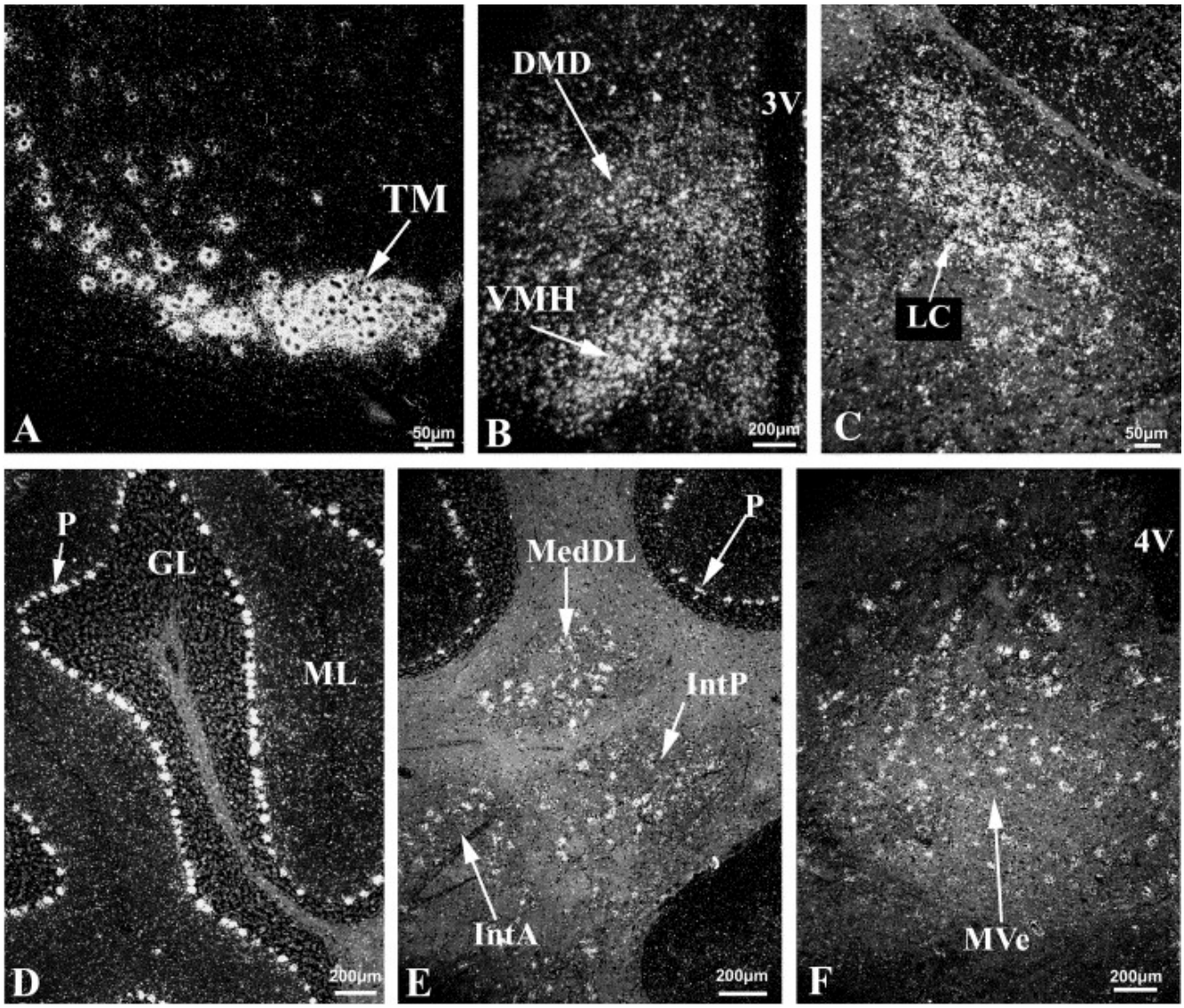

Fig. 9. Cellular localization of *H_3R* gene transcripts in the hypothalamus and caudal areas. Localization of *H_3R* mRNAs in the TM (A), ventromedial and dorsomedial hypothalamic nuclei (B), locus coeruleus (C), cerebellar cortex (D), central cerebellar nuclei (E) and medial vestibular nucleus (F).

Autoradiographic distribution of [^{125}I]iodoproxyfan binding sites

A heterogeneous labelling of sections was observed (Fig. 1) which contrasted with the extremely faint non-specific background, as evidenced in the presence of 1 µM (R)-α-methylhistamine (Fig. 2A).

The highest receptor densities were found in the cerebral cortex, tenia tecta, anterior olfactory nucleus, nucleus accumbens, caudate putamen, olfactory tubercles, external and internal segments of the globus pallidus, amygdala, substantia nigra pars reticulata and TM (Fig. 1 and Tables 1, 2, 4 and 5). In the cerebral cortex, H_3R binding sites were abundant in all areas. However, their laminar distribution was clearly apparent in several areas of the neocortex such as the somatosensory cortex and the auditory and visual cortices, in which a much lower density of binding sites was detected in layer V (Table 1). A laminar pattern of labelling was also observed in the hippocampal formation, a moderate binding being detected in the pyramidal layer and stratum lacunosum moleculare of the CA1 field and in the

molecular layer of the dentate gyrus and subiculum (Table 1). A highly heterogeneous labelling was observed in the thalamus, with the highest binding being found in the lateral posterior, laterodorsal, paraventricular, intermediodorsal, reuniens and rhomboid nuclei (Table 3). A rather dense binding was also found in some hypothalamic nuclei, including the medial preoptic, paraventricular, supraoptic, arcuate, ventromedial, medial tuberal, terete, mammillary and tuberomammillary nuclei (Table 4). In the lower brainstem, moderate labelling was detected in the colliculi, pontine nuclei and periaqueductal gray (Table 5).

Autoradiographic distribution of H_3 receptor gene transcripts

The distribution of H_3R mRNAs was analyzed on adjacent sections from the same levels by *in situ* hybridization. Well-contrasted autoradiograms were generated with the ^{33}P-labelled antisense RNA probe, whereas a very faint background was seen with the corresponding sense probe (Fig. 2E). In addition, the cellular localization of *H_3R* gene transcripts was analyzed in various brain regions by *in situ* hybridization in sections dipped in a photographic emulsion (Figs. 7–9).

The highest level of expression was found in the cerebral cortex, hippocampus, tenia tecta, nucleus accumbens, caudate putamen, olfactory tubercles, amygdala, thalamus, some hypothalamic nuclei, cerebellum and vestibular nuclei (Fig. 1, Tables 1–5).

In the cerebral cortex, a laminar distribution pattern was found, the expression being stronger in the intermediate and deep layers than in the superficial layers. In all areas of the neocortex, layer V displayed an intense mRNA labelling, strong signals being observed within pyramidal cells (not shown). Strong signals were also found in pyramidal layer III in the secondary motor cortex, granular layer IV in the auditory cortex and layer VI in the somatosensory and auditory cortices (Table 1 and Fig. 7B–F). An intense mRNA labelling was also observed in the archeocortex and the distribution pattern in the cingulate and retrosplenial cortices was laminar, with a lower expression in superficial layers (Table 1).

In the hippocampic formation, a strong mRNA expression was found in CA1 and ventral CA3 pyramidal layers of Ammon's Horn as well as in the pyramidal layer of the subiculum (Table 1, Fig. 7A). A very low density of signals was detected in the stratum lacunosum moleculare of the CA1 field and no signals were detected in the other layers of Ammon's Horn. In the dentate gyrus, mRNA signals were rather dense in the granular layer, lower in the polymorph layer and not detected in the molecular layer (Table 1, Fig. 7A). An intense labelling was found in the entorhinal cortex, particularly in layers I, V and VI, and in the tenia tecta (Table 1).

In the olfactory system, a strong mRNA expression was found in the anterior olfactory nucleus. Dense signals were also observed in the amygdala and in the bed nucleus of the stria terminalis (Table 2). In the basal forebrain, a strong and homogeneous expression was detected in the caudate putamen and in the core and shell subdivisions of the nucleus accumbens (Fig. 8A and B). A high expression was found in the olfactory tubercles. A rather dense mRNA labelling was detected in the lateral and medial septal nuclei as well as in the subthalamic nucleus (Table 2). The signals were very low in the globus pallidus and substantia innominata. H_3R gene transcripts were not detected in islands of Calleja (Table 2, Fig. 8C, D).

In the thalamus, strong signals were evidenced in all sensory nuclei, except the medial part of the medial geniculate nucleus, and in all motor and association nuclei (Table 3). All midline and intralaminar nuclei also expressed high mRNA densities, although a lower density was

evidenced in the parafascicular nucleus. Rather dense signals were also found in the reticular nucleus (Table 3). A low to moderate mRNA expression was detected in the habenula and zona incerta (Table 3).

In the hypothalamus, a highly heterogeneous mRNA expression was observed (Table 4). In the preoptic region, high signals were observed in the medial preoptic nucleus. Rather dense signals were also evidenced in the medial preoptic area, the lateral and magnocellular preoptic nuclei. In the anterior region, a dense mRNA labelling was found in the paraventricular nucleus and in the anterior part of the anterior area. A low to moderate density of signals was observed in the supraoptic and suprachiasmatic nuclei (Table 4). In the tuberal region, a strong mRNA expression was observed in the ventromedial and terete nuclei. Rather dense signals were also found in the dorsomedial and medial tuberal nuclei (Table 4, Fig. 9B). In the mammillary region, strong signals were evidenced in the TM and rather dense signals were found in the posterior hypothalamic area and supramammillary nucleus (Table 4, Fig. 9A).

In the caudal areas, dense mRNA signals were evidenced within the locus coeruleus and the various vestibular nuclei (Table 5, Fig. 9C, F). In the cerebellum, a strong expression of H_3R mRNAs was found in most, if not all, Purkinje cells of the cortex and a rather high expression was also detected in several central cerebellar nuclei including the medial cerebellar nucleus and the interposed cerebellar nuclei (Table 5, Fig. 9D, E). A moderate density of hybridization signals was detected in the periaqueductal gray, in several nuclei of the brainstem, e.g. the dorsal raphe, solitary tract, dorsal cochlear, facial and pontine nuclei, and in the colliculi and inferior olive (Table 5). Hybridization signals were low in the substantia nigra pars compacta and pars reticulata and were not detected in the ventral tegmental area (Table 5, Fig. 8E, F).

Discussion

The aim of the present work was to rate and compare the detailed autoradiographic distributions of the H_3R and its gene transcripts on adjacent brain sections from the same rats, using two selective probes, i.e. the radioligand [^{125}I]iodoproxyfan (Ligneau et al., 1994) and a ^{33}P-labelled riboprobe corresponding to a partial coding sequence (~600 bp) of the rat H_3R and detecting all the previously cloned functional isoforms of the receptor (Morisset et al., 2001; Héron et al., 2001). The detailed distribution that we have reported using [^{3}H](R)α-methylhistamine indicated already that the highest concentrations of the H_3R were observed in the cerebral cortex and basal ganglia (Pollard et al., 1993). However, the relative H_3R densities within cell layers of the cerebral cortex, cerebellum and hippocampus and in nuclei of thalamus, hypothalamus and brainstem could be determined with much more details with [^{125}I]iodoproxyfan (Figs. 3–6), leading to the identification of neuronal populations expressing the H_3R. Recent *in situ* hybridization studies have already reported a strong expression of H_3R mRNAs not only within the TM, in which histaminergic perikarya are located (Panula and Airaksinen, 1991; Tohyama et al., 1991; Wouterlood and Steinbusch, 1991), but also in other regions of the rat brain (Lovenberg et al., 1999; Tardivel-Lacombe et al., 2000; Morisset et al., 2001; Drutel et al., 2001). However, these studies were not as detailed as the present one, the signals obtained were not rated and some of these studies used oligoprobes for which an inherently lower sensitivity, due to a lower specific activity, is expected when compared to that of a riboprobe. The main finding of this study is that the localization of the H_3R parallels that of its mRNAs in some areas, but that interesting discrepancies were observed in numerous other brain regions, revealing the expression of many H_3Rs on neuronal dendrites and/or projections.

In the cerebral cortex, H_3R mRNA expression is found in all layers but displays a laminar pattern which varies within the different areas (Table 1). Previous studies suggested that these mRNAs predominantly encode for isoforms with 445 and 397 amino acids (Drutel et al., 2001; Morisset et al., 2001). The strong mRNA labelling that we constantly observed at the cellular level in pyramidal cells of layer V (Fig. 7B–F; Tardivel-Lacombe et al., 2000), contrasts with the low binding found in this layer (except in the motor cortex). This suggests that a part of the dense binding in layers I–IV corresponds to H_3Rs located upon apical dendrites of pyramidal cells present in layer V. Such an apical sorting of the H_3R is supported by the intense H_3R immunoreactivity recently reported within the soma and apical dendrites of layer V pyramidal cells in the mouse brain (Chazot et al., 2001). In addition, H_3Rs may be located on granule cells, at least in layer IV, in which we easily detect mRNAs, particularly in the auditory cortex (Fig. 7E). In layer VI, the mRNA expression probably accounts for the binding in this layer (Fig. 7 and Table 1). All these findings indicate that H_3Rs are present on various types of cortical neurons, in agreement with the strong decrease in cortical binding induced by quinolinic or kainic acid lesions (Cumming et al., 1991; Pollard et al., 1993). However, several of our findings are consistent with the expected presence of presynaptic H_3Rs on afferents. In contrast with the moderate to strong expression previously reported in layer II with an oligoprobe (Lovenberg et al., 1999; Drutel et al., 2001), a low mRNA expression was observed in this layer as well as in layer I with the riboprobe (Fig. 7B–F). The binding observed in these layers may therefore largely correspond to autoreceptors modulating histamine release from the dense network of histaminergic fibers innervating these layers (Takeda et al., 1984; Steinbusch and Mulder, 1984; Arrang et al., 1985) and to presynaptic heteroreceptors known to modulate noradrenaline and serotonin release in the cerebral cortex (Schlicker et al., 1994). In addition, the strong mRNA expression (but very low binding) in thalamic sensorimotor nuclei and the dense binding in cortical layer IV, a major destination of glutamatergic thalamocortical projections, suggest that H_3Rs are presynaptically located upon, and may regulate the activity of, thalamic relay neurons. All these cortical H_3Rs may act in a synergic manner to regulate cortical activation known to be induced by histaminergic neurons, either directly by their ascending cortical projections, or indirectly via the thalamocortical system (Lin, 2000). Besides H_3 auto- and heteroreceptors, H_3Rs on cortical pyramidal neurons and thalamic relay neurons, that are both depolarized by histamine (Steriade et al., 1993; Reiner and Kamondi, 1994), may be involved in the arousal and improvement of attention induced by H_3R antagonists (or inverse agonists) (Lin et al., 1990; Ligneau et al., 1998).

In Ammon's Horn of the hippocampus, the binding is moderate and restricted to the CA1 field, in agreement with the low binding previously reported in this region (Cumming et al., 1991; Pollard et al., 1993). The small labelling of the pyramidal layer indicates the presence of some H_3Rs on pyramidal cell perikarya. However, the very high mRNA expression in pyramidal layers of the CA1 and ventral CA3 fields, which may predominantly encode for the longer H_3R isoform (Drutel et al., 2001; Morisset et al., 2001), indicates that H_3Rs are more abundant on the efferent projections of pyramidal cells. In agreement, a dense binding is observed within the target areas of the latter, i.e. the perirhinal cortex, tenia tecta, anterior olfactory nucleus, accumbens nucleus and anterior and dorsomedial hypothalamic nuclei. The binding in the stratum lacunosum moleculare of the CA1 field may represent receptors located upon afferents including perforant pathway fibers (Amaral and Witter, 1989) and histaminergic nerve endings (Panula et al., 1989). *In the dentate gyrus*, H_3R mRNAs are found in the granule cell layer (Fig. 7A), whereas binding is restricted to the molecular layer (Table 1), suggesting the insertion of H_3Rs in the dendrites of granule cells. It should be noted that H_3Rs may be colocalized with H_1 and H_2 receptors in most, if not all, granule cells, since

H_1 and H_2 receptor gene expression and binding occur with a similar pattern (Traiffort et al., 1994; Vizuete et al., 1997). However, whether H_3Rs regulate the activity of granule cells and mossy fibers remains unknown. Whereas a prominent H_3R immunoreactivity was detected in the granular and molecular layer of the CA3 field in the mouse hippocampus (Chazot et al., 2001), we detect a very low binding, if any, in the CA3 field, including its stratum lucidum, which rather suggests that mossy fibers are not endowed with presynaptic H_3Rs. A part of the binding in the molecular layer of the dentate gyrus is expected to represent autoreceptors on histaminergic projections (Panula et al., 1989) and heteroreceptors inhibiting glutamate release from the perforant path (Brown and Reymann, 1996; Brown and Haas, 1999). The latter is formed by axons of stellate and pyramidal cells of layers II and III of the entorhinal cortex, respectively, where we found a clear mRNA expression (Table 1).

In the basal ganglia, high densities of both H_3R binding sites and mRNAs are found in the caudate putamen, nucleus accumbens and olfactory tubercles. High densities of H_3R mRNAs were already reported in the striatum from various species including rat (Lovenberg et al., 1999), guinea-pig (Tardivel-Lacombe et al., 2000) and human (Anichtchik et al., 2001) and may predominantly encode for the shorter H_3R isoform (Morisset et al., 2001; Drutel et al., 2001). Consistent with lesion experiments indicating that the vast majority of striatal H_3Rs were present on intrinsic neurons (Cumming et al., 1991; Pollard et al., 1993; Ryu et al., 1994a,b, 1995, 1996; Anichtchik et al., 2000), H_3R mRNAs are densely and homogeneously distributed within the caudate putamen and nucleus accumbens when analyzed at the cellular level (Fig. 8A, B). This observation suggests that most H_3Rs expressed in the striatal complex are present on GABAergic medium-sized spiny neurons which represent over 90% of striatal neuronal cells and project to the pallidum and substantia nigra (Gerfen, 1992; Parent and Harati, 1995; Kawaguchi, 1997). Consistent with this hypothesis, the high densities of H_3Rs found in the human striatum and globus pallidus (Martinez-Mir et al., 1990;Anichtchik et al., 2001) are significantly lower in Huntington's disease (Goodchild et al., 1999), which is characterized by degeneration of medium-sized, spiny projection neurons in the striatum (Sharp and Ross, 1996). However, the presence of H_3Rs on other striatal elements cannot be ruled out. Recently, an intense bipolar H_3R immunoreactivity was reported at the level of medium-sized neuronal cell somata, possibly indicating the presence of presynaptic H_3Rs located on the inputs from the cholinergic interneurons (Chazot et al., 2001). Moreover, some binding sites are expected from functional studies to represent presynaptic H_3Rs upon striatal afferents. H_3autoreceptors are known to modulate histamine release from rat striatal slices (Arrang et al., 1985). The very high mRNA expression that we observe in cortical layer V may encode for presynaptic H_3Rs regulating glutamate release from corticostriatal neurons (Doreulee et al., 2001). Although previous studies using an oligoprobe failed to detect any significant H_3R mRNA expression within the dopaminergic neurons (Drutel et al., 2001; Anichtchik et al., 2001), mRNA hybridization signals were clearly observed at the cellular level with our riboprobe in the substantia nigra pars compacta (Fig. 8E), suggesting that H_3Rs previously reported to modulate dopamine synthesis (Molina-Hernandez et al., 2000) and release (Schlicker et al., 1993) are presynaptically located on nigrostriatal afferents. However, the apparent absence of any signal in the ventral tegmental area (VTA) (Fig. 8F) shows that not all dopaminergic neurons express H_3Rs. This observation is consistent with the previous finding that H_3Rs may have no important role in the regulation of dopamine neuron activity *in vivo* (Oishi et al., 1990; Imaizumi and Onodera, 1993). In addition, in contrast with the high expression observed in the caudate putamen and nucleus accumbens, the absence of mRNA signals in islands of Calleja suggests that neurons targeted by mesolimbic dopaminergic pathways in this projection area do not either express H_3Rs.

Consistent with the hypothesis that H_3Rs are present on spiny, striatal projection neurons, a dense binding was detected not only in the substantia nigra pars reticulata, but also in both external and internal segments of the globus pallidus. In addition, this binding was always accompanied by a low mRNA expression which contrasted with the prominent mRNA expression recently reported in the human globus pallidus (Anichtchik et al., 2001). This finding is consistent with the H_3R-mediated inhibition of [^{3}H]GABA release in rat substantia nigra pars reticulata (Garcia et al., 1997) and suggests the presence of H_3Rs presynaptically located not only on striatonigral, but also on striatopallidal projections of both the 'direct' and 'indirect' movement pathways. In addition, the very low mRNA expression in the pallidum and the very low binding in the subthalamic nucleus and motor thalamic nuclei, the target structures of external and internal pallidum, respectively (Tables 1 and 3), reveal a very low density of H_3Rs on pallidal efferents. However, the activity of the 'indirect' movement pathway may also be modulated by presynaptic H_3Rs present on excitatory projections from the subthalamic nucleus to the substantia nigra, two regions that express mRNAs and very dense binding, respectively.

In the amygdaloid complex, where no data were yet available, the high mRNA expression that we observe suggests that not all binding sites correspond to autoreceptors, in spite of a dense histaminergic innervation (Panula and Airaksinen, 1991; Wouterlood and Steinbusch, 1991; Tohyama et al., 1991), and that a major part is located upon amygdala projections. In addition to its role in emotional learning, amygdala plays a key role in the coordinated regulation of attentional processes via projections to cholinergic systems (Gallagher and Holland, 1994; Davis et al., 1994). The latter, located in the forebrain and mesopontine tegmentum, are activated by histamine (Khateb et al., 1995; Lin et al., 1996) and are involved in the maintenance of cortical activation and wakefulness by histaminergic neurons (Lin, 2000). H_3Rs on both amygdala and hypothalamic projections to cholinergic neurons may therefore be involved in the arousal and attention induced by H_3R inverse agonists (Ligneau et al., 1998; Morisset et al., 2000a) and may account for the binding observed in the substantia innominata, pontine nuclei (Tables 2 and 5) and lateral dorsal tegmentum (Cumming et al., 1994).

In the thalamus, the heterogeneous binding that we described using [^{3}H](R)α-methylhistamine (Pollard et al., 1993) is highlighted with [^{125}I]iodoproxyfan which allows a much more detailed analysis. Although the distribution of binding sites roughly parallels histaminergic innervation (Steinbusch and Mulder, 1984; Inagaki et al., 1988; Panula et al., 1989), strong discrepancies in most nuclei between a low binding and very high mRNA expression reveal that many H_3Rs are located upon projections such as the thalamocortical projections (Table 3). However, the binding in dorsal association nuclei may correspond to presynaptic H_3Rs located upon corticothalamic neurons emanating from cortical layers V or VI, in which we detect a high mRNA expression (Fig. 6). In addition, the dense binding in the laterodorsal and lateroposterior nuclei suggests the involvement of H_3Rs in visual systems. This hypothesis is further strengthened by the presence of binding sites in the main retinal primary projection areas, e.g. the superior colliculus and dorsal lateral geniculate nucleus (Tables 3 and 5), that are dramatically up-regulated following visual deprivation (Nakagawa et al., 1994).

In the hypothalamus, the highest mRNA expression was found in the TM where it was suggested to encode for all isoforms of the receptor (Drutel et al., 2001). We also confirm that this expression is accompanied by a high density of binding sites (Pollard et al., 1993). Some of these sites seem to be located upon the histaminergic perikarya, since histamine synthesis is regulated in the posterior hypothalamus (Arrang et al., 1987b) and somatodendritic H_3 autoreceptors inhibit the firing of TM neurons (Haas, 1992; Diewald et al., 1997) by

modulating high voltage-activated calcium channels (Takeshita et al., 1998). However, it is very likely that a significant fraction of the binding that we observe in the TM is located on afferent connections. In agreement, mRNA expression is rather high in several brain areas projecting to the TM, e.g. the subiculum, septum–diagonal band complex, preoptic region, lateral hypothalamus, and raphe nuclei (Ericson et al., 1989, 1991; Wouterlood and Steinbusch, 1991) (Tables 1, 2, 4 and 5). However, further studies are needed to know if mRNAs that we detected in the ventrolateral preoptic nucleus and lateral hypothalamic area encode for H_3Rs regulating the activity of sleep-active GABAergic (Sherin et al., 1996, 1998; Yang and Hatton, 1997) and orexin-containing (Chemelli et al., 1999; Lin et al., 1999; Lu et al., 2000) projections, respectively, two major inputs to the TM involved in the control of arousal (Schwartz and Arrang, 2002). The mRNA expression level in the other hypothalamic nuclei parallels the density of binding sites, suggesting the presence of H_3Rs on perikarya, dendrites and/or short axons. However, many of these nuclei exhibit a very dense histaminergic innervation and it is therefore hard to predict the presynaptic and/or postsynaptic localization of hypothalamic H_3Rs. For example, the rather high mRNA expression suggests that the binding sites that we detect in the supraoptic and paraventricular nuclei correspond to H_3Rs coexpressed in magnocellular neurons with H_1Rs involved in the regulation by histamine of vasopressin and oxytocin secretion (Kjaer et al., 1995; Luckman and Larsen, 1997). However, these binding sites may also correspond to presynaptic autoreceptors known to regulate the activation of these magnocellular neurons by endogenous histamine (Vizuete et al., 1995). The function and cellular distribution of the receptors corresponding to the dense binding and strong mRNA expression that we detect in several nuclei of the tuberal region, such as the ventromedial nucleus, requires also further investigation.

In the lower brainstem, strong discrepancies are observed between the distributions of the H_3R and its mRNAs (Table 5). The densest histaminergic innervation is found in the nucleus of the solitary tract (Inagaki et al., 1988) where we detect a fairly high mRNA expression but very low binding which contrasts with the rather dense binding previously reported with a radiolabelled agonist (Cumming et al., 1994). Histaminergic fibers also innervate vestibular nuclei, particularly the medial vestibular nucleus (Takeda et al., 1987; Steinbusch, 1991). In agreement with the very low binding (Table 5), previous studies suggested the presence of H_3Rs only on these projections and not on vestibular perikarya themselves (Serafin et al., 1993; Yabe et al., 1993; Wang and Dutia, 1995). However, the high level of mRNAs that we observe in the vestibular nuclei, including the medial vestibular nucleus, suggests the presence of H_3Rs on their projections. Besides autoreceptors, these H_3Rs may explain that systemic administrations of H_3R antagonists or inverse agonists strongly decrease the horizontal vestibular ocular reflex in the guinea-pig (Yabe et al., 1993) and facilitate vestibular compensation in the cat (Tighilet et al., 1995), thereby suggesting the potential interest of these compounds as anti-vertigo or anti-motion sickness drugs. The high mRNA expression in the locus coeruleus and raphe nuclei is consistent with previous findings and was recently suggested to encode for the shorter isoforms of the receptor (Lovenberg et al., 1999; Drutel et al., 2001). However, the very low binding observed in both areas (Table 5) suggests that presynaptic rather than somatodendritic heteroreceptors are involved in the H_3R-mediated inhibition of noradrenaline and serotonin release, respectively (Schlicker et al., 1994). In addition, the rather high mRNA expression in the facial nuclei (Table 5) may encode for H_3Rs present on the chorda tympani nerve, a branch of the facial nerve which is involved in the activation of histaminergic neurons by leptin (Morimoto-Ishizuka et al., 2001).

In the cerebellum, hybridization performed at the cellular level shows a strong mRNA expression not only in most, if not all, Purkinje cells, but also in several central cerebellar

nuclei, suggesting an important functional role of H_3Rs in this brain region. The signals observed in Purkinje cells may correspond to the shorter isoform (Drutel et al., 2001) and accounts for the pronounced immunoreactivity detected in Purkinje cell bodies (Chazot et al., 2001). The hybridization signals in the granule cells were hardly detectable, making doubtful the high level of expression reported in these cells with an oligoprobe (Drutel et al., 2001). However, the high mRNA expression that we observed in the guinea-pig (Tardivel-Lacombe et al., 2000) and low H_3R immunoreactivity reported in the mouse (Chazot et al., 2001) suggest that the H_3R expression in cerebellar granule cells varies among animal species. The very low binding, if any, that we observe in the cerebellar cortex suggests that H_3Rs present on Purkinje cells are expressed at the level of their efferent projections rather than on their soma or dendrites. In addition, although they express the three gene transcripts, H_3Rs may not colocalize with H_1 and H_2 receptors on Purkinje cells, since a high density of H_1 and H_2 receptors (Traiffort et al., 1994; Vizuete et al., 1997), but a low H_3R binding and immunoreactivity (Chazot et al., 2001), are observed in the molecular layer which contains Purkinje cell dendrites.

Conclusion

The present study provides evidence for the expression of H_3Rs upon many neuronal cell types. If some localizations are consistent with previous functional studies, the physiological role of many of the receptors present on neuronal perikarya, dendrites and/or projections remains to be understood. Moreover, many of these localizations do not parallel the histaminergic innervation, and the question then arises as to how these receptors are activated. We showed recently that native H_3Rs in rodent brain display high constitutive activity (Morisset et al., 2000). It can be therefore hypothesized that the receptors present upon the various neuronal populations described in this study, may be spontaneously active in the absence of histamine. In addition, as already mentioned, the various H_3R isoforms are recognized by [^{125}I]iodoproxyfan and the riboprobe used in this study (Ligneau et al., 2000; Morisset et al., 2001) but the expression pattern and relative ratios of their transcripts vary among brain regions (Morisset et al., 2001; Drutel et al., 2001). When available, selective probes for the corresponding proteins will allow to investigate the distributions of these H_3R isoforms and thus to identify further the neuronal populations in which they are expressed.

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