The Journal of Neuroscience, July 1994, 14(7): 4252–4268

# Distribution of $\alpha_1$ Adrenoceptors in Rat Brain Revealed by *in situ* Hybridization Experiments Utilizing Subtype-Specific Probes

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**The distribution of neurons in the rat CNS that synthesize mRNA for the $\alpha_{1A/D}$ and $\alpha_{1B}$ adrenoceptors was revealed by the *in situ* hybridization method. Forty-eight–mer DNA probes were synthesized to two different and unique regions of both the $\alpha_{1A/D}$ and $\alpha_{1B}$ mRNAs. Tissue sections from all levels of the CNS and some peripheral ganglia were incubated in a hybridization cocktail containing one of these four probes. The two mRNAs were expressed in a discrete and often complementary manner to each other, and identical hybridization patterns were seen for the probes directed against the same mRNA.**

**The $\alpha_{1A/D}$ probes hybridized heavily with neurons in the internal granular and internal plexiform layers of the olfactory bulb, in layers II–V of most areas of the cerebral cortex, and in the lateral aspect of the lateral amygdaloid nucleus, with pyramidal neurons of CA1–CA4 regions, hilar and granular neurons of the dentate gyrus, and neurons in the reticular thalamic nucleus, cranial and spinal motor nuclei, and the inferior olivary nucleus. Light labeling was seen in a variety of other regions in the brain and spinal cord.**

**The $\alpha_{1B}$ probes hybridized heavily with neurons in the mid layers of cerebral cortex and with virtually all neurons in the thalamus, except the reticular and habenular nuclei. In addition, labeling was seen in the lateral and central amygdaloid nuclei, in brainstem and spinal motor nuclei, over most neurons of the dorsal and medullary raphe nuclei and neurons of the intermediolateral cell column in the spinal cord. Light labeling was seen in the septal nucleus, the horizontal limb of the diagonal band, the paraventricular and lateral hypothalamic nuclei, the pontine and medullary reticular formation, and in most laminae in the spinal cord.**

**The patterns of labeling obtained with the $\alpha_{1B}$ probes resemble the labeling seen in previous autoradiographic ligand binding studies utilizing "general" $\alpha_1$ ligands, while the labeling patterns seen with the $\alpha_{1A/D}$ probes do not correspond to any published $\alpha_1$ receptor distribution pattern, indicating that this mRNA likely encodes for a novel adrenoceptor. The present findings further expand the heterogeneity of adrenoceptor mRNAs presented in two accompanying studies (Nicholas et al., 1993a,b). This differential distribution of adrenoceptors subtypes provides a framework for the functional diversity to the apparently widespread, diffuse, and rather homogeneous noradrenergic innervation of the CNS.**

***[Key words: adrenaline, noradrenaline, epinephrine, norepinephrine, adrenergic, locus coeruleus, cardiovascular]***

Central noradrenergic (Foote et al., 1983) and adrenergic (Hökfelt et al., 1984) neurotransmission is important in a variety of different neuronal functions including arousal, sleep, mood, cardiovascular regulation, feeding, pain, and motor output. Pharmacological manipulations of these two brain catecholamine systems are used in the clinical treatment of a variety of diseases including depression and cardiovascular disorders. Noradrenaline-containing fibers are found throughout the entire neuraxis innervating areas of the brain involved in all the above types of CNS functions (Dahlström and Fuxe, 1964; Fuxe, 1965a,b; Ungerstedt, 1971; Swanson and Hartman, 1975; Moore and Card, 1985), whereas the adrenergic system has a more limited distribution (Hökfelt et al., 1984). The majority of noradrenergic fibers in the CNS arise from a single source in the pons, the locus coeruleus (LC; A6 according to Dahlström and Fuxe, 1964). However, neuronal activity in LC is highly homogeneous; that is, these neurons respond in unison to a variety of afferent inputs (Foote et al., 1980, 1983; Aston-Jones and Bloom, 1981). Therefore, the multitude of effects resulting from noradrenergic activation is likely a result of different postsynaptic responses to noradrenaline. In fact, it is possible that the various functional modalities attributed to noradrenaline and adrenaline are a result of a differential distribution of adrenoceptors.

NA mediates its effects in the CNS via several different G-protein–linked receptors broadly classified as $\alpha_1$, $\alpha_2$, and $\beta$ adrenoceptors (Ahlquist, 1948; Berthelsen and Pettinger, 1977; Raymond et al., 1991). The present study will focus on the CNS distribution of only one class of these receptors, the $\alpha_1$ receptors. The $\alpha_1$ adrenoceptors have been subdivided into $\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{1C}$, and possibly $\alpha_{1D}$ receptors, based upon their differential affinities to a variety of agonists and antagonists, their differential susceptibility to covalent inactivation by chlorethylclonidine (CEC), and their activation of different second messenger cascades (McGrath, 1982; Morrow et al., 1985; Hieble et al., 1986; Morrow and Creese, 1986; Han et al., 1987a; Johnson and Minneman, 1987; McGrath and Wilson, 1988; Minneman, 1988; Raymond et al., 1990; Schwinn et al., 1990; Han and Minneman, 1991).

Received Aug. 2, 1993; revised Dec. 28, 1993; accepted Jan. 13, 1994.

We acknowledge the superb technical assistance of Ms. K. Friberg, Ms. S. Nilsson, Ms. K. Åman, and W. Hiort. V.A.P. was supported by a grant from the National Science Foundation (INT-8908720) and the Fogarty International Center (1 F20 TWO 1586-01). A.P.N. was supported by grants from The Jeane B. Kempner Foundation of the University of Texas Medical Branch at Galveston and by the National Heart, Lung and Blood Institute of the NIH (F32HL08494-01$\alpha$1). Å.D. was supported by grants from Svenska Sällskapet för Medicinsk Forskning, Stiftelsen Sigurd och Elsa Goljes Minne, Anders Otto Swärds Stiftelse, Svenska Läkaresällskapet and Karolinska Institutets Fonder. Grants from the Swedish Medical Research Council (04X-2887) and the NIMH (MH 43230) are also gratefully acknowledged.

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Numerous studies employing the tissue section ligand binding autoradiography technique developed by Young and Kuhar (1979a) have examined the distribution of $\alpha_1$ adrenoceptors in the CNS (Young and Kuhar, 1979b, 1980; Unnerstall et al., 1982; Dashwood, 1983; Rainbow and Biegon, 1983; Jones et al., 1985; Palacios et al., 1987; Unnerstall, 1987; Chamba et al., 1991). Three ligands have been preferentially used in these studies, $^3$H-WB4101, $^3$H-prazosin, and $^{125}$I-HEAT. Recent studies have utilized predominately prazosin and HEAT, because WB4101 was found not be specific for $\alpha_1$ adrenoceptors (Hoffman and Lefkowitz, 1980; Lyon and Randall, 1980; Rehavi et al., 1980). While the binding patterns of prazosin and HEAT have generally given comparable results, HEAT is preferred for a variety of reasons (see Unnerstall, 1987). However, neither of these compounds binds to only one subtype of the $\alpha_1$ adrenoceptor family. Furthermore, the low cellular resolution of the autoradiographic ligand binding technique does not allow accurate identification of the cells in the CNS that express a specific $\alpha_1$ adrenoceptor subtype. In addition, the autoradiographic labeling seen with HEAT has often contradicted biochemical studies on the localization of $\alpha_1$ receptors. For example, such studies have indicated that $\alpha_1$ receptors are present in the hippocampus, while many autoradiographic studies utilizing HEAT and prazosin binding have consistently failed to identify substantial binding in the hippocampus (Unnerstall et al., 1982, 1985; Dashwood, 1983; Rainbow and Biegon, 1983; Jones et al., 1985; Palacios et al., 1987; Unnerstall, 1987; Marks et al., 1990; Chamba et al., 1991; but see Zilles et al., 1991).

Utilizing a biochemical method that combines the preferential covalent modification of $\alpha_{1A}$ adrenoceptors by CEC with competitive ligand binding, Wilson and Minneman (1989) have reported regional differences in the distribution of $\alpha_{1A}$ and $\alpha_{1B}$ adrenoceptors. These investigators have found that $\alpha_{1A}$ receptors predominate in the hippocampus, pons–medulla, and spinal cord, while $\alpha_{1B}$ receptors predominate in the thalamus, hypothalamus, and cerebellum.

After the first cloning of an adrenoreceptor by Lefkowitz and collaborators (Cotecchia et al., 1988), the genetic sequences of a number of adrenoceptors have been determined (Buckland et al., 1990; Machida et al., 1990; Schwinn et al., 1990; Shimomura and Terada, 1990; Voigt et al., 1990, 1991; Zeng et al., 1990; Flordellis et al., 1991; Lanier et al., 1991; Lomasney et al., 1991). This has made it possible to utilize the *in situ* hybridization technique (see Young, 1990) to examine the distribution of cells in the CNS that synthesize mRNA to these various adrenoceptors (Nicholas et al., 1991), revealing distinctly differential distribution patterns for the $\alpha_2$- and $\beta$-adrenergic receptor mRNAs (Nicholas et al., 1993a,b). In addition to specificity, these types of studies offer a cellular resolution difficult to obtain with traditional biochemical or autoradiographic techniques. On the other hand, the *in situ* hybridization technique only reveals the cell bodies in which the receptors are synthesized and not the distribution of the receptor protein in the dendritic and axonal ramifications. Thus, information from the two techniques is complementary and together provides important clues to the functional role of receptors.

A receptor initially termed the $\alpha_{1A}$ adrenoceptor was recently cloned from a rat brain cDNA library (Lomasney et al., 1991). However, this mRNA does not seem to code for the classic $\alpha_{1A}$ adrenoceptor but rather a heretofore unidentified "$\alpha_{1D}$" (Perez et al., 1991) or "$\alpha_{1A/D}$" adrenoceptor (Schwinn and Lomasney, 1992). This receptor (here termed $\alpha_{1A/D}$) has some but not all the properties of the $\alpha_{1A}$ adrenoceptor. Additionally, a cDNA clone was isolated coding for a receptor with the pharmacological properties of the classic $\alpha_{1B}$ adrenoceptor (Voight et al., 1990). The present study reveals a unique and widespread distribution pattern for $\alpha_{1A/D}$ and $\alpha_{1B}$ adrenoceptor mRNAs.

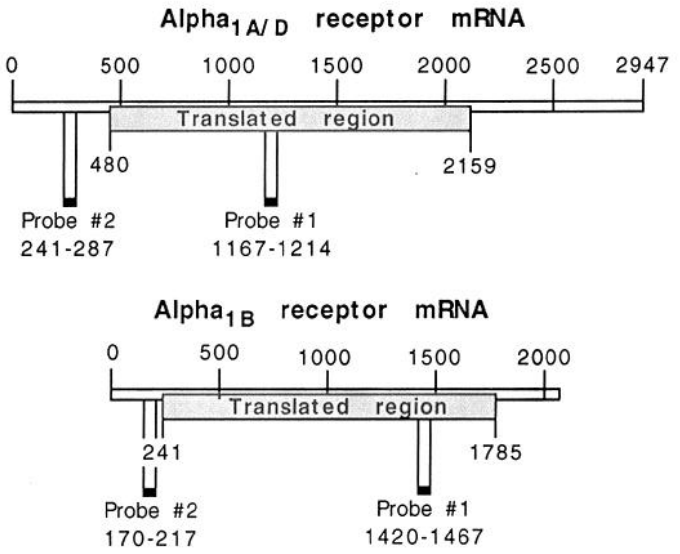


*Figure 1.* A schematic diagram of the $\alpha_{1A/D}$ and $\alpha_{1B}$ receptor mRNAs indicating the locations of the sequences from which the four oligonucleotide probes were derived. Two 48-mer reverse and complementary probes were made to each of the mRNAs. For each of the mRNAs, one probe was made to the region of the mRNA that was translated into the receptor protein while another was made against the 5′ untranslated region of the mRNA. Each of the probes was used on alternate sections in some experiments and the labeling obtained compared (Fig. 12).

## Materials and Methods

*Preparation of the oligonucleotide probes.* In efforts to develop probes specific for either of the two subtypes of the rat $\alpha_1$ adrenoceptors sequenced so far, nonhomologous regions of the mRNA were used in generating the probes. Two probes directed against each of the receptors were constructed, two of which were complementary to regions in the translated hydrophilic domain (third intracellular loop) of each of the mRNAs and two complementary to the 5′ untranslated region of each of the mRNAs (Fig. 1). Using MACVECTOR software (IBI, New Haven, CT), 48-nucleotide segments were selected based on a determined optimum ratio for total G/C content (60–65%) and minimal homology (not greater than 80%) with GenBank-entered eukaryotic gene sequences (as of August 1991) and specifically nonhomologous with the other adrenergic receptors mRNA ($\alpha_{2A,B,C}$ and $\beta_{1,2}$) sequences (not greater than 65% homology; Cotecchia et al., 1988; Buckland et al., 1990; Machida et al., 1990; Schwinn et al., 1990; Shimomura and Terada, 1990; Zeng et al., 1990; Voigt et al., 1991; Lanier et al., 1991). Reversed and complementary DNA probes were made (Scandinavian Gene Synthesis AB, Köping, Sweden) to the following sequences: 241–287 and 1167–1214 of rat $\alpha_{1A/D}$ receptor (Lomasney et al., 1991) and 170–217 and 1420–1467 of rat $\alpha_{1B}$ receptor (Voigt et al., 1990) mRNAs. Eighty nanograms (5 pmol) of oligonucleotide probe were labeled at the 3′-end using terminal deoxynucleotidyltransferase (Amersham) and $^{35}$S-dATP (New England Nuclear) to a specific activity of 1–2 × $10^6$ cpm/ng.

*Preparation of tissue and* in situ *hybridization.* Male Sprague–Dawley rats ($n$ = 8; 250–350 gm) were decapitated, and the brain, spinal cord and superior cervical, dorsal root (L5), and nodose ganglia were rapidly removed and frozen with gas produced from liquid carbon dioxide. Serial coronal and sagittal cryostat sections (14 $\mu$m) were melted onto Fisher Probe-On slides (Fisher Scientific, Pittsburgh, PA). *In situ* hybridization experiments were performed as previously described (Schalling et al., 1988; Schalling, 1990; Young, 1990; Dagerlind et al., 1992). Briefly, adjacent unfixed tissue sections (Dagerlind et al., 1992) were air

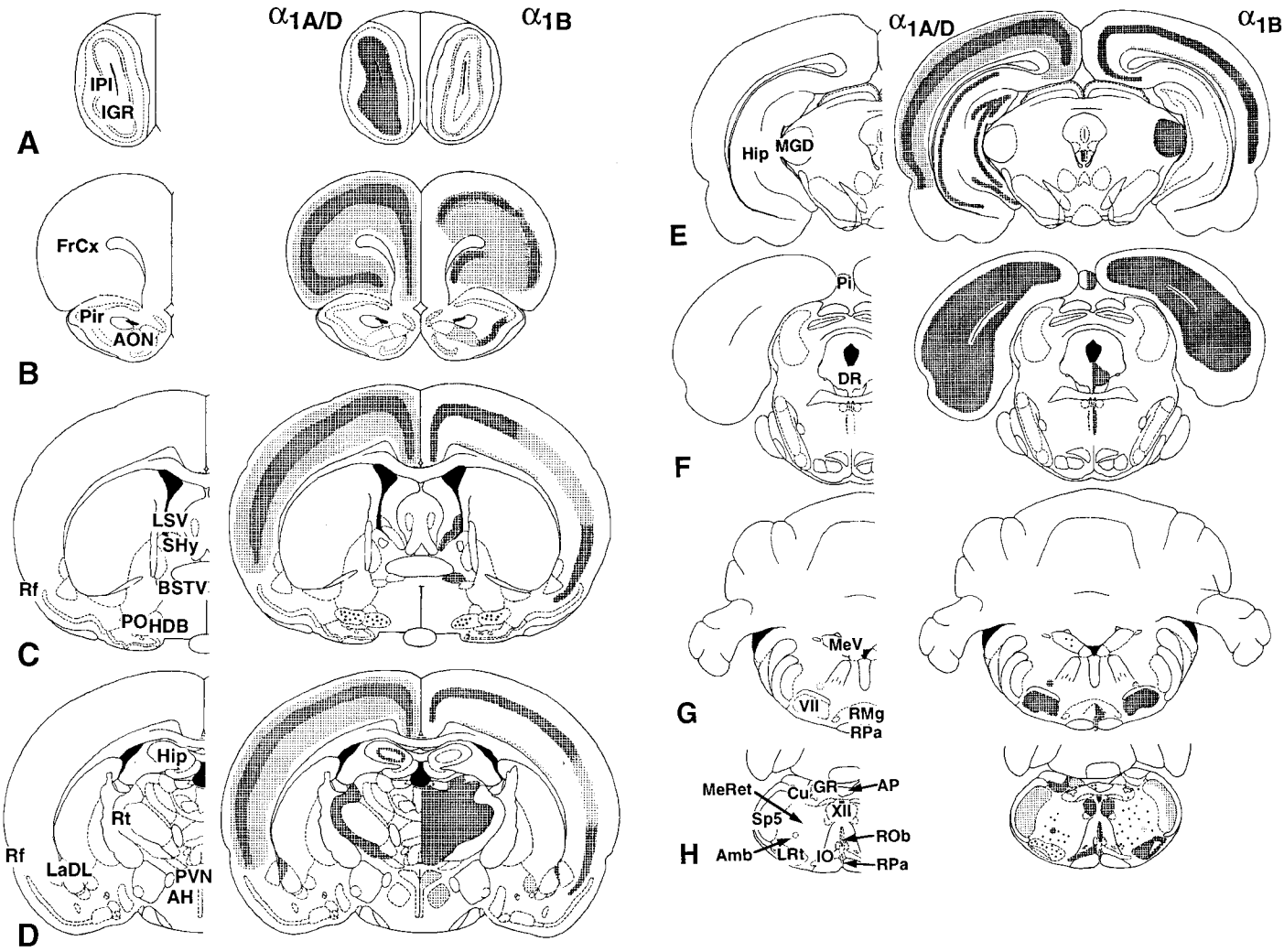


*Figure 2.* *A–H*, Schematic diagrams indicating the brain regions containing cells that hybridized with the $\alpha_{1A/D}$ and $\alpha_{1B}$ receptor mRNA probes. The *sections on the right* indicate the areas of the brain to which the $\alpha_{1A/D}$ (right side, left hemisection) and $\alpha_{1B}$ (right side, right hemisection) probes hybridized. The *hemisections on the left side* indicate the cytoarchitectonic boundaries and nomenclature. Two patterns are used to indicate cells exhibiting heavy hybridization (*dark shading*) versus light hybridization or lightly hybridized cells (*light shading*) with either of the probes. In addition, areas that contained low number of isolated labeled cells are indicated by *large dots*. The boundaries and nomenclature are derived mainly from Paxinos and Watson (1991). *AH*, anterior hypothalamic nucleus; *Amb*, nucleus ambiguus; *AON*, anterior olfactory nucleus; *AP*, area postrema; *BSTV*, bed nucleus of the stria terminalis (ventralis); *Cu*, cuneate nucleus; *DR*, dorsal raphe; *FrCx*, frontal cortex; *GR*, gracile nucleus; *HDB*, horizontal limb of the diagonal band; *Hip*, hippocampus; *IGR*, internal granular cell layer of the olfactory bulb; *IO*, inferior olivary complex; *IPl*, internal plexiform layer of the olfactory bulb; *LaDL*, lateral amygdaloid nucleus, dorsolateral part; *LRt*, lateral reticular nucleus; *LSV*, lateral septal nucleus (ventralis); *MeRet*, median reticular formation; *MeV*, medial vestibular nucleus; *MGD*, medial geniculate body; *Pi*, pineal gland; *Pir*, piriform cortex; *PO*, preoptic nucleus; *PVN*, paraventricular nucleus; *Rf*, rhinal fissure; *RMg*, raphe magnus; *ROb*, raphe obscurus; *RPa*, raphe pallidus; *Rt*, reticular thalamic nucleus; *SHy*, septohypothalamic nucleus; *Sp5*, spinal trigeminal nucleus; *VII*, facial nucleus; *XII*, hypoglossal nucleus.

dried and incubated at 42°C for 16–18 hr with 0.5–1.0 ng of the labeled probe per 100 μl of hybridization cocktail consisting of 50% deionized formamide, 4× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate), 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 1% sarkosyl, 0.02 M sodium phosphate (pH 7.0), 10% dextran sulfate, 200 mM dithiothreitol, and 500 μg/ml of heat-denatured salmon testis DNA. For competitive binding control experiments, radiolabeled probes were mixed with an excess (100×) of the respective nonradiolabeled probe. Sections were then washed in 1× SSC buffer (4 × 15 min) at 55°C, slowly cooled to room temperature over 30 min while in the final rinse, briefly transferred through distilled water, and dehydrated in 60% and then 90% ethanol. Following this procedure, sections were either opposed to autoradiographic film (Hyperfilm-βmax; Amersham) or dipped in Kodak NTB2 autoradiography emulsion (50% in water). Following 4–17 weeks exposure at −20°C, films were developed with LX 24 (Kodak) for 2 min, rinsed in distilled water (30 sec), and fixed in AL 4 (Kodak) for 15 min. Sections dipped in photographic emulsion were allowed to expose 10–17 weeks in light-free metal containers at −20°C, and then developed in Kodak D19 (3 min), fixed in Kodak 3000 A and B (6–7 min), and coverslipped in glycerol. Some tissue sections were counterstained with 0.1% toluidine blue with 0.5% sodium borate and coverslipped with Entellan (Merck). All sections were examined under light- or dark-field illumination with a Nikon Microphot-FX microscope. Photomicrographs were taken with Kodak T-Max 100 black-and-white film. In some cases photographic prints were made directly from the autoradiographic films.

*Evaluation of results.* Labeled structures were evaluated in sections that had been counterstained with toluidine blue. The distribution of the two mRNA is shown in several coronal planes and represents a subjective integration of cell density and intensity of labeling.

*Northern blot analysis.* Approximately 500 mg of frozen tissue (cerebral cortex or thalamus/hypothalamus) was placed in 4 M guanidine isothiocyanate, 0.1 M β-mercaptoethanol, 0.025 M sodium citrate (pH 7.0) and immediately homogenized with a Polytrone. Each tissue ho-

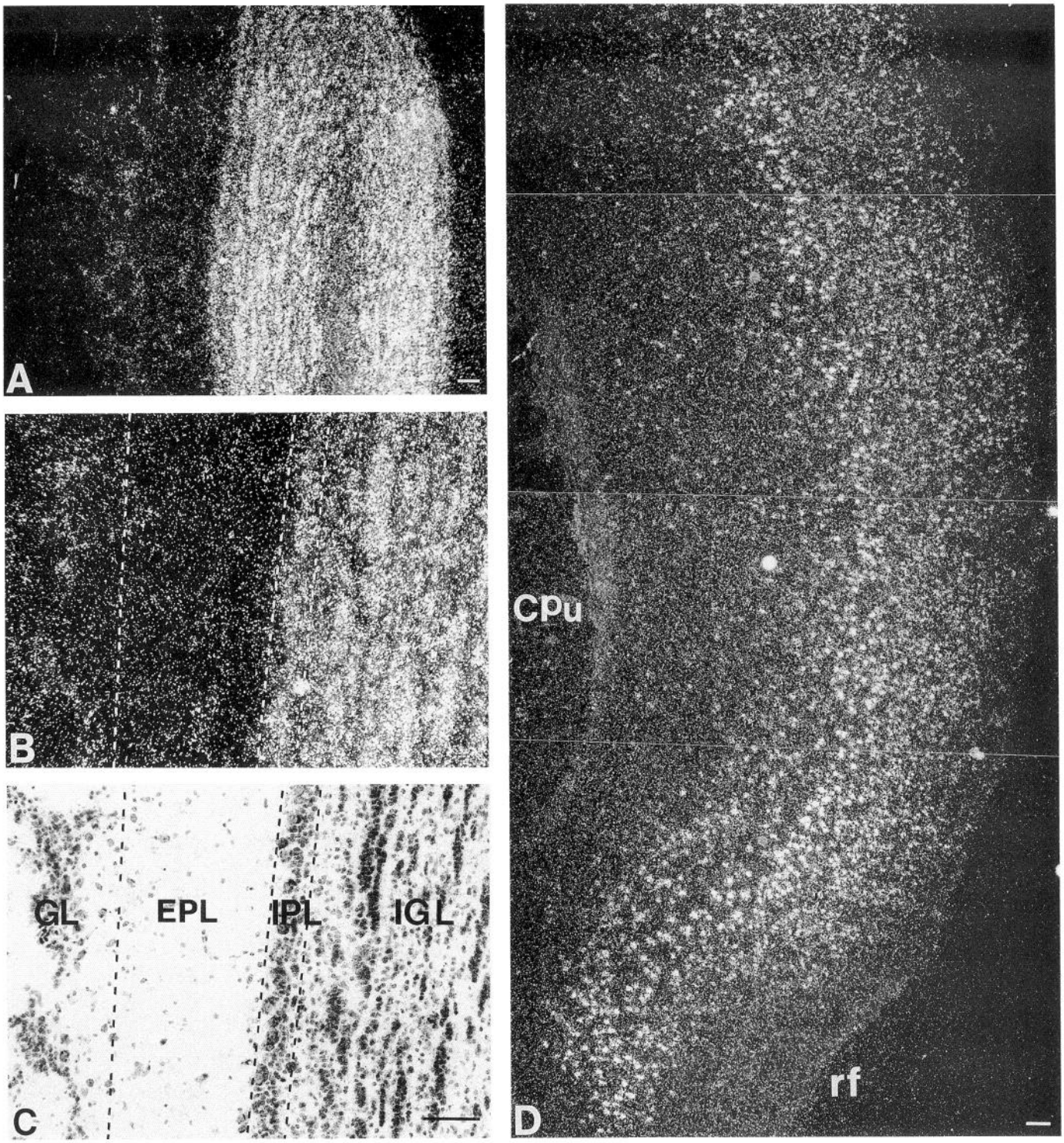


*Figure 3.* Distribution of $\alpha_{1A/D}$ mRNA hybridization in the forebrain. *A–C*, Heavy hybridization is seen in the olfactory bulb predominantly in the internal granule cell (*IGL*) and internal plexiform (*IPL*) layers of the olfactory bulb. Levels are just at background in the external plexiform (*EPL*) and glomerular (*GL*) layers. Occasionally, it appears that labeling in glomerular layer was slightly above background (*A, B*). *A* and *B* are dark-field photomicrographs of emulsion-dipped sections, while *C* shows the same region as in *B* under bright-field illumination to reveal the Nissl staining. *D*, A dark-field photomontage of the parietotemporal cortex showing the widespread distribution of cells expressing $\alpha_{1A/D}$ adrenoceptor mRNA. Labeling is found in layers II–V, while layer I is unlabeled. A few weakly positive cells are seen in layer VI. The ventral border of the labeling was generally the rhinal fissure (*rf*). *CPu*, nucleus caudatus putamen. Scale bars, 100 μm.

mogenate was layered over a 4 ml cushion of 5.7 M CsCl in 0.025 M sodium citrate (pH 5.5) and centrifuged at 20°C in a Beckman SW41 rotor at 35,000 rpm for 18 hr. The amount of total RNA was quantified spectrophotometrically at 260 nm and 15 μg of total RNA from each sample and 4 μg RNA size marker (Promega, Madison, WI) were separated on a 1% agarose gel containing 0.7% formaldehyde, blotted onto Hybond-N membranes (Amersham), and cross-linked by UV illumination. Membranes were prehybridized at 42°C overnight in a solution

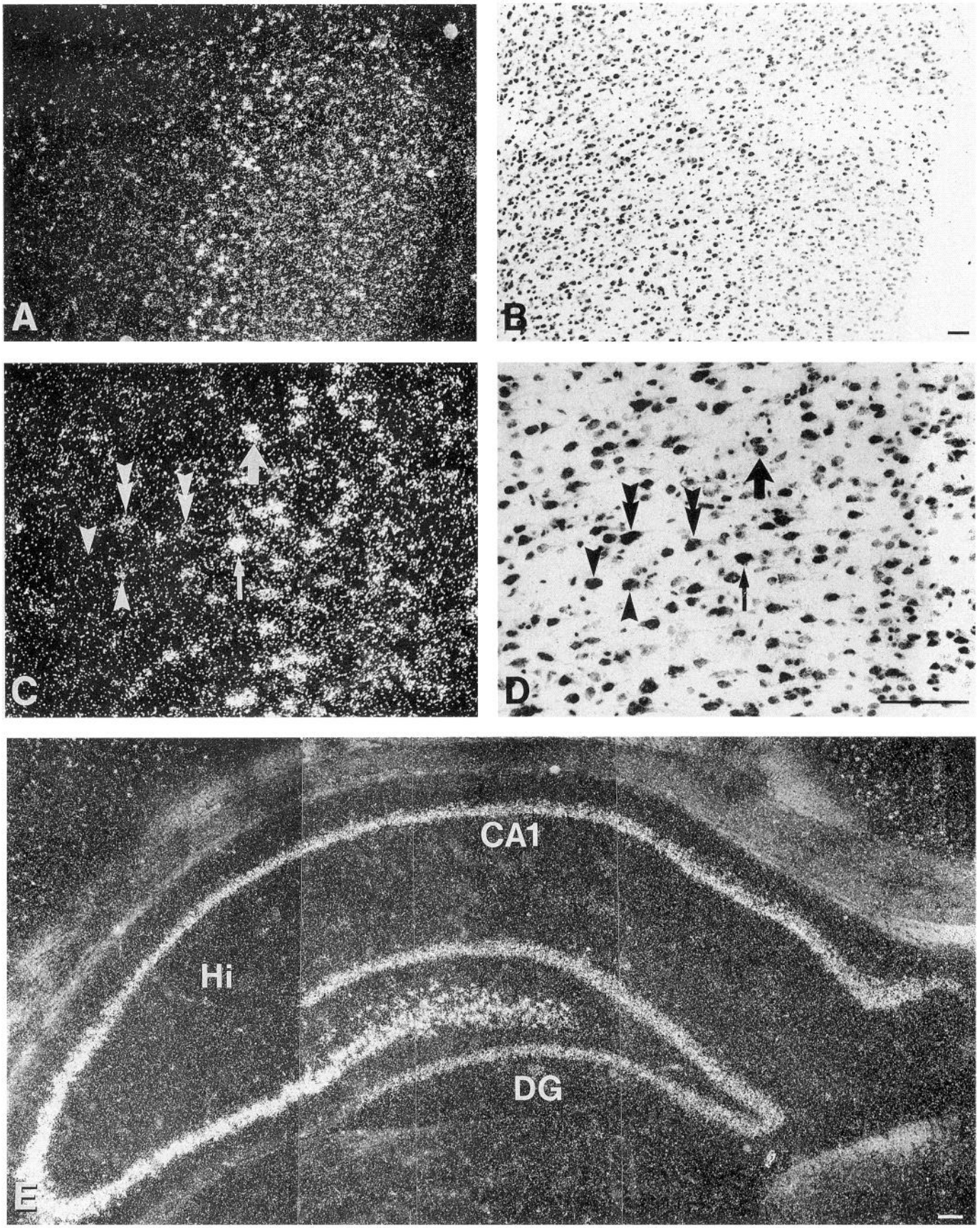


*Figure 4.* *A–E*, Distribution of $\alpha_{1A/D}$ mRNA hybridization in cortex (*A–D*) and hippocampus (*E*). *A–D*, Numerous cells, mainly in layers II–V, are labeled, with low numbers of weakly positive cells in layer VI. At a higher magnification it can be seen that many cells in outer layer V (*arrows*)

containing 50% formamide (J. T. Baker BV, Deventer, The Netherlands), 5× SSPE (1× SSPE = 0.15 M sodium chloride, 0.01 M sodium dihydrogen phosphate monohydrate, 1 mM EDTA) pH 7.7, 0.02 M sodium phosphate buffer pH 7.0, 0.1% sodium dodecyl sulfate (SDS), 1× Denhardt's (0.02% each of polyvinylpyrrolidone, Ficoll, bovine serum albumin; Sigma), and 100 μg/ml of sheared and heat denatured salmon testis DNA (Sigma). Eight nanograms of $^{32}$P-labeled (1–2 × $10^6$ cpm/ng) $\alpha_{1A/D}$ or $\alpha_{1B}$ oligonucleotide probe were added per milliliter of hybridization solution, and the incubation was continued for an additional 18–20 hr at 42°C. Membranes were washed in 2× SSPE and 0.1% SDS (2 × 10 min at 55°C), 1× SSPE and 0.1% SDS (1 × 20 min at 55°C), and 0.1× SSPE and 0.1% SDS (2 × 10 min at room temperature = 25°C) and exposed to film (New England Nuclear Reflection) with intensifying screens (New England Nuclear Reflection) at 4°C for 3 d.

## Results

The $\alpha_{1A/D}$ and $\alpha_{1B}$ receptor mRNAs had a wide distribution in the rat CNS (Figs. 2–11). Nissl-counterstained tissue sections revealed that the labeling of both mRNAs was accumulated over neuron-like somata, although glial labeling can not be excluded (Stone and Ariano, 1989; Aoki, 1992). However, hybridization with the probe for the untranslated region of the $\alpha_{1B}$ adrenoceptor mRNA resulted, in addition, in a diffuse signal over white matter (see Fig. 12*D,E*). Therefore, the probes directed against the translated regions of each mRNA were used for the mapping study. Generally very long exposure times (8–12 weeks) were required to visualize labeling of both mRNAs. The distribution patterns are demonstrated in schematic drawings (Fig. 2*A–H*) reproduced from the atlas of Paxinos and Watson (1991) and in micrographs of bright- and dark-field autoradiograms of emulsion-dipped, toluidine blue–counterstained sections (Figs. 3–11).

### $\alpha_{1A/D}$ receptor mRNA distribution

*Telencephalon.* Pronounced labeling was seen in the internal granular and plexiform cell layers of the olfactory bulb (Fig. 3*A–C*). This labeling was found uniformly throughout the bulb but did not extend into the external plexiform layer or the mitral cell layer (Fig. 3*B,C*). Light labeling was seen in the glomerular layer over the periglomerular neurons (Fig. 3*B,C*). No labeling was seen in the anterior olfactory nucleus.

Neurons throughout the cerebral cortex labeled extensively and heavily with the $\alpha_{1A/D}$ probes (Figs. 3*D*, 4*A–D*). The most pronounced labeling was seen in the prefrontal, cingulate, and occipital cortex, while less labeling was seen in the temporal cortex. The piriform cortex was generally unlabeled. Ventrally, the labeling generally ended at the level of the rhinal fissure (Figs. 3*D*, 6*A*). Labeled neurons were seen in all layers of the cortex except layer I, and only a few labeled neurons were observed in layer VI. Some superficial pyramidal neurons in layer V were often heavily labeled, whereas others had only low or no detectable labeling (Fig. 4*A–D*). In Nissl-stained sections of the cingulate cortex most neurons in layer II–V were labeled.

In the hippocampal formation the pyramidal and granular cell layers were heavily labeled with the $\alpha_{1A/D}$ probes (Figs. 4*E*, 5*A*; see also Fig. 12*A,B*). The dorsal and the ventral hippocampus were equally labeled.

The dorsolateral aspect of the lateral amygdaloid nucleus was strongly labeled (Fig. 6*A*), while the remainder of the amygdala was unlabeled. Numerous lightly labeled neurons were seen in the medial septum/diagonal band complex, and scattered neurons were found in the vertical and horizontal limbs of the diagonal band and the magnocellular preoptic nucleus (Fig. 2*C*). The basal ganglia including the caudate putamen, the globus pallidus, and the substantia nigra were all unlabeled.

*Diencephalon.* The entire hypothalamus and thalamus were unlabeled, with the exception of the reticular thalamic nucleus, which was heavily labeled throughout its entire extent (Figs. 5*A*, 6*B*).

*Mesencephalon and rhombencephalon.* Virtually all of the cranial motor nuclei were labeled (Fig. 2*G,H*), including the oculomotor, facial, hypoglossal, ambiguus, and motor trigeminal nuclei. The dorsal motor nucleus of the vagus was, however, unlabeled. A small collection of lightly labeled cells was consistently seen in the area of the lateral dorsal tegmental nucleus in the pontine central gray matter. In addition, numerous scattered labeled cells were seen throughout the reticular formation of the pons and medulla.

The most intensely labeled structure in the brainstem was the inferior olivary complex (Fig. 6*C*). Virtually all neurons in all subdivision of the inferior olive were heavily labeled (Fig. 6*D*). The labeling extended from the most rostral to the most caudal extent of the structure. Another heavily labeled structure in the caudal medulla was the cuneate nucleus (Fig. 2*H*), while the external cuneate nucleus was unlabeled. The gracile nucleus was lightly labeled (Fig. 2*H*). Scattered positive cells could also be seen in the medial vestibular nucleus, the area postrema and the lateral reticular nucleus (Fig. 2*H*). Light but distinct labeling was seen in the superficial layers of the trigeminal spinal nucleus (Fig. 2*H*).

*Spinal cord.* The most prominently labeled cells in the spinal cord were the ventral horn motor neurons at all levels. In the remaining laminae of the spinal cord a large number of scattered neurons was seen in the dorsal aspects of the ventral horn.

*Peripheral tissues.* The pineal gland was unlabeled with the $\alpha_{1A/D}$ probes, as were the superior cervical ganglia, lumbar dorsal root ganglia and the nodose ganglia.

### $\alpha_{1B}$ receptor mRNA distribution

*Telencephalon.* The olfactory bulb was virtually unlabeled with the $\alpha_{1B}$ probe. A very light labeling was consistently observed only in the internal plexiform layer. Distinct though light labeling was seen within the ventral division of the anterior olfactory nucleus and within the rostral piriform cortex (Fig. 2*B*).

The cerebral cortex was strongly labeled with the $\alpha_{1B}$ probe but not to the extent seen with the $\alpha_{1A/D}$ probes (Figs. 7, 8*A,B*). The strongest labeling was observed in layers III–V, while the superficial layers were unlabeled. Single labeled cells formed a thin band in deep layer VI overlying the dorsal aspects of the corpus callosum (Fig. 2*C,D*). As with the $\alpha_{1A/D}$ probe the large pyramidal neurons of layer IV were distinctly labeled with the $\alpha_{1B}$ probe. Although the piriform cortex within the accessory olfactory bulb was lightly labeled, the remainder of it was unlabeled. Generally the cortical labeling with the $\alpha_{1B}$ probes ended

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are strongly labeled and some of them are pyramidal cells. Other pyramidal cells are either very weakly labeled (*double arrowheads*) or not labeled at all (*arrowheads*). *B* and *D* show the same region as in *A* and *C*, respectively, under bright-field illumination and after Nissl staining. *E*, The pyramidal cell layers of CA1–CA4 and the granular cell layers of the dentate gyrus (*DG*) are heavily labeled. Scale bars, 100 μm.

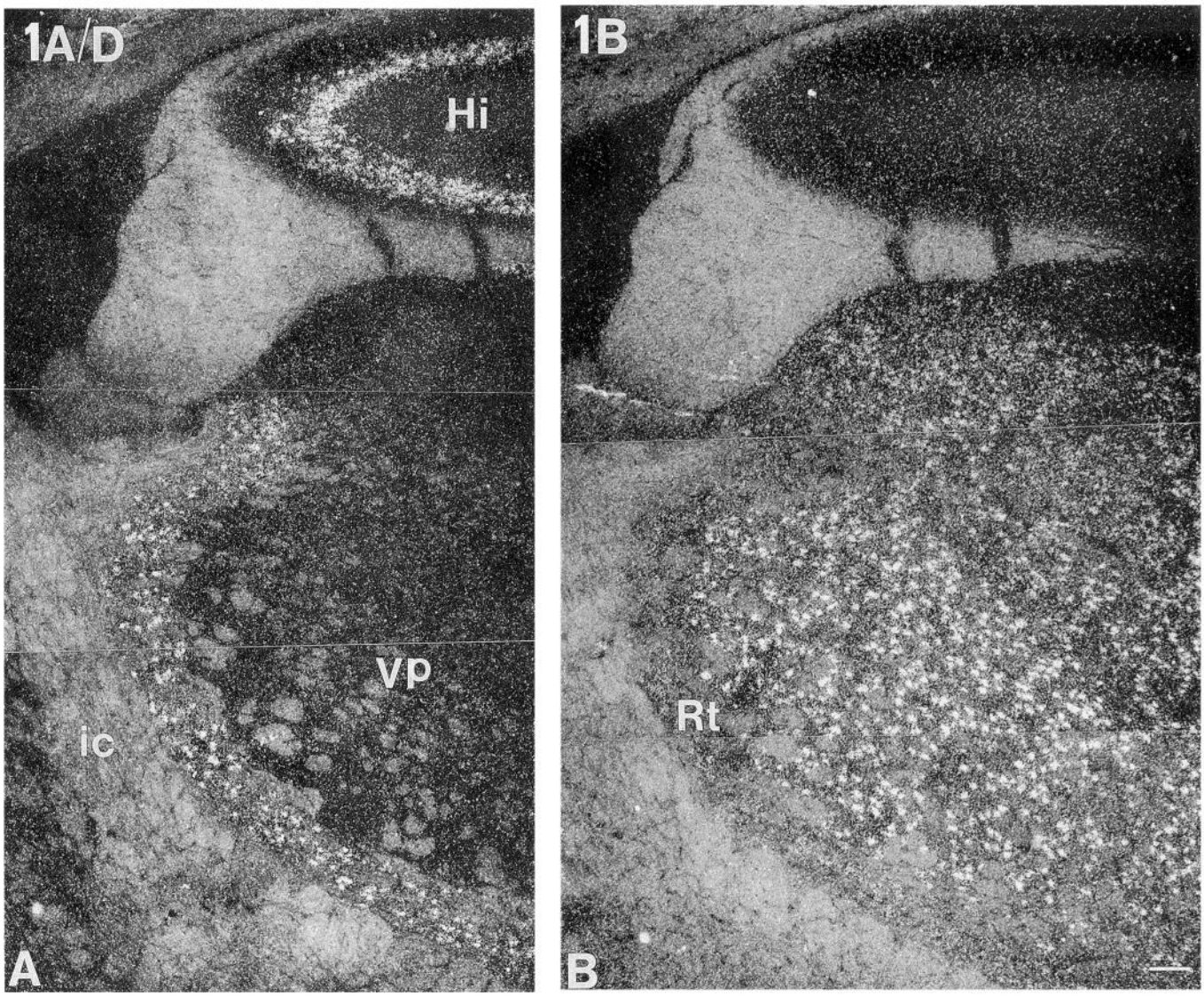


*Figure 5.* *A* and *B*, Distribution of $\alpha_{1A/D}$ (*A*) and $\alpha_{1B}$ (*B*) mRNA labeling in the dorsal thalamus and dorsal lateral hippocampus (*Hi*). *A* and *B* show adjacent sections. Strong $\alpha_{1A/D}$ labeling is seen in the thalamic reticular nucleus (*Rt*) but not in the remaining thalamus, for example, in the adjacent ventral posterior nucleus (*VP*) (*A*). Instead, the remaining thalamus is rich in $\alpha_{1B}$ mRNA–expressing cells; however, they are not found in the reticular nucleus (*B*). Note strong $\alpha_{1A/D}$ labeling in the pyramidal cells in the hippocampus (*A*) while no $\alpha_{1B}$ labeling is seen in the hippocampus in the adjacent section (*B*). Scale bar, 100 $\mu$m.

just ventral to the rhinal fissure (Fig. 2). Area 1 of the parietal cortex was less labeled than the surrounding cortex. As with the $\alpha_{1A/D}$ probe, the dorsolateral division of the lateral amygdaloid nucleus was heavily labeled. However, unlike the $\alpha_{1A/D}$ probe, the central amygdaloid nucleus was lightly labeled with the $\alpha_{1B}$ probe, as was the bed nucleus of the stria terminalis and the ventral septal area (Fig. 2*C*,*D*). The vertical and horizontal limbs of the diagonal band were labeled (Fig. 2*C*). There was no discernible signal in any of the basal ganglia structures.

*Diencephalon.* One of the most striking and distinct labeling patterns was seen in the thalamus (Figs. 5*B*, 9*A–C*). With the exceptions of the reticular and habenular nuclei, the entire thalamus was heavily labeled. All of the medial and lateral tier of thalamic nuclei (except the reticular nucleus) (Fig. 5*B*), including the geniculate bodies (Fig. 9*A–C*), was heavily labeled. In Nissl-stained sections of the thalamus it appeared that virtually every neuron in the labeled nuclei expressed $\alpha_{1B}$ receptor mRNA (Fig. 9*C*). In striking contrast to the labeling pattern seen with the $\alpha_{1A/D}$ probe, no certain $\alpha_{1B}$ labeling was seen in the reticular thalamic nucleus (Fig. 5*B*).

The lateral hypothalamic nucleus and the paraventricular nucleus both exhibited very light but significant labeling (Fig. 2*D*).

*Mesencephalon and rhombencephalon.* The most pronounced labeling in the brainstem was seen in the dorsal raphe complex (Fig. 10*A*). Virtually all neurons in the dorsal raphe (including the lateral wings) were labeled. This labeling extended ventrally to include the central superior nucleus and caudally to the medulla oblongata including the raphe magnus, pallidus, and obscurus groups. Prominent labeling was seen over cranial motor neurons (Fig. 8*B*) and in the lateral reticular nucleus. Motor neurons in the facial (Fig. 8*B*), hypoglossal, ambiguus, and motor trigeminal nuclei were all well labeled. However, the dorsal motor nucleus of the vagus was not labeled. The lateral reticular nucleus was also heavily labeled throughout its rostrocaudal

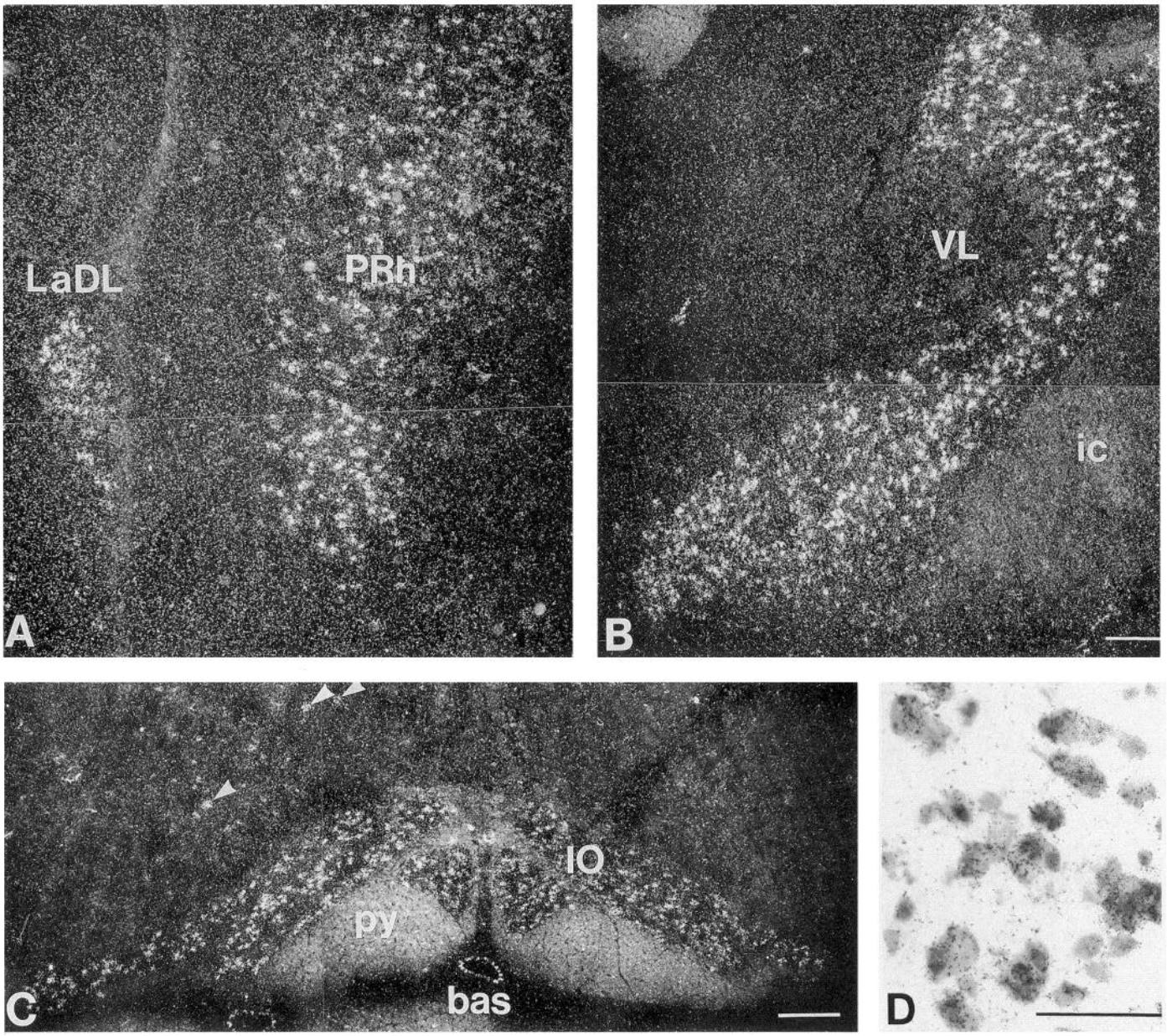


*Figure 6.* *A–D*, $\alpha_{1A/D}$ mRNA labeling of the amygdala and cortex (*A*), thalamus (*B*), and inferior olivary complex (*C* and *D*). *A*, In the amygdala only the lateral aspect of the lateral amygdaloid nucleus is labeled, and the labeling is very strong. *B*, The reticular thalamic nucleus was the only labeled structure in the thalamus. This nucleus was heavily labeled throughout its rostrocaudal extent. *C* and *D*, The entire inferior olivary complex with all of its subdivisions was heavily labeled (*C*). Upon Nissl counterstaining it appeared that virtually all neurons were labeled (*D*). *Arrowheads* in *C* represent isolated cells labeled in the medullary reticular formation. Scale bars: *A–C*, 200 μm; *D*, 50 μm.

extent (Fig. 2*G,H*). In addition, the external cuneate nucleus was lightly labeled, as were neurons in the spinal trigeminal nucleus. A large number of scattered, positive cells were seen in the pontine and medullary reticular formation, including a well-defined group in the paragigantocellularis nucleus region immediately adjacent to the inferior olive (Fig. 1*B*). A very faint signal was detected in the cerebellar cortex upon prolonged exposure. The labeling was generally over the granule and Purkinje cell layers.

*Spinal cord.* Spinal motor neurons were heavily labeled (Fig. 10*C,D*), and scattered labeled cells were seen throughout the various laminae in the remainder of the grey matter of the spinal cord. Presumably preganglionic neurons in the intermediolateral cell columns of the thoracic spinal cord were also labeled.

*Peripheral tissues.* The pineal gland was very heavily labeled with the $\alpha_{1B}$ probe (Fig. 8*C*), while the peripheral ganglia showed no labeling.

### *Control experiments*

Three types of control experiments were performed. First, to control for the nonspecific binding of the probe to cellular components, incubations of alternate sections with probes against the translated regions were done in the presence of an excess (100×) of the respective unlabeled probe. In all cases, sections incubated with an excess of unlabeled probe showed no hybridization (not shown). Second, to exclude "nonspecific" hybridization to unknown related RNA, additional probes were generated to the 5′ untranslated regions of either the $\alpha_{1A/D}$ or $\alpha_{1B}$

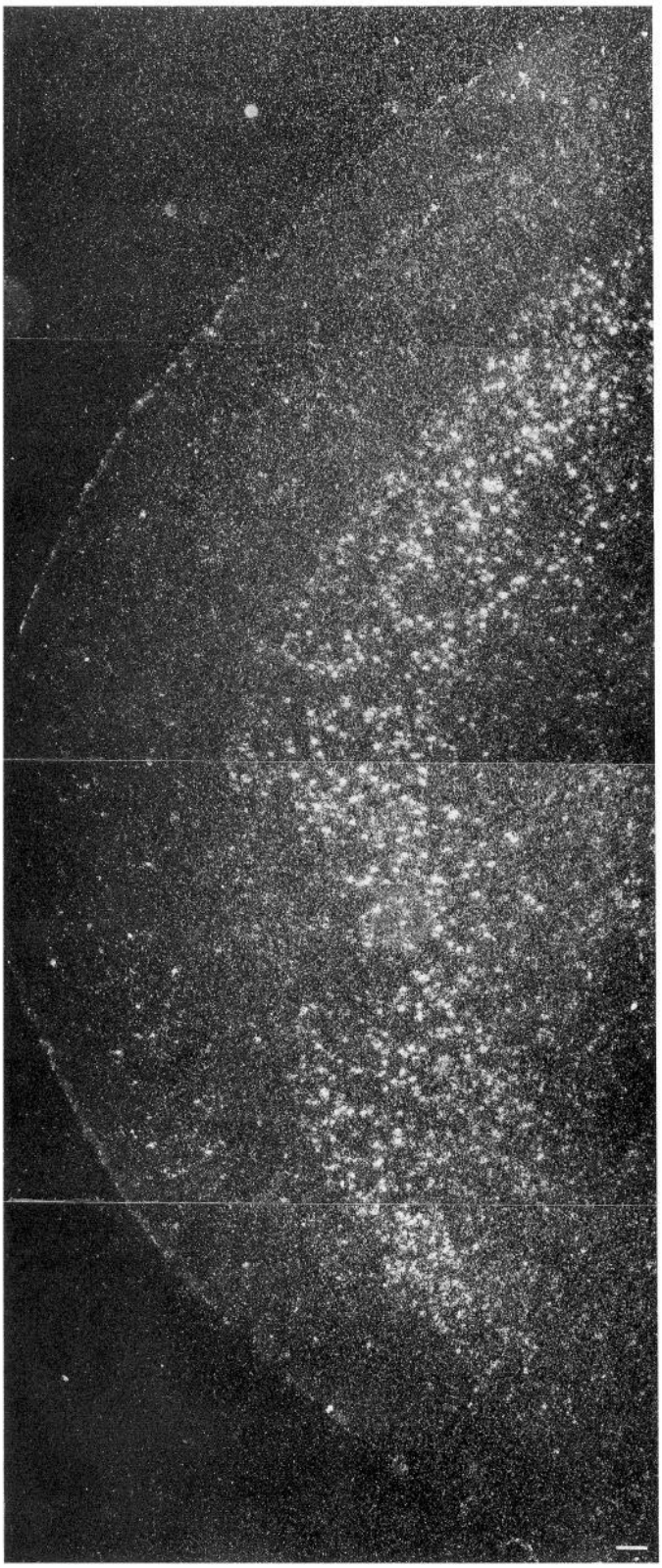

*Figure 7.* Cortical labeling with the $\alpha_{1B}$ mRNA probe shown in a dark-field photomontage of the lateral frontal lobe. Intense $\alpha_{1B}$ labeling is seen in the intermediate layers of the cortex, while the deep (VI) and superficial layers (I and II) are very weakly labeled. Scale bar, 100 $\mu$m.

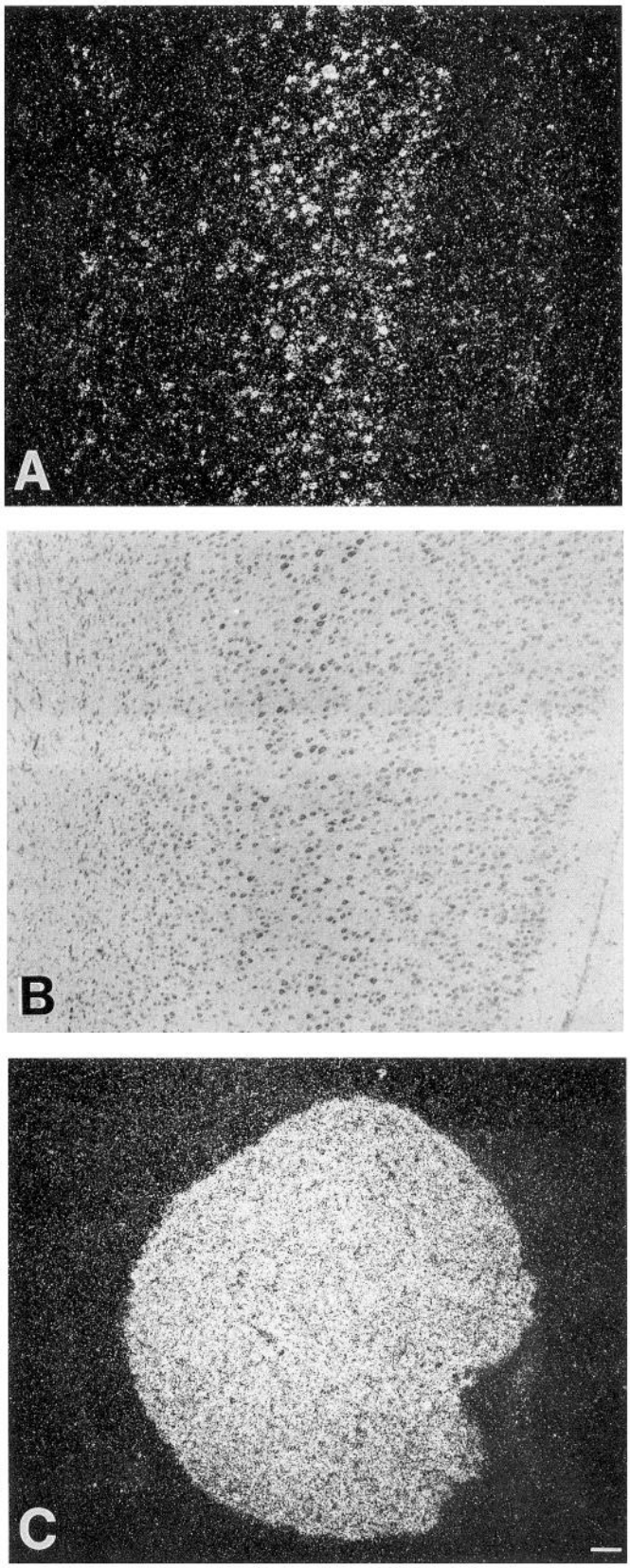


*Figure 8.* *A–C*, $\alpha_{1B}$ mRNA labeling of cortex (*A*) and pineal gland (*C*). *A* and *B*, The comparison of the dark-field (*A*) and bright-field (*B*) micrographs show that most labeled cells are seen in layers IV and V, with more weakly labeled cells in layer VI. *C*, A strong signal is seen in the pineal gland. Scale bar, 100 $\mu$m.

mRNAs, that is, regions that normally contain very low sequence homologies between even closely related mRNAs. These probes were used on adjacent sections to those hybridized with the original probes, and the patterns of labeling were compared. Probes made against the untranslated or the translated region gave identical labeling patterns (Fig. 12*A–E*). However, the $\alpha_{1B}$ probe against the untranslated mRNA region in addition showed a signal over the white matter (Fig. 12*D,E*). Finally, Northern blot (Fig. 13) of total RNA was used to ensure that a single major RNA species was being detected with the *in situ* probes and that the size of the transcript compared to published reports (Lomasney et al., 1991; McCune and Voigt, 1991). Both probes labeled only a single band in each lane, a 3.0 kb transcript for the $\alpha_{1A/D}$ probe and a 2.4 kb transcript for the $\alpha_{1B}$ probe. A greater amount of $\alpha_{1A/D}$ mRNA was seen in the cerebral cortex than in the thalamus, while the $\alpha_{1B}$ probe revealed an opposite pattern, with a greater amount of $\alpha_{1B}$ mRNA transcript seen in the thalamus and less transcript seen in the cerebral cortex. The Northern blot data therefore agree with the *in situ* hybridization results in the thalamus and cerebral cortex and reveal a single transcript of correct size for each of the probes.

## Discussion

Using *in situ* hybridization histochemistry the cellular localization of $\alpha_{1A/D}$ and $\alpha_{1B}$ mRNA has been analyzed. Results show both complementary and overlapping distribution patterns for the two receptors mRNAs. Since earlier autoradiographic ligand binding studies have mainly identified $\alpha_{1B}$ receptors (for references, see below), the present results on the $\alpha_{1A/D}$ receptors are of particular interest. However, the cDNA encoding the true $\alpha_{1A}$ adrenoceptor has likely not yet been cloned. Conversely, it does not appear that the $\alpha_{1C}$ adrenoceptor is present in the CNS. However, it is possible that other $\alpha_1$-like adrenoceptors will be cloned in the future.

The term "$\alpha_{1A/D}$ adrenoceptor" is used here, because the biochemical classification of the receptor has been equivocal. In the initial cloning report (Lomasney et al., 1991), the properties of this receptor in COS-7 cells mostly resembled those of the classical $\alpha_{1A}$ adrenoceptor. In another study (Perez et al., 1991), apparently the same cDNA sequence (with two base pair differences that were likely due to an initial sequencing mistake) was isolated, cloned, and also expressed in COS-7 cells. The resulting receptors properties appeared to represent an, as yet unidentified, $\alpha_1$ adrenoceptor, which was termed the $\alpha_{1D}$ adrenoceptor and which, only in some respects, resembled the $\alpha_{1A}$ adrenoceptor (Perez et al., 1991). Subsequently, authors of the first report suggested that, in fact, the receptor they had cloned was likely a novel $\alpha_1$ adrenoreceptor and termed it the "$\alpha_{1A/D}$ adrenoceptor" (Schwinn and Lomasney, 1992). Our results indicate that this mRNA likely encodes for a novel adrenoceptor (and not the $\alpha_{1A}$ adrenoceptor).

### *Specificity of the* in situ *method*

It is well known that members of the G-protein–linked receptor family in general share a great homology (Raymond et al., 1990), making it difficult to distinguish between different types of adrenergic receptors using hybridization techniques. We have therefore used several probes directed against different parts of each individual transcript of the $\alpha_{1A/D}$ and $\alpha_{1B}$ receptor mRNAs and compared their labeling patterns. Our results show that the two probes raised against $\alpha_{1A/D}$ and $\alpha_{1B}$ mRNA, respectively, show identical labeling, thus supporting specificity of the results.

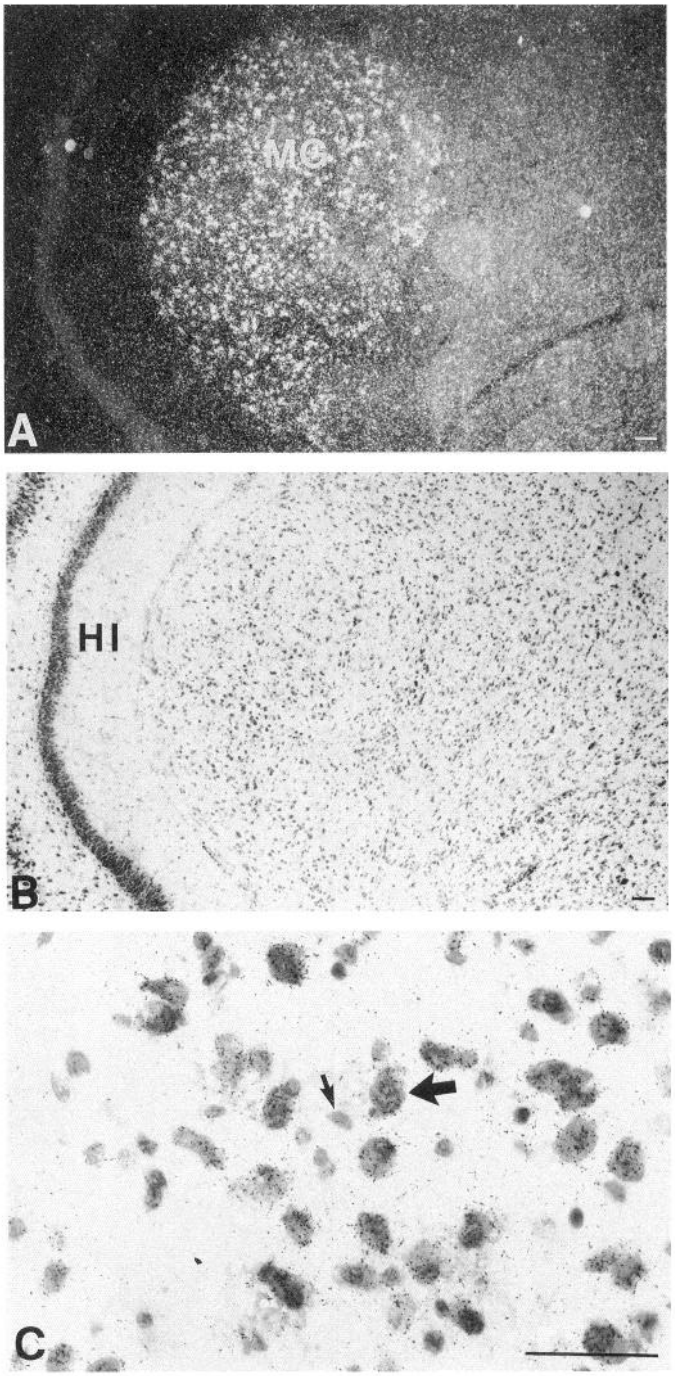


*Figure 9.* *A–C*, $\alpha_{1B}$ mRNA labeling of the medial geniculate body. *A*, Intense labeling is seen in the thalamic nuclei including the medial geniculate bodies (*MG*). *B*, In a bright-field view of the same region it is evident that the labeling is restricted to the medial geniculate body and does not include the neighboring hippocampus (*HI*) and midbrain gray matter. *C*, At higher power, the geniculate body labeling can be seen over most large cells, presumably representing neurons (*large arrow*) but many small cells are unlabeled (*small arrow*). It is not possible to state whether these cells represent neurons or glia. Scale bars: *A* and *B*, 100 μm; *C*, 50 μm.

Furthermore, Northern blot analysis using the same oligonucleotide probe as in the *in situ* hybridization experiments showed a single mRNA specimen correlating well with the previously reported sizes for the individual mRNAs (Lomasney et al., 1991;

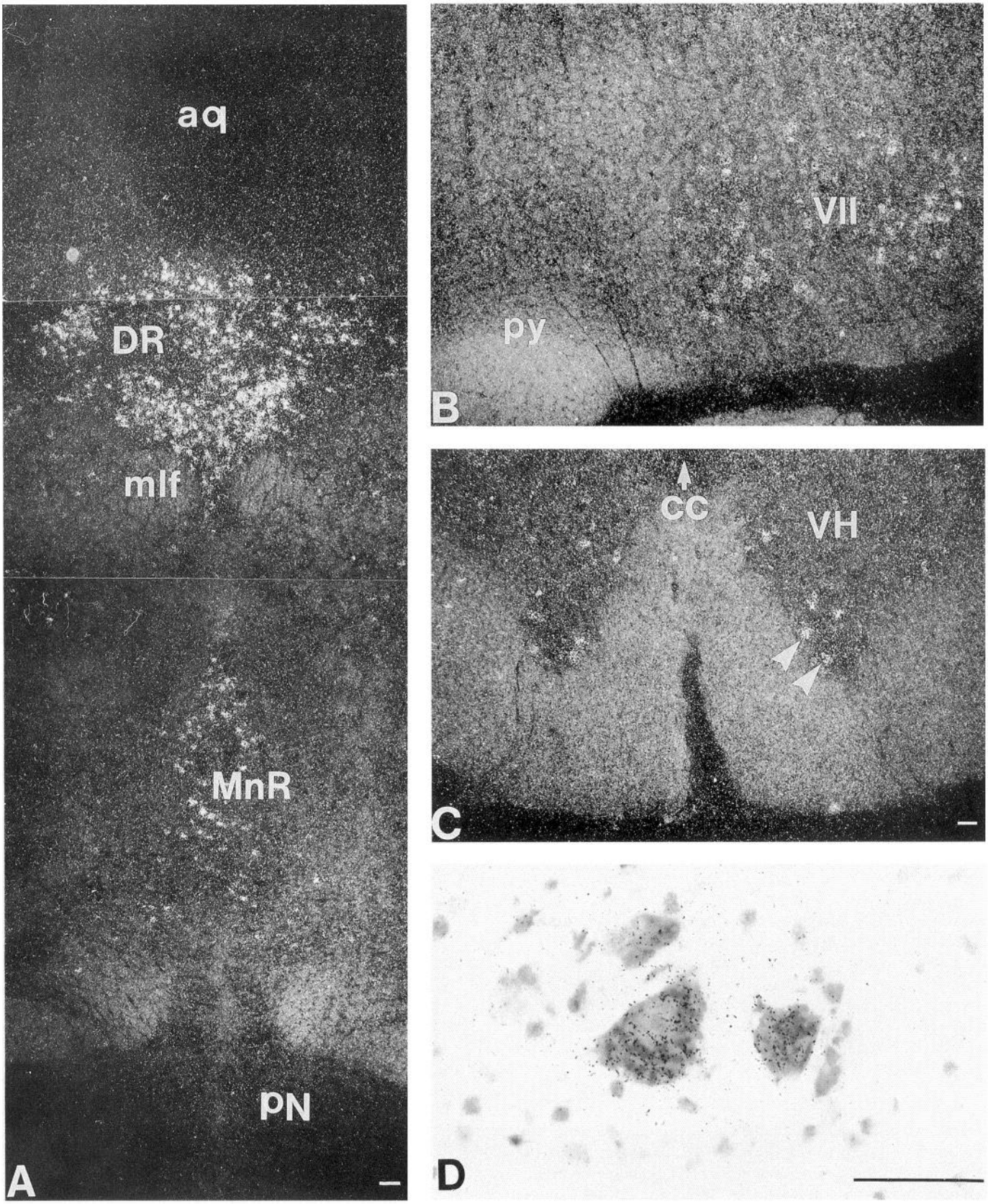


*Figure 10.* Raphe nucleus and motor neuron labeling with the $\alpha_{1B}$ mRNA probe. *A*, The most heavily labeled structure in the brainstem is the raphe complex. In *A* both the dorsal (*DR*) and median (*MnR*) raphe nuclei are intensely labeled. Virtually all neurons in the dorsal raphe are labeled, both in the central and "lateral wing" zones. *B–D*, Motor neurons in the facial (*B*) and the spinal ventral horn (*C*) are labeled, as is evident also after Nissl staining (*D*). *aq*, midbrain aqueduct; *cc*, central canal; *DR*, dorsal raphe; *mlf*, medial longitudinal fasciculus; *MnR*, median raphe nucleus; *PN*, pontine nuclei; *py*, pyramidal tracts; *VH*, ventral horn; *VII*, facial nucleus. Scale bars: *A–C*, 100 μm; *D*, 50 μm.

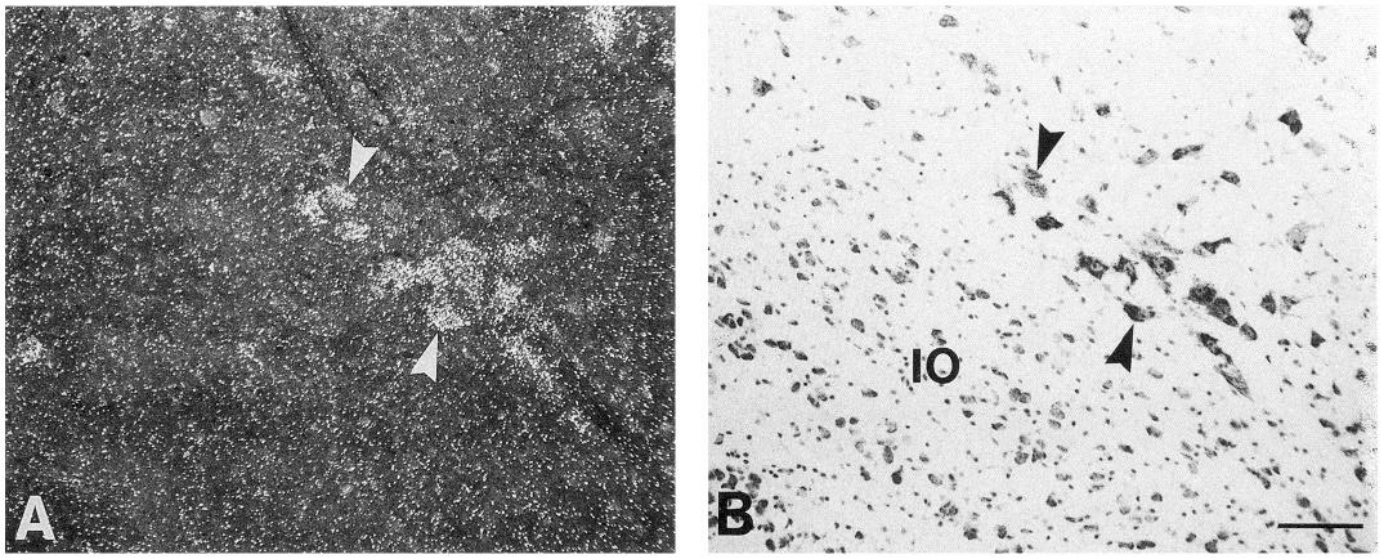


*Figure 11.* *A* and *B*, Labeling with $\alpha_{1B}$ mRNA probe in the ventral gigantocellular reticular nucleus at the rostral level of the inferior olive (*IO*). A small group of cells immediately dorsolateral to the inferior olive is strongly labeled (*arrowheads*) as can be seen, when comparing the dark-field (*A*) and bright-field (*B*) micrographs of the same tissue section. Scale bar, 100 $\mu$m.

McCune and Voigt, 1991; but see Pieribone et al., 1992). Finally, after incubation with an excess of unlabeled probe, the hybridization patterns were abolished, ruling out unspecific hybridization to cellular components other than mRNA.

*Localization of receptor mRNA versus receptor protein*

Several caveats must be made when interpreting *in situ* hybridization data. First, the lack of detectable mRNA levels in a particular area of the CNS does not exclude presence of the mRNA of interest, since the levels may be below the detection limit of our method. It is therefore possible that $\alpha_1$ receptor–synthesizing neurons have a more widespread distribution than shown here. Second, the technique does not prove translation into a functional receptor protein. Finally, the presence of receptor mRNA in the neuronal soma does not indicate where on the cell's surface the receptor protein is deployed. A cell may preferentially express a particular receptor on its presynaptic terminal that would only be visible in the target area using the autoradiographic receptor ligand binding technique (Young and Kuhar, 1979a) or an immunohistochemical procedure utilizing antibodies directed against the receptor (Aoki et al., 1987).

*Comparison with studies using Northern blot analysis of CNS $\alpha_1$ adrenoceptor mRNAs*

Several studies have examined CNS concentrations of the various $\alpha_1$ adrenoceptor mRNAs by Northern blot analysis of mRNA extracted from various brain regions (Schwinn et al., 1990; Lomasney et al., 1991; McCune and Voigt, 1991). McCune and Voigt (1991) and Lomasney et al. (1991) have presented conflicting results about the distribution of $\alpha_{1B}$ receptor mRNA in the rat CNS. McCune and Voigt (1991) report that $\alpha_{1B}$ receptor mRNA is present in comparable amounts in the cerebral cortex, cerebellum, hippocampus, and hypothalamus, while 20% less was seen in the midbrain and brainstem. In contrast, Lomasney et al. (1991) report $\alpha_{1B}$ receptor mRNA only in the brainstem and cerebral cortex and not in the hippocampus or cerebellum. Our results are in agreement with the findings of Lomasney et al. (1991) on $\alpha_{1B}$ receptor mRNA distribution patterns in the CNS, but could not confirm the McCune and Voigt (1991) findings of $\alpha_{1B}$ receptor mRNA in the hippocampus, and we only find a weak signal in the cerebellum.

Schwinn et al. (1990) have cloned and expressed an $\alpha_1$-like adrenoceptor from a bovine brain cDNA library. This receptor, termed $\alpha_{1C}$, although pharmacologically similar to the $\alpha_{1A}$ receptor, is more sensitive to CEC inactivation (68% vs 15% inactivation of each receptor, respectively). However, $\alpha_{1C}$ is inactivated by CEC to a lesser degree than in the $\alpha_{1B}$ receptor (68% vs 95%). These authors also state that Southern blot analysis shows that rats have a gene analog to the bovine $\alpha_{1C}$ receptor and different from the $\alpha_{1A/D}$ or $\alpha_{1B}$ receptors, but they found no expression of a bovine $\alpha_{1C}$-like receptor mRNA in rat cerebral cortex, hippocampus, or brainstem or in bovine cerebral cortex (Schwinn et al., 1990).

*In situ versus autoradiographic ligand localization of $\alpha_1$ adrenoceptors*

Numerous autoradiographic studies have examined the distribution of $\alpha_1$ adrenoceptors in the CNS. Early studies (Young and Kuhar, 1979b, 1980) utilizing $^3$H-WB4101 as a ligand showed very limited and weak binding in the granule cell layer of the hippocampus and dentate gyrus as well as within the periaqueductal gray. These early studies have been difficult to interpret (Unnerstall, 1987), since $^3$H-WB4101 is not specific for $\alpha_1$ adrenoceptors but also binds to 5-HT receptors (see below and Hoffman and Lefkowitz, 1980; Lyon and Randall, 1980; Rehavi et al., 1980; Morrow and Creese, 1986; Unnerstall, 1987).

The ligands now in most common use are $^{125}$I-HEAT (IBE 2254), $^3$H-WB4101, and $^3$H-prazosin (Unnerstall et al., 1982, 1985; Dashwood, 1983; Rainbow and Biegon, 1983; Jones et al., 1985; Palacios et al., 1987; Unnerstall, 1987; Marks et al., 1990; Chamba et al., 1991). While HEAT and prazosin are reported to bind to both $\alpha_{1A}$ and $\alpha_{1B}$ sites in homogenates (Han et al., 1987b; Morrow and Creese, 1986; Minneman, 1988), autoradiographic studies utilizing these ligands have shown very little labeling in the hippocampus or reticular thalamic nucleus. These areas contain exclusively $\alpha_{1A}$ receptors, according to pre-

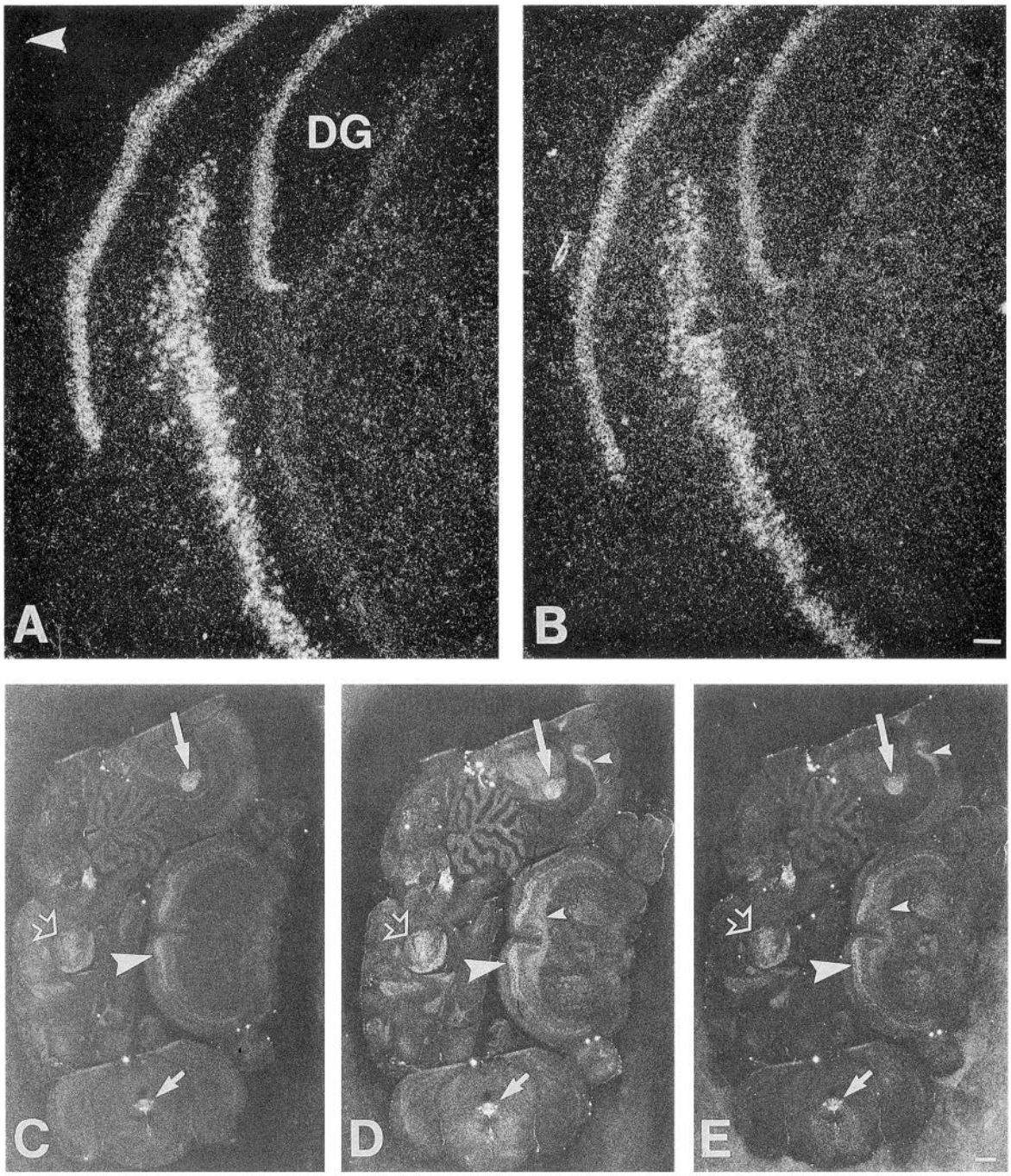


*Figure 12.* *A–E*, Comparison of hybridization obtained with the four different probes directed against the $\alpha_{1A/D}$ and $\alpha_{1B}$ mRNAs. *A* and *B*, Labeling is seen in two adjacent sections of the ventral hippocampus hybridized with the oligonucleotide probes, one (*A*) complementary to a region in the translated domain of the $\alpha_{1A/D}$ mRNA (probe 1 in Fig. 1, top) and the other one (*B*) to a region in the 3′ untranslated region of the mRNA (probe 2 in Fig. 1, top). Note that in both regions the probes give an identical pattern of labeling. *C–E*, Direct prints from film autoradiograms of three adjacent tissue sections including sections from several brain regions hybridized with the oligonucleotide probe complementary to the translated region of the $\alpha_{1B}$ mRNA (*C*; probe 1 in Fig. 1, bottom), the untranslated 5′ region of the mRNA (*E*; probe 2 in Fig. 1, bottom) or a combination of both probes (*D*). Note that the medial geniculate body (*long arrows*), the thalamus (*open arrows*), the dorsal raphe nucleus (*short arrows*), and the cortex (*large arrowheads*) are all labeled with both the translated and untranslated probes. However, the probe to the untranslated 3′ region also gives a signal over white matter (*small arrowheads*) (*E*), which is accentuated after hybridization with the mixture of the two probes (*D*). Scale bar: 100 μm for *A* and *B*, 1000 μm for *C–E*.

vious studies (Morrow et al., 1985; Morrow and Creese, 1986; Wilson and Minneman, 1989; however, see also Zilles et al. 1991). It appears that while these ligands are useful in biochemical assays to identify $\alpha_{1A}$ receptors, they are not as suitable for this purpose in autoradiographic studies.

Recently, WB4101 has been "rediscovered" as a potential $\alpha_{1A}$ ligand (Morrow and Crease, 1986; Blendy et al., 1990, 1991; Grimm et al., 1992). When $^3$H-WB4101 is combined with unlabeled 5-HT (to avoid its binding to 5-HT$_{1A}$ sites), it is reported to label only $\alpha_{1A}$ sites (Morrow and Crease, 1986; Blendy et al.,

1990, 1991; Grimm et al., 1992). Oddly, when one compares the "$\alpha_{1A}$-specific" $^3$H-WB4101 binding with the "$\alpha_{1B}$-specific" $^3$H-prazosin binding (preabsorbtion of tissue with cold WB4101), the patterns look rather similar (Blendy et al., 1990, 1991; Grimm et al., 1992). These studies point to further problems using this ligand. For example, the medial thalamus shows greater $\alpha_{1A}$ labeling than either the hippocampus or the reticular thalamic nucleus, which does not agree with previous biochemical studies showing low $\alpha_{1A}$ binding in the thalamus and higher binding in the hippocampus (Wilson and Minneman, 1989). Wilson and Minneman (1989) used CEC inactivation combined with the competitive binding of WB4101 to distinguish between $\alpha_{1A}$ and $\alpha_{1B}$ receptors. The $\alpha_{1A}$ receptor, which is insensitive to inactivation by CEC, is found to predominate in the hippocampus, brainstem, and spinal cord, while CEC-sensitive sites ($\alpha_{1B}$) are found in the thalamus, hypothalamus, and cerebellum. While $^3$H-WB4101 appears to label some $\alpha_{1A/D}$ sites it may not adequately reflect the total $\alpha_{1A}$ receptor distribution. In some obvious cases, however, HEAT may label $\alpha_{1A/D}$ receptors. For example, the inferior olivary complex and the olfactory bulb, which both contain a large amount of $\alpha_{1A/D}$ mRNA (and no $\alpha_{1B}$ mRNA), were also heavily labeled with $^{125}$I-HEAT (Jones et al., 1985; Unnerstall et al., 1985; Palacios et al., 1987; Unnerstall, 1987).

Previously it has been shown that the locus coeruleus contains a high density of $\alpha_1$ receptors as determined by receptor autoradiography (Jones et al., 1985; Palacios et al., 1987; Unnerstall, 1987; Chamba et al., 1991). However, physiological experiments both *in vivo* and *in vitro* have failed to identify any $\alpha_1$-like responses in adult locus coeruleus neurons (Cederbaum and Aghajanian, 1977; Egan et al., 1983). In the present study, neither $\alpha_{1A/D}$ nor $\alpha_{1B}$ mRNA was found in neurons of the locus coeruleus. It is possible that $\alpha_1$ binding seen in the locus coeruleus represents presynaptic receptors or perhaps $\alpha_{1A}$ receptors (Schwinn et al., 1990).

In conclusion, it is obvious that the available ligands used to study the autoradiographic distribution of $\alpha_1$ adrenoceptors are not specific for either the $\alpha_{1A}$ or $\alpha_{1B}$ adrenoceptors and definitely do not recognize the receptor encoded by the recently identified $\alpha_{1A/D}$ mRNA. Complementary *in situ* hybridization studies may therefore help to localize the many types of closely related adrenoceptors that are expressed in the CNS.

### *Functional roles for different $\alpha_1$ receptors*

In many CNS areas only one of the two $\alpha_1$ subtype receptors was found (Table 1). The most striking examples were the reticular thalamic nucleus, on the one hand, and the remaining thalamus, the raphe nuclei, the inferior olivary nucleus, the pineal gland, and the hippocampus, on the other. In each of these cases, only one of the probes heavily labeled neurons in each respective area. Conversely, in some other areas both probes showed hybridization, for example, in the midlayers of the cortex, the dorsal division of the lateral amygdaloid nucleus, and in the brainstem and spinal motor neurons. These differences in distribution of receptor mRNAs do not seem simply to reflect the differential input from the endogenous adrenoceptor ligands, adrenaline and noradrenaline. Moreover, these two catecholamines are not likely to reflect the regional differences seen, since as noted above the noradrenaline-producing locus coeruleus is the major afferent source of the endogenous ligand for both receptor subtypes evaluated in the present study, in which adrenaline has a very restricted distribution (Ungerstedt, 1971; Swanson and Hartman, 1975; Jones and Moore, 1977; Hökfelt et al., 1984; Moore and Card, 1984; Jones and Yang, 1985). However, in retrospect it may be possible to determine what the physiologic effects of stimulating the $\alpha_{1A/D}$ adrenoceptor are, since previous studies have examined the physiological actions of $\alpha_1$ adrenoceptor agonists in areas shown in the present study to be enriched in $\alpha_{1A/D}$ adrenoceptors (Baraban and Aghajanian, 1980; Bradshaw et al., 1981; Fung and Barnes, 1981; Aghajanian, 1985; Freedman and Aghajanian, 1987; McCormick and Prince, 1988; McCormick and Wang, 1988; McCormick, 1992).

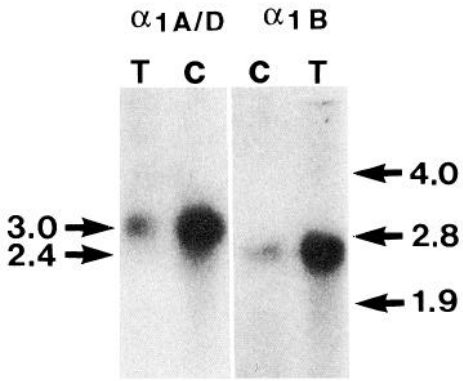


*Figure 13.* *A*, Northern blot of total RNA extracted from rat thalamus/hypothalamus (*T*) and cerebral cortex (*C*) probed with the oligonucleotide probes for the $\alpha_{1A/D}$ (*left side*) and $\alpha_{1B}$ (*right side*) mRNA. The size of the $\alpha_{1A/D}$ mRNA is approximately 3.0 kb, whereas the size of $\alpha_{1B}$ mRNA is approximately 2.4 kb. In agreement with the *in situ* hybridization results, there is higher expression of $\alpha_{1A/D}$ mRNA in cerebral cortex than there is in the thalamus/hypothalamus, while the reverse is true for the $\alpha_{1B}$ mRNA. The migration of three RNA size markers (4.0, 2.8, and 1.9 kb, respectively; Promega) is indicated to the right.

Based on biochemical studies of brain homogenates, the two original $\alpha_1$ receptor subtypes, $\alpha_{1A}$ and $\alpha_{1B}$ adrenoceptors, differ from each other with regard to the second messenger cascades that they activate (Johnson and Minneman, 1986, 1987; Han et al., 1987a; Minneman, 1988; Raymond et al., 1990). The $\alpha_{1A/D}$ receptor is linked via a G-protein directly to a dihydropyridine-sensitive $Ca^{2+}$ channel. The $\alpha_{1B}$ receptor, on the other hand, activates phospholipase C to hydrolyze phosphoinositol into inositol triphosphate and diacylglycerol to activate $Ca^{2+}$ influx (Minneman and Johnson, 1984; Schoepp et al., 1984; Johnson and Minneman, 1986, 1987; Han et al., 1987a; Minneman, 1988; Michel et al., 1990). While these are the effects observed in biochemical studies, they are not the major effects seen physiologically in the CNS by activation of $\alpha_1$ receptors. In most studied areas of the brain, including the cerebral cortex, medial and lateral geniculate nuclei, the reticular thalamic nucleus, dorsal raphe and spinal motor neurons, $\alpha_1$ receptor activation causes strictly excitatory responses both *in vivo* and *in vitro* (Baraban and Aghajanian, 1980; Bradshaw et al., 1981, 1985; Fung and Barnes, 1981; Aghajanian, 1985; Freedman and Aghajanian, 1987; McCormick and Prince, 1988; McCormick and Wang, 1991; McCormick, 1992; for review, see Szabadi, 1979; Foote et al., 1983; Szabadi and Bradshaw, 1987). However, it appears that this activation is due largely to a decrease in resting $K^+$ conductance and not to a robust increase in $Ca^{2+}$ conductance (Baraban and Aghajanian, 1980; Aghajanian, 1985; McCormick and Prince, 1988; McCormick and Wang, 1991; McCormick, 1992). In all these cases, this $K^+$ conductance reduction is not

**Table 1. Differential distribution of $\alpha_{1A/D}$ and $\alpha_{1B}$ receptor mRNAs and $\alpha_1$ ligand binding**

| Structure | $\alpha_{1A/D}$ mRNA | $\alpha_{1B}$ mRNA | $\alpha_1$ ligand binding |
|---|---|---|---|
| Predominately $\alpha_{1A/D}$ receptor mRNA | | | |
| Internal granule cell layer of the olfactory bulb | +++ | − | + |
| Hippocampus | +++ | − | + |
| Reticulated thalamic nucleus | +++ | − | + |
| Inferior olivary complex | +++ | − | ++ |
| Cuneate nucleus | ++ | − | − |
| Predominately $\alpha_{1B}$ receptor mRNA | | | |
| Paraventricular hypothalamic nucleus | − | + | ++ |
| Thalamus (except ret. thal. nuc.) | − | +++ | +++ |
| Raphe nuclei | − | +++ | ++ |
| External cuneate nucleus | − | ++ | ++ |
| Intermediolateral column in the spinal cord | − | + | + |
| Pineal gland | − | +++ | ++ |
| High levels of $\alpha_1$ receptor binding but little mRNA | | | |
| External plexiform layer of the olfactory bulb | − | − | +++ |
| Locus coeruleus | − | − | +++ |
| Overlapping distribution of $\alpha_{1A/D}$ and $\alpha_{1B}$ receptor mRNAs and $\alpha_1$ ligand binding | | | |
| Cerebral cortex (layers II–V) | +++ | +++ | +++ |
| Dorsolateral lateral amygdala | +++ | +++ | +++ |
| Cranial and spinal motor neurons | +++ | +++ | +++ |
| Sensory trigeminal nucleus | + | + | + |
| Pontine and medullary reticular formation | + | + | + |

This table shows the differential distribution of $\alpha_{1A/D}$ and $\alpha_{1B}$ mRNA in selected regions of the CNS as compared to previous ligand binding studies (Unnerstall et al., 1982, 1985; Dashwood, 1983; Rainbow and Biegon, 1983; Jones et al., 1985; Palacios et al., 1987; Unnerstall, 1987; Marks et al., 1990; Chamba et al., 1991). This is meant for comparison only and is relative and subjective. Single pluses indicate low, two pluses indicate moderate, and three pluses indicate high intensity of the hybridization signal found over neurons in the *in situ* experiments or the autoradiographic grain density in the binding studies.

dependent on extracellular $Ca^{2+}$ entering the cell. In CNS areas such as the reticular thalamic nucleus, that apparently contain only $\alpha_{1A/D}$ receptors, which should directly activate a $Ca^{2+}$ current, no such activation has been reported (McCormick and Wang, 1991; McCormick, 1992). In the case of raphe neurons, shown in the present study to contain a large amount of $\alpha_{1B}$ receptor mRNA, $\alpha_1$ agonists do, in addition to reducing a resting A current, activate a slow $Ca^{2+}$-dependent $K^+$ current (Baraban and Aghajanian, 1980; Aghajanian, 1985). However, this secondary effect seen in the raphe neurons is opposite to the effect seen by $\alpha_{1B}$ receptor activation in the medial thalamic nuclei, including the geniculate bodies (McCormick and Wang, 1991; McCormick, 1992). In the geniculate bodies, shown here to also be rich in $\alpha_{1B}$ but not in $\alpha_{1A/D}$ receptors, $\alpha_1$ activation, in addition to reducing a resting A current, causes a reduction in a slow $Ca^{2+}$-dependent $K^+$ current (McCormick and Prince, 1988). In these two different areas, which are enriched in only one of the two different $\alpha_1$ receptor subtypes, $\alpha_1$ activation leads to the same "primary" effect on resting $K^+$ conductance but causes opposing "secondary" effects on the afterhyperpolarization current. Taken together, it appears that the most robust effect of either $\alpha_{1A/D}$ or $\alpha_{1B}$ activation is a reduction in resting $K^+$ conductance, while the secondary effects can include a reduction or an enhancement of a $Ca^{2+}$ and/or $Ca^{2+}$-dependent $K^+$ current. Thus, although the $\alpha_{1A/D}$ and $\alpha_{1B}$ receptors often have a different localization, the effects of activation of these two subtypes by a catecholamine seem to be similar.

*Conclusions*

In conclusion, we have found that two $\alpha_1$ receptor mRNAs are expressed partly in different and partly in the same regions of the rat brain, suggesting involvement of these receptors in many CNS functions. These findings imply that pharmacological agents specific for $\alpha_{1A/D}$ and $\alpha_{1B}$ receptors are likely to have different effects related to their regional distribution. Depressive disorders (Blendy et al., 1990, 1991) or depression-mimicking conditions (Grimm et al., 1992) seem to be linked mainly to $\alpha_{1B}$ receptor, and revealing the distribution patterns of the cells synthesizing this receptor in the human brain may in the future give further clues to the substrate for these mental disorders.

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