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Autoradiographic Differentiation of Mu, Delta, and Kappa Opioid Receptors in the Rat Forebrain and Midbrain

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While there is an abundance of pharmacological and biochemical evidence to suggest the existence of multiple opioid receptors, their precise localization within the brain is unclear. To help clarify this issue, the present study examined the distributions of the mu, delta, and kappa opioid receptor subtypes in the rat forebrain and midbrain using *in vitro* autoradiography. Mu and delta receptors were labeled with the selective ligands ^{3}H-DAGO (Tyr- D-Ala-Gly-MePhe-Gly-ol), and ^{3}H-DPDPE (D-Pen^2, D-Pen^5-enkephalin), respectively, while the kappa receptors were labeled with ^{3}H-(−)bremazocine in the presence of unlabeled DAGO and DPDPE. Based on previous findings in our laboratory, the labeling conditions were such that each ligand selectively occupied approximately 75% of each of the opioid sites. The results demonstrated that all 3 opioid receptor subtypes were differentially distributed in the rat brain. Mu binding was dense in anterior cingulate cortex, neocortex, amygdala, hippocampus, ventral dentate gyrus, presubiculum, nucleus accumbens, caudate putamen, thalamus, habenula, interpeduncular nucleus, pars compacta of the substantia nigra, superior and inferior colliculi, and raphe nuclei. In contrast, delta binding was restricted to only a few brain areas, including anterior cingulate cortex, neocortex, amygdala, olfactory tubercle, nucleus accumbens, and caudate putamen. Kappa binding, while not as widespread as observed with mu binding, was densely distributed in the amygdala, olfactory tubercle, nucleus accumbens, caudate putamen, medial preoptic area, hypothalamus, median eminence, periventricular thalamus, and interpeduncular nucleus. While all 3 opioid receptor subtypes could sometimes be localized within the same brain area, their precise distribution within the region often varied widely. For example, in the caudate putamen, mu binding had a patchy distribution, while delta and kappa sites were diffusely distributed, with delta sites being particularly dense ventrolaterally and kappa sites being concentrated ventromedially. These results support the existence of at least 3 distinct opioid receptors with possibly separate functional roles.

There is an abundance of evidence to suggest the existence of multiple opioid receptors (Martin et al., 1976; Lord et al., 1977; Chang and Cuatrecasas, 1981; Wood, 1982; Pasternak et al., 1983; Robson et al., 1983; Goldstein and James, 1984; James and Goldstein, 1984; Zukin and Zukin, 1984). Despite this apparent consensus, the nature and distribution of these sites are still controversial. Some investigators (Quirion et al., 1981; Bowen and Pert, 1982; Rothman and Pert, 1984) have suggested the existence of 2 subtypes, referred to as types I and II. The type I opioid receptor is suggested to be conformationally labile and, depending on experimental conditions, will bind mu, delta, and kappa agonists, while the type II receptor is suggested to have a fixed conformation selective for enkephalin-like compounds. These receptors were characterized further on the basis of their anatomical distribution, with type I receptors being discretely localized in various CNS nuclei and type II receptors being more diffusely distributed (Bowen et al., 1981; Olgiati et al., 1982).

This scheme has recently been challenged with the advent of more selective ligands and protection experiments (Robson and Kosterlitz, 1979; Smith and Simon, 1980; Goldstein and James, 1984; James and Goldstein, 1984). Presently, there is some agreement as to the existence of at least 3 opioid receptor subtypes; a mu subtype, similar to the type I receptor but selective for morphine-like compounds; a delta subtype, similar to the type II receptor and selective for enkephalin-like peptides; and a kappa subtype selective for benzomorphans and dynorphin-like peptides. Some investigators have delineated these subtypes even further as mu_1 and mu_2 (Pasternak et al., 1983) and $kappa_1$ and $kappa_2$ (Attali et al., 1982; Gouaderes et al., 1983) on the basis of pharmacological and receptor binding studies. In addition to these receptor sites, some investigators have postulated the existence of epsilon and lambda opioid receptors (Schulz et al., 1981; Garzon et al., 1984; Grevel et al., 1985) selective for β-endorphin and naloxone, respectively. While recognizing there may be other subtypes or subclasses of opioid receptor sites, this paper deals most directly with the mu, delta, and kappa subtypes.

One of the lines of evidence in support of the multiplicity of opioid receptors has been their differential localization in the nervous system (Goodman et al., 1980; Duka et al., 1981; Lewis et al., 1983; Maurer et al., 1983; Quirion et al., 1983b). While the results are most compelling for a differential distribution of mu and delta receptors, numerous inconsistencies have ap-

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peared in the literature. For instance, some investigators (Duka et al., 1981; Lewis et al., 1983) have suggested that delta opioid receptors are diffusely localized in rat cortex, while others (Goodman et al., 1980; Quirion et al., 1983b) report that their density varies with cortical lamina. Similarly, some studies (Duka et al., 1981; Moskowitz and Goodman, 1984) have suggested that the hypothalamus contains both mu and delta sites, while others report either a predominance of mu sites (Goodman et al., 1980) or sparse, if any, opioid binding sites (Herkenham and Pert, 1980; Quirion et al., 1983b).

In the case of kappa opioid receptors, there is even less agreement concerning their distribution. Goodman and Snyder (1982) have reported that kappa sites are uniquely distributed in the guinea pig, with dense labeling of the deep layers (V and VI) of cortex and diffuse binding in the caudate putamen. Mu binding, on the other hand, was found to be predominantly localized in "patches" in the caudate putamen and in layers I and IV of frontal cortex. Quirion et al. (1983a) confirmed Goodman and Snyder's findings in the guinea pig but failed to find similar results in the rat. They reported that mu and kappa receptors did not differ in their distribution in the rat and suggested that their results were due to species differences. These latter findings have not, however, been confirmed by more recent studies (Lynch et al., 1985; Mansour et al., 1986a), and preliminary results from our laboratory have demonstrated that the 3 opioid receptor subtypes are uniquely distributed in the rat nervous system.

A major source of confusion concerning the distribution of the opioid receptor subtypes may have been due to the lack of selective opioid ligands. The recent advent of selective ligands, such as the mu agonist ^{3}H-DAGO (Tyr-D-Ala-Gly-MePhe-Gly-ol) and the delta agonist ^{3}H-DPDPE (D-Pen^2, D-Pen^5-enkephalin), has substantially reduced this problem (Kosterlitz and Paterson, 1980; Handa et al., 1981; Mosberg et al., 1983a, b; James and Goldstein, 1984). There continues to be a problem, however, in selectively labeling kappa receptors. Until recently the only commercially available kappa radioligand has been ^{3}H-ethylketocyclazocine (EKC), a nonselective ligand that labels all the opioid subtypes under receptor binding conditions. While some investigators have used ^{3}H-EKC in the presence of saturating concentrations of mu and delta agonists (Quirion et al., 1983a), determining the blocking concentrations to use remains problematic. This lack of selective kappa ligands is exacerbated further in tissues that contain relatively small proportions of these sites such as rat brain, which has primarily mu and delta receptors.

Another possible source of confusion may have been due to the varying concentrations of radioligand used to label the receptor sites. Because of the varying affinities of the ligands for each of the opioid receptor subtypes, the labeling concentration is of primary importance when comparing these subtypes, as it would be otherwise impossible to determine whether the results were due to differences in the proportion of sites occupied or to anatomical differences in their distribution. In addition, labeling with concentrations of radioligands that are well below a ligand's K_d is difficult, as it provides a mapping of only a small proportion of the available sites.

The present paper takes into consideration the above concerns and extends our earlier findings by providing a detailed comparative mapping of the distribution of the mu, delta, and kappa opioid receptors in the rat forebrain and midbrain using *in vitro* autoradiography. In the following studies, we have chosen a concentration equal to 3 times the K_d value of each of the labeling ligands in order to occupy approximately 75% of the available sites. These values were based on previous findings (Mansour et al., 1986a) obtained using slide-mounted tissue sections, and allow the labeling of the greatest number of sites without sacrificing specific binding. In addition, the ligands and incubation conditions chosen have been determined on the basis of equilibrium analysis and competition studies (Mansour et al., 1986a) to selectively label the mu, delta, and kappa subtypes under the autoradiographic conditions used in the present study.

Materials and Methods

Tissue preparation and incubation medium. Three adult male Sprague-Dawley rats were sacrificed by decapitation; their brains were quickly removed and frozen in liquid isopentane (−30°C) for 30 sec. Frozen brains were sectioned in a Bright cryostat (25 μm) and thaw-mounted on precleaned and subbed microscope slides. The brain sections were then dried overnight at 4°C under reduced pressure in a glass desiccator and stored in a −80°C freezer. Immediately prior to using the tissue, the slide-mounted sections were gradually brought up to room temperature and incubated with various tritiated opioid ligands in 50 mM Tris buffer (pH 7.5 at 25°C). The tritriated opioids used were DAGO (60 Ci/mmol) and DPDPE (35 Ci/mmol) purchased from Amersham, and (−)bremazocine (41.4 Ci/mmol) purchased from New England Nuclear. The mapping concentrations used were as follows: DAGO (4.9 nM), DPDPE (36.0 nM), and bremazocine (0.9 nM). As bremazocine does not selectively bind to kappa receptors (Gillan and Kosterlitz, 1982), all ^{3}H-bremazocine binding was conducted in the presence of 100 nM DAGO and DPDPE in order to block the mu and delta sites. The mapping concentrations chosen were approximately 3 times the K_d value for each of the ligands as determined by previous studies (Mansour et al., 1986a) and represent a 77–78% receptor occupancy. In the case of ^{3}H-bremazocine, some sections were incubated with a series of concentrations (0.09–40.0 nM) in the presence of 100 nM DAGO and DPDPE to determine whether the distribution of these sites varied with labeling concentration.

Autoradiographic procedures. After being brought up to room temperature, the brain sections were placed in incubation chambers designed to maintain ambient temperature (25°C) and humidity (60–80%) and were incubated with 200 μl of ^{3}H-ligand and buffer. The slides were incubated for 60 min, drained, and washed in 4 consecutive 250 ml, 50 mM Tris washes (pH 7.6, 4°C). Slides incubated with ^{3}H-DAGO and ^{3}H-DPDPE were given four 30 sec washes, while those incubated with ^{3}H-bremazocine were given four 4 min washes. Following the Tris washes, all slides were rinsed (2 sec) in a 250 ml wash of distilled water (4°C) and quickly dried with a portable hair dryer set to "cool." Nonspecific binding was evaluated by treating a parallel set of slides with the same concentrations of ^{3}H-ligand with a 1 μM final concentration of an unlabeled competitor: levorphanol to displace ^{3}H-DAGO, DSLET (Tyr-D-Ser-Gly-Phe-Leu-Thr) to displace ^{3}H-DPDPE, and UM 1071 [M_R 2034; (−)-(1R,5R,9R 2″S)-5,9-dimethyl-2-tetrahydrofurfuryl-2′-hydroxyl-6,7-benzomorphan] to displace ^{3}H-bremazocine. Slides used for autoradiography were either arranged in X-ray cassettes and apposed to tritium-sensitive LKB Ultrofilm or set aside for dipping in liquid emulsion (Herkenham and Pert, 1982). The LKB films were exposed at room temperature and developed in Kodak D-19 (4 min, 19°C), agitated in 2% acetic acid (30 sec), and fixed in Kodak Rapidfix (5 min). The films were then washed in running water (30 min) before air-drying. The film exposure time varied with ^{3}H-ligand; sections labeled with ^{3}H-DAGO and ^{3}H-DPDPE for general mapping were apposed to LKB film for 34 d, while those used for mapping kappa sites (^{3}H-bremazocine, 0.9 nM) were apposed for 92 d. The other kappa autoradiograms presented here (sections labeled with 0.36 and 1.6 nM bremazocine in the presence of 100 nM DAGO and DPDPE) were apposed for 225 d in order to photograph the original autoradiogram. The autoradiograms produced with the LKB film are presented as either dark-field images using the LKB film itself as the negative or as computer enhancements produced by the Loats Image Analysis System (Westminster, MD).

The sections set aside for liquid emulsion autoradiography were incubated and dried as above and fixed by exposure to paraformaldehyde vapors (80°C, 2 hr) in an evacuated desiccator. This procedure is suitable for fixing ^{3}H-DAGO and ^{3}H-DPDPE but not ^{3}H-bremazocine. The

Table 1. Relative distribution of mu, delta, and kappa receptors

Brain area	Mu	Delta	Kappa
Telencephalon			
Cingulate cortex			
Anterior			
Layer I	++++	+	+
Layer II	+	++	+
Layer III	+++	++	++
Layer V	+++	++++	++
Layer VI	++	++	++
Posterior			
Layer I	+	+	0
Layers II–VI	0	++	0
Frontal parietal cortex			
Layer I	+++	++	0
Layers II and III	+	+++	++
Layer IV	+++	++	0
Layers V and VI	+	+++	++
Piriform cortex			
Layer I	+	+	++
Layers II and III	++	++	++
Entorhinal cortex			
Layer I	+	+	+
Layers II–VI	++	++	++
Temporal cortex			
Layer I	+++	++	0
Layers II and III	++	+++	0
Layer IV	+++	+	0
Layers V and VI	++	+++	+
Striate cortex			
Layer I	++	0	0
Layers II and III	+	++	0
Layer IV	+++	+	0
Layers V and VI	++	+++	++
Endopiriform nucleus	+++	++++	++++
Amygdala			
Medial nucleus	+++	++	++
Central nucleus	0	0	++
Cortical nucleus	++++	++	++
Lateral	++++	+++	+++
Basolateral	++++	+++	+++
Hippocampal formation			
Hippocampus			
Pyramidal cell layer (CA1, CA2, CA3)	+++	++	+
Stratum moleculare	+	0	0
Stratum lacunosum-moleculare	+++	++	+
Stratum oriens	0	0	0
Stratum radiatum	0	0	0
Dentate gyrus			
Dorsal			
Molecular layer	0	0	0
Granular layer	0	0	0
Ventral			
Molecular layer	++++	+	+
Granular layer	+++	+	+
Presubiculum	++++	+++	+
Olfactory tubercle (islands of Calleja)	+	+++	+++
Nucleus accumbens	++++ (patchy)	++++	+++ (vent.)

Table 1. Continued

Brain area	Mu	Delta	Kappa
Caudate putamen	++++ (patchy)	++++ (vent. lat.)	+++ (vent. med.)
Globus pallidus	+	+	+
Septum			
Lateral nucleus	0	++	++
Medial nucleus	+++	+	+
Diagonal band	++	++	+++
Subfornical organ	++	+	+++
Stria terminalis	++++	+++	0
Bed nucleus stria terminalis	++	++	+++
Medial preoptic area	+	+	++++
Diencephalon			
Hypothalamus			
Anterior hypothalamic area	0	0	++
Supraoptic nucleus	0	0	++
Paraventricular nucleus	0	0	++
Periventricular nucleus	0	0	+++
Arcuate	0	0	++
Median eminence	0	0	+++
Ventromedial nucleus	0	+	+++
Dorsomedial nucleus	+	0	+++
Lateral hypothalamic area	+	0	++
Medial mammillary nucleus	+++	0	++
Mammillary peduncle	++	0	+
Thalamus			
Periventricular nucleus	0	0	+++
Mediodorsal nucleus	++++	+	++
Intermediodorsal	++++	+	++
Laterodorsal nucleus	++	0	0
Central medial nucleus	++++	+	++
Central lateral nucleus	0	0	0
Paracentral nucleus	++++	+	++
Rhomboid nucleus	++++	+	++
Reuniens nucleus	++++	+	++
Gelatinosus nucleus	++	0	0
Ventromedial nucleus	++++	+	+
Lateral posterior nucleus	++++	0	0
Posterior group	++++	0	0
Ventrolateral nucleus	0	0	0
Ventral posteromedial nucleus	0	0	0
Ventral posterolateral nucleus	++	++	0
Medial geniculate nucleus	++++	0	+
Zona incerta	0	0	++
Habenula			
Medial	+++	+	+++
Lateral	0	0	0
Fasciculus retroflexus	+++ (patchy)	0	0
Mesencephalon			
Interpeduncular complex			
Central nucleus	++++	+++	+++
Paramedian	++++	+	++
Inner posterior	+++	0	++
Substantia nigra			
Pars compacta	+++	0	0
Pars reticulata	++	+	+
Ventral tegmental area	++	0	+

Table 1. Continued

Brain area	Mu	Delta	Kappa
Medial terminal nucleus of accessory optic tract	++++	0	0
Periaqueductal gray (rostral)			
Ventral	+	0	++
Dorsal	++	0	+
Periaqueductal gray (caudal)			
Ventral	0	0	++
Dorsal	+	0	++
Superior colliculus			
Superficial gray layer	++++	+	++
Intermediate gray layer	++	0	+
Deep gray layer	++	0	+
Inferior colliculus			
Central dorsomedial nucleus	++++	+	++
Central ventrolateral nucleus	+	0	0
Raphe nuclei			
Caudal linear nucleus	+++	0	++
Median raphe nucleus	+++	0	++

Density: ++++, very dense; +++, dense; ++, moderate; +, light; 0, undetectable.

slides were placed in a hood overnight, and the lipids were removed by a series of aqueous ethanol (70–100%) washes, 5 xylene washes, and a second series of ethanol solutions (100–70%), followed by washes in distilled water. The slides were then dried overnight and dipped under safelight conditions into molten (41°C) Kodak NTB2 emulsion diluted 1:1 with a solution of 0.1% Dreft detergent dissolved in distilled water. The slides were dipped individually, dried vertically for 2 hr in an atmosphere of 60–80% humidity, and sealed in lighttight boxes containing Drierite packets. These boxes were stored at 4°C for either 38 d (^{3}H DAGO) or 44 d (^{3}H DPDPE), developed in Kodak D-19 (2 min, 17°C), washed in distilled water (20 sec), fixed in Kodak Rapidfix (3 min), rewashed in running water (30 min), lightly stained for cell bodies using thioin, and coverslipped with Permount. The slides were then cleaned with acid–alcohol and photographed using a Wild Heerbrugg stereomicroscope.

Results

The distributions of the mu, delta, and kappa receptor subtypes varied widely in the forebrain and midbrain of the rat. In the following paragraphs we describe each of these distributions (see Table 1), but before doing so a brief explanation of the enhancement procedure would be appropriate. Figure 1 compares the dark-field images of coronal rat brain sections produced with LKB film (left) and their black-and-white computer enhancements (right). Notice that the basic distributions of the receptor subtypes are not altered by this amplification. The relative distribution of mu sites in the thalamus or the kappa sites in the hypothalamic area are not appreciably changed by the computer enhancement. The Loats imaging system can further process the different optical densities of the LKB film from a series of gray tones as depicted in Figure 1 to a series of color tones shown in Figure 2. The brain images shown in column D of Figure 2 are, in fact, the color enhancements of those presented in Figure 1. As can be seen by comparing these 2 sets of images, each computer manipulation does not appear to alter the relative distributions of each of the subtypes. We would like to emphasize that these autoradiographic enhancements are meant to be qualitative, providing a relative measure of binding within a receptor distribution, and are not designed to give absolute estimates of binding. Further, as the kappa autoradiograms were, of necessity, exposed to the LKB film longer than the mu and delta autoradiograms, optical density alone gives an overestimation of binding.

An additional word of caution is necessary in interpreting the color-enhanced autoradiograms. An inherent problem in using such images is that small differences in optical density may be amplified to an extent that may not be meaningful or have any functional significance. By comparing the black-and-white and color-enhanced images, one can get a sense of this within our data. However, as the goal of this study is to demonstrate how each of the opioid receptor subtypes varies in a qualitative sense in its distribution, this is less of a problem here. We have therefore chosen to present both computer enhancements as well as the original autoradiograms to provide a more sensitive analysis of the receptor distributions.

In describing the relative density of a particular receptor subtype, we have elected to use the following terminology in the text and table: very dense (++++), dense (+++), moderate (++), light (+), and undetectable (0). The grading system was determined as follows: Given the 16 tone color scale produced by the Loats system, we referred to those areas shaded in white (having the highest optical density) as "very dense," red tones were referred to as "dense," yellow and green tones as "moderate," blue tones as "light," and black as "undetectable." These terms are not designed to be quantitative in an absolute sense and are only designed to facilitate the description of the receptor distributions. In fact, since the color scales vary between the mu, delta, and kappa distributions, areas that are referred to as very dense for kappa binding may be equivalent in receptor numbers to areas of light mu or delta binding. Careful quantification to determine B_{max} and K_d in each brain region is necessary to give a more accurate estimate of each of the binding sites, but this is beyond the scope of this paper. The anatomical nomenclature is that of Paxinos and Watson (1982), and the

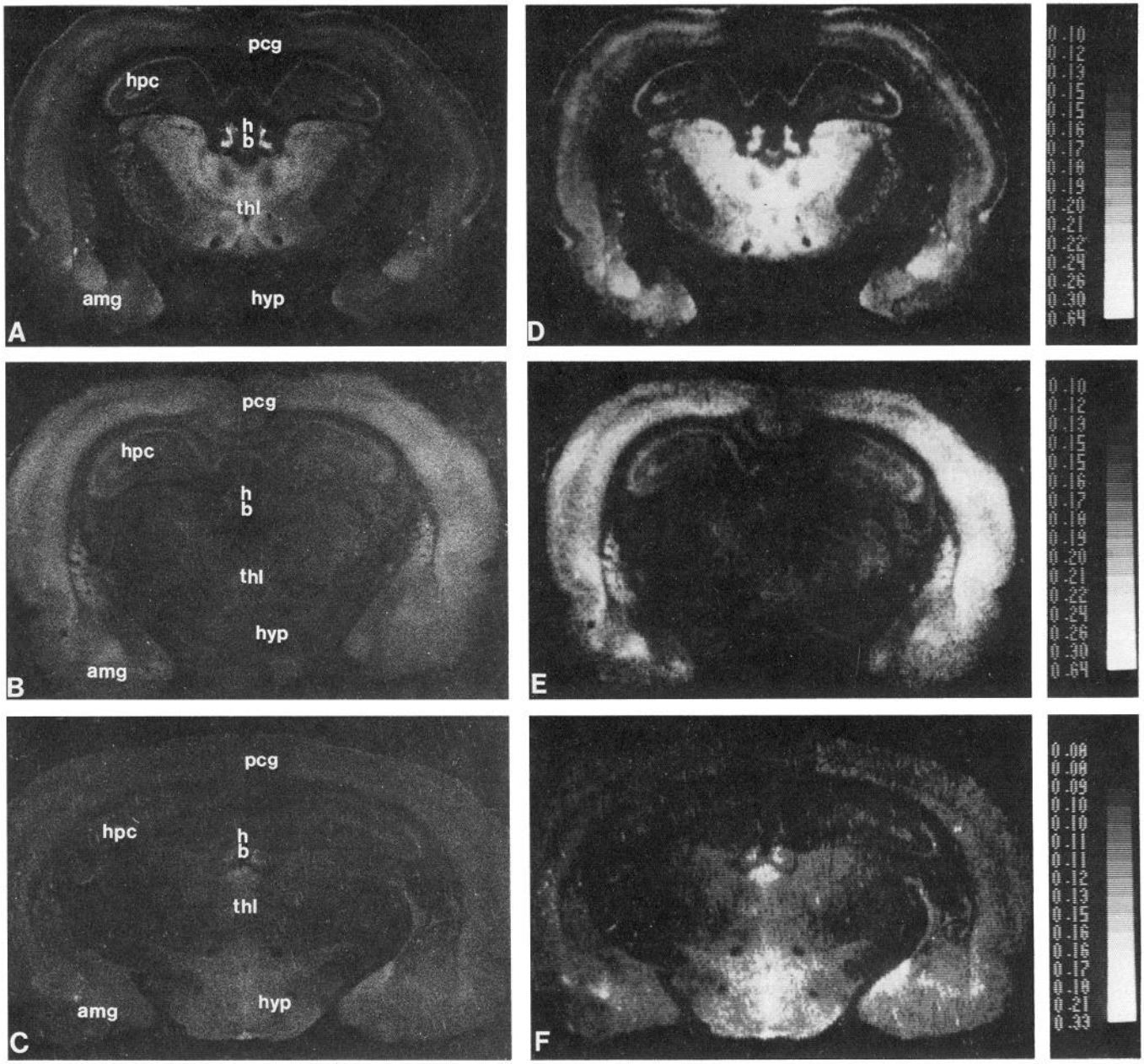


Figure 1. Dark-field autoradiograms produced with LKB film (*left column*) and their corresponding black-and-white computer-enhanced images (*right column*). The distributions of the mu (*A, D*), delta (*B, E*), and kappa (*C, F*) subtypes are compared. The black-and-white color bars to the right are computer-generated and designed to give a measure of relative optical density within an autoradiogram. See Appendix for abbreviations used here and for all other figures.

anatomy was identified either by thioin staining the sections themselves (emulsion-dipped slides) or sections adjacent to those used in the LKB film autoradiograms.

In addition to the computer enhancements, we have included autoradiograms of mu and delta binding in which the sections have been dipped in NTB2 emulsion, instead of being apposed to LKB film to provide higher-resolution images. Since the emulsion-dipped sections are defatted prior to dipping, the problem of differential quenching observed in the nondefatted sections exposed to LKB Ultrofilm is avoided. Six anatomical levels of mu binding using dipped sections are provided in Figure 3 and can be compared to the LKB film images. Further anatomical detail is provided in Figures 4–6, in which particular brain areas of the sections depicted in Figure 3 have been further enlarged. We did not include a similar set of delta autoradiograms because their distribution was more limited and the information they incorporated repeated that provided in the computer-generated images. As indicated earlier, ^{3}H-bremazocine cannot be efficiently fixed with formaldehyde vapors, making it impossible to dip into NTB2 emulsion without substantial loss of the ^{3}H-ligand.

Telencephalon

In the cerebral cortex, mu, delta, and kappa binding sites exhibit striking regional as well as laminar differences in their distributions (Fig. 2, Table 1). In the anterior cingulate cortex (Fig. 2, *A–C*), mu binding is densest in layers I, III, and V. By contrast, delta binding is most pronounced in layer V, while light to

moderate kappa binding is seen throughout. The binding of mu and kappa ligands is uniformly light or undetectable in the posterior cingulate cortex (Fig. 2*D*), with light to moderate delta binding, suggesting that the 2 divisions of cingulate may be functionally distinct. In the frontal parietal cortex (Fig. 2, *B–D*), mu binding is dense in layers I and IV, while delta binding is dense in layers II, III, V, and VI. Kappa binding is less pronounced than either mu or delta binding and is moderate in layers II, III, V, and VI. In the piriform and entorhinal cortex (Fig. 2, *D–H*), some mu, delta, and kappa binding is evident in the superficial layers and deeper layers in almost equal but light to moderate densities. In the temporal cortex (Fig. 2*F*), mu and delta binding is similar to that in the frontal parietal cortex, i.e., dense in layers I and IV (mu binding) or in layers II, III, V, and VI (delta binding). Kappa binding is light to undetectable throughout temporal cortex. In the striate cortex (Fig. 2, *G, H*), the densest concentration of mu binding is seen in layer IV, while the densest delta binding is in layers V and VI. Moderate kappa binding is also seen in layers V and VI. Last, the endopiriform nucleus exhibits dense to very dense binding with all 3 opioid receptor subtypes, with delta and kappa binding being particularly prominent (Fig. 2, *B, C*).

In amygdala, the central nuclei (Fig. 2*D*) exhibit moderate kappa binding and no detectable mu and delta binding. In contrast, the cortical, lateral, and basolateral nuclei exhibit very dense mu binding (Figs. 2, *D–F*; 3, *C* and *D*; and 4*D*), and moderate to dense delta and kappa binding (Fig. 2, *D–F*). Similarly, the medial amygdala shows dense mu binding and moderate delta and kappa binding (Fig. 2*D*). As can also be seen from Figure 4*D*, there are 2 dense islands of mu binding (intercalated nuclei) medial and lateral to the lateral amygdala. These areas of dense binding correspond to dense clusters of cells found in this region and are selectively labeled with mu ligands.

In the hippocampal formation, mu binding is dense in the pyramidal cell layer of areas CA1, CA2, and CA3 (Figs. 2*D*; 3, *C* and *D*; and 4*E*). Delta and kappa binding is moderate to light, respectively, in these same areas (Fig. 2*D*). Of the remaining areas of the hippocampus proper, only stratum lacunosum-moleculare exhibits dense mu binding (Figs. 2*D*, 4*E*) and moderate to light delta and kappa binding. The remaining strata, including stratum moleculare, oriens, and radiatum are uniformly poor in all 3 receptor subtypes. In the dentate gyrus, both molecular and granular cell layers exhibit dense to very dense mu binding, but only in the ventral dentate gyrus. Dorsally, virtually no binding is seen in the dentate gyrus. Compare, for instance, Figure 4, *E* and *F*, where the ventral portion of the dentate gyrus has dense binding (*F*) compared to the poor mu binding in the dorsal portion (*E*). Comparatively, only light delta and kappa binding is seen in the ventral dentate gyrus. The presubiculum also exhibits very dense mu binding, dense delta binding, and moderate kappa binding (Fig. 2*G*).

In the olfactory tubercle, the islands of Calleja exhibit light mu binding (Figs. 2, *B, C*; 3*A*). By contrast, both delta and kappa binding are dense in this region. More dorsally, in the nucleus accumbens (Figs. 2*B*; 3*A*; 5, *A–C*), very dense patches of mu binding can be seen throughout this nucleus. However, the equally dense delta binding is more diffusely distributed throughout this nucleus (Figs. 2*B*; 5, *D–F*). Kappa binding is also dense in this region but is densest in its more ventral aspects (Fig. 2*B*).

In the striatum (Figs. 2, *B, C*; 3*A*; 5, *A–C*), the nuclei caudate putamen exhibit very dense, patchy mu binding, in addition to a distinct subcallosal streak. The equally dense delta binding and somewhat less dense kappa binding of the caudate putamen are diffuse and more pronounced in the ventrolateral (delta) and ventromedial (kappa) aspects of this region, respectively (Figs. 2, *B* and *C*; 5, *D* and *E*). Mu and delta binding also vary rostrocaudally in the caudate putamen. As can be seen from Figure 5, the number of mu patches diminishes in the caudate putamen in a rostrocaudal gradient (left column, *A–C*) and are almost completely absent in the caudal portion of this structure (Fig. 3*B*). Delta binding, on the other hand, becomes denser rostrocaudally in the caudate putamen (Fig. 5, right column, *D–F*). By contrast, only light mu, delta, and kappa binding is seen in the entire globus pallidus (Fig. 2*C*).

In other areas of telencephalon, some distinct receptor subtype distributions are noteworthy. For example, in the septum, moderate delta and kappa binding is seen in the lateral nucleus, while dense mu and light delta and kappa binding is localized in the medial nucleus (Fig. 2*B*). The diagonal band also exhibits moderate mu and delta, and denser kappa binding (Fig. 2*C*). In the subfornical organ, delta, mu, and kappa binding are light, moderate, and dense, respectively (Fig. 2*C*). A striking difference in receptor binding is seen in the bed nucleus of stria terminalis and the stria terminalis itself. Mu and delta binding are dense to very dense in the stria terminalis and only moderate in the bed nucleus. Kappa binding, on the other hand, is dense in the bed nucleus of stria terminalis and undetectable in the stria terminalis. Last, in the preoptic area, mu and delta binding are light, while relatively very dense kappa binding is seen in the medial preoptic area (Fig. 2*C*).

Diencephalon

A large number of diencephalic structures exhibit 1, 2, or all 3 opioid receptor subtypes. Generally, kappa binding is densest in the hypothalamus, while mu binding is densest in the thalamus. By contrast, light delta binding, if any, is seen throughout the diencephalic structures.

In the hypothalamus (Fig. 2, *D, E*), the following nuclei and areas exhibit dense kappa binding: periventricular nucleus, ventromedial nucleus, dorsomedial nucleus, and median eminence. Areas that show moderate kappa binding include anterior hypothalamic area, the magnocellular supraoptic and paraventricular nuclei, arcuate nucleus, lateral hypothalamic area, and medial mammillary nucleus. By contrast, mu binding is dense only in the medial mammillary nucleus, with light binding in the dorsomedial nucleus and lateral hypothalamic area. Light delta binding in hypothalamus is restricted to the ventromedial nucleus only.

A more striking pattern of receptor binding is seen in the thalamus. Numerous nuclei exhibit very dense mu binding (Figs. 2, *D, E*; 3, *C, D*; 4, *A–C*). These include mediodorsal, intermediodorsal, central medial, paracentral, rhomboid, reuniens, ventromedial, lateral posterior, medial geniculate nuclei, and the posterior group. Moderate mu binding is also seen in the laterodorsal, gelatinosus, and ventral posterolateral nuclei. Dense kappa binding in thalamus is restricted to the periventricular nucleus (Fig. 2*D*), an area devoid of mu and delta binding. Other thalamic nuclei containing moderate kappa binding are the mediodorsal, intermediodorsal, central medial, paracentral, rhomboid, and reuniens nuclei, all areas with very dense mu binding. The only other nuclei with light kappa binding are the ventromedial (Fig. 2*D*), and medial geniculate nuclei (Fig. 2, *E, F*).

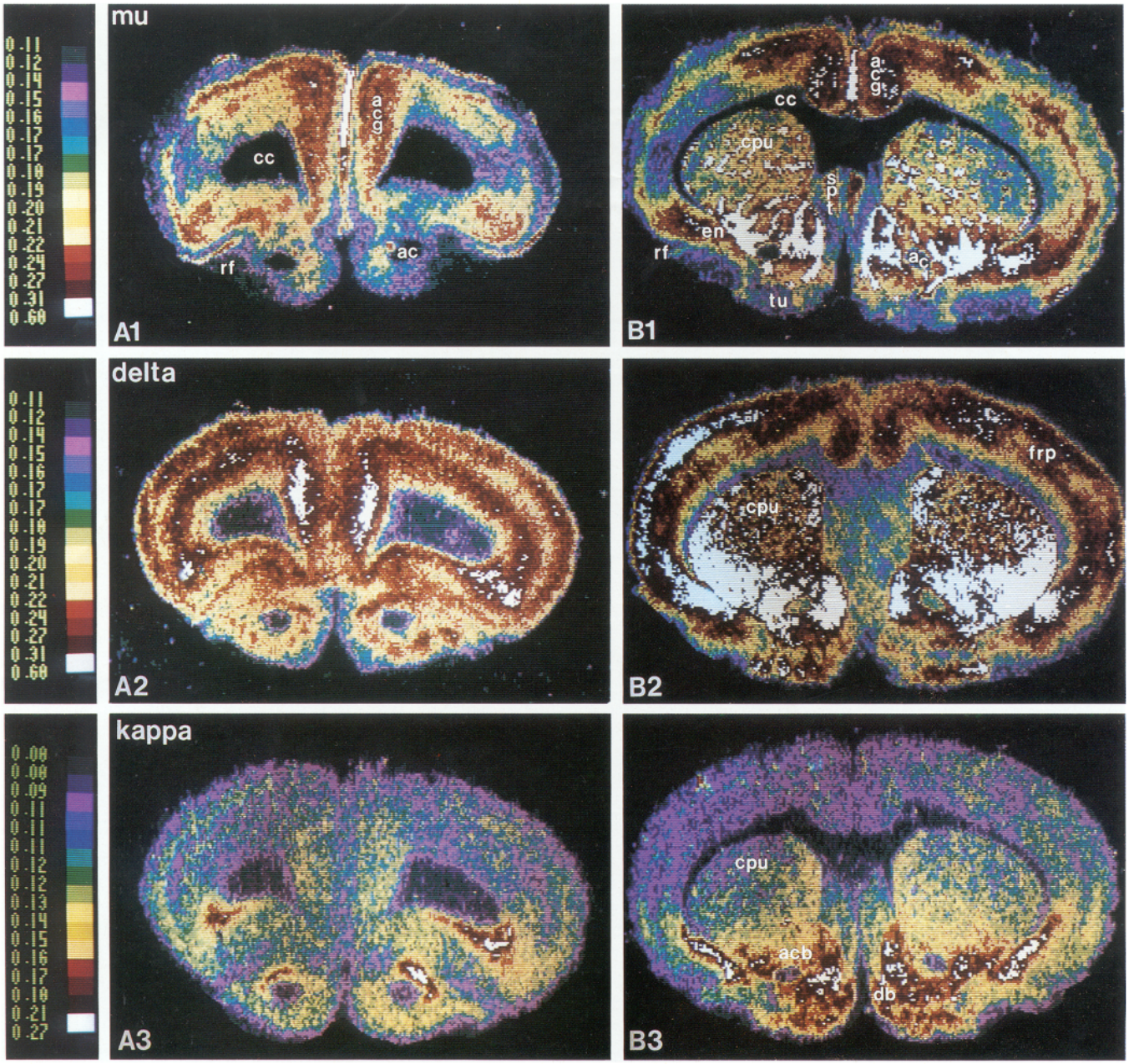


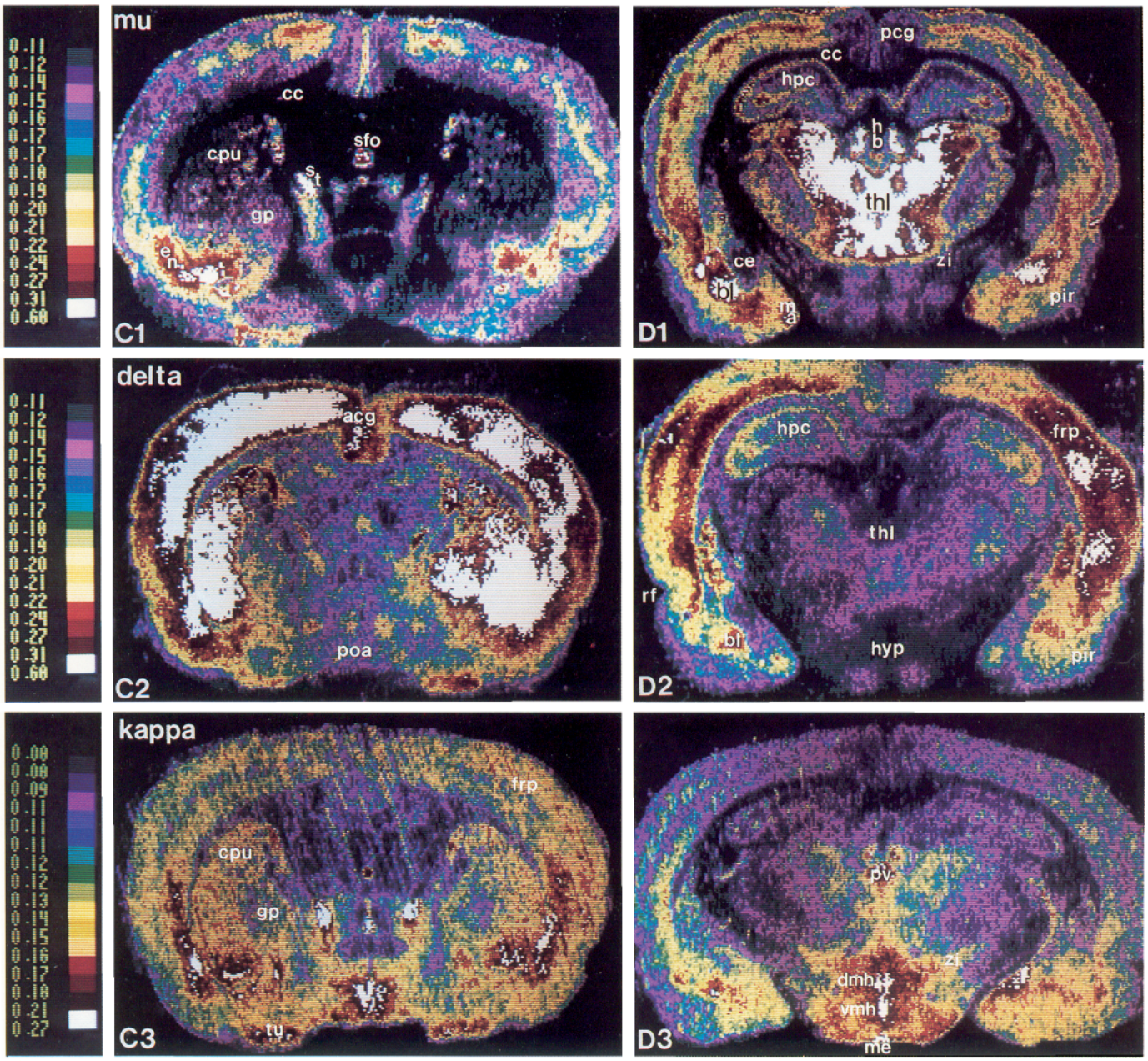
Figure 2. Computer-enhanced color autoradiograms of coronal rat brain section at various fore- and midbrain levels. All the images in the *top row* represent mu (^{3}H-DAGO) binding, while those in the *middle* and *bottom rows* depict delta (^{3}H-DPDPE) and kappa (^{3}H-bremazocine in the presence of 100 nM DAGO and DPDPE) binding, respectively. The color bars to the left are computer-generated and are designed to give a relative measure of optical density within a binding condition. Prior to photographing, all images were shade-corrected for uneven illumination. The ratio

As mentioned earlier, delta binding is very poor in both the hypothalamus and thalamus. In the latter, light delta binding is seen in mediodorsal, intermediodorsal, central medial, paracentral, rhomboid, reuniens, ventromedial, and ventral posterolateral nuclei. Other thalamic and hypothalamic nuclei mentioned that contain mu or kappa binding, contain almost no delta binding. Similarly, in the zona incerta, there is moderate kappa binding and almost no delta or mu binding (Fig. 2*D*).

In habenular nuclei, the medial nucleus contains dense mu and kappa binding but light delta binding. By contrast, the lateral habenular nucleus is devoid of all 3 receptor subtypes (Figs. 2*D*, 3*C*, 4*B*). Interestingly, dense mu binding is seen in the form of patches in the fasciculus retroflexus, which connects the habenula to the interpeduncular nucleus (Figs. 4*C*, 6*A*) and is consistent with the opiatergic pathway described by Herkenham and Pert (1980) using ^{3}H-naloxone.

Mesencephalon

As in the diencephalon, mu and kappa binding predominate in the mesencephalon, with delta binding being restricted to only a few structures. For example, in the interpeduncular nucleus, mu binding is dense to very dense throughout all the subnuclei, such as the central, paramedian, and inner posterior (Figs. 2*G*, 3*F*, 6*B*). Kappa binding is similar to mu binding but less pronounced, i.e., moderate to dense. Delta binding, on the other hand, is dense in the central nucleus but light or nonexistent in



of specific to nonspecific binding was determined using scintillation counting and varied with ligand; DAGO (88%), DPDPE (67%), and bremazocine in the presence of mu and delta blockers (79%). As nonspecific binding tends to be diffuse and homogenous across a brain section, these are fairly good estimates of specific labeling.

the paramedian and inner posterior nuclei, respectively.

In the substantia nigra, dense mu binding is seen in the pars compacta, an area devoid of either delta or kappa binding (Figs. 2*F*, 6*A*). The comparatively little kappa binding observed in the rat substantia nigra is in contradistinction to the findings of Foote and Maurer (1983) in the guinea pig and may represent genuine species differences, an interpretation supported by direct comparative studies (Lewis et al., 1985b). The pars reticulata exhibits moderate mu binding and only light delta and kappa binding. Likewise, mu binding is moderate in the ventral tegmental area (Fig. 6*B*), with kappa binding being only light in this area. Medial to the substantia nigra, a very dense distribution of mu binding is selectively seen in the medial terminal nucleus of the accessory optic tract (Figs. 2*F*, 6*A*).

In the rostral periaqueductal gray, moderate to light mu binding is seen dorsally (Fig. 2, *E*, *F*). The pattern of kappa binding in this region is different than that seen with mu binding, with moderate to light kappa binding seen in the ventral areas. In the caudal periaqueductal gray (Fig. 2, *G*, *H*), kappa receptors are present in most of this tissue at moderate concentrations. While previous reports (e.g., Wood et al., 1981) had suggested that the analgesia induced by kappa drugs is spinally mediated, these data would suggest the potential for a supraspinal kappa-mediated analgesic system (Steinfels and Cook, 1985). No delta binding is distinguishable in any area of the periaqueductal gray.

In the colliculi, distinct regional patterns of receptor binding

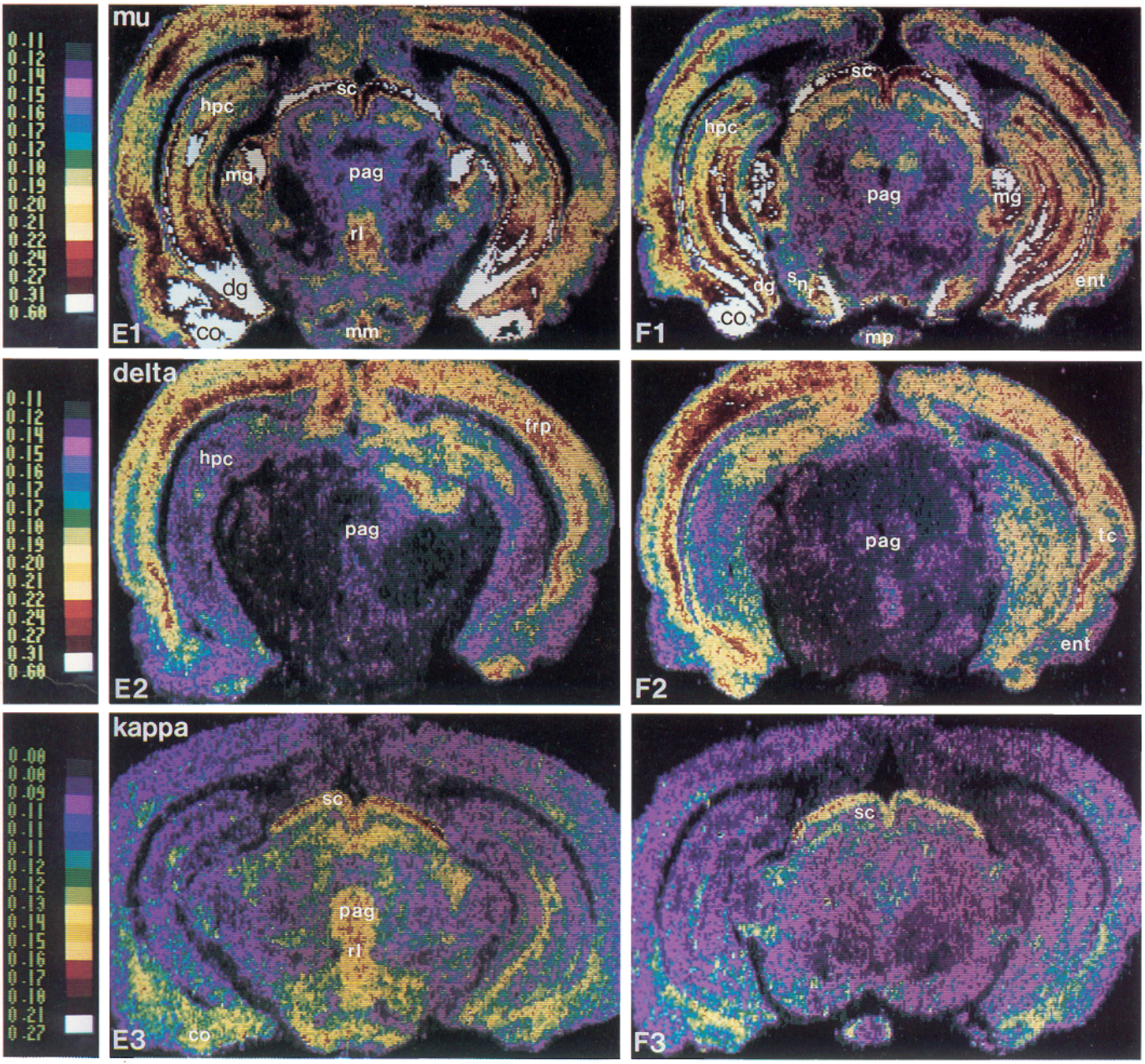


Figure 2. Continued.

are seen again in both the superior and inferior colliculi (Fig. 2, *E–H*). In the superior colliculus, very dense mu binding is seen in the superficial gray layer, while moderate mu binding is seen in the intermediate and deep gray layers (Figs. 3*E*, 6*C*). In the inferior colliculus, very dense mu binding is seen in the central dorsomedial nucleus, with only light binding seen ventrolaterally (Figs. 3*F*, 6*D*). While delta binding is light to undetectable in both the superior and inferior colliculi, moderate kappa binding is seen in the superficial gray layer of the superior colliculus and central dorsomedial nucleus of the inferior colliculus. Elsewhere in the superior colliculus, kappa binding is light, e.g., in the intermediate and deep gray layers.

Last, in midbrain raphe nuclei, dense mu binding is seen in both the caudal linear and median raphe nuclei. Kappa binding is only moderate in the latter nuclei, while no delta binding is seen in either of the raphe nuclei.

As the results of a previous study (Mansour et al., 1986a) suggested that the concentration of ^{3}H-bremazocine was critical in selectively labeling this site, we compared autoradiographic patterns produced with sections labeled with approximately 1 or 5 times the K_d of ^{3}H-bremazocine in the presence of 100 nM DAGO and DPDPE. As a point of reference, the present mapping study was done with a concentration of ^{3}H-bremazocine that is approximately 3 times its K_d. As can be seen from Figure 7, the autoradiographic patterns of ^{3}H-bremazocine change with the higher labeling concentration, demonstrating more mu and delta patterning and suggesting that the 100 nM blocking concentrations may become insufficient to completely prevent interactions with the mu and delta sites. For example, the patches in the caudate putamen and the thalamic nuclei that are labeled with the higher ^{3}H-bremazocine concentration correspond to the mu patterns observed with ^{3}H-DAGO. In contrast, the sections labeled with lower concentration of ^{3}H-bremazocine show the same autoradiographic patterns presented in the color maps.

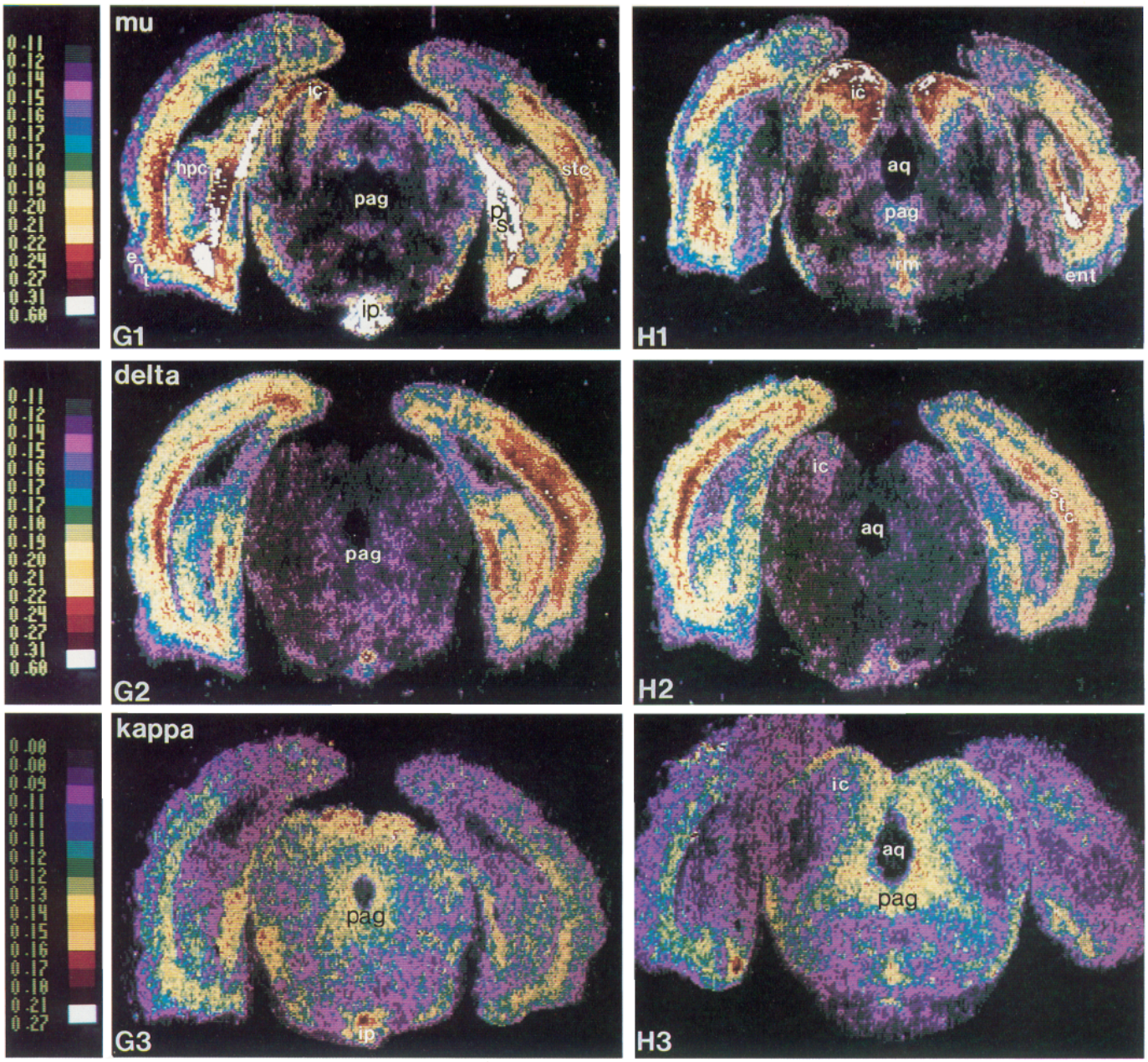


Figure 2. Continued.

Presently, concentrations of 0.5 nM bremazocine (in the presence of 200 nM DAGO and DPDPE) are used to routinely label kappa sites.

Discussion

While previous mapping studies have suggested that the opioid receptor subtypes are uniquely distributed, many have been flawed by insufficiently selective ligands and binding conditions. The present study employs highly selective ligands and conditions, providing an extensive anatomical description of the 3 opioid receptor subtypes in the rat. While there is some evidence to suggest that the 3 receptor subtypes are differentially distributed in the guinea pig (Goodman and Snyder, 1982; Palacios and Maurer, 1984) and human (Maurer et al., 1983) nervous systems, the relative paucity of information available concerning the distribution of opioid receptor subtypes in these and in other animals precludes extensive cross-species comparisons. Further, such comparisons would require the labeling of an equivalent percentage of sites across all species studied, a project that has thus far not been carried out.

Focusing on the rat, the overall distributions of the mu, delta, and kappa sites varied widely in the fore- and midbrain. Mu binding sites were the most widespread and could be observed throughout the tel- and mesencephalon. In contrast, the distribution of delta sites was more restricted: These sites were primarily observed in the neocortex, paleocortex, olfactory tubercle, caudate putamen, and nucleus accumbens. Kappa sites, on the other hand, were observed in an intermediate number of brain areas, including the paleocortex, olfactory tubercle, caudate putamen, nucleus accumbens, hypothalamus, amygdala, and periacqueductal gray.

While earlier studies had suggested that mu and delta receptors were differentially distributed in the neocortex, it is only

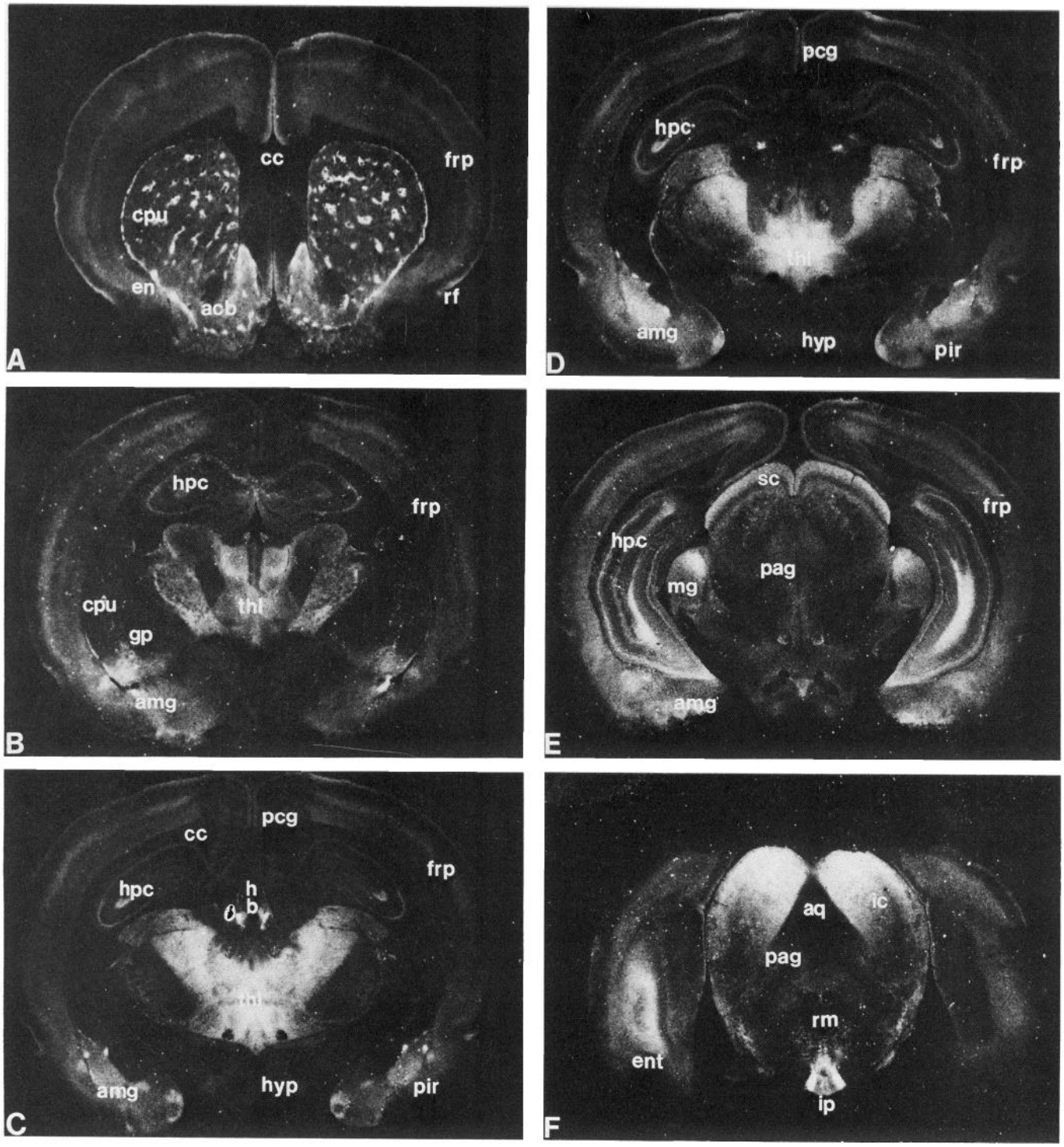


Figure 3. NTB2 film emulsion dark-field autoradiograms of mu binding at 6 anatomical levels (*A–F*).

recently, with the advent of more selective ligands, that the complementary nature of these distributions has become evident. Early reports by Duka et al. (1981) and Lewis et al. (1983) using D-Ala^2-D-Leu^5-enkephalin (DADL), for instance, had suggested that delta receptors are diffusely distributed throughout cortex. However, since DADL can bind to both mu and delta sites (Chang and Cuatrecasas, 1979), a diffuse binding pattern in cortex would result from labeling of receptors in complementary laminae. Studies conducted with more selective binding conditions (Goodman et al., 1980; Quirion et al., 1983b), as well as this study, suggest this interpretation is correct. Similarly, previous reports using such ligands as ^{3}H-dihydromorphine (Goodman et al., 1980), ^{3}H-etorphine (Duka et al., 1981), and ^{3}H-naloxone (Lewis et al., 1983) to label mu sites had suggested dense labeling in layer VI of neocortex. This was generally not observed in the present study with ^{3}H-DAGO, a more selective mu ligand.

In addition to cortical areas, dense opioid receptor labeling

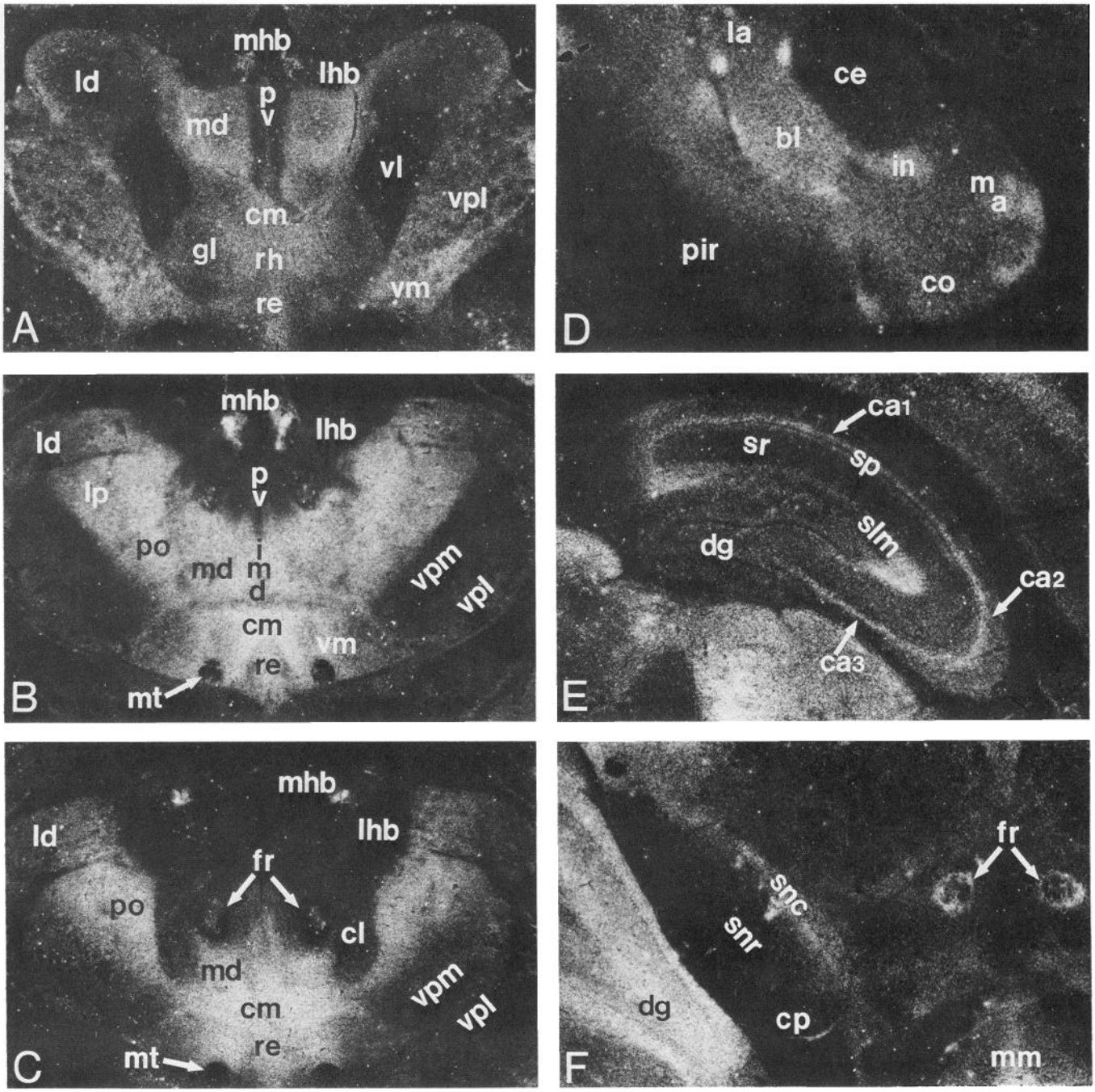


Figure 4. NTB2 film emulsion dark-field autoradiograms. The panels above depict mu binding in the thalamus (*A–C*), amygdala (*D*), hippocampus (*E*), and ventral dentate gyrus and substantia nigra (*F*).

was observed throughout the limbic system. Within the hippocampal formation, for example, the pyramidal cell layer and the striatum lacunosum-moleculare are particularly enriched in opioid receptors. In general, there appears to be a preponderance of mu sites, a moderate number of delta sites, and few kappa sites. The relatively low density of kappa binding observed is discordant with the rich dynorphin immunoreactivity found within the hippocampus (Khachaturian et al., 1985). Similar observations of rich mu labeling in the hippocampus have been made by others (Goodman et al., 1980; Duka et al., 1981; Moskowitz and Goodman, 1984; Lewis et al., 1985a), suggesting that dynorphin may function primarily at mu sites in the rat hippocampus. This suggestion is consistent with membrane homogenate binding studies, which demonstrate that dynorphin effectively labeled mu sites in the rat but not in the guinea pig and monkey (Young et al., 1986). Within the dentate gyrus, marked differences were observed between the ventral and dorsal aspects, with dense to moderate mu and delta binding being observed in the ventral portion of the dentate gyrus, and no detectable opioid binding in the dorsal portion, suggesting separate functional roles.

More rostrally, the caudate putamen and nucleus accumbens show complex opioid receptor anatomy. Mu binding was found predominantly in the subcallosal region and the familiar striatal

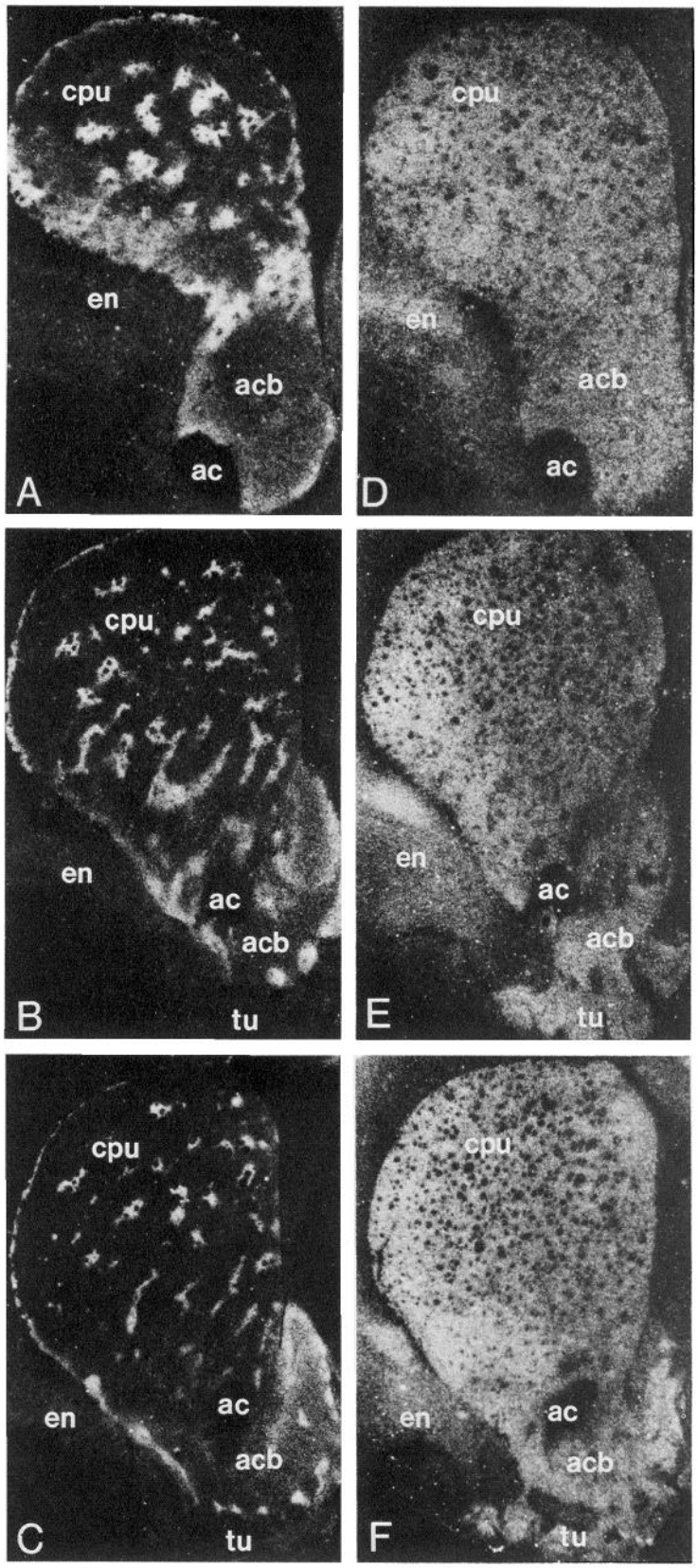


Figure 5. NTB2 film emulsion dark-field autoradiograms of mu (*left column*) and delta (*right column*) binding in the striatum and nucleus accumbens. Sections progress rostrocaudally from the top to the bottom of each column. Notice that the patches in the caudate putamen become more sparse rostrocaudally, while delta binding either remains the same or becomes more dense laterally.

"patches" (Atweh and Kuhar, 1977; Herkenham and Pert, 1981, 1982), which have been a source of controversy in the literature. Quirion et al. (1983a) suggested that the striatal patches have both mu and kappa receptor sites, while our data and those of Lynch et al. (1985) indicate that mu and kappa sites are differentially distributed in the striatum. Moreover, the diffuse kappa pattern observed here in the rat is similar to the diffuse kappa binding pattern observed in the caudate putamen of the guinea

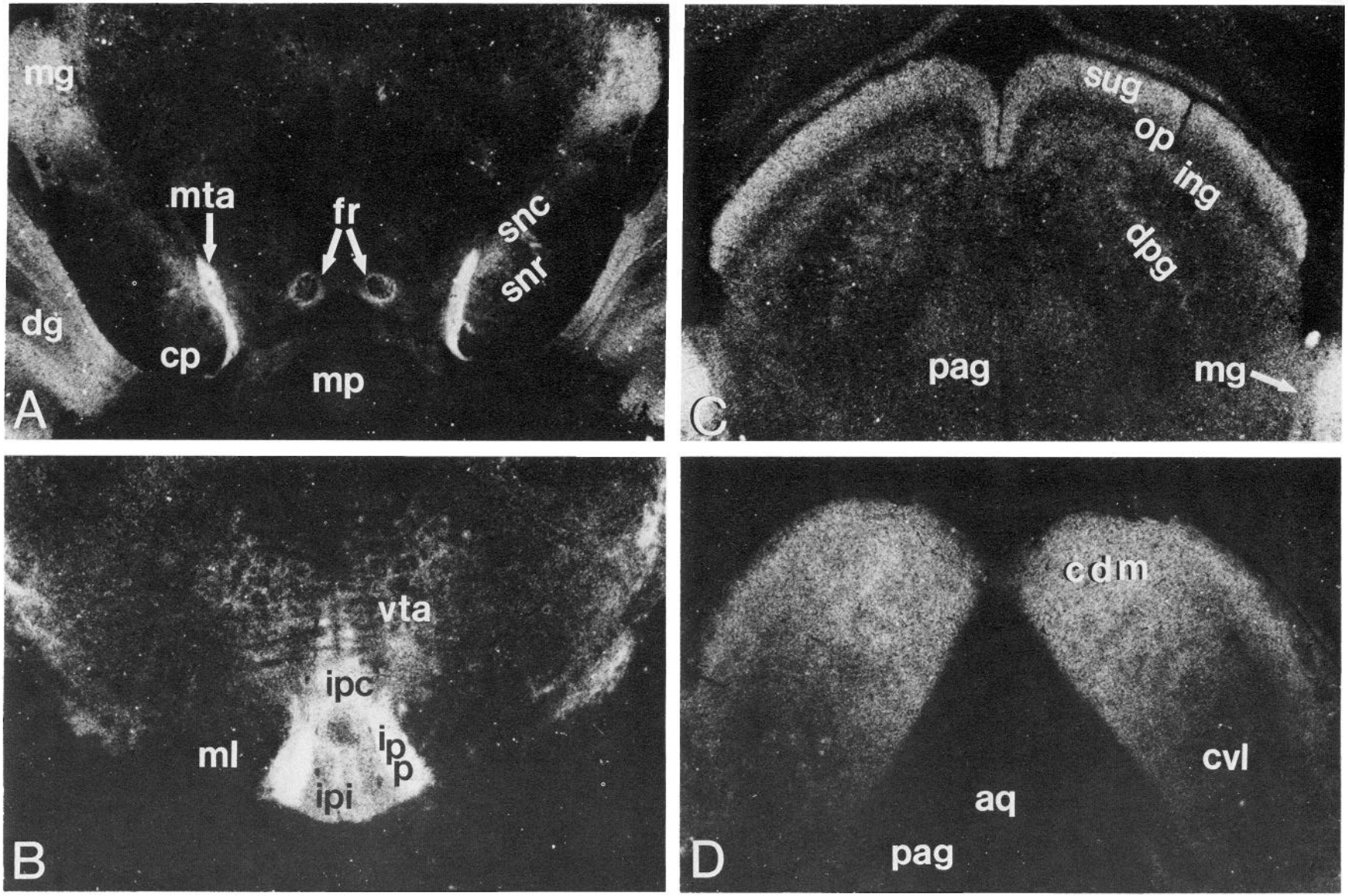


Figure 6. NTB2 film emulsion dark-field autoradiograms of mu binding in midbrain. *A,* Mu binding in the substantia nigra. *B,* Subdivisions of the interpeduncular nucleus and ventral tegmental area. The superior and inferior colliculi are displayed in *C* and *D,* respectively.

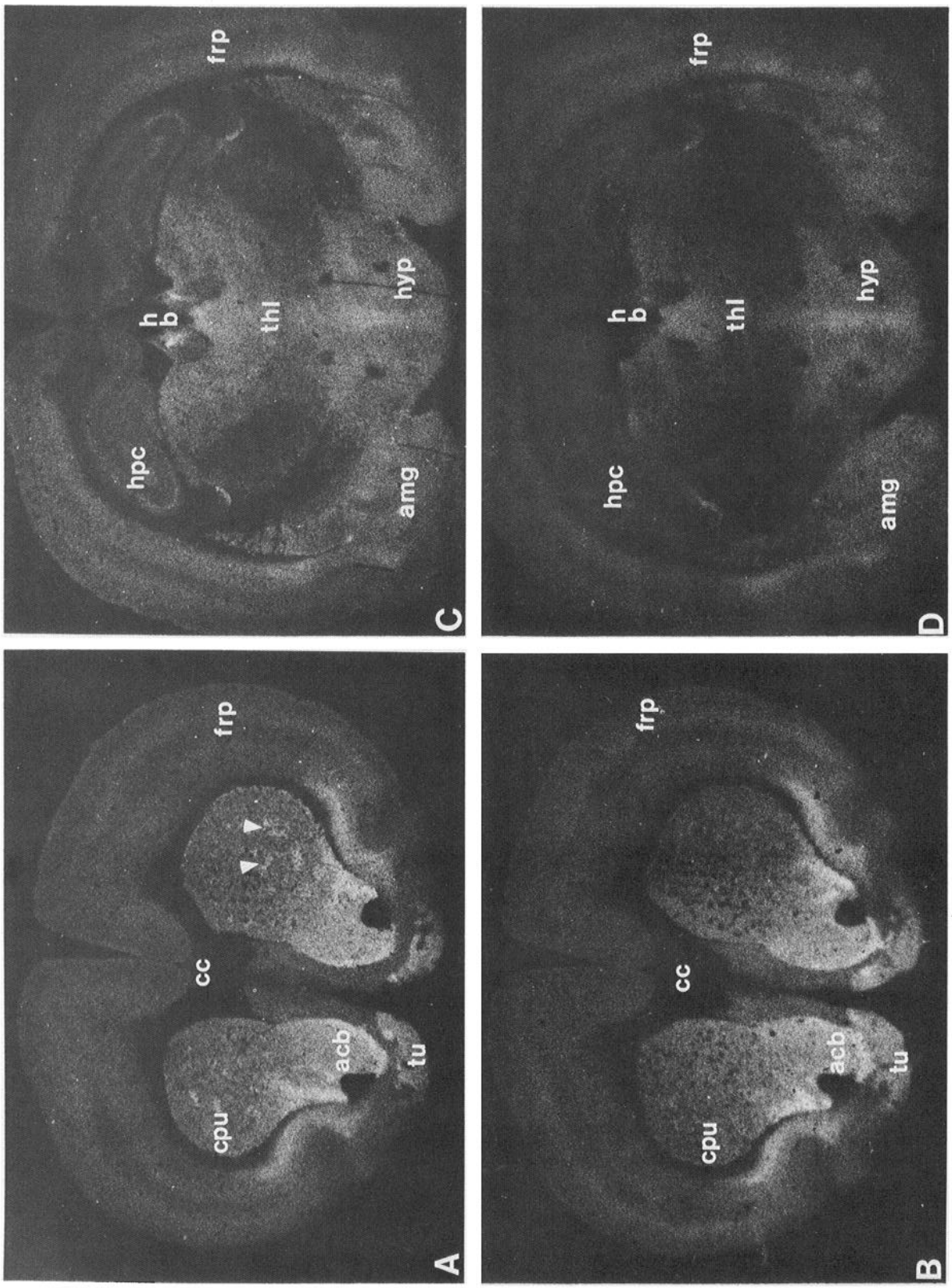


Figure 7. Dark-field autoradiograms produced with 2 concentrations of ^{3}H-bremazocine. Sections in *B* and *D* were incubated with 0.36 nM ^{3}H-bremazocine, while those in *A* and *C* were labeled with 1.6 nM. All sections were coincubated with a 100 nM DAGO and DPDPE. Notice that the higher ^{3}H-bremazocine concentration labels brain areas similar to those seen with ^{3}H-DAGO and ^{3}H-DPDPE. For example, patches are observed in the caudate putamen (*A*) and a wider range of thalamic nuclei are labeled (*C*).

pig (Quirion et al., 1983a). The discrepancy between our results and those of Quirion et al. (1983a) point to the difficulty in selectively labeling kappa sites in the rat.

The converse situation is observed in the hypothalamus. Goodman et al. (1980) suggested that the hypothalamus has primarily mu sites, while Duka et al. (1981) suggested that it contains both mu and delta sites, and others have reported (Herkenham and Pert, 1980; Quirion et al., 1983b) negligible levels of either mu or delta sites. Our findings of relatively poor mu and delta binding and relatively rich kappa binding would suggest that the earlier studies may have inadvertently labeled kappa sites in addition to the possible mu and delta sites present. If this is the case, then both the selectivity of a ligand and the relative proportions of opioid receptor subtypes present are critical factors in selectively labeling a subpopulation of sites. Thus, a relatively nonselective ligand may appear to be selective if the tissue is enriched with that site but may label other sites if its primary receptor is not present or present in relatively low amounts. Both these factors have come into play, for example, in the labeling of the opioid receptors in the neural lobe of the pituitary. Early studies with ^{3}H-naloxone (Simantov and Snyder, 1977; Lightman et al., 1983), carried out under conditions that were reported to be mu selective, reported mu sites in the neural lobe. More recent studies with ^{3}H-DAGO and ^{3}H-bremazocine (Bunn et al., 1985; Mansour et al., 1986b) suggest that these same sites are actually kappa sites. So while ^{3}H-naloxone may appear to be selective when used in the presence of an abundance of mu sites, it will label other opioid sites if they predominate. Such nonselectivity can be quantitatively shown by the marked shift in IC_{50} of a nonselective ligand across different neural regions that contain varying proportions of opioid receptor subtypes (Hewlett, 1982). The advent of selective ligands, such as DAGO and DPDPE, has substantially reduced this problem and has allowed the labeling of select subpopulations of mu and delta receptor sites.

Selective receptor labeling is still difficult for the kappa subtype. Since a selective radiolabeled kappa ligand is not commercially available, investigators have had to use 1 of 3 procedures to study kappa receptors. One procedure involves the application of mathematical modeling to determine the number of sites labeled and their relative proportions. While this may be suitable for homogenate binding, it is presently impractical for receptor autoradiography. A second procedure that some investigators have used has been to suppress binding at the mu and delta sites by initially selectively eliminating these sites, by alkylation, for example, prior to incubating with the kappa radioligand (McLean et al., 1985). The difficulty here lies in the necessity of destroying all the mu and delta sites without harming the kappa sites. This problem is exacerbated by the comparatively low number of kappa sites found in the rat, so that the remainder of even a small percentage of mu and delta sites will distort the autoradiograms. A third procedure currently used in labeling kappa receptors has been the concurrent incubation of saturating concentrations of mu and delta agonists with the radiolabeled kappa ligand. This procedure suffers from the same problem described above, namely, determining what concentrations of mu and delta ligands to use to saturate these sites without affecting the kappa sites. Some studies have employed a constant final concentration of mu and delta agonists to saturate these sites. However, we have observed (Mansour et al., 1986a) that when this is done with the rat brain over a wide range of ^{3}H-bremazocine concentrations, computer analysis of the saturation data reveals a high-affinity site (approx. 0.2–0.3 nM) and a low-affinity one (20–40 nM). The low-affinity site is selectively eliminated, however, if a constant ratio of ^{3}H-bremazocine and mu and delta agonists are maintained across all the tritiated ligand concentrations (0.1–6.2 nM). These results indicate that the low-affinity binding resulted from the interaction of ^{3}H-bremazocine with mu and delta sites, despite the coincubation with 100 nM DAGO and DPDPE, concentrations conventionally thought to saturate the mu and delta sites. The concentrations of ^{3}H-bremazocine and the blocking mu and delta ligands used in this study were selected to label the high-affinity site primarily. Higher concentrations of ^{3}H-bremazocine in the presence of 100 nM DAGO and DPDPE will label mu and delta sites. This is clearly evident by comparing autoradiograms produced with a concentration approximately 5–6 times the K_d of bremazocine at the high-affinity site (1.6 nM) to those produced with a concentration that is 1–2 times its K_d. The autoradiograms produced with the lower concentration of ^{3}H-bremazocine appear to be similar to those seen in the mapping study, while those produced with the higher concentration of ^{3}H-bremazocine appear to label kappa and mu sites. Several reports in the literature have used these relatively high concentrations of labeled kappa agonists to map the kappa sites and may not have used a sufficient concentration of mu and delta blockers (Maurer et al., 1983; Quirion et al., 1983a).

While we have sought in this study to maximize the selectivity of kappa labeling, the possibility exists that we also labeled some mu and delta sites with the ^{3}H-bremazocine. Based on the K_d's of DPDPE and DAGO, a 100 nM concentration of these drugs would occupy at most approximately 91–93% of the available delta sites and 98% of the mu sites. ^{3}H-Bremazocine would, therefore, label the kappa sites and the remaining mu and delta sites. While this does not appear to be a major problem in the present study, since the three distributions appear to be distinct from one another, ^{3}H-bremazocine-labeled brain areas with overlapping sites should be viewed with caution as the labeled sites may not represent solely kappa receptors. Higher concentrations of DAGO and DPDPE were not used in order not to lose any possible kappa sites. Selective kappa radioligands need to become available to evaluate this issue properly.

One of the primary aims in anatomically differentiating the opioid receptor subtypes is to get some insight into the functional significance of these receptors. While the scope of this paper does not permit a detailed discussion of all possible functional roles of the receptor subtypes, we will discuss some of the possible functions of these sites. One function that appears likely is that of sensory integration (Lewis et al., 1981). Consistent with this, mu binding was observed in a wide number of neural areas involved in the sensory processing of visual (superior colliculus, lateral geniculate, and accessory optic tract), auditory (inferior colliculus and medial geniculate), olfactory (olfactory bulb and amygdala), and nociceptive (thalamus, periaqueductal gray, raphe, and substantia gelatinosa of the spinal cord) information. Kappa receptors, while differentially distributed, were observed in the same neural systems, suggesting that these sites may also be important for sensory processing. Delta receptors were more limited in their distribution and were found in sensory areas involved in olfaction, including olfactory bulb, olfactory tubercle, and amygdala. The dense delta binding observed in cortex and caudate putamen suggests that these sites

may be important in sensorimotor integration. The dense mu binding observed in the complementary layers of cortex, the nigrostriatal system, and thalamus similarly suggests that these receptors may be involved not only in the primary perception of stimuli, but also in their sensory and motor integration.

Perhaps the most striking observation that was made in this study was the relative abundance of kappa sites in the hypothalamus and median eminence. These findings, in addition to those demonstrating kappa receptors in the neural lobe of the pituitary (Bunn et al., 1985; Mansour et al., 1986b), suggest that kappa receptors may play a major role in neuroendocrine regulation. We should emphasize that the hypothalamus does not have exclusively kappa sites, but of the areas that were labeled with ^{3}H-bremazocine in the presence of mu and delta blockers, the hypothalamus was one of the structures most densely labeled. The localization of these sites and the opioid precursors in the hypothalamus (Khachaturian et al., 1985) provides an anatomical framework for understanding the effects of endogenous opioids, as well as opiate drugs on the regulation of the hypothalamic–pituitary axis.

In addition to their neuroendocrine role, kappa receptors may also be integral in homeostatic behaviors. Several behavioral studies have suggested that kappa receptors may be important in the regulation of eating and drinking (Leander and Hynes, 1983; Morley et al., 1983; Lynch et al., 1985), and their localization within gustatory-related neural areas certainly supports this contention. The colocalization of dynorphin with vasopressin (Watson et al., 1982) and the diuretic effects of kappa drugs (Slizgi et al., 1984) are consistent with a prominent homeostatic function.

In addition to the functional implications of our findings, the differential distribution of the opioid receptor subtypes is important in further understanding the nature of these sites. Previous reports (Quirion et al., 1981; Bowen and Pert, 1982; Rothman and Pert, 1984) suggesting a conformationally labile site (type I) able to bind mu, delta, or kappa agonists were based on the use of nonselective ligands such as D-Ala^2-D-Leu^5-enkephalin. It is conceivable that the binding conditions differentially alter the affinity of these nonselective drugs such that they are able to bind to the same opioid receptor without having to postulate a site that is conformationally labile. One line of evidence in support of a conformationally labile type I receptor has come from the labeling of striatal patches by mu, delta, and kappa ligands. As can be seen from the findings presented here, the striatal patches were labeled by ^{3}H-DAGO, a mu agonist. ^{3}H-DPDPE failed to label these sites even at high nanomolar concentrations, and ^{3}H-bremazocine showed primarily a diffuse distribution in the caudate putamen. In order to demonstrate the existence of conformationally labile type I receptors, the early studies supporting this interpretation need to be repeated with the selective labeling conditions now available.

In agreement with the findings of protection experiments (Robson and Kosterlitz, 1979; Smith and Simon, 1980; Goldstein and James, 1984; James and Goldstein, 1984) our findings suggest the existence of 3 distinct opioid receptors that are differentially distributed in the nervous system. Consistent with an abundance of pharmacological and behavioral evidence, the differential localization of these subtypes suggests that they have different functional roles. Further research is needed both in the rat and in other species in order to understand more completely the comparative anatomy of these receptor systems and their evolutionary and functional significance. The present study was a step towards this goal and provides an anatomical framework from which to pursue issues of opioid receptor regulation and peptide colocalization in the CNS and PNS.

Appendix
List of abbreviations used in the figures:

ac, anterior commissure
acb, nucleus accumbens
acg, anterior cingulate cortex
amg, amygdala
aq, cerebral aqueduct
bl, basolateral amygdaloid nucleus
ca 1, field CA1 of Ammon's horn
ca 2, field CA2 of Ammon's horn
ca 3, field CA3 of Ammon's horn
cc, corpus callosum
cdm, central dorsomedial nucleus, inferior colliculus
ce, central amygdaloid nucleus
cl, centrolateral thalamic nucleus
cm, central medial thalamic nucleus
co, cortical amygdaloid nucleus
cp, cerebral peduncle
cpu, caudate putamen
cvl, central ventrolateral nucleus, inferior colliculus
db, diagonal band
dg, dentate gyrus
dmh, dorsomedial hypothalamic nucleus
dpg, deep gray layer, superior colliculus
en, endopiriform nucleus
ent, entorhinal cortex
fr, fasciculus retroflexus
frp, frontoparietal cortex
gl, gelatinosus thalamic nucleus
gp, globus pallidus
hb, habenula
hpc, hippocampal formation
hyp, hypothalamus
ic, inferior colliculus
imd, intermediodorsal thalamic nucleus
ing, intermediate gray layer, superior colliculus
ip, interpeduncular nucleus
ipc, interpeduncular nucleus, central
ipi, interpeduncular nucleus, inner posterior subnucleus
ipp, interpeduncular nucleus, paramedian
la, lateral amygdaloid nucleus
ld, laterodorsal thalamic nucleus
lhb, lateral habenular nucleus
lp, lateral posterior thalamic nucleus
ma, medial amygdaloid nucleus
md, mediodorsal thalamic nucleus
me, median eminence
mg, medial geniculate nucleus
mhb, medial habenular nucleus
ml, medial leminscus
mm, medial mammillary nucleus
mp, mammillary peduncle
mt, mammillothalamic tract
mta, medial terminal nucleus, accessory optic tract
op, optic nerve layer, superior colliculus
pag, periaqueductal gray
pcg, posterior cingulate cortex
pir, piriform cortex

po, posterior thalamic nuclear group
poa, preoptic area
ps, presubiculum
pr, periventricular thalamic nucleus
re, reuniens thalamic nucleus
rf, rhinal fissure
rh, rhomboid thalamic nucleus
rl, nucleus raphe linearis
sc, superior colliculus
sfo, subfornical organ
slm, stratum lacunosum-moleculare, hippocampus
snc, substantia nigra, pars compacta
snr, substantia nigra, pars reticulata
sp, stratum pyramidal, hippocampus
spt, septal nuclei
sr, stratum radiatum, hippocampus
st, stria terminalis
stc, striate cortex
sug, superficial gray layer, superior colliculus
tc, temporal cortex
thl, thalamus
tu, olfactory tubercle
vl, ventrolateral thalamic nucleus
vm, ventromedial thalamic nucleus
vmh, ventromedial hypothalamic nucleus
vpl, ventral posterolateral thalamic nucleus
vpm, ventral posteromedial thalamic nucleus
vta, ventral tegmental area
zi, zona incerta.

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