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Mu, Delta, and Kappa Opioid Receptor mRNA Expression in the Rat CNS: An In Situ Hybridization Study

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ABSTRACT

The μ, δ, and κ opioid receptors are the three main types of opioid receptors found in the central nervous system (CNS) and periphery. These receptors and the peptides with which they interact are important in a number of physiological functions, including analgesia, respiration, and hormonal regulation. This study examines the expression of μ, δ, and κ receptor mRNAs in the rat brain and spinal cord using in situ hybridization techniques. Tissue sections were hybridized with ^{35}S-labeled cRNA probes to the rat μ (744–1,064 b), δ (304–1,287 b), and κ (1,351–2,124 b) receptors. Each mRNA demonstrates a distinct anatomical distribution that corresponds well to known receptor binding distributions. Cells expressing μ receptor mRNA are localized in such regions as the olfactory bulb, caudate-putamen, nucleus accumbens, lateral and medial septum, diagonal band of Broca, bed nucleus of the stria terminalis, most thalamic nuclei, hippocampus, amygdala, medial preoptic area, superior and inferior colliculi, central gray, dorsal and median raphe, raphe magnus, locus coeruleus, parabrachial nucleus, pontine and medullary reticular nuclei, nucleus ambiguus, nucleus of the solitary tract, nucleus gracilis and cuneatus, dorsal motor nucleus of vagus, spinal cord, and dorsal root ganglia. Cellular localization of δ receptor mRNA varied from μ or κ, with expression in such regions as the olfactory bulb, allo- and neocortex, caudate-putamen, nucleus accumbens, olfactory tubercle, ventromedial hypothalamus, hippocampus, amygdala, red nucleus, pontine nuclei, reticulotegmental nucleus, motor and spinal trigeminal, linear nucleus of the medulla, lateral reticular nucleus, spinal cord, and dorsal root ganglia. Cells expressing κ receptor mRNA demonstrate a third pattern of expression, with cells localized in regions such as the claustrum, endopiriform nucleus, nucleus accumbens, olfactory tubercle, medial preoptic area, bed nucleus of the stria terminalis, amygdala, most hypothalamic nuclei, median eminence, infundibulum, substantia nigra, ventral tegmental area, raphe nuclei, paratrigeminal and spinal trigeminal, nucleus of the solitary tract, spinal cord, and dorsal root ganglia. These findings are discussed in relation to the physiological functions associated with the opioid receptors.

Key words: endorphins, endorphin receptors, localization, opiates, peptide receptors

Several lines of research have demonstrated that the opioid receptors can be classified into three main types referred to as mu (μ), delta (δ), and kappa (κ; Martin et al., 1976; Lord et al., 1977; Chang and Cuatrecasas, 1979; Smith and Simon, 1980). The μ and κ receptor types were initially differentiated by Martin and colleagues (1976) in the chronic spinal dog preparation and are the sites at which alkaloids, such as morphine, and benzomorphans, such as ethylketazocine, bind to produce their respective physiological effects. The δ receptors, named for the mouse vas deferens preparation from which they were initially described (Lord et al., 1977), differ from the μ and κ binding sites and have a higher affinity for proenkephalin-derived peptides. These early studies suggesting multiple opioid receptor types have been extended by numerous anatomical, pharmacological, biochemical, and behavioral studies (Gillan and Kosterlitz, 1982; Magnun et al., 1982; Wood, 1982; Pasternak et al., 1983; Goldstein and James, 1984; James and Goldstein, 1984; Zukin and Zukin, 1984; McLean et al., 1986; Mansour et al., 1987; Goldstein and Naidu,

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1989; Sharif and Hughes, 1989) demonstrating distinct opioid receptor types in the central nervous system (CNS) and periphery and have led to the cloning of at least three opioid receptor cDNAs that correspond to the pharmacologically defined μ, δ, and κ receptors (Evans et al., 1992; Kieffer et al., 1992; Chen et al., 1993; Fukada et al., 1993; Meng et al., 1993; Thompson et al., 1993; Wang et al., 1993; Yasuda et al., 1993).

The three cloned opioid receptors are members of the seven transmembrane superfamily of G-protein coupled receptors and are homologous to one another at both the amino acid and the nucleic acid levels, particularly in regions spanning the putative transmembrane domains and cytosolic loops. The most divergent regions of these receptors are the N- and C-termini, extracellular loops 2 and 3, and transmembrane 4 (Chen et al., 1993; Fukada et al., 1993; Meng et al., 1993; Thompson et al., 1993; Wang et al., 1993; Yasuda et al., 1993). Northern blot analysis suggests that the opioid receptors are encoded by comparatively large mRNAs that range from 4.5–11 kb for the rat δ receptor (Fukada et al., 1993) to 10–16 kb for the rat μ receptor (Fukada et al., 1993; Thompson et al., 1993; Wang et al., 1993). The mouse κ receptor appears to be encoded by a single mRNA transcript that is 5.2 kb in length (Yasuda et al., 1993). The relatively large size of opioid receptor mRNAs is primarily due to their long 3′-untranslated regions; the protein-coding and 5′-untranslated regions range from 1.2–1.5 kb.

Single genes have been suggested for the μ, δ, and κ receptors (Evans et al., 1992; Thompson et al., 1993; Yasuda et al., 1993). These genes contain multiple introns that may result in the transcription of alternatively spliced mRNA forms of each receptor type. The function of these long 3′-untranslated regions and the multiple mRNA tran-

Abbreviations

3	oculomotor nucleus
3V	third ventricle
10	dorsal motor nucleus of vagus
12	hypoglossal nucleus
ac	anterior commissure
Acb	nucleus accumbens
AHPM	amygdalohippocampal area, posteromedial
AI	agranular insular cortex
Amb	ambiguus nucleus
AOD	anterior olfactory nucleus, dorsal
AOL	anterior olfactory nucleus, lateral
AOM	anterior olfactory nucleus, medial
AOV	anterior olfactory nucleus, ventral
Arc	arcuate hypothalamic nucleus
ATg	anterior tegmental nucleus
BL	basolateral amygdala
BM	basomedial amygdala
CA1	field CA1 of Ammon's horn
CA2	field CA2 of Ammon's horn
CA3	field CA3 of Ammon's horn
cc	central canal
Ce	central amygdala
CG	central gray
Cg	cingulate cortex
Cl	claustrum
CLi	caudal linear raphe
CM	centromedial thalamus
CPu	caudate-putamen
Cu	nucleus cuneatus
DB	diagonal band of Broca
DG	dentate gyrus
DMH	dorsomedial hypothalamus
DPGi	dorsal paragigantocellular nucleus
DpMe	deep mesencephalic nucleus
DR	dorsal raphe
DRG	dorsal root ganglia
ECu	external cuneate nucleus
En	endopiriform nucleus
Ent	entorhinal cortex
Fr	frontal cortex
Gi	gigantocellular reticular nucleus
Gl	glomerular layer olfactory bulb
Gr	nucleus gracilis
HPC	dorsal hippocampus
IC	inferior colliculus
IGr	internal granular layer of olfactory bulb
In	intercalated nuclei, amygdala
InfS	infundibular stem
IPR	interpeduncular nucleus, rostral
IRt	intermediate reticular nucleus
LC	locus coeruleus
LD	laterodorsal thalamus
LH	lateral hypothalamus
Li	linear nucleus, medulla
LL	nucleus of the lateral lemniscus
LO	lateral orbital cortex
LRt	lateral reticular nucleus
LS	lateral septum
Md	medullary reticular nucleus
ME	median eminence
Me	medial amygdala
MG	medial geniculate, thalamus
MH	medial habenula
Mi	mitral cell layer olfactory bulb
Mn	median raphe
MnR	median raphe
MPA	medial preoptic area
MS	medial septum
Oc	occipital cortex
Or	oriens layer, hippocampus
OX	optic chiasm
Pa	paraventricular hypothalamus
Pa5	paratrigeminal nucleus
Par	parietal cortex
PB	parabrachial nucleus
Pir	piriform cortex
PMCo	posteromedial cortical amygdala
Pn	pontine nuclei
PnRt	pontine reticular
PrH	prepositus hypoglossal nucleus
PrS	presubiculum
PS	parastrial nucleus
PV	paraventricular thalamus
Py	pyramidal cell layer, hippocampus
Rad	stratum radiatum, hippocampus
Re	reuniens
RMC	red nucleus, magnocellular
RMg	raphe magnus
ROb	raphe obscurus
RtTg	reticulotegmental nucleus, pons
S	subiculum
SC	superior colliculus
SCh	suprachiasmatic nucleus
SHy	septohypothalamic nucleus
SN	substantia nigra
SNC	substantia nigra, pars compacta
SNR	substantia nigra, pars reticulata
SO	supraoptic nucleus
Sol	nucleus solitary tract
SolC	nucleus solitary tract, commissural
SolR	nucleus solitary tract, rostral
Sp5	spinal trigeminal nucleus
SpVe	spinal vestibular nucleus
Te	temporal cortex
Tu	olfactory tubercle
VMH	ventromedial hypothalamus
VP	ventral pallidum
VPL	ventral posterolateral thalamic nucleus
VTA	ventral tegmental area
ZI	zona incerta

scripts that are possible from a single receptor gene are presently unknown.

The focus of this study is to compare the anatomical localization of the μ, δ, and κ receptor mRNAs in the rat CNS using in situ hybridization techniques. Previous studies examining the distribution of μ and κ receptor mRNAs in the rat (Mansour et al., 1994a,b) and δ receptor mRNA in the mouse (Mansour et al., 1993) have compared opioid receptor mRNA and receptor binding. The emphasis here is comparison of all three receptor mRNAs simultaneously in the rat CNS to gain further insights into their specific receptor distributions and their diverse functions. This analysis also provides the first anatomical description of δ receptor mRNA expression in the rat CNS.

MATERIALS AND METHODS

Tissue preparation

Adult Sprague-Dawley rats (Charles River; n = 3, 250–300 g) were humanely killed by decapitation, and their brains were removed, frozen in liquid isopentane (−30°C) for 30 seconds, and transferred to dry ice. In addition to whole rat brains, a thoracic portion (1.5 cm) of spinal cord with attached dorsal roots and dorsal root ganglia was dissected and similarly frozen. Brain and spinal cord tissue were stored at −80°C until sectioning on a Bright cryostat (15 μm). The sections were thaw mounted on polylysine-subbed microscope slides and stored at −80°C.

In situ hybridization

Adjacent series of frozen brain and spinal cord sections were removed from storage at −80°C and placed into 4% formaldehyde for 60 minutes (22°C) prior to processing for in situ hybridization (Mansour et al., 1993). Following three 5 minute rinses in 2× SSC (300 mM sodium chloride, 30 mM sodium citrate, pH 7.2), sections were treated with proteinase K (1 μg/ml in 100 mM Tris, pH 8.0, 50 mM EDTA) for 10 minutes at 37°C. Slides were then rinsed in water followed by 0.1 M triethanolamine, pH 8.0, and treated with a mixture of 0.1 M triethanolamine, pH 8.0, and acetic anhydride (400:1, vol/vol) for 10 minutes. The sections were rinsed again in water, dehydrated through graded alcohols, and allowed to air dry.

Brain and spinal cord sections were hybridized with ^{35}S-UTP-and ^{35}S-CTP-labeled riboprobes generated either to the rat μ, δ, or κ receptors. A polymerase chain reaction (PCR) fragment that corresponded to the transmembrane III–VI (744–1,064 b; Thompson et al., 1993) of the μ receptor was used to prepare a cRNA probe. A cDNA fragment spanning from extracellular loop 1 to transmembrane domain VII of the rat δ receptor (304-1287 b; Fukuda et al., 1993) and a fragment corresponding to 45 bp of the terminal coding region and the 3′-untranslated region of the rat κ receptor (1,351–2,124 b; Meng et al., 1993) were similarly cloned in Bluescript transcriptional vectors and used in preparing cRNA probes to the δ and κ receptors, respectively. The cRNA probes were diluted in hybridization buffer (75% formamide, 10% dextran sulfate, 3× SSC, 50 mM Na_2PO_4, pH 7.4, 1× Denhardt's, 0.1 mg/ml yeast tRNA, 10 mM dithiothreitol) to result in a final concentration of 1–2 × 10^6 cpm/50 μl. Volumes of 50 μl of diluted probe were applied to coronal brain and spinal cord sections. Glass coverslips were placed over sections to keep hybridization buffer in contact with tissue. The slides were then transferred to sealed humidifying chambers containing 50% formamide and hybridized overnight at 55°C.

The next day, the slides were rinsed in 2× SSC (5 minutes) and treated with RNase A (200 μg/ml in 100 mM Tris, pH 8.0, and 0.5 M NaCl) for 60 minutes at 37°C. Subsequently, the sections were rinsed in 2× SSC for 5 minutes (22°C) and 0.1× SSC for 60 minutes (65°C). Following the low-salt wash, sections were rinsed in water, dehydrated through graded alcohols, and air dried. Sections were apposed to Kodak XAR-5 X-ray film for 7 (δ), 10 (μ), or 11 (κ) days and dipped in NTB2 film emulsion. Brain sections used to visualize μ and κ receptor mRNAs were developed following a 35-day exposure to NTB2 emulsion, whereas those used for localizing δ receptor mRNA in the brain were exposed for 24 days. Sections used to visualize the opioid receptor mRNAs in the spinal cord and dorsal root ganglia were developed following an 8 (μ)-, 11 (κ)-, or 15 (δ)-day exposure. Exposure times were chosen to maximize the detection of specific in situ hybridization grains and were empirically determined by the periodic development of test slides. Following development of the NTB2 film emulsion, all slides were washed in running water (30 minutes, 22°C) and Nissl counterstained.

RESULTS

The following in situ hybridization results demonstrate that cells expressing μ, δ, or κ opioid receptor mRNA are differentially distributed in the rat brain and spinal cord. Each mRNA has a distinct anatomical distribution that corresponds well to known receptor binding distributions described with receptor autoradiography. μ Receptor mRNA expression is widespread and localized in such regions as the striatal patches of the caudate-putamen, hippocampus, several thalamic nuclei, amygdala, locus coeruleus, parabrachial nucleus, and the nucleus of the solitary tract. In contrast, cells expressing δ receptor mRNA are found in such regions as the neocortex, pontine nuclei, caudate-putamen and nucleus accumbens, ventromedial hypothalamus, hippocampus, amygdala, and linear nucleus of the medulla. Cells expressing κ receptor mRNA demonstrate a third distribution predominantly localized in midline and ventral structures, including the claustrum, endopiriform nucleus, nucleus accumbens, olfactory tubercle, hypothalamus, median eminence, paraventricular thalamus, substantia nigra, ventral tegmental area, and nucleus of the solitary tract.

The anatomical results are organized as low-magnification darkfield micrographs (Figs. 1–11) illustrating the general distributions of the μ, δ, and κ receptor mRNAs in the rat brain. Figures 12–20 provide high-resolution cellular images of μ, δ, and κ receptor mRNAs in selected brain and spinal cord regions using emulsion-dip autoradiography. Given that cRNA transcript lengths and exposure times vary somewhat with receptor type, the anatomical description provided is qualitative, and any reference to mRNA expression level applies within a particular receptor mRNA distribution, without implying quantitation across receptors. The anatomical nomenclature is that of Paxinos and Watson (1982).

Telencephalon

The distribution of cells expressing μ, δ, and κ receptor mRNAs are easily differentiated within the layers of the olfactory bulb. High levels of μ receptor mRNA expression

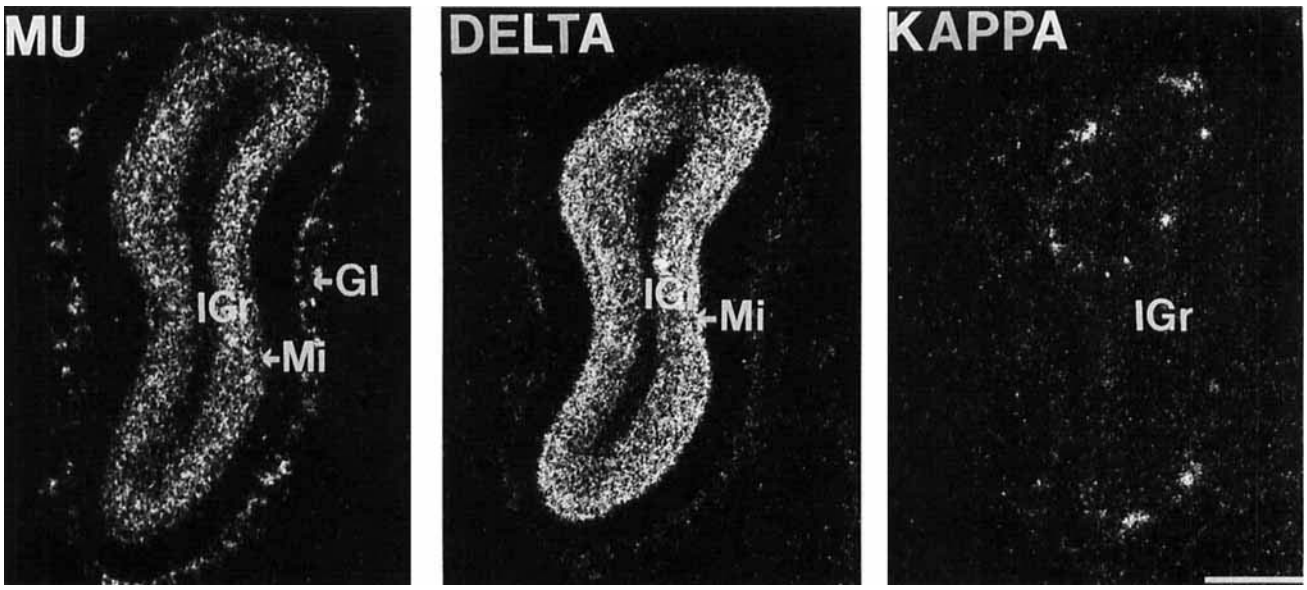

Fig. 1. Darkfield autoradiograms comparing the μ, δ, and κ receptor mRNA distributions in the rat olfactory bulb. Cells expressing μ receptor mRNA are localized in the internal granular layer, mitral cell layer, and glomerular cell layer. High levels of δ receptor mRNA are also localized in the internal granular and mitral cell layers, but none can be detected in the glomerular cell layer. A few cells expressing κ receptor mRNA are scattered in the internal granular cell layer. Scale bar = 1,000 μm.

are observed in the densely packed cells of the internal granular layer, in the cells of the mitral cell layer, and in scattered cells of the glomerular layer (Fig. 1, left). High levels of δ receptor mRNA expression are also observed in the cells of the internal granular and mitral cell layers, but little if any δ receptor mRNA expression is observed in the cells of the glomerular layer (Fig. 1, center). In contrast to the high numbers of cells expressing μ and δ receptor mRNAs in the olfactory bulb, only a few widely scattered cells in the internal granular layer express relatively high levels of κ receptor mRNA, with no cells detected in the mitral or glomerular layers (Fig. 1, right).

In addition to the main olfactory bulb, cells of the accessory olfactory bulb express high levels of μ receptor mRNA, with no detectable δ or κ receptor mRNA expression. Regions such as the bed nucleus of the accessory olfactory tract and posterior mediocortical amygdala that have direct reciprocal projections to the accessory olfactory bulb also display high levels of μ receptor mRNA expression, further emphasizing the importance of μ receptors in the processing of olfactory information.

Cells of the anterior olfactory nucleus express μ, δ, and κ receptor mRNAs; however, their distributions differ with nuclear subdivisions. In the dorsal, ventral, medial, and lateral anterior olfactory nucleus, μ receptor mRNA expression is low, with comparatively few cells observed scattered in these nuclei (Fig. 2, top). More caudally, however, in the external and posterior divisions of the anterior olfactory nucleus, high levels of μ receptor expression are observed. In contrast, δ receptor expression is high in most of the divisions of the anterior olfactory nucleus including the dorsal, medial, lateral, ventral, and posterior divisions (Fig. 2, center). Cells expressing κ receptor mRNA show a high level of expression, predominantly in the dorsal and lateral anterior olfactory nucleus, with comparatively few cells detected in the medial, ventral, and posterior divisions (Fig. 2, bottom).

In neocortical and allocortical regions, cells expressing δ receptor mRNA are particularly prominent, with high levels of expression in the agranular insular, lateral orbital, prefrontal, cingulate, piriform, entorhinal, frontal, parietal, occipital, and temporal cortices. Generally, cells expressing δ receptor mRNA show a bilaminar distribution in neocortical areas, with highest levels of expression in layers 2, 3 and 5, 6 (Figs. 2–8, center). A third peak of δ receptor mRNA expression is seen in a thin layer of cells located in the deepest portion of layer 6 of most neocortical areas. Allocortical areas also show specific laminar expression patterns with high levels of expression, for example, in the piriform cortex localized in the densely packed cellular layer. Similarly, in the prefrontal cortex, δ receptor expression is highest in layers 2 and 5 and the superficial portion of layer 6.

In contrast, cells expressing μ receptors show comparatively low levels of expression in most neocortical and allocortical areas. Within the frontal, parietal, occipital, and temporal cortices, cells expressing μ receptors are scattered in layers 2 and 3, with a moderate level of expression in cells found in the deepest portion of layer 6 immediately adjacent to the corpus callosum. Differences in laminar distributions are also observed in allocortical regions and often demonstrate a complementary pattern of expression compared to cells expressing δ receptor mRNA. For example, in the piriform cortex, cells expressing μ receptors are localized in a layer of cells superficial to those expressing δ. Similarly, in the prefrontal cortex, cells expressing μ receptor mRNA are localized in a thin column of cells in layer 2 and in cells deeper to those expressing δ in layer 6. Other allocortical areas expressing μ receptor mRNA include the agranular insular and lateral orbital cortex, where cells have a scattered distribution and expression levels are low (Fig. 2, top).

Cells expressing κ receptor mRNA show yet a third pattern of expression in the neo- and allocortex. High levels of κ receptor mRNA expression are observed in the lateral

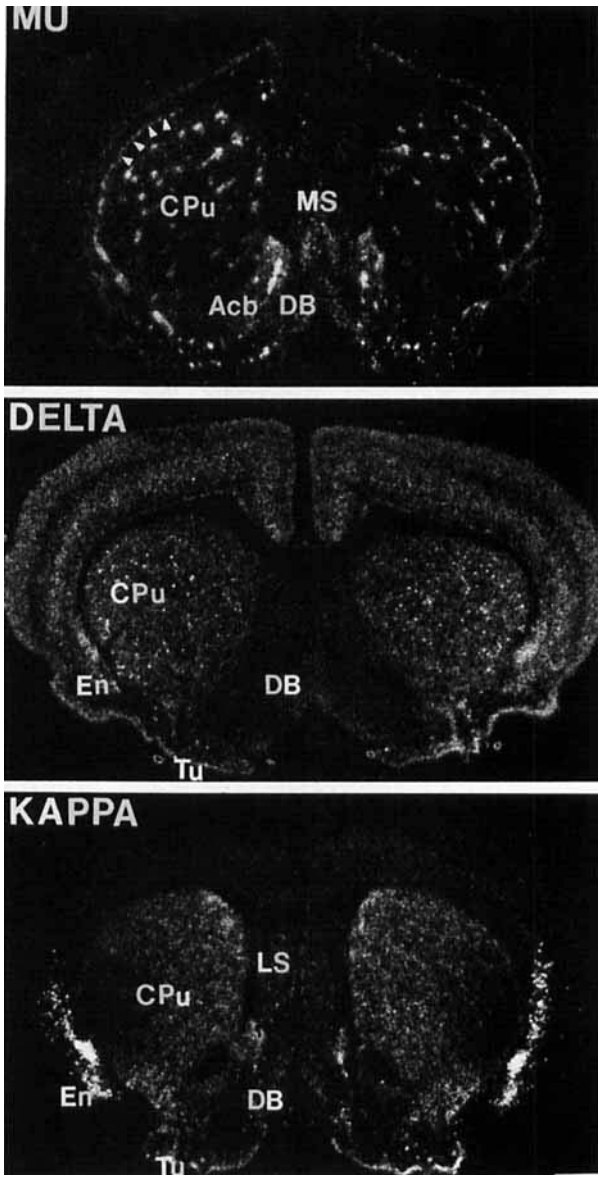

Fig. 2. Darkfield autoradiograms comparing μ, δ, and κ receptor mRNA expression in the rostral cortex and the anterior olfactory nucleus. Cells expressing δ receptor mRNA are localized in the piriform, cingulate, frontal, agranular insular, and lateral orbital cortices as well as the medial, dorsal, ventral, and lateral divisions of the anterior olfactory nucleus. Cells expressing κ receptor mRNA have a more restricted distribution at this level and are localized predominantly in the lateral orbital and agranular insular cortices and in the dorsal and lateral divisions of the anterior olfactory nucleus. Scattered cells expressing μ receptor mRNA are observed in frontal cortex and in the dorsal, ventral, medial, and lateral divisions of the anterior olfactory nucleus. Low levels of μ receptor mRNA expression are also detected in the piriform and cingulate cortices. Scale bar = 1,000 μm.

orbital cortex, with cells fanning out into the agranular insular cortex (Fig. 2, bottom). In contrast to μ and δ receptor distributions, no κ receptor mRNA can be detected in the piriform and prefrontal cortices, but high levels are seen in the entorhinal cortex (Figs. 2, 8, bottom). Within neocortical regions, cells expressing κ receptor mRNA are localized in layers 5 and 6 of the parietal, temporal, and

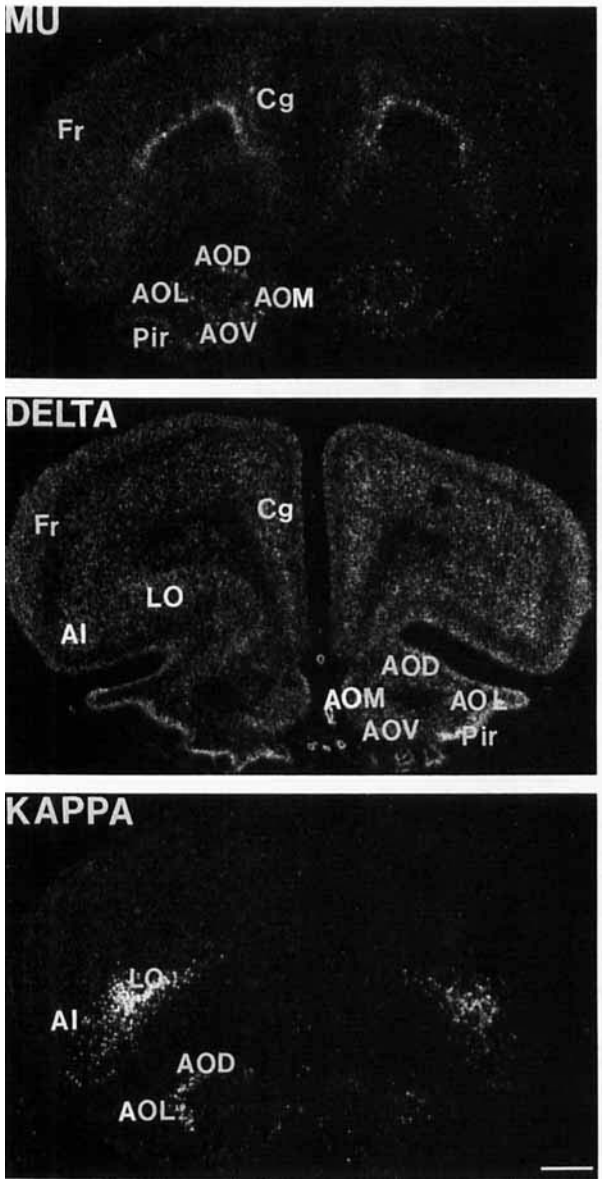

Fig. 3. Darkfield autoradiograms comparing μ, δ, and κ receptor mRNA expression at the level of the striatum. Cells expressing μ receptor mRNA are localized in patches in the caudate-putamen and nucleus accumbens, in a layer of cells medial to the corpus callosum referred to as the subcallosal streak (arrowheads), the diagonal band of Broca, and the medial septum. Cells expressing δ receptor mRNA have a more homogeneous distribution in the caudate-putamen and nucleus accumbens, with a higher number of cells expressing δ receptor mRNA observed in the lateral caudate-putamen. In addition, individual large cells in the CPu demonstrate particularly high levels of δ receptor mRNA. Cells expressing δ receptor mRNA are also localized in the cingulate cortex, frontal and parietal cortices, endopiriform nucleus, claustrum, and olfactory tubercle. In contrast to the μ and δ receptor mRNA distributions, cells expressing κ receptor mRNA are localized in the medial and ventral CPu, avoiding the lateral portion of the nucleus. κ Receptor mRNA expression is also found in the endopiriform, claustrum, olfactory tubercle, diagonal band of Broca, and lateral septum. Higher resolution images of the cells expressing μ, δ, and κ receptor mRNAs in the striatum are provided in Figure 13. Scale bar = 1,000 μm.

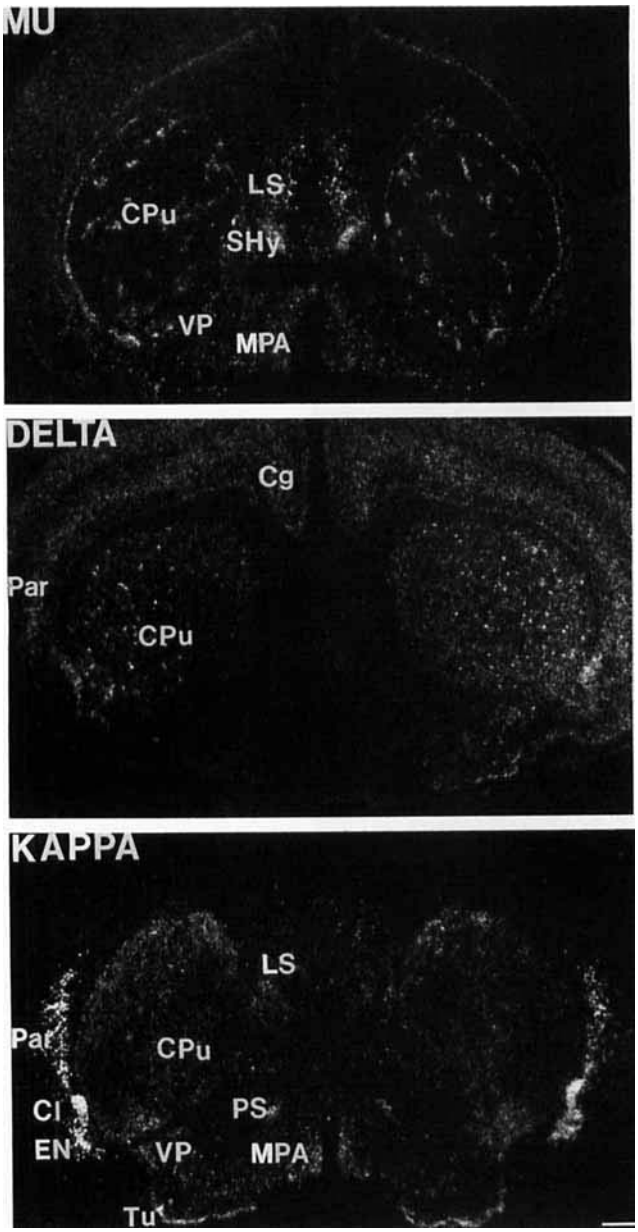

Fig. 4. Darkfield autoradiograms comparing μ, δ, and κ mRNA expression at the level of the crossing of the anterior commissure. Cells expressing μ receptor mRNA are localized in the patches of caudate-putamen, medial preoptic area, ventral pallidum, lateral septum, and the septohypothalamic nucleus. At this level, cells expressing κ receptor mRNA in the MPA are localized more medially compared to the more widespread distribution of cells expressing μ receptor mRNA. Other regions expressing κ receptor mRNA include the ventral portion of parietal cortex, claustrum, endopiriform, ventral pallidum, olfactory tubercle, lateral septum, bed nucleus stria terminalis, and parastrial nucleus. In contrast, cells expressing δ receptor mRNA are localized in the cingulate, parietal cortex, and in the caudate-putamen. Scale bar = 1,000 μm.

occipital cortices. Few, if any, cells expressing κ receptors are detected in the frontal cortex. Surprisingly, cells in the parietal, temporal, and occipital cortices expressing κ receptors are localized only in the ventral extents of layers 5 and 6 (Figs. 4–7, bottom) and do not extend dorsally as observed with most receptors found in the neocortex, including μ and δ.

In the dorsal hippocampus and dentate gyrus, only μ and δ receptor mRNA expression is observed. Cells expressing δ receptor mRNA are localized predominantly in the pyramidal cell layer, with occasional cells observed in the stratum oriens (Figs. 5–7, center). In addition to the expression of δ receptor mRNA within most pyramidal cells, scattered cells within the CA1–CA3 pyramidal layer express particularly high levels of δ receptor mRNA. A few isolated cells in the pyramidal layer expressing high levels of δ receptor mRNA are presented in Figure 12B. It is unclear whether these high-expressing cells are pyramidal cells or interneurons. High levels of δ receptor mRNA are also found in the dentate gyrus (Figs. 5–7, center), with expression localized in the granule cell layer.

In contrast to the relatively large number of cells in the hippocampus and dentate gyrus expressing δ receptor mRNA, far fewer cells express μ receptor mRNA in these regions. These cells are large, express relatively high levels of μ receptor mRNA, and are scattered predominantly in the pyramidal cell layer, with occasional cells observed in stratum oriens and radiatum (Fig. 12A) of the hippocampus. Similarly, in the dentate gyrus, cells expressing μ receptor mRNA are scattered in the granular cell layer and have a high level of expression. Rostral-caudal differences are observed with higher levels of μ and δ receptor observed in the caudal or temporal hippocampus and dentate gyrus. κ Receptor mRNA expression is also observed in the temporal hippocampus, with expression localized in the stratum radiatum.

Cells expressing μ and δ mRNAs have a scattered distribution in the subiculum, with high levels of expression observed (Figs. 6, 7). In contrast, only a low level of κ mRNA expression is detected in the subiculum, with a few scattered, high-expressing cells observed. In other divisions of the subicular complex, however, such as the parasubiculum, cells express predominantly κ receptor mRNA, with little μ and δ expression. Similarly, in the presubiculum, a selective expression of μ receptor mRNA is observed, with little if any δ or κ receptor mRNA detected (Fig. 7).

In the amygdala, cells expressing μ, δ, or κ receptor mRNA are differentially localized within nuclear groups. High levels of μ receptor mRNA expression are found in the intercalated nuclei of the basolateral amygdala (Fig. 12C), the posterior portion of the medial amygdala, and the posterior medial cortical amygdala (Fig. 6, top). Moderate levels of μ receptor mRNA expression are found in the medial, basomedial, and centromedial nuclei, with low levels of expression in the anterior, basolateral, and anterior cortical amygdaloid nuclei. In contrast, the pattern of δ receptor mRNA expression differs with the highest levels of δ receptor mRNA in the lateral, basolateral, medial, and basomedial nuclei (Figs. 5, center; 12D), with moderate levels in the posterior medial cortical nucleus. Yet a third pattern is observed with those cells expressing κ receptor mRNA, with high levels in the central lateral, basolateral, and medial nuclei (Figs. 5, bottom; 12E,F). Particularly high levels of κ receptor mRNA expression are also observed in the posterior portion of the medial nucleus and the posteromedial amygdalohippocampal transition area (Fig. 6, bottom). High levels of κ receptor mRNA expression are also observed in the nucleus of the lateral olfactory tract, which is thought to be a part of the extended amygdala

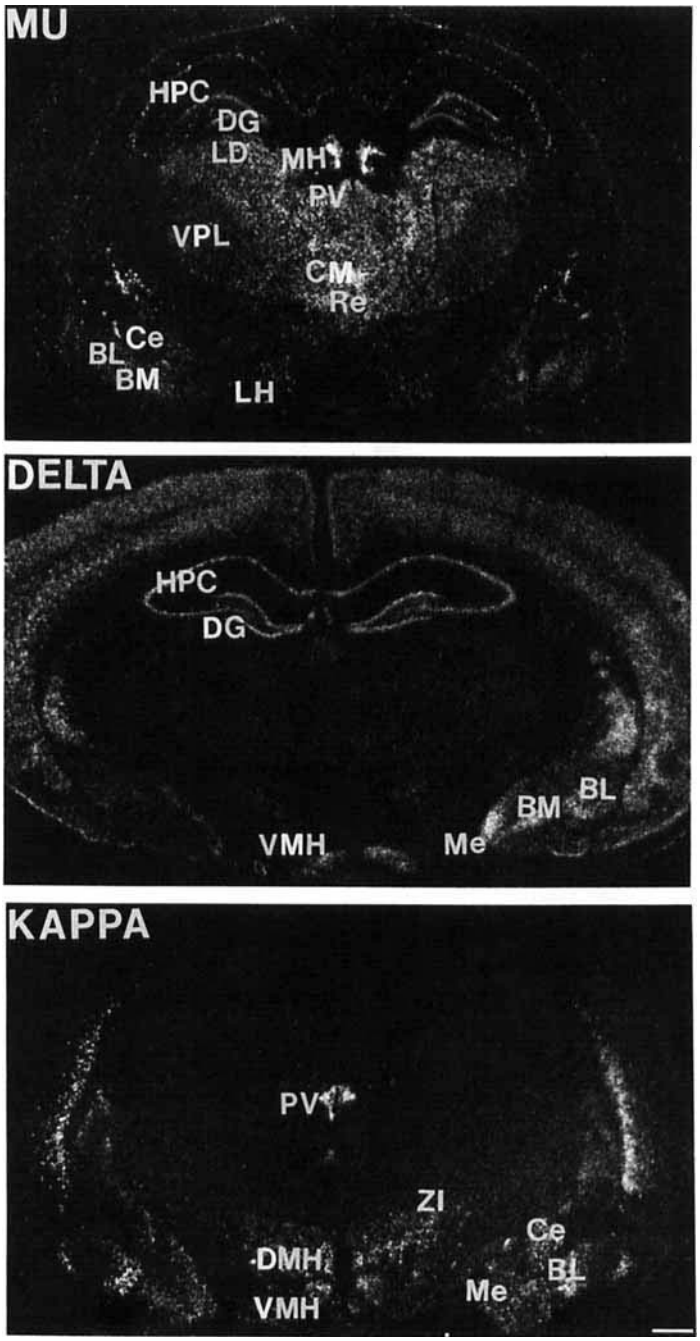

Fig. 5. Darkfield autoradiograms comparing μ, δ, and κ receptor mRNA expression at the level of the diencephalon. High levels of μ receptor mRNA expression are observed in several thalamic nuclei, including the medial habenula, laterodorsal, paraventricular, centromedial, and reuniens nuclei. In contrast, little δ receptor mRNA expression is observed in the nuclei of the thalamus, and κ receptor mRNA expression is limited to such nuclei as the paraventricular and to the zona incerta. Within the hypothalamus, cells expressing κ receptor are prominent in several nuclei, including the dorsomedial and ventromedial, whereas those expressing δ receptor mRNA are restricted to the ventromedial nucleus. For higher resolution cellular images of cells expressing κ receptor mRNA in other hypothalamic nuclei, see Figure 14. Although no κ receptor mRNA can be detected in the dorsal hippocampus and dentate gyrus, cells expressing μ and δ receptor mRNAs are observed. Cells expressing κ receptor mRNA are prominent, however, in the amygdaloid nuclei, where κ receptor mRNA expression is observed in the medial, basolateral, and central nuclei. High levels of δ receptor mRNA expression are also observed in the basolateral, basomedial, and medial nuclei of the amygdala. Higher resolution images of cells expressing μ, δ, and κ receptor mRNA in the hippocampus and amygdaloid nuclei are provided in Figure 12. Scale bar = 1,000 μm.

involved in processing olfactory information. Somewhat lower levels of κ receptor mRNA expression are observed in the centromedial and basomedial nuclei.

The cells of the bed nucleus stria terminalis, also thought to be part of the extended amygdala, express μ, δ, and κ receptor mRNAs. Whereas μ, δ, and κ receptor mRNA

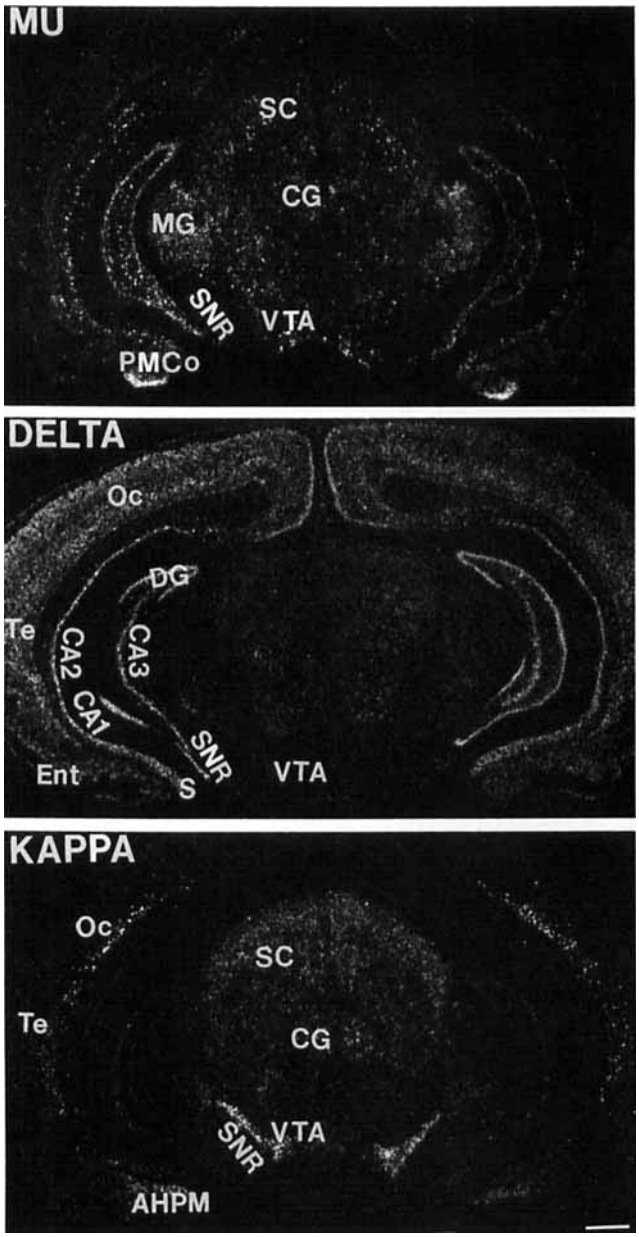

Fig. 6. Darkfield autoradiograms comparing μ, δ, and κ receptor mRNA expression at the level of the midbrain. Cells expressing κ receptor mRNA are localized in the substantia nigra (pars compacta and pars reticulata), ventral tegmental area, posteromedial amygdalo-hippocampal area, central gray, superior colliculus, and temporal and occipital cortices. In contrast, only scattered cells in the substantia nigra, pars compacta, and VTA express μ and δ receptor mRNAs. Although they cannot be seen in these low-magnification autoradiograms, cells expressing μ and δ receptor mRNAs are also localized in the substantia nigra, pars reticulata (see Fig. 15). Cells expressing δ receptor mRNA are prominent, however, in the pyramidal hippocampal fields, dentate gyrus, entorhinal, and temporal and occipital cortices as well as the subiculum. μ Receptor mRNA expression is high in the posteromedial cortical amygdala, in the medial geniculate nucleus of the thalamus, and in large scattered cells of the superior colliculus. Higher resolution images of the cells expressing μ, δ, and κ receptor mRNA in the substantia nigra and ventral tegmental area are provided in Figure 15. Scale bar = 1,000 μm.

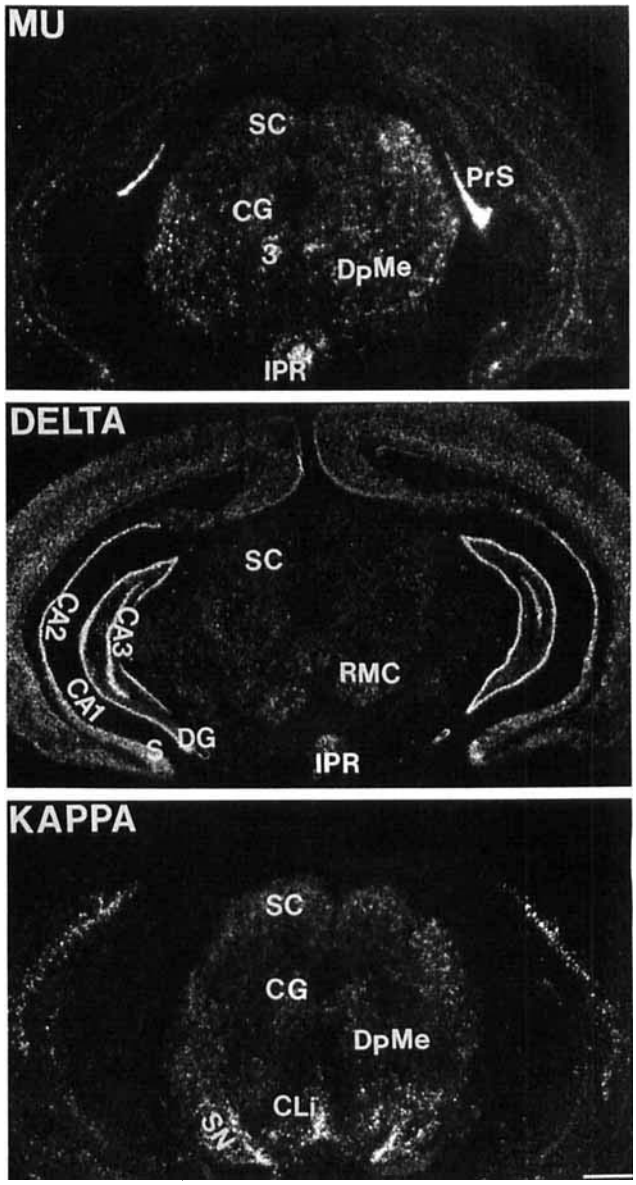

Fig. 7. Darkfield autoradiograms comparing μ, δ, and κ receptor mRNA expression at the level of the caudal midbrain. μ Cellular expression is localized in the presubiculum, rostral interpeduncular nucleus, oculomotor nucleus, scattered large cells of the superior colliculus, and deep mesencephalic nucleus. Cells expressing δ receptor mRNA are similarly localized in the rostral interpeduncular nucleus in addition to the magnocellular neurons of red nucleus. Cells expressing κ receptor mRNA have a more widespread distribution at this level, however, with κ receptor mRNA expression in the caudal linear raphe nucleus, substantia nigra (pars reticulata and pars compacta), central gray, superior colliculus, and DpMe. A high-resolution image of cells in the caudal linear raphe that express κ receptors is provided in Figure 16. Scale bar = 1,000 μm.

expression levels are moderate in most divisions of the bed nucleus stria terminalis, high levels of κ receptor mRNA are seen in the medial posterior division. Small cellular groups embedded within the stria terminalis also express μ and κ receptor mRNAs.

The distribution of cells expressing opioid receptor mRNAs varies markedly in the striatum. In the caudate-

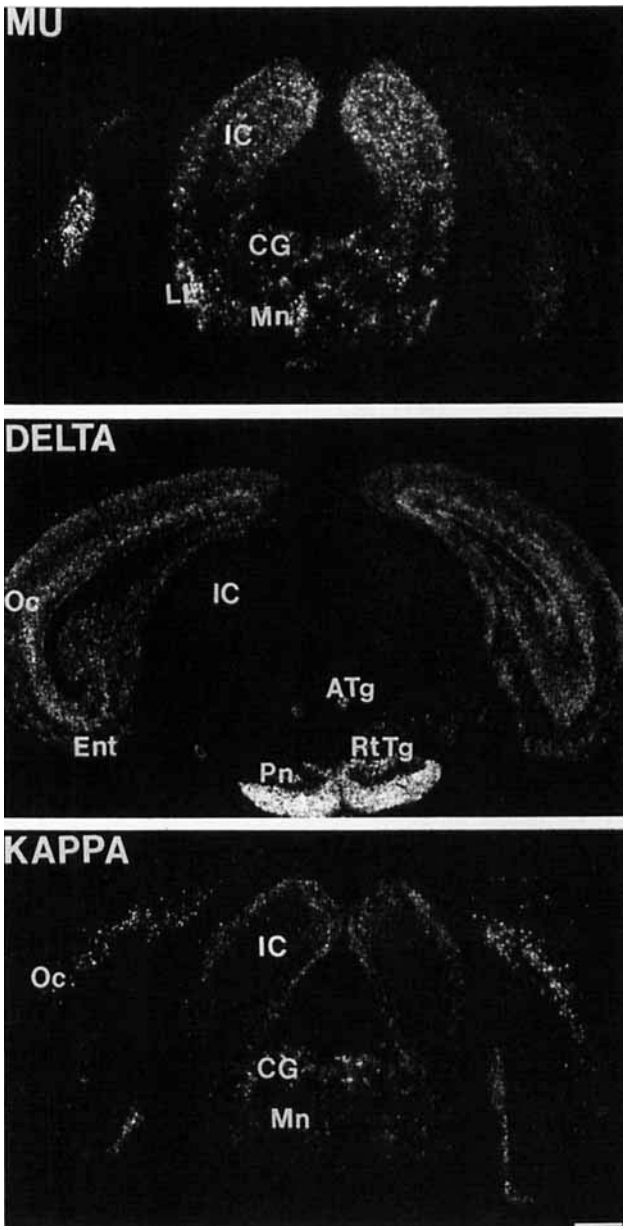

Fig. 8. Darkfield autoradiograms comparing μ, δ, and κ receptor mRNA expression at the level of the pontine nuclei. δ Receptor mRNA expression is prominent in the pontine nuclei, reticulotegmental nucleus of the pons, and anterior tegmental nucleus, all regions not demonstrating μ or κ receptor mRNA expression. As was shown in preceding figures, cells expressing δ receptor mRNA are also prominent in the occipital and entorhinal cortices. Cells expressing μ receptor mRNA are localized in the central and external cortices of the inferior colliculus, pontine central gray, lateral lemniscus, and median raphe. In contrast, cells expressing κ receptor mRNA are restricted to the dorsal and external cortices of the inferior colliculus. Other regions expressing κ receptor mRNA include layers 5 and 6 of the occipital cortex, pontine central gray, and median raphe. A higher resolution image of the cells in the median raphe expressing μ receptors is provided in Figure 16. Scale bar = 1,000 μm.

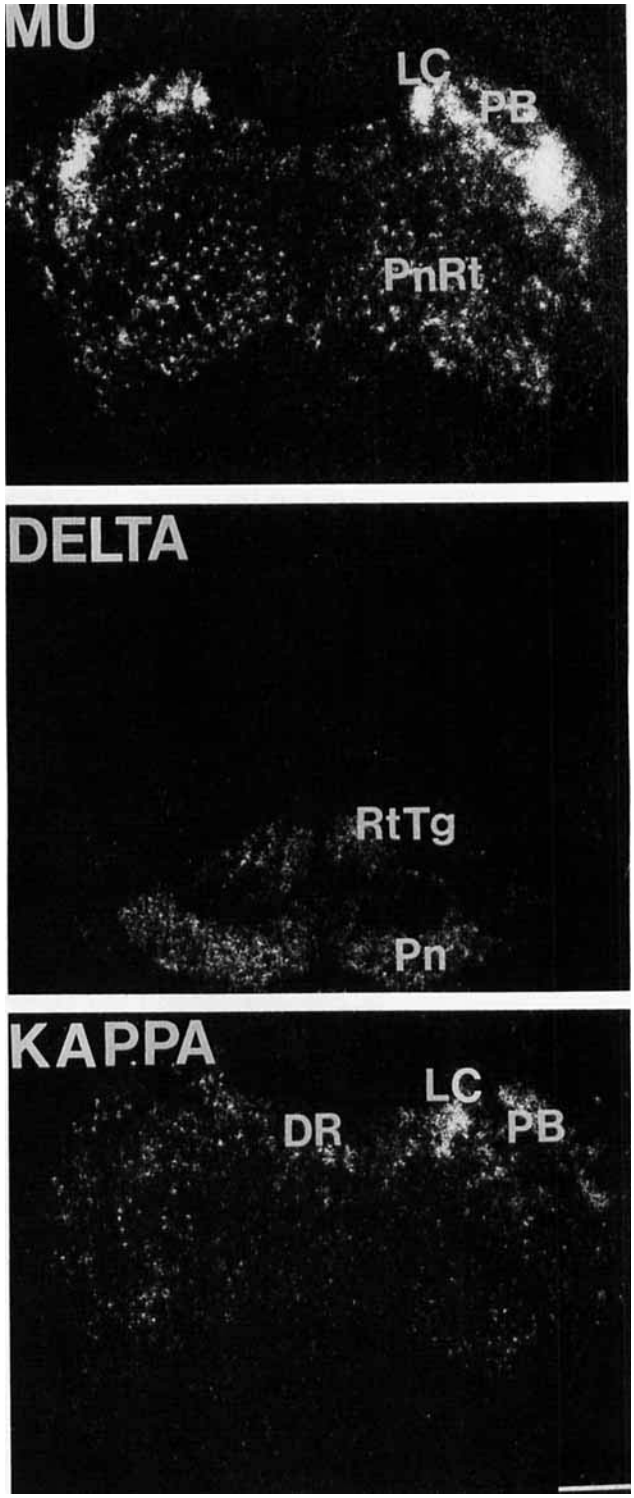

Fig. 9. Darkfield autoradiograms comparing μ, δ, and κ mRNA expression at the level of the locus coeruleus and parabrachial nucleus. μ And κ receptor mRNA expression is observed in LC and PB nucleus, regions of no or low δ receptor mRNA expression. In contrast, cells expressing δ receptor mRNA are localized in the pontine nuclei and the reticulotegmental nucleus of the pons. Cells expressing μ and κ receptor mRNA are also observed in the large scattered cells of the pontine reticular nucleus and in the dorsal raphe. Scale bar = 500 μm.

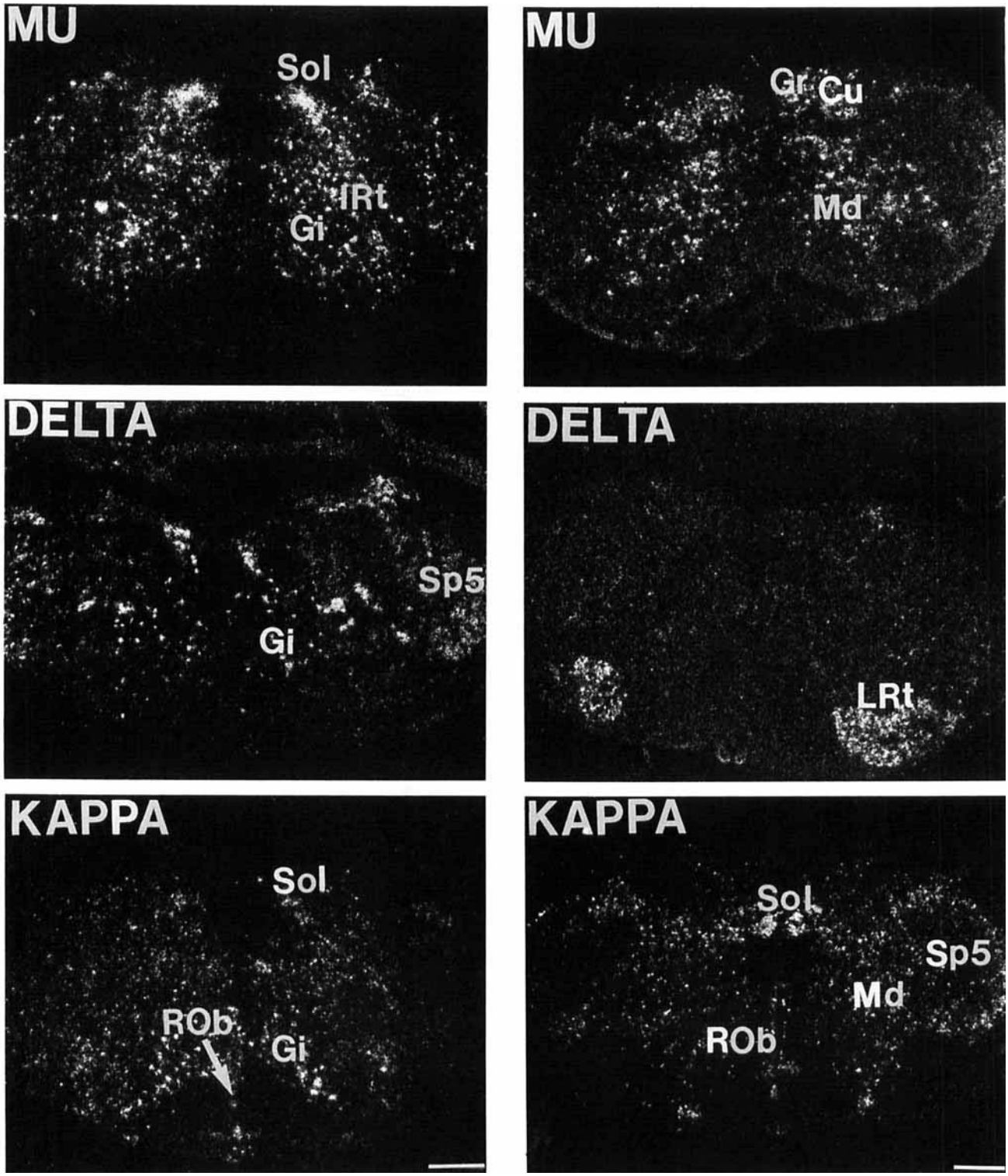

Fig. 10. Darkfield autoradiograms comparing μ, δ, and κ receptor mRNA expression in the rat brainstem. Cells expressing μ receptor mRNA are prominent in the rostral portion of the nucleus of the solitary tract and in scattered cells of the intermediate and gigantocellular reticular nuclei. Cells expressing δ and κ receptor mRNAs are similarly distributed in the intermediate and gigantocellular reticular nuclei, with δ expression also high in the cells of the spinal trigeminal nucleus and κ receptor expression found in the raphe obscurus. Cells in the rostral nucleus of the solitary tract also express κ receptor mRNA, but their levels of expression are lower than those observed for μ. Higher resolution images of opioid receptor mRNA expression in the raphe obscurus, nucleus of the solitary tract, and gigantocellular reticular field are provided in Figures 16, 18, and 19. Scale bar = 500 μm.

Fig. 11. Darkfield autoradiograms comparing μ, δ, and κ receptor mRNA expression in the caudal brainstem. Cells expressing μ receptor mRNA are localized in nucleus gracilis, nucleus cuneatus, and dorsal motor nucleus of vagus in addition to scattered cells of the medullary reticular field. Cells expressing δ receptor mRNA have a more limited distribution, with high levels in the lateral reticular nucleus. In contrast to the low number of cells in the commissural division of the nucleus of the solitary tract that express μ receptor mRNA, cells expressing κ receptor mRNA are prominent at this level of the solitary tract nucleus. Other regions expressing κ receptor mRNA include the spinal trigeminal, dorsal motor nucleus of vagus, raphe obscurus, and medullary reticular field. Higher resolution images of opioid receptor mRNA expression in raphe obscurus and nucleus of the solitary tract are provided in Figures 16 and 19. Scale bar = 500 μm.

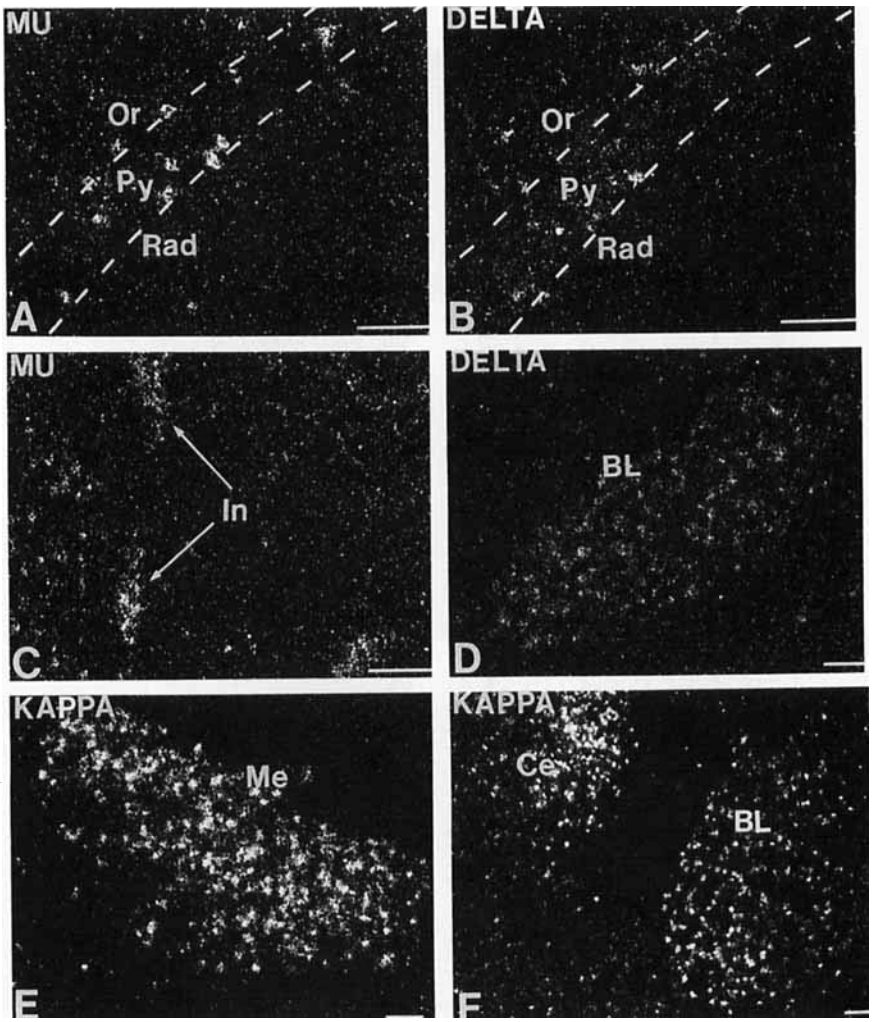

Fig. 12. Darkfield autoradiograms demonstrating the cellular expression of μ, δ, and κ receptor mRNAs in the hippocampus and amygdaloid nuclei. Within the hippocampus, cells expressing μ receptor mRNA (**A**) are scattered in the pyramidal cell layer, with occasional large cells observed in stratum oriens and radiatum. A few isolated, scattered cells expressing high levels of δ receptor mRNA are visible in **B**. Within the amygdala, μ receptor mRNA expression is high in the intercalated nuclei of the basolateral amygdala (**C**), whereas cells expressing κ receptor mRNA are found in the central and basolateral nuclei (**F**) as well as the posterior division of the medial nucleus (**E**). Cells expressing δ receptor mRNA are localized in several nuclei of the amygdala including the basolateral nucleus shown above (**D**). Scale bars = 100 μm.

putamen, cells expressing μ receptor mRNA are organized in cellular groups or patches (Figs. 3, 4, top; 13A) that extend ventrally into the nucleus accumbens. These cellular μ receptor patches vary in cell number and demonstrate a three-dimensional distribution, with the highest density of patches found in the rostral and dorsal striatum. Medial-lateral differences are also seen with a higher number of patches observed laterally. In addition to μ receptor patches, a high level of μ receptor mRNA expression is observed in a cell layer immediately adjacent to the corpus callosum, referred to as the subcallosal streak (Atweh and Kuhar, 1977c). Surrounding the μ patches are scattered cells in the striatal matrix that generally express lower levels of μ receptor mRNA. In the caudal portion of the caudate-putamen, higher levels of μ, δ, and κ receptor mRNA expression are observed in the fundus striati.

In the nucleus accumbens, cell clusters as defined by Herkenham et al. (1984) also express high levels of μ receptor mRNA (Fig. 13C). These cellular clusters, which can be identified by Nissl staining, are found predominantly in the nucleus accumbens shell. Both the core and shell compartments of the nucleus accumbens express μ receptors; however, expression levels are higher in the accumbens shell and septal pole regions (Fig. 3, top). Farther ventrally, scattered cells expressing μ receptor mRNA are found in the region of the olfactory tubercle; however, they are not localized to the islands of Calleja or the olfactory tubercle proper.

In contrast to the patch- and cluster-like distribution of cells expressing μ receptor mRNA, cells expressing δ receptors have a more homogeneous distribution in the caudate-putamen and nucleus accumbens (Figs. 3, 4, center; 13B). Although rostral-caudal differences in δ receptor expression are not prominent, higher numbers of cells expressing δ receptor mRNA are observed in the lateral caudate-putamen that likely contribute to the higher amounts of δ

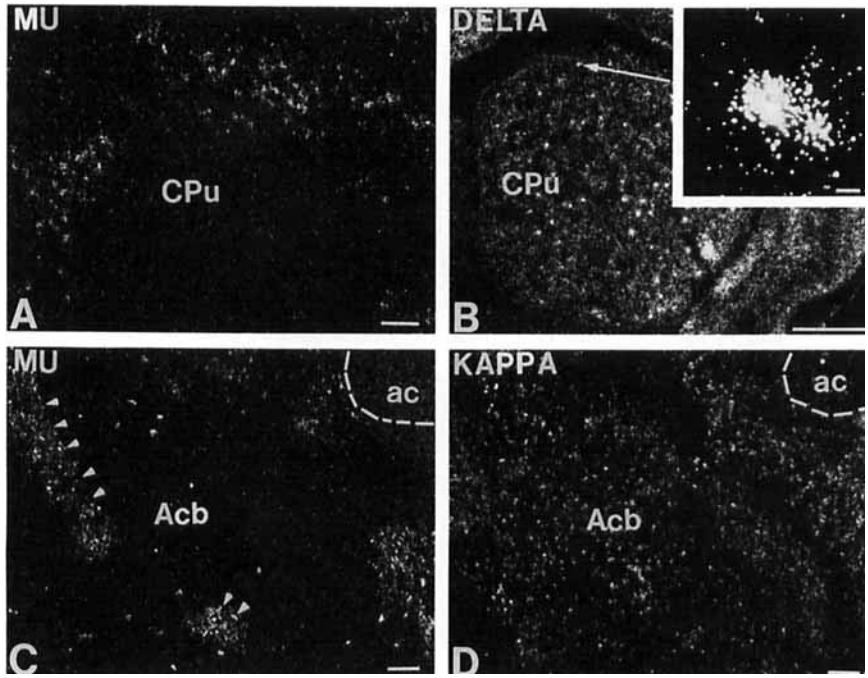

Fig. 13. Darkfield autoradiograms demonstrating the cellular expression of μ, δ, and κ receptor mRNAs in the caudate-putamen and nucleus accumbens. Cells expressing μ receptor mRNA are distributed in patches in the CPu (**A**) or as cellular groups or clusters in the Acb (**C**). Regions highlighted by arrowheads represent cell clusters in the nucleus accumbens that can be identified with Nissl staining (C). Similar but more densely organized clusters of cells in the nucleus accumbens express κ receptor mRNA (**D**). In contrast, cells expressing δ receptor mRNA are more homogeneously distributed in the CPu (**B**), with higher numbers of cells in the lateral CPu. In addition, a small proportion of caudate-putamen cells, possibly cholinergic, expresses particularly high levels of δ receptor mRNA. An example of one of these cells has been included as the **inset** in B. The dashed lines in C and D define the border of the anterior commissure. Scale bars = 100 μm in A,C,D, 1,000 μm in B, 10 μm in inset.

receptor binding seen in the lateral striatum. Scattered among the cells expressing a moderate level of δ receptor mRNA is a small population of large cells that contain high levels of δ receptor mRNA (Fig. 13B). These cells are predominantly localized in the caudate-putamen and may be the large cholinergic neurons found in the striatum. Although not apparent from Figure 3, higher levels of δ receptor mRNA are observed in the accumbens shell and septal pole regions as compared to the core. More ventrally, high levels of δ receptor mRNA expression are also observed in the olfactory tubercle (Fig. 3, center).

Cells expressing κ receptor mRNA have a different distribution in the striatum, with high levels of expression in the nucleus accumbens and olfactory tubercle. In the nucleus accumbens, κ receptor expression is not homogenous, with cells expressing κ receptors organized into clusters (Fig. 13D) that can be identified in Nissl-counterstained sections. Differences in expression levels are also seen in the compartments of the nucleus accumbens, with higher levels seen in the shell and septal pole regions (Fig. 3, bottom). In the dorsal striatum, cells expressing κ receptor mRNA are localized in the medial and ventral caudate-putamen, avoiding the lateral quadrant where δ receptor mRNA expression is highest. Higher κ receptor mRNA expression is also observed in the dorsomedial portion of the caudate-putamen, which is thought to be the rodent equivalent of the caudate nucleus.

Cells of the globus pallidus and ventral pallidum express μ, δ, and κ receptor mRNAs. In the globus pallidus, scattered large cells express high levels of μ and κ receptor mRNAs. Farther caudally in the globus pallidus at the level of the thalamus, cells expressing μ and κ receptors are less widely distributed and have a more clustered appearance. In the rostral ventral pallidum, cells expressing μ and κ receptors are localized in small groups ventral to the nucleus accumbens and become more widely scattered in the caudal ventral pallidum. Despite a similar distribution in the globus pallidus and ventral pallidum, colocalization studies are necessary to determine whether μ and κ mRNAs are localized in the same cells. Delta receptor mRNA expression is comparatively low in the globus pallidus and ventral pallidum, with a few scattered cells detected in both regions.

In the septal nuclei, cells expressing μ receptor mRNA are localized in the medial and lateral divisions as well as the septohypothalamic nucleus, where expression levels are high (Figs. 3, 4, top). μ Receptor mRNA levels are high throughout the rostral-caudal extent of the medial septum; however, differences are seen in the lateral septum, where μ receptor mRNA expression is higher in the caudal portion of the intermediate lateral septum (Fig. 4, top). In contrast, cells expressing κ receptor mRNA are widely distributed in the lateral and medial septum and have a moderate level of expression (Figs. 3, 4, bottom), whereas those expressing δ receptors have comparatively low levels of expression in the medial and lateral septum, with only a few cells detected. Ventral to the septum, cells of the diagonal band of Broca contain μ, δ, and κ receptor mRNAs (Fig. 3). Moderate levels of μ, δ, and κ receptor expression are seen in scattered cells in the vertical and horizontal limbs of the diagonal band of Broca.

Other regions in the telencephalon that express opioid receptors include the endopiriform nucleus, claustrum, and parastrial nucleus (Figs. 3, 4), where high levels of κ receptor mRNA are observed. Whereas the cells in the parastrial nucleus express exclusively κ receptors, cells in the claustrum also demonstrate high levels of δ and low levels of μ receptor mRNA, and those in the endopiriform nucleus express low to moderate levels of μ and δ receptor mRNAs.

Diencephalon

There are marked differences in the distribution of cells expressing μ, δ, and κ receptor mRNAs in the diencephalon. μ Receptor mRNA expression is high in most thalamic nuclei, whereas cells expressing κ receptors have a more limited distribution and are primarily localized in the medial thalamic nuclei. In the hypothalamus, where κ receptor mRNA expression predominates, cells expressing μ receptor mRNA maintain a widespread distribution, but high levels of expression are limited to a few nuclei. Cells expressing δ receptor mRNA also have a limited distribution in the hypothalamus, and expression levels are comparatively low in most regions except for the ventromedial hypothalamus.

High levels of μ receptor expression are observed in the medial habenula, paraventricular, mediodorsal, laterodorsal, centromedial, paracentral, interanteromedial, gelatinosus, reuniens, rhomboid, ventrolateral, ventromedial, parafascicular, and posterior thalamic nuclei (Fig. 5, top). Little, if any, μ receptor mRNA could be detected in the anterior thalamic nuclear groups of the anterodorsal and anteroventral nuclei or in the lateral habenula. Rostral-caudal differences in μ receptor expression are observed in the reticular nucleus, where higher expression levels are detected in its most rostral portion. Lower levels of μ receptor mRNA expression are seen in several other nuclei including the ventral posterior lateral nucleus and the zona incerta. Differences are also seen in the geniculate bodies, where high levels of μ receptor mRNA expression are observed in the medial and subgeniculate nuclei (Fig. 6, top), whereas only moderate levels are detected in the lateral geniculate body.

In contrast, high levels of κ receptor expression in the thalamus are limited to the paraventricular, centromedial (Fig. 5, bottom), paracentral, and parafascicular nuclei, with moderate levels of expression in the lateral habenula, laterodorsal nucleus, rhomboid, zona incerta, and the ventral lateral geniculate body. Expression of δ receptor mRNA in the thalamus is low, with cells localized in the laterodorsal, reticular, and parafascicular nuclei as well as the zona incerta and lateral geniculate body.

In the rostral hypothalamus, cells expressing μ and κ receptor mRNAs are observed in the medial preoptic area (Fig. 4), with high levels of μ receptor mRNA expression also seen in the medial preoptic nucleus. Cells expressing μ and κ receptor mRNAs are widely distributed, with scattered cells in the medial preoptic, median preoptic, and lateral preoptic areas. Differences are seen within the medial preoptic area, where higher levels of μ receptor expression are observed in the lateral portion, whereas cells expressing κ receptor mRNA have a more medial localization (Fig. 4). In contrast, only low levels of δ receptor mRNA expression can be detected in scattered cells of the medial preoptic area and are observable only at high magnification

More caudally within the hypothalamus, high levels of κ receptor mRNA expression are observed in the paraventricular, periventricular, supraoptic, and suprachiasmatic nuclei of the hypothalamus (Fig. 14A,C,E,G). In the paraventricular nucleus, cells in the anterior parvocellular, medial parvocellular, and magnocellular divisions express κ receptor mRNA, with particularly high levels of expression in the magnocellular neurons. The scattered distribution of the magnocellular neurons in the paraventricular and supraoptic nuclei that express κ receptor mRNA (Fig. 14A,C) suggests that this represents only a subpopulation of magnocellular neurons. Similarly, by comparing Nissl-counterstained sections, it is clear that only the cells localized in the ventral suprachiasmatic nucleus express κ receptor mRNA (Fig. 14E,G). In contrast, comparatively low levels of μ receptor mRNA expression are observed in scattered cells of the paraventricular, periventricular, and supraoptic nuclei, with no cells detected in the suprachiasmatic nucleus. δ Receptor mRNA expression is undetectable in the periventricular and suprachiasmatic nuclei of the hypothalamus, with only low levels of expression observed in the paraventricular and supraoptic nuclei.

High levels of κ receptor mRNA expression are also seen in the cells of the arcuate nucleus, median eminence, and infundibular stem (Fig. 14B,D,E), where κ receptors may serve to regulate neuroendocrine release. Within the median eminence, κ receptor mRNA is localized in the ependymal cells immediately adjacent to the floor of the third ventricle and in scattered neurons in the inner and outer cell layers (Fig. 14D). Low levels of μ receptor mRNA expression are seen in the arcuate nucleus, with no detectable μ or δ expression in the cells of the median eminence and infundibulum. Opioid receptor mRNA expression extends to the anterior and lateral hypothalamic nuclei, with scattered cells expressing moderate levels of μ and κ mRNAs. Only low levels of δ receptor mRNA expression are seen in scattered cells of the lateral hypothalamus.

Cells expressing high levels of κ receptor mRNA are localized in both the dorsomedial and the ventromedial nuclei, whereas those expressing δ receptors are localized in the ventromedial nucleus of the hypothalamus, with no cells detected in the dorsomedial nucleus. Within the ventromedial nucleus of the hypothalamus, cells expressing κ mRNA have a more limited distribution, predominantly localized in the dorsomedial subdivision. Cells expressing μ receptor mRNA demonstrate a third pattern, with low levels of expression in dorsomedial nucleus and no detectable cells in the ventromedial nucleus of the hypothalamus. Farther caudally, high levels of μ and κ receptor expression are observed in the posterior nucleus of the hypothalamus, where no δ receptor mRNA expression can be detected.

In the mammillary complex, cells with high levels of μ receptor mRNA are observed in the supramammillary nucleus, with only low levels in the medial median mammillary and premammillary nuclei. In contrast, δ receptor mRNA expression cannot be detected in the mammillary nuclei, and κ receptor expression is limited to the ventral premammillary and supramammillary nuclei. No opioid mRNA expression could be detected in the lateral mammillary nucleus of the hypothalamus.

Mesencephalon

Cells expressing μ, δ, and κ receptor mRNAs are localized in the superior and inferior colliculi (Figs. 6–8). In the superior colliculus, a laminar pattern is seen, with the

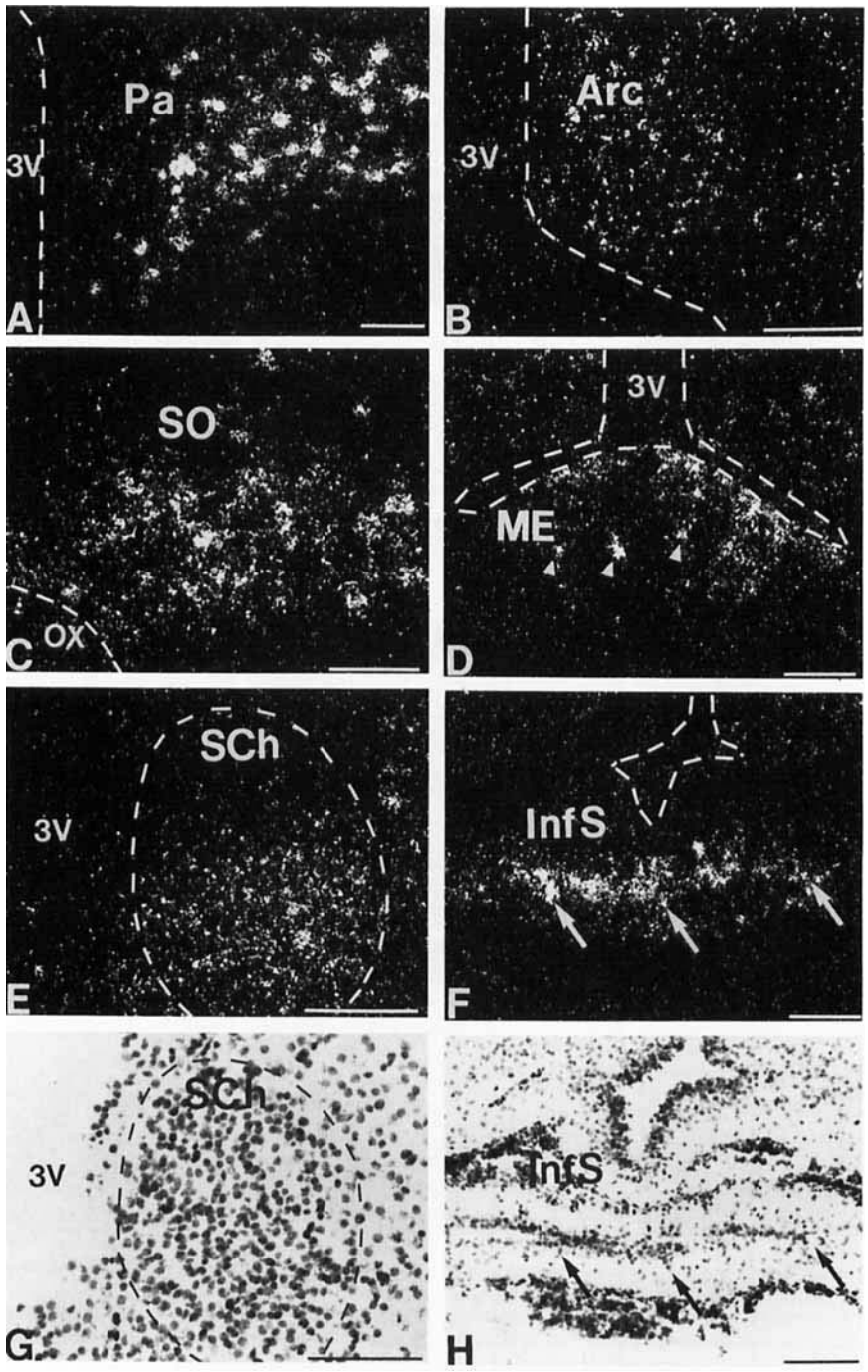

Fig. 14. κ Receptor mRNA expression in the nuclei of the hypothalamus, median eminence, and infundibulum. Darkfield autoradiograms demonstrate that the cells expressing κ receptor mRNA are localized in the paraventricular (**A**), supraoptic (**C**), and suprachiasmatic (**E**) nuclei of the hypothalamus, where they may play a role in response to stress and in maintaining electrolyte balance. As can be seen from the corresponding brightfield image (**G**), only the cells in the ventral portion of the suprachiasmatic nucleus express κ receptor mRNA. Similarly, only a subpopulation of magnocellular neurons in the paraventricular and supraoptic nuclei expresses κ receptor mRNA. Darkfield images show that cells in the arcuate nucleus (**B**), median eminence (**D**), and infundibular stem (**F**) also express κ receptor mRNA and may be of importance in hormonal regulation. Within the median eminence, both ependymal cells immediately adjacent to the base of the third ventricle (3V) and scattered cells, as indicated by arrowheads (D), in the inner and outer layers of the median eminence express κ receptor mRNA. Similarly, in comparing the brightfield (**H**) and darkfield (**F**) images, it appears that cells in the infundibular stem express κ receptors. The same cells are highlighted by arrows in F (darkfield) and H (brightfield). Scale bars = 100 μm.

highest level of μ receptor mRNA expression detected in the large scattered cells in the intermediate and deep layers. Lower levels of μ receptor expression are seen in the superficial gray layer, with no detectable μ receptor mRNA expression in the optic nerve layer. Cells expressing δ receptor mRNA are also localized to the intermediate and

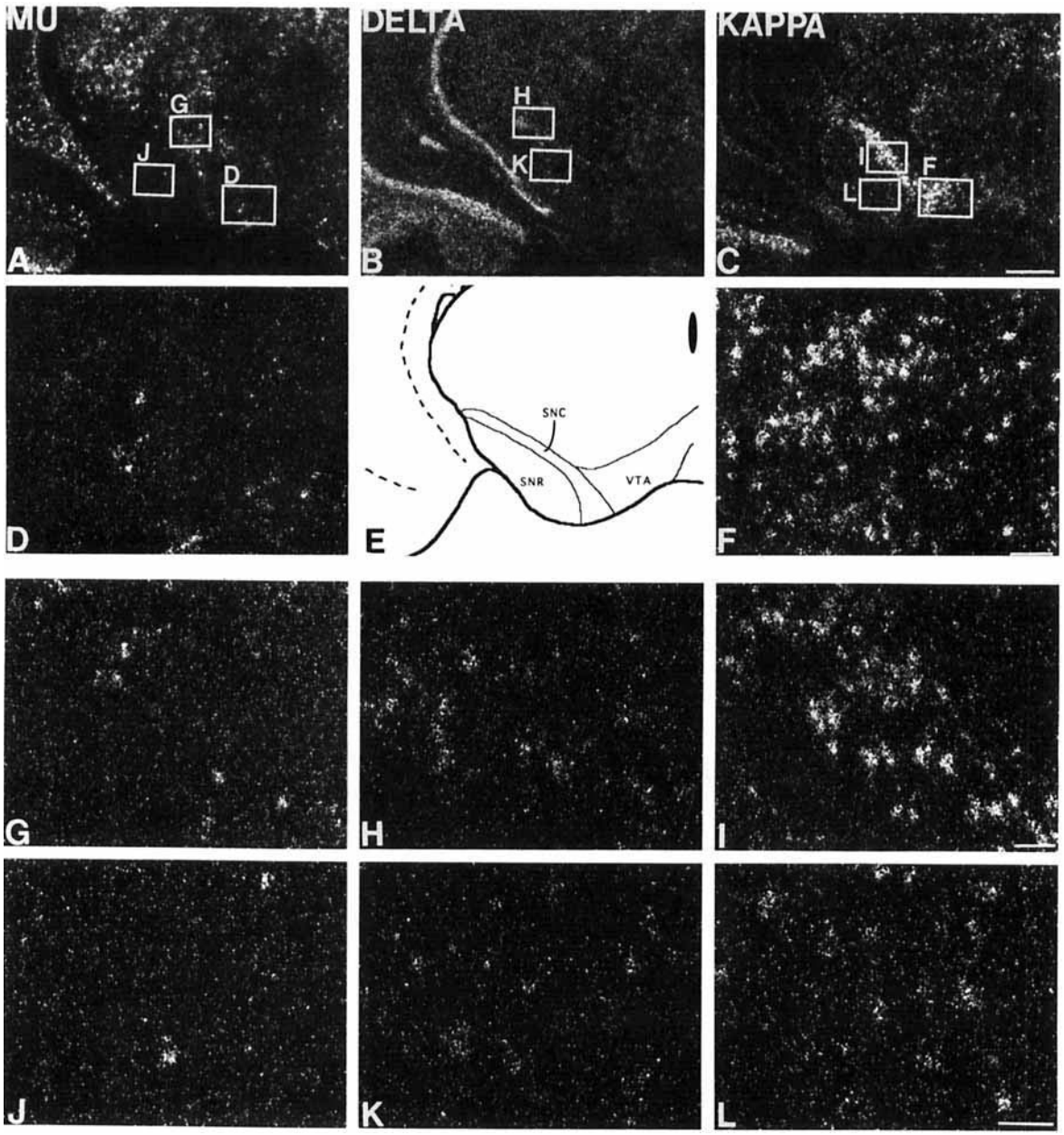

Fig. 15. Darkfield autoradiograms showing the cellular expression of μ, δ, and κ receptor mRNAs in the substantia nigra and ventral tegmental area. Regions indicated by boxes in **A–C** have been magnified and presented in **D,F–L**. A schematic drawing is provided in **E** that illustrates the divisions of the substantia nigra and the level of the ventral tegmental area examined. Cells expressing κ receptor mRNA are prominent in the ventral tegmental area (F); substantia nigra, pars compacta (I); and substantia nigra, pars reticulata (L). Within the substantia nigra, far fewer cells expressing κ receptor mRNA are observed in the pars lateralis, a region where δ receptor mRNA expression is observed (H). Scattered cells expressing δ receptor mRNA are also observed in the substantia nigra, pars reticulata, and are more abundant in the dorsolateral portion (K). In contrast, comparatively few cells expressing μ receptor mRNA are seen in the ventral tegmental area (D) and substantia nigra, pars compacta (G) and pars reticulata (J). Scale bars = 500 μm in C, 100 μm in F,I,L.

deep layers of the superior colliculus; however, comparatively fewer cells and lower levels of expression are observed. In contrast to the laminar distribution of cells expressing μ and δ receptor mRNAs, cells expressing κ receptors have a more homogeneous distribution in the superior colliculus, with moderate levels of expression in the superficial gray, optic nerve, intermediate gray, and deep gray layers. In the inferior colliculus, cells expressing μ and δ receptor mRNAs are localized in the dorsal, central, and external cortex, whereas cells expressing κ receptors have a more limited distribution, localized primarily in the dorsal and external cortex (Fig. 8). Despite a similar anatomical distribution of μ and δ receptor mRNAs within the inferior colliculus, δ expression levels are low compared to μ.

In the rostral central gray, moderate μ receptor mRNA expression levels are observed, with cells localized predominantly in the ventrolateral portion. Cells expressing κ

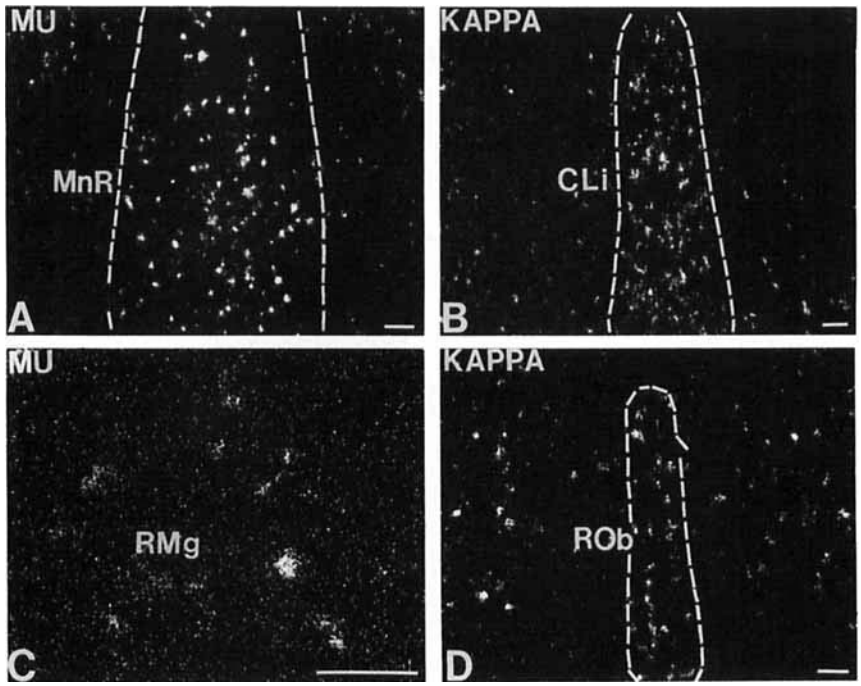

Fig. 16. Darkfield autoradiograms demonstrating the cellular expression of μ and κ receptor mRNAs in the raphe nuclei. Cells expressing μ receptor mRNA are localized in the median raphe (**A**) and raphe magnus (**C**), whereas cells expressing κ receptor mRNA are localized in the caudal linear raphe (**B**) and raphe obscurus (**D**). Scale bars = 100 μm.

receptor mRNA are more widely distributed in the central gray, with moderate levels of expression in the dorsal and ventrolateral central gray. μ And κ receptor mRNA expression extends to the pontine central gray, where expression levels are higher. In contrast, only low levels of δ receptor mRNA can be detected in the rostral central gray with little, if any, observed in the pontine central gray.

κ Receptor mRNA expression is prominent in the cells of the substantia nigra and the ventral tegmental area (Figs. 6, 15). In the substantia nigra, cells expressing high levels of κ receptor mRNA are predominantly localized in the medial portion of the pars compacta (Fig. 15I), with comparatively fewer cells and lower levels of expression in the pars lateralis. Moderate levels of κ receptor expression are also seen in large scattered cells of the pars reticulata, with higher expression levels in the caudal portion of the pars reticulata (Figs. 6 vs. 7; 15L). In the ventral tegmental area, cells expressing high levels of κ receptor mRNA are most dense immediately adjacent to the substantia nigra, pars compacta, having a more scattered distribution in the remaining tegmental area. δ Receptor expression is comparatively low in the substantia nigra and ventral tegmental area, with cells localized predominantly in the lateral pars compacta (Fig. 15H) and widely scattered in the ventral tegmental area. Cells of the pars reticulata also express δ receptor mRNA, with moderate levels of expression in the lateral pars reticulata (Fig. 15K). Moderate levels of κ and low levels of δ receptor mRNA expression are observed in the subthalamic nucleus, where they may serve to regulate nigrostriatal systems.

In contrast, relatively few cells expressing μ receptor mRNA are seen in the ventral tegmental area and in the pars compacta and pars reticulata of the substantia nigra (Fig. 15D,G,J).Within the pars compacta, a higher number of cells expressing μ receptor mRNA are localized in the lateral pars compacta, a region of comparatively lower κ receptor mRNA expression. Rostral-caudal differences in μ receptor mRNA expression are observed in the ventral tegmental area and substantia nigra, pars reticulata, with higher levels of μ receptor expression observed caudally.

In the interpeduncular complex, μ, δ, and κ receptor mRNAs are expressed (Fig. 7). High levels of μ receptor mRNA are observed in the cells of the rostral interpeduncular nucleus, with a few cells detected in the caudal nucleus demonstrating high levels of expression. Only moderate levels of μ receptor expression are seen in the intermediate subdivision of the interpeduncular nucleus. Cells expressing δ receptor mRNA are primarily localized in the rostral interpeduncular, whereas those expressing low levels of κ receptor mRNA are scattered in the rostral, caudal, and intermediate subdivisions.

Other regions in the mesencephalon that express high levels of opioid receptor mRNAs are the red nucleus, the oculomotor nucleus, and the nucleus of the lateral lemniscus. The magnocellular division of the red nucleus is one of the few regions in the midbrain of relatively high δ receptor mRNA expression. This region contains no μ or κ receptor mRNA expression and suggests the importance of δ receptors in the corticorubrospinal pathway. High levels of μ mRNA and moderate levels of δ mRNA are observed in the cells of the oculomotor nucleus, where they may be important in eye muscle control (Fig. 7). The cells of the lateral lemniscus (Fig. 8) predominantly express μ receptor mRNA, however, with only a low level of δ and κ expression in the ventral nucleus of the lateral lemniscus.

Cells expressing μ, δ, and κ mRNAs are observed scattered in the deep mesencephalic nucleus (Fig. 7). Cells expressing high levels of μ, δ, and κ mRNA extend caudally into the brainstem, where large numbers of cells are seen

scattered throughout the pontine and medullary reticular nuclei (Figs. 8–11).

Met- and mylencephalon

The cells of the pontine nuclei contain the highest levels of δ receptor mRNA found in the rat brain (Figs. 8, 9). High δ receptor mRNA expression is not limited to the pontine nuclei but extends to the reticulotegmental nucleus of the pons, with lower levels of expression in the anterior tegmental nucleus. These regions demonstrate predominantly δ receptor mRNA expression, with no detectable μ or κ receptor mRNA expression. Low levels of μ and κ receptor mRNA expression are seen, however, in the cells of the dorsal tegmental nucleus, with κ receptor mRNA expression extending to the laterodorsal tegmental nucleus.

Cells of the raphe nuclei, on the other hand, predominantly express κ and μ receptor mRNAs. High levels of κ receptor mRNA are observed in the caudal linear and dorsal raphe, as well as the raphe obscurus (Figs. 7, 9–11, 16B,D), whereas moderate levels are seen in the median raphe (Fig. 8, bottom). μ Receptor mRNA expression differs in the raphe nuclei, with high levels of expression in the median raphe and in scattered large cells of the raphe magnus (Figs. 8; 16A,C). Lower levels of μ receptor expression are seen in the caudal portion of the dorsal raphe, and, in the caudal linear raphe, only a few cells expressing μ receptor mRNA can be detected. In comparison, low levels of δ mRNA are seen in the median raphe and raphe magnus, and moderate levels are observed in raphe pallidus.

Cells in the locus coeruleus and parabrachial nucleus express μ and κ receptor mRNAs (Fig. 9). High levels of μ receptor mRNA expression are seen in the locus coeruleus and in the lateral, medial, and ventral divisions of the parabrachial nucleus. κ Receptor mRNA expression is also high in the locus coeruleus; however, the number of cells expressing κ receptor mRNA is lower compared to those expressing μ. Similarly, cells expressing κ receptor mRNA in the parabrachial nucleus are predominantly localized in the lateral division, with fewer cells in the medial and ventral parabrachial nucleus. No δ mRNA expression can be detected in the locus coeruleus, and low levels of expression are observed in the parabrachial nucleus.

In the trigeminal nuclei, δ receptor mRNA expression is high in the cells of the motor and spinal trigeminal (Fig. 10), with moderate levels of expression in the principal sensory nucleus. Comparatively few cells expressing low levels of μ and κ receptor mRNAs are seen in the principal sensory and motor trigeminal. In the spinal trigeminal nucleus, where μ receptor mRNA expression is low, cells expressing κ receptors have a high level of expression in the superficial portion of the caudal spinal trigeminal nucleus (Fig. 11, bottom). High levels of κ receptor mRNA are also seen in the paratrigeminal nucleus (Fig. 17C).

μ Receptor mRNA expression is high in the olivary complex. Scattered cells in the medial ventral, lateral ventral, superior paraolivary, and lateral superior olive express high levels of μ receptor mRNA, with no cells detected in the inferior olive. Comparatively, low levels of δ and κ receptor mRNA expression is seen in the medial ventral, lateral ventral, superior paraolivary, and lateral superior olive, with moderate levels of δ expression in the rostral preolivary nucleus. In the inferior olive, no δ receptor mRNA expression can be observed, but cells expressing κ receptor mRNA are localized in the β and α subunits.

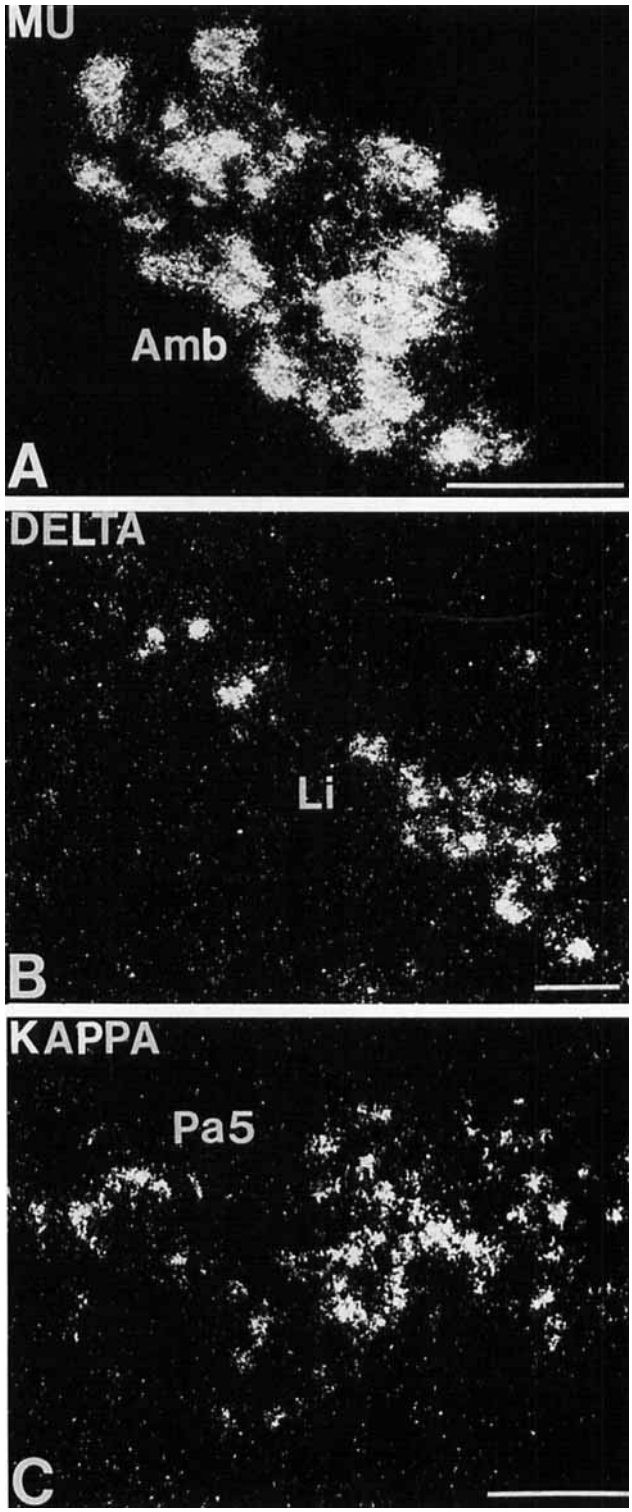

Fig. 17. Darkfield autoradiograms showing the cellular expression of μ, δ, and κ receptor mRNAs in brainstem nuclei. High levels of μ receptor mRNA expression are observed in nucleus ambiguus (**A**), whereas cells expressing δ or κ receptor mRNA are localized in the linear nucleus of the medulla (**B**) and the paratrigeminal (**C**) nucleus, respectively. Scale bars = 100 μm.

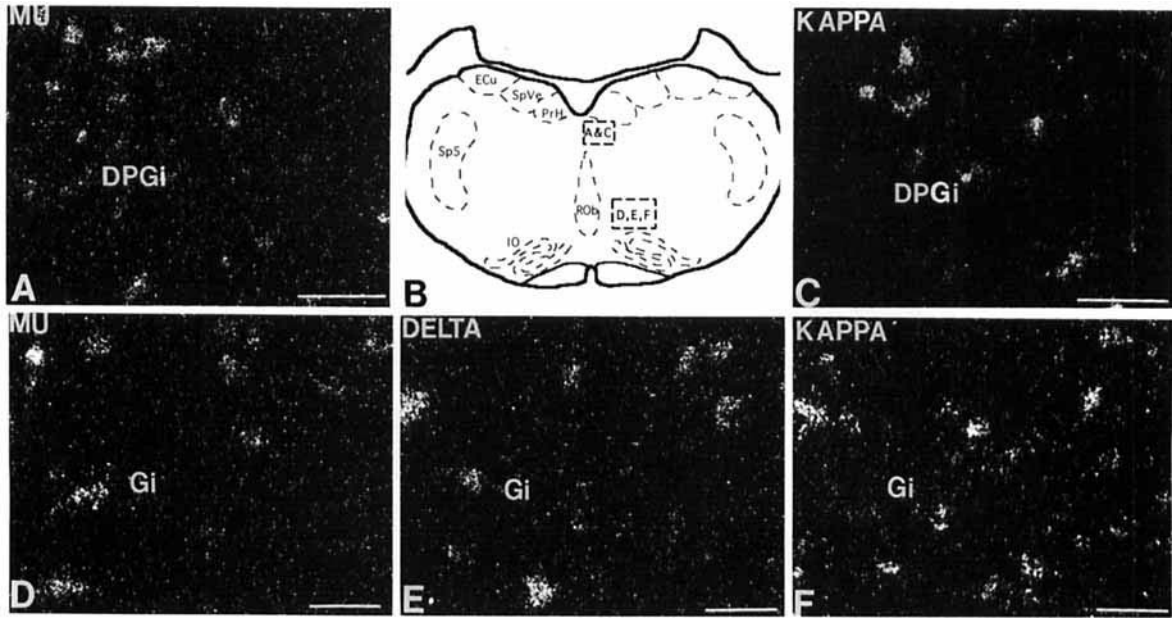

Fig. 18. Darkfield autoradiograms demonstrating the cellular expression of μ, δ, and κ receptor mRNAs in the dorsal paragigantocellular and the gigantocellular reticular nuclei. Regions highlighted in **B** indicate the areas of the brainstem examined in **A,C–F**. Large cells in the dorsal paragigantocellular nucleus express μ (A) and κ (C) receptor mRNAs. More ventrally, gigantocellular reticular nucleus cells expressing μ (D), δ (E), and κ (F) receptor mRNAs are observed. Scale bars = 100 μm.

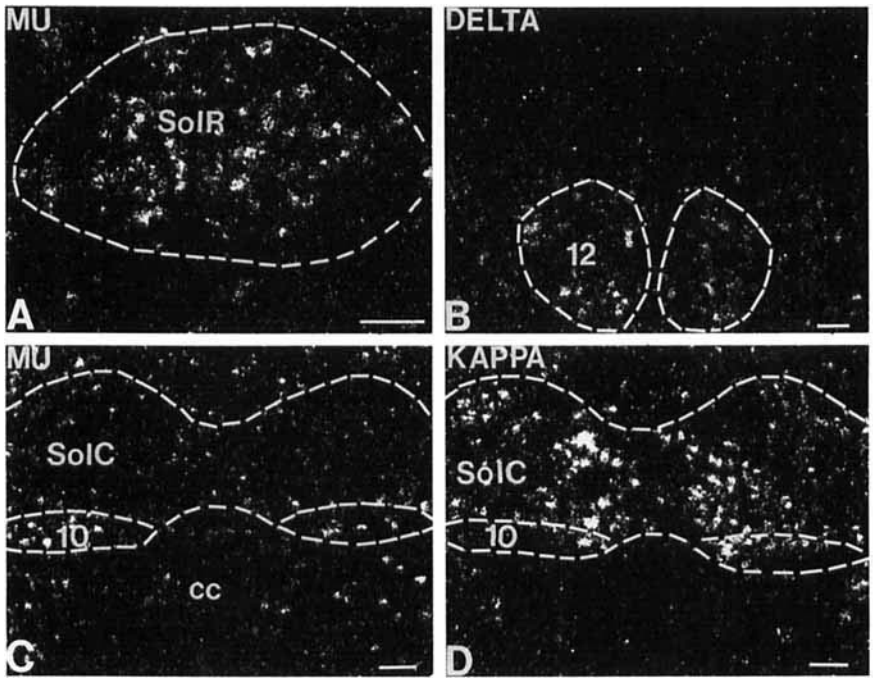

Fig. 19. Darkfield autoradiograms demonstrating the cellular expression of μ, δ, and κ receptor mRNAs in the nucleus of the solitary tract and the hypoglossal nucleus. Rostral-caudal differences in μ and κ receptor mRNA expression are observed in the nucleus of the solitary tract, with μ receptor mRNA expression predominantly in the rostral division (**A** vs. **C**), whereas cells expressing κ receptor mRNA are found in the commissural division (**D**). In addition to the nucleus of the solitary tract, cells of the dorsal motor vagus nucleus express μ (C) and κ (D) receptor mRNAs. **B** shows cells of the hypoglossal nucleus that express exclusively δ receptors. Scale bars = 100 μm.

Moderate levels of δ and κ receptor mRNA expression are observed in the cochlear nucleus. A few cells expressing μ receptor mRNA are also observed in the cochlear nucleus; however, their levels of expression are low. Similarly, in the vestibular nuclei, moderate levels of δ expression are seen in the medial and lateral nuclei, whereas only low levels of μ and κ receptor mRNA expression can be detected.

Scattered in the brainstem, the gigantocellular cells express high levels of μ, δ, and κ mRNAs (Fig. 18). Gigantocellular cells expressing μ, δ, and κ mRNA are

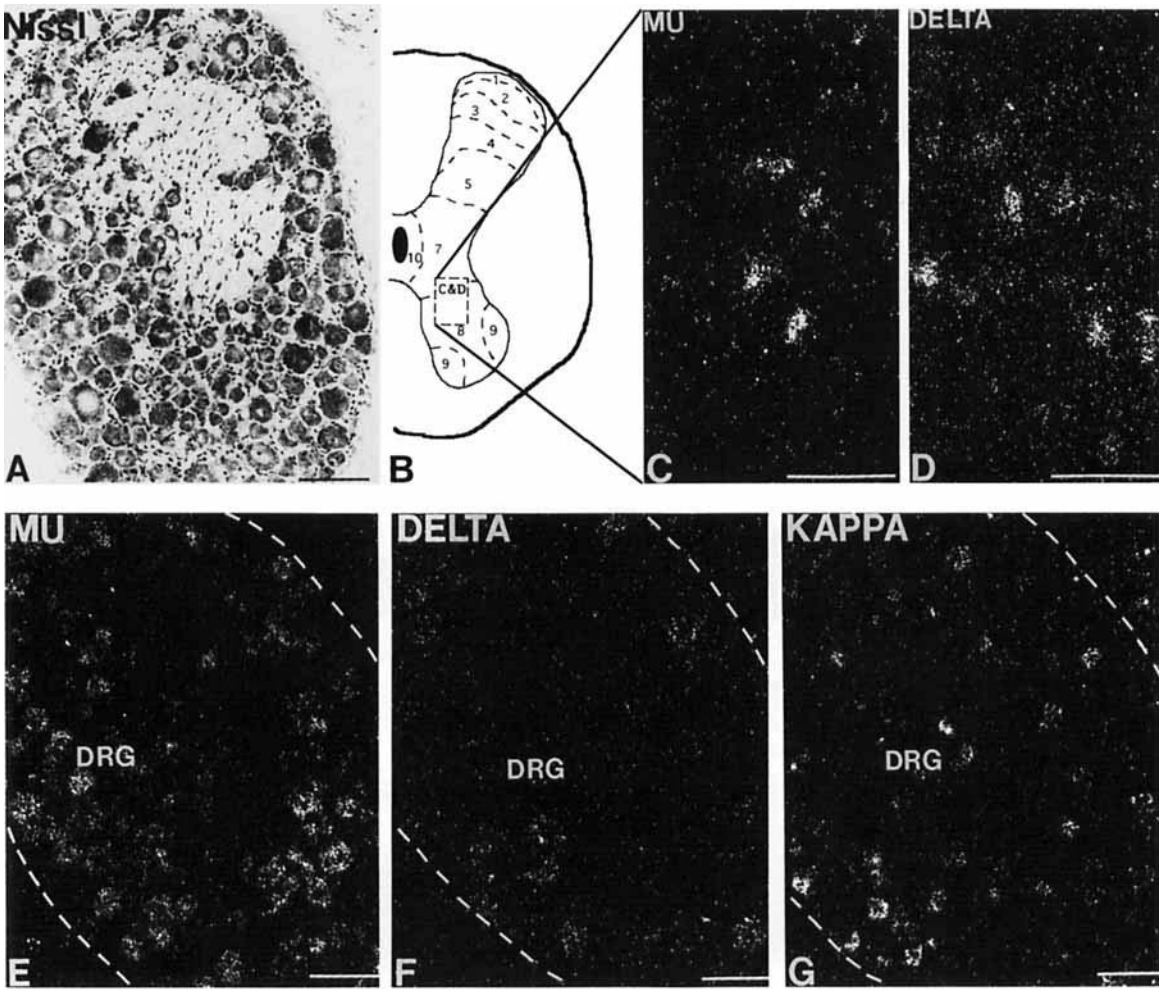

Fig. 20. Darkfield autoradiograms demonstrating the cellular expression of μ, δ, and κ receptor mRNAs in the dorsal root ganglia and thoracic spinal cord (**C–G**). A large number of dorsal root ganglia cells express μ and κ receptor mRNAs (E,G) compared to those expressing δ receptor mRNA (F). In addition, the subpopulation of dorsal root ganglia cells expressing the opioid receptor mRNAs appears to differ with δ receptors expressed in large-diameter cells, μ expressed in both medium- and large-diameter cells, and κ predominantly expressed in medium-diameter neurons. A Nissl-stained dorsal root ganglia section is provided in **A** and a schematic drawing of the thoracic spinal in **B** for comparison. C and D demonstrate the large neurons expressing either μ or δ receptor mRNAs in the ventral horn of the spinal cord. Scale bars = 100 μm.

widely distributed and are also seen in the dorsal paragigantocellular nucleus (Fig. 18A,C). μ, δ, And κ mRNA expression extends laterally to the intermediate reticular nucleus and caudally into the medullary reticular nucleus, where high levels of expression are observed (Figs. 10, 11).

Marked rostral-caudal differences are observed in the expression of opioid receptor mRNAs in the nucleus of the solitary tract. In the rostral nucleus of the solitary, μ expression levels are higher compared to the commissural division, where far fewer cells expressing μ mRNA are observed (Figs. 10 vs. 11; 19A,C). Cells expressing κ receptor mRNA, on the other hand, are predominantly localized in the commissural division of the nucleus of the solitary tract, with comparatively fewer cells expressing κ receptor mRNA in the rostral portion of the nucleus of the solitary tract (Figs. 10 vs. 11; 19C). In contrast, little if any δ receptor mRNA expression can be observed in the rostral or commissural nucleus of the solitary tract.

In the caudal brainstem, μ receptor mRNA expression is high in the nucleus ambiguus, dorsal motor nucleus of vagus, nucleus cuneatus, and nucleus gracilis (Figs. 10, 11, 17A, 19C). The cells in the nucleus ambiguus express exclusively μ receptor mRNA, with no δ or κ receptor expression observed. Similarly, in the nucleus cuneatus, μ receptor expression predominates, with only a few scattered cells expressing δ and κ receptor mRNAs observed. δ Receptor mRNA expression is high, however, in the external cuneate nucleus, where μ and κ expression is not detected. Cells of the dorsal motor nucleus of vagus, in addition to demonstrating high levels of μ receptor mRNA, also express high levels of κ receptor mRNA, with no detectable δ receptor expression. Low κ receptor mRNA expression is also seen in the nucleus gracilis.

Several regions in the brainstem exclusively express δ receptor mRNA. These nuclei include the trapezoid nucleus, linear nucleus of the medulla (Fig. 17B), hypoglossal nucleus (Fig. 19B), and lateral reticular nucleus (Fig. 11), where moderate to high levels of δ receptor mRNA expression are observed, with no μ or κ receptor mRNAs detected. Cells in the prepositus hypoglossal nucleus also express high levels

of δ receptor mRNA and moderate levels of κ receptor mRNA.

In the cerebellum, cells in the granular cell layer express δ receptor mRNA, with no μ or κ receptor mRNA detected. In addition to the lobules of the cerebellum, cells in the interposed and medial cerebellar nuclei express moderate levels of δ receptor mRNA, with low levels of μ and κ receptor mRNA expression. Cells in the lateral cerebellar nucleus, however, fail to show any μ, δ, or κ receptor mRNA expression.

Spinal cord and dorsal root ganglia

In the spinal cord, cells expressing μ, δ, and κ receptor mRNA are distributed in the laminae of the dorsal and ventral horns. Cells expressing μ receptor mRNA are localized predominantly in laminae 4, 5, 7, 8, and 10, with fewer cells observed in laminae 2 and 3 of the thoracic spinal cord (Fig. 20B,C). Cells expressing δ receptor mRNA are also scattered in the dorsal and ventral horn and are localized in laminae 4, 5, and 7–10, with a few cells in lamina 3 of the thoracic cord. In contrast, comparatively few cells expressing κ receptor mRNA are detected in the thoracic spinal cord, localized predominantly in laminae 4, 5, 7, and 10, with no cells detected in laminae 1–3 and 9. The localization of μ and δ receptor mRNA in large cells of the ventral horn suggests an opioid receptor expression in motor neurons of the spinal cord.

μ, δ, And κ receptor mRNA expression is also observed in the cells of the dorsal root ganglia; however, the population of ganglia neurons in which the opioid mRNAs are localized may differ (Fig. 20). μ Receptor mRNA expression, which is prominent in the dorsal root ganglia, is localized in medium- and large-diameter ganglion cells, whereas κ receptor expression is localized in smaller diameter neurons (Figs. 20A,E,G). Comparatively fewer dorsal root ganglia neurons express δ receptor mRNA, with δ receptor expression localized predominantly in large-diameter neurons (Fig. 20F).

DISCUSSION

The in situ hybridization results demonstrate a differential localization of μ, δ, and κ mRNAs in the central nervous system, consistent with the unique physiological and pharmacological functions associated with each of the opioid receptors. These findings complement previous receptor autoradiographic studies demonstrating a differential distribution of the μ, δ, and κ binding sites in the CNS (McLean et al., 1986; Mansour et al., 1987, 1988; Sharif and Hughes, 1989) and provide insights into the cellular localization of the opioid receptor mRNAs encoding these receptors and the neuronal circuits they may modulate. Cells expressing μ receptor mRNA have a widespread distribution and are localized in such regions as the olfactory bulb, caudate-putamen, nucleus accumbens, lateral and medial septum, diagonal band of Broca, most thalamic nuclei, hippocampus, amygdala, medial preoptic nucleus, superior and inferior colliculi, central gray, dorsal and median raphe, raphe magnus, locus coeruleus, parabrachial nucleus, pontine, intermediate gigantocellular and medullary reticular nuclei, nucleus ambiguus, nucleus of the solitary tract, nucleus gracilis and cuneatus, dorsal motor nucleus of vagus, spinal cord, and dorsal root ganglia. Cellular expression of δ receptor mRNA differs, with high levels of expression in such regions as the olfactory bulb, allo- and neocortical areas, caudate-putamen, nucleus accumbens, olfactory tubercle, ventromedial hypothalamus, hippocampus, amygdala, red nucleus, pontine nuclei, reticulotegmental nucleus, motor and spinal trigeminal, linear nucleus of the medulla, pontine and medullary reticular nuclei, lateral reticular nucleus, hypoglossal nucleus, spinal cord, and dorsal root ganglia. Cells expressing κ receptor mRNA demonstrate a third pattern of expression, with cells localized in such regions as the lateral orbital and agranular insular cortices; entorhinal cortex; layers 5 and 6 of parietal, temporal, and occipital cortices; claustrum; endopiriform nucleus; nucleus accumbens; olfactory tubercle; medial preoptic area; bed nucleus stria terminalis; amygdala; most hypothalamic nuclei; median eminence; infundibulum; paraventricular and centromedial nuclei of the thalamus; zona incerta; substantia nigra; ventral tegmental area; central gray; dorsal and laterodorsal tegmental nuclei; raphe nuclei; pontine and medullary reticular nuclei; locus coeruleus; parabrachial nucleus; paratrigeminal and spinal trigeminal nuclei; nucleus of the solitary tract; spinal cord; and dorsal root ganglia.

Given the high nucleic acid and amino acid homologies of the cloned μ, δ, and κ receptors, the potential exists that a cRNA probe chosen to identify a specific mRNA may cross-hybridize to another opioid receptor type. This risk is mitigated by the facts that 1) three distinct mRNA distributions are observed that correspond to well-known opioid receptor binding distributions and 2) when cRNA probes directed to different regions of the μ or κ receptors are used to identify their respective mRNA distributions, distinct μ or κ mRNA expression patterns are observed (Mansour et al., 1994a,b). These μ and κ mRNA expression patterns differ from those seen with δ receptor cRNA probes presented here.

Although it is unlikely, then, that the cRNA probes used under the present conditions are cross-hybridizing to another cloned receptor, it is possible that they are labeling multiple mRNAs. The intronic organization of the μ, δ, and κ genes suggests that alternative splicing may produce multiple mRNA forms and is consistent with the multiple mRNA bands observed with Northern blots of the μ and δ receptors (Evans et al., 1992; Fukada et al., 1993; Thompson et al., 1993). Insofar as the nucleic acid sequences of these alternatively spliced mRNAs have not been identified, it is difficult to know whether the in situ hybridization probes used in this study hybridize to one or more of these mRNAs. Clearly, this is an important consideration for future studies, in that the levels of receptor mRNA expression and their response to regulatory stimuli can vary markedly between alternatively spliced forms of the same receptor (Arnauld et al., 1991; Neve et al., 1991). In addition, pharmacological and receptor binding studies suggest the presence of subtypes for each class of receptors (Wolozin et al., 1981; Iyengar et al., 1986; Clark et al., 1989; Rothman et al., 1989; Jiang et al., 1991; Sofuoglu et al., 1991), and it is unclear whether these represent alternatively spliced forms, distinct gene products, or differential posttranslational processing.

A finding not emphasized in the results that needs to be discussed is that, although expression levels of each of the receptor mRNA differ between brain regions, their overall levels of expression in the rat CNS are quite comparable. This is evidenced from the similar film exposure times used to identify the μ, δ, and κ mRNAs and by similar results obtained with Northern blot analysis (Fukada et al., 1993;

Thompson et al., 1993; Yasuda et al., 1993). This is interesting, in that the numbers of κ receptor binding sites in the rat brain are significantly lower than numbers of μ or δ sites as measured by binding studies (Gillan and Kosterlitz, 1982), and it suggests a differential translation or transcriptional regulation of the κ opioid receptors. Clearly, more research is necessary to confirm this suggestion, and controls for cRNA length, exposure time, and tissue penetration are necessary.

A major advantage in using in situ hybridization techniques over traditional receptor autoradiography to localize receptors is the cellular resolution that can be achieved. Specific mRNAs can be localized to single cells, often aiding in characterizing their function. The identification of κ receptor mRNA in the cells of substantia nigra (SN) and ventral tegmental areas (VTA), for example, suggests a localization in dopamine-synthesizing cells and a possible modulation of dopamine release. This conclusion is supported by 6-hydroxydopamine lesion studies demonstrating a complete loss of κ receptor mRNA in the SN and VTA following the selective ablation of dopamine neurons (Mansour et al., unpublished observations) and by pharmacological studies showing that κ agonists selectively decrease presynaptic dopamine release in the rat striatum (Mulder et al., 1984; Di Chiara and Imperato, 1988; Spanagel et al., 1990, 1992).

Similarly, the distinct δ receptor mRNA distribution in the caudate-putamen is suggestive of a specific localization in cholinergic neurons. A moderate level of δ receptor mRNA expression is observed in most medium-sized neurons of the caudate-putamen, with a small proportion of large, widely scattered neurons expressing high levels of δ receptor mRNA. This small population of large neurons has anatomical and morphological properties similar to those of the cholinergic neurons found in the striatum and suggests that δ agonists may modulate release of acetylcholine. Pharmacological studies have demonstrated that selective δ and μ agonists inhibit acetylcholine release from the striatum, consistent with this observation (Wood and Rackham, 1981; Mulder et al., 1984).

Other examples can be seen in the distribution of μ receptor mRNA. Cells expressing μ mRNA are localized in the locus coeruleus, suggesting a μ modulation of norepinephrine (NE) release. The mechanism of action of μ opioids on NE release is unclear; electrophysiological studies (Aghajanian and Wang, 1986; North et al., 1987) suggest that μ agonists selectively modulate the firing of locus coeruleus neurons, whereas other studies using cortical slice preparations (Mulder et al., 1987; Werling et al., 1987; Schoffelmeer et al., 1988) suggest an inhibition of NE release from cortical nerve terminals via a presynaptic mechanism. In that μ receptor binding sites and mRNA are localized in the locus coeruleus and cortex, both mechanisms of action are possible. Given the comparatively lower number of cells in the locus coeruleus that express κ compared to μ, the ineffectiveness of κ agonists to inhibit release (Mulder et al., 1987; Werling et al., 1987) may be due to a localization of κ receptors in subpopulation noradrenergic neurons that do not project as extensively to the rat cortex. δ Agonists are likely unable to directly inhibit NE release due to the absence of δ receptors in the locus coeruleus. Furthermore, receptor binding and mRNA data suggest that δ receptors are not on NE terminals in the cortex but may have a postsynaptic localization.

In the hippocampus, the scattered nature of the μ-expressing cells in the pyramidal cell layer is consistent with a large body of electrophysiological findings suggesting that these are inhibitory γ-aminobutyric acidergic (GABAergic) interneurons (Nicoll et al., 1977, 1980; Zieglgänsberger et al., 1979; Madison and Nicoll, 1988). The distribution of cells expressing δ receptor mRNA differs, with a moderate level of expression in most pyramidal cells and with occasional cells (possibly interneurons) expressing high levels of δ receptor mRNA. Cells expressing κ receptor mRNA are not observed in the dorsal hippocampus but are localized to the stratum radiatum of the temporal hippocampus.

In the case of serotonin regulation, the anatomical evidence suggests that μ and κ receptors may be primarily involved. Although not all cells in the raphe nuclei contain serotonin, the high levels of κ receptor mRNA expression in the caudal linear raphe, dorsal raphe, and raphe obscurus and of μ receptor mRNA expression in the median raphe, dorsal raphe, and raphe magnus are consistent with a role of κ and μ receptors in regulating serotonin release (Yaksh and Tyce, 1979; VonVoigtlander et al., 1983). This is consonant with pharmacological data suggesting that serotonergic mechanisms are important in antinociceptive responses mediated by μ and κ agonists (Yaksh, 1979; VonVoigtlander et al., 1984; Ho and Takemori, 1989). A comparatively lesser role is likely played by δ receptors in serotonin release, with a few cells localized in the raphe magnus and median raphe.

The role of opioid receptors in modulating neuropeptide release and, particularly, the release of opioid peptides is less clear. The localization of κ-expressing cells in such regions as the paraventricular magnocellular neurons, nucleus accumbens, and centrolateral nucleus of the amygdala, all of which contain prodynorphin-expressing cell bodies, suggests a possible κ autoreceptor function. κ Agonists have been demonstrated to inhibit the release of the vasopressin (AVP) and oxytocin (OXY; Slizgi and Ludens, 1982; Carter and Lightman, 1984; Bicknell et al., 1985; Falke, 1988) and, presumably, the prodynorphin and proenkephalin peptides with which they are colocalized (Martin and Voigt, 1981; Watson et al., 1982; Whitnall et al., 1983). However, given that prodynorphin and proenkephalin cleavage products are able to bind μ, δ, and κ receptors (Quirion and Pert, 1981; Wüster et al., 1981; Schultz et al., 1982; Quirion and Weiss, 1983; Shook et al., 1988) and the often overlapping receptor and peptide distributions observed in many brain regions (Khachaturian et al., 1982, 1983; Fallon and Leslie, 1986), the precise relationships between the opioid peptides and potential autoreceptors are difficult to predict at present. Clearly, more studies are necessary to colocalize the opioid peptides and opioid receptors in multiple brain regions before one can evaluate their precise relationships and to determine whether opioid peptides can modulate their own release.

The localization of opioid receptor mRNAs within well-characterized circuits and neuronal systems can shed light on their functions. For example, both receptor autoradiographic (Atweh and Kuhar, 1977a–c; Herkenham and Pert, 1980; Mansour et al., 1987) and in situ hybridization results presented here strongly suggest that opioid receptors are intimately involved in the processing of sensory, proprioceptive, and vestibular information. The type of opioid receptor predominantly involved is dependent on sensory modality and level of anatomical processing. In the olfactory system, for example, μ and δ receptors are likely important in the primary processing and integration of olfactory information. μ- And δ-containing cells are localized in the main olfactory bulb and primary olfactory cortex

as well as more integrative olfactory processing regions such as the anterior olfactory nuclei, diagonal band of Broca, bed nucleus of the stria terminalis and endopiriform nucleus, amygdala, and entorhinal cortex. κ Receptors, on the other hand, may play a more modulatory role, not localized to a large degree in the main and accessory olfactory bulbs and piriform cortex but expressed in regions that receive primary and secondary olfactory afferents such as the anterior olfactory nucleus, olfactory tubercle, amygdala, entorhinal cortex, nucleus of the lateral olfactory tract, and bed nucleus stria terminalis. The localization of cells expressing μ receptor mRNA in the accessory olfactory bulb, bed nucleus of the accessory olfactory tract, medial amygdala, and posteromedial cortical amygdala suggests that predominantly μ receptors process information from the cells in the vomeronasal organ, which may be important in sexual and aggressive behaviors (Winans and Powers, 1977; Lehman and Winans, 1982).

In the visual system, μ, δ, and κ receptor mRNAs are localized in the cells of the superior colliculus, lateral and ventral lateral geniculate, and visual cortex, suggesting that all three receptors may be involved in the processing of visual information. In the superior colliculus, cells expressing μ and δ mRNA have a distinct laminar distribution, whereas those expressing κ mRNA are more homogeneously distributed, possibly reflecting differential processing of visual information such as orientation to visual stimuli. In addition to μ and κ, the localization of δ receptor mRNA in the intermediate and deep layers of the superior colliculus is particularly interesting in view of the projections of these layers to the pontine nuclei and lateral reticular nucleus, regions of high δ mRNA expression, suggesting a δ receptor circuit. The dorsal lateral geniculate nucleus of the thalamus, which receives primary visual projections from the retina and provides the main afferent projections to the visual cortex (Ribak and Peters, 1975), expresses predominantly μ mRNA, whereas the cells in ventral lateral geniculate express both μ and κ and may be involved in brightness discrimination (Legg and Cowey, 1977). Cells expressing μ, δ, and κ mRNAs are also localized in specific laminae of the visual cortex, where they may play a role in visual discrimination. Cells of the claustrum, which express high levels of δ and κ but low levels of μ mRNA, project to the visual cortex (LeVay and Sherk, 1981), potentially influencing the processing of visual information.

In the auditory system, the cochlear and trapezoid nuclei, regions of primary and secondary auditory processing, express predominantly δ receptor mRNA, with moderate levels of κ mRNA expression in the cochlear nucleus. However, higher sites of auditory processing such as the superior olivary complex, the nuclei of the lateral lemniscus and medial geniculate bodies express predominantly μ receptor mRNA. In the inferior colliculus, cells expressing μ and δ receptor mRNAs are localized in the dorsal, central, and external cortex, whereas those expressing κ are localized only in the dorsal and external cortex. Taken together, the results suggest that δ and κ receptors may be involved in the primary processing of auditory information, whereas μ receptors may be involved in further integration of the information prior to relaying it to the auditory cortex.

In the somatosensory systems, cells expressing opioid receptor mRNAs are observed in the dorsal root ganglia, dorsal horn of the spinal cord, nucleus gracilis and cuneatus, sensory and spinal trigeminal, ventromedial, posterior and intralaminar nuclei of the thalamus, and the parietal cortex. The opioid receptor mRNAs are differentially expressed in the dorsal root ganglia, with μ receptor mRNA localized in medium-sized and large cells, κ in smaller diameter neurons, and δ in predominantly large-diameter cells. This differential distribution may be related to the differential processing of thermoceptive, mechanoceptive, and nociceptive responses mediated by these neurons. Concordant with this suggestion, opioid analgesics vary in their effectiveness depending on type and intensity of nociceptive stimulus used and receptor type activated (Schmauss and Yaksh, 1984; Millan, 1986, 1990). κ-Selective analgesics are effective in modulating visceral and low-intensity thermal and mechanical nociceptive responses, whereas δ analgesics are potent in inhibiting a range of thermal and mechanical stimuli and are relatively ineffective in inhibiting visceral nociception. μ Analgesics have a broader range of action on thermal, mechanical, and visceral stimuli that might be related to the larger number of medium- and large-diameter dorsal root ganglia neurons that express μ receptor mRNA. Although cell diameter is a discriminating feature in DRG neurons, colocalization studies with neuropeptides such as somatostatin, enkephalin, substance P, and dynorphin are needed to characterize these cells properly.

Afferent fibers from cells in the DRG send collaterals that synapse in the dorsal horn of the spinal cord, where μ-, δ-, and κ-expressing cells are observed, and the nucleus gracilis and cuneatus, where cells expressing μ receptor mRNA predominate. This, in addition to the high levels of μ receptor mRNA expression observed in the posterior, ventromedial, and intralaminar nuclei of the thalamus, suggests that μ receptors may regulate much of the somatosensory information received from the body. The δ and κ mRNA expression observed in the spinal and sensory trigeminal nuclei, however, argues that these sites may be more involved in receiving and processing information from the face and nasal mucosa as well as in higher order processing in the somatosensory cortex.

In addition to directly inhibiting sensory afferents entering the spinal cord, opioid analgesics produce their effects by modulating descending pain-inhibitory pathways originating in the midbrain and brainstem (Fields and Basbaum, 1978; Yaksh and Rudy, 1978). The central gray, the midline raphe nuclei, and the mesencephalic and gigantocellular reticular nuclei, which have been implicated in these pathways, are regions of μ, δ, and κ receptor mRNA expression. Although there is agreement that μ analgesics produce their effects at both supraspinal and spinal sites of actions, much debate surrounds δ and κ analgesics; some have argued for an exclusively spinal site of action (Piercy et al., 1982; Kaneko et al., 1983; Fang et al., 1986). The anatomical findings of predominantly μ and κ mRNA expression in the central gray and raphe nuclei and μ, δ, and κ expression in the mesencephalic and gigantocellular reticular nuclei support the view that μ, δ, and κ agonists can produce their effects at both the supraspinal and the spinal levels (Porreca et al., 1987; Millan et al., 1989).

Opioid agonists, in addition to being potent analgesics, produce significant effects on respiratory, cardiovascular, and gastrointestinal functions (Pfeiffer et al., 1983a; Porreca et al., 1984; Fox and Burks, 1988; Kiritsy-Roy et al., 1989). Many of these effects are likely coordinated by the nucleus of the solitary tract (Carter and Lightman, 1985; Rhim et al., 1993), nucleus ambiguus, and dorsal motor nucleus of vagus, regions that express predominantly μ and/or κ receptor mRNA. The caudal portion of the nucleus

of the solitary tract, which has been implicated in cardiovascular function, contains predominantly κ, with some μ, mRNA expression, whereas the rostral portion, which has been associated with gustatory functions, contains predominantly cells expressing μ receptor mRNA. The paratrigeminal, which receives visceral input from the nasal mucosa, expresses exclusively κ receptor mRNA. These anatomical findings suggest that predominantly μ and κ receptors mediate the cardiovascular, respiratory, and gastrointestinal responses observed with opioid administration.

Among the better characterized effects of opioid agonists are their actions on hormonal release. Several studies (Pang et al., 1977; Slizgi and Ludens, 1982; Pfieffer et al., 1983b, 1985; Carter and Lightman, 1984; Bicknell et al., 1985; Krulich et al., 1986; Laedem and Yagenova, 1987; Iyengar et al., 1987; Manzanares et al., 1990) have demonstrated that opioid agonists increase the release of prolactin (PRL), growth hormone (GH), proopiomelanocortin peptides (POMC), and corticosteroids (CORT), while decreasing the release of luteinizing hormone (LH), OXY, and AVP. The relative involvement of the specific receptor types in these hormonal effects and the anatomical site of action is, however, less clear. Most of the inconsistencies observed surround the involvement of δ receptors, with some studies demonstrating δ receptor-mediated effects on PRL, POMC, and LH secretion and others reporting no effect (Pfeiffer et al., 1983b; Wiesner et al., 1985; Iyengar et al., 1987; Leadem and Yagenova, 1987; Kehoe et al., 1993). Such inconsistencies are likely due to a previous lack of δ-selective agonists, which may have confused the interpretation of the results. In addition, the relatively low levels of δ receptor binding and mRNA expression observed in the neuroendocrine systems mediating these effects suggest that δ receptors may play a minor role. μ And κ agonists similarly increase PRL, POMC, and CORT and decrease LH, suggesting a wide range of actions on both hypothalamic-pituitary-gonadal as well as adrenal axes. The inhibition of OXY and AVP is selective for κ receptor agonists (Carter and Lightman, 1984; Bicknell et al., 1985; Leander et al., 1985, 1987), suggesting a specific role of κ receptors in lactation, uterine contraction, and water balance. Some studies report an inhibition of AVP and OXY release with μ agonists (Van de Heijning et al., 1991).

The hormonal effects described above demonstrate that opioid peptides can modulate the release of hormones from the anterior (ACTH, PRL, GH, LH), intermediate (α-melanocyte-stimulating hormone; α-MSH), and neural (OXY, AVP) lobes of the pituitary. The anterior and intermediate lobes of the pituitary are not, however, the primary sites of action; agonists fail to affect hormonal release in pituitary cultures (Buckingham, 1982), and receptor autoradiographic studies have localized only κ binding sites in the neural lobe, with no μ or δ binding observed in the other lobes of the pituitary (Herkenham, 1986). The focus has, therefore, shifted to the cells of the hypothalamus that project to the median eminence and influence the release of anterior pituitary hormones to explain the μ receptor-mediated effects (Wiesner et al., 1984; Kapoor and Willoughby, 1990). For example, μ and κ expression in the medial preoptic area and μ expression in the medial preoptic nucleus, where high numbers of luteinizing hormone-releasing hormone (LHRH) neurons are localized (for review, see Swanson, 1987), suggest that these regions may be important in opioid-mediated inhibition of LH secretion. Localization of cells expressing μ and κ mRNAs in the parvocellular paraventricular hypothalamic nucleus suggests a possible colocalization with CRH and may be related to the increased release of corticosteroids following opioid administration. Opioid mediation of prolactin and α-MSH release, which are dependent on tuberoinfundibular and tuberohypophysial dopamine, respectively, may be regulated by μ-and κ-expressing cells in the arcuate nucleus (Manzanares et al., 1990, 1992). Similarly, the localization of β-endorphin and growth hormone-releasing factor in the cells of the arcuate nucleus suggests that this may be the site of action of μ- and κ-mediated effects on corticosteroids and GH. Corticosteroid levels may be influenced by β-endorphin neurons projecting to the median eminence to affect the pituitary directly or via their projections to the paraventricular nucleus. Colocalization studies examining the distribution of the opioid receptor mRNAs in relation to the hypothalamic releasing hormones as well as microinjection of specific opioid agonists in selective nuclei are clearly necessary to verify these suggestions.

Although we have emphasized potential hypothalamic nuclei in mediating these opioid-hormone effects, other regions in the CNS, particularly in the midbrain and brainstem, that project to the hypothalamus likely play critical roles. The nucleus of the solitary tract and parabrachial nucleus, which project to paraventricular hypothalamic and arcuate nuclei, for example, may be important sites of hormonal regulation by μ agonists, especially given the comparatively low levels of μ receptor mRNA expression found in the hypothalamic nuclei.

The localization of cells in the median eminence and infundibulum that exclusively express κ receptor mRNA and binding (Mansour et al., 1987) suggests that κ agonists, unlike μ, may regulate hormone release by their action on cell terminals at the level of the median eminence in addition to modulating activity of hypothalamic neurons. The identification of ependymal cells as well as neurons in the median eminence suggests that these cells may monitor levels of opioid peptides in the cerebrospinal fluid (CSF) to regulate subsequent release. Colocalization studies with glial-and neuronal-specific markers are necessary to characterize the κ receptor-expressing cells in the median eminence and infundibular stem. A few scattered neurons expressing β-endorphin have been identified in the median eminence (Ishikawa et al., 1992), and it will be interesting to see if they also express κ receptors.

Conflicting results have emerged with regard to the site of action of κ agonists on OXY and AVP release. Some studies using isolated terminals from rat neural lobe suggest that κ receptors are localized on oxytocin terminals and not vasopressin (Falke and Martin, 1988), whereas others demonstrate that κ receptors are on both OXY and AVP terminals and are able to inhibit the release of both neurophysins (Zhao et al., 1988). The failure to detect cells expressing κ receptor mRNA in the neural lobe (Mansour et al., 1994a) and the high levels of κ receptor mRNA in the magnocellular paraventricular and supraoptic nuclei suggest that the κ receptor binding sites are synthesized in these hypothalamic nuclei and are transported to terminals in the neural lobe. Colocalization studies in the paraventricular and supraoptic nuclei are, however, necessary to determine whether there is a preferential localization with OXY or AVP.

Opioid receptors have been implicated in the control of feeding behavior (Morley and Levine, 1983; Leibowitz, 1985; Gosnell et al., 1986, 1987; Cooper et al., 1988). Generally, μ and κ agonists such as morphine, DAMGO, and U50,488 produce an increase in feeding, whereas opiate

antagonists reduce food intake. The majority of studies support a μ and κ receptor involvement in feeding, although some studies suggest that δ receptors may also be involved (Bakshi and Kelly, 1993). The localization of cells expressing μ and κ receptor mRNAs in the gustatory-related nuclei of the CNS, including the nucleus of the solitary tract, parabrachial nucleus, bed nucleus stria terminalis, central amygdala, and lateral and paraventricular hypothalamus, is supportive of a broad role of μ and κ receptors in maintaining feeding behavior. The findings that μ and δ agonists can elicit a feeding response in the nucleus accumbens (Bakshi and Kelly, 1993) suggest that this site may be involved in the rewarding properties of food. On the other hand, the high levels of δ and κ receptor mRNA expression in the ventromedial nucleus of the hypothalamus suggest that these receptor types may be important in long-term food intake and satiety. In that feeding is a complex behavior, it is not surprising that all three opioid receptor types may be differentially involved depending on the site of injection, the neural system activated, and the physiological state of the animal.

In contrast to most opiate-mediated behaviors, opioid agonists produced markedly different effects on locomotion. At low doses, μ and δ agonists produce an increase in gross motor activity, although, at high doses, μ agonists produce catalepsy and profound muscular rigidity in the rat (Iwamoto et al., 1981). κ Agonists, on the other hand, fail to produce an increase in locomotion at any dose and have predominantly sedative effects (Martin et al., 1976). These behavioral differences in response to opioids have been associated with a differential release of nigrostriatal dopamine, with μ and δ agonists increasing, and κ agonists decreasing, striatal dopamine release (Mulder et al., 1984; Di Chiara and Imperato, 1988). The distribution of opioid binding sites and receptor mRNAs in the nigrostriatal system suggests that the opioid receptors may be differentially localized within the nigrostriatal loop, in part explaining these effects on dopamine release. The high levels of κ mRNA expression in the substantia nigra, pars compacta, in conjunction with little κ receptor binding (Mansour et al., 1987; 1994a), suggest that these sites are synthesized in the pars compacta and are transported to presynaptic terminals, where they may directly affect dopaminergic release. On the other hand, the presence of μ receptor binding sites in the substantia nigra, pars reticulata, with few cells expressing μ receptor mRNA, suggests that these binding sites are on terminals originating, at least in part, in the striatum. Given that μ agonists iontophoretically applied to the substantia nigra, pars compacta, fail to affect dopaminergic cell firing (Hommer and Pert, 1983), the increase in dopamine release following systemic μ agonists is likely indirect. The anatomical data suggest that μ receptors localized in striatal cells bodies that project to the dopaminergic cells of the substantia nigra may mediate the increase in dopamine release and the observed increase in locomotion with μ agonists. The presence of δ receptor mRNA and binding in the substantia nigra, pars reticulata, suggests a local synthesis of δ receptors and an indirect modulation of dopamine release via inhibition of an inhibitory interneuron (Collingridge and Davies, 1982; Hommer and Pert, 1983). The visualization of κ binding sites and mRNA in the substantia nigra, pars reticulata, similarly suggests a local synthesis; however, these receptors are localized in a population of cells different from those that express δ. This is supported by the finding that intranigral injections of κ agonists produce circling that is independent of dopamine systems, whereas circling induced by δ agonists requires intact dopamine neurons (Matsumoto et al., 1988).

Opposing effects of opioids are also observed with regard to their positive reinforcing or hedonic properties. μ And δ agonists generally act as reinforcers, being self-administered into specific brain areas and positively associated in conditioned place-preference paradigms (Bozarth and Wise, 1981, 1984; Shippenberg et al., 1987). κ Agonists, on the other hand, are aversive, reportedly producing dysphoria in humans (Pfeifer et al., 1986) and conditioned place aversion in rats in paired-drug paradigms (Bals-Kubik et al., 1989). These behavioral differences have been linked to the effects of opioid agonists on mesolimbic dopamine systems, with μ and δ agonists stimulating release of dopamine in the nucleus accumbens and κ agonists decreasing release, paralleling the effects observed in the nigrostriatal system (Di Chiara and Imperato, 1988). Recent electrophysiological studies (e.g., Johnson and North, 1992) have generally been supportive of this position, demonstrating that μ agonists hyperpolarize dopamine neurons in the ventral tegmental area indirectly via a secondary GABAergic interneuron. Cells expressing μ receptor mRNA were detected in the ventral tegmental area in the present study and, given these electrophysiological results, may be localized on GABA neurons. Because it is difficult to compare these anatomical findings directly to the electrophysiological findings presented above, colocalization studies of the opioid receptor and GABA mRNAs are necessary to confirm these observations.

In addition to gross motor movements, the localization of opioid receptor mRNAs in brainstem motor nuclei that innervate the head and tongue suggests that opioid receptors mediate a broad range of motor functions. The selective expression of δ receptor mRNA in the hypoglossal nucleus and μ receptor in the nucleus ambiguus, for example, suggests that both of these receptors may be important in controlling tongue movements. Similarly, the high levels of δ receptor mRNA expression and lower levels of κ and μ expression in the motor trigeminal nucleus suggest that predominantly δ receptors may be involved in controlling the muscles of mastication. A further role of μ and δ receptors in modulating motor responses is suggested by a localization of μ and δ receptor mRNAs in the oculomotor nucleus and the ventral horn of the spinal cord.

Detection of δ receptor mRNA in the lobules of the cerebellum is somewhat surprising given the lack of specific δ receptor binding. This may reflect a dissociation of transcriptional and translational control of δ receptors in the cerebellum, so, although the mRNA is present, it is not effectively translated into a functional binding protein. Alternatively, the relatively high levels of nonspecific binding observed with selective δ ligands, such as DPDPE, may not allow the detection of low levels of δ receptor binding.

It is tempting to speculate that, given the levels of δ receptor mRNA expression in all cortical regions, the pontine nuclei, red nucleus, lateral reticular nucleus, cerebellum, and spinal cord, a primary function of δ receptors may be that of sensory-motor integration. Much of the somatosensory motor, visual, and auditory cortices project to the pontine nuclei, which, in turn, project to the cerebellum, which is in the position to influence descending pathways. This pathway, in addition to the corticospinal, corticorubrospinal, and the reticulocerebellar pathways, provides the major connections between cortical areas and either the cerebellum or the spinal cord. These pathways are impor-

tant in initiating voluntary movements and may represent specific δ receptor circuits.

In conclusion, this study provides the first comparative analysis of the μ, δ, and κ receptor mRNA distributions in the CNS. It lays the foundation for future tract-tracing, colocalization, and regulatory studies that will further define the specific circuits and functions mediated by the opioid receptors. These studies in combination with those designed to visualize the opioid peptides will provide insight into the organization of the opioid systems and a better understanding of the opioid receptors with which they interact.

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