Anatomy of CNS opioid receptors

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There is a wide body of evidence to suggest the existence of at least three distinct opioid receptor types in the CNS, referred to as μ, δ, and κ. This paper reviews some of the findings that have led to this conclusion and the anatomical distributions of these sites in the rat brain. Their relation to the opioid peptides and some of the proposed functions mediated by these receptor systems are also discussed.

Largely because of their clinical significance and recent technological advances, the opioid systems are among the best understood peptide systems in the CNS. While it is difficult to pinpoint precisely what has caused this interest, it is clear that the key elements included the discovery of opioid peptides in the CNS[1], the description of their genes, mRNAs and precursors[2–4], the characterization of their receptors[5], and the finding that these peptides are released following certain forms of stimulation[6]. Since it is well beyond the scope of this paper to review this field comprehensively, we have chosen to concentrate our efforts on the anatomical distribution of the opioid receptors in the CNS and their relationships with the opioid peptide systems. Having presented this anatomical information, we will then discuss some of the functions thought to be mediated by these multiple receptors.

Historically, the discovery of opioid receptors[7] preceded the isolation and characterization of the opioid peptides. Once we knew of only one opiate receptor – now there have been suggestions of as many as nine[8–12]. This review will focus on the three widely accepted opioid receptor types which are referred to as μ, δ and κ, and will discuss their possible interactions with the pro-opiomelanocortin (POMC), pro-enkephalin and pro-dynorphin peptide systems.

Several lines of evidence support the existence of multiple opioid receptors. One of the earliest findings to suggest this multiplicity was the demonstration by Martin and his colleagues[13] that different classes of opiate drugs produced distinct behavioral syndromes and that tolerance to one group of opiates did not result in cross-tolerance to another class of opiate drugs. On the basis of these findings, Martin and his colleagues proposed the existence of three types of opioid receptors: μ for morphine-like compounds, κ for ketocyclazocine-like drugs and σ for drugs such as SKF 10,047. Further support for this position came from studies using peripheral organ bioassays where the relative potencies of several opioids and opiate antagonists varied with tissue system[14], again suggesting a heterogeneity of receptors. In addition, investigators using these tissue systems suggested the existence of yet another receptor type, referred to as δ, named for the mouse vas deferens bioassay, where enkephalin peptides were found to be particularly potent[5]. The findings from these early pharmacological experiments were further supported

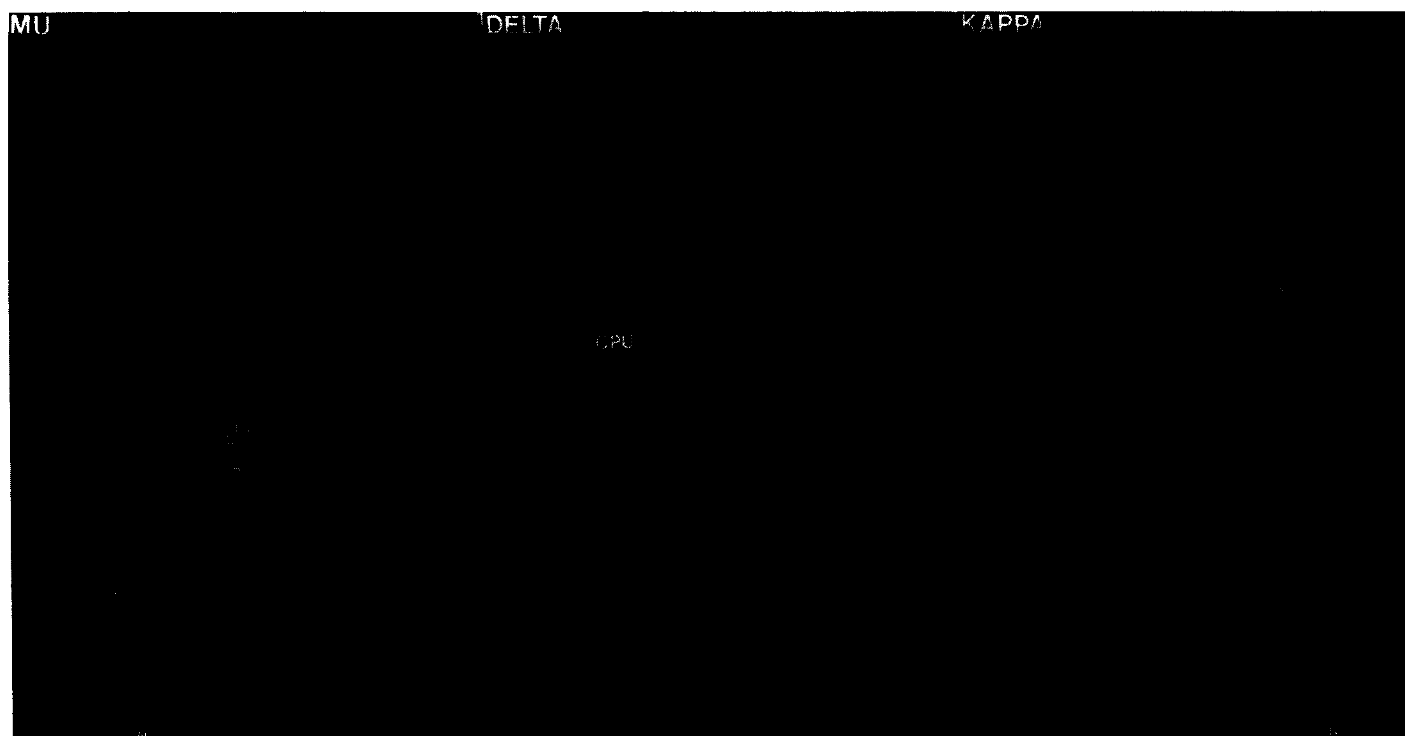

Fig. 1. *Darkfield autoradiograms of mu, delta and kappa receptors using horizontal rat brain sections. Note the three distinct receptor distributions. μ sites were labelled with [^{3}H]DAGO (Tyr-D-Ala-Gly-MePhe-Gly-ol), δ sites were labelled with [^{3}H]DPDPE (D-Pen^2, D-Pen^5-enkephalin), and κ sites were labelled with [^{3}H]bremazocine in the presence of saturating concentrations of DAGO and DPDPE. The binding conditions were such that approximately 75% of each receptor site was occupied[19,20]. These conditions were used to produce all the autoradiograms presented in this review. Abbreviations not listed in the centrefold include: CG, central gray; DR, dorsal raphe; f, fornix; Pir, piriform cortex; PRTX, parietal cortex; THL, thalamus; 4V, fourth ventricle.*

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by homogenate binding and autoradiographic studies demonstrating μ, δ and κ receptors as distinct opioid binding sites[15–23], with σ receptors being non-opioid in nature. In the following sections we present some of the anatomical evidence that has led to this conclusion and the functional implications of these results.

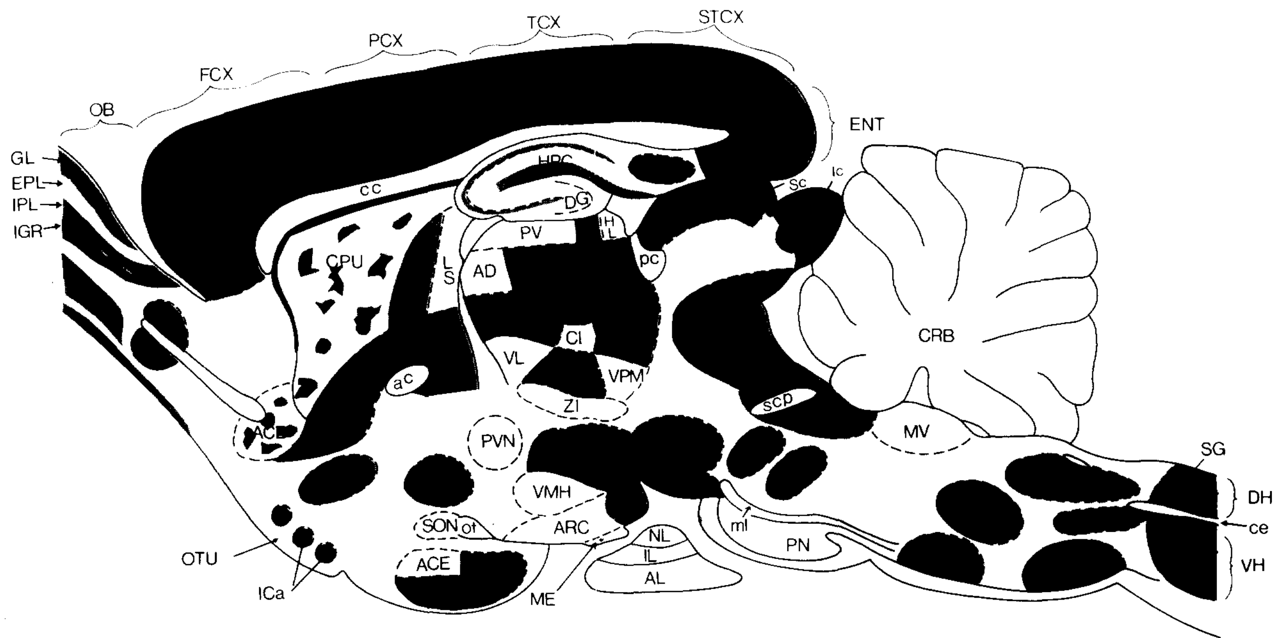
Opioid 'receptor' distribution

Opioid receptors are widely distributed throughout the neuraxis with particularly dense binding observed in limbic structures, thalamic nuclei and neural areas important for visceral functioning. To aid in the discussion of these sites and their comparative distributions in other animals, we have provided parasagittal drawings (centerfold) and horizontal darkfield autoradiograms (Fig. 1) that summarize the distribution of the opioid receptors in the rat brain. The parasagittal drawings are based on autoradiographic studies and represent qualitative differences

TABLE I. Distribution of opioid receptors and peptides in the rat brain

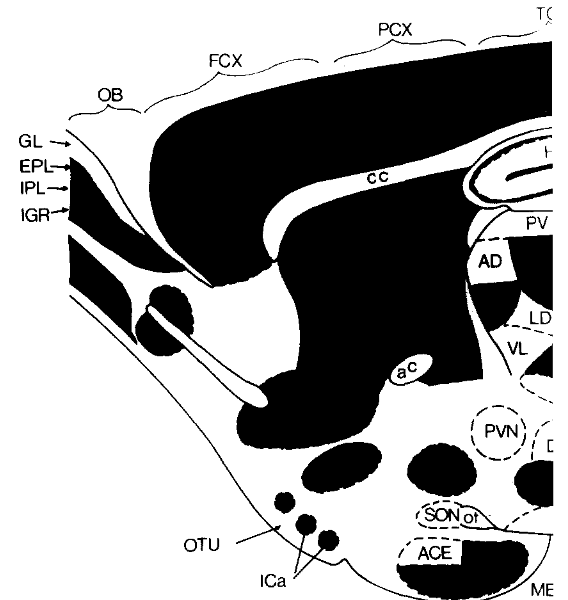
CNS Region	Receptors			Peptides		
	μ	δ	κ	POMC	Pro-Enk	Pro-Dyn
I. Telencephalon						
Frontal cortex (laminar)	+++	++	+	0	++	+
Piriform cortex (laminar)	++	++	++	0	++	+
Entorhinal cortex (laminar)	++	++	++	0	+++	+
Amygdala						
Central nucleus	0	0	++	++++	++++	++
Medial nucleus	+++	++	++	+++	+++	+
Lateral nucleus	++++	+++	+++	++	+++	+
Hippocampal formation						
Hippocampus (laminar)	+++	++	+	0	++	+++
Dentate gyrus (laminar)	+++	+	+	0	++	+++
Olfactory tubercle	+	+++	+++	0	++	++
Nucleus accumbens	++++ (patchy)	++++	+++ (ventral)	+	+++	++
Caudate-putamen	++++ (patchy)	++++ (vent-lat)	+++ (vent-med)	0	+++ (patchy)	++
Globus pallidus	+	+	+	0	++++	+++
Medial septum	+++	+	+	+++	+++	0
Bed nucleus stria terminalis	++	++	+++	++++	+++	++
Preoptic area	+	+	++++	+++	+++	++
II. Diencephalon						
Hypothalamus						
Supraoptic nucleus	0	0	++	0	+	+++
Paraventricular nucleus	0	0	++	++++	++++	++++
Arcuate nucleus	0	0	++	++++	+++	++
Ventromedial nucleus	0	+	+++	+	+++	++
Dorsomedial nucleus	+	0	+++	++++	++	++
Lateral hypothalamic area	+	0	++	+++	++	+++
Thalamus						
Periventricular nucleus	0	0	+++	++++	+++	+
Central–medial nucleus	++++	+	++	0	+++	0
Reuniens nucleus	++++	+	++	0	++	0
Medial habenula	+++	+	+++	0	+++	0
III. Mesencephalon						
Interpeduncular nucleus (central)	++++	+++	+++	0	+++	0
Substantia nigra						
Pars compacta	+++	0	0	+	++	+
Pars reticulata	++	+	+	0	+	++++
Ventral tegmental area	++	0	+	++	++	+
Periaqueductal gray (rostral–ventral)	+	0	++	++++	+++	++
Sup./Inf. colliculi	++++	+	++	++	+++	+
Dorsal raphe nucleus	++	0	++	+++	++	+
IV. Pons/medulla						
Parabrachial nucleus	+++	0	++	+++	+++	++
Nucleus raphe magnus	++	0	+	+	+++	++
Nucleus reticular gigantocellularis	+	0	+	++	+++	+
Nucleus tractus solitarius (caudal part)	++++	+	+++	+++	+++	+++
Lateral reticular nucleus	+	0	+	+++	+++	+
Spinal trigeminal nucleus	+++	0	++	++	++++	+++
V. Spinal cord						
Substantia gelatinosa	+++	+	++	++	++++	+++

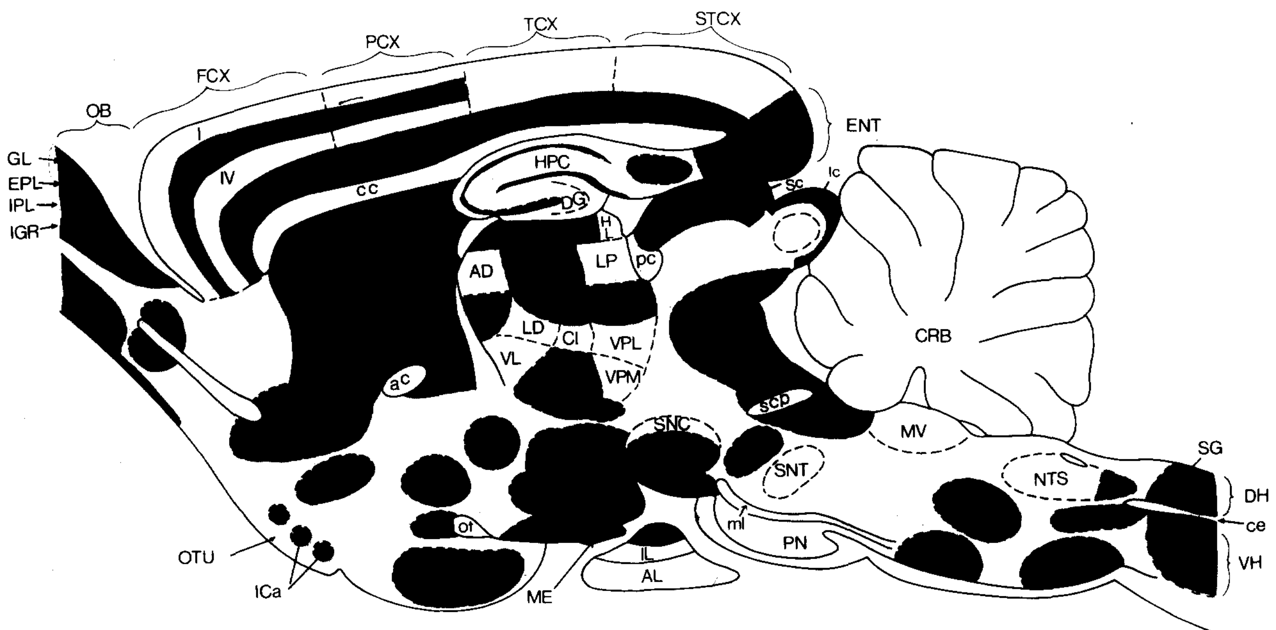
++++ = very dense; +++ = dense; ++ = moderate; + = low; 0 = undetectable.



DELTA OPIOID RECEPTORS

delta opoid receptors are more restricted in their distribution and appear densest in olfactory-related neural areas, neocortex, caudate-putamen, nucleus accumbens, and amygdala. Little or no delta binding can be observed in the thalamus, hypothalamus and brainstem. The function of these opioid receptor sites is unclear, but they may play a role in motor integration, olfaction and cognitive functioning.

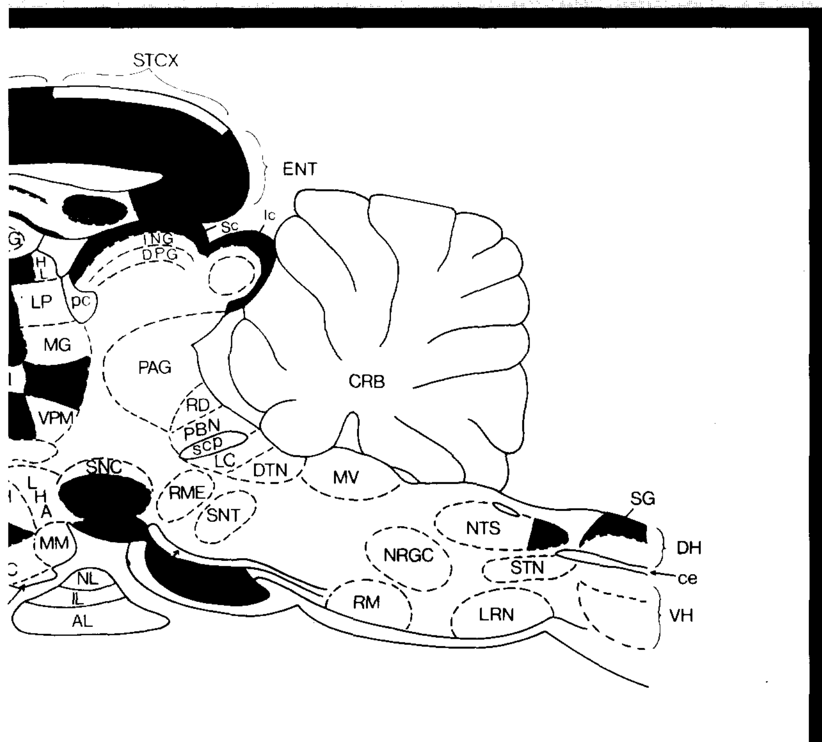




OPIOID RECEPTOR DISTRIBUTION

MU OPIOID RECEPTORS

mu opioid receptors are widely distributed throughout the forebrain, midbrain and hindbrain. mu opioid receptor sites are most dense in the neocortex, caudate-putamen, nucleus accumbens, thalamus, hippocampus, amygdala, inferior and superior colliculi, nucleus tractus solitarius, spinal trigeminal nucleus and dorsal horn.
A moderate density of mu receptors is observed in the periaqueductal gray and raphe nuclei, while relatively little binding is seen in the hypothalamus, preoptic area and globus pallidus. The distribution of opioid receptors corresponds well with their putative role in pain regulation and sensorimotor integration.



KAPPA OPIOID RECEPTORS

kappa opioid receptors are localized in an intermediate number of brain areas with the densest areas of binding observed in the caudate-putamen, nucleus accumbens, amygdala, hypothalamus, neural lobe of the pituitary, median eminence, and nucleus tractus solitarius. Moderate amounts of kappa sites are found in the periaqueductal gray, raphe nuclei, spinal trigeminal nucleus and dorsal horn. The distribution of kappa opioid receptors is consistent with their probable role in regulating water balance, food intake, pain perception and neuroendocrine functioning.

Schematic representation of mu, delta and kappa opioid receptors in the rat brain as determined by receptor autoradiography techniques. To facilitate the description of these distributions, the receptor densities are colour- coded, with red= very dense (++++), orange = dense (+++), green = moderate (++), and blue = light (+). These terms are not meant to be quantitative and only provide a relative measure within a receptor distribution. In fact, since there are relatively few kappa sites in the rat, areas referred to as dense for kappa binding may be equivalent in receptor number to areas of light or moderate mu or delta binding. Each map represents mutiple parasagittal levels through the rat brain and was reconstructed using the atlas of G. Paximos and C.Watson (*The Rat Brain in Stereotaxic Coordinates*, Academic Press, 1986).

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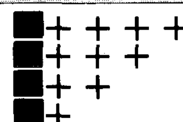
ABBREVIATIONS

ABL, basolateral amygdaloid nucleus; **AC**, anterior commissure; **ACB**, nucleus accumbens; **ACE**, central amygdaloid nucleus; **AD**, anterodorsal thalamus; **AL**, anterior lobe, pituitary; **AME**, medial amygdaloid nucleus; **AON**, anterior olfactory nucleus; **ARC**, arcuate nucleus, hypothalamus; **BST**, bed nucleus stria terminalis; **cc**, corpus callosum; **ce**, central canal; **CL**, centrolateral thalamus; **CM**, centromedial thalamus; **CPU**, caudate-putamen; **CRB**, cerebellum; **DG**, dentate gyrus; **DH**, dorsal horn, spinal cord; **DMH**, dorsomedial hypothalamus; **DPG**, deep grey matter, superior colliculus; **FCX**, frontal cortex; **ICa**, Islands of Calleja; **IGR**, intermediate granular layer, olfactory bulb; **IL**, intermediate lobe; **IMD**, intermediodorsal thalamus; **ING**, intermediate gray layer, superior colliculus; **IP**, interpeduncular nucleus; **IPL**, intermediate plexiform layer, olfactory bulb; **LC**, locus coeruleus; **LD**, laterodorsal thalamus; **LHA**, lateral hypothalamic area; **LP**, latero-posterior thalamus; **LRN**, lateral reticular nucleus; **LS**, lateral septum; **MD**,dorsomedial thalamus; **ME**, median eminence; **MG**, medial geniculate; **ML**, medial lemniscus; **MM**, medial mammillary nucleus; **MS**, medial septum; **MV**, medial vestibular nucleus; **NDB**, nucleus diagonal band; **NL**, neural lobe, pituitary; **NRGC**, nucleus reticularis gigantocellularis; **NTS**, nucleus tractus solitarius; **OB**, olfactory bulb; **OT**, optic track; **OTV**, olfactory tubercle; **PBN**, parabrachial nucleus; **PC**, posterior commissure; **PCX**, parietal cortex; **PN**, pons; **POA**, preoptic area; **PrS**, presubiculum; **PV**, para-ventricular thalamus; **PVN**, para-ventricular hypothalamus; **RD**, dorsal raphe; **RE**, reuniens thalamus; **RM**, raphe magnus; **RME**, median raphe; **SC**, superior colliculus; **scp**, superior cerebellar peduncle; **SG**, substantia gelatinosa; **SNC**, substantia nigra, pars compacta; **SNR**, substantia nigra, pars reticulata; **SNT**, sensory nucleus trigeminal; **SON**, supraoptic nucleus; **STCX**, striate cortex; **STN**, spinal trigeminal nucleus; **SUG**, superficial gray layer, superior colliculus; **TCX**, temporal cortex; **VH**, ventral horn, spinal cord; **VL**, ventrolateral thalamus; **VM**, ventromedial thalamus; **VMH**, ventromedial hypothalamus; **VP**, ventral pallidus; **VPL**, ventroposteriolateral thalamus; **VPM**, ventroposteriormedial thalamus; and **ZI**, zona incerta.



trends in NEUROSCIENCES

in relative densities within a receptor distribution and are not meant to indicate absolute densities of a receptor type within a structure. Rather, they indicate that within a receptor distribution, there is a relative abundance of this site in these structures. As can be seen from these figures, the distributions of the opioid receptors vary markedly in both their relative abundance across brain regions and their specific localization.

μ binding is observed in numerous nuclei and at several levels of the neuraxis, including the neocortex, caudate-putamen, septum, thalamus, hippocampus, substantia nigra, inferior and superior colliculi, locus coeruleus and nucleus tractus solitarius. The distribution of δ receptors, on the other hand, is more restricted and predominantly in forebrain structures, such as neocortex, caudate-putamen, and amygdala. κ receptor autoradiography demonstrates yet a third receptor pattern, with sites localized in the preoptic area, hypothalamus, median eminence, caudate-putamen, amygdala and nucleus tractus solitarius. While there is some overlap in the localization of each of these receptor types, their precise anatomical distributions vary markedly. Since a comprehensive summary of each of the opioid receptor distributions would be impractical in this format, only the most salient comparisons will be discussed. More detailed information is provided in the figures and tables of this review, as well as in several recent papers[19,22,23].

The pattern of receptor binding in the neocortex demonstrates a number of features seen repeatedly in opioid receptor anatomy. For instance, while both μ and δ sites are prominent in this tissue, their precise distributions differ and generally appear complementary. Although there are regional differences in lamination, μ sites are most prominent in layers I and III/IV of frontal, parietal, and temporal cortex, whereas δ receptors tend to be diffusely localized in layers II, III, V, and VI. A similar complementary pattern is observed in the olfactory bulb, where μ binding is prominent in the glomerular layer and δ binding is densest in the external plexiform layer. κ receptors are not as prominent in cortex and are light to moderate in density in layers II, III, V, and VI of frontal and parietal cortex.

Limbic system structures, like neocortex, have a rich distribution of opioid receptors. For example, in the hippocampal formation there is a relative abundance of μ receptors in the pyramidal cell layer, stratum lacunosum-moleculare, and the molecular and granular cell layers of the ventral dentate gyrus. Comparatively moderate to light δ and κ labelling is observed in these areas. The relatively low number of κ sites observed in the hippocampus is in marked contrast to the fairly rich dynorphin innervation observed in this region and is consistent with other evidence that dynorphin peptides may be active at μ receptors in this region of rat brain[24].

In contrast to the situation in the hippocampus, all three receptor types are densely distributed within the caudate-putamen and nucleus accumbens, two major catecholaminergic projection regions. μ receptors occur predominantly in the sub-callosal region of the caudate-putamen and in receptor 'patches' that extend from the striatum to the nucleus accumbens. δ and κ receptors, in contrast, are diffusely distributed within these structures, being particularly dense ventrolaterally (δ) or ventromedially (κ).

TABLE II. Relative proportions of the opioid receptor types in several species[a]

Species	μ	δ	κ
Rat	52.5 (41%)	64.1 (50%)	12.6 (9%)
Guinea-pig	17.1 (25%)	17.7 (25%)	34.4 (50%)
Mouse	32.5 (25%)	81.0 (62%)	17.6 (13%)
Pigeon	17.7 (14%)	12.4 (10%)	100.2 (76%)
Human	22.7 (29%)	27.0 (34%)	29.2 (37%)

[a] Opioid receptor binding in several species as determined by Scatchard analysis. The upper number of each pair refers to fmol per mg tissue. μ receptors were labelled with [^{3}H]DAGO, δ sites with [^{3}H]DPDPE and κ receptors with [^{3}H]bremazocine in the presence of a 300-fold excess of unlabelled DAGO and DPDPE. Given the total amount of opioid binding, the numbers beneath in parentheses are the relative abundance of these sites within brain tissue. The values reported here reflect the binding of human frontal cortex and forebrain tissue of the rat, guinea-pig, mouse and pigeon.

By comparison with the telencephalon, diencephalic structures show a predominance of μ and κ binding. In the thalamus, there is a predominance of μ receptors in most of the nuclear divisions with the exception of the zona incerta, periventricular, central lateral and ventral posteromedial nuclei. Moderate to dense κ labelling is seen in the medial regions of the thalamus including the periventricular, mediodorsal, and reuniens nuclei. In the hypothalamus, the distribution appears to be reversed with moderate to dense κ labelling observed throughout most of the hypothalamic nuclei and little or no μ binding observed. A notable exception is the mammillary nucleus which contains dense numbers of μ receptors and a moderate density of κ sites.

More caudally in the mesencephalon and brainstem, μ and κ receptors show largely parallel distributions. Both μ and κ receptors are observed in the periaqueductal gray, superior and inferior colliculi, interpeduncular nucleus, raphe nuclei, locus coeruleus, parabrachial nucleus, nucleus tractus solitarius, spinal trigeminal nucleus, and substantia gelatinosa of the spinal cord. The distributions vary markedly in the substantia nigra where dense μ binding is observed in the pars compacta, an area devoid of κ receptors in the rat. Relatively low levels of δ and κ binding are observed in the pars reticulata. No opioid receptors are detected in the rat cerebellum.

Opioid 'peptide–receptor' interactions

Given the complexity of the distributions of both the opioid receptors and peptide systems in the CNS[25–27], it may be premature to attempt to correlate a particular opioid peptidergic system with a receptor type. Early investigators examining this issue made the assumption that based on the relative selectivities of the opioid peptides, there should be a correspondence between a particular opioid peptide and receptor. For example, β-endorphin is known to bind selectively to both μ and δ receptors, while dynorphin A_{1-17} and [leu]enkephalin show affinity for κ and δ receptors, respectively. However, the problem with this line of reasoning is that pro-enkephalin and pro-dynorphin peptides can bind to μ, δ or κ receptors depending on the specific peptide product[28,29] and

species. Therefore, since there are many potential areas of peptide–receptor interaction, the more logical questions to be asked about any particular CNS region are what peptide forms are present, and what are their receptor affinities.

The relative densities of μ, δ and κ receptors are compared in Table I with the relative densities of POMC, pro-enkephalin and pro-dynorphin in each of several CNS regions. This summary table provides a qualitative indication of several good matches and many mismatches in the 'peptide–receptor' co-distribution. For example, in cortex, amygdala and caudate-putamen, there is a general match between the distribution of δ and κ receptors on the one hand, and pro-enkephalin and pro-dynorphin peptides on the other. Likewise, in the hypothalamus, there is a good correlation between pro-dynorphin and κ sites in almost every nucleus. Furthermore, the μ receptor distribution in some amygdaloid nuclei, in the septum, parabrachial nucleus, and nucleus tractus solitarius corresponds well with the distribution of POMC in these same nuclei. Conversely, there is a striking lack of correlation between the distribution of POMC peptides and μ receptors in the cerebral cortex, hippocampus, caudate-putamen and thalamus, to name a few. The same can be said about the distribution of δ receptors and pro-enkephalin in the globus pallidus, hypothalamus, substantia nigra, periaqueductal gray and parabrachial nucleus. In the globus pallidus, thalamus and substantia nigra, there is a further lack of correlation between the distribution of pro-dynorphin and κ receptors.

From the above, it appears that with the present state of knowledge on opioid peptide–receptor interactions, it is unwise to try to correlate a given peptide precursor system with a particular receptor type. Instead, the differential processing of each of the three precursors in each CNS nucleus may be a crucial regulatory mechanism whereby opioid peptides of varying lengths and receptor affinities are released presynaptically and act as selective ligands at the μ, δ or κ receptors, depending upon the relative abundance of these receptor types. While this view may account for the complex anatomical interrelationships between the multiple opioid systems and receptor types, there still remains the problem of the frequent inconsistencies in areas where there are opioid receptors and no detectable peptides or the reverse case of opioid peptides and no detectable receptors. Several possible reasons for peptide–receptor mismatches have been extensively discussed by other investigators and might include limitations in immunocytochemial detection of peptides in fine 'synaptic' terminals, non-functional or spare receptors, unrecognized low affinity or occupied receptors or diffusion as part of the mechanism of opioid peptide action[27,30–32].

Species differences in opioid receptor distributions

The complexity of the opioid receptor and peptide interactions is magnified when species differences are considered. At the simplest level, species vary dramatically in the relative abundance of each of these sites. As can be seen from Table II, κ sites in the rat comprise approximately 10% of the total number of opioid receptor sites in the forebrain, whereas in most other species, such as guinea-pig, monkey and human, they represent at least a third of the total opioid receptor population. Forebrain tissue varies across species, demonstrating predominantly κ receptors in pigeon, predominantly δ receptors in mouse, and a mixture of receptor sites in the guinea-pig and human.

Anatomically, species differences can be observed within the distributions of each of the receptor types and at several levels of the neuraxis. In general, the distribution of opioid receptor types is well conserved across species in brainstem and spinal cord areas and varies most markedly in forebrain and midbrain structures. Examples of these differences are provided in Table III. When comparing rat and monkey at the level of the hypothalamus (Fig. 2), for instance, the differences in the μ and κ distributions are quite striking. In the rat hypothalamus and median eminence there is a relative abundance of κ sites and few, if any, μ and δ receptors, while in monkey, μ sites are prominent in these areas. Similarly, in the rat there is little κ binding in the neocortex, whereas there is dense κ binding in monkey cortex. These differences extend to other neural areas including the nigrostriatal system, hippocampus and cerebellum. While species differences are also observed in the distribution of δ receptors, their overall distribution appears to be far more conserved across mammalian species, with dense binding found primarily in the cerebral cortex, caudate-putamen, and amygdala and low binding observed in most of the midbrain and brainstem.

As there appears to be a relatively low abundance of κ receptors in the rat, numerous investigators have used other animals such as the guinea-pig[32,33] to study these sites. Several studies have, in fact, shown that the distribution of κ sites in the guinea-pig more closely parallels that observed in the monkey, with dense labelling in deep layers of cortex, substantia nigra, hippocampus and cerebellum, possibly making the guinea-pig a better model for studying κ receptor–peptide interactions.

Functional roles of the opioid receptor types

Despite a plethora of studies on the physiological

TABLE III. Species differences in selected brain regions

Receptor	Brain region	Rat	Monkey
μ	Central nuc. amygdala	0	+++
	Caudate	++++ (patchy)	++ (diffuse)
	Hypothalamus	+	++++
	Median eminence	0	+++
	Periventricular thalamus	0	++++
δ	Central nuc. amygdala	0	++
	Hippocampus (striatum moleculare)	+	++++
	Median eminence	0	+++
κ	Frontal cortex	+	+++
	Hippocampus (striatum lacunosum-moleculare)	+	+++
	Caudate	+++ (diffuse)	++ (patchy)
	Globus pallidus	+	++
	Medial mammillary nucleus	++	0
	Cerebellum	0	+++
	Median eminence	++++	+++

Relative densities within a species: ++++ = very dense, +++ = dense, ++ = moderate, + = light, 0 = not detectable.

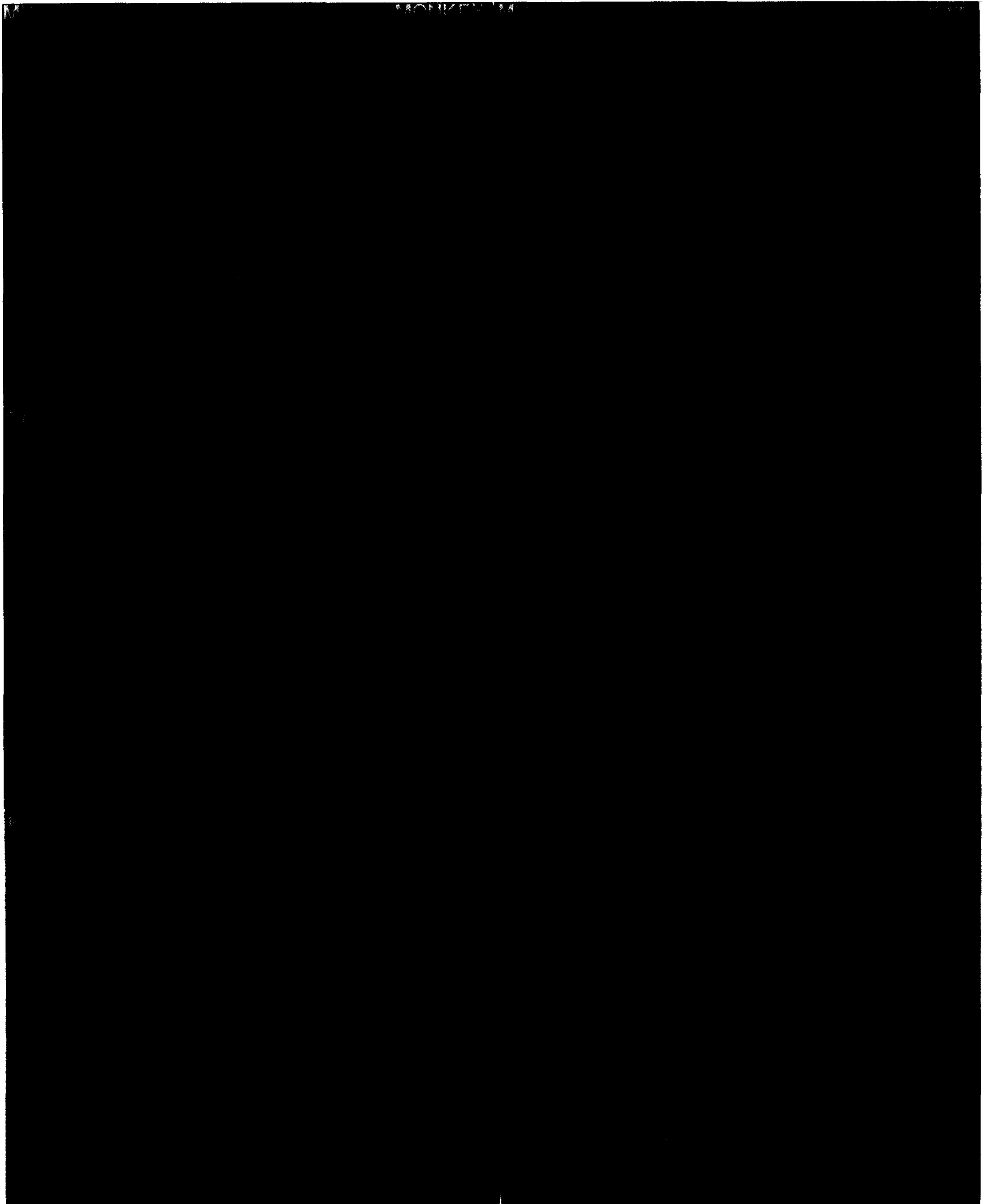


Fig. 2. *Darkfield autoradiograms of monkey and rat frontal sections taken at the level of the diencephalon. Note the marked species differences observed in μ binding in the cortex and hypothalamus. In contrast, the distribution of δ sites is well conserved in these divergent species. Abbreviations not listed in centrefold include: AMG, amygdala; CA1,2,3, fields 1, 2, and 3 of Ammon's horn; CAU, caudate; Cg, cingulate cortex; CL, claustrum; EN, endopiriform nucleus; f, fornix; FPCX, frontal parietal cortex; HB, habenula; HYP, hypothalamus; LA, lateral amygdala; LH, lateral hypothalamus; mt, mammillothalamic tract; PCg, posterior cingulate cortex; PUT, putamen; VA, anterior ventral thalamus.*

and behavioral effects of administering diverse opioid peptides, alkaloids and antagonists[34], it is still difficult to draw firm conclusions regarding the functional roles of different opioid receptor types in any neuronal system. For example, the neural circuitry involved in the central control of cardiovascular function has been intensely studied, and the loci of opioid neurons and receptors within this circuitry is well defined. However, it has proven very difficult to isolate the roles of the different opioid systems and/or receptors within this circuitry because the physiological effects of opioids are influenced by pharmacological variables such as the dose, stability, site of administration, and receptor specificity of the peptide or alkaloid given, and by organismic variables, such as whether the animal has been stressed, injured, or anaesthetized[35].

TABLE IV. Proposed functions of the opioid receptor types[a]

Function	Receptor types	Anatomy
Appetite modulation, eating behavior	μ, δ and κ	Ventral tegmental area
Cardiovascular regulation	μ, δ and κ	Nucleus tractus solitarius
Fluid balance	κ: diuresis μ: antidiuresis	Hypothalamus and/or pituitary; also possibly kidney (κ)
Endocrine responses		Hypothalamus, possibly pituitary
Stimulatory effects		
Growth hormone	δ	
ACTH	μ and κ	
Prolactin	μ and κ	
Inhibitory effects		
Luteinizing hormone	μ and δ (?)	
Vasopressin	κ	(also nucleus tractus solitarius)
Oxytocin	μ and κ	
Pain inhibition	μ and δ	Supraspinal, spinal
	δ	medullary reticular formation
	κ	spinal
Respiration	μ and δ: may mediate respiratory depression	Brainstem
Locomotor behavior	μ: increased activity	A9, A10 DA systems
	κ: sedation	A10 DA systems (?)
Thermoregulation	μ: may mediate hypothermia	Hypothalamus
	δ: may mediate hyperthermia	

[a] This table, which is not a complete summary, is modified from Holaday (1985) *Endogenous Opioids and Their Receptors*, Upjohn Publications.

While similar problems have plagued many functional studies of opioid receptor types, significant progress has been made in several systems (see Table IV). For example because of the immense background of knowledge of the anatomy and functions of the mesencephalic dopaminergic neuronal systems, the study of their modulation by opioids began early[36] and has been a particularly active area of research. While there has been debate concerning which dopamine (DA) neurons (A9 versus A10) are involved in the motor stimulation effects of morphine in rodents, it is still clear that both systems are powerfully influenced by opiate administration, and that the opioid receptor types are differentially involved. For example, direct administration of morphine into the A10 region results in a DA-dependent increase in locomotor activity[37], which is probably μ-dependent since similar administration of DAGO (a μ-selective compound) produces activation at doses 100–1000 times less than those required for DPDPE (a δ-selective drug)[38]. These functional findings agree with the observation of greater μ than δ radioligand binding in the A10 region (ventral tegmental area; Table I). Based on studies of the interacting effects of differentially selective opiates on DA metabolism, κ receptors have been suggested to modulate μ receptor activation of A10 neurons[39].

Electrophysiological studies of A9 neurons point to μ and κ opiates having opposite effects on cellular firing rates[40], which parallel the opposite effects of these opiates on motor behavior, i.e. activation and sedation, respectively. The inhibitory actions of κ opiates on A9 neurons appear to be mediated at the level of the striatum rather than the substantia nigra[40], which is consistent with the relative density of κ radioligand binding in these structures (Table I).

Opposing actions of κ and μ agonists have also been found on fluid regulation, these compounds having diuretic[41] and antidiuretic effects[42], respectively. κ diuresis appears to be due to inhibition of vasopressin secretion[43], probably via pituitary or hypothalamic κ receptors, although a diuretic effect via renal κ receptors[44] cannot be ruled out. Since the antidiuretic effects of μ agonists do not appear to involve vasopressin secretion[43], these opposing physiological actions are presumably mediated by μ and κ receptors coupled to different systems.

The μ, δ and κ opioid receptor types have all been implicated in the mediation of analgesia, but the details of their differential involvement are a source of lively debate. Supraspinal mediation of opioid analgesia has frequently been attributed exclusively to μ receptor activation[45,46], but recent evidence points to the involvement of δ receptors as well[47,48], particularly in the medullary reticular formation[49]. At the spinal level, selective μ, δ and κ agonists are all active in visceral analgesia testing[48]. However, κ agonists appear to be distinct in that they block mechanical nociceptive responses; this contrasts with the mediation of thermal nociceptive responses by μ and/or δ receptors[50]. These differential actions may be related to differences in the intraspinal distribution of the multiple opioid receptor types[51].

Unfortunately, autoradiographic mapping studies are not invariably consistent with functional studies, a discrepancy that may be regarded as another form of 'mismatch'. An instructive example is given by the finding that μ, δ and κ agonists stimulate feeding equipotently when microinjected into the ventral tegmental area[52], a result which would not have been predicted from autoradiographic studies since the ventral tegmental area shows moderate μ binding, but little κ and no detectable δ binding (Table I). Such functional mismatches may arise because autoradiographic studies cannot discriminate between uncoupled binding sites and fully functional receptors. Likewise, the relative 'efficiency' of functional coupling of the different receptor types cannot be determined from autoradiographic studies.

Thus, while it is clear that autoradiographic maps of receptors may be useful in suggesting sites for functional studies, it is also apparent that mapping

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studies have so far been of limited value in predicting specific functional consequences of activating different opioid receptor types. Nevertheless, since the anatomical distribution of the multiple receptor types is now clearly delineated, it may be worthwhile to use this information to explicitly develop and test specific hypotheses regarding the functional consequences of activating these receptors via microinjections of receptor-selective, stable agonists or antagonists into areas with known peptide and receptor content.

Future directions

Given the ability to selectively label each of the opioid receptor types, the fundamental cell biological issue of differential receptor regulation, e.g. in response to physiological and pharmacological manipulations[53], will be increasingly answered. These studies will be aided greatly by the ability to study the genomic regulation of receptor expression, and the recent cloning of the δ receptor[54] provides the first in a new series of molecular tools to explore this level of regulation. As molecular biological tools are further applied to the study of the opioid receptors, several fundamental questions will be answered. For example, as indicated earlier, binding studies[8–12] suggest that there may be more than the three opioid receptor types described here. Molecular biological techniques will aid in determining the number of receptor types present and whether they are derived from several genes or are the product of differential post-translational processing of a single gene product. The use of these tools at the anatomical level, via *in-situ* hybridization histochemistry, will permit the identification of populations of neurons that express the different receptors, and by strategic combination of different methodologies it will be possible to identify their possible synaptic inputs and their regulation at the single cell level. Where will this approach lead us? Perhaps by obtaining a new understanding of how opioid receptors can be synaptically regulated, together with deeper insight into the anatomical basis of different opioid actions, we may ultimately be able to define a new type of opioid pharmacology – the selective expression of different opioid receptor types in specific brain areas for the purpose of modifying the functions of the endogenous opioid peptide systems, e.g. to alleviate pain without the danger of addiction or respiratory depression. Such fanciful goals may be less distant than they seem if future molecular and functional studies of opioid receptors are thoughtfully integrated in the context of the chemical neuroanatomy of the CNS.

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