THE JOURNAL OF COMPARATIVE NEUROLOGY 374:223–229 (1996)

Opioid Receptor Ligand Binding in the Human Striatum: II. Heterogeneous Distribution of Kappa Opioid Receptor Labeled With [^{3}H]Bremazocine

HARALD E. VONKEMAN, PIETER VOORN, LINDA S. BRADY, HENK W. BERENDSE, AND ERIC K. RICHFIELD

Department of Anatomy and Embryology, Research Institute Neurosciences, Vrije Universiteit, 1081 BT Amsterdam (H.E.V., P.V.) and Department of Neurology, Academic Hospital of the Vrije Universiteit, 1081 HV Amsterdam (H.W.B.), The Netherlands; Section of Functional Neuroanatomy, National Institute of Mental Health, Bethesda, Maryland 20892 (L.S.B.); Departments of Neurology and Pharmacology (H.E.V., P.V., H.W.B., E.K.R.) and Division of Neuropathology (E.K.R.), University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

ABSTRACT

Selective kappa opioid receptor autoradiography with [^{3}H]bremazocine (BRM) was used to examine regional and subregional kappa receptor distribution patterns at five rostrocaudal levels through the human striatum. [^{3}H]BRM binding densities were measured in the individual striatal nuclei and in subregions therein. The distribution of [^{3}H]BRM binding sites was found to have a strongly heterogeneous character. At the regional level a rostral-to-caudal decrease in [^{3}H]BRM binding densities was observed. Also, a dorsal-to-ventral differentiation was seen, with higher values in the ventral striatum, especially in the nucleus accumbens, and lower values in the dorsal parts of the caudate nucleus and putamen. These findings suggest an association of kappa receptor function with limbic-related processes in the ventral striatum. Along the ventral edge of the nucleus accumbens and putamen, specific domains with extremely high [^{3}H]BRM binding values were identified. © 1996 Wiley-Liss, Inc.

Indexing terms: basal ganglia, nucleus accumbens, receptor autoradiography, kappa opioid receptor

The endogenous opioids have been implicated in a broad range of central nervous system functions including modulation of neurotransmitter release, pain perception, and motor control (Yaksh and Rudy, 1978; Bozarth and Wise, 1984; Schoffelmeer et al., 1988; Cunningham and Kelley, 1992). The different opioid receptor subtypes, mu, kappa, and delta, appear to form distinct functional populations within the central nervous system (McLean et al., 1986; Mansour et al., 1988, 1995). Studies using selective opioid receptor agonists and antagonists have shown that the different receptor subtypes possess unique pharmacological properties and that activation of the respective subtypes produces different or even opposite effects in animal behavioural experiments (Shippenberg et al., 1987; Bals-Kubik et al., 1989; Goldstein and Naidu, 1989; Bot et al., 1992; Mansour et al., 1995).

In the basal ganglia, endogenous opioids appear to modulate the activity of several neurotransmitter systems, including some that have been implicated in the pathophysiology of basal ganglia–related disorders. For instance, excessive opioid activity has been associated with diseases including tardive dyskinesia, progressive supranuclear palsy, and Gilles de la Tourette syndrome (Sandyk, 1985). It is, therefore, important to determine the localization of the opioid peptides as well as their receptors in the human basal ganglia.

The opioid peptides in the striatum, the input-processing part of the basal ganglia, are thought to be produced in the striatal projection neurons. With respect to the neuroanatomical distribution of the mu, kappa, and delta opioid receptor, it has been found in the rat brain that the striatum contains high binding densities for all three opioid receptors. The patterns of receptor binding vary between the three receptor subtypes, however. The density of the

Accepted May 17, 1996.

Address reprint requests to Eric K. Richfield, M.D., Ph.D., Division of Neuropathology, University of Rochester, School of Medicine and Dentistry, Box 626, 601 Elmwood Avenue, Rochester, NY 14642.
E-mail: erichfield@pathology.rochester.edu

© 1996 WILEY-LISS, INC.

kappa receptor is especially high in the ventral part of the nucleus accumbens and in the ventromedial part of caudate-putamen (Tempel and Zukin, 1987; Mansour et al., 1987, 1995; Nock et al., 1988; Sharif and Hughes, 1989). The mu receptor binding pattern has a mosaic-like appearance, whereas the delta receptor binding densities are more homogeneously distributed (Tempel and Zukin, 1987; Mansour et al., 1987, 1995; Sharif and Hughes, 1989). In primates, the pattern of opioid receptor binding in the striatum appears to be different. Contrary to the situation in the rat, the caudate nucleus of the rhesus monkey shows a patchy pattern of kappa receptor binding and a more diffuse mu receptor binding. Furthermore, in rhesus monkeys both kappa and mu receptor binding densities appear to be higher in the dorsal than in the ventral part of the caudate nucleus (Lewis et al., 1984; Mansour et al., 1988).

Although there have been a few studies on the neuroanatomical distribution of the opioid receptors in the human brain (Maurer et al., 1983; Pilapil et al., 1986; Hurd and Herkenham, 1995), no quantitative analysis of the opioid receptor distribution has been reported thus far. As part of a comprehensive analysis of the localization of neurotransmitter markers in the human striatum (Berendse and Richfield, 1993; Voorn et al., 1994), the present study focuses on the kappa receptor distribution patterns in the different striatal nuclei, using [^{3}H]bremazocine and quantitative autoradiography.

MATERIALS AND METHODS

Human brain tissue was obtained at autopsy from three men and one woman, ages 46.0 ± 19.4 years (mean ± SD), with no history of neurologic or psychiatric disorders or of substance abuse. Postmortem delay was 12.2 ± 3.5 hours (mean ± SD). The left hemisphere was fixed for routine neuropathology. The right hemisphere was cut in 1-cm-thick coronal slices and stored at −75°C. Striatal tissue was dissected from these slices and sectioned at 20 μm on a cryostat. Sections were thaw-mounted onto gelatine-coated glass slides, dried at room temperature, and stored at −32°C. Neuropathologic examination was normal for all brains used.

Labeling of kappa and mu receptors was performed as previously described (Brady et al., 1988; Voorn et al., 1994). Slides for kappa opioid receptors were preincubated for 30 minutes at 0°C in 15 mM potassium phosphate buffer (KPB), pH 7.4, containing 150 mM NaCl and 0.1% bovine serum albumin (BSA) and then rinsed for 5 minutes at 25°C in 50 mM MOPS buffer, pH 7.4 containing 3 mM $MnCl_2$ and 1 μM 2-(*p*-ethoxybenzyl)-1-diethylaminoethyl-5-isothiocyanato-benzimidizole (BIT) and 1 mM N-phenyl-N-[1-(2-(*p*-isocyanato) phenylethyl)-4-piperidinyl]propanamide (FIT), alkylating agents which selectively block binding to mu and delta opioid receptors, respectively (Rice et al., 1983). The slides were then rinsed 3 × 10 minutes at 25°C in 15 mM KPB, pH 7.4, containing 150 mM NaCl and 0.1% BSA to remove excess BIT and FIT. Slides were then incubated for 4 hours at 0°C in 50 mM KPB containing 400 mM NaCl, 0.1% BSA, and 1.9 nM [^{3}H]bremazocine (BRM, 30.0 Ci/mmol), rinsed 4 × 1 minute at 0°C in 50 mM KPB, pH 7.4, and dried. Nonspecific binding was determined with 10 μM naloxone. [^{3}H]DAMGO (59.0 Ci/mmol) was used at a concentration of 1.8 nM to label mu receptors. Nonspecific binding was determined with 10 μM levallorphan. Specific binding for both opioid receptors was greater than 90% in the striatum.

Film handling

^{3}H-Labeled dried sections were placed in an X-ray cassette with ^{14}C plastic standards previously calibrated with ^{3}H brain paste sections in units of decaus per minute (DPM)/mg protein (Pan et al., 1983; Richfield et al., 1987) and exposed to Amersham Hyperfilm ^{3}H at 25°C for 3 to 16 weeks depending on assay. All films were developed in Kodak D19 for 4 minutes at room temperature, stopped in 1.5% acetic acid, fixed in Kodak Rapid Fix for 3.5 minutes, and washed in running tap water for 20 minutes.

Densitometry

Film data were obtained using densitometry (MCID BRS, Imaging Research, Inc. M1-v3.61) from portions of the striatum. Regional density of binding in units of DPM/mg protein was made using a calibration curve generated from data of the co-exposed ^{14}C standards with a 4th degree polynomial fit procedure. Subsequently, these radioactivity values (DPM/mg protein) were converted to fmol ligand bound/mg protein using the specific activity of the ligand. Each region or subregion of interest was measured in one of two ways. To measure the density of an entire region or subregion, an autosample tool was used to outline the entire area, separated from adjacent white matter based on the difference in density, and separated from an adjacent area in the striatum by a user drawn line. To measure areas not measurable using the autosample tool, a mouse driven cursor was used to outline the area, and the density was measured within the outline. Measurements for each region and subregion were obtained from two adjacent sections and averaged together for each case. A single measurement from one section was used to determine nonspecific binding. Specific binding was obtained by subtracting the total binding from the nonspecific binding.

Digitized images

Figure 1 represents computer-generated images from sections. Digital images were captured using a 256-gray level camera with 512 × 512 pixel resolution and saved as a TIFF file. The images were cropped, positioned, and annotated in a page layout program. Stray marks outside the brain regions were digitally removed.

Materials

[^{3}H]BRM was obtained from New England Nuclear/Du Pont (Wilmington, DE), and [^{3}H]DAMGO was from Amersham (Arlington Hts, IL). BIT and FIT were generously donated by Dr. Kenner Rice, National Institute of Diabetes, Digestive and Kidney Diseases, National Institute of Health. All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

RESULTS

In each brain, regional binding densities were measured at five rostrocaudal levels through the striatum (Fig. 1A–E). Because level 2 measurements could be obtained from only one brain, the data for levels 1 and 2 were combined. Levels 1 and 2 (Fig. 1A,B) in our series of sections are approximately 1 mm apart, whereas the distance between levels 2 and 3 (Fig. 1B,C) is approximately 2

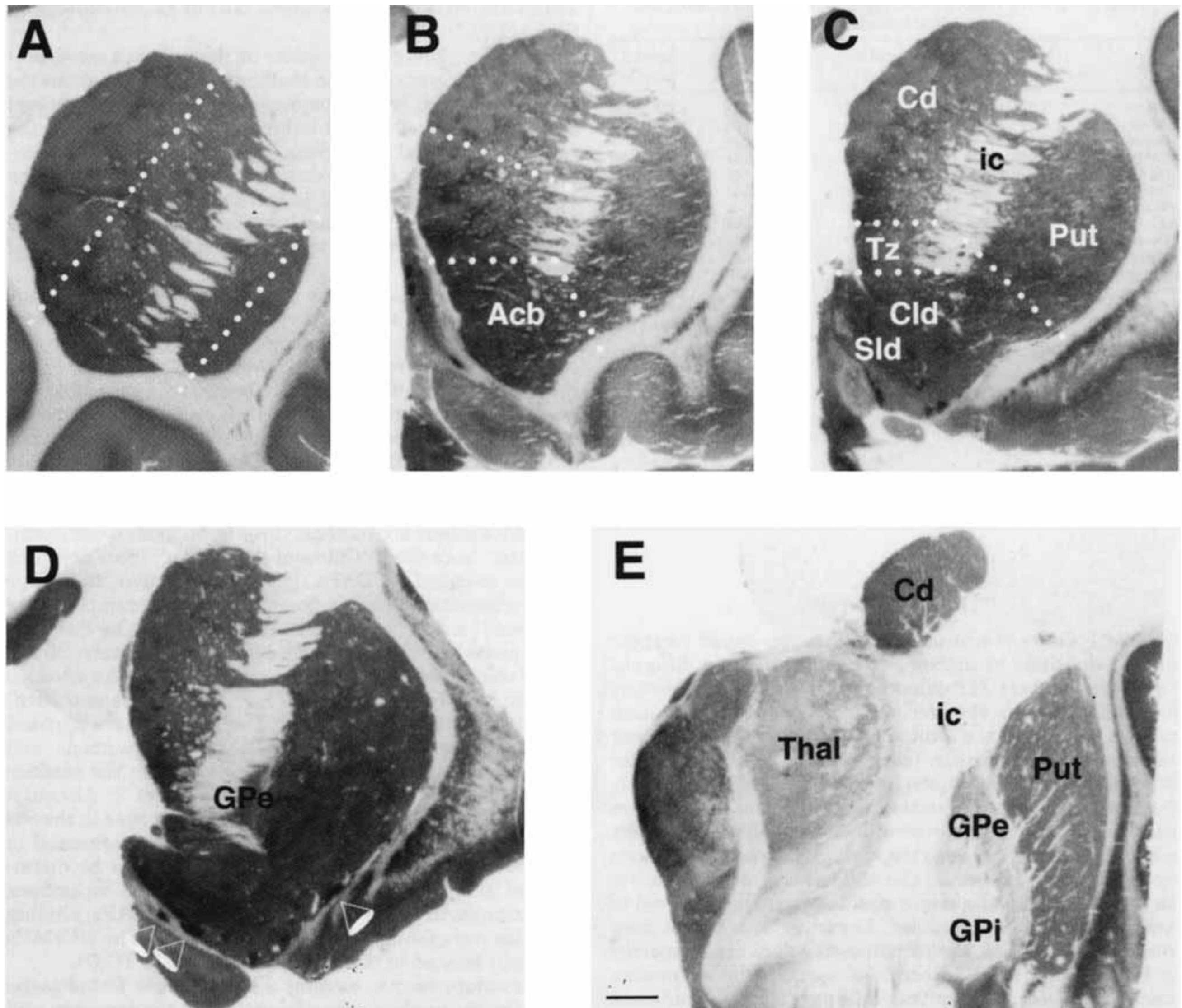

Fig. 1. Five frontal sections through the human striatum illustrating the heterogeneous distribution of [^{3}H]bremazocine (BRM) binding at the different rostrocaudal levels 1 to 5 (**A–E**). Lateral is to the right in all figures. Receptor binding is high in the nucleus accumbens (Acb) and lower in the dorsal parts of the caudate nucleus (Cd) and putamen (Put). Straight, dotted lines in A (level 1) show the medial-lateral division of the Cd and Put. Straight, dotted lines in B (level 2) mark the boundaries between the Cd, the Put, and the Acb, and the division of the Cd into dorsal and ventral parts. Lines in C (level 3) delineate the dorsal part and the ventral "transition zone" (Tz) of the Cd, the ventral one-third and dorsal two-thirds of the Put, and the "core-like" division (Cld) and "shell-like" division (Sld) of the Acb. The NUDAPs are pointed out by arrowheads in D (level 4). GPe, globus pallidus pars externa; GPi, globus pallidus pars interna; ic, internal capsule; Thal, thalamus. Scale bar = 5 mm.

mm. These levels will be referred to as levels 1 and 2 (see Table 1). At the various levels, the different striatal nuclei and subregions within these nuclei were delineated using the criteria previously described by Berendse and Richfield (1993). Straight lines were drawn across the caudate nucleus and the putamen, running perpendicular to the internal capsule, to divide the caudate nucleus and putamen into dorsal and ventral parts. Straight lines, running parallel to the internal capsule were used to divide the caudate nucleus and putamen into medial and lateral parts. The putamen was also divided into a ventral one-third and a dorsal two-thirds, again using a straight line running perpendicular to the internal capsule, as an approximation of the gross anatomical division of the striatum into "dorsal" and "ventral" parts (Heimer and Wilson, 1975; see Berendse and Richfield, 1993). In the caudate nucleus, a "transition zone" (Berendse and Richfield, 1993) was distinguished by drawing a line across the caudate nucleus, at the point where the internal capsule and the caudate nucleus bend laterally (Fig. 1C). The nucleus accumbens was divided into a "core-like" division (Cld) and a "shell-like" division (Sld) using the pattern of [^{3}H]DAMGO binding, as previously described (Voorn et al., 1994) (Fig. 1C).

Qualifications of high vs. low binding density values are not meant in an absolute sense in comparison to other studies, but serve for internal comparison of densities only.

TABLE 1. (^{3}H)BRM Binding (fmol/mg) in Basal Ganglia (Sub)regions at Five Rostrocaudal Levels[1]

	Levels 1 & 2 (n = 3)	Level 3 (n = 2)	Level 4 (n = 3)	Level 5 (n = 1)
Striatum	1,928 ± 176	1,818 ± 621	1,745 ± 188	1,218 ± 127
Caudate	1,777 ± 177	1,409 ± 475	1,640 ± 112	1,488 ± 160
Dorsal	1,553 ± 230	1,256 ± 459	1,497 ± 124	1,306 ± 173
Ventral	2,083 ± 129	1,597 ± 493	1,851 ± 121	1,705 ± 160
Medial	1,898 ± 153	1,601 ± 563	1,831 ± 119	1,699 ± 167
Lateral	1,689 ± 177	1,246 ± 398	1,474 ± 147	1,312 ± 160
Trans. zone		1,878 ± 549		
Putamen	1,836 ± 238	1,770 ± 704	1,820 ± 265	1,192 ± 133
Dorsal 2/3	1,740 ± 236	1,566 ± 667	1,684 ± 269	1,181 ± 147
Ventral 1/3	2,146 ± 229	2,338 ± 793	2,228 ± 291	1,249 ± 95
Dorsal	1,658 ± 253	1,449 ± 655	1,603 ± 248	1,173 ± 150
Ventral	2,106 ± 211	2,190 ± 758	2,098 ± 308	1,236 ± 114
Medial	1,793 ± 227	1,838 ± 824	1,854 ± 230	1,260 ± 134
Lateral	1,958 ± 261	1,765 ± 626	1,791 ± 293	1,154 ± 133
Accumbens	2,661 ± 159	2,454 ± 628		
Core		2,395 ± 579		
Shell		2,505 ± 681		
NUDAPs		3,387 ± 587	3,796 ± 327	2,482 ± 116
G. pallidus				
p. externa			1,812 ± 189	609 ± 146
p. interna				832 ± 295
Background	39 ± 5	33 ± 10	38 ± 4	40 ± 3

[1]Tissue was obtained as indicated in the text. Labeling of kappa receptors was performed using [^{3}H]bremazocine (30.0 Ci/mmol) at a concentration of 1.9 nM. Data were collected using densitometry. Values are means ± SEM.

Densitometry

Table 1 shows the mean regional kappa opioid receptor binding densities in individual striatal nuclei at different rostrocaudal levels. It further shows differences in binding densities between the various subregions within each nucleus. In view of the limited number of brains it was not considered appropriate to carry out statistical tests for differences in binding values between (sub)regions. Strictly speaking, the absence of statistics and the small sample size make it difficult to assess whether differences, specifically subtle differences, between the various striatal (sub)regions are significant. However, the differences and trends reported below were of a major size and could be observed in every individual brain studied. Moreover, it becomes clear from the figures that the densitometry data are supported by the distributional patterns as seen in the autoradiograms. The description refers to the data from all four cases as presented in Table 1. The binding pattern is illustrated in one brain.

The striatum as a whole shows a pattern of highest binding density in the rostral levels 1 and 2 which decreases more caudally in levels 3 and 4. At the most caudal level 5, the kappa binding density drops sharply (Fig. 1A–E; Table 1).

In the individual striatal nuclei, this pattern is not as apparent. In all nuclei, binding densities are highest at levels 1 and 2 and lowest at level 5. However, at level 3, both the caudate nucleus and the putamen show relatively low binding densities, lower than at level 4. The nucleus accumbens, which shows the highest binding density of all three nuclei, is present only in the first three levels (Fig. 1A–C). At level 4, the absence of the nucleus accumbens causes the binding density for the striatum as a whole to fall below that of level 3, thus creating a pattern of a gradual rostral-to-caudal decrease of binding values. The sharp drop in binding densities from level 4 to level 5 is only really prominent in the putamen. In the caudate nucleus, the binding density at this level stays relatively consistent. The decrease in binding values in the caudate nucleus at level 3 and in the putamen at level 5 is explained by the relative abundance of low-density areas within these subregions (Fig. 1C,E).

The subregions of the caudate nucleus show a consistent pattern of differentiation in binding density throughout the five rostrocaudal levels, the binding densities being lowest in the dorsal and lateral subregions and highest in the ventral and medial subregions. This difference was particularly prominent at levels 1 and 2 and 5, but can also be seen in the autoradiograms of other levels (Fig. 1A–E). The binding values in the transition zone were found to be relatively high, setting it apart from the remainder of the caudate nucleus.

Within the putamen, as in the caudate nucleus, the binding densities also show consistent differences among the subregions. Lowest binding values were found in the dorsal and dorsal-two-thirds subregions and highest in the ventral and ventral-one-third subregions. This difference was especially prominent at levels 3 and 4. In contrast to the situation in the caudate nucleus, differences between medial and lateral parts of the putamen were small. At level 5, the putamen was found to be fairly homogeneous with respect to subregional binding densities (Fig. 1C–E).

In the nucleus accumbens, three subregions were examined: the "core-like" (Cld) and "shell-like" (Sld) divisions and the so-called NUDAPs. The latter acronym stands for "neurochemically unique domains of the accumbens and putamen," a descriptive term that refers to the fact that these areas stand out in the distribution pattern of the opioid mu receptor and the dopamine D_1 receptor because of their high binding density (Fig. 2; Berendse and Richfield, 1993; Voorn et al., 1994). The NUDAPs are situated along the ventral edge of the nucleus accumbens and putamen (Fig. 1C,D). The Cld and Sld within the nucleus accumbens could only be measured at level 3. Although binding densities were found to be slightly higher in the Sld than in the Cld, these differences seem inconsequential in view of the high SEM values. NUDAPs could be distinguished along the ventral edge of the nucleus accumbens and putamen in levels 3, 4, and 5. In the NUDAPs, binding densities were found to be highest at level 4. The NUDAPs can easily be seen in the autoradiograms (Fig. 1C,D).

In absolute terms, binding densities were found to be higher in the nucleus accumbens than in the putamen and caudate nucleus. The binding densities in the NUDAPs were found to be highest of all striatal (sub)regions.

DISCUSSION

For kappa agonists, the existence of several different binding sites has been demonstrated (Nock et al., 1988; Zukin et al., 1988; Rothman et al., 1992). Also, for each binding site, two receptor subtypes have been identified. The binding conditions using [^{3}H]BRM for the kappa opioid receptor employed in the present study have been demonstrated to label the kappa$_2$ subtype in man (Rothman et al., 1992). Both kappa$_{2a}$ and kappa$_{2b}$ receptors are labeled and cannot be separated in this assay. In humans, kappa$_2$ receptors exceed kappa$_1$ receptors by about a 6:1 ratio (Rothman et al., 1992). Some of the heterogeneities seen in the striatum may be due to differences in the density or affinity of kappa$_2$ receptor subtypes for [^{3}H]BRM. Studies using [^{3}H]BRM in the presence of kappa$_{2a}$ or kappa$_{2b}$ selective (or preferring) ligands will be needed to determine if that is the case. The role of kappa$_1$ receptors in the human ventral striatum remains to be established.

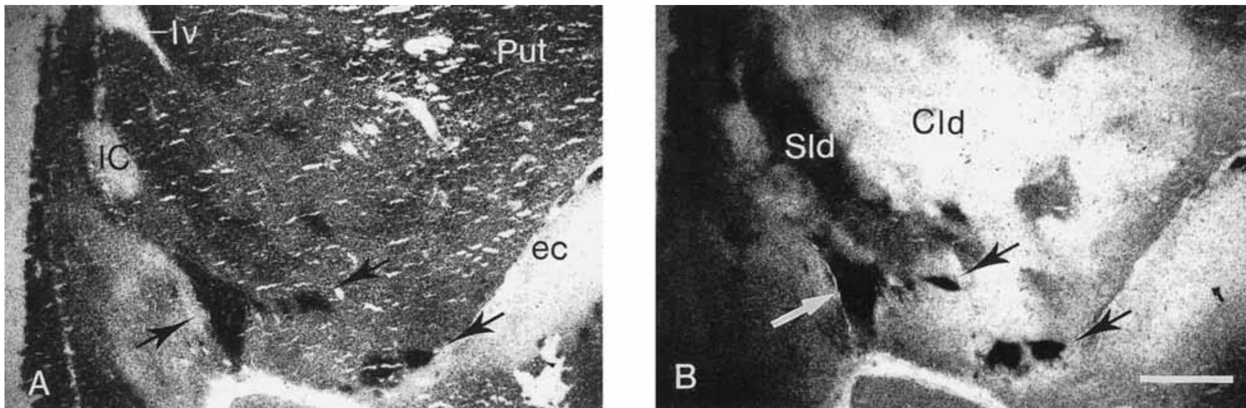

Fig. 2. Pair of aligned, closely adjacent sections through the nucleus accumbens at level 3 (Fig 1C) from one brain showing the NUDAPS (arrows) in the distribution of (**A**) [^{3}H]BRM binding (κ receptor) and (**B**) [^{3}H]DAMGO binding (μ receptor). Note the identical positions of the high-density areas, i.e., the NUDAPs (arrows), in the patterns of both receptors. ec, external capsule; IC, major island of Calleja; lv, lateral ventricle. See Figure 1 for other abbreviations. Scale bar = 2.5 mm.

The present results show that the distribution of kappa receptors as visualized with [^{3}H]BRM has a strongly heterogeneous character with regional and subregional differences in the striatum. At the regional level, changes in binding values were seen along the rostrocaudal axis of the striatum. In addition, a marked dorsal-to-ventral differentiation in [^{3}H]BRM binding density was observed, with higher values in the ventral striatum, especially in the nucleus accumbens, and lower values in the dorsal parts of the caudate nucleus and putamen. The pattern of kappa receptor binding in the human striatum appears to be comparable to that seen in the striatum of the rat. Unlike the pattern seen in the rhesus monkey, in both the human and rat striatum, kappa binding is not patchy and shows a dorsal-to-ventral, low-to-high gradient.

If we compare the dorsal-to-ventral gradient in kappa receptor binding density with the functional organization of the striatum, an interesting correlation becomes apparent. The striatum can be divided into functionally distinct parts on the basis of the corticostriatal projection patterns. Various anterograde fiber degeneration and tract-tracing studies have shown a topological relationship between individual cortical areas and (sub)regions of the striatum. The ventral parts of the striatum, including the nucleus accumbens and the ventral parts of the caudate nucleus and putamen, by virtue of major inputs from hippocampus and amygdala, form a limbic-related area. A second region is formed by the ventral half of the putamen and most of the caudate nucleus, which receive mainly projections from associative cortical areas. Finally, the dorsal parts of the putamen and caudate nucleus are related to sensorimotor cortical areas (Selemon and Goldman-Rakic, 1985; McGeorge and Faull, 1989; Parent, 1990). Comparison of the pattern of kappa receptor binding with this corticostriatal pattern of organization shows a positive correlation between, on the one hand, regions with high kappa receptor density and limbic-related areas, and on the other hand, regions with low receptor density and sensorimotor areas. This would suggest a stronger association of kappa receptor function with limbic-related processes than with sensorimotor function. In a parallel study (Voorn et al., 1996), we demonstrate a dorsal-to-ventral gradient in the distribution of the mu opioid receptor that is the exact opposite of the kappa receptor pattern, suggesting a stronger involvement in sensorimotor than limbic functions for the mu receptor.

In the nucleus accumbens, the most remarkable subregional heterogeneities in the distributional pattern are formed by the NUDAPs. These areas display the highest [^{3}H]BRM binding values of the entire striatum. No substantial differences in binding values for [^{3}H]BRM were observed between the Cld and Sld of the nucleus accumbens. These divisions can be readily appreciated in the mu opioid receptor distribution and also in a comparison of the mu and kappa opioid receptor patterns (Voorn et al., 1994). The reason for not being able to discriminate between Cld and Sld on the basis of the present data may be that the visual boundaries constitute steep, local changes in binding density which do not show up in the densitometry measurements, as binding values were averaged over the entire subdivisions of core and shell. The function of the NUDAPs is at present unknown. The high concentrations of several receptor types in the NUDAPs suggest an important regulatory role for these regions. Their location along the ventral edge of the nucleus accumbens and putamen might imply this regulation to be limbic-related.

Differences in receptor density can be seen as a reflection of functional differences in opioid neurotransmission. From pharmacological and animal-behavioural studies it has become clear that activation of different opioid receptors in the central nervous system can produce different or opposite effects. At low doses, systemically applied kappa receptor agonists prove to be inhibitory, whereas mu (and delta) receptor agonists produce stimulatory locomotor effects. Furthermore, kappa agonists produce aversion in the place-preference paradigm and will not be self-administered, whereas mu and delta agonists appear to act as positive reinforcers in place-preference and self-administration experiments (Shippenberg et al., 1987; Bals-Kubik et al., 1989). In humans, kappa agonists induce dysphoric states, whereas mu receptor agonists produce euphoria (Pfeiffer, 1986). Interaction between the opioid receptors and the mesolimbic dopamine system is thought to play an important role in the mediation of both motivational and locomotor effects (Stinus et al., 1980; Kalivas et al., 1983; Spyraki et al., 1983; Wise, 1983; Di Chiara and Imperato, 1988;

Herz and Shippenberg, 1989; Bals-Kubik et al., 1993). The kappa receptors modulate neural activity by presynaptic inhibition of neurotransmitter (dopamine) release, and possibly by postsynaptic reduction of responses of receptors for other neurotransmitters (Yuan et al., 1992). The specific functional consequences of postsynaptic kappa receptor stimulation in the striatum are not clear. The present study does not distinguish between pre- or postsynaptic localization of kappa receptors. However, most kappa receptors within the ventral striatum are thought to be presynaptic, thereby directly inhibiting dopamine release.

The presently demonstrated dorsal-to-ventral, low-to-high gradient in [^{3}H]BRM binding density in the striatum suggests a relatively strong relationship with ventral striatum-related processes. The pharmacobehavioural data on the effects of kappa opioid receptor stimulation, such as inhibition of locomotor activity and aversion in place preference, confirm this hypothesis. Interestingly, findings by Koob (1992) indicate that the ventral striatum is not only involved in locomotor control but also in mediating the psychomotor effects of psychopharmacological drugs. In the present study strong subregional differences in binding density were found, such as a medial–lateral difference in the caudate nucleus and a high density in the NUDAPs of the nucleus accumbens. This suggests an even further functional differentiation based on relationships between kappa receptor stimulation and specific functions invested in these regions.

ACKNOWLEDGMENTS

This work was supported by grants to H.E.V. from the Netherlands Foundation for Pharmacological Research and to P.V. from the Netherlands Organization for Scientific Research (NWO) and the Royal Dutch Academy of Sciences (KNAW). We thank the University of Rochester Clinical Research Center for the Psychopathology of the Elderly (MH 40381) for providing us with human brain tissue, Ms. Jennifer Gilmore and Mr. Dirk de Jong for technical assistance, and Professor Henk J. Groenewegen for critical comments on the manuscript.

LITERATURE CITED

Bals-Kubik, R., A. Herz, and T.S. Shippenberg (1989) Evidence that the aversive effects of opioid antagonists and kappa-agonists are centrally mediated. Psychopharmacology *98:*203–206.

Bals-Kubik, R., A. Ableitner, A. Herz, and T.S. Shippenberg (1993) Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. J. Pharmacol. Exp. Ther. *264:*489–495.

Berendse, H.W., and E.K. Richfield (1993) Heterogeneous distribution of dopamine D1 and D2 receptors in the human ventral striatum. Neurosci. Lett. *150:*75–79.

Bot, G., L.A. Chahl, P.J. Brent, and P.A. Johnston (1992) Effects of intracerebroventricularly administered mu-, delta- and kappa-opioid agonists on locomotor activity of the guinea pig and the pharmacology of the locomotor response to U50,488H. Neuropharmacology *31:*825–833.

Bozarth, M.A., and R.A. Wise (1984) Anatomically distinct opiate receptor fields mediate reward and physical dependence. Science *224:*516–517.

Brady, L.S., R.B. Rothman, and M. Herkenham (1988) Physiological regulation of neurohypophyseal kappa-opiate receptors. Brain Res. *443:*398–402.

Cunningham, S.T., and A.E. Kelley (1992) Opiate infusion into nucleus accumbens, contrasting effects on motor activity and responding for conditioned reward. Brain Res. *588:*104–114.

Di Chiara, G., and A. Imperato (1988) Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. J. Pharmacol. Exp. Ther. *244:*1067–1080.

Goldstein, A., and A. Naidu (1989) Multiple opioid receptors: ligand selectivity profiles and binding site signatures. Mol. Pharmacol. *36:*265–272.

Heimer, L., and R.D. Wilson (1975) The subcortical projections of the allocortex: similarities in the neural associations of the hippocampus, the piriform cortex, and the neocortex. In M. Santini (ed): Golgi Centennial Symposium. Proceedings. New York: Raven Press, pp. 177–193.

Herz, A., and T.S. Shippenberg (1989) Neurochemical aspects of addiction: opioids and other drugs of abuse. In A. Goldstein (ed): Molecular and Cellular Aspects of Drug Addiction. New York: Springer-Verlag, pp. 111–141.

Hurd, Y.L., and M. Herkenham (1995) The human neostriatum shows compartmentalization of neuropeptide gene expression in dorsal and ventral regions: an in situ hybridization histochemical analysis. Neuroscience *64:*571–586.

Kalivas, P.W., E. Widerlov, D. Stanley, G. Breese, and A.J. Prange (1983) Enkephalin action on the mesolimbic system: a dopamine-dependent and a dopamine-independent increase in locomotor activity. J. Pharmacol. Exp. Ther. *227:*229–237.

Koob, G.F. (1992) Drugs of Abuse, Anatomy, Pharmacology and Function of Reward Pathways. Trends Pharmacol. Sci. *13:*177–184.

Lewis, M.E., H. Khachaturian, H. Akil, and S.J. Watson (1984) Anatomical relationship between opioid peptides and receptors in rhesus monkey brain. Brain Res. Bull. *13:*801–812.

Mansour, A., H. Khachaturian, M.E. Lewis, H. Akil, and S.J. Watson (1987) Autoradiographic differentiation of mu, delta, and kappa opioid receptors in the rat forebrain and midbrain. J. Neurosci. *7:*2445–2464.

Mansour, A., H. Khachaturian, M.E. Lewis, H. Akil, and S.J. Watson (1988) Anatomy of CNS opioid receptors. Trends Neurosci. *11:*308–314.

Mansour, A., C.A. Fox, H. Akil, and S.J. Watson (1995) Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. Trends Neurosci. *18:*22–29.

Maurer, R., R. Cortes, A. Probst, and J.M. Palacios (1983) Multiple opiate receptors in human brain: an autoradiographic investigation. Life Sci. *33:*231–234.

McGeorge, A.J., and R.L.M. Faull (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience *29:*503–537.

McLean, S., R.B. Rothman, and M. Herkenham (1986) Autoradiographic localization of mu- and delta-opiate receptors in the forebrain of the rat. Brain Res. *378:*49–60.

Nock, B., A. Rajpara, L.H. O'Connor, and T.J. Cicero (1988) Autoradiography of [^{3}H]U-69593 binding sites in rat brain: evidence for kappa opioid receptor subtypes. Eur. J. Pharmacol. *154:*27–34.

Pan, H.S., Frey, K.A., Young, A.B., and Penney, J.B. (1983) Changes in [^{3}H]muscimol binding in substantia nigra, entopeduncular nucleus, globus pallidus and thalamus after striatal lesions as demonstrated by quantitative receptor autoradiography. J. Neurosci. *3:*1189–1198.

Parent, A. (1990) Extrinsic connections of the basal ganglia. Trends Neurosci. *13:*254–258.

Pfeiffer, A., V. Brantl, A. Herz, and H.M. Emrich (1986) Psychotomimesis mediated by kappa opiate receptors. Science *233:*774–776.

Pilapil, C., S. Welner, J. Magnan, S. Gauthier, and R. Quirion (1987) Autoradiographic distribution of multiple classes of opioid receptor binding sites in human forebrain. Brain Res. Bull. *19:*611–615.

Rice, K.C., A.E. Jacobson, T.R. Burke, B.S. Bajwa, R.A. Streaty, and W.A. Klee (1983) Irreversible ligands with high selectivity towards μ or κ opioid receptors in the rat forebrain and midbrain. J. Neurosci. *7:*2445–2464.

Richfield, E.K., A.B. Young, and J.B. Penney (1987) The distribution of D-1 and D-2 dopamine receptors in the basal ganglia of turtles, pigeons, rats, cats, and monkeys. J. Comp. Neurol. *262:*446–463.

Rothman, R.B., V. Bykov, B.G. Xue, H. Xu, B.R. De Costa, A.E. Jacobson, K.C. Rice, J.E. Kleinman, and L.S. Brady (1992) Interaction of opioid peptides and other drugs with multiple kappa receptors in rat and human brain. Evidence for species differences. Peptides *13:*977–987.

Sandyk, R. (1985) The endogenous opioid system in neurological disorders of the basal ganglia. Life. Sci. *37:*1655–1663.

Schoffelmeer, A.N., K.C. Rice, A.E. Jacobson, J.G. Van Gelderen, F. Hogenboom, M.H. Heijna, and A.H. Mulder (1988) Mu-, delta- and kappa-opioid receptor-mediated inhibition of neurotransmitter release and adenylate cyclase activity in rat brain slices: studies with fentanyl isothiocyanate. Eur. J. Pharmacol. *154:*169–178.

Selemon, L.D., and P.S. Goldman-Rakic (1985) Longitudinal topography and interdigitation of corticostriatal projections in the Rhesus monkey. J. Neurosci. *5:*776–794.

Sharif, N.A., and J. Hughes (1989) Discrete mapping of brain Mu and delta opioid receptors using selective peptides: quantitative autoradiography, species differences and comparison with kappa receptors. Peptides *10:*499–522.

Shippenberg, T.S., R. Bals-Kubik, and A. Herz (1987) Motivational properties of opioids: evidence that an activation of delta-receptors mediates reinforcement processes. Brain Res. *436:*234–239.

Spyraki, C., H.C. Fibiger, and A.G. Phillips (1983) Attenuation of heroin reward in rats by disruption of the mesolimbic dopamine system. Psychopharmacology *79:*278–283.

Stinus, L., G.F. Koob, N. Ling, F.E. Bloom, and M. Le Moal (1980) Locomotor activation induced by infusion of endorphins into the ventral tegmental area: evidence for opiate-dopamine interactions. Proc. Natl Acad. Sci. U.S.A. *77:*2323–2327.

Tempel, A., and R.S. Zukin (1987) Neuroanatomical patterns of the mu, delta, and kappa opioid receptors of rat brain as determined by quantitative in vitro autoradiography. Proc. Natl Acad. Sci. U.S.A. *84:*4308–4312.

Voorn, P., L.S. Brady, A. Schotte, H.W. Berendse, and E.K. Richfield (1994) Evidence for two neurochemical divisions in the human nucleus accumbens. Eur. J. Neurosci. *6:*1913–1916.

Voorn, P., Brady, L.S., Berendse, H.W. and E.K. Richfield (1996) Densitometrical analysis of opioid receptor ligand binding in the human striatum: I. distribution of mu opioid receptor defines shell and core of the ventrical striatum. Neuroscience (in press).

Wise, R.A. (1983) Brain neuronal systems mediating reward. In J.E. Smith, and J.D. Lane (eds): The Neurobiology of Opiate Reward Processes. Amsterdam: Elsevier Biomedical, pp. 405–428.

Yaksh, T.L., and T.A. Rudy (1978) Narcotic analgetics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. Pain *4:*299–359.

Yuan, X.R., S. Madamba, and G.R. Siggins (1992) Opioid peptides reduce synaptic transmission in the nucleus accumbens. Neurosci. Lett. *134:*223–228.

Zukin, R.S., M. Eghbali, D. Olive, E.M. Unterwald, and A. Tempel (1988) Characterization and visualization of rat and guinea pig brain kappa opioid receptors: evidence for kappa 1 and kappa 2 opioid receptors. Proc. Natl Acad. Sci. U.S.A. *85:*4061–4065.