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Discrete Mapping of Brain Mu and Delta Opioid Receptors Using Selective Peptides: Quantitative Autoradiography, Species Differences and Comparison With Kappa Receptors

N. A. SHARIF[1] AND J. HUGHES

Parke-Davis Research Unit, Addenbrookes Hospital Site, Cambridge, CB2 2QB UK

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SHARIF, N. A. AND J. HUGHES. *Discrete mapping of brain mu and delta opioid receptors using selective peptides: Quantitative autoradiography, species differences and comparison with kappa receptors.* PEPTIDES **10**(3) 499–522, 1989.—The opioid peptides, [^{3}H]DAGO and [^{3}H]DPDPE, bound to rat and guinea pig brain homogenates with a high, nanomolar affinity and to a high density of mu and delta receptors, respectively. [^{3}H]DAGO binding to mu receptors was competitively inhibited by unlabelled opioids with the following rank order of potency: DAGO > morphine > DADLE > naloxone > etorphine >> U50488 >> DPDPE. In contrast, [^{3}H]DPDPE binding to delta receptors was inhibited by compounds with the following rank order of potency: DPDPE > DADLE > etorphine > dynorphin(1–8) > naloxone >> U50488 >> DAGO. These profiles were consistent with specific labelling of the mu and delta opioid receptors, respectively. In vitro autoradiographic techniques coupled with computer-assisted image analyses revealed a discrete but differential anatomical localization of mu and delta receptors in the rat and guinea pig brain. In general, mu and delta receptor density in the rat exceeded that in the guinea pig brain and differed markedly from that of kappa receptors in these species. However, while mu receptors were distributed throughout the brain with "hotspots" in the fore-, mid- and hindbrain of the two rodents, the delta sites were relatively diffusely distributed, and were mainly concentrated in the forebrain with particularly high levels within the olfactory bulb (OB), n.accumbens and striatum. Notable regions of high density of mu receptors in the rat and guinea pig brain were the accessory olfactory bulb, striatal "patches" and "streaks," amygdaloid nuclei, ventral hippocampal subiculum and dentate gyrus, numerous thalamic nuclei, geniculate bodies, central grey, superior and inferior colliculi, solitary and pontine nuclei and s.nigra. Tissues of high delta receptor concentration included, OB (external plexiform layer), striatum, n.accumbens, amygdala and cortex (layers I–II and V–VI). Delta receptors in the guinea pig were, in general, similarly distributed to the rat, but in contrast to the latter, the hindbrain regions such as the thalamus, geniculate bodies, central grey and superior and inferior colliculi of the guinea pig were apparently more enriched than the rat. These patterns of mu and delta site distribution differed dramatically from that of the kappa opioid sites in these species studied with the peptide [^{125}I]dynorphin(1–8).

Peptides Opioids Quantitative autoradiography Mu receptors Delta receptors Kappa receptors [^{3}H]DAGO [^{3}H]DPDPE

OPIOID receptors in the mamalian central nervous system (CNS) have been classified into three subtypes, namely mu, delta and kappa, based on extensive in vivo and in vitro pharmacological and behavioral studies [see Paterson *et al.* (31); Kosterlitz and Paterson (19) for reviews]. The mu-type has a high affinity for morphine-like nonpeptide compounds and Met-enkephalin-like peptides; the delta-type is selectively activated by enkephalin-like (in particular Leu-enkephalin) peptides; the kappa-type has a high affinity for benzomorphans, arylacetamides, oripavines and dynorphin-like peptides.

Although mu and delta receptors have been radiolabelled and visualized by autoradiography and biochemically characterized and differentiated into pharmacologically distinct moieties, the majority of these studies have employed relatively nonselective radioligands of relatively low affinity and the autoradiographic studies have been primarily qualitative. Thus, mu receptors have been studied using the nonselective agonist [^{3}H]etorphine, and the nonselective antagonist [^{3}H]diprenorphine (2, 46, 47) and [^{3}H]morphine and [^{3}H]dihydromorphine (9, 30, 34), often with conflicting results. However, even when more specific radiolabels

[1]Requests for reprints should be addressed to Dr. N. A. Sharif at his present address: Syntex Research, Institute of Experimental Pharmacology, 3401 Hillview Avenue, Palo Alto, CA 94303.

TABLE 1

SATURATION ANALYSES OF MU AND DELTA OPIOID RECEPTORS IN RODENT BRAIN

	Binding Parameters			
	Delta Receptors		Mu Receptors	
Species	K_d	B_{max}	K_d	B_{max}
Rat	1.8 ± 0.6	2.2 ± 0.1	0.7 ± 0.1	10.3 ± 1.8
Guinea Pig	5.1 ± 0.3	4.4 ± 1.5	1.1 ± 0.2	7.9 ± 0.9
Mouse	0.8 ± 0.1	2.7 ± 0.3	ND	ND

The data are means ± SEM of 3–4 experiments conducted at 4°C using brain homogenates. Delta receptors were studies using [^{3}H][D-Pen^{2,5}]-enkephalin and mu receptors were labelled with [^{3}H]DAGO as described in the text. K_d = dissociation constant, nM; B_{max} = apparent maximum binding capacity, pmol/g wet weight. ND = not determined, Kappa receptors labeled with [^{3}H]PD117302 in guinea pig brain yielded K_ds of 0.21 nM and 2.7 nM and B_{max}s of 8.6 and 3.4 pmol/g wet weight, respectively. ND = not determined.

TABLE 2

PHARMACOLOGICAL SPECIFICITY OF DELTA OPIOID RECEPTORS IN RODENT BRAIN HOMOGENATES

	Inhibition Constants (K_i, nM)		
Compound	Rat	Guinea Pig	Mouse
DPDPE	3.2 ± 0.3	5.5 ± 0.7	1.4 ± 0.5
DADLE	4.8 ± 0.9	2.6 ± 0.5	2.1 ± 0.3
Naloxone	28.7 ± 14.0	16.3 ± 1.5	21.8 ± 1.4
Etorphine	4.7 ± 0.7	4.2 ± 0.4	2.9 ± 0.2
U50488	221.6 ± 56.4	654.1 ± 251.3	257.3 ± 79.1
Dynorphin(1–8)	ND	16.4 ± 1.0	32.7 ± 1.7
DAGO	>1 μM	>1 μM	>1 μM

The data are means ± SEM of 3–8 experiments conducted on brain homogenates to 4°C using [^{3}H]DPDPE as the radioligand in the presence of 5 mM $MgCl_2$, 0.1% BSA and 20 μg/ml bacitracin. Note the similarity of the pharmacological profile of delta receptors in the three species. ND = not detrmined. The Hill coefficients for the majority of the competitors were 0.88–1.2, except for DAGO which were 2.6–3.4.

have been used, for example [^{3}H]naloxone (16), [^{125}I]DAGO (14,23) and [^{3}H]DAGO (33,35), the majority of these studies have not fully characterized the binding and pharmacological properties of the radioligands. Furthermore, species comparisons for the autoradiographic distribution of mu and delta receptors have not been performed in parallel or have been described incompletely and in a nonquantitative manner. Although Mansour *et al.* (25,26) have tried to tackle some of these issues, their studies have not addressed the question of detailed quantitative autoradiographic analyses of mu and delta receptors across a number of rodent species in parallel assays, but instead they have concentrated on the rat fore- and midbrain.

The problems with studying delta receptors in homogenate- and autoradiographic-based paradigms have been even more complicated and confusing than those for the mu receptors. Here, [^{3}H]DADLE (10,12), [^{3}H]DSLET and [^{3}H]DTLET (33, 45, 47) have been used to label delta receptors, primarily in the rat brain. However, these probes are essentially not very selective and have been used under a variety of assay conditions leading to conflicting results and conclusions [see Wamsley (46); Kosterlitz and Paterson (19)]. The problem of the lack of the delta-selective ligands has been overcome recently with the commercial availability of [^{3}H][D-Pen^2-D-Pen^5]-enkephalin ([^{3}H]DPDPE) (1,29), a conformationally restricted enkephalin analog possessing only minimal cross-reactivity with mu and kappa receptors. However, only a limited number of biochemical (1,9) and *qualitative* autoradiographic (16, 21, 25, 26) studies have been conducted using this radioligand. The bulk of these studies have concentrated on the rat brain and thus detailed information on the biochemical/pharmacologcal and *quantitative* autoradiographic localization profile of [^{3}H]DPDPE-labelled delta receptors in the CNS of other rodent species is therefore not available in the literature.

The aims of the present studies were therefore: 1) to characterize the delta and mu receptor pharmacology and binding properties of [^{3}H]DPDPE and [^{3}H]DAGO in the rat, guinea pig and mouse brain using a combination of parallel assays conducted on brain homogenates and slide-mounted sections; 2) to study the *quantitative* autoradiographic distribution of mu and delta receptors in the rat and guinea pig CNS using parallel assays conducted with [^{3}H]DAGO and [^{3}H]DPDPE at saturating concentrations and to compare this profile with that of kappa receptors; 3) to employ specific neurotoxin-induced lesions to ascertain the neuronal elements on which the mu and delta receptors are located in the rat striatum.

METHOD

Brain Homogenate Preparations

Male Dunkin-Hartley guinea pigs (250–350 g) and male Sprague-Dawley rats (200–250 g) (Interfauna plc, UK) were killed by cervical dislocation. Whole brains minus cerebellae were removed rapidly and homogenized in 10 vol. Tris HCl (50 mM, pH 7.4 at 4°C) with a Brinkman Polytron (setting 6 for 15 sec) and the homogenate centrifuged at 49,000 × g for 10 min at 4°C. The resultant pellets were resuspended in 10 vol. of fresh 50 mM Tris HCl buffer and incubated at 37°C for 45 min. After this time the homogenates were recentrifuged as above, the supernatants discarded and the pellets resuspended in ice-cold buffer at a concentration of 25–50 mg original wet weight/ml (8, 38–41).

Receptor Binding Assays

Membrane aliquots (0.4 ml) were incubated in a final volume of 0.5 ml with competing unlabelled compounds and either [^{3}H]DAGO (1 nM) or [^{3}H]DPDPE (1–3 nM containing 5 mM $MgCl_2$, 0.1% BSA and 20 μg/ml bacitracin). Nonspecific binding was defined using 1 μM unlabelled DAGO (for mu assays) and DPDPE (for delta assays) and represented 10–20% of the total binding. The assays were conducted at 4°C for 90 min and terminated by rapid filtration through Whatman GF/B glass fiber filters on a Brandell M-48 cell harvester. The filters were washed with 3 × 2 ml of ice-cold 50 mM Tris HCl buffer and the radioactivity determined by liquid scintillation spectrometry at 48% efficiency (8, 38–41).

Lesion Procedures

Male Sprague-Dawley rats (200–250 g) were anesthetized with sodium pentobarbitone (30 mg/kg IP) and placed in a Kopf stereotaxic frame. Following surgery, animals were left to recover for 3 weeks to allow neuronal degeneration to occur. Stereotaxic coordinates were defined using the most appropriate atlas for a particular lesion site. However, all autoradiographic sections were cut and analyzed according to the atlas of Paxinos and Watson (32).

6-OHDA lesion of the nigrostriatal dopamine pathway. Rats were infused with 6-hydroxydopamine hydrobromide (6-OHDA;

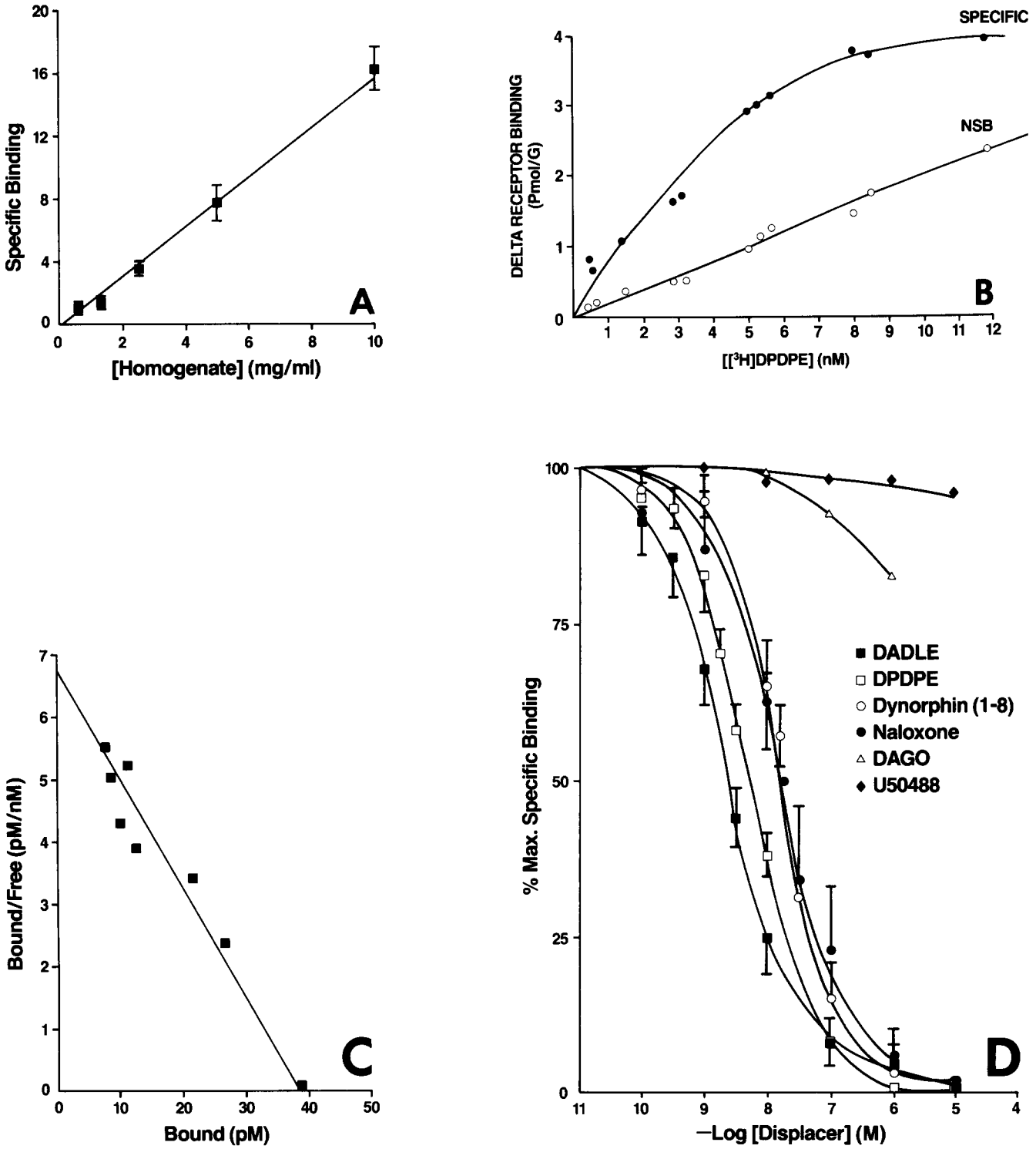

FIG. 1. Delta opioid receptor binding to rat and guinea pig brain homogenates using [^{3}H]DPDPE. (A) Tissue linearity of delta binding in guinea pig brain; y-axis units are pmol/g tissue; (B) saturation analysis of [^{3}H]DPDPE binding in guinea pig brain; (C) Scatchard plot of a saturation curve in the rat brain; (D) pharmacological specificity of [^{3}H]DPDPE binding in guinea pig brain. Note the relative inactivity of the mu and kappa agonists, DAGO and U50488.

Sigma Chemical Co.; 8 μg in 4 μl 0.9% w/v saline containing 0.02% ascorbic acid) at a rate of 1 μl/min into the left medial forebrain bundle (MFB) (A: 3.0, L: 1.2, V: 7.3; all coordinates in mm, anterior coordinates from the interauricular line, lateral coordinates from the midline, ventral coordinates from the surface of the dura). All animals were given desmethylimipramine (25 mg/kg IP; Sigma Chemical Co.) 30 min prior to 6-OHDA to protect noradrenergic neurones. The effectiveness of the lesion

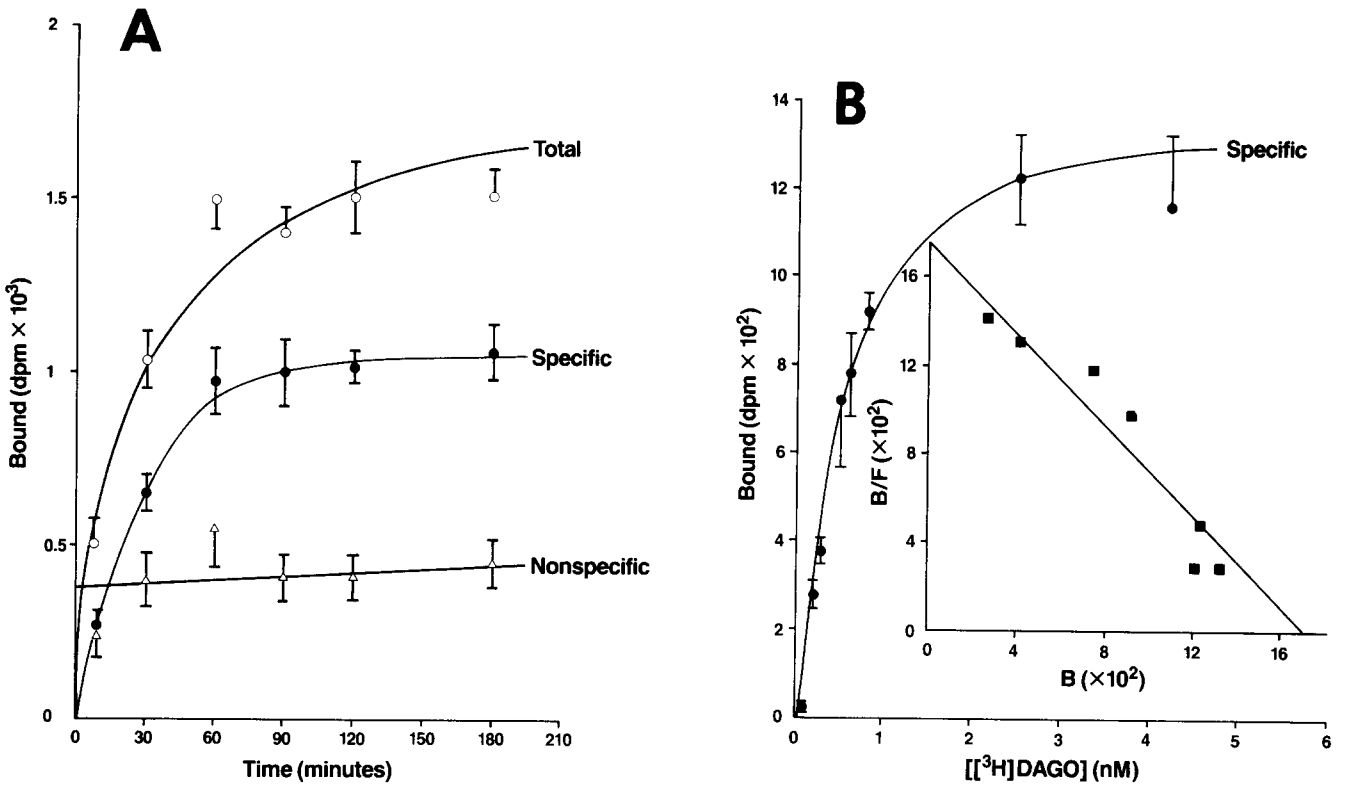

FIG. 2. Mu opioid receptor binding on sections of rat brain cut at the striatal level. (A) Time course to equilibrium study; (B) saturation analysis of [^{3}H]DAGO binding, with the inset showing Scatchard analysis of the saturation data.

was tested by assessment of the ipsilateral rotation response to d-amphetamine sulphate (5 mg/kg IP) 7 days postlesion and the contralateral rotation response to apomorphine hydrochloride (0.5 mg/kg SC; McFarlen-Smith) 14 days postlesion. All the lesioned rats displayed >8 contralateral rotations/minute after an apomorphine injection, indicating >97% depletion of striatal dopamine as shown previously (3).

Ibotenic acid lesion of the striatum. Rats were unilaterally infused with ibotenic acid (IA; 10 μg in 1 μl phosphate buffer, pH 7.4; Sigma Chemical Co.) at a rate of 1 μl/min into 2 stereotaxically defined sites in the caudate putamen (1 μl/injection site; A: 1.4, L: 3.0, V: 4.6; and A: 2.6, L: 2.7, V: 4.7, all coordinates in mm, anterior and lateral coordinates from Bregma, ventral coordinates from the surface of the dural) (3).

Autoradiography and Image Analysis

Cryostat sections (10 μm) were cut at −15°C from rat and guinea pig brains, thaw-mounted on gelatinized slides and stored at −20°C for up to 1 week. For the receptor binding experiments, the sections were thawed at 22°C, preincubated at 37°C for 45 min in 50 mM Tris HCl (pH 7.4) and then allowed to cool to 4°C over 15 min. Aliquots (1 ml) of [^{3}H]DAGO (3 nM), [^{125}I]dynorphin (1–8) (0.2 nM + 200 nM DAGO and DADLE), [^{3}H]DPDPE (8 nM containing 5 mM $MgCl_2$, 0.1% BSA and 20 μg/ml bacitracin), in the presence or absence of 1 μM unlabelled peptides, were spread evenly over the sections (37–41). The incubation with the radioligands was terminated after 90 min at 4°C by rinsing the slides in 500 ml of fresh ice-cold Tris HCl buffer for a total of 15 min at 4°C followed by a rapid rinse (2 sec) in ice-cold distilled water and the sections dried rapidly under a stream of cold air. Following overnight dessication the slides were apposed tightly to a tritium-sensitive film, together with 8–10 tritium brain paste radiation standards or [^{125}I] containing standards, for 2–12 weeks. Subsequently, the autoradiograms were developed and analyzed on the Quantimet 920 image analysis system (37–41). Briefly, quantification of the autoradiograms was achieved by simultaneous exposure of the film to tissue sections and the brain paste radiation standards. Computer-assisted linear calibration graphs of ln optical density vs. ln radioactivity were determined and stored on the computer. Anatomically distinct brain regions were then selected from images of total binding brain sections using a digitizing tablet and the optical densities, and subsequently the molar receptor content, determined automatically by the computer using the stored calibration graphs. Specific binding of the radioligands in the regions of interest was obtained by automatic digital subtraction of the nonspecific binding images from those of total binding after superimposing the former over the outlines of the latter images on the computer monitor. All the quantitative data, including the correlation coefficients (normal range 0.96–0.98) of

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FIG. 3. [^{3}H]DADLE binding to mu and delta receptors in rat brain sections (A), and [^{3}H]DPDPE binding (in absence of $MgCl_2$) to delta receptors (B) in adjacent sections to those shown in (A).

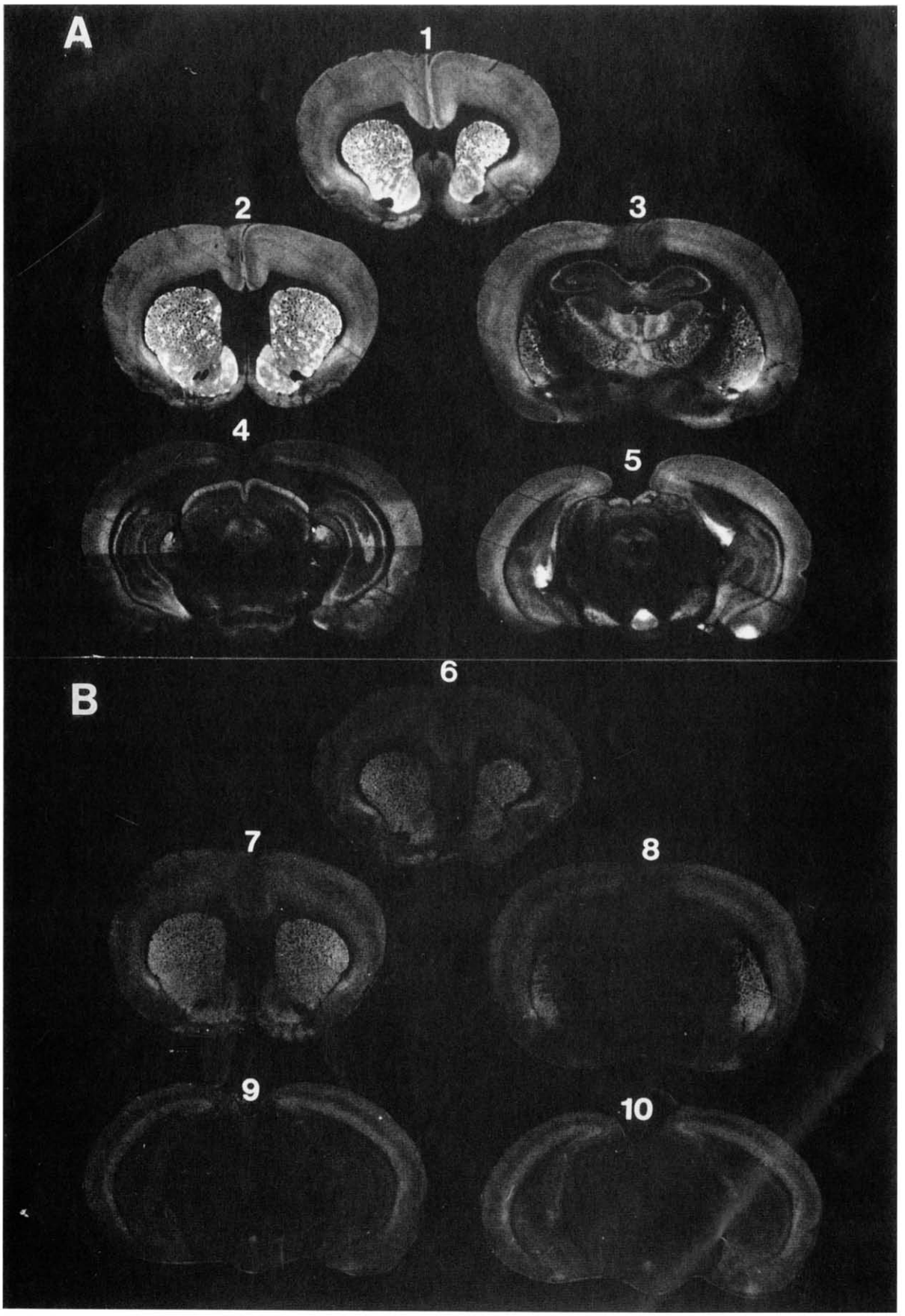
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TABLE 3

PHARMACOLOGICAL SELECTIVITY OF [^{3}H]DAGO BINDING TO MU OPIOID RECEPTORS

Compound	Inhibition Constants (K_i, nM) Rat	Guinea Pig
DAGO	3.7 ± 0.4 (3)	1.9 (2)
DADLE	24.3 ± 3.7 (3)	22.3 (2)
DPDPE	>1 μM (3)	>1 μM (2)
Morphine	3.9 ± 0.2 (3)	2.1 (2)
Naloxone	3.7 ± 1.3 (3)	2.9 (2)
Etorphine	2.3 ± 0.4 (3)	1.7 (2)
U50488	183 ± 20 (3)	810.0 (2)

Data are means of 2–3 experiments (<5% variation between results for guinea pig) conducted in duplicate at 4°C for each compound using 1–1.5 nM[^{3}H]DAGO as the radioligand. The Hill coefficients for the majority of the competing compounds in both species were 0.86–1.2, except for DPDPE which were 2.7–3.1.

the calibration graphs, were then printed out in an annotated format (37–41).

For initial method validation studies the labelled and washed sections from above were wiped with damp filter discs and the radioactivity counted by liquid scintillation counting at 48% efficiency (37–41).

RESULTS

Homogenate Assays

Specific [^{3}H]DPDPE binding to delta receptors in guinea pig brain homogenates at 4°C comprised 72–80% of total binding. Thus, in general, total binding amounted to 823 ± 125 dpm and nonspecific binding (1 μM unlabelled DPDPE or DADLE) amounted to 233 ± 32 dpm (at 1 nM [^{3}H]DPDPE). Specific [^{3}H]DPDPE binding increased linearly with tissue concentration (Fig. 1A) and exhibited full saturability, apparently approaching saturation at ≈8 nM (Fig. 1B). Scatchard analyses (Fig. 1C) of [^{3}H]DPDPE saturation data (Fig. 1B) indicated the presence of a single population of high-affinity (nanomolar dissociation constants (K_ds, range 0.84–5.1 nM) binding sites of variable maximal densities (B_{max}, range 2.2–4.4 pmol/g tissue) in the guinea pig, rat and mouse brain (Table 1). Other workers have prevously reported delta receptor K_ds of 3.3 nM (1), 5.2 nM (9) for rat and 1.6 nM (9) for the guinea pig brain using [^{3}H]DPDPE. However, using [^{3}H]DADLE, values of 1.1–1.7 nM (9, 12, 35) and 0.6 nM (9) have been described for the rat and guinea pig delta sites, respectively, while [^{3}H]DSLET labelled a 1.2 nM K_d delta receptor in the guinea pig brain (9). In addition, [^{3}H]DPDPE binding to delta receptors was readily inhibited by unlabelled DPDPE and other agents known to have a high affinity for delta binding. These included DADLE, Leu- and Met-enkephalin, bremazocine and etorphine (Table 2; Fig. 1D), the latter two being nonselective agents. [^{3}H]DPDPE binding was weakly inhibited by the mu- and kappa-selective agents such as DAGO, U50488 and dynorphin(1–8). Naloxone also displaced [^{3}H]DPDPE binding with a high affinity in brain homogenates (Table 2; Fig. 1D), confirming the opioid nature of these receptors. In general, the pharmacology of [^{3}H]DPDPE-labelled delta binding was similar for the rat, mouse and guinea pig brain (Table 2).

Mu opioid receptor binding was studied in rat and guinea pig homogenates and was linearly related to tissue concentration (data not shown). Specific [^{3}H]DAGO binding comprised 80–90% of the total binding. Thus, total binding amounted to 4003 ± 781 dpm and nonspecific binding (NSB, with 1 μM DAGO) amounted to 824 ± 99 dpm at 0.8 nM [^{3}H]DAGO in guinea pig brain and totals of 9300 ± 981 dpm, NSB = 1081 in rat brain at 1.05 nM [^{3}H]DAGO. [^{3}H]DAGO interacted with a single class of high-affinity (K_d = 0.7 ± 0.1 nM for rat; K_d = 1.1 ± 0.2 nM for guinea pig) and high-capacity (B_{max} = 10.3 ± 1.8 pmol/g for rat; B_{max} = 7.9 ± 0.9 pmol/g for guinea pig) mu binding sites in the rodent brain (Table 1). These values compare well with those published previously (10, 12, 25, 35). Specific [^{3}H]DAGO binding was reversible, being readily inhibited in a concentration-dependent manner in rat and guinea pig brain homogenates by unlabelled mu-selective agents such as DAGO and morphine, and by other nonselective agents known to possess a relatively high affinity for mu binding sites such as DADLE and etorphine. [^{3}H]DAGO binding was also inhibited by nanomolar levels of naloxone, but less so by the kappa-selective agent, U50488 (Table 3).

Assays on Brain Sections

[^{3}H]DAGO binding to 10 μm sections of rat brain cut at the level of the striatum (or hippocampus) was of high affinity, was ≥80% of the total specific binding, attained equilibrium within 60–90 min at 4°C (Fig. 2A) and bound to a single population of mu receptors (Fig. 2B) [K_d = 0.67 ± 0.1 nM (n = 4); B_{max} = 10.8 ± 1.3 pmol/g wet weight]. Therefore, the saturation data obtained from brain sections compared well with that from brain homogenates above. Using a concentration of [^{3}H]DAGO close to the K_d (1 nM), the level of mu binding observed at the rat striatal level was 4.4 ± 0.35 (n = 22) pmol/g wet weight. Unlabelled DAGO competed for [^{3}H]DAGO binding to mu receptors in a competitive manner yielding a K_i value (drug concentration producing 50% inhibition of binding) of 1.48 nM (n = 2).

Quantitative Autoradiography

Initial autoradiographic studies to specifically label delta receptors using [^{3}H]DADLE on its own were unsuccessful since this peptide showed considerable binding to both mu and delta sites (Fig. 3A). Similarly, when [^{3}H]DPDPE was used on its own, in the absence of $MgCl_2$ or BSA, delta receptors were labelled, but the radioligand yielded faint autoradiographic images (Fig. 3B). Inclusion of 5 mM $MgCl_2$ and 0.1% BSA to the incubation buffer containing [^{3}H]DPDPE, in the absence of any mu and kappa blockers, potentiated the binding and led to highly specific labelling of delta receptors and well resolved autoradiograms (Fig. 4A). These latter conditions were used for all subsequent experiments with [^{3}H]DPDPE.

Delta receptors in the rat and guinea pig CNS (Figs. 4–8; Table 4) were, in general, mostly concentrated in the forebrain regions with particular enrichment in the olfactory tissues, striatum,

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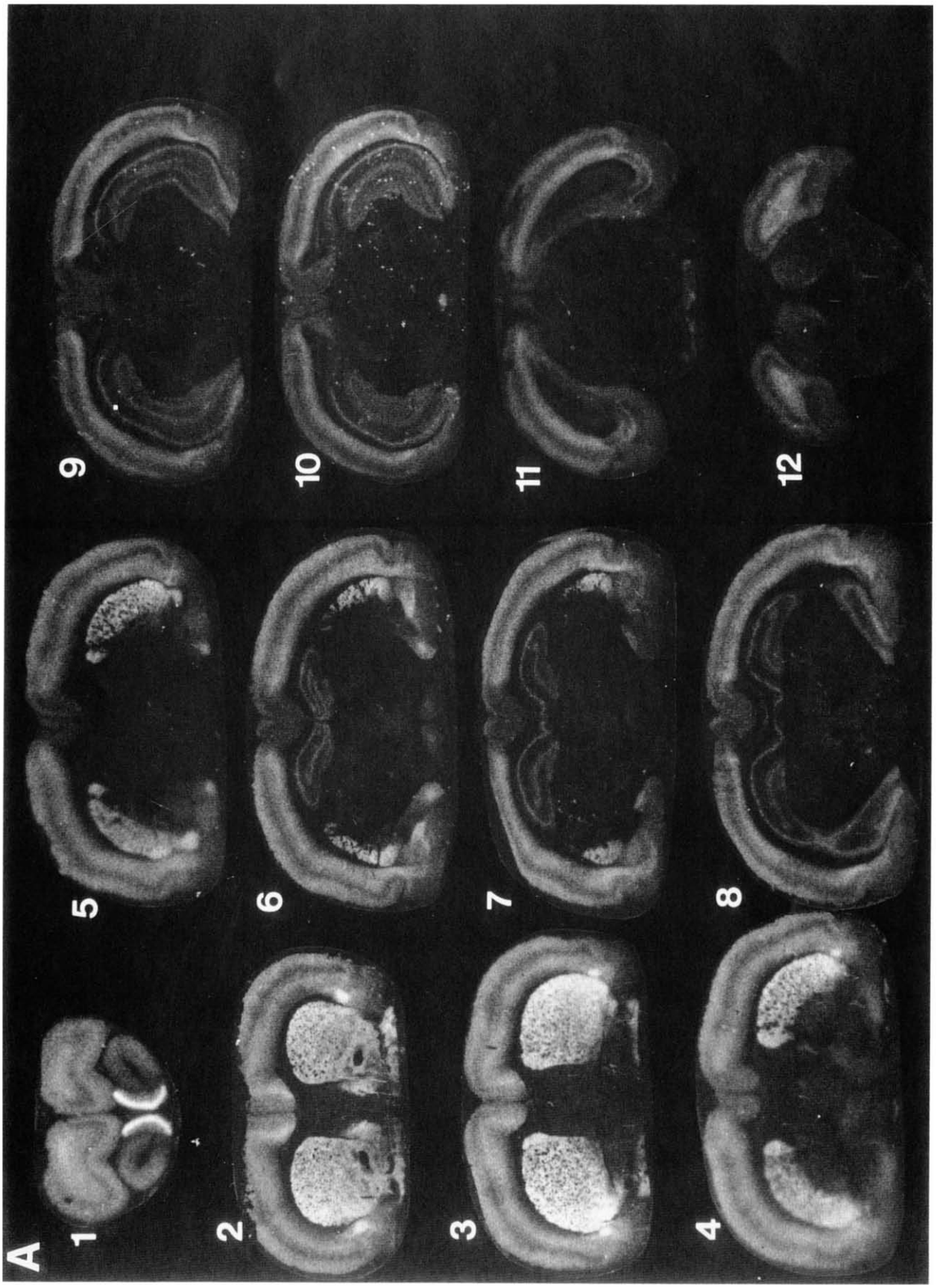
FIG. 4. (A) Anatomical distribution of delta receptors in serial coronal sections of rat brain labelled with [^{3}H]DPDPE in the presence of 5 mM $MgCl_2$, 0.1% BSA and 20 μg/ml bacitracin. (B) Distribution of mu receptors in rat brain using [^{3}H]DAGO. (C) Distribution of kappa receptors in rat brain using 0.2 nM [^{125}I]-dynorphin(1–8) in the presence of 200 nM DAGO and DADLE. (D) Delta receptor profile in guinea pig brain using [^{3}H]DPDPE.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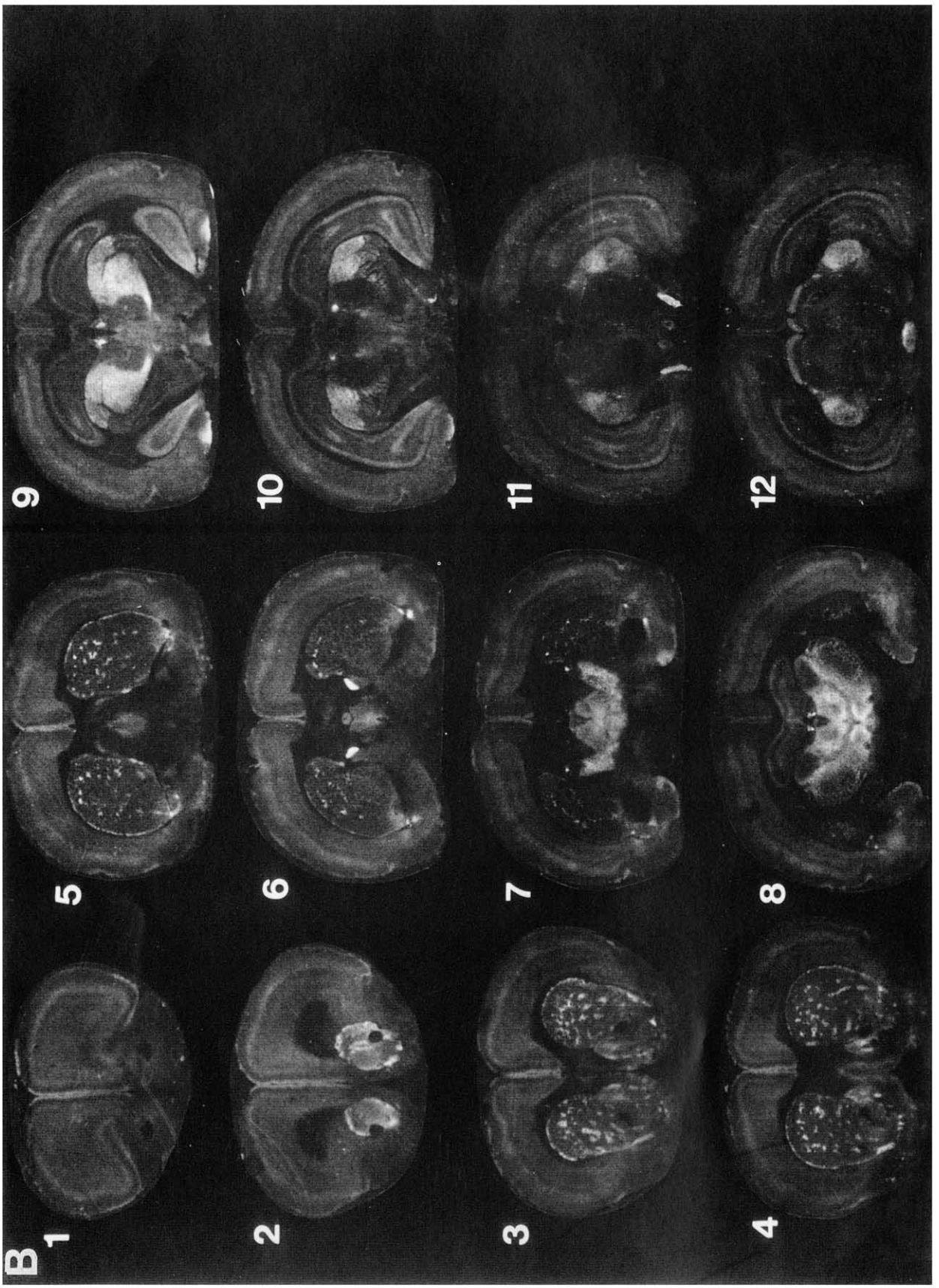
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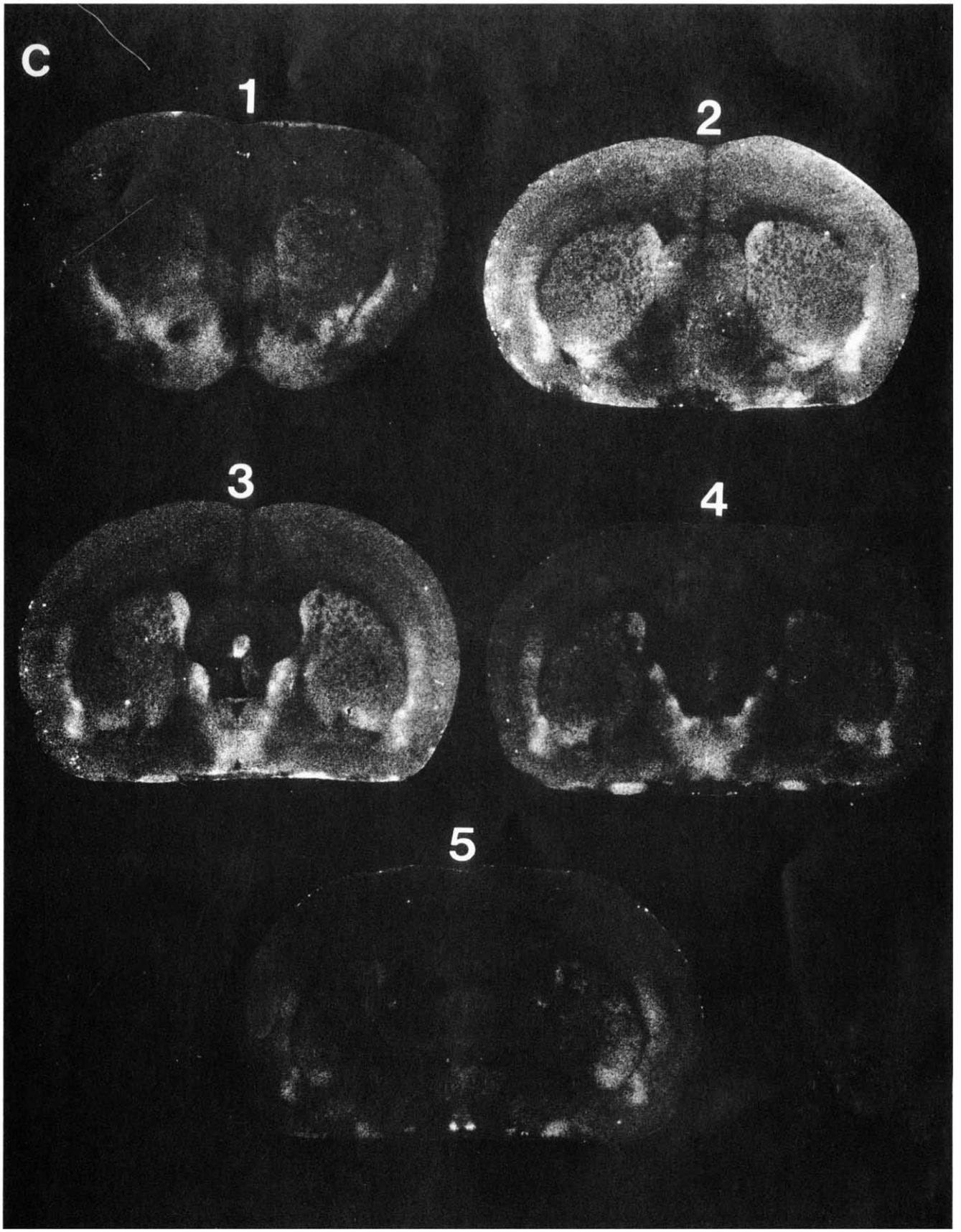
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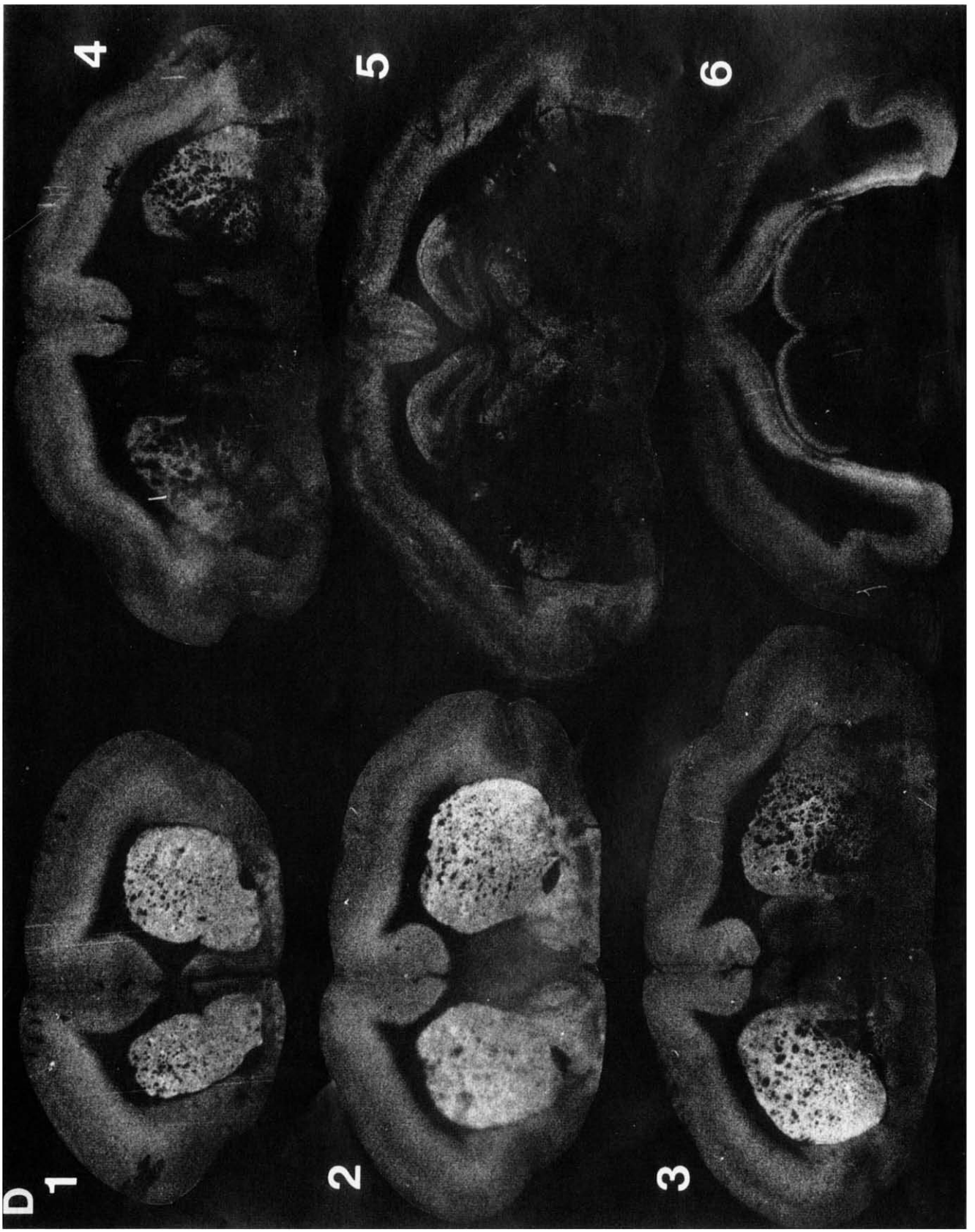
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TABLE 4

QUANTITATIVE AUTORADIOGRAPHY OF [^{3}H]DPDPE-LABELLED DELTA OPIOID RECEPTORS IN RAT AND GUINEA PIG CNS

Brain Region	Specific Binding (amol/mm^2) Rat	Guinea Pig
Olfactory tubercule	592 ± 22	275 ± 11
Accessory olfactory bulb	40 ± 10	38 ± 12
Olfactory bulb		
Glomerular layer	49 ± 13	30 ± 13
External plexiform layer	625 ± 112	396 ± 131
Granular layer	181 ± 90	172 ± 62
Striatum	306 ± 30	152 ± 24
N.acumbens	597 ± 22	327 ± 17
C.cortex		
Layers I–II	265 ± 89	250 ± 16
Layers III—IV	204 ± 22	121 ± 16
Layers V–VI	313 ± 60	280 ± 12
Stria terminalis	33 ± 4	84 ± 7
Amygdala		
Basolateral nucleus	298 ± 81	112 ± 39
Medial nucleus	310 ± 69	131 ± 43
Cortical nucleus	272 ± 89	132 ± 61
Lateral nucleus	245 ± 72	129 ± 74
Central nucleus	59 ± 21	40 ± 11
Hypothalamus	49 ± 6	82 ± 21
Habenula	50 ± 8	90 ± 10
Hippocampus pyramidal layer	83 ± 3	65 ± 5
Globus pallidus	50 ± 13	111 ± 12
Thalamus	31 ± 5	162 ± 13
Substantia nigra	32 ± 6	18 ± 2
Lat. Geniculate bodies	30 ± 4	116 ± 13
Central grey	33 ± 4	78 ± 9
Superior colliculus	33 ± 4	197 ± 18
Inferior colliculus	68 ± 8	94 ± 8
Spinal cord		
S.gelatinosa	36 ± 6	58 ± 7
V.grey	7 ± 1	19 ± 5

The autoradiographic experiments were performed with 8 nM [^{3}H]DPDPE as described in the text. The above data are means ± SEM of results from three animals of each species. Specific[^{3}H]DPDPE binding was 80–90% of the total binding. Olf = olfactory; EP = external plexiform layer; S.gelatinosa = substantia gelatinosa; V.grey = ventral grey; ND = not determined.

TABLE 5

QUANTITATIVE AUTORADIOGRAPHIC LOCALIZATION OF MU OPIOID RECEPTORS IN RAT AND GUINEA PIG BRAIN

Brain Region	Specific Binding (amol/mm^2) Rat	Guinea Pig
Olfactory tubercule	121 ± 38	ND
Accessory olfactory bulb	547 ± 123	620 ± 160
Olfactory bulb		
Glomerular layer	361 ± 98	393 ± 112
External plexiform layer	340 ± 79	421 ± 109
Granular layer	109 ± 39	113 ± 79
Amygdala		
Baslolateral nucleus	296 ± 27	210 ± 45
Medial nucleus	240 ± 21	201 ± 39
Cortical nucleus	287 ± 30	289 ± 36
Lateral nucleus	267 ± 59	253 ± 46
Central nucleus	50 ± 21	71 ± 36
Septohippocamp. N.	113 ± 19	99 ± 31
Subfornical organ	195 ± 18	ND
Stria terminalis	250 ± 51	181 ± 62
Striatum (non patch)	239 ± 64	261 ± 80
(non patch) head	106 ± 23	81 ± 25
(non patch) body	188 ± 69	110 ± 30
(non patch) tail	227 ± 35	139 ± 12
Striatum patches	628 ± 74	723 ± 95
Striatum streaks	422 ± 155	616 ± 186
N.accumbens	398 ± 71	501 ± 139
Hippocampus		
Molecular layer	176 ± 19	150 ± 87
Pyramidal layer	211 ± 25	49 ± 13
Dentate gyrus (vent.)	265 ± 13	169 ± 38
Spinal cord		
Substantia gelatinosa	ND	119 ± 10
Dorsal/ventral grey	ND	30 ± 7
Cerebellum	12 ± 4	18 ± 3

The data (amol/sq. mm) are means ± SEM from 3–4 animals of each species using 3.0 ± 0.2 nM [^{3}H]DAGO and 1 μM DAGO for defining nonspecific binding. ND = not determined.

n.accumbens, cerebral cortex (layers I–II and V–VI) and the hippocampal formation. The rat brain appeared to contain a slightly higher overall density of delta sites than the guinea pig (Fig. 4A, D; Table 4), but whereas the rat mid- and hindbrain appeared to be relatively deficient in delta sites this was not the case for the guinea pig since an appreciable level of [^{3}H]DPDPE binding was associated with the thalamus, lateral geniculate bodies, central grey and superior and inferior colliculus (Fig. 4A, D; Table 4) in the latter species compared to the rat. Although quantitative data were not obtained for the mouse brain, initial qualitative observations (Fig. 8A) indicated that the relative brain delta site distribution in this species resembled that of the guinea pig and the rat. However, the pattern of delta receptor localization in the three rodents was totally different to that found for mu (Figs. 4B, 5B, 6; Table 5) and kappa (Fig. 4C; Table 6) opioid sites in these species.

Mu receptors in the rat brain (Figs. 4B, 5B, 6A; Table 5) were ubiquitously distributed in the fore-, mid- and hindbrain, and this profile also resembled, in general, that of guinea pig (Figs. 5C, 6B; Table 5) and mouse (Fig. 8B) brain. Brain regions of high mu receptor density included the olfactory tissues, amygdaloid complex, n.accumbens, striatal patches and streaks, hippocampal pyramidal layer and dentate gyrus, thalamic nuclei, medial cortical laminae, inferior and superior colliculi, central grey, geniculate bodies, substantia nigra and interpeduncular nucleus. The cerebel-

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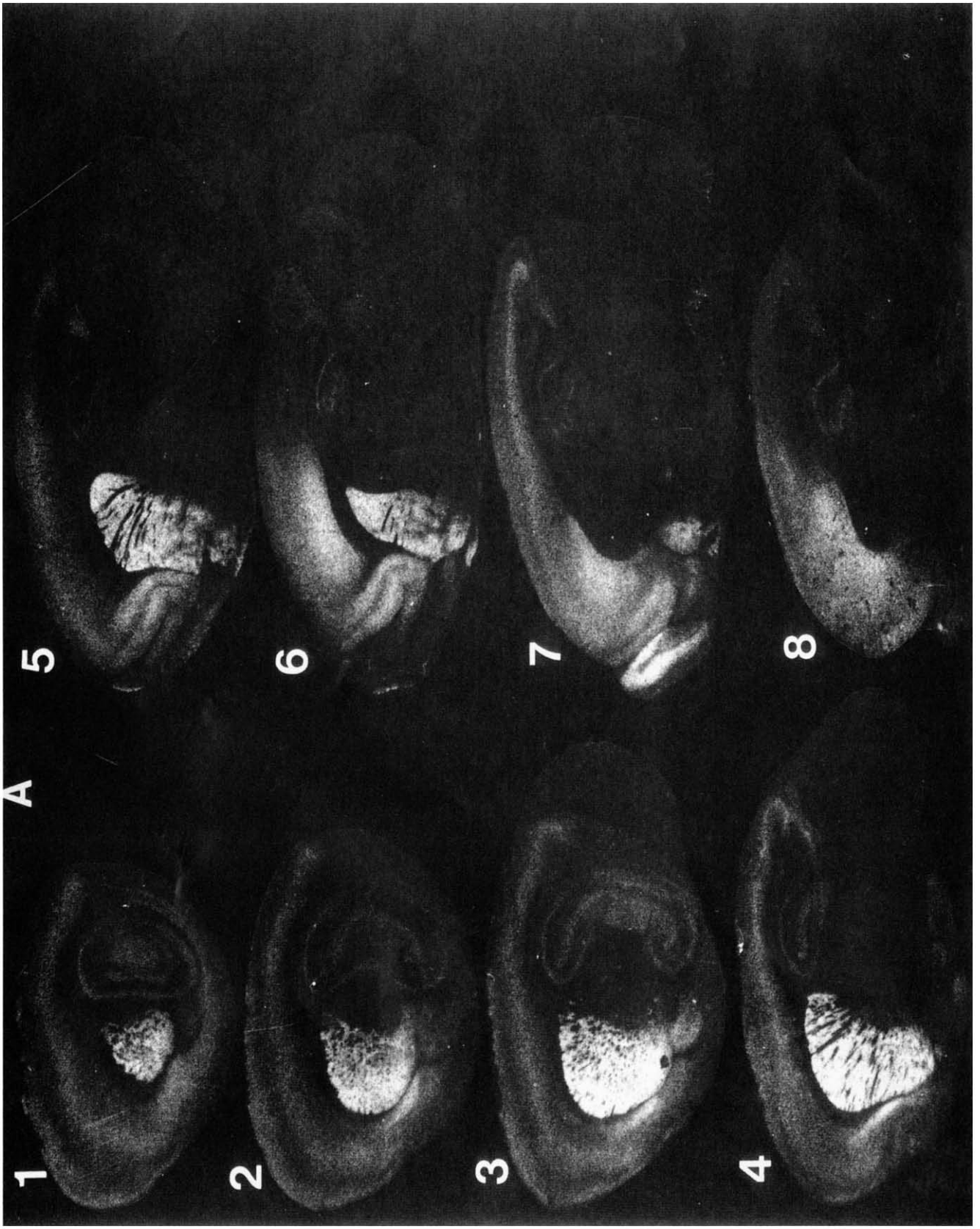
FIG. 5. (A) Distribution of delta receptors in sagittal sections of rat brain using [^{3}H]DPDPE. (B) Distribution of mu receptors in sagittal sections of rat brain. (C) Distribution of mu (sections 1–4) and delta (sections 5–7) receptors in guinea pig brain. Section 8 = mu; 9 = kappa and 10 = delta receptors in guinea pig spinal cord.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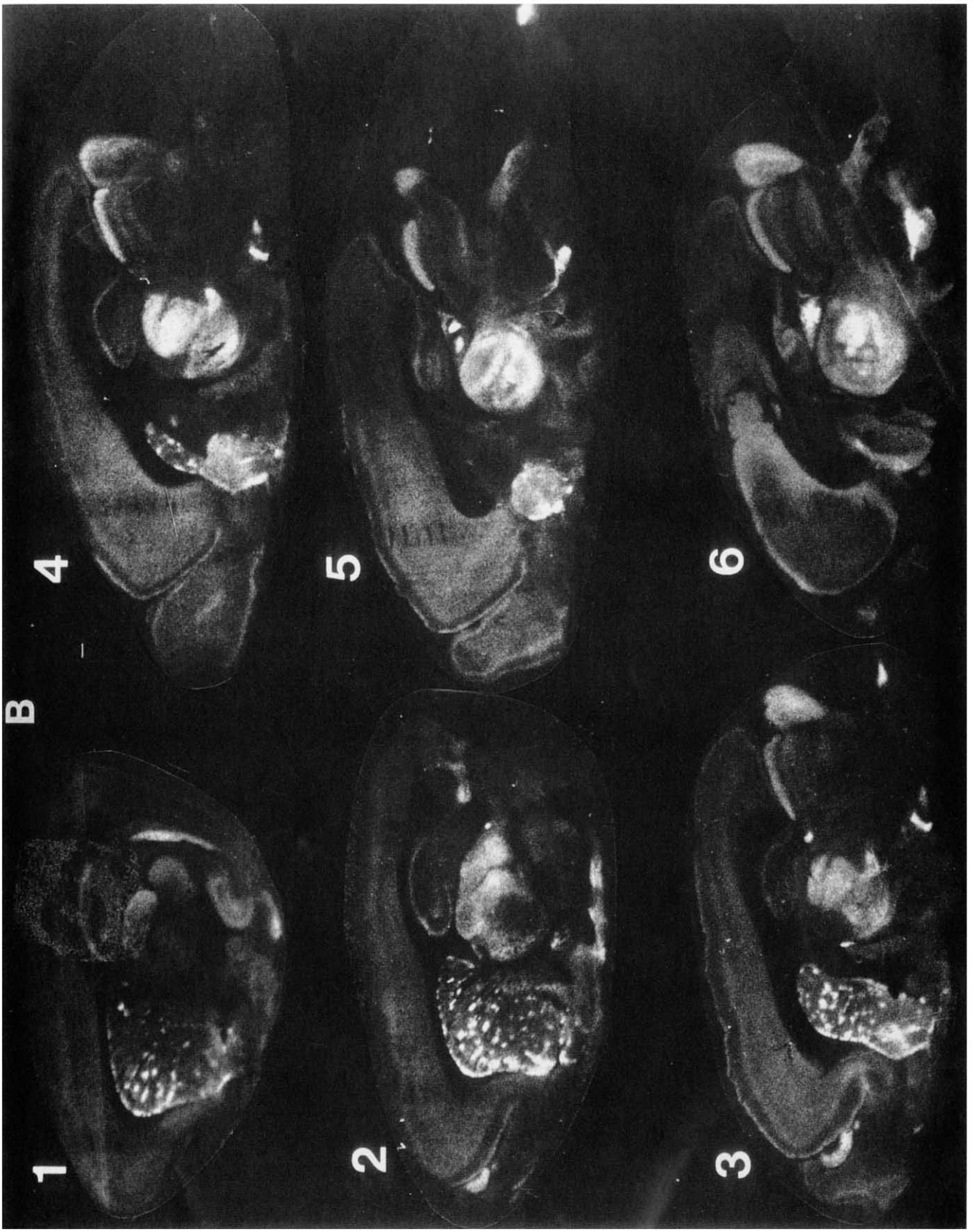
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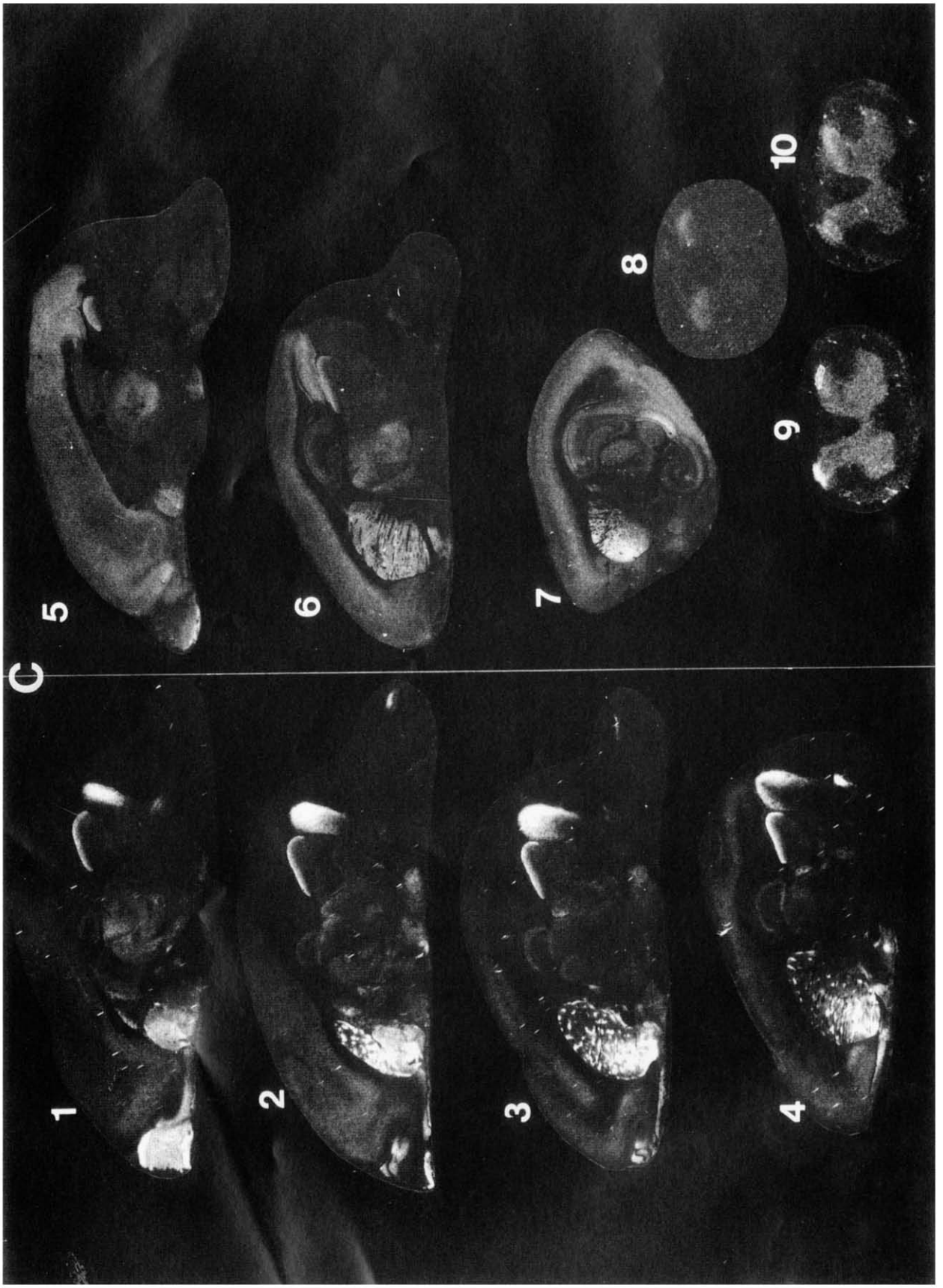
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TABLE 6

QUANTITATIVE AUTORADIOGRAPHIC DISTRIBUTION OF KAPPA OPIOID RECEPTORS USING THE PEPTIDE [^{125}I]DYNORPHIN(1–8)

Brain Region	Specific K-Binding (amol/mm^2) Rat	Guinea Pig
Olfactory bulb	ND	1.8 ± 0.3
Olfactory tubercle	3.1 ± 0.4	2.7 ± 0.2
Amygdaloid complex	1.6 ± 0.2	1.4 ± 0.3
Globus pallidus	1.3 ± 0.1	4.9 ± 0.4
Subfornical organ	2.5 ± 0.2	0.3 ± 0.1
N.accumbens	2.2 ± 0.2	4.1 ± 0.2
Striatum		
Head	1.8 ± 0.1	4.1 ± 0.7
Body	0.9 ± 0.1	3.6 ± 0.2
Tail	2.3 ± 0.2	6.2 ± 0.7
Hippocampus		
Molecular layer	0.4 ± 0.2	2.0 ± 0.2
Granular layer	0.3 ± 0.1	0.6 ± 0.1
Thalamus		
Centromedial N.	0.8 ± 0.1	0.5 ± 0.1
Reticular N.	1.9 ± 0.2	0.4 ± 0.1
Hypothalamus		
Dorsomedial N.	1.2 ± 0.2	0.6 ± 0.2
Ventromedial N.	1.1 ± 0.1	2.0 ± 0.4
Medial preoptic area	2.8 ± 0.3	0.3 ± 0.1
Suprachiasmatic N.	2.3 ± 0.2	0.5 ± 0.1
Cerebral Cortex		
Layers I–II	0.9 ± 0.1	1.8 ± 0.3
Layers III–IV	0.6 ± 0.1	1.4 ± 0.2
Layers V–VI	1.9 ± 0.3	5.1 ± 0.3
Central grey	1.9 ± 0.2	1.4 ± 0.3
Substantia nigra	2.2 ± 0.4	2.2 ± 0.3
Cerebellum		
Molecular layer	0.4 ± 0.1	2.6 ± 0.2
Granular layer	0.3 ± 0.1	0.9 ± 0.2
Posterior pituitary gland	ND	1.9 ± 0.3
Anterior pituitary gland	ND	0.3 ± 0.1

Data are means ± SEM of selected tissues from rat and guinea pig brains (n = 3–10 of each species) using 0.2 nM [^{125}I]dynorphin(1–8) in the presence of 200 nM DAGO and 200 nM DADLE to block mu and delta receptors. Nonspecific binding was defined with 1 μM etorphine and accounted for 20–35% of the total binding in these tissues. ND = not determined.

lum of all three rodents contained almost undetectable levels of specific mu sites.

The rat brain exceeded the guinea pig brain in its content of mu receptors (Figs. 4B, 5B, C, 6A, B; Table 5). Specifically, the following brain structures of the rat appeared more enriched in mu sites than the equivalent guinea pig tissues: olfactory bulb, hippocampal formation, various thalamic and hypothalamic nuclei and cortex (layers III–IV) (Table 5).

Following ibotenate-induced lesions of the striatum a marked (45–50%) reduction in mu and delta receptors was observed in the rat ipsilateral striatum as compared to the contralateral control striatum (Fig. 7A). Similarly, after 6-hydroxydopamine-induced degeneration of the nigro-striatal pathway, a 30% loss of striatal mu receptors was noted (Fig. 7B) and a 28% decrease in the delta receptors in this tissue (data not shown). The loss of mu receptors after both types of lesions appeared to be due to a decrease in labelling of striatal "patches."

DISCUSSION

Quantitative autoradiographic analyses of the relative brain regional distribution of mu, delta and to a limited extent, kappa receptors, in the rat and guinea pig brain demonstrated marked species and subregional localization patterns for these receptor subtypes. For simplicity and conciseness these aspects will be discussed on a tissue basis.

Limbic System

The olfactory bulb (OB) and the associated tubercle were highly enriched in mu, delta and kappa receptors. Mu and delta sites were also abundant in the mouse olfactory tissues (Fig. 8). Delta receptors predominated in the exteral plexiform layer (EPL) of the OB and the tubercle and exhibited a lower concentration in the accessory olfactory bulb (AOB) and the remainder of the OB in the rat and guinea pig (Table 4). In contrast, mu receptors were highly concentrated in the AOB, EPL of the bulb and the glomerular layer of the bulb (Table 5). Kappa sites displayed a similar labelling profile to the mu receptors (Table 6). While the overlapping localization of the subtypes of opioid receptor in the olfactory tissues indicates an overall involvement of opioids in modulating/transmitting olfactory sensations, the divergent patterns of mu, delta and kappa site binding suggest that these subtypes may also have unique functions in these tissues. For instance, the mu receptors appear to be located in the glomerular layer and the EPL of the OB, sites where they may modulate primary sensory and centrifugally-derived information, respectively. However, the functional relevance of the differential opioid receptor distribution in the rodent olfactory tissues remains to be determined.

A high density of mu, delta and kappa receptors was present in the rat and guinea pig amygdaloid complex. The rat exceeded the guinea pig in its content of delta sites in this limbic structure (Table 4) but the relative distribution was similar in both species. Of the five amygdaloid nuclei examined, the central nucleus appeared to express the lowest density of both mu and delta sites in the rat and guinea pig (Tables 4, 5). Although these observations corroborate the qualitative observations made in the rat and monkey (21–23, 25, 26) they are at variance with the earlier data of Goodman *et al.* (13,14) who reported that only delta receptors predominated in the amygdala. The reason for this discrepancy could be due to the use of rather nonselective radioligands and the lack of quantification of data by the latter authors. In any case, we have now provided *quantitative* data for the amygdaloid complex in the rat and guinea pig. The delta receptor activation in the limbic regions, such as the amygdala, has the functional correlates of limbic seizures (43) and reward behaviors (42).

Within the hippocampal formation marked variations were found in the regional and species localization patterns of opioid receptors. While delta and mu receptors were primarily associated

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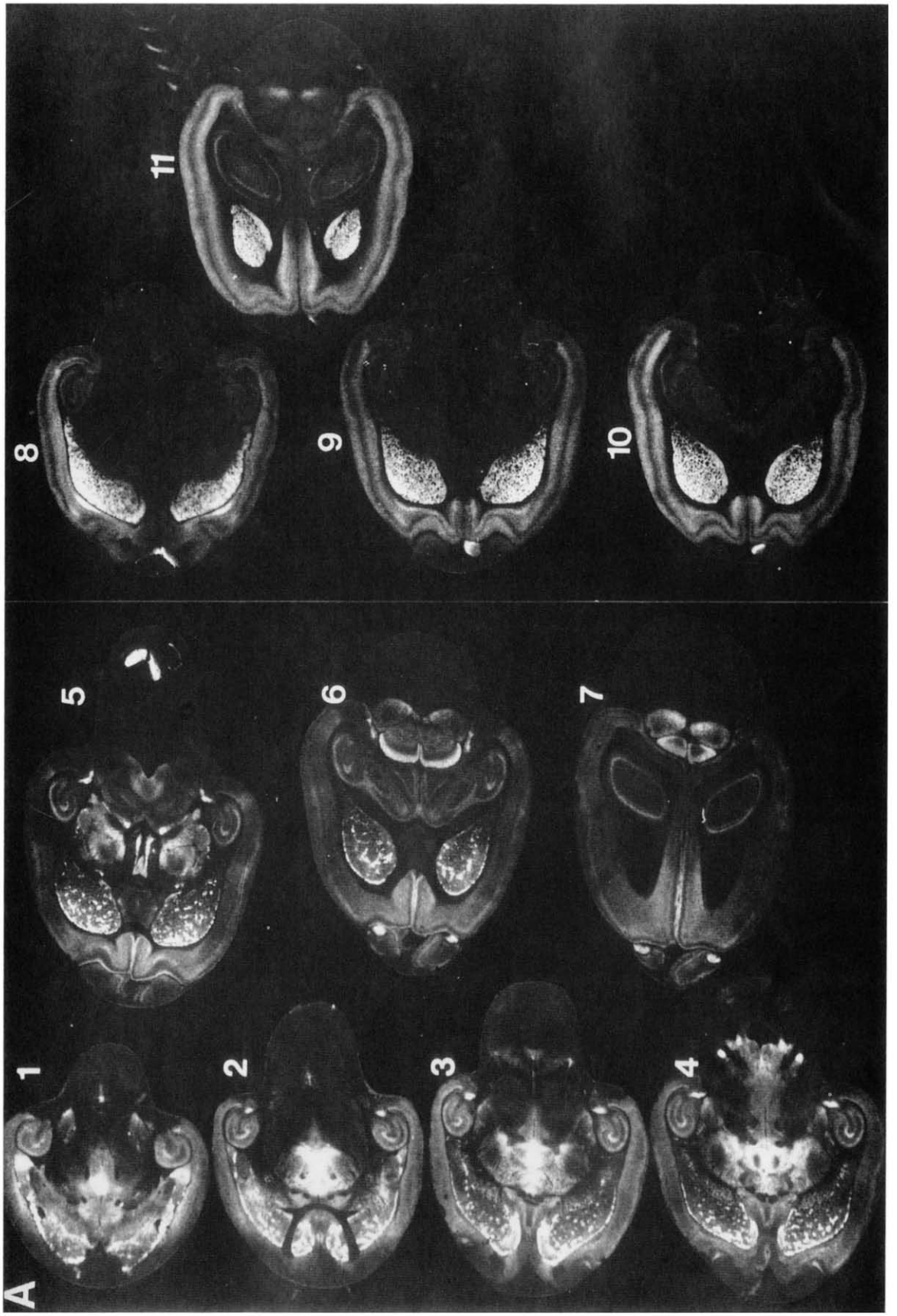
FIG. 6. (A) Distribution of mu (sections 1–7) and delta (sections 8–11) receptors in horizontal sections of the rat brain. Note the dissimilar localization profiles of these receptor systems. (B) Mu receptor distribution in horizontal sections of guinea pig brain.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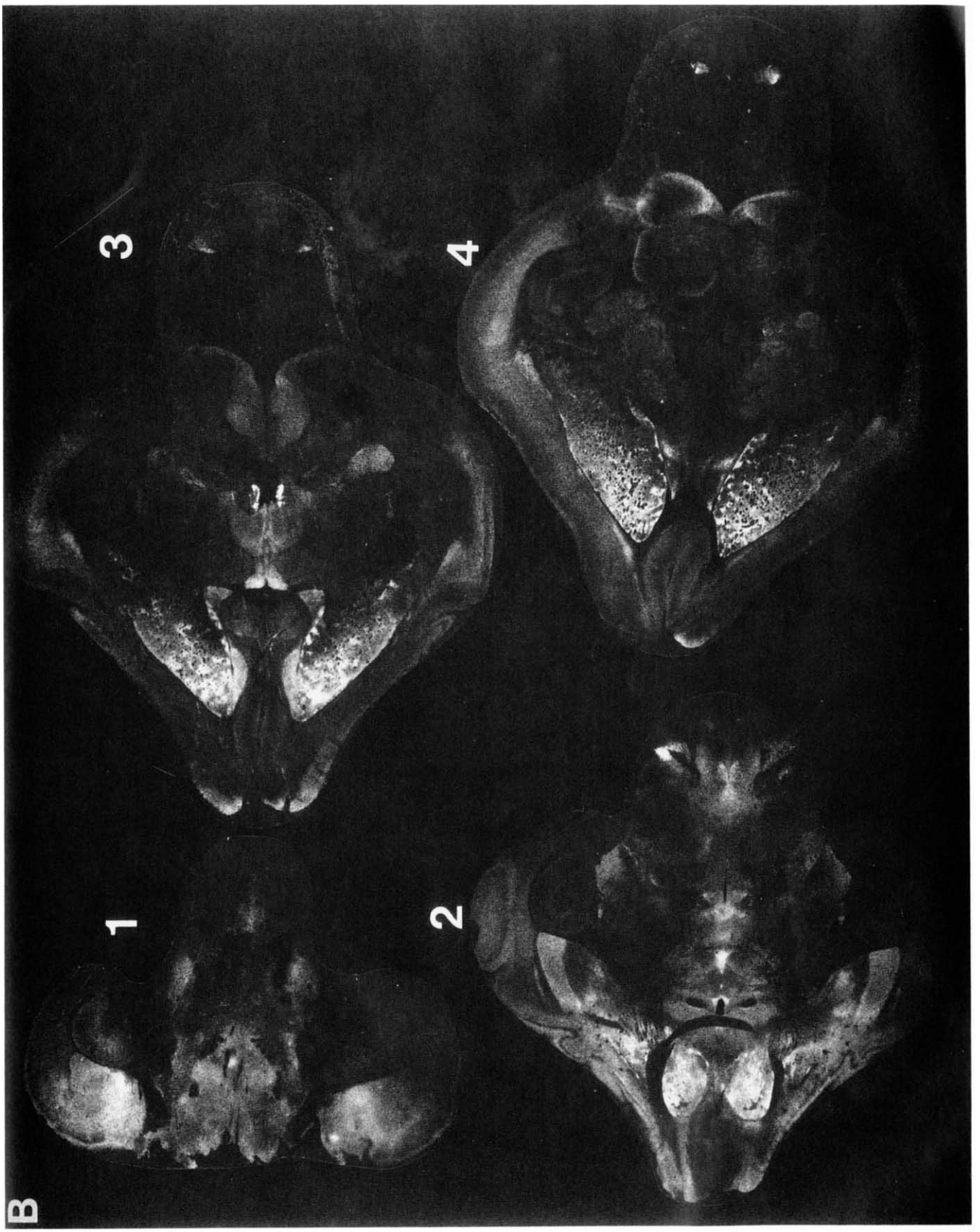
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with the pyramidal cell layer in the rat, kappa sites were diffusely labelled in this region and the rest of the hippocampus. In contrast, in the guinea pig a higher density of mu receptors was found in the molecular layer as opposed to the pyramidal layer, and kappa sites were mainly localized to the molecular and granular layers. All three opioid receptor subtypes exhibited a high concentration in the ventral dentate gyrus of the hippocampus (Figs. 3–8) that exceeded that observed in the dorsal hippocampus. While the rat apparently exceeded the guinea pig in its content of mu sites, the opposite profile was apparent for the kappa sites in the hippocampus, indicating that perhaps the rat is a good model to study mu opioid action while the guinea pig represents an equally good model for studying kappa agonist action at this anatomical level. Similar findings to the above in the rat hippocampus have been previously reported by other authors (23, 24, 48).

The n.accumbens of the rat exceeded the guinea pig tissue in its enrichment of delta (Table 4) and kappa (Table 6) sites, but the reverse was true for the mu sites (Table 5).

Extrapyramidal System

Mu, delta and kappa receptors were present in high concentrations in the striatum of the rat, guinea pig and mouse. However, while the delta and kappa sites exhibited a relatively uniform distribution here, the mu sites were discretely localized in distinct patches (Figs. 4–6). Of the "non-patchy" striatal mu sites the bulk seemed to be localized to the ventrolateral striatum ("tail") (Table 5). Interestingly, the kappa receptors in the rat and guinea pig striatum showed a similar high density in the "head" and "tail" of this structure (39).

In general, a relatively good correlation has been found between the distribution of mu and delta receptors and that of enkephalinase in the rat striatum (44,45), thus providing some support for the physiological functions of mu and delta receptors in the striatum. With respect to the latter, mu and delta agonists modulate striatal dopamine release (7) and high opiate doses have been shown to lead to cataleptic behavior with muscular rigidity (2). The fact that the rodent substantia nigra also contains a high density of mu receptors (Table 5) suggests that the latter are principally involved in modulating the dopaminergic transmission at the nigrostriatal pathway.

Neurotoxin-induced lesions of the rat striatum (Fig. 7A, B) indicated that an almost equal proportion of mu and delta sites is located on the striatal neurones and on the presynaptic terminals of the nigrostriatal dopamine neurones, thus providing further evidence for the possible physiological role of mu and delta receptors in this tissue. Similar decreases in striatal mu (40–50% of controls) and delta (22–51% of controls) receptors have been previously observed following kainate and 6-hydroxydopamine-induced lesions in the rat brain using a variety of radioligands (5, 11, 45). An antinociceptive role of striatal mu receptors has also been demonstrated in the rat (18) and in the monkey (22).

While the globus pallidus of the rat and guinea pig appeared to contain a very low density of mu and delta sites, this region was highly endowed with kappa receptors, particularly in the guinea pig (Table 6). Curiously, the globus pallidus is very rich in enkephalins and relatively deficient in dynorphins (21,46), thus highlighting a classical transmitter-receptor mismatch.

Sensory Systems

Opioid receptor subtypes were present at most cortical levels that exhibited differential localization patterns (Figs. 3–8). While the delta receptors were predominantly localized within the outermost and innermost cortical laminae (layers I–II and V–VI) in the rat, guinea pig and mouse brain, the mu sites were highly concentrated within the medial (layers III–VI) laminae in these species. Kappa sites, on the other hand, were mainly found within layers V–VI (Fig. 4C; Table 6) and exhibited a greater density in the guinea pig than the rat. The distribution of mu sites can be correlated with the efferents from the thalamic nuclei to the midcortical layer cells (21, 23, 25, 26) and delta receptor localization has been correlated with the terminal zones of vertical interlaminar local circuit cells. Thus, while mu receptors appear to be involved in modulating thalamocortical information, the delta sites probably influence the intracortical information processing. The deep cortical localization sites of kappa receptors have been suggested as the neuronal substrates for the mediation of the sedative and analgesic effects of kappa agonists (13), at least in the guinea pig.

Mu, delta and kappa receptors have been visualized in the substantia gelatinosa of the rat and guinea pig spinal cord in the present study (Fig. 5C; Tables 4–6) and similar qualitative observations have been previously reported for the rat (15). These receptors are of course well positioned for modulating the sensory information that is relayed to the brainstem, thalamus and the higher cortical centers, and much evidence supports the role of these spinal opioid receptors in producing the antinociceptive effects of mu, delta and kappa opioid drugs (2,36). In view of the loss of opioid receptors after dorsal rhizotomies (20) and the opioid mediated inhibition of substance P from the rat trigeminal nucleus (17), the majority of the opioid receptors in the spinal cord dorsal grey appear to be presynaptic in nature. Since high levels of enkephalins are also found in the dorsal grey of the cord, this suggests that these may subserve a neurotransmitter function and probably activate the mu and delta receptors observed here (Fig. 5C).

An interesting dichotomy was apparent in relation to the relative presence/absence of the opioid receptor subtypes in the thalamic complex. Thus, while mu receptors could be visualized within the majority of the rat and guinea pig thalamic nuclei (Figs. 4B, 5B; Table 5), these subregions were poorly labelled by both delta and kappa radioligands (Figs. 4A, C, D, 5A; Tables 4 and 6). In a similar vein, the superior and inferior colliculi of the rat and guinea pig appeared to be more enriched in mu receptors than delta and kappa receptors (Tables 4–6). The pattern of opioid receptor distribution/density in the thalamus, brainstem and the colliculi has been best correlated with the specific site of mediation of the sedative and respiratory depressant actions of opioids, in particular mu agonists (2,46), in addition to their analgesic properties. The density of mu and kappa sites in the rat thalamic nuclei exceeded that of the guinea pig, whilst the reverse was apparent for the delta receptors (Tables 4–6). Once again these data may indicate that the rat may be a more useful species to study the mu actions of opioid

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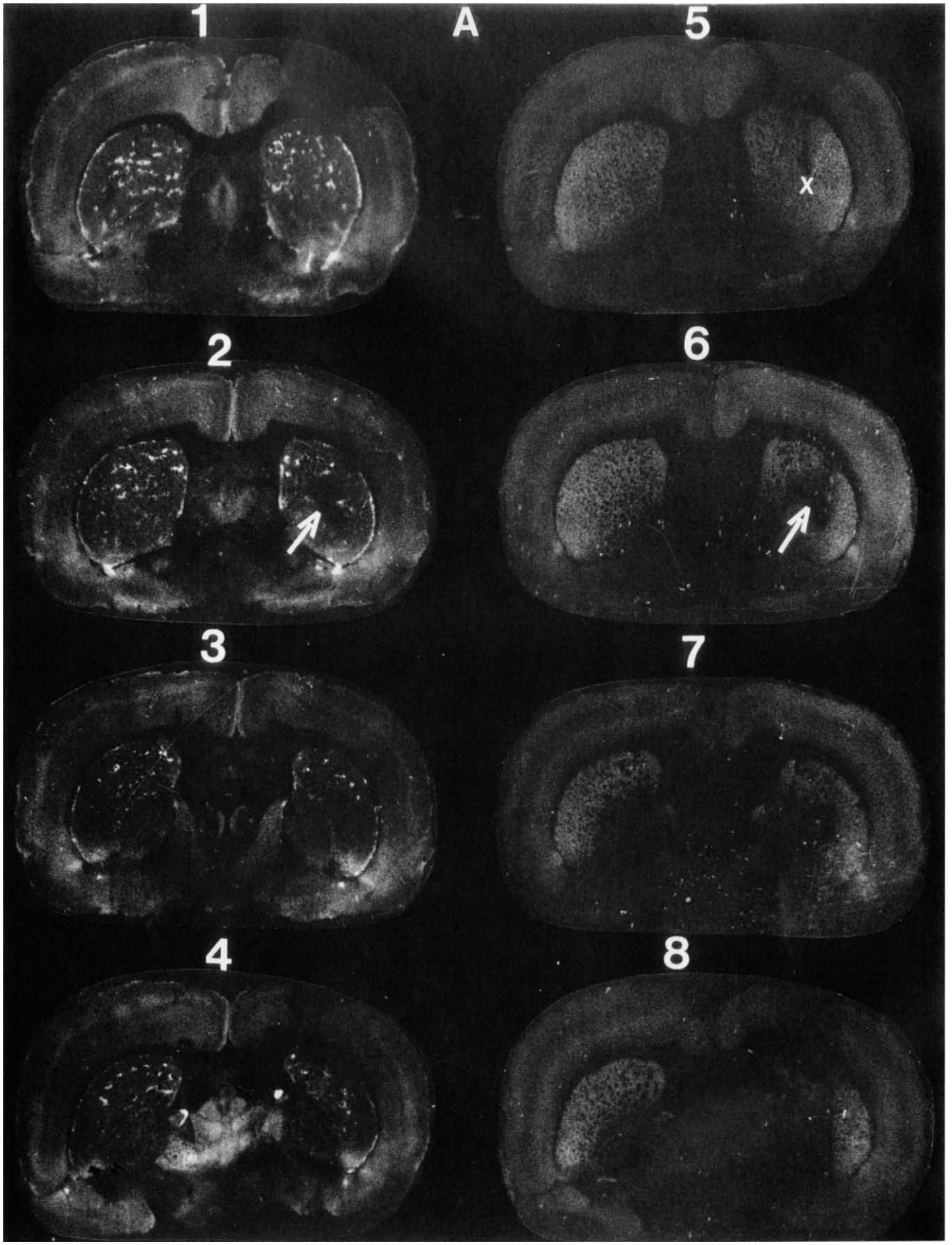
FIG. 7. (A) Ibotenate-induced lesions of rat striatum. Note the marked reductions in mu receptors (sections 1–4) and delta receptors (sections 5–8) in the lesioned striatum (site of the toxin injection marked with an "X" and the receptor loss indicated by arrows) as compared with unlesioned contralateral striatum. (B) Mu receptor distribution in coronal rat brain sections after destruction of the nigrostriatal pathway. Note the loss of mu receptors on the lesioned striatum (arrows) compared to the control side.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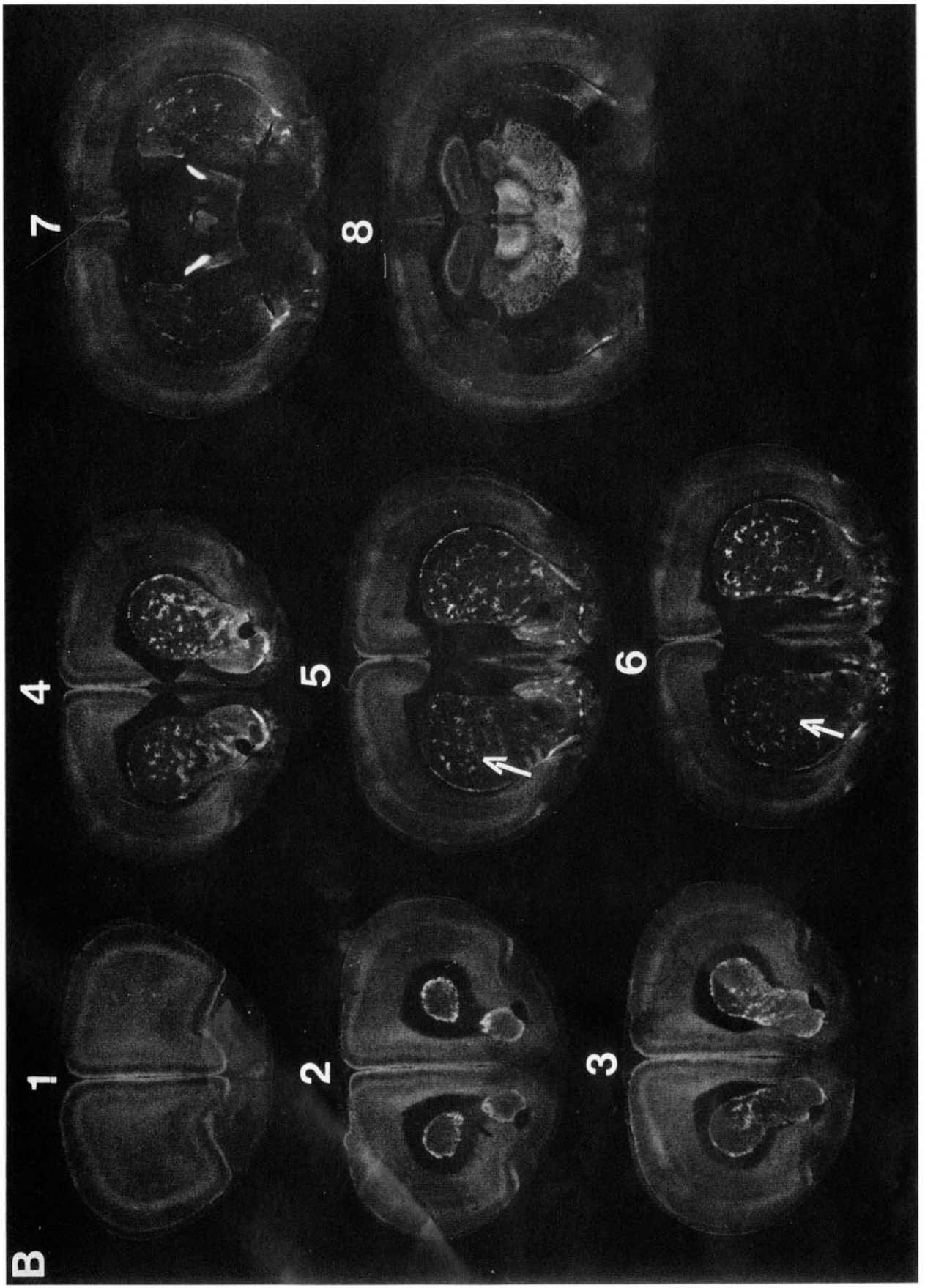
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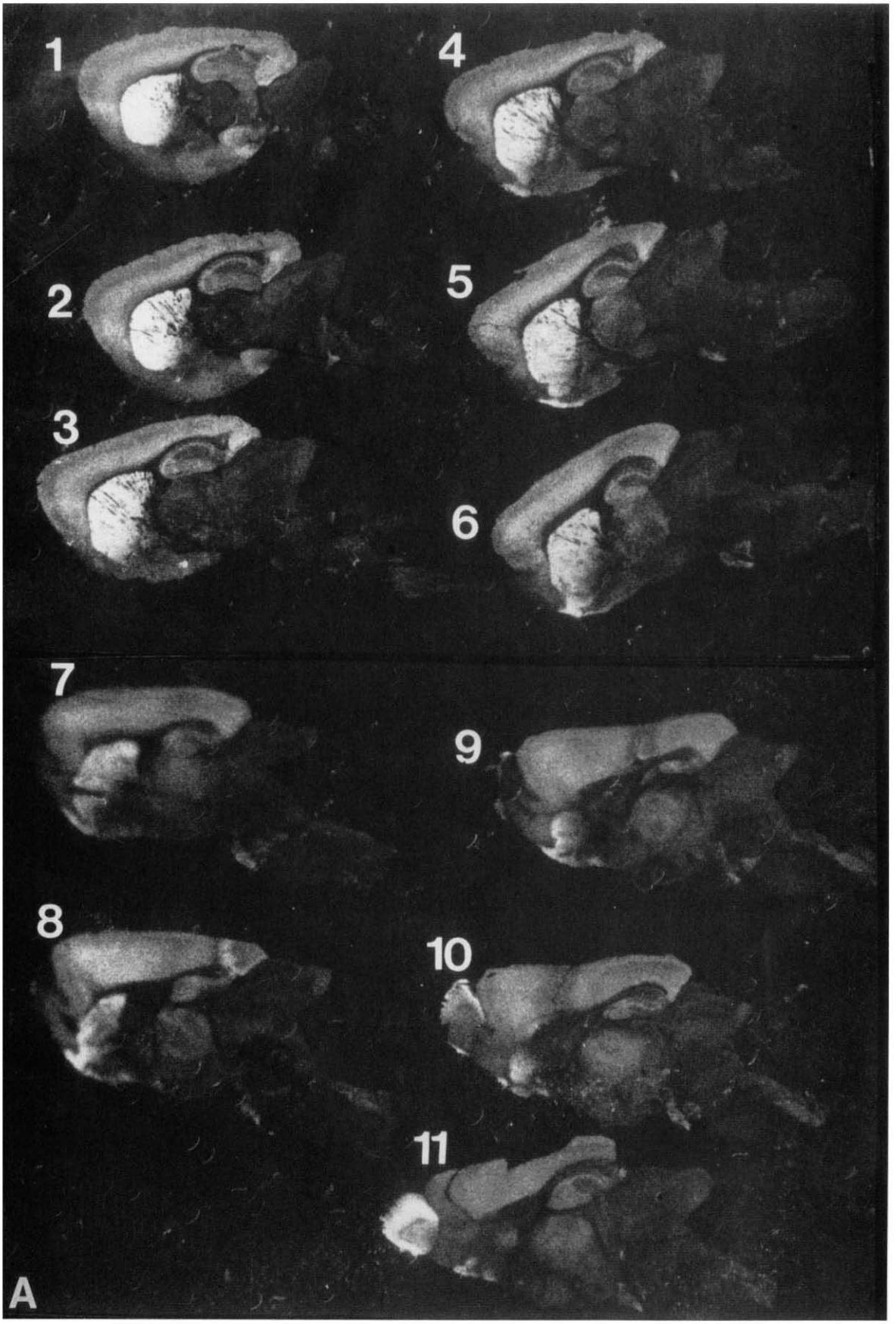
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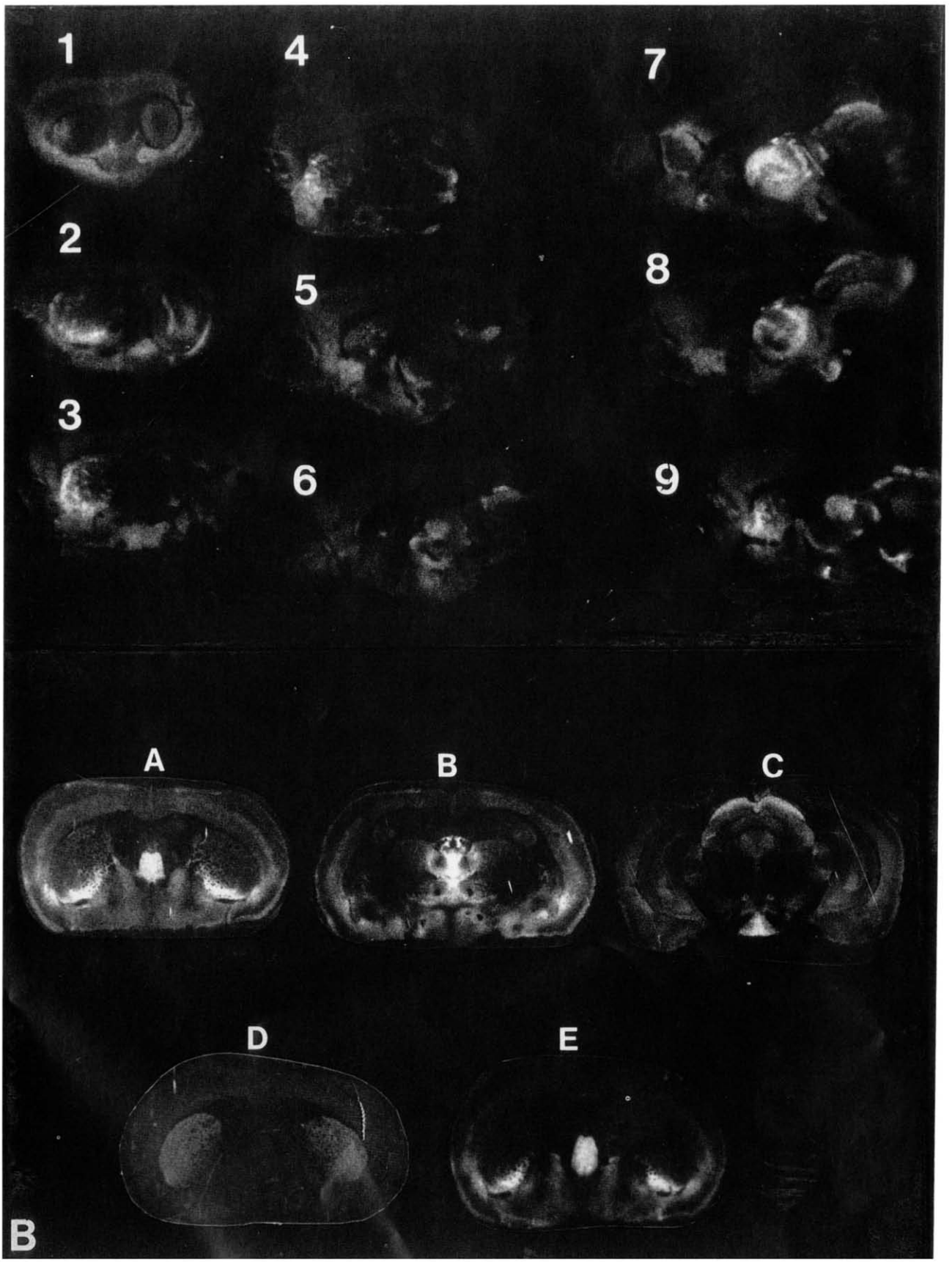
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FIG. 8. (A) Distribution of delta receptors in sagittal sections of mouse brain using [^{3}H]DPDPE. Note the similarity of labelling profile in the rat (Fig. 5A) and mouse (this figure) brain. (B) Distribution of mu receptors in sagittal sections (1–9), and in coronal sections (A–C) of the mouse brain. Sections D and E show the relative distribution of delta and mu receptors in adjacent mouse brain sections, respectively.

drugs than the guinea pig.

The presence of a relatively high density of mu and kappa receptors in the central grey, and brainstem nuclei such as solitary, spinal trigeminal and pontine nuclei, of the rat and guinea pig (Tables 4 and 6) suggests that these receptor types may also affect some of their analgesic and autonomic functions through these central loci (2). Once again delta receptors appeared to have a low density within the above-mentioned structures in the rat and guinea pig hindbrain (Table 5).

Neuroendocrine and Optical Systems

Opioids are known to exert pronounced effects on hormonal secretion at the CNS, pituitary and peripheral tissue levels (27,28). In agreement with these observations kappa and mu receptors have been detected in various hypothalamic nuclei and the posterior (neural) pituitary gland (kappa only in the latter tissue) (Tables 5, 6; Fig. 4B, C). Thus, the supraoptic/suprachiasmatic nuclei of the hypothalamus, that contain neurones projecting to the posterior pituitary, contain a high density of kappa and mu receptors in the rat, and to a lesser degree, in the guinea pig (Tables 5, 6). In addition, the medial preoptic area and dorsomedial and ventromedial nuclei of the rat and guinea pig hypothalamus contain an appreciable number of kappa receptors. Kappa receptors in the latter nuclei and the posterior pituitary are suitably located to influence hormonal secretion and indeed much evidence supports the inhibitory function of kappa opioid agonists on vasopressin release leading to the well known diuretic actions of kappa agonists (4,46). Interestingly, the rat and guinea pig posterior and anterior pituitary glands and the hypothalamus are relatively deficient in delta opioid receptors (Table 4; Fig. 4A), but the reason behind these differences is not clear at present. Other authors (6) have previously demonstrated the presence of kappa receptors in the rat posterior pituitary which undergo down-regulation after chronic osmotic stimulation.

Since opioids are known to be involved in the diurnal regulation of hormonal secretion (27,28), it was of some significance that mu receptors were highly concentrated in the rat and guinea pig lateral/medial geniculate bodies and inferior and superior colliculi (Table 5), areas that receive sensory information from the retina. Again, these tissues were relatively devoid of delta opioid receptors in the rat brain, although the guinea pig did appear to express a moderate density of these sites in these tissues (Table 4). Kappa receptors may also be involved in the circadian rhythms since at least the colliculi of the guinea pig had a relatively high density of kappa receptors (39). The presence of a high concentration of mu and kappa receptors in the rat superior colliculus and the preoptic area of the midbrain has been linked to the meiotic effects of opioids since these brain regions are known to control the pupillary muscles of the eye (2).

The relatively high levels of mu and kappa receptors within the hypothalamus have also been correlated with the hypothalamic and aphagic effects of opiates (2,28), thus reinforcing the wide spectrum of actions of these drugs which appear to be mediated at different anatomical levels within the mammalian brain.

In conclusion, opioid receptor subtypes have been visualized and quantified in discrete brain regions of the rodent brain, using peptide radioligands, and shown to have different anatomical localization profiles and to exhibit species variations. Since the rat brain appears to contain a greater overall density of mu receptors than the guinea pig, whilst the opposite is indicated for the kappa receptors, we can suggest that the rat be used as an animal model to study the mu and delta effects and that the guinea pig be used to study the kappa effects of novel opioid agonist/antagonist drugs.

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