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Autoradiographic study on the pharmacological characteristics of [^{3}H]3-OH-PCP binding sites in rat brain

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Abstract

The pharmacological characteristics and the regional distribution of [^{3}H]3-OH-PCP (1-[1(3-hydroxyphenyl)-cyclohexyl]piperidine) binding were investigated in rat brain by quantitative autoradiography. Kinetic analysis of [^{3}H]3-OH-PCP binding revealed fast and slow components, in the association and dissociation studies. The regional distribution of binding closely corresponded to those of binding sites labeled by [^{3}H]*N*-[1-(2-thienyl)-cyclohexyl]3,4-piperidine (TCP) and [^{3}H](+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (MK 801). High densities of [^{3}H]3-OH-PCP binding sites were found in the stratum radiatum and oriens of field CA1 in the hippocampus and in the outer layers of cerebral cortices. In contrast, low levels of binding were seen in the brain stem and the granular cell layer of the cerebellum. [^{3}H]3-OH-PCP binding was strongly inhibited by MK 801 and 3-OH-PCP, while the potency of (+)-SKF 10047 in inhibiting [^{3}H]3-OH-PCP binding was less in the cerebral cortex and hippocampus. The antagonists for the glutamate, glycine and polyamine recognition sites at the NMDA/PCP receptor complex displaced [^{3}H]3-OH-PCP binding sites with a potency similar to that of [^{3}H]MK 801. These findings suggest that the [^{3}H]3-OH-PCP binding site is similar or identical to the PCP binding site labeled by [^{3}H]TCP and [^{3}H]MK 801.

Keywords: 3-OH-PCP (1-[1(3-hydroxyphenyl)-cyclohexyl]piperidine); Phencyclidine; NMDA receptor; Autoradiography; Brain, rat

1. Introduction

Phencyclidine (PCP), a dissociative anesthetic, is known to produce severe mental disturbances characterized by delusions, hallucinations, psychomotor excitation and concreteness of thought (Bakker and Amini, 1961; Snyder, 1980). The symptoms of PCP resemble both positive and negative symptoms of schizophrenia, unlike amphetamine-induced psychosis (Javitt and Zukin, 1991). The administration of PCP also exacerbates psychotic symptoms in acute and chronic schizophrenic patients (Ban et al., 1961; Itil et al., 1967).

The psychotomimetic effects of PCP are mediated through a site located within the ion channel formed by the NMDA receptor complex. PCP and related drugs inhibit non-competitively NMDA-mediated glutamergic neurotransmission (Lodge and Anis, 1982; Anis et al., 1983; Coan and Collingridge, 1987; Kemp et al., 1987). MK 801 is also known to interact competitively with a PCP recognition site in the NMDA/PCP receptor complex (Largent et al., 1986; Wong et al., 1986). The regional distribution of PCP binding sites labeled by [^{3}H]*N*-[1-(2-thienyl)-cyclohexyl]3,4-piperidine (TCP) and [^{3}H](+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (MK 801) correlates well with that of the NMDA receptor labeled by [^{3}H]glutamate (Maragos et al., 1986, 1988; Bowery et al., 1988), [^{3}H]glycine and [^{3}H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) (Jarvis et al., 1987). The PCP site labeled by [^{3}H]TCP and [^{3}H]MK 801 has two different binding components in brain (Vignon et al., 1986; Haring et al., 1987; Chicheportiche et al., 1988; Javitt and Zukin, 1989a,b). High affinity binding sites labeled by [^{3}H]TCP are exclusively located

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in the forebrain, while only the low affinity sites are present in the hindbrain and spinal cord (Vignon et al., 1986). A subsequent study has suggested that high affinity sites linked to NMDA-gated ionic channels, while low affinity TCP sites represent σ-binding site or dopamine uptake complex (Vignon et al., 1989). In addition, the NMDA-coupled PCP receptors are enhanced by glutamate (Loo et al., 1986), NMDA (Reynolds and Miller, 1988) and glycine (Snell et al., 1987; Thomas et al., 1988). Although the low affinity sites labeled by [^{3}H](+)*N*-allylnormetazocine ((+)-[^{3}H]SKF 10047) are similar in their distribution as [^{3}H]TCP labeled sites (Largent et al., 1986), (+)-SKF 10047 has relatively less potency for inhibiting [^{3}H]TCP-labeled PCP binding than PCP and its analog (Zhou et al., 1991). Thus, the PCP site associated with the NMDA receptor complex is referred to as the PCP_1 site on a pharmacological profile, while a lower affinity site not linked to the NMDA receptor complex is called the PCP_2 site (Zhou et al., 1991). These receptors are also regulated by additional agents including magnesium and polyamines, such as spermine and spermidine (Ransom and Stec, 1988; Williams et al., 1989, 1990, 1991). Polyamines act at a polyamine recognition site of the NMDA receptor complex and affect the recognition sites for glutamate and glycine (Ransom and Stec, 1988).

1-[1(3-Hydroxyphenyl)-cyclohexyl]piperidine (3-OH-PCP), the metaphenolic derivative of PCP, has been demonstrated to have higher affinity for [^{3}H]PCP binding sites than does PCP itself in competition binding assays (Kamenka et al., 1982; Itzhak, 1987; Zhou et al., 1991). Also, disturbances in the rotarod test of forced motor activity are found in mice injected subcutaneously with 3-OH-PCP (Kamenka et al., 1982) which correlate with the inhibitory effect of 3-OH-PCP on [^{3}H]3-OH-PCP. Recently, Itzhak (1989) reported that a low affinity [^{3}H]3-OH-PCP binding site is regulated by NMDA receptor agonists and antagonists, and may correspond to the NMDA/PCP receptor ion channel complex. Moreover, a high affinity [^{3}H]3-OH-PCP binding site is sensitive to (+)-SKF 10047 in rat brain membrane, but was not affected by L-glutamate, NMDA, or the competitive antagonists (Itzhak, 1987, 1988, 1989). Thus, they proposed a high affinity PCP recognition site distinct from the NMDA-regulated PCP recognition site. However, no additional pharmacological properties have yet been investigated for the high affinity [^{3}H]3-OH-PCP binding site. We now report the regional localization and pharmacological characteristics of [^{3}H]3-OH-PCP binding sites in rat brain, using quantitative autoradiographic techniques.

2. Materials and methods

2.1. Drugs

[^{3}H]3-OH-PCP (21.6 Ci/mmol) and 3-OH-PCP were generously provided by Du Pont/New England Nuclear (Boston, MA, USA). L-Glutamate, glycine, spermine, MK 801 and (+)-SKF 10047 were purchased from Research Biochemicals International (Natick, MA, USA). 7-Chlorokynurenic acid and 2-amino-5-phosphonopentanoic acid (D-AP5) were obtained from Tocris Cookson (Bristol, UK). Arcaine and diethylenetriamine and the other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were analytical grade.

2.2. Tissue preparation for autoradiography

Adult male Wistar rats weighing 300–350 g were killed by decapitation and the brains were rapidly removed. Tissue sections were prepared as previously described (Suzuki and Moroji, 1989; Suzuki et al., 1993). In brief, after freezing the brains at −70°C, coronal sections were cut at a thickness of 20 μm on a microtome cryostat and thaw-mounted onto a gelatin-coated glass slide. The tissue sections were stored at 4°C for no more than 24 h and then left at room temperature for about 5 min before assay (Meibach, 1982).

2.3. Autoradiographic studies

Sections were prewashed in a buffer containing 5 mM Tris-HCl (pH 7.5) for 30 min at room temperature to remove endogeneous amino acids in tissue sections and then blown dry under a stream of cool air. For the binding of [^{3}H]3-OH-PCP, the experimental conditions were determined during preliminary experiments with minor modifications of the methods by Gundlach et al. (1985, 1986a) and Largent et al. (1986). Namely, the sections were incubated with 3 nM [^{3}H]3-OH-PCP for 120 min at room temperature in 5 mM Tris-HCl buffer in the presence or absence of 100 μM 3-OH-PCP. The slides were then washed 3 times for 1 min at 4°C in the same buffer, followed by one rinse with distilled water, and rapidly dried at 4°C. Dried tissue sections were juxtaposed to tritium-sensitive film, together with a set of commercial tritium standards (^{3}H-Microscale RPA 510, Amersham International, Buckinghamshire, UK), in a X-ray exposure holder. The films were exposed at −80°C for 21 days. After exposure, the film was developed in Kodak D-19 at 20°C for 5 min and fixed for 10 min.

Autoradiograms were analyzed as previously described (Suzuki et al., 1993). They were converted to colour-coded images using a computer-assisted image device (MCID, Imaging Research, Ontario, Canada). After conversion, a non-specific binding image was subtracted from its corresponding total binding image in an adjacent section. Specific binding was taken as the difference between total binding and that observed in the presence of 100 μM 3-OH-PCP, using ^{3}H-Microscale RPA 510 as a reference. Optical density was determined by positioning a square cursor over each subregion. The density in each region was recorded for between 4 and 12 areas per region on bilateral

sides of the brain, depending on the shape and size of the region examined. Each value was the mean of duplicate determinations from a single experiment. The mean values were determined from five rats and were expressed as means ± S.E.M. in fmol/mg dry tissue weight equivalent for intact brain grey matter. Adjacent brain sections were stained with cresyl violet for histological verification of structures according to the rat stereotaxic atlas. Anatomical structures of the rat brain, including cortical regions, were identified with reference to the brain atlas defined by Paxinos and Watson (1982) and Zilles (1985).

For saturation analysis, experiments were carried out using coronal sections with nine to ten concentrations of radioligand, over a range of approximately 0.25–10.0 nM for [^{3}H]3-OH-PCP at room temperature for 120 min. Binding of [^{3}H]3-OH-PCP was measured at the level of A 8920 μm for the caudate-putamen (medial and lateral parts) and at the level of A 4620 μm for the frontoparietal cortex (inner and outer layers), hippocampus (the dentate gyrus and the stratum radiatum of field CA1) and thalamus (ventral posterolateral nuclei), according to the stereotaxic atlas of König and Klippel (1963).

In the inhibition studies, the tissue sections were incubated with 3 nM [^{3}H]3-OH-PCP in the presence or absence of various concentration of drugs (3-OH-PCP, MK 801, (+)-SKF 10047, arcaine, diethylenetriamine, D-AP5, 7-chlorokynurenic acid) at room temperature for 120 min.

2.4. Data analysis

Kinetic parameters were calculated using the iterating curve-fitting program KINETICS (McPherson, 1985). Observed association (K_{obs}) and dissociation (K_2) rate constants were obtained from the least-squares fit of the data. The association rate constant (K_1) was determined using the equation ($[K_{obs} - K_2]/[^3$H]3-OH-PCP). Scatchard transformation of the data from each region showed a single linear plot. Each value of the binding capacity (B_{max}) and the equilibrium apparent dissociation constants ($K_{d(app)}$) was determined by least-square regression analysis. IC_{50} values and Hill coefficients (n_H) for competitors of [^{3}H]3-OH-PCP were also analyzed using LIGAND. Apparent K_i values were determined by the method of Cheng and Prusoff (1973) using the formula $K_i = IC_{50}/[1 + ([L]/[K_d])]$. All the experiments were performed in duplicate and repeated at least 4–5 times. The data are expressed as the means ± S.E.M. calculated from 3–6 experiments. Statistical significance of the results was analyzed by ANOVA (one-way analysis of variance).

3. Results

3.1. Kinetics of [^{3}H]3-OH-PCP

The experiments in the present study were performed as described in our previous publication (Suzuki et al., 1993).

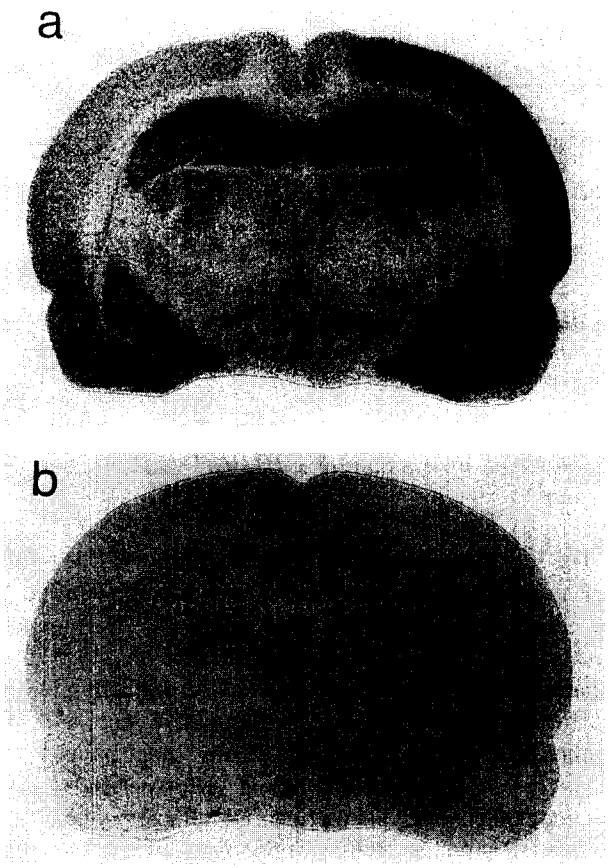

Fig. 1. Autoradiograms show the total (upper (a)) and non-specific (lower (b)) binding in the coronal brain sections at the level of hippocampal formations. The tissue sections were incubated with 3.0 nM [^{3}H]3-OH-PCP for 120 min at room temperature.

Slide-mounted sections of rat brain were incubated with 3 nM [^{3}H]3-OH-PCP for various times at room temperature. The assay conditions with a rinse for 5 min twice afforded the highest signal to noise ratio. Non-specific [^{3}H]3-OH-PCP binding was approximately 20–25% of total binding in the stratum radiatum of hippocampal formation (Fig. 1). Under control conditions (no added glutamate and glycine), the specific binding was time-dependent and reached equilibrium at about 120 min. Therefore, an incubation time of 120 min was used in all subsequent experiments. The association rate constant (k_{+1}) for [^{3}H]3-OH-PCP was $1.21 \pm 0.44 \times 10^8$ $min^{-1} \cdot M^{-1}$ (mean ± S.E.M.) in the fast association phase and $3.64 \pm 0.61 \times 10^6$ $min^{-1} \cdot M^{-1}$ in the slow association phase (Table 1 and Fig. 2A). The dissociation of [^{3}H]3-OH-PCP from the slide-mounted tissue sections was initiated by the addition of 100 μM 3-OH-PCP and was also biphasic. Th dissociation rate constant was 16.1 ± 1.6 $min^{-1} \cdot 10^{-3}$ in the fast dissociation phase and 1.28 ± 0.3 $min^{-1} \cdot 10^{-3}$ in the slow dissociation phase (Table 1 and Fig. 2B). The kinetic K_d values calculated from the ratio of the dissociation and association rate constants (k_{+1}/k_{-1}) were 0.133 ± 0.01 nM ($K_{d(H)}$) and 0.353 ± 0.04 nM ($K_{d(L)}$), respectively.

Table 1
Kinetic parameters of [^{3}H]3-OH-PCP binding to the stratum radiatum of field CA1 of hippocampal formation in the slide-mounted tissue sections

	Fast	Slow
Association constant (k_{+1}) ($M^{-1} \cdot min^{-1}$)	$1.21 \pm 0.44 \times 10^8$	$3.64 \pm 0.61 \times 10^6$
Dissociation constant (k_{-1}) ($min^{-1} \cdot 10^{-3}$)	16.1 ± 1.6	1.28 ± 0.3
K_d (k_{-1}/k_{+1}) (nM)	0.133 ± 0.01 ($K_{d(H)}$)	0.353 ± 0.04 ($K_{d(L)}$)

The tissue sections were incubated with 3.0 nM [^{3}H]3-OH-PCP for 180 min at room temperature. Each value is the mean ± S.E.M. of values from three separate experiments.

3.2. Regional distribution of [^{3}H]3-OH-PCP binding sites in brain

The specific binding sites labeled with 3 nM [^{3}H]3-OH-PCP were distributed in a heterogeneous manner throughout the brain (Fig. 3(1,2) and Table 2). The greatest density of specific binding was observed in the stratum radiatum and stratum oriens of field CA1 in the hippocampal formation. In contrast, the stratum pyramidale and stratum lacunosum moleculare of the hippocampus had relatively low densities of binding. The specific binding was moderately high in the anterior olfactory nuclei and the dentate gyrus, as well as in the outer layers of cerebral cortices, except for the retrosplenial cortex. Moderate densities of binding were found in the external plexiform layer of the olfactory bulb, the olfactory systems (olfactory tubercle, accumbens nucleus), the inner layers of cerebral cortices, stratum oriens and stratum radiatum of fields CA2/3 in hippocampus and the subiculum. The thalamus and amygdala contained moderate and low densities of specific binding sites, respectively. The basolateral and medial-cortical nucleus had moderate densities of binding in amygdala. Compared to the levels of the above regions, lower binding densities were seen in the lateral septal nucleus, bed nucleus of stria terminalis and the superficial gray layer of superior colliculus. The caudate-putamen also showed moderate to low densities of binding. Low densities of binding were observed in the dorsomedial nucleus of hypothalamus, the granule cell layer of the cerebellum and brain stem including substantia nigra, interpeduncular nucleus and central gray. There was very little binding of [^{3}H]3-OH-PCP in the globus pallidus, medial septal nucleus or the nucleus of the vertical limb of diagonal band. The white matter tract (e.g., corpus callosum and anterior commissure) was especially devoid of [^{3}H]3-OH-PCP binding.

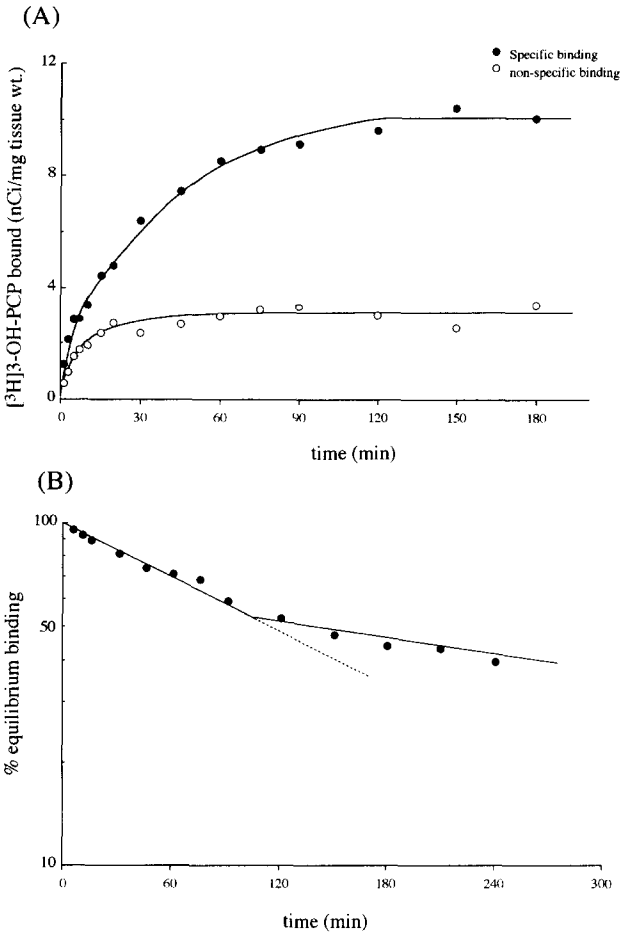

Fig. 2. (A) The association of specific and non-specific [^{3}H]3-OH-PCP binding to the stratum radiatum of the CA1 field in the slide-mounted tissue sections was examined. Open and solid circles indicate the specific and non-specific [^{3}H]3-OH-PCP binding, respectively. The tissue sections were incubated with 3.0 nM [^{3}H]3-OH-PCP for up to 180 min at room temperature. (B) The dissociation of specific [^{3}H]3-OH-PCP binding to the stratum radiatum of the CA1 field in the slide-mounted tissue sections was examined. 3.0 nM [^{3}H]3-OH-PCP was first allowed to associate with the slide-mounted sections for 120 min at room temperature. The tissue sections were then transferred to the buffer for various length of time for up to 4 h.

Fig. 3. (1) Autoradiograms showing the distribution of binding sites labeled by [^{3}H]3-OH-PCP in coronal sections of rat brain (1). Abbreviations: Fr, frontal cortex; IGr, internal granular layer of bulb; EPl, external plexiform layer of bulb; Gl, glomerular layer of bulb; AOL, anterior olfactory nucleus, lateral; AOM, anterior olfactory nucleus, medial; Cg, cingulate cortex; Is, insular cortex; Tu, olfactory tubercle; CPu, caudate putamen; Acb, accumbens nucleus; cp, choroid plexus; LS, lateral septal nucleus; BST, bed nucleus stria terminalis; Par, parietal cortex; Pir, piriform cortex; sm, stria medullaris thalamus; AD, anterodorsal thalamic nucleus; AM, anteromedial thalamic nucleus; GP, globus pallidus; f, fornix. (2) St-O, stratum oriens; St-P, stratum pyramidale; St-R, stratum radiatum; DG, dentate gyrus; Hb, habenular nucleus; LD, lateral dorsal thalamic nucleus; VP, ventropostero thalamic nucleus; MD, mediodorsal thalamic nucleus; CL, centrolateral thalamic nucleus; G, gelatinosus thalamic nucleus; mt, mammillothalamic nucleus; DM, dorsomedial hypothal nucleus; BL, basolateral amygdaloid nucleus; Me-Co, medial-cortical amygdaloid nucleus; CA1, fields CA1 of Ammon's horn; CA3, fields CA3 of Ammon's horn; LG, lateral geniculate nucleus; Rs, retrosplenial cortex; Oc, occipital cortex; Te, temporal cortex; Ent, entorhinal cortex; SuG, superficial gray layer of superior colliculus; MG, medial geniculate nucleus; SN, substantia nigra; IP, interpeduncular nucleus.

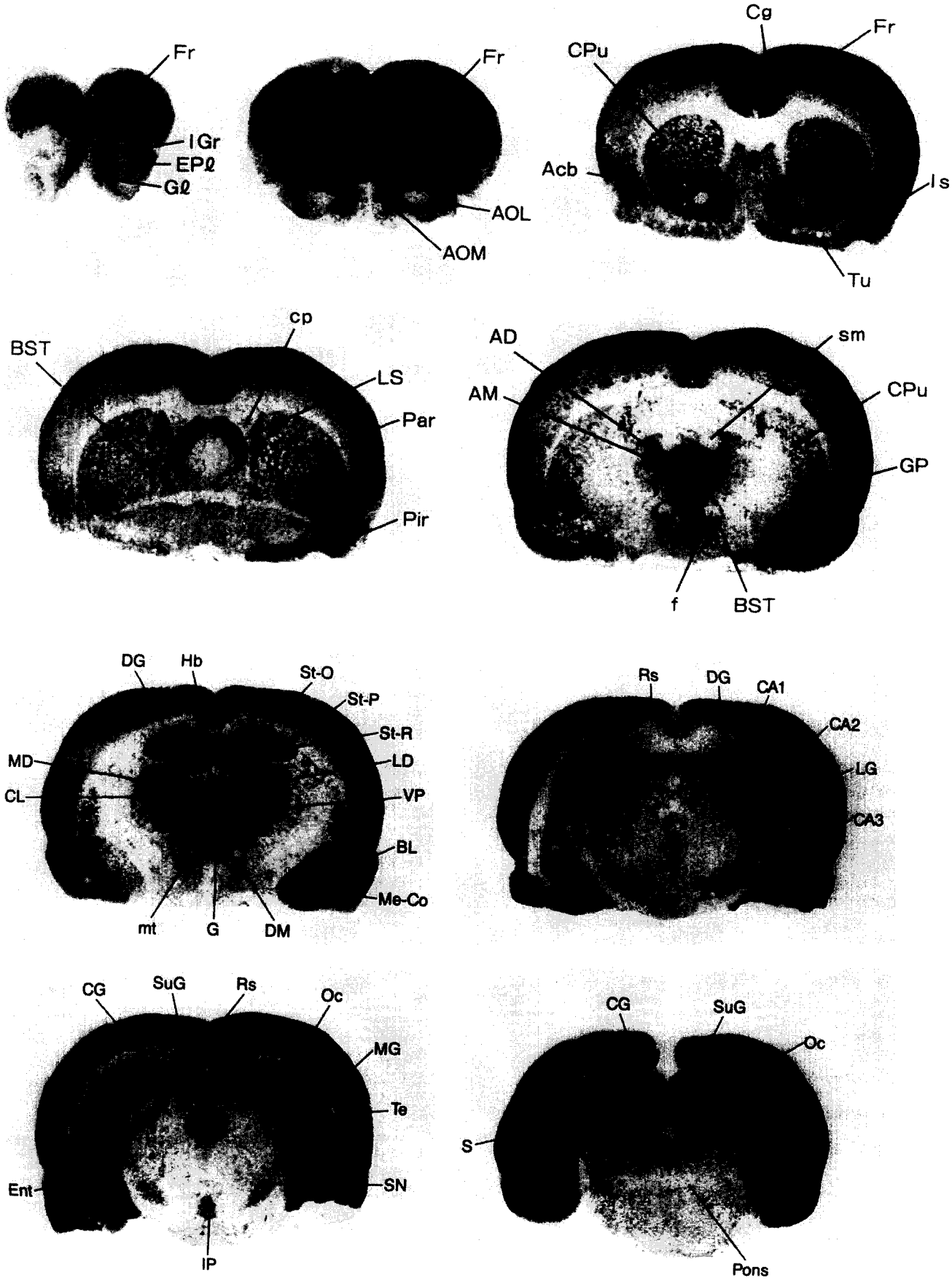
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Table 2
Regional density of [^{3}H]3-OH-PCP binding sites in rat brain

Brain region		Bound (fmol/mg tissue weight)	A (%)
Olfactory bulb			
Glomerular layer		83.3 ± 2.0	17
External plexiform layer		240.7 ± 12.3	49
Internal granular layer		121.1 ± 9.5	25
Anterior olfactory nucleus	Medial	334.1 ± 25.3	68
	Lateral	316.8 ± 33.3	65
Olfactory systems			
Olfactory tubercle		250.0 ± 17.6	51
Accumbens nucleus		231.5 ± 16.3	47
Basal ganglia			
Caudate-putamen	Anterior	166.7 ± 12.5	34
	Posteriomedial	148.1 ± 11.9	30
	Posterolateral	160.7 ± 17.2	33
Globus pallidus		27.8 ± 4.5	6
Basal forebrain			
Lateral septal nucleus, intermediate		185.2 ± 23.6	38
Bed nucleus of the stria terminalis		175.9 ± 24.3	36
Medial septal nucleus		50.9 ± 7.3	10
Nucleus of the vertical limb of the diagonal band		55.6 ± 6.3	11
Choroid plexus		117.1 ± 3.7	24
Hippocampal formation			
Field CA1 of Ammon's horn			
Stratum oriens		453.7 ± 5.2	92
Stratum pyramidale		305.6 ± 21.5	62
Stratum radiatum		490.7 ± 17.8	100
Stratum lacunosum moleculare		250.0 ± 15.1	51
Stratum moleculare		387.9 ± 9.2	79
Field CA2/3 of Ammon's horn			
Stratum oriens		259.3 ± 19.8	53
Stratum pyramidale		138.9 ± 20.3	28
Stratum radiatum		296.3 ± 20.3	60
Dentate gyrus			
Molecular layer		311.8 ± 15.8	64
Polymorph layer		388.9 ± 18.6	79
Subiculum		232.2 ± 25.3	47
Cerebral cortex			
Frontal cortex	Outer layer	305.6 ± 32.4	62
	Inner layer	190.4 ± 8.0	39
Anterior cingulate cortex	Outer layer	298.3 ± 20.1	61
	Inner layer	222.2 ± 9.9	45
Anterior insular cortex	Outer layer	324.1 ± 26.6	66
	Inner layer	263.5 ± 20.0	54
Posterior insular cortex	Outer layer	351.9 ± 36.7	72
	Inner layer	266.5 ± 21.6	53
Piriform cortex		300.9 ± 20.4	61
Retrosplenial cortex		138.9 ± 18.4	28
Entorhinal cortex		305.8 ± 12.2	62
Anterior frontoparietal cortex	Outer layer	314.8 ± 28.6	64
	Inner layer	185.2 ± 11.3	38
Posterior frontoparietal cortex	Outer layer	324.0 ± 23.3	66
	Inner layer	166.7 ± 13.3	34
Temporal cortex	Outer layer	314.8 ± 15.3	64
	Inner layer	208.7 ± 17.1	43
Occipital cortex	Outer layer	287.0 ± 24.4	58
	Inner layer	213.0 ± 24.8	43

Table 2 (continued)

Brain region	Bound (fmol/mg tissue weight)	A (%)
Hypothalamus		
Dorsomedial nucleus	129.6 ± 14.0	26
Amygdala		
Basolateral nucleus	268.5 ± 14.9	55
Medio-cortical nucleus	273.5 ± 14.7	56
Thalamus		
Habenular nucleus	64.8 ± 7.3	13
Anterodorsal nucleus	203.0 ± 21.1	41
Anteromedial nucleus	254.6 ± 36.1	52
Mediodorsal nucleus	250.0 ± 16.8	51
Laterodorsal nucleus	240.7 ± 21.3	49
Ventral posterolateral nucleus	194.4 ± 7.5	40
Gelatinosus	259.3 ± 21.9	53
Medial geniculate nucleus	200.7 ± 10.6	41
Lateral geniculate nucleus	203.7 ± 10.4	42
Brain stem		
Substantia nigra	92.6 ± 5.2	19
Interpeduncular	92.5 ± 7.7	19
Central gray	74.1 ± 7.4	15
Superior colliculus		
Superficial gray layer	194.4 ± 11.8	40
Other structures	76.1 ± 7.9	16
Cerebellum		
Granular cell layer	111.1 ± 12.5	23
White matter	27.6 ± 4.5	6

Mean value in each brain region was determined from five to six experiments and was expressed in fmol/mg dry tissue eighwt equivalent for intact brain grey matter. *A* (%) represents a percentage of [^{3}H]3-OH-PCP binding in the different brain regions versus the binding in the stratum radiatum in fields CA1 of hippocampus with the highest value.

3.3. Binding parameters (B_{max} and $K_{d(app)}$) of [^{3}H]3-OH-PCP binding

Scatchard transformation showed a single component of [^{3}H]3-OH-PCP binding in all 11 rat brain regions that were studied (Fig. 4A). Specific binding of [^{3}H]3-OH-PCP to the slide-mounted tissue sections was saturable with increasing concentrations of the radioligand in the range 0.25–10.0 nM (Fig. 4B). The $K_{d(app)}$ values ranged from 5.50 ± 0.50 nM in the accumbens nucleus to 3.51 ± 0.34 nM in the dentate gyrus (Table 3). There was no significant difference in the $K_{d(app)}$ values among the 11 brain regions examined (ANOVA). The affinities of [^{3}H]3-OH-PCP binding sites in all brain regions were relatively high, and similar to those of high affinity binding sites of [^{3}H]TCP described by Vignon et al. (1986), and to our data obtained from the brain homogenates (data not shown).

The B_{max} of [^{3}H]3-OH-PCP binding sites in the measured regions ranged from 1169.4 ± 56.8 fmol/mg tissue weight in the stratum radiatum of field CA1 to 303.6 ± 19.0 fmol/mg tissue weight in the medial part of caudate-putamen. The B_{max} in the outer layer of cerebral cortex was higher than that in the inner layer of cortex. In the caudate-putamen, [^{3}H]3-OH-PCP binding sites were more numerous in the lateral part rather than in the medial part.

Table 3
Saturation binding parameter of [^{3}H]3-OH-PCP binding to the slide-mounted tissue sections in various regions of rat brain

		B_{max} (fmol/mg tissue weight)	$K_{d(app)}$ (nM)
Hippocampus			
CA1, stratum radiatum		1144.4 ± 54.6	4.33 ± 0.45
Dentate gyrus		784.8 ± 48.6	3.51 ± 0.34
Cerebral cortex			
Frontal cortex	Outer layer	589.5 ± 64.0	5.23 ± 0.68
	Inner layer	312.5 ± 26.2	4.96 ± 0.69
Parietal cortex	Outer layer	714.2 ± 29.8	5.37 ± 0.31
	Inner layer	421.9 ± 34.5	4.02 ± 0.30
Caudate putamen			
Medial part		309.7 ± 18.1	4.21 ± 0.23
Lateral part		386.5 ± 33.5	5.21 ± 0.65
Nucleus accumbens		475.5 ± 49.5	5.50 ± 0.50
Thalamus			
Posterolateral nucleus		533.4 ± 46.9	4.01 ± 0.43
Amygdala			
Cortico-basolateral nucleus		623.7 ± 40.7	4.00 ± 0.57

The data shows the B_{max} and $K_{d(app)}$ values of [^{3}H]3-OH-PCP binding sites in the 11 discrete brain regions. Each value represents the mean ± S.E.M. calculated from the five to six rats.

Table 4
IC_{50} values, Hill coefficients (n_H) and K_i in competitive inhibition of [^{3}H]3-OH-PCP specific binding to the slide-mounted tissue sections in various regions of rat brain by 3-OH-PCP, MK 801 and (+)-SKF 10047 (1)

		3-OH-PCP		MK 801		(+)-SKF 10047	
		IC_{50} (nM) K_i (nM)	n_H	IC_{50} (nM) K_i (nM)	n_H	IC_{50} (nM) K_i (nM)	n_H
Hippocampus		15.2 ± 2.6	0.90 ± 0.05	4.1 ± 0.4	0.83 ± 0.15	378.4 ± 21.5	0.85 ± 0.03
(CA1, stratum radiatum)		9.4 ± 1.1		3.2 ± 0.4		215.0 ± 12.2	
Cerebral cortex							
Frontal cortex	Outer layer	12.0 ± 1.3	0.80 ± 0.09	10.5 ± 0.9	0.67 ± 0.04	308.1 ± 38.3	0.73 ± 0.01
		8.5 ± 0.7		6.8 ± 0.3		201.5 ± 17.7	
	Inner layer	16.0 ± 2.7	0.75 ± 0.07	13.4 ± 0.8	0.70 ± 0.04	323.9 ± 39.2	0.78 ± 0.05
		10.1 ± 1.3		8.8 ± 0.1		206.2 ± 16.6	
Parietal cortex							
	Outer layer	11.8 ± 1.8	0.82 ± 0.01	9.0 ± 1.3	0.68 ± 0.07	337.3 ± 38.5	0.78 ± 0.04
		7.6 ± 0.9		5.3 ± 0.6		217.1 ± 16.6	
Caudate-putamen							
Medial part		15.7 ± 4.6	0.78 ± 0.03	11.3 ± 0.1	0.62 ± 0.03	290.2 ± 33.1	0.61 ± 0.02
		7.4 ± 1.0		6.4 ± 0.1		174.7 ± 15.2	
Lateral part		14.4 ± 1.6	0.76 ± 0.04	13.8 ± 1.1	0.64 ± 0.04	280.0 ± 69.6	0.64 ± 0.03
		9.3 ± 0.7		9.3 ± 0.3		211.0 ± 35.9	
Nucleus accumbens		17.9 ± 1.8	0.77 ± 0.04	11.2 ± 1.9	0.64 ± 0.05	250.3 ± 27.8	0.63 ± 0.01
		12.3 ± 0.8		7.6 ± 1.1		162.9 ± 14.1	
Thalamus							
Postero-lateral nucleus		17.5 ± 2.2	0.86 ± 0.03	15.4 ± 2.2	0.76 ± 0.06	640.3 ± 90.2	0.75 ± 0.03
		10.2 ± 0.8		9.7 ± 0.2		363.9 ± 26.6	

Each value of IC_{50} and K_i is the mean ± S.E.M. of values from four experiments, each performed in duplicate. n_H represents the mean of Hill coefficients.

The rank order of B_{max} among brain regions were correspondent to that obtained from the regional distribution data described in Table 2.

3.4. Competitive inhibition of [^{3}H]3-OH-PCP binding sites by 3-OH-PCP, MK 801 and (+)-SKF 10047

3-OH-PCP and MK 801 inhibited [^{3}H]3-OH-PCP binding in a regionally similar manner. MK 801 exhibited a high binding potency for the NMDA/PCP receptor ion channel complex labeled by [^{3}H]3-OH-PCP with an IC_{50} value of 10.5 ± 0.9 nM in the outer layer of the frontal cortex and of 4.1 ± 0.4 nM in the stratum radiatum of field CA1 of hippocampus (Table 4). 3-OH-PCP was somewhat less potent than MK 801 in inhibiting [^{3}H]3-OH-PCP binding in all the brain regions, i.e., IC_{50} values of 12.0 ± 1.3 nM in the frontal cortex and 15.2 ± 2.6 nM in the hippocampus. In contrast, [^{3}H]3-OH-PCP binding was displaced by (+)-SKF 10047 with an IC_{50} value of 308.1 ± 38.3 nM in the outer layer of frontal cortex and 378.4 ± 21.5 nM in the stratum radiatum of CA1. The potency of (+)-SKF 10047 in inhibiting [^{3}H]3-OH-PCP binding was approximately 20 times less than those of 3-OH-PCP and MK 801 in all brain regions examined (Fig. 5). The Hill coefficient values (n_H) of [^{3}H]3-OH-PCP binding sites for all the regions ranged from 0.75 ± 0.07 to 0.90 ± 0.05 for 3-OH-PCP, from 0.62 ± 0.03 to 0.83 ± 0.15 for MK 801 and from 0.61 ± 0.02 to 0.85 ± 0.03 for (+)SKF 10047.

Table 5
Inhibition of [^{3}H]3-OH-PCP binding to the slide-mounted tissue sections in rat brain by D-AP5, 7-chlorokynurenic acid, arcaine and diethylenetriamine

	D-AP5		7-Chlorokynurenic acid		Arcaine		Diethylenetriamine	
	IC_{50} (μM) K_i (μM)	n_H	IC_{50} (μM) K_i (μM)	n_H	IC_{50} (μM) K_i (μM)	n_H	IC_{50} (μM) K_i (μM)	n_H
Hippocampus	36.3 ± 1.7	0.96 ± 0.02	5.3 ± 1.3	0.87 ± 0.05	4.5 ± 0.5	0.97 ± 0.05	6.5 ± 1.1	1.13 ± 0.08
(stratum radiatum, CA1)	21.7 ± 1.0		4.2 ± 0.7		2.6 ± 0.3		3.8 ± 0.5	
Cerebral cortex	20.8 ± 1.4	0.94 ± 0.07	7.3 ± 1.6	0.86 ± 0.10	4.0 ± 0.2	1.02 ± 0.06	5.6 ± 1.3	1.10 ± 0.03
Outer layer	13.3 ± 0.9		4.0 ± 0.5		2.5 ± 0.3		3.6 ± 0.8	

Each value of IC_{50} and K_i is the mean ± S.E.M. of values from four experiments, each performed in duplicate. n_H represents the mean of Hill coefficients. Abbreviations: D-AP5, 2-amino-5-phosphonopentanoic acid; 7-Cl-KYNA, 7-chlorokynurenic acid; DET, diethylenetriamine.

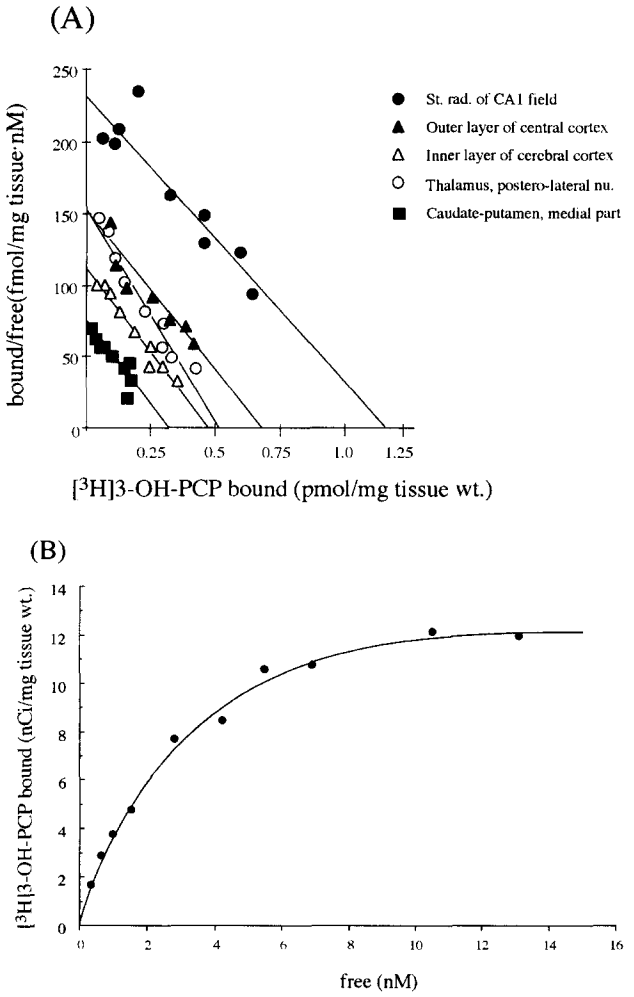

Fig. 4. (A) Representative Scatchard plots of [^{3}H]3-OH-PCP binding to five discrete brain regions (stratum radiatum of CA1 field of hippocampus (solid circle), outer layer of cerebral cortex (solid triangle), inner layer of cerebral cortex (open triangle), thalamus, postero-lateral nucleus (open circle), caudate-putamen, medial part (solid square)). The tissue sections were incubated with 0.25–10.0 nM [^{3}H]3-OH-PCP for 120 min at room temperature. Each point is the mean of duplicate determinations from a typical experiment. (B) The tissue sections at the level of dentate gyrus of hippocampal formations were incubated with 0.25–10.0 nM [^{3}H]3-OH-PCP for 120 min at room temperature.

3.5. Competitive inhibition of [^{3}H]3-OH-PCP binding sites by polyamines

D-AP5, 7-chlorokynurenic acid, arcaine and diethylenetriamine inhibited [^{3}H]3-OH-PCP binding in the outer layer of the cerebral cortex and in the stratum radiatum of field CA1. The IC_{50} values for 7-chlorokynurenic acid, arcaine and diethylenetriamine ranged from 4.0 ± 0.2 to 7.3 ± 1.6 μM in the outer later of cerebral cortex and from 4.5 ± 0.5 to 6.5 ± 1.1 μM in the stratum radiatum of field CA1 of hippocampus (Table 5). D-AP5 was 4–6 times less potent in inhibiting [^{3}H]3-OH-PCP binding than the other drugs. Thus, the rank order of potency (the IC_{50} value) to inhibit [^{3}H]3-OH-PCP binding in the cerebral cortex and hippocampus was arcaine > 7-chlorokynurenic acid > diethylenetriamine > D-AP5. The Hill coefficients (n_H) of the above four drugs were approximately unity.

4. Discussion

Both the association and dissociation curves of [^{3}H]3-OH-PCP binding (Table 1) were best fit by a two-site kinetic model, while Scatchard analysis suggested a single class of binding site (Fig. 2). Previous kinetic studies with [^{3}H]MK 801 binding sites in rat brains has also revealed two binding components under baseline conditions, or in the presence of combined L-glutamate and glycine (Javitt and Zukin, 1989a). Although there is some discrepancy with regard to the kinetic property of [^{3}H]MK 801 binding among previous studies (Bonhaus and McNamara, 1988; Kloog et al., 1988a,b; Reynolds and Miller, 1988), the biexponential fashion of [^{3}H]3-OH-PCP obtained here closely resembles several reports (Javitt and Zukin, 1989a,b). The biexponential kinetics of [^{3}H]MK 801 binding sites has been disclosed only in extensively washed rat cortical membranes (Javitt and Zukin, 1989a,b). The use of extensively washed membranes and a long time of incubation are suggested to be critically important in these kinetic experiments (Javitt and Zukin, 1989a). In our slide-mounted tissue sectins, we also performed kinetic measurement following a 30 min preincubation period, and then with a relatively long time (2 h) of incubation. Thus, these assay conditions may account for the biexponential fashion of our kinetic data. One possible explanation is the existence of multiple affinity components of the PCP receptor (Javitt and Zukin, 1989a). Indeed, PCP-like agents have been speculated to gain access to their recognition site via two distinct paths, namely fast hydrophilic and slow hydrophobic paths (Javitt and Zukin, 1989a).

In contrast to the kinetic measurement, the Scatchard analysis of [^{3}H]3-OH-PCP binding sites displayed a single linear plot (Fig. 4). Several lines of evidence suggest the existence of two different affinities of [^{3}H]3-OH-PCP binding site (Itzhak, 1987, Itzhak, 1989). In addition, the kinetic K_d values of ≤ 1 nM, calculated from the association and dissociation rate constants, were not in agreement with the $K_{d(app)}$ values in Scatchard plots, which values were about 10–20-fold greater than the kinetic K_d values. Such a tendency in kinetic characteristics was also reported in previous autoradiographic study with [^{3}H]MK 801 (Sakurai et al., 1991). Hammond and Clanachan (1985) have proposed that there are two possible reasons for inconsistencies between Scatchard analysis and kinetic studies. Linear Scatchard plots may be obtained if the radioligand binds to two distinct sites but has a less than 10-fold difference in $K_{d(app)}$ values. Our experiments show less than a 10-fold difference in K_d values calculated from kinetic parameters. Another interpretation is that one of the two binding sites constitute a relatively small portion of

the total number of sites. The other possible explanation is a difference in assay conditions, i.e. relatively high concentrations of L-glutamate, glycine or the other endogenous substances stimulating NMDA/PCP receptor complexes could not be excluded from the tissue sections in the autoradiographic studies. In addition, the possibility that the concentration range 0.25–10.0 nM in the saturation study may have been sufficient to observe only one binding component, whereas the kinetic study is more sensitive to both binding components. Thus, these factors may have led to a single site of PCP receptor laleled by [^{3}H]3-OH-PCP in the Scatchard analysis.

Previous reports on the distribution of PCP binding sites have utilized [^{3}H]TCP and [^{3}H]MK 801 as radioligands. Especially, the PCP receptor labeled by [^{3}H]TCP, which is a more selective analog for PCP binding sites than PCP itself (Vignon et al., 1982, 1983, 1986), has been investigated in rat brain using autoradiographic techniques (Sircar and Zukin, 1985; Contreras et al., 1986; Gundlach et al., 1986a,b; Largent et al., 1986; Maragos et al., 1986, 1988; Vignon et al., 1986; Jarvis et al., 1987). The distribution of [^{3}H]MK 801 binding sites are correspondent to that of the NMDA/PCP receptor complex labeled by [^{3}H]TCP, except for in the cerebellum (Bowery et al., 1988; Sakurai et al., 1991, 1993; Subramanian and McGonigle, 1991). [^{3}H]MK 801 shows a high density of binding sites in the cerebral cortices and in the CA1 field of the hippocampal complex, while a low level of binding is found in the brain stem and caudate putamen. The binding sites labeled by [^{3}H]TCP and [^{3}H]MK 801 demonstrate high affinity for NMDA/PCP receptor complex and a relatively low affinity for the σ receptor (Vignon et al., 1989). Other reports (Quirion et al., 1987; Zhou et al., 1991) have indicated that PCP binding sites have high affinity for PCP, TCP, and MK 801, moderate affinity for (+)-SKF10047, and very low affinity for σ receptor drugs such as 3-[3-hydroxyphenyl]-*N*](1-propyl)piperidine ((+)3-PPP), 1,3-di-*o*-tolyl-guanidine (DTG) and haloperidol.

In the binding characteristics of [^{3}H]3-OH-PCP, it has been reported an existence of high and low affinity sites in whole brain (Itzhak, 1987, 1988). High affinity site of [^{3}H]3-OH-PCP binding is potently inhibited by (+)-SKF 10047 (Itzhak, 1987, 1988). Furthermore, 3-OH-PCP potently inhibits (+)-[^{3}H]SKF 10047 binding in a competitive manner, supporting a presence of a common binding site shared by both 3-OH-PCP and (+)-SKF 10047 (Itzhak, 1987). In the subsequent study, [^{3}H]3-OH-PCP binding to the high affinity site is found to be not affected by NMDA nor competitive NMDA receptor antagonists (Itzhak, 1989). It suggests that high affinity [^{3}H]3-OH-PCP binding site is not coupled to NMDA/PCP receptor complex. In contrast, the low affinity site of [^{3}H]3-OH-PCP binding site is reduced competitively by NMDA receptor antagonists and enhanced by glutamate (Itzhak, 1989). The non-competitive NMDA receptor antagonist MK801 also inhibits potently [^{3}H]3-OH-PCP binding to the low affinity site. Thus, low affinity may be coupled to the NMDA/PCP receptor complex. However, these findings have not been confirmed definitely (Zhou et al., 1991).

In the present study, there were significant correlations between the distribution of [^{3}H]3-OH-PCP binding sites and the sites labelled by [^{3}H]MK 801 (Sakurai et al., 1991; Subramanian and McGonigle, 1991), [^{3}H]TCP (Gundlach et al., 1986a, Maragos et al., 1988), and [^{3}H]L-glutamate

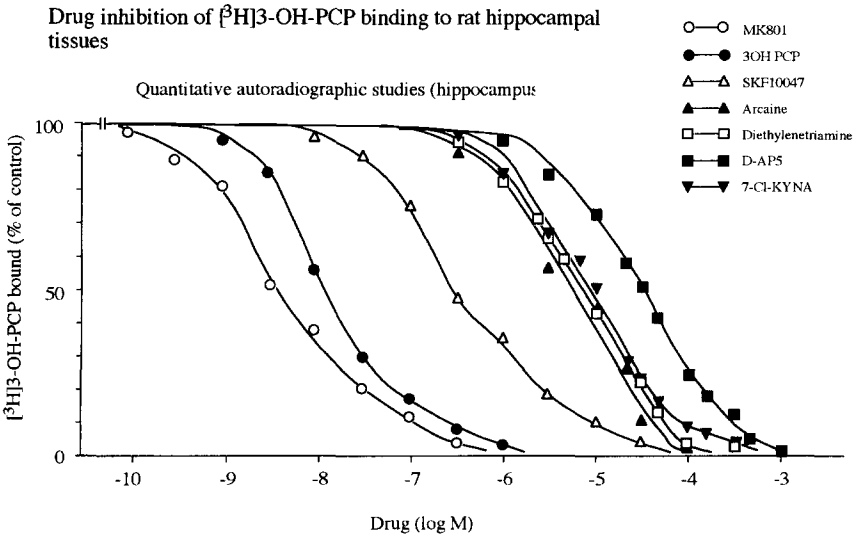

Fig. 5. The tissue sections were incubated with 3.0 nM [^{3}H]3-OH-PCP and increasing concentrations of MK 801 (open circle), 3-OH-PCP (solid circle), (+)-SKF 10047 (open triangle), arcaine (solid triangle), diethylenetriamine (open square), D-AP5 (solid square) and 7-chlorokynurenic acid (solid inverted triangle) for 120 min at room temperature. Each point is the mean of duplicate determinations from a typical experiment.

(Monaghan and Cotman, 1985; Maragos et al., 1988) (the Pearson's correlation coefficients were as follows, between [^{3}H]3-OH-PCP binding site distribution and [^{3}H]MK 801 binding sites, $r = 0.889$ (Sakurai et al., 1991), $P < 0.01$; $r = 0.856$ (Subramanian and McGonigle, 1991), $P < 0.01$; [^{3}H]TCP binding sites, $r = 0.881$ (Gundlach et al., 1986a), $P < 0.01$, $r = 0.906$ (Maragos et al., 1988), $P < 0.01$ and [^{3}H]L-glutamate binding sites, $r = 0.882$ (Monaghan and Cotman, 1985), $P < 0.01$; $r = 0.898$ (Maragos et al., 1988), $P < 0.01$). The significance of these correlations indicates a close similarity or identity between the distribution of [^{3}H]3-OH-PCP binding sites and that of the NMDA/PCP receptor complex labeled by [^{3}H]MK 801 and [^{3}H]TCP, and [^{3}H]L-glutamate.

It has been suggested that the higher affinity site labeled by (+)-[^{3}H]SKF 10047 is displaced preferentially by (+)3-PPP and DTG, and the lower affinity site is displaced by PCP-like drugs (Tam and Cook, 1984; Largent et al., 1986; Sircar et al., 1986). Autoradiographic studies using (+)[^{3}H]3-PPP (Gundlach et al., 1986b; Largent et al., 1986) and (+)-[^{3}H]SKF 10047 (Sircar and Zukin, 1985; Largent et al., 1986; Sircar et al., 1986) for σ binding sites in rat brain have shown a differential localization from [^{3}H]TCP binding sites. The cerebellum, brain stem, hypothalamus and septal areas possess high densities of high affinity (+)[^{3}H]SKF10047 binding sites, but few PCP sites (Gundlach et al., 1985; Contreras et al., 1986; Largent et al., 1986). In fact, no significant correlation has been observed in the regional distribution between (+)-[^{3}H]SKF 10047 binding and the [^{3}H]3-OH-PCP binding site obtained here ($r = 0.431$) (Gundlach et al., 1986b). Considering the characteristics of regional distribution of [^{3}H]3-OH-PCP in brain, this suggests that [^{3}H]3-OH-PCP binding sites are distinct from the σ binding sites labeled by (+)-[^{3}H]SKF 10047 or (+)[^{3}H]3-PPP.

3-OH-PCP and MK 801 exhibited a high binding potency for the [^{3}H]3-OH-PCP binding site. The IC_{50} values for these compounds were 12.0 nM and 10.5 nM, respectively, in the outer layer of the frontal cortex. In contrast, the potency of (+)-SKF 10047 for inhibiting binding was approximately 30 times less than those of 3-OH-PCP and MK 801. No regional difference in the IC_{50} and K_i values was found among eight brain regions examined in each inhibition study with 3-OH-PCP, MK 801 or (+)-SKF 10047. These results indicate MK 801 and 3-OH-PCP bind to the PCP binding site within NMDA receptor ion-channel complex with higher affinity than (+)-SKF 10047 does. The Hill coefficients of 3-OH-PCP, MK 801 and (+)-SKF 10047 for the inhibition of [^{3}H]3-OH-PCP binding were less than 1.0, suggesting a negative allosteric cooperativity of [^{3}H]3-OH-PCP binding.

Compounds with high affinity for NMDA receptor complex were very competitive with [^{3}H]3-OH-PCP binding. D-AP5, which is a potent, selective and competitive antagonist of the glutamate recognition site at the NMDA receptor complex (Davies et al., 1981; Olverman et al., 1984; Javitt et al., 1987), completely inhibited [^{3}H]3-OH-PCP binding. 7-Chlorokynurenic acid is a selective inhibitor at the strychnine-insensitive glycine binding site of the NMDA receptor complex (Kemp et al., 1988; Sircar et al., 1989; Tacconi et al., 1993). It displaced [^{3}H]3-OH-PCP binding with a higher potency for inhibition than D-AP5. No significant differences in the IC_{50} value of D-AP5 and 7-chlorokynurenic acid were observed between two different brain regions, i.e., the cerebral cortex and hippocampus. Previous autoradiographic studies with [^{3}H]MK 801 (Sakurai et al., 1991, 1993; Tacconi et al., 1993) are similar to the data obtained here. Both D-AP5 and 7-chlorokynurenic acid were reported to decrease the NMDA agonist-induced enhancement of [^{3}H]MK 801 binding (Nussenzveig et al., 1991; Sircar and Zukin, 1991) and to mediate its inhibitory effects on [^{3}H]TCP binding via an interaction at a single site of the NMDA receptor (Kloog et al., 1990; Hatta et al., 1992). Thus, our present findings support a close relationship between [^{3}H]3-OH-PCP binding sites and PCP binding site in the NMDA/PCP receptor complex.

The endogenous polyamines spermine and spermidine have been reported to increase the binding of [^{3}H]MK 801 to the NMDA receptor complex (Ransom and Stec, 1988; Williams et al., 1990, 1991). Arcaine, a putative antagonist at the polyamine site on the NMDA receptor complex, competitively antagonizes the spermine-induced increase in PCP binding sites labeled by [^{3}H]MK 801 (Williams et al., 1989; Reynolds, 1990) and [^{3}H]TCP (Sacaan and Johnson, 1991; Nagase et al., 1994). The present study demonstrated IC_{50} values of 4.0–4.5 μM for inhibition by arcaine on [^{3}H]3-OH-PCP binding in the cerebral cortex and hippocampus. These values are correspondent to those observed in previous studies using radioligands of [^{3}H]TCP (Sacaan and Johnson, 1991) and [^{3}H]MK 801 (Reynolds, 1990) in membrane binding. Diethylenetriamine has also been reported to selectively inhibit an increase in the binding of [^{3}H]MK 801 to the NMDA/PCP receptor complex by spermine or spermidine (Williams et al., 1991), but has no effect on the [^{3}H]MK 801 binding enhanced in the presence of L-glutamate or glycine (Williams et al., 1989, 1990). In the present study, diethylenetriamine also antagonized [^{3}H]3-OH-PCP binding at a polyamine site with a potency close to that of arcaine. The Hill coefficients of the above four drugs were almost at unity, in agreement with a previous autoradiographic study of [^{3}H]MK 801 binding (Sakurai et al., 1993). This may reflect that each polyamine acts at a single site and inhibits [^{3}H]3-OH-PCP binding in a similar manner as that of [^{3}H]MK 801 binding.

In conclusion, [^{3}H]3-OH-PCP appears to bind with a high affinity to the NMDA/PCP receptor complex rather than to σ receptors labeled by (+)-[^{3}H]SKF 10047. Our distribution and inhibition studies corresponded closely with those observed in previous studies using [^{3}H]MK 801 and [^{3}H]TCP. [^{3}H]3-OH-PCP demonstrated little advan-

tage to discriminate between the NMDA-coupled and the other NMDA-uncoupled PCP binding site in comparison with that of [^{3}H]TCP.

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