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Anatomic Correlation of NMDA and ^{3}H-TCP-Labeled Receptors in Rat Brain

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Using quantitative autoradiography, we have compared the regional distribution of *N*-methyl-D-aspartate (NMDA) receptors labeled with ^{3}H-glutamate and dissociative anesthetic binding sites labeled with ^{3}H-*N*-(1-[2-thienyl]cyclohexyl)3,4-piperidine (^{3}H-TCP). Binding of both ligands was highest in the hippocampal formation, with high concentrations in a number of cortical and olfactory regions. Intermediate amounts of binding for both ligands were measured in several thalamic and basal telencephalic structures. Very little binding was observed in the hypothalamus, some deep forebrain regions, and most brain-stem structures. Linear-regression analysis comparing the binding at both sites revealed a marked concordance ($R = 0.95$; $p < 0.001$; Pearson product–moment). The granule cell layer of the cerebellum was the only region in which this concordance was not observed. Scatchard analysis of ^{3}H-glutamate binding to NMDA receptors in stratum radiatum of hippocampal formation revealed an apparent single binding site with a B_{max} of 9.78 ± 0.84 pmol/mg protein and K_D of 158 ± 37 nM. ^{3}H-TCP also bound to an apparent single site with a B_{max} of 2.07 ± 0.16 pmol/mg protein and K_D of 127 ± 30 nM. Our results are consistent with the hypothesis that the dissociative anesthetic binding site is linked to the NMDA receptor, and the data suggest that there are approximately 4–5 NMDA binding sites for each dissociative anesthetic binding site.

The dissociative anesthetics—e.g., phencyclidine (PCP), ketamine, and related drugs—comprise a novel group of drugs that were originally used as surgical anesthetics (Greifenstein et al., 1958; Johnstone et al., 1959; Corssen and Domino, 1966). More recently, these drugs have become major substances of abuse (Burns and Lerner, 1976). Drugs of this class produce psychotomimetic effects in humans, including thought disorders, distortions of body image, depersonalization, mania, and sometimes catatonia (Luby et al., 1959; Domino, 1964). These behaviors are similar to those seen in chronic schizophrenia, and this observation had led some to postulate a common underlying mechanism.

Recently, electrophysiological studies have indicated that the dissociative anesthetics antagonize the effects of *N*-methyl-D-aspartate (NMDA)-sensitive glutamate receptors in the cerebral cortex (Harrison and Simmonds, 1985; Thomson et al., 1985), hippocampal formation (Raja and Guyenet, 1982), and spinal cord (Anis et al., 1983). The NMDA receptor is one of at least 3 receptor subtypes at which the excitatory amino acid, glutamate, is believed to exert its action (Watkins and Evans, 1981). NMDA receptors occur throughout the mammalian CNS (Greenamyre et al., 1985; Monaghan and Cotman, 1985). Although the exact behavioral role of these receptors is unknown, they appear to be involved in learning (Morris et al., 1986), memory (Collingridge, 1985), and possibly epilepsy (Meldrum, 1985). At the molecular level, NMDA receptors are linked to a voltage-dependent cation channel (Engberg et al., 1978; MacDonald et al., 1982; Flatman et al., 1983). This channel is gated by Mg^{2+} (Mayer et al., 1984; Nowak et al., 1984), which has been shown to inhibit the actions of NMDA noncompetitively (Harrison and Simmonds, 1985). The dissociative anesthetics exert inhibitory actions on the channel similar to Mg^{2+} (Duchen et al., 1985; Honey et al., 1985), perhaps through an allosteric site on the NMDA receptor-channel complex (Harrison and Simmonds, 1985; Martin and Lodge, 1985).

If NMDA receptors and dissociative anesthetic (PCP) recognition sites are linked, one prediction is that they should have a similar anatomical distribution. Recently, autoradiographic assays have been developed in several laboratories to visualize both NMDA (Greenamyre et al., 1985; Monaghan and Cotman, 1985) and dissociative anesthetic receptor distribution (Quirion et al., 1981; Sircar and Zukin, 1985; Gundlach et al., 1986; Vignon et al., 1986). Using ^{3}H-glutamate and ^{3}H-*N*-(1-[2-thienyl]cyclohexyl)3,4-piperidine (^{3}H-TCP), we have applied the technique of quantitative autoradiography to investigate the distribution of NMDA and dissociative anesthetic recognition sites, respectively, in the rat CNS (Maragos et al., 1986). ^{3}H-TCP was selected as the ligand since it binds with higher affinity (Vignon et al., 1983) and 50–100 times greater specificity (Largent et al., 1986) to the dissociative anesthetic site than PCP or other PCP-like drugs.

Materials and Methods

Male Sprague-Dawley rats weighing approximately 200 gm were used in this study. On the day of the experiment, animals were decapitated, and the brains were rapidly removed and immediately frozen on dry ice. The frozen brains were mounted on aluminum chucks and equilibrated to the cryostat temperature (−17°C) for a half-hour prior to sectioning. Assays of each receptor class were carried out on alternate 20 μm sections thaw-mounted onto gelatin-coated slides.

^{3}H-TCP binding assay. ^{3}H-TCP binding using autoradiographic techniques has been examined by several groups (Sircar and Zukin, 1985; Gundlach et al., 1986; Largent et al., 1986). In these studies, the pharmacology, distribution, and physiochemical properties of binding have been examined, and they fit closely with the known physiology and

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behavior of dissociative anesthetics. In initial experiments, we found that the association rate of ^{3}H-TCP with the receptor at 4°C was 6.1 ± 2.0 × 10^3 M^{-1} sec^{-1}. The dissociation rate was determined as 8.8 ± 3.0 × 10^{-4} sec^{-1}. The ratio of k_{-1}/k_1 corresponded closely to the equilibrium-saturation constant, K_D = 127 ± 30 nM. This finding suggested that the labeled ligand was binding via a bimolecular reaction and was unlikely to be sequestered by transport processes, as has been suggested for the binding of some labeled ligands (Kessler et al., 1987). The off-rate of ^{3}H-TCP was slower than that of ^{3}H-glutamate, and rinse times of 3 × 1 min optimized the specific to nonspecific ratios. We also confirmed the pharmacological specificity of ^{3}H-TCP binding as described by others (Largent et al., 1986; W. F. Maragos, unpublished observations).

For routine assays, sections were washed for 30 min in cold 50 mM Tris-acetate, pH 7.4, and dried. For regional distribution studies, sections were incubated for 45 min in the same buffer at 4°C, containing 1 mM magnesium acetate and 20 nM ^{3}H-TCP (52.9 Ci/mmol, New England Nuclear Corporation). Magnesium acetate was added to the incubation medium, as this has been shown in preliminary experiments to enhance binding (D. C. M. Chu, unpublished observations). Nonspecific binding was determined by incubating slides with ^{3}H-TCP in the presence of 20 μM unlabeled PCP. Saturation studies were carried out in 2 parts. For the lower concentrations, sections were incubated for 45 min in cold buffer containing concentrations of ^{3}H-TCP ranging from 1 to 20 nM. Nonspecific binding was determined for each point in the presence of 20 μM PCP. For higher concentrations, sections were incubated for 45 min in 20 nM ^{3}H-TCP diluted with varying amounts of nonradioactive TCP ranging from 30 to 980 nM. For this group of sections, nonspecific binding was determined in 20 nM ^{3}H-TCP in the presence of 20 μM PCP. Following incubation, sections were washed 3 times for 1 min each in cold buffer containing magnesium and rapidly dried.

NMDA receptor assay. NMDA receptors were labeled with L-^{3}H-glutamate under conditions that have been shown to select for binding to NMDA receptors (Greenamyre et al., 1985). Under these conditions, the kinetics of glutamate binding were identical to those determined for ^{3}H-glutamate binding in the presence of calcium and chloride (Greenamyre et al., 1983). For regional distribution studies, sections were washed for 30 min in cold 50 mM Tris-acetate, pH 7.4, and dried. Sections were then incubated in the same buffer at 4°C containing 200 nM L-^{3}H-glutamate (specific activity, 5.24 Ci/mmol; Amersham Corporation) and 1 μM quisqualic acid. This concentration of quisqualate has been determined to inhibit >90% of the non-NMDA glutamate receptors. For saturation studies, sections were incubated at 4°C in buffer containing 26 nM L-^{3}H-glutamate (39 Ci/mmol) diluted with varying concentrations of nonradioactive glutamate ranging from 25 nM to 10 μM. Following a 45 min incubation all sections were rapidly rinsed 3 times with 3 ml of cold buffer followed by 1 rinse with a solution of cold glutaraldehyde and acetone (1:19, vol/vol) and rapidly dried. The total rinse time of this assay did not exceed 10 sec. Nonspecific binding was determined in the presence of 100 μM NMDA or 1 mM glutamate, and values were similar using either displacer. Depending on the region, nonspecific binding varied from 5 to 50% of total glutamate binding.

Autoradiography and data analysis. Sections were placed in an x-ray cassette and apposed to a piece of Ultrofilm ^{3}H (LKB). A complete set of radioactive standards calibrated against brain paste with known amounts of tritium was co-exposed with each film. Following a 3 week exposure at 4°C, films were developed in Kodak D19, fixed, and air-dried. Autoradiographic analysis was carried out using previously described methods (Pan et al., 1983). At least 15 readings were made for each region studied. Anatomical areas were determined using the atlases of Paxinos and Watson (1982) and Zilles (1985). Scatchard analyses of saturation data were constructed using the computer program LIGAND (Munson and Rodbard, 1980). Binding of the 2 ligands to the various areas of the brain was compared with the Pearson product–moment coefficient and linear-regression analysis.

Results

The binding pattern of L-^{3}H-glutamate-labeled NMDA receptors and ^{3}H-TCP recognition sites was nearly identical in all forebrain regions (Fig. 1). When the bound values for NMDA and TCP receptors were normalized to the stratum radiatum of the CA1 region of the hippocampal formation, the ratio of NMDA and TCP binding sites in the forebrain and brain stem never exceeded 1.2, while in the cerebellar granule cell layer this ratio was greater than 6. On plotting the number of NMDA and TCP receptors against each other and using linear regression, a highly significant correlation was observed ($R = 0.95, p < 0.001$) (Fig. 2).

The stratum radiatum of the CA1 division of the hippocampal formation contained the highest number of both NMDA and PCP receptors in the CNS. Scatchard analysis of L-^{3}H-glutamate binding revealed a single binding site with a maximum bound value (B_{max}) of 9.78 ± 0.84 pmol/mg protein and an association constant (K_D) of 158 ± 37 nM (Fig. 3*A*). ^{3}H-TCP binding displayed an apparent single binding site with a B_{max} of 2.07 ± 0.16 pmol/mg protein and K_D of 127 ± 30 nM (Fig. 3*B*). When the B_{max} of NMDA receptors was compared with that of ^{3}H-TCP labeled receptors, a stoichiometry of 4–5 NMDA to 1 TCP site was determined. Similar values were observed when saturation analyses of binding to layers I–II of cortex were examined.

Comparative distribution of ^{3}H-glutamate-sensitive NMDA and ^{3}H-TCP-labeled PCP binding sites

A distinctly "NMDA-like" laminar distribution was recognized for both ligands in the hippocampal formation (Fig. 1*E–G*). Binding was highest in stratum radiatum, followed by stratum oriens of the CA1/CA2 region (Table 1). Binding in these 2 regions was highly delineated since both of these structures abut the stratum pyramidale, a region of relatively low binding. There was a relatively small number of receptors for both ligands within stratum oriens and stratum radiatum of CA3, which made the boundary between CA1/CA2 and CA3 readily apparent. Both blades of the molecular layer of the dentate gyrus possessed very high densities of binding, while the stratum moleculare/lacunosum of CA1 exhibited intermediate levels.

Densities of binding ranged widely in the cerebral cortex (Table 1). The visual cortex clearly possessed the greatest number of NMDA and TCP receptors, being approximately ¾ as high as the binding in the stratum radiatum (Fig. 1*H*). High densities were also observed in layers I and II of somatosensory and motor cortices, while layers V and VI of these regions had less than half of the number of binding sites observed in superficial layers (Fig. 1*D*). In the somatosensory cortex, but not the motor region, an intermediate level of binding was observed as a narrow band in layer IV (Fig. 1*D*). The anterior cingulate (Fig. 1*C*) and entorhinal regions (Fig. 1*H*) possessed equally high levels of binding, which were more than 2 times higher than those in either the retrosplenial cortex (Fig. 1*G*) or the posterior cingulate gyrus (Fig. 1*E*).

The basal ganglia and precommissural basal forebrain also revealed heterogeneous patterns of binding. In these regions, however, binding never exceeded 50% of that observed in the densest region of the hippocampus. In the striatum binding was of intermediate density, while the adjacent globus pallidus possessed virtually no binding sites (Fig. 1*D*). Similarly, the nucleus accumbens and lateral septum possessed nearly equal, intermediate levels of binding, while the closely situated medial septum and horizontal limb of the diagonal band were almost devoid of receptors (Fig. 1*C*).

Numerous connections have been said to exist between various olfactory regions and the amygdala (Fig. 1, *A–G*). In this study, with only several exceptions, these regions appeared to possess intermediate to high levels of NMDA and TCP binding. Relatively low levels of binding were measured in the internal granule layer of the olfactory bulb and medial amygdaloid nu-

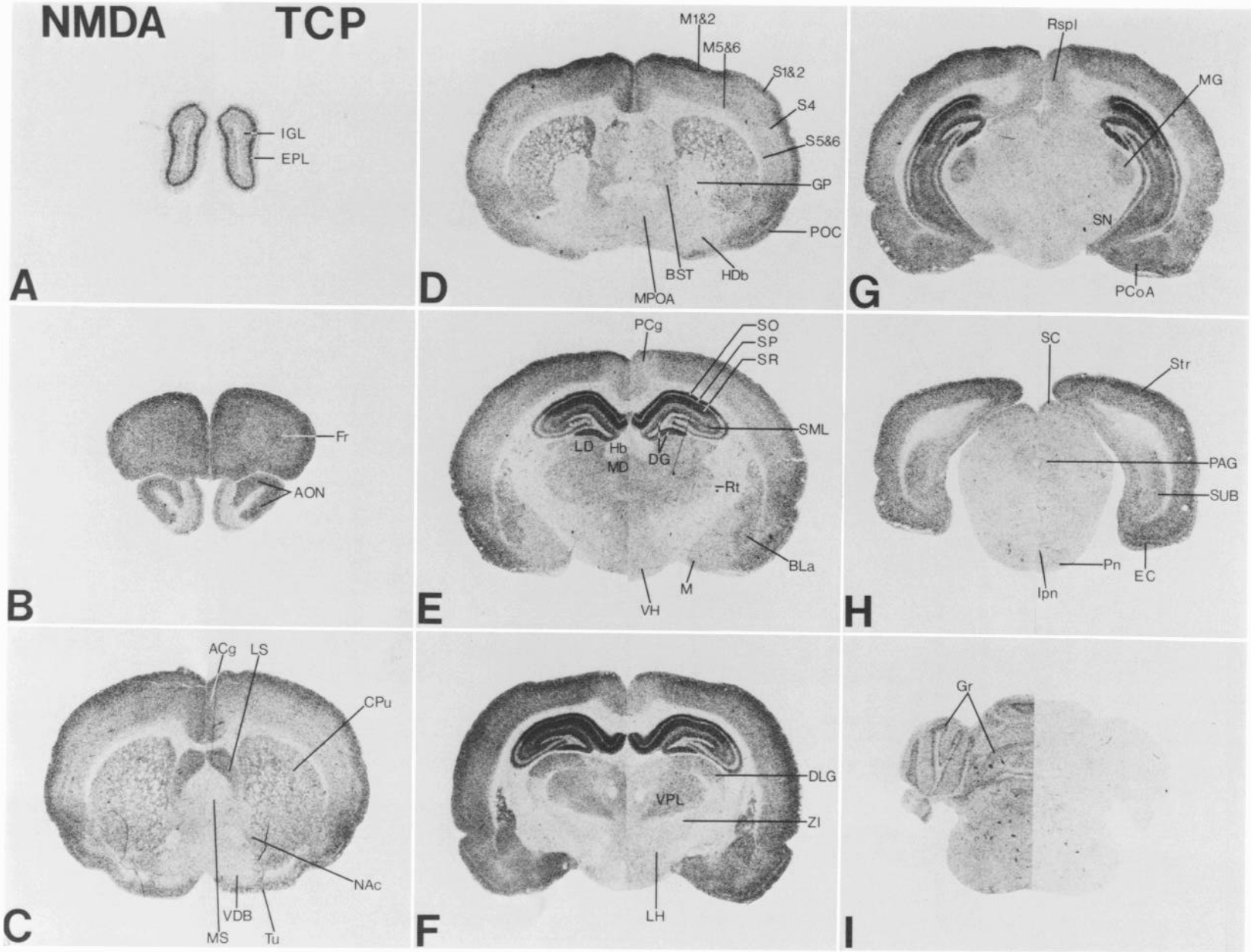

Figure 1. Regional distribution of ^{3}H-glutamate-labeled NMDA receptors (*left half of brain*) and ^{3}H-TCP-labeled PCP receptors (*right half*) in rat. NMDA receptors were labeled with 200 nM ^{3}H-glutamate in Tris-acetate buffer containing 1 μM quisqualic acid. ^{3}H-TCP binding (20 nM) was carried out on serially adjacent sections in Tris-acetate buffer containing 1 mM Mg^{2+}. All sections are from left hemisphere, but the TCP autoradiograms were reversed before printing. For abbreviations see Table 1.

Table 1. Binding of ^{3}H-TCP to PCP sites and of ^{3}H-glutamate to NMDA sites in rat brain

Area	Ligand bound (fmol/mg protein)		Binding relative to stratum radiatum of CA1 (%)	
	NMDA	TCP	NMDA	TCP
Olfactory region				
External plexiform layer (EPL)	1069 ± 159	189 ± 46	33	52
Internal granule layer (IGL)	488 ± 115	57 ± 6	15	16
Anterior olfactory nucleus (AON)	1368 ± 346	211 ± 43	42	58
Amygdala				
Basolateral, anterior (BLa)	1056 ± 203	141 ± 24	32	39
Medial (M)	506 ± 165	70 ± 14	15	19
Posterior cortical (PCoA)	1346 ± 330	176 ± 48	41	49
Basal ganglia				
Caudate-putamen (CPu)	695 ± 161	92 ± 18	21	25
Globus-pallidus (GP)	75 ± 29	18 ± 6	2	5
Septal area				
Lateral septum (LS)	1060 ± 184	136 ± 25	32	38
Medial septum (MS)	130 ± 35	35 ± 11	4	10
Ventral pallidum				
Nucleus accumbens (NAc)	849 ± 241	106 ± 18	26	29
Olfactory tubercle (TU)	1135 ± 290	198 ± 35	35	54
Cortex				
Somatosensory, layers I&II (51 & 2)	2160 ± 381	251 ± 34	66	68
Somatosensory, layer III (53)	1184 ± 216	128 ± 19	36	35
Somatosensory, layers V&VI (55 & 6)	1038 ± 257	97 ± 18	32	26
Motor, layers I&II (M1 & 2)	1932 ± 315	251 ± 36	59	69
Motor, layers V&VI (M5 & 6)	713 ± 162	97 ± 18	22	27
Anterior cingulate (ACg)	1830 ± 472	198 ± 34	56	54
Posterior cingulate (PCg)	752 ± 119	70 ± 15	23	19
Entorhinal (EC)	1553 ± 299	242 ± 28	48	64
Primary olfactory (POC)	1548 ± 311	216 ± 33	47	59
Retrosplenial (Rspl)	884 ± 197	79 ± 15	27	22
Striate (Str)	2416 ± 256	268 ± 27	74	73
Hippocampal formation				
Dentate gyrus (DG)	2473 ± 262	251 ± 36	76	68
CA1, stratum radiatum (SR)	3238 ± 367	365 ± 34	100	100
CA1, stratum oriens (SO)	2886 ± 327	312 ± 38	89	85
CA3, stratum radiatum	1060 ± 169	176 ± 26	32	48
CA3, stratum oriens	946 ± 305	101 ± 19	29	27
CA1/CA3, stratum pyramidale (SP)	792 ± 200	97 ± 16	24	27
Stratum lacunosum/moleculare (SML)	1839 ± 268	216 ± 33	56	59
Subiculum, dorsalis (SUB)	1074 ± 141	97 ± 19	33	26
Thalamus				
Habenula (Hb)	75 ± 44	13 ± 6	2	3
Lateral dorsal (LD)	902 ± 167	88 ± 17	27	24
Medial dorsal (MD)	977 ± 168	84 ± 17	30	23
Medial geniculate (MG)	968 ± 239	92 ± 15	29	25
Dorsolateral geniculate (DLG)	858 ± 153	106 ± 21	26	28
Ventral posterior lateral/medial (VPL)	647 ± 178	88 ± 11	19	24
Reticular (Rt)	308 ± 119	31 ± 3	9	8
Other				
Bed nucleus stria terminalis (BST)	497 ± 127	53 ± 11	15	14
Medial preoptic area (MPOA)	286 ± 119	40 ± 14	8	11
Lateral hypothalamus (LH)	48 ± 27	18 ± 7	1	4
Ventromedial hypothalamus (VH)	84 ± 31	31 ± 10	2	8
Diagonal band, horizontal limb (HDb)	190 ± 67	35 ± 9	6	9
Zona incerta (ZI)	31 ± 13	13 ± 8	1	4

Table 1. Continued

Area	Ligand bound (fmol/mg protein)		Binding relative to stratum radiatum of CA1 (%)	
	NMDA	TCP	NMDA	TCP
Brain stem				
Superior colliculus (SC)	616 ± 132	53 ± 10	18	15
Periaqueductal gray (PAG)	242 ± 65	40 ± 6	8	10
Interpeduncular nucleus (IPn)	198 ± 144	26 ± 8	6	7
Pontine nuclei (Pn)	110 ± 101	18 ± 4	3	5
Substantia nigra (SN)	163 ± 87	13 ± 7	5	4
Cerebellum				
Granule layer (Gnl)	633 ± 97	13 ± 5	19	3

Values represent the mean ± SEM of 7 animals. ^{3}H-glutamate concentration was 200 nM. ^{3}H-TCP concentration was 20 nM. Autoradiography and quantification of autoradiograms was carried out as described in text.

cleus. The external plexiform layer of the olfactory bulb, the lateral anterior olfactory nucleus, the olfactory tubercle, pyramidal layer of cortex, and the basolateral and primary olfactory/posterior amygdaloid nuclei contained 30–60% of the maximum number of NMDA and TCP recognition sites.

In the thalamus (Fig. 1*E–G*), homogeneous binding was observed in the laterodorsal and mediodorsal nuclei, as well as in the medial and dorsolateral geniculate and the ventroposterolateral nuclei. This density of binding is in sharp contrast to that in the medial habenula, reticular nuclei, and ventrolateral geniculate nuclei (data not shown), which were nearly devoid of binding. Very low levels of binding were also observed in other diencephalic structures (Fig. 1, *E, F*), including the zona incerta, and the lateral and ventromedial hypothalamus. The telencephalic medial preoptic area (Fig. 1*D*), which is the anterior continuation of the hypothalamus, contained low but slightly higher levels than the hypothalamus (Fig. 1*E*). The bed nucleus of the stria terminalis contained slightly higher levels than the medial preoptic area (Fig. 1*D*).

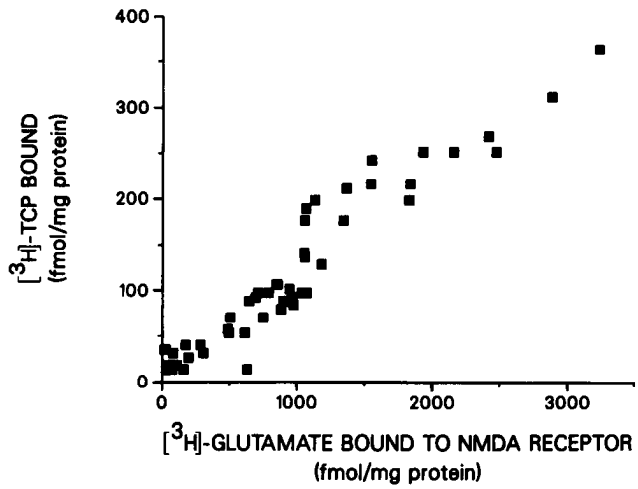

Figure 2. Scatter-plot of bound values (in fmol/mg protein) of ^{3}H-TCP (20 nM) versus bound values for NMDA receptors (^{3}H-glutamate, 200 nM as above). Linear-regression analysis yielded a correlation coefficient of 0.95 ($p < 0.001$, Pearson product–moment).

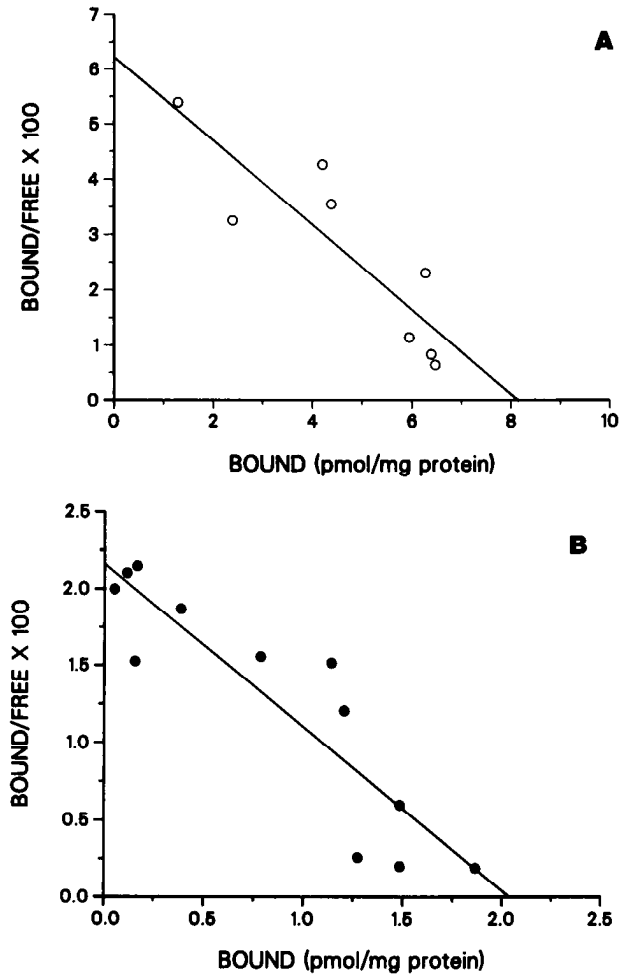

Figure 3. Representative Scatchard plots of ^{3}H-glutamate binding (*A*) and ^{3}H-TCP binding (*B*) in stratum radiatum of the CA1 subfield of the hippocampal formation. Autoradiography for each ligand was performed as described in Materials and Methods. Each point represents specific binding (average of 15 readings minus readings from nearly adjacent sections) incubated in either the presence of 100 μM NMDA for ^{3}H-glutamate or 20 μM PCP for ^{3}H-TCP binding.

Brain stem and cerebellum

In the brain stem, significant densities were observed only in the superior colliculus and periaqueductal gray region (Fig. 1*H*). Interestingly, as has been previously noted, the granule layer of the cerebellum had about 20% of the highest number of NMDA binding sites but had very few TCP binding sites (Fig. 1*I*).

Discussion

The described distribution of NMDA receptors in the rat brain is in agreement with previous reports (Greenamyre et al., 1985; Monaghan and Cotman, 1985). Each region evaluated receives putative excitatory amino acid inputs (for a review, see Fagg and Foster, 1983). In hippocampal formation, for instance, NMDA receptors are densest in the stratum radiatum of the CA1 division, which has dense neuropil consisting of the apical dendrites of CA1 pyramidal neurons (Lorente de Nó, 1934) upon which terminate fibers originating from the ipsi- and contralateral CA3 regions (Laurberg, 1979; Taxt and Storm-Mathisen, 1984). Both neurochemical (Storm-Mathisen and Iversen, 1979; Skrede and Malthe-Sorenssen, 1981) and physiological (Collingridge et al., 1983a) studies indicate that these pathways use either glutamate or aspartate as their transmitter. In physiological studies, NMDA, quisqualate- and kainate-sensitive glutamate receptors exist on the apical dendrites of CA1 pyramidal neurons (Collingridge et al., 1983b). NMDA elicits long-lasting, delayed responses suited for involvement in synaptic plasticity (Collingridge, 1985). Excitatory stimulation of CA1 *in vitro* induces long-term potentiation (Alger and Tyler, 1976; Dunwiddie and Lynch, 1978), a model of information storage (Swanson et al., 1982). Dendritic responses elicited by excitation of the CA1 input pathways and long-term potentiation are inhibited by specific NMDA antagonists (Collingridge et al., 1983a; Harris et al., 1984; Wigstrom and Gustafsson, 1984).

Various cortical regions are also densely populated with NMDA receptors. Layers I and II of somatosensory cortex, for instance, have more than twice as many NMDA receptors as intermediate and deep layers. Inter- and intrahemispheric corticocortical association fibers, which are thought to be primarily glutamatergic (Stone, 1979; Fonnum et al., 1981; Streit, 1984; Manzoni et al., 1986; Peinado and Mora, 1986), synapse heavily in the superficial and, to a lesser degree, the deep layers (Lorente de Nó, 1933). The excitation of apical dendrites elicited by stimulation of these pathways is inhibited by NMDA antagonists (Thomson et al., 1985), suggesting a role for NMDA receptors in cortical synaptic transmission.

The striatum receives a rich glutamatergic innervation from the cortex (Spencer, 1976; McGeer et al., 1977; Young et al., 1981; Young and Bradford, 1986), which synapses in part on cholinergic interneurons where NMDA and its antagonists modulate the release of ACh (Scatton and Lehmann, 1982; Snell and Johnson, 1986). The hypothalamic nuclei and most brain-stem structures have very few NMDA receptors. L-^{3}H-glutamate binds sites in these areas (Greenamyre et al., 1984; Halpain et al., 1984); thus, they likely mediate actions of other classes of glutamate receptors, such as the quisqualate or kainate receptors.

Dissociative anesthetics affect a number of chemically and physiologically defined systems in the CNS. For instance, they compete at both muscarinic and opiate receptors (Vincent et al., 1978), as well as inhibit high-affinity reuptake of dopamine (Snell and Johnson, 1986), 5-HT (Smith et al., 1977), and norepinephrine (Gear and Heath, 1976). Moreover, PCP interacts with ion channels such as that associated with the nicotinic receptor (Eldefrawi et al., 1982) and certain potassium channels (Albuquerque et al., 1981). The effects of dissociative anesthetics on so many different chemical systems is not surprising in light of the myriad behavior effects they produce.

Until recently, the dissociative anesthetics and the so-called sigma opiates—the compound SKF 10,047 being the prototype) (Martin et al., 1976)—were thought to act at the same site, the PCP/sigma opiate receptor. This assertion was based on binding (Zukin et al., 1983; Mendelsohn et al., 1985) and drug discrimination studies (Herling et al., 1981; Brady et al., 1982). However, recent data have shown that SKF 10,047 binds to 2 separate sites (Largent et al., 1986; Sircar et al., 1986). The high-affinity site, or "sigma receptor," has a distinct pharmacology, and drugs such as haloperidol and 3-[3-hydroxyphenyl]-*N*-(1-propyl)piperidine display marked inhibitory actions at this site. The low-affinity site has an anatomical distribution distinct from the sigma site. The dissociative anesthetics, including TCP, are very potent inhibitors at this so-called dissociative anesthetic (PCP) site.

Several groups have found that ^{3}H-TCP binding sites are dense in the dendritic zones of CA1 of the hippocampal formation, with binding highest in the stratum radiatum (Gundlach et al., 1986; Sircar et al., 1986). Minimal binding was noted in the pyramidal cell layer of the hippocampal formation, which possesses a high concentration of non-PCP sigma opiate binding sites (Largent et al., 1986). Very high ^{3}H-TCP binding was also found in the dentate gyrus, where dissociative anesthetics inhibit the actions of NMDA (Raja and Guyenet, 1982). ^{3}H-TCP binding in these and other regions paralleled the distribution of NMDA receptors in the cerebral cortex, striatum, and thalamus. The hypothalamus and brain-stem structures, with the exception of the superior colliculus and periaqueductal gray zone, revealed very little binding of either ligand. The greater density of binding in telencephalic versus the more primitive hypothalamic and brain-stem structures is compatible with the cognitive and behavioral abnormalities produced by PCP.

Physiologically, PCP and PCP-like drugs inhibit noncompetitively the excitatory properties of NMDA in the cerebral cortex (Harrison and Simmonds, 1985) and spinal cord (Martin and Lodge, 1985). Like NMDA antagonists, the dissociative anesthetics inhibit long-term potentiation *in vitro* (Stringer et al., 1983) and *in vivo* (Stringer et al., 1983; Morris et al., 1986). The dissociative anesthetics also inhibit NMDA-mediated neural destruction (Olney et al., 1986; Weiss et al., 1986) and attenuate NMDA-enhanced release of ACh in the cortex (Lodge and Johnston, 1985). Behavioral studies indicate a unique relationship between NMDA receptors and PCP recognition sites. For instance, animals given the NMDA antagonist 2-amino-5-phosphonovalerate behave as if treated with PCP (Koek et al., 1986). Moreover, animals generalize to NMDA antagonists as PCP-like in drug discrimination tests (Willets et al., 1986). These data support the concept that NMDA and PCP bind to the same receptor complex.

The cerebellum was the only region in the rat brain where NMDA and ^{3}H-TCP receptors were mismatched. NMDA receptors are concentrated in the granule cell layer and to a lesser extent in the molecular layer. Quinolinic acid, a tryptophan metabolite, preferentially stimulates NMDA receptors in the cerebral cortex, compared with those in the cerebellum or spinal cord (Perkins and Stone, 1983). Based on this observation, the

existence of 2 NMDA receptor subtypes has been postulated. The loss of concordance between ^{3}H-TCP and NMDA binding sites in the cerebellum adds further support to this hypothesis and suggests that the quinolinate-insensitive site may not be linked to a TCP-regulated site. The few ^{3}H-TCP sites in the granular layer may represent labeling to the lower-affinity TCP site described by Vignon et al. (1986).

The precise mechanism whereby the dissociative anesthetics inhibit the action of the NMDA receptor is unclear. NMDA causes the opening of a voltage-dependent cation channel that is gated by Mg^{2+} (Mayer et al., 1984; Nowak et al., 1984). Like PCP, Mg^{2+} inhibits noncompetitively the action of NMDA (Nowak et al., 1984; Harrison and Simmonds, 1985). Pharmacological evidence suggests that Mg^{2+} and the dissociative anesthetics bind at allosteric sites on the receptor-channel molecule and that both of these sites are distinct from the NMDA recognition site (Harrison and Simmonds, 1985; Martin and Lodge, 1985).

The 4–5:1 stoichiometry of NMDA to ^{3}H-TCP-labeled receptors suggests that several molecules of glutamate are needed to fully activate the receptor and that the dissociative anesthetics exert their inhibitory influences somewhere near the channel. Like Mg^{2+}, ketamine and PCP produce a voltage-dependent blockade of the channel (Honey et al., 1985). Furthermore, it has been shown that ^{3}H-TCP binding is enhanced in the presence of glutamate (Loo et al., 1986). Thus, the dissociative anesthetics, like Mg^{2+}, may bind preferentially near the open channel site. However, pharmacological evidence indicates that Mg^{2+} and the dissociative anesthetic binding sites are not identical (Harrison and Simmonds, 1985; Martin and Lodge, 1985).

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