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Quisqualate- and NMDA-Sensitive [^{3}H]Glutamate Binding in Primate Brain

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Excitatory amino acids (EAA) such as glutamate and aspartate are probably the neurotransmitters of a majority of mammalian neurons. Only a few previous studies have been concerned with the distribution of the subtypes of EAA receptor binding in the primate brain. We examined NMDA- and quisqualate-sensitive [^{3}H]glutamate binding using quantitative autoradiography in monkey brain *(Macaca fascicularis)*. The two types of binding were differentially distributed. NMDA-sensitive binding was most dense in dentate gyrus of hippocampus, stratum pyramidale of hippocampus, and outer layers of cerebral cortex. Quisqualate-sensitive binding was most dense in dentate gyrus of hippocampus, inner and outer layers of cerebral cortex, and molecular layer of cerebellum. In caudate nucleus and putamen, quisqualate- and NMDA-sensitive binding sites were nearly equal in density. However, in globus pallidus, substantia nigra, and subthalamic nucleus, quisqualate-sensitive binding was several-fold greater than NMDA-sensitive binding. In thalamus, [^{3}H]glutamate binding was generally low for both subtypes of binding except for the anterior ventral, lateral dorsal, and pulvinar nuclei. In the brainstem, low levels of binding were found, and strikingly the red nucleus and pons, which are thought to receive glutamatergic projections, had approximately 1/20 the binding observed in cerebral cortex.

These results demonstrate that NMDA- and quisqualate-sensitive [^{3}H]glutamate binding are observed in all regions of primate brain, but that in some regions one subtype predominates over the other. In addition, certain areas thought to receive glutamatergic projections have low levels of both types of binding.

Key words: primate, excitatory amino acid receptors, autoradiography

In the last 15 years, excitatory amino acids (EAA) such as glutamate and aspartate have been identified as the probable neurotransmitters of a large percentage of neurons in mammalian brain (Watkins et al., 1990; Young and Fagg, 1990). Because EAA are involved in general cellular metabolic functions, they were not originally considered to be likely neurotransmitter candidates. Electrophysiological studies demonstrated convincingly the potency of these substances as depolarizing agents and eventually discerned specific subtypes of EAA receptors that responded preferentially to EAA analogs (Dingledine et al., 1988). Coincident with the electrophysiological studies, biochemical studies indicated that EAA were released from slice and synaptosome preparations in a calcium-dependent fashion and were accumulated in synaptosome preparations by a high-affinity transport system. With these methodologies, EAA release and uptake were found to be selectively decreased in the projection regions of a variety of neuronal pathways after the pathways were lesioned. Studies using immunocytochemical methods to stain conjugated-glutamate or aspartate have also demonstrated the wide distribution of presumed glutamatergic and aspartergic neurons (Otterson and Storm-Mathisen, 1987; Giuffrida and Rustioni, 1989; Conti et al., 1988). Initial experiments in cerebellum indicated that the granule cells use glutamate as a putative neurotransmitter (Young et al., 1974). Cortico-cortical association pathways were also linked to glutamate (Fonnum et al., 1981; Barbaresi et al., 1987). Subsequently, numerous pathways, including afferents, intrinsic neurons, and efferents of the hippocampal formation, were found to use EAA as neurotransmitters (Fagg and Foster, 1983; Fonnum, 1984; Storm-Mathisen, 1977). Cortical inputs to subcortical regions such as thalamus, caudate/putamen, red nucleus, pons, and spinal cord were shown to use EAA as probable neurotransmitters (Bernays et al., 1988; Christie et al., 1986; Fosse and Fonnum, 1987; Giuffrida and Rustioni, 1988; Rouzaire-Dubois and Scarnati, 1987a,b; Young et al., 1981, 1983). Most of these pathways have been

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examined in rodents and cats, but some have been investigated in primate (Young et al., 1981 and 1983).

Electrophysiological studies now indicate the presence of at least four subtypes of EAA receptors in brain (Watkins et al., 1990; Young and Fagg, 1990). Three of these receptors are linked to ion channels: the kainate, AMPA, and N-methyl-D-aspartate (NMDA) receptors. The fourth receptor type modulates inositol triphosphate metabolism and is sensitive to quisqualate (as in the AMPA site) but is not particularly sensitive to other selective ion channel-activating AMPA or quisqualate agonists (Nicoletti et al., 1985; Rescasens et al., 1987). Thus, quisqualate-sensitive [^{3}H]glutamate binding represents binding to both the AMPA and metabotropic receptors (Cha et al., 1990). Biochemical studies in homogenates and autoradiographic studies on tissue sections have demonstrated NMDA-, quisqualate-sensitive [^{3}H]glutamate binding sites that have pharmacological properties similar to those of the physiologically defined NMDA and quisqualate receptors, respectively (Young and Fagg, 1990).

Only a limited number of studies have been performed showing the distribution of receptor subtypes in the primate brain (Geddes et al., 1989; Jansen et al., 1989). In this study, we examined the regional distribution of NMDA-sensitive and quisqualate-sensitive [^{3}H]glutamate binding in many regions of monkey brain as part of an ongoing series of studies of plasticity of the motor system in monkeys (Dauth et al., 1985; Gilman et al., 1987; Shimoyama et al., 1988).

MATERIALS AND METHODS

Four adult *Macaca fascicularis* monkeys weighing 2.5–3.5 kg were anesthetized with pentobarbital and killed by an intravenous injection of saturated KCl. The brain was rapidly removed and frozen in isopentane on dry ice. Coronal sections of 20 μm were taken serially from the rostral caudate through the deep cerebellar nuclei of the brain and assayed for [^{3}H]glutamate binding within 36 hours of sectioning. Two sections were taken for histological analysis every 20 sections. One section was stained with cresyl violet for cell bodies, the other with osmium tetroxide for myelin. Sections for [^{3}H] glutamate autoradiography were taken at the level of the rostral caudate, anterior commissure, medial globus pallidus, subthalamic nucleus, ventral anterior thalamic nucleus, substantia nigra, and deep cerebellar nuclei. Sections were given three 5 minute prewashes at 4°C in 50 mM Tris-HCl buffer containing 2.5 mM $CaCl_2$ at pH 7.2. Duplicate sections were then incubated with 20 nM [^{3}H]glutamate (specific activity 40 Ci/mmol) in 50 mM Tris-HCl buffer with 2.5 mM $CaCl_2$ in the presence or absence of 2.5 μM quisqualate, 1 mM NMDA, both 2.5 μM quisqualate, and 1 mM NMDA or 1 mM glutamate. After a 45 minute incubation at 4°C, sections were rinsed three times with cold buffer and once in cold acetone with 2.5% glutaraldehyde (v/v) and then rapidly dried. Rinse times were less than 10 seconds. Sections were apposed to tritium-sensitive LKB Ultrofilm ^{3}H for 3 weeks along with plastic standards from American Radiolabeled Chemical, Inc. that had been calibrated to dpm/μg protein in our laboratory. After exposure the films were developed in D-19 and analyzed for binding density with computerized densitometry.

The BRS2 quantitative imaging program from Imaging Research, Inc. (St. Catherine, Ontario, Canada) was used to digitize the autoradiograms. The program converted the density readings to dpm/μg protein by comparison to the standards (Pan et al., 1983). The data from the BRS2 program were then converted to fmol/mg protein.

Five to 20 individual readings were taken from each structure bilaterally and from each level at which the structure appeared. In cortex, readings were taken from both the outer (I–III) and inner IV–VI) layers. The individual readings from each structure along with identifying information were incorporated into a database using the SYSTAT statistical analysis program. All subsequent transformations, merging and averaging of data, both within and across subjects, was accomplished with SYSTAT. A mean value for each structure was computed from the data from duplicate sections. Specific binding data were calculated as described below. The mean values of the data were then computed by structure across all section levels of the brain for each structure in each animal. The values for each structure from each animal were used to calculate the means and standard errors for each structure listed in the tables (Tables I, II).

The cortical outer layer/inner layer (O/I) binding ratios were calculated from the specific binding under each displacer condition, i.e., total binding minus binding in the presence of 2.5 μM quisqualate and total binding minus binding in the presence of 1 mM NMDA. Mean values of these data were then computed and tabulated as described above (Table III).

The specific binding for the glutamate subtypes was calculated from the total binding data in the following manner:

$$S(\text{NMDA}) = B(^3\text{H-glutamate}) - b(\text{NMDA})$$
$$S(\text{quis}) = B(^3\text{H-glutamate}) - b(\text{quisqualate})$$

where S ( ) = specific subtype binding, B ( ) = total binding, b ( ) = nonspecific binding as defined by binding in the presence of 1 mM NMDA (for NMDA-sensitive binding) or 2.5 μM quisqualate (for quisqualate-sensitive binding). With these equations, it was

possible to determine specific NMDA-sensitive and quisqualate-sensitive glutamate binding.

RESULTS

NMDA-sensitive and quisqualate-sensitive sites were differentially distributed (Tables I, II; Figs. 1, 2). Binding was highest for each of the subtypes in hippocampus and cerebral cortex (Table I).

NMDA-sensitive binding was most dense in hippocampal formation and cerebral cortex. In the hippocampal region, NMDA-sensitive binding was dense in dentate gyrus, stratum pyramidale of CA1, and entorhinal cortex. In cerebral neocortex, NMDA-sensitive binding was highest in the outer layers, with Brodmann areas 8, 12, and 6 having the highest binding. Inner layers showed variable densities depending on the region. There was an area of decreased apparent binding associated with the increased white matter in layer IV. A thin band of higher density NMDA-sensitive binding was observed at the border between layers IV and V. Primary motor cortex, which had no band of increased binding, showed the highest ratio of O/I binding in cerebral cortex. Temporal association cortex (Brodmann areas 21, 22, and 24), entorhinal, and insular cortex showed the lowest O/I ratios (Table III).

Quisqualate-sensitive binding was most dense in dentate gyrus and stratum pyramidale of CA1 in hippocampal formation with intermediate densities in subiculum and entorhinal cortex. In cerebral cortex, quisqualate binding was highest in density in outer layers of prefrontal cortex (Brodmann areas 45 and 46) and insular cortex. There was less of a band of increased binding in layer V and the O/I ratios of quisqualate-sensitive binding were lower than those for NMDA-sensitive binding. In motor cortex, the O/I ratios were approximately 1.3–2.3 (compared to 2.2–3.2 for NMDA-sensitive binding).

The basal ganglia showed marked regional heterogeneity of NMDA-sensitive and quisqualate-sensitive binding (Table II). In caudate/putamen quisqualate-sensitive binding was somewhat higher than NMDA-sensitive binding. Globus pallidus binding of both types was considerably lower than caudate/putamen. In external globus pallidus quisqualate-sensitive binding was higher than NMDA-sensitive binding by 2:1 and in internal globus pallidus by nearly 8:1. Similar relationships held for the substantia nigra pars reticulata and subthalamic nucleus.

In the thalamus, binding was generally only of low density. The exceptions were the anterior ventral nucleus, which had a NMDA:quisqualate binding ratio of 2:3; the pulvinar, which displayed a high density of NMDA-sensitive binding; the lateral and medial dorsal nuclei, where quisqualate-sensitive binding was denser than the NMDA-sensitive binding; and the parvocellular portion of the ventral posteromedial nucleus, which had mainly quisqualate-sensitive binding. The overall density of [^{3}H]glutamate binding was substantially less in other thalamic regions than in the cerebral cortex. In particular, very low densities of binding were found in the motor thalamus and the geniculate nuclei.

In the brainstem, binding densities were again low except for the central gray zones, superior colliculus, and vestibular nuclei. In the central gray, NMDA-sensitive binding predominated. In superior colliculus, quisqualate-sensitive binding was more dense. In the granule cell layer of the cerebellum, quisqualate-sensitive binding was nearly equal to NMDA-sensitive binding. In the molecular layer, however, quisqualate-sensitive binding was four times higher than NMDA-sensitive binding, and quisqualate-sensitive binding in the molecular layer was twice as dense as that in the granule cell layer. The deep cerebellar nuclei displayed low binding densities for both subtypes.

DISCUSSION

Several different techniques for measuring NMDA- and quisqualate-sensitive [^{3}H]glutamate binding have been reported (Young and Fagg, 1990). Some methods are more selective than others and new assays are being developed continually. [^{3}H]Glutamate can be used to label all the EAA receptor subtypes, although the subtypes must be identified by competition studies because glutamate itself does not distinguish between the sites. NMDA appears to interact with a homogeneous group of [^{3}H]glutamate binding sites whereas quisqualate interacts with receptors linked to ion channels and receptors linked to inositol triphosphate metabolism (Monaghan et al., 1989; Young and Fagg, 1990). Quisqualate-sensitive [^{3}H]glutamate binding is highly chloride sensitive and is stimulated at least four-fold in the presence of 40 mM chloride and 2.5 mM calcium chloride (Cha et al., 1988). In the presence of calcium, chloride, and KSCN, the quisqualate agonist AMPA will compete for a subset of quisqualate-sensitive sites, presumably those on the ion channel linked quisqualate receptor (Cha et al., 1988). This so-called "metabotropic" quisqualate receptor is insensitive to AMPA (Nicoletti et al., 1985; Palmer et al., 1989; Rescasens et al., 1987). Quisqualate-sensitive [^{3}H]glutamate binding probably measures binding to both quisqualate-linked EAA subtypes, i.e., the ion channel and the metabotropic receptor (Young and Fagg, 1990).

Quisqualate inhibits [^{3}H]glutamate binding in a biphasic manner with a high-affinity component in the low nanomolar range and a low-affinity component in the high micromolar range (Greenamyre et al., 1985; Cha et

TABLE I. NMDA-Sensitive and Quisqualate-Sensitive [^{3}H]Glutamate Binding in *Macaca fascicularis* Cerebral Cortex*

Brodmann area	Inner (I) and outer (O) layers[a]	NMDA-sensitive, fmol/mg protein, mean (SEM)[b]	Quisqualate-sensitive, fmol/mg protein, mean (SEM)[c]
Frontal motor			
4	I	108 (26)	132 (19)
4	O	350 (64)	300 (53)
6	I	162 (49)	202 (47)
6	O	405 (58)	298 (52)
Supplementary motor	I	179 (52)	218 (31)
Supplementary motor	O	368 (49)	300 (37)
Association			
8	I	257 (154)	203 (49)
8	O	550 (190)	307 (58)
12	I	198 (10)	249 (32)
12	O	434 (80)	325 (21)
45	I	NA	290 (37)
45	O	NA	434 (70)
46	I	NA	312 (70)
46	O	NA	392 (96)
Anterior cingulate			
24	I	211 (48)	247 (35)
24	O	324 (65)	302 (44)
Insular cortex	I	263 (33)	293 (46)
Insular cortex	O	387 (43)	344 (44)
Parietal sensory			
1&2	I	201 (57)	185 (39)
1&2	O	351 (91)	246 (59)
3	I	180 (54)	164 (31)
3	O	373 (85)	258 (59)
Association			
5	I	176 (107)	165 (54)
5	O	346 (171)	275 (85)
7	I	154 (94)	182 (58)
7	O	296 (165)	264 (106)
Temporal			
Auditory			
AI	I	151 (43)	161 (43)
AI	O	301 (84)	247 (69)
Association			
21	I	175 (48)	202 (42)
21	O	295 (58)	265 (57)
22	I	209 (37)	254 (43)
22	O	324 (53)	294 (61)
Hippocampal region			
Entorhinal cortex	I	155 (52)	218 (46)
Entorhinal cortex	O	244 (66)	270 (61)
Subiculum		210 (57)	233 (38)
Presubiculum		207 (81)	243 (60)
Stratum moleculare, CA1		243 (56)	247 (26)
Stratum pyramidale, CA1		310 (68)	301 (45)
Stratum pyramidale, CA3		228 (46)	229 (27)
Stratum molecular dentate gyrus		382 (87)	363 (55)

*Values are the mean (SEM) of 4 monkeys. NA = not available.

[a]Inner layers (I) were layers IV–VI, outer layers (O) were layers I–III.

[b]NMDA-sensitive [^{3}H]glutamate binding was determined in 20 nM ligand in 50 mM Tris-HCl buffer plus 2.5 mM $CaCl_2$; binding in 1 mM NMDA was subtracted from total [^{3}H]glutamate binding.

[c]Quisqualate-sensitive [^{3}H]glutamate binding was determined by subtracting binding in the presence of 2.5 μM quisqualate from total [^{3}H]glutamate binding in 20 nM ligand, 50 mM Tris-HCl buffer plus 2.5 mM $CaCl_2$.

TABLE II. NMDA-Sensitive and Quisqualate-Sensitive [^{3}H]Glutamate Binding in Subcortical Regions of *Macaca fascicularis

Structure	NMDA-sensitive, fmol/mg protein, mean (SEM)[a]	Quisqualate-sensitive, fmol/mg protein, mean (SEM)[b]
Claustrum	145 (29)	170 (29)
Septum	242 (36)	259 (23)
Basal ganglia		
Caudate	160 (50)	219 (26)
Caudate, ventral tail	163 (41)	196 (23)
Putamen	147 (30)	216 (23)
Globus pallidus, external	38 (24)	66 (10)
Globus pallidus, internal	4 (10)	31 (6)
Substantia nigra pars compacta	17 (14)	37 (11)
Substantia nigra pars reticulata	14 (21)	48 (15)
Subthalamic nucleus	18 (19)	49 (18)
Thalamus		
Anteroventral	159 (105)	227 (91)
Centrum medianum, parafascicular	6 (14)	28 (20)
Lateral dorsal	61 (16)	77 (42)
Lateral geniculate	−21 (10)[c]	6 (10)
Medial dorsal	27 (31)	66 (19)
Medial geniculate	−11 (—)	62 (—)
Pulvinar	529 (—)	267 (50)
Ventral anterior	22 (21)	56 (19)
Ventral lateral, medial	2 (17)	44 (19)
Ventral, lateral, oral	17 (30)	54 (32)
Ventral posteroinferior	−9 (12)	11 (10)
Ventral posterior	9 (12)	31 (14)
Ventral posteromedial	15 (13)	39 (14)
Ventral posteromedial, parvocellular	56 (27)	103 (12)
Brainstem		
Central gray	181 (—)	114 (42)
Superior colliculus	91 (—)	101 (40)
Inferior colliculus	−2 (—)	46 (22)
Pontine nuclei	−9 (12)	13 (12)
Red nucleus	1 (21)	22 (25)
Vestibular nuclei	NA	66 (48)
Cerebellum		
Granular layer, cerebellar cortex	205 (58)	233 (27)
Molecular layer, cerebellar cortex	119 (57)	437 (63)
Dentate nucleus	0 (13)	29 (11)
Fastigial nucleus	−5 (1)	6 (1)
Interposed nucleus	−12 (3)	8 (1)

*Values are the mean (SEM) of data from 4 monkeys except where indicated.
(—) = Estimate based on 1 animal. NA = not available.
[a]NMDA-sensitive [^{3}H]glutamate binding was determined in 20 nM ligand in 50 mM Tris-HCl buffer plus 2.5 mM $CaCl_2$; binding in 1 mM NMDA was subtracted from total [^{3}H]glutamate binding.
[b]Quisqualate-sensitive [^{3}H]glutamate binding was determined by subtracting binding in the presence of 2.5 μM quisqualate from total [^{3}H]glutamate binding in 20 nM ligand, 50 mM Tris-HCl buffer plus 2.5 mM $CaCl_2$.
[c]Negative values occur when binding in the presence of NMDA (or quisqualate) was actually slightly higher than total binding.

al., 1988). The high-affinity quisqualate-sensitive sites appear biochemically to resemble quisqualate-linked ion channels and quisqualate-linked metabotropic receptors. The low-affinity quisqualate-sensitive sites are probably equivalent to the NMDA-sensitive sites (Greenamyre et al., 1985). Approximately 95% of the high-affinity quisqualate-sensitive sites can be blocked by 2.5 μM quisqualate. This concentration of quisqualate has minimal effects on the low-affinity site. Quisqualate receptors can also be selectively measured in Tris-chloride buffer with calcium in the presence of 1 mM NMDA and 1 μM kainate.

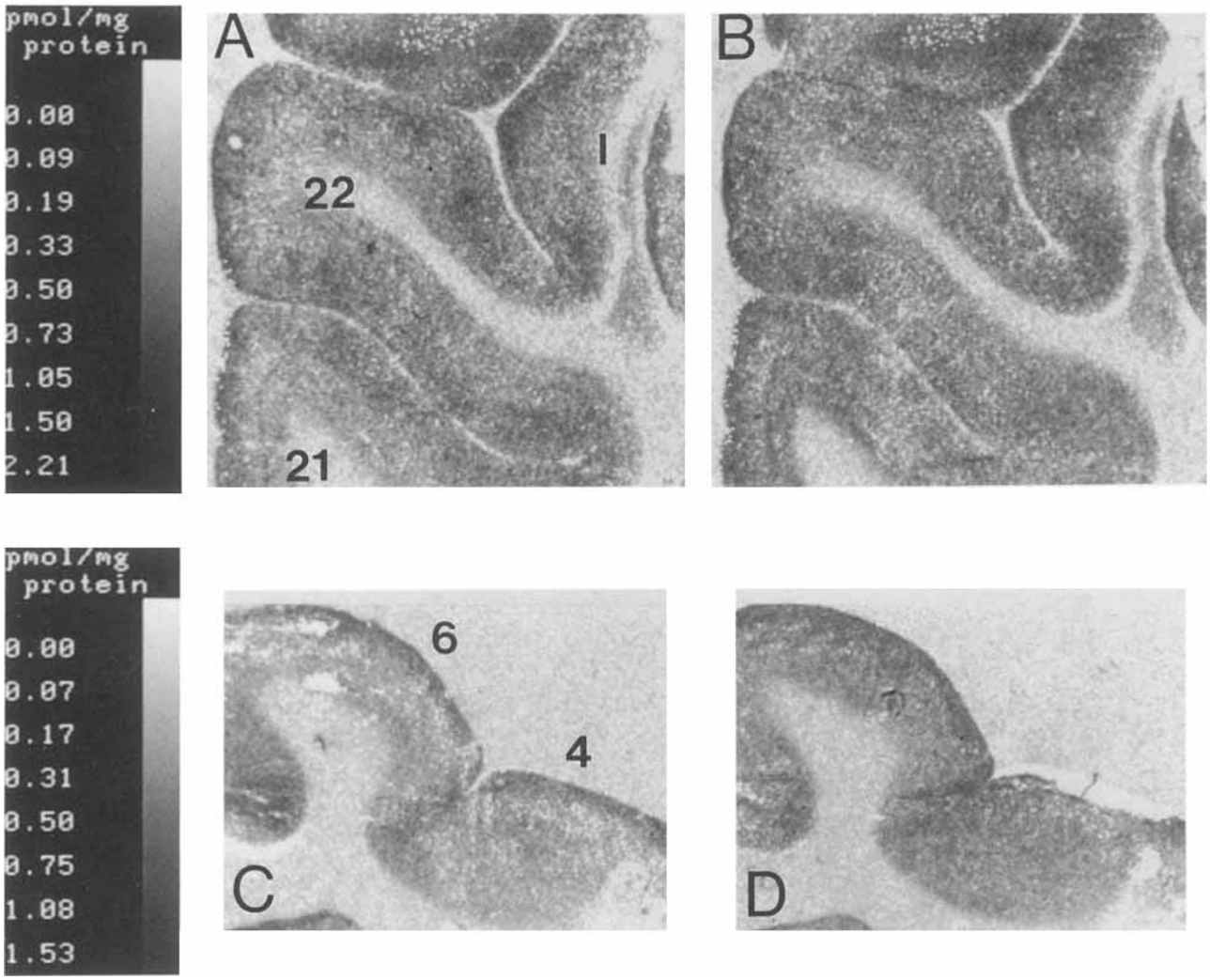

Fig. 1. Computerized images of coronal sections demonstrating NMDA-sensitive **(left)** and quisqualate-sensitive **(right)** binding in association **(A,B)** and motor **(C,D)** cortices of primate brain. The images are subtraction images of specific binding. They were obtained by subtracting from total [^{3}H]glutamate binding the binding remaining in either 1 mM NMDA (to measure NMDA-sensitive binding) or 2.5 μM quisqualate (to measure quisqualate-sensitive binding). The subtractions were carried out on a computerized image processing system from Image Research, Inc., St. Catherine's, Ontario, Canada. The bars on the left indicate the density scales for the images to the right of the bar. Note that NMDA-sensitive sites are denser in outer layers of motor cortex than inner cortex and no dense band is seen in layer IV of motor cortex. In area 21, a dense band of binding is observed in layer IV. Abbreviations: 4, 6, 21, and 22 refer to the respective Brodmann areas; I is insular cortex.

NMDA-sensitive [^{3}H]glutamate binding is not chloride sensitive and can be measured adequately in the absence of chloride or calcium (Greenamyre et al., 1985; Monaghan et al., 1989; Young and Fagg, 1990). Thus, NMDA-sensitive receptors can be measured in Tris-acetate with 2.5 μM quisqualate and 1 μM kainate. Alternatively, NMDA-sensitive [^{3}H]glutamate binding can be measured in the presence of calcium and chloride by examining only the binding that is sensitive to a high concentration of NMDA.

The assay used in this study is less selective than some current binding assays, but selective information was obtained by analyzing NMDA- and quisqualate-sensitive binding alone. In this study, however, quisqualate-sensitive [^{3}H]glutamate binding probably represents binding to both the quisqualate-sensitive ion channel and the metabotropic receptor. In addition, not all [^{3}H]glutamate binding in primate brain was inhibited by 1 mM NMDA and 2.5 μM quisqualate; in some regions considerable binding remained.

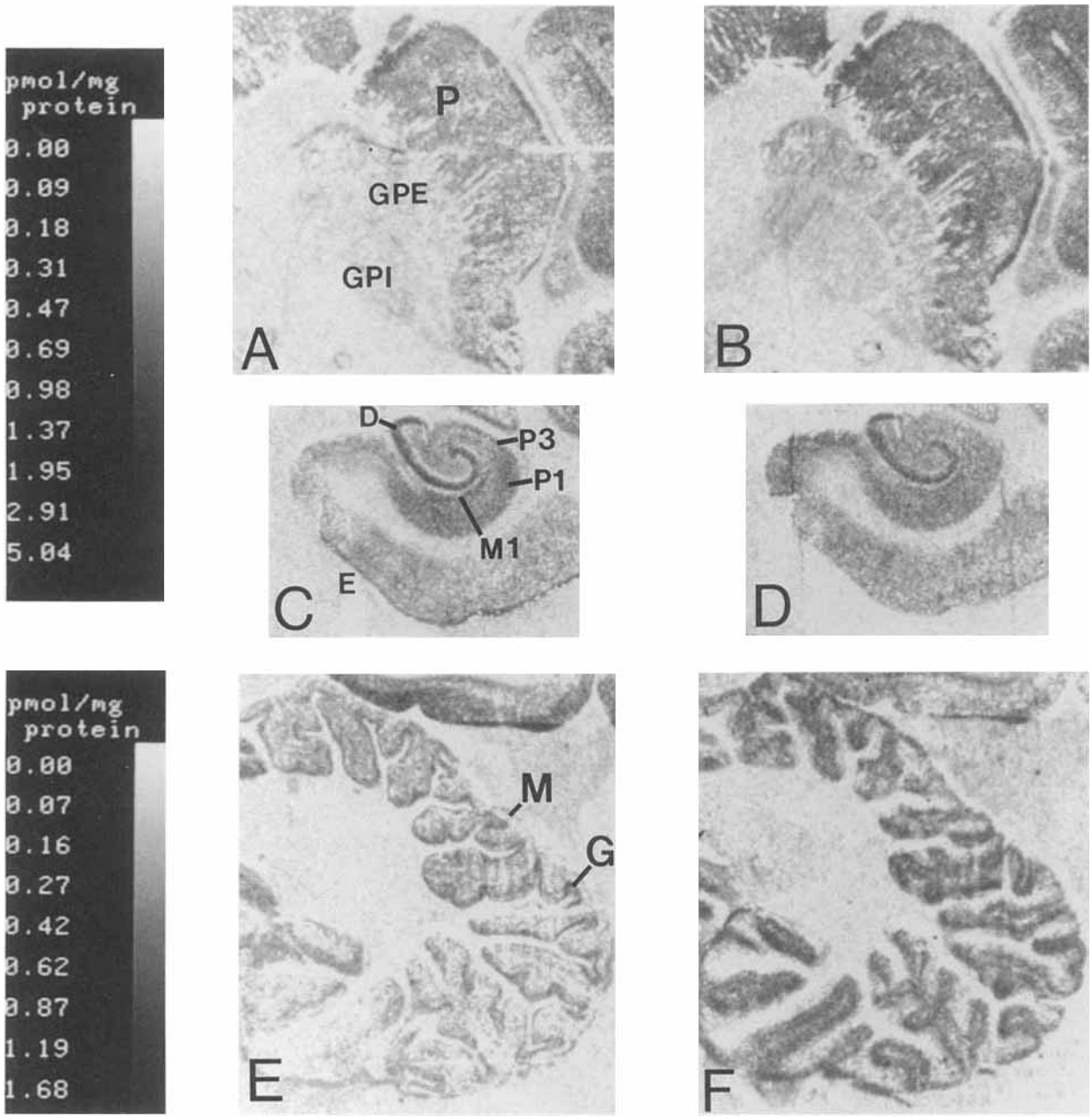

Fig. 2. Computerized images of NMDA-sensitive **(left)** and quisqualate-sensitive **(right)** binding in coronal sections of basal ganglia **(A,B),** hippocampus **(C,D),** and cerebellum **(E,F).** The images are subtraction images of specific binding and were obtained as described in Figure 1. The bars indicate the density scales for the images to the right of the bar. The same bar applies to basal ganglia and hippocampus. Abbreviations: P, putamen; GPE, external globus pallidus; GPI, internal globus pallidus; E, entorhinal cortex; D, molecular layer of dentate gyrus; M1, stratum moleculare of CA1; P1, stratum pyramidale of CA1; P3, stratum pyramidale of CA3; M, molecular layer of cerebellum; G, granule cell layer of cerebellum.

Most biochemical studies of EAA receptor subtypes have been carried out in rodent tissue where they display strikingly independent regional distributions (Young and Fagg, 1990). Quisqualate- and NMDA-sensitive [^{3}H]glutamate binding was also differentially distributed in the primate brain. Although both binding subtypes were present in each area of brain examined, in many areas one subtype predominated over the other.

NMDA receptors are gated by magnesium in a voltage-dependent fashion and thus are maximally acti-

TABLE III. Cortical NMDA-Sensitive and Quisqualate-Sensitive Binding: Outer/Inner Layers*

Cortical area	Mean outer/inner ratios	
	NMDA	Quisqualate
4	3.24	2.27
6	2.50	1.47
Supplementary motor area	2.06	1.38
8[a]	2.14	1.51
12	2.20	1.30
45	NA	1.50
46	NA	1.25
24	1.54	1.22
1&2	1.75	1.32
3	2.07	1.57
5	1.97	1.67
7	1.92	1.45
Auditory cortex	1.99	1.53
21	1.69	1.31
22	1.55	1.16
Insular cortex	1.47	1.17
Entorhinal cortex	1.57	1.24

*Ratios of values in Table 1. NA = not available.
[a]Brodmann areas.

vated in situations in which other neurotransmitters depolarize the neuron. The availability of selective NMDA receptor antagonists has made it possible to examine the role of these receptors in normal behavior. NMDA receptor blockade has been associated with disruption of long-term potentiation, a model of memory formation (Collingridge and Singer, 1990). The high density of NMDA-sensitive [^{3}H]glutamate binding in forebrain, especially hippocampus and cerebral cortex, is consistent with the proposed role these receptors have in learning and memory (Collingridge and Singer, 1990). NMDA antagonists have been found to be anticonvulsants, which is consistent with the high density of NMDA binding in cerebral cortex, the presumed site of action of anticonvulsants (Meldrum et al., 1983; Schwarcz and Meldrum, 1985).

In cerebral cortex, NMDA-sensitive binding was most dense in outer layers (layers I to III). There was an apparent zone of decreased binding in layer IV. This finding may be an artifact due to quenching of the tritium emissions by the increased white matter in this layer (Rainbow et al., 1984; Geary and Wooten, 1985; Kuhar and Unnerstall, 1985). Intermediate densities were observed in layers V and VI. In neocortical areas, except for motor cortex, a thin band of NMDA-sensitive binding was observed at what appeared to be the junction between layers IV and V. The differential distribution of these binding sites may be related to specific cortical afferent systems. Nonspecific afferents from thalamus synapse on the distal dendrites of layer III pyramidal cells and specific thalamocortical projections synapse heavily in layer IV (Brodal, 1981). Feedforward association fibers from primary sensory cortex synapse in layer IV of association cortex (Pons et al., 1987). These feedforward fibers mediate fast synaptic transmission and pyramidal cell excitation. Feedback pathways from other areas of cerebral cortex, however, synapse on layer III pyramidal dendrites and modulate neuronal activity (Pons et al., 1987). NMDA receptors are activated in an apparent voltage-dependent manner because of their voltage-dependent blockade by magnesium. Thus, the strength of these responses is likely to be modulated by the activity of other excitatory inputs. The high density of NMDA binding in outer layers of cerebral cortex and at the layer IV–V junction is consistent with the presence of NMDA modulatory function in these areas of mammalian cortex (Thomson, 1986; Addae and Stone, 1986).

In the hippocampal formation, both NMDA- and quisqualate-sensitive binding were present in high densities. This is in keeping with prior studies indicating that the entorhinal pathway to dentate gyrus and stratum pyramidale and moleculare of CA1 is EAAergic, as are the projections from dentate granule cells to CA3 neurons, from CA3 neurons to CA1 pyramidal cells, and from CA1 pyramidal cells to subiculum (Fagg and Foster, 1983; Storm-Mathisen, 1977). This series of EAAergic pathways is thought to be important in learning and memory (Collingridge and Singer, 1990). Long-term potentiation in hippocampal formation is disrupted by both quisqualate and NMDA antagonists.

The neocortical and hippocampal distribution of EAA receptors is very similar to those found by Geddes et al. (1989) who studied NMDA receptors in the baboon and Jansen et al. (1989) who studied NMDA, AMPA, and kainate binding sites in human cortex. All groups find the same hippocampal and outer cortical binding. Geddes et al. do not comment on the apparent zone of decreased binding in layer IV. Whether this is due to a species difference or a difference in the methodologies is not clear.

NMDA receptor antagonists affect motor function, resulting in decreased muscle tone and ataxia (Greenamyre, 1986; Schwarcz and Meldrum, 1985). These effects are probably due to NMDA receptor blockade in structures such as pons, spinal cord, and cerebellum. NMDA binding in these regions is low even though electrophysiological and behavioral studies indicate an important role for these receptors in motor function. Mismatches between binding densities and presynaptic neurotransmitter pathways have been noted in other systems (Kuhar, 1985). Whether some receptors are spare receptors in certain regions is unclear but striking mismatches do appear to exist in the EAA system.

Quisqualate receptors are thought to be responsible for EAA-induced fast synaptic transmission (Dingledine et al., 1988; Watkins et al., 1990). Thus, the finding that most regions of brain contain high densities of quisqualate binding was expected. Certain areas of brain contain predominantly quisqualate binding. Notably, the subthalamic nucleus, which plays an important role in motor function, has a moderate density of quisqualate-sensitive binding sites and a low density of NMDA binding. Subthalamic neurons receive an excitatory input from cerebral cortex that is mediated by quisqualate receptors (Rouzaire-Dubois and Scarnati, 1987a,b). Quisqualate-sensitive binding sites are also higher than NMDA-binding in globus pallidus and substantia nigra pars reticulata. These two regions receive presumed excitatory input from the subthalamic nucleus (Smith and Parent, 1988). Based on these findings, quisqualate antagonists would be expected to have potentially beneficial effects in the treatment of basal ganglia disorders (Klockgether and Turski, 1989). The recent development of selective quisqualate receptor antagonists will allow examination of these receptors in basal ganglia function.

In the cerebellar cortex, quisqualate-sensitive binding predominates in the molecular layer (Garthwaite and Beaumont, 1989; Olson et al., 1987; Blackstone et al., 1989; Cha et al., 1990; Kano et al., 1988). Studies of cerebellar Purkinje cell-deficient rodents indicate that quisqualate-sensitive sites are localized on Purkinje cells (Olson et al., 1987; Cha et al., 1990). This binding upregulates when deprived of granule cell afferent input. Studies in cerebellar cultures also suggest that quisqualate binding is localized predominantly on Purkinje cells (Joels et al., 1989). NMDA binding is most dense in the granule cell layer and decreases in density in rodents with a granule cell-deficient cerebellum (Olson et al., 1987). NMDA receptors may mediate afferent input from the inferior olives and certain studies suggest that this pathway uses aspartate as a neurotransmitter.

In conclusion, NMDA- and quisqualate-sensitive [^{3}H]glutamate binding sites are widely distributed in the central nervous system of *Macaca fascicularis*. The binding pattern is very similar to that seen in human and baboon brains. Areas of high binding correspond to areas of high EAA innervation. However, some areas of high EAA innervation (notably red nucleus and pons) do not have high densities of binding sites.

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