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Properties of Quisqualate-Sensitive L-[^{3}H]Glutamate Binding Sites in Rat Brain as Determined by Quantitative Autoradiography

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Abstract: Quisqualate, a glutamate analogue, displaced L-[^{3}H]glutamate binding in a biphasic manner, corresponding to "high-affinity" and "low-affinity" binding sites. High-affinity quisqualate sites were termed "quisqualate-sensitive L-[^{3}H]glutamate" binding sites. Quisqualate-sensitive L-[^{3}H]glutamate binding was regionally distributed, with the highest levels present in the cerebellar molecular layer. This binding was stimulated by millimolar concentrations of chloride and calcium. The stimulatory effects of calcium required the presence of chloride ions, whereas chloride's stimulatory effects did not require calcium. All of the L-[^{3}H]glutamate binding stimulated by chloride/calcium was quisqualate sensitive and only weakly displaced by *N*-methyl-D-aspartate, L-aspartate, or kainate. At high concentrations (1 m*M*), the anion blockers 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid and 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid both reduced, by 41 and 43%, respectively, the stimulatory effects of chloride. At concentrations of 100 μM, kynurenate, L-aspartate, (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and L-2-amino-4-phosphonobutyric acid (L-APB) failed to displace quisqualate-sensitive L-[^{3}H]-glutamate binding in the cerebellar molecular layer. In the presence of KSCN, however, 100 μM AMPA displaced 44% of binding. Quisqualate-sensitive L-[^{3}H]glutamate binding was not sensitive to freezing, and, in contrast to other chloride- and calcium-dependent L-[^{3}H]glutamate binding sites that have been reported, quisqualate-sensitive binding observed by autoradiography was enhanced at 4°C compared with 37°C. Quisqualate-sensitive L-[^{3}H]glutamate binding likely represents binding to the subclass of postsynaptic neuronal glutamate receptors known as quisqualate receptors, rather than binding to previously described APB receptors, chloride-driven sequestration into vesicles, or binding to astrocytic membrane binding sites. **Key Words:** Glutamate—Quisqualate—Receptor—Autoradiography—Chloride—Calcium. **Cha J. J. et al.** Properties of quisqualate-sensitive L-[^{3}H]glutamate binding sites in rat brain as determined by quantitative autoradiography. *J. Neurochem.* **51,** 469–478 (1988).

Glutamate is a major excitatory neurotransmitter in the mammalian CNS. Electrophysiological responses to glutamate appear to be mediated by at least three classes of receptors (Watkins and Evans, 1981). According to the most common classification, these receptors are named for the glutamate agonists that preferentially excite them: *N*-methyl-D-aspartic acid (NMDA), quisqualate, and kainate. Receptor binding studies in homogenates reveal binding sites for excitatory amino acids that appear to correspond to electrophysiologically defined receptors (Foster and Fagg, 1984). Receptor binding studies using quantitative autoradiography have also characterized binding sites for L-[^{3}H]glutamate that correspond to electrophysiologically defined receptors (Greenamyre et al., 1985*a*; Monaghan et al., 1985).

The most well-characterized class of glutamate receptor is the NMDA receptor (Cotman and Iversen,

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Abbreviations used: AMPA, (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; L-APB, L-2-amino-4-phosphonobutyric acid; DIDS, 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; ETC, ethacrynic acid; GAMS, γ-aminomethylsulfonic acid; NAAG, *N*-acetylaspartylglutamic acid; NAALADase, *N*-acetylated-α-linked acidic dipeptidase; NMDA, *N*-methyl-D-aspartic acid; SITS, 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid; Tris-Ac, Tris-acetate buffer.

1987), owing in large part to the existence of compounds that specifically block NMDA responses and interact with NMDA binding sites (Watkins and Olverman, 1987). In contrast, elucidation of the non-NMDA receptors, the quisqualate and kainate receptors, has been hampered by the lack of such compounds. The clearest evidence that separate binding sites actually do exist is that NMDA, quisqualate, and kainate binding sites have unique anatomical distributions (Monaghan et al., 1983*a*; Greenamyre et al., 1985*a*). We have taken advantage of the specific regional distribution of quisqualate-sensitive L-[^{3}H]-glutamate binding sites to study specifically this class of binding sites. In addition, manipulation of ionic conditions, particularly the absence or presence of chloride and/or calcium ions, favors binding of L-[^{3}H]glutamate to certain sites while inhibiting binding to others, making the study of different subclasses of L-[^{3}H]glutamate binding sites possible.

The nature of the subclass of glutamate receptor that mediates responses to quisqualate has not been elucidated. We report here that quisqualate-displaceable L-[^{3}H]glutamate binding is dependent on chloride and calcium ions. In addition, this chloride- and calcium-stimulated binding observed by autoradiography most likely represents binding to neuronal postsynaptic glutamate receptors and is distinct from other chloride- and calcium-stimulated binding sites that have been reported.

MATERIALS AND METHODS

Materials

L-[^{3}H]Glutamic acid (specific activity = 22–53 Ci/mmol) was obtained from Amersham (Arlington Heights, IL, U.S.A.). (*RS*)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) was obtained from Cambridge Research Biochemicals (Valley Stream, NY, U.S.A.). γ-Aminomethylsulfonic acid (GAMS) and L-2-amino-4-phosphonobutyric acid (L-APB) were obtained from Tocris Neuramin (Essex, U.K.). *N*-Acetylaspartylglutamic acid (NAAG) was obtained from Bachem (Torrance, CA, U.S.A.). Ethacrynic acid (ETC) was obtained from Merck (Rahway, NJ, U.S.A.). Quisqualic acid was a generous gift from Dr. J. Collins or obtained from Sigma (St. Louis, MO, U.S.A.). $CaCl_2$ was obtained from Fisher (Fairlawn, NJ, U.S.A.). All other compounds were purchased from Sigma.

Tissue preparation

Male Sprague-Dawley rats (weighing 175–250 g) were decapitated, and the brains were quickly removed, mounted on cryotome pedestals with Lipshaw embedding matrix, and frozen under powdered dry ice. Sections (20 μm thick) were cut on a Lipshaw cryostat and thaw-mounted onto gelatin-coated slides. Sections were either used directly or stored for <24 h at −20°C. No differences in L-[^{3}H]glutamate binding were observed in slides that were stored frozen. To remove endogenous glutamate, all sections underwent a prewash for 30 min at 2°C in either 50 m*M* Tris-HCl buffer containing 2.5 m*M* $CaCl_2$ (pH 7.20) or 50 m*M* Tris-acetate (Tris-Ac) buffer (pH 7.20). The buffer used in the prewash was always the same as that used in subsequent binding experiments, as described below. Sections were blown dry under a stream of room temperature air.

Autoradiography

A detailed description of the method for glutamate receptor autoradiography has been published (Greenamyre et al., 1984, 1985*a,b*). In brief, in competition studies, tissues were incubated for 45 min at 2°C with various competitors in the presence of 200 n*M* L-[^{3}H]glutamate (specific activity = 4.2–7.3 Ci/mmol) in a total volume of 8 ml. All solutions were adjusted to pH 7.2 with Tris base, acetic acid, or HCl before use. Nonspecific binding was defined as that binding occurring in the presence of 1 m*M* unlabeled glutamate and represented <10% of the total binding of L-[^{3}H]glutamate. In experiments examining the effects of calcium or chloride ions, these ions were added as acetate and Tris salts, respectively, and nonspecific binding was determined under each different ionic condition.

After the incubation, sections were rinsed quickly three times with cold buffer and then rinsed with cold 2.5% (vol/vol) glutaraldehyde in acetone. Sections were blown dry with warm air. The rinse/drying procedure took no more than 10 s. Dried sections were placed in x-ray cassettes with appropriate radioactive standards (Pan et al., 1983) and apposed to LKB Ultrofilm ^{3}H. The film was exposed to the tissue sections for 14–21 days at 4°C and then developed, fixed, and dried. The optical densities of the resultant film images were determined using a computer-assisted microdensitometer (Dauth et al., 1984). Sixteen to 25 readings were averaged from each area of interest in quadruplicate sections. The radioactivity was determined by a computer-generated polynomial regression analysis that compared film densities produced by the tissue sections to those produced by the radioactive standards. All data presented were analyzed densitometrically from autoradiographic images.

K_I values were generated by the nonlinear regression program LIGAND (Munson and Rodbard, 1980).

RESULTS

Quisqualate displacement of L-[^{3}H]glutamate binding

In 50 m*M* Tris-HCl containing 2.5 m*M* $CaCl_2$, quisqualate resolved two distinct binding sites for L-[^{3}H]glutamate, producing a biphasic displacement curve (Fig. 1). The K_I value for the "high-affinity" site in striatum was 26.02 ± 4.35 n*M* (mean ± SEM). The K_I value for the "low-affinity" site ranged from 26.97 ± 6.96 to 277.57 ± 53.65 μ*M*, depending on the batch of quisqualate used (Cha et al., 1987). The high-affinity site K_I values were not significantly different among different batches, using an unpaired *t* test ($p > 0.05$). K_I values in the molecular layer of cerebellum were essentially identical to those values obtained in the striatum. For the purposes of this article, "quisqualate-sensitive L-[^{3}H]glutamate binding" is defined as that binding displaced by 2.5 μ*M* quisqualate.

Regional distribution of quisqualate-sensitive L-[^{3}H]glutamate binding

Quisqualate-sensitive L-[^{3}H]glutamate was nonuniformly distributed in the rat brain (Fig. 2). Quis-

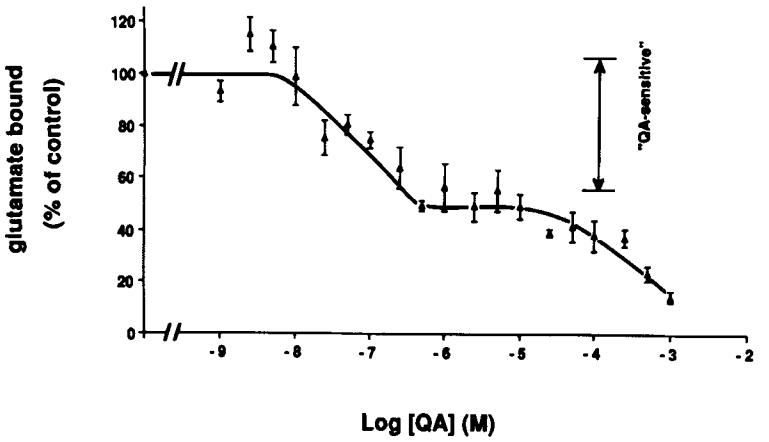

FIG. 1. Displacement of L-[^{3}H]glutamate by quisqualate (QA). Measurements were made in the striatum. Data are mean ± SEM (bars) values from four individual animals.

qualate displaced L-[^{3}H]glutamate binding from different brain areas in the following order (most binding displaced to least): molecular layer of the cerebellum > outer layers of parietal cortex, area 2 (Paxinos and Watson, 1986) > stratum moleculare of the dentate gyrus of the hippocampus, entorhinal cortex, stratum radiatum of the CA_1 region of the hippocampus > granule cell layer of the cerebellum, striatum > inner layers of parietal cortex (Fig. 3). There was minimal, but detectable, quisqualate-sensitive binding in the kidney. In addition to having the highest levels of quisqualate-sensitive L-[^{3}H]-glutamate binding of any area examined, the molecular layer of the cerebellum had the highest proportion of quisqualate-sensitive sites relative to total L-[^{3}H]-glutamate binding. In the cerebellar molecular layer, 79% of L-[^{3}H]glutamate binding was displaceable by 2.5 μM quisqualate. Thus, to study quisqualate-sensitive L-[^{3}H]glutamate binding, we concentrated on the cerebellar molecular layer, where both the absolute levels and the proportion of quisqualate-sensitive L-[^{3}H]glutamate was highest.

Effect of chloride and calcium ions on quisqualate-sensitive L-[^{3}H]glutamate binding

Chloride ions markedly stimulated L-[^{3}H]-glutamate binding in the cerebellar molecular layer (Fig. 4). Although all concentrations of chloride tested stimulated L-[^{3}H]glutamate binding, binding was stimulated more in the presence of 40 mM chloride than in the presence of 20 or 86 mM chloride. Calcium ions also displayed a biphasic effect on L-[^{3}H]glutamate binding, but only in the presence of chloride ions. Under zero chloride conditions, calcium exerted no stimulatory effect. For each concentration of chloride tested, the maximal amount of stimulation by calcium occurred at 10 mM. No stimulation of L-[^{3}H]glutamate binding occurred at concentrations of calcium <1 mM. The stimulation of L-[^{3}H]glutamate binding by chloride did not require the presence of calcium.

The stimulatory effects of chloride ions were weakly sensitive to chloride anion channel blockers (Fig. 5). In Tris-HCl, both 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS) and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) at a concentration of 1 mM significantly reduced L-[^{3}H]glutamate binding to 59 and 57% of control, respectively. ETC had no effect on binding in either Tris-Ac or Tris-HCl. None of the three anion blockers, however, even at a relatively high concentration of 1 mM, eliminated the stimulatory effect of chloride ions.

Pharmacological profile of chloride/calcium-stimulated L-[^{3}H]glutamate binding

The increased L-[^{3}H]glutamate binding observed in the presence of chloride and calcium ions was displaceable by quisqualate. In the cerebellar molecular layer, there was no increase in binding in Tris-HCl buffer with 2.5 mM $CaCl_2$ (final chloride concentration = 45 mM) compared with Tris-Ac buffer if 2.5 μM quisqualate was included in each condition (Fig. 6). Thus, all the chloride- and calcium-stimulated L-[^{3}H]glutamate binding appeared to be sensitive to quisqualate. Similarly, 2.5 μM quisqualate abolished the stimulatory effects of chloride and calcium in the granule cell layer of the cerebellum and in the stratum moleculare of the dentate gyrus and stratum radiatum of the CA_1 region of the hippocampus (Table 1). Conversely, no chloride or calcium enhancement of quisqualate-insensitive L-[^{3}H]glutamate binding was observed.

Although the chloride/calcium-stimulated binding was fully displaceable by quisqualate, other glutamate compounds were far less effective. Whereas 2.5 μM quisqualate displaced >83% of L-[^{3}H]glutamate in the cerebellar molecular layer, NMDA (at a concentration of 100 μM), L-aspartate (10 μM), and kainate (10 μM) all failed to displace >15% of the binding, a result reflecting the predominance of quisqualate-sensitive binding sites in this anatomical structure. In other areas where the proportion of quisqualate-insensitive L-[^{3}H]glutamate binding was higher, NMDA and L-aspartate were more effective displacers. In the presence of calcium and chloride

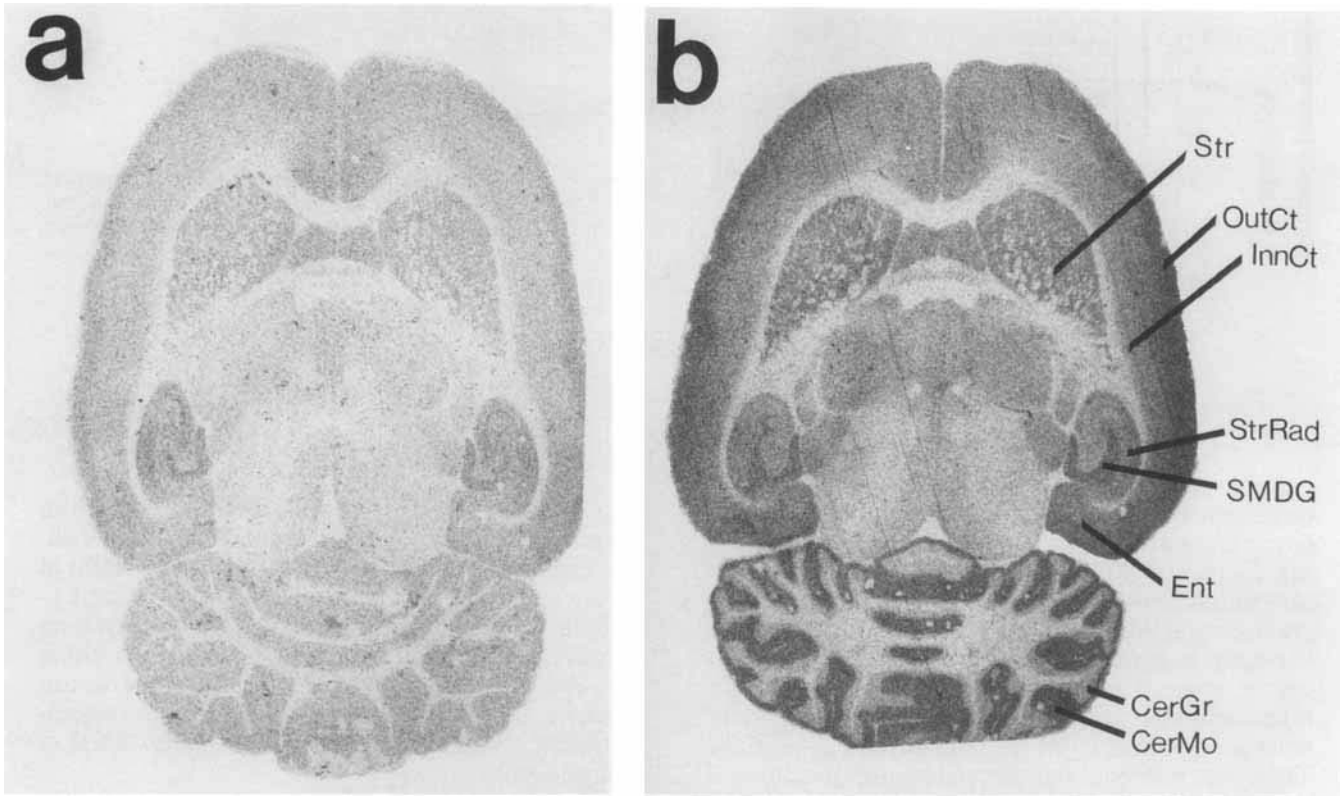

FIG. 2. Autoradiograms of L-[^{3}H]glutamate binding sites in rat brain. Both sections were incubated with L-[^{3}H]glutamate in the presence of 100 μM NMDA: (**a**) in Tris-Ac and (**b**) in Tris-HCl plus 2.5 mM $CaCl_2$. In (a), some of the L-[^{3}H]glutamate binding is to kainate sites, which are inhibited by Ca^{2+} and thus are not labeled in (b). In (b), chloride- and calcium-stimulated binding is nonuniformly distributed. As noted in the text, all chloride/calcium-stimulated binding is quisqualate sensitive. Str, striatum; OutCt, outerlayers of parietal cortex; InnCt, inner layers of parietal cortex; StrRad, stratum radiatum; SMDG, stratum moleculare of the dentate gyrus; Ent, entorhinal cortex; CerGr, granule cell layer of the cerebellum; CerMo, molecular layer of the cerebellum.

ions, kainate displaced <13% of the binding in all areas tested.

When tested at 100 μM, L-aspartate, kynurenate, GAMS, and L-APB failed to displace any L-[^{3}H]-glutamate binding from the cerebellar molecular layer (Fig. 7). Essentially the same results were obtained if the competition experiment was conducted in the presence of 1 mM unlabeled NMDA (data not shown). NMDA itself at 100 μM displaced small but significant amounts of binding. L-Serine-*O*-sulfate and the dipeptide NAAG displaced 61 and 45%, respectively, of binding at 100 μM. AMPA (100 μM) displaced only small amounts of binding in Tris-HCl with 2.5 mM $CaCl_2$, but in the presence of 100 mM KSCN, AMPA became an effective displacer (Fig. 8) (Nielsen et al., manuscript in preparation). KSCN had no effect by itself on the overall binding of L-[^{3}H]glutamate.

Quisqualate-sensitive L-[^{3}H]glutamate binding at 37 versus 4°C

Autoradiographic images obtained from tissue sections that had been incubated with L-[^{3}H]glutamate at 37°C were qualitatively similar to those obtained from sections that had been incubated at 4°C. Tissue sections incubated at 4°C exhibited 59% more ($p < 0.02$ by unpaired t test) specific binding in all regions examined than sections incubated at 37°C (data not shown). There was a small increase in the amount of nonspecific binding observed with an increase in temperature.

DISCUSSION

Quisqualate receptors are believed to mediate fast synaptic transmission at excitatory synapses (Crunelli et al., 1983; Jahr and Jessell, 1985; Nelson et al., 1986). Quisqualate-activated channels are voltage independent and selective for monovalent cations (MacDonald and Porietis, 1982). Patch clamp experiments have demonstrated that quisqualate preferentially caused small conductance (<20 pS) channel openings (Cull-Candy and Usowicz, 1987; Jahr and Stevens, 1987). In addition to its ability to depolarize neuronal membranes, quisqualate has also been shown to elevate cyclic GMP production (McCaslin and Morgan, 1987) and to stimulate phosphoinositide metabolism (Sladeczek et al., 1985; Nicoletti et al., 1986). Whether all of these actions are mediated by a single type of quisqualate receptor is not known.

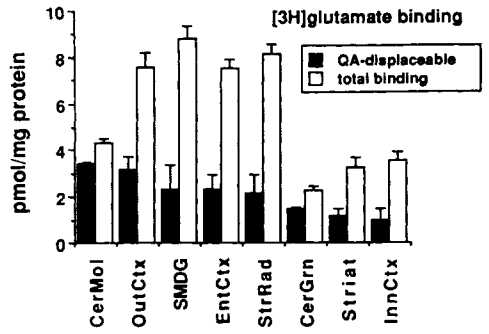

FIG. 3. Regional distribution of quisqualate (QA)-sensitive L-[^{3}H]-glutamate binding sites. Solid columns represent the amount of L-[^{3}H]glutamate binding displaced by 2.5 μM quisqualate [mean ± SEM (bars)]. Open columns represent the total amount of L-[^{3}H]glutamate binding present in Tris-HCl plus 2.5 mM $CaCl_2$. CerMol, molecular layer of the cerebellum; OutCtx, outer layers of parietal cortex; SMDG, stratum moleculare of the dentate gyrus; EntCtx, entorhinal cortex; StrRad, stratum radiatum; CerGrn, granule cell layer of the cerebellum; Striat, striatum; InnCtx, inner layers of parietal cortex.

Functionally, quisqualate receptors have been implicated in a form of synaptic plasticity (Kano and Kato, 1987) and may also mediate neurotoxic events (Silverstein et al., 1986). Injections of quisqualate cause seizures in the rat hippocampus (Fukuda et al., 1985), and the putative kainate/quisqualate receptor antagonist GAMS prevents seizures in mice (Croucher et al., 1984). In Alzheimer's brains, quisqualate receptors appear to be one of the glutamate receptor subtypes, determined by autoradiographic receptor binding, that is decreased in number in the hippocampus (Greenamyre et al., 1987*a*).

Unlabeled glutamate competed for L-[^{3}H]-glutamate binding, as measured by quantitative autoradiography, in a monophasic fashion, consistent with the possibility that glutamate interacts with a single class of receptors (Greenamyre et al., 1983, 1984, 1985*a*; Halpain et al., 1983). The displacement curve produced by quisqualate, on the other hand, confirms that at least two binding sites for L-[^{3}H]-glutamate exist that differ in their affinity for quisqualate. The low-affinity component of the quisqualate displacement curve likely represents binding to NMDA receptors, because drugs that interact with the NMDA receptor compete for this binding, and the proportion of this site decreases if unlabeled NMDA is included in the incubation medium (Greenamyre et al., 1985*a*; Foster and Fagg, 1987). The fact that high concentrations of quisqualate can compete for this binding could be explained in two ways. The first is that at high concentrations, quisqualate is a nonselective NMDA agonist and, thereby, acts directly at NMDA receptors. Alternatively, binding to NMDA receptors could be displaced by free glutamate, which may appear as a contaminant of commercially available quisqualate (Cha et al., 1987). The level of contamination of quisqualate, aspartate, or both is typically <1%. Thus, at a concentration of 2.5 μM quisqualate, the combined contaminant level of glutamate/aspartate would not be expected to exceed 25 nM, ~1/10th the K_D of glutamate. It is, therefore, unlikely that contamination significantly affects quisqualate-sensitive binding.

Non-NMDA receptors comprise both quisqualate and kainate receptors. Quisqualate binds to both these receptor subclasses, whereas kainate interacts preferentially with the kainate subclass of receptors (Greenamyre et al., 1985*a*). Quisqualate-sensitive sites are far more numerous (at pmol/mg of protein) than are binding sites for [^{3}H]kainate (fmol/mg of protein) (Beaumont et al., 1979). Glutamate binding to kainate receptors is inhibited by calcium ions (Monaghan et al., 1983*a*; Greenamyre et al., 1985*a*). Thus, most chloride/calcium-stimulated quisqualate-sensitive binding represents binding to quisqualate receptors. This interpretation is borne out by the observation that NMDA and kainate are weak dis-

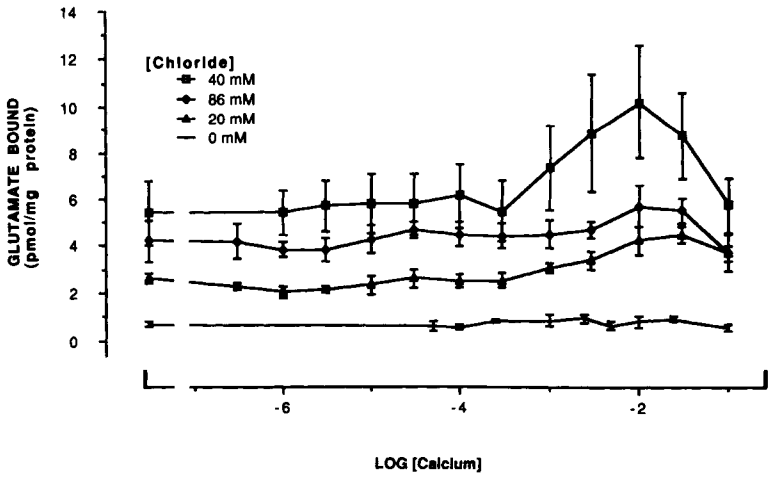

FIG. 4. Effects of chloride and calcium ions on L-[^{3}H]glutamate binding in the molecular layer of the cerebellum. Data are mean ± SEM (bars) values for specific binding from four individual animals.

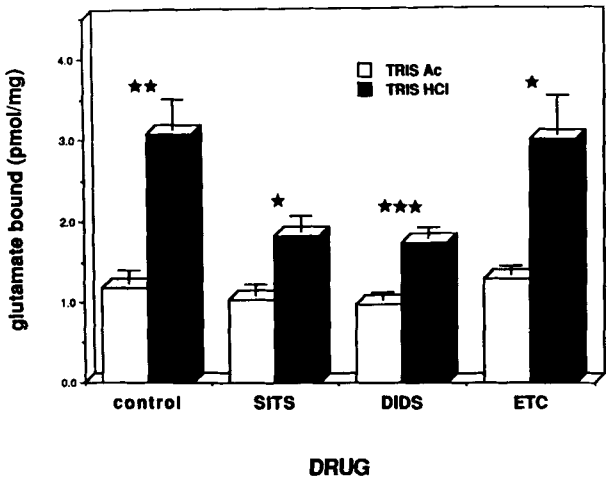

FIG. 5. Effects of anion channel blockers on chloride stimulation of L-[^{3}H]glutamate binding sites. Each drug was tested at 1 m*M*. Data are mean ± SEM (bars) values from four individual animals. Significant differences between buffer conditions are indicated: *p < 0.05, **p < 0.01, ***p < 0.005. SITS and DIDS inhibited the stimulation of binding by chloride (p < 0.02 by one-way analysis of variance).

placers of binding to the high-affinity quisqualate site.

That GAMS and AMPA, compounds that are believed to interact with the quisqualate receptor based on electrophysiological evidence (Krogsgaard-Larsen et al., 1980; Davies and Watkins, 1985), were weak displacers of L-[^{3}H]glutamate binding to the high-affinity quisqualate site was unexpected. At the relatively high concentration of 100 μM, NAAG displaced nearly 50% of quisqualate-sensitive binding, a finding that may reflect a low grade of contamination of NAAG by free glutamate.

The ability of AMPA to displace quisqualate-sensitive L-[^{3}H]glutamate binding was greatly increased by addition of KSCN. KSCN did not affect specific glutamate binding or high-affinity quisqualate displacement thereof. It is of interest to note that KSCN has been reported to increase the binding of [^{3}H]AMPA to rat brain cortical membranes (Honoré and Nielsen, 1985; Murphy et al., 1987). Although the mechanism of action by which KSCN increases AMPA binding is not known with certainty, it may increase the affinity of a low-affinity AMPA sensitive site.

Quisqualate-sensitive L-[^{3}H]glutamate binding sites were found to be widely distributed in the rat brain and were examined in detail in striatum, hippocampus, and cerebellum. In all areas examined in detail, quisqualate-sensitive glutamate binding had a very similar affinity for quisqualate and displayed an identical pharmacological profile and sensitivity to stimulation by ions. Notable concentrations of quisqualate-sensitive L-[^{3}H]glutamate binding sites were found in the cerebellum, cortex, striatum, and hippocampus, structures that are believed to possess extensive glutamatergic circuitry. For example, excitatory amino acids are the putative transmitters of cortical association fibers (Giuffrida and Rustioni, 1987), corticofugal pathways (Young et al., 1981), mossy fiber afferents to cerebellum (Beitz et al., 1986), and the cerebellar parallel fiber system (Young et al., 1974). It is of interest to note that the distribution of quisqualate-sensitive L-[^{3}H]glutamate binding sites was anatomically different from the distribution of kainate and NMDA binding sites (Greenamyre et al., 1985*a*; Maragos et al., 1986). It has recently been suggested that NMDA, quisqualate, and kainate receptors may share a common ion channel (Cull-Candy and Usowicz, 1987; Jahr and Stevens, 1987). Because each of the receptors has a unique anatomical distribution, however, they may not share a common ion channel in all areas. Or, conceivably, the stoichiometry of the multireceptor-ion channel complex may vary in different areas.

An interesting aspect of the binding described in this report is that there is apparently more binding at 4°C than at 37°C. The mechanism of enhanced binding at 4°C remains to be elucidated. The increased binding could simply reflect a slower dissociation rate at 4°C. After a 37°C incubation, [^{3}H]glutamate has a $t_{1/2}$ of dissociation of 0.38 min (Greenamyre et al., 1984). Thus, at a given constant rinse time, there would be more apparent binding when the assay and rinse were performed at 4°C, presumably due to a slowing of the dissociation rate. Regardless of the mechanism, it is clear that binding is not reduced at 4°C, in contradistinction to other chloride-dependent glutamate binding sites (see below).

The quisqualate-sensitive binding site versus other L-[^{3}H]glutamate binding sites

In the adult rat, all of the binding stimulated by addition of chloride and calcium ions was displace-

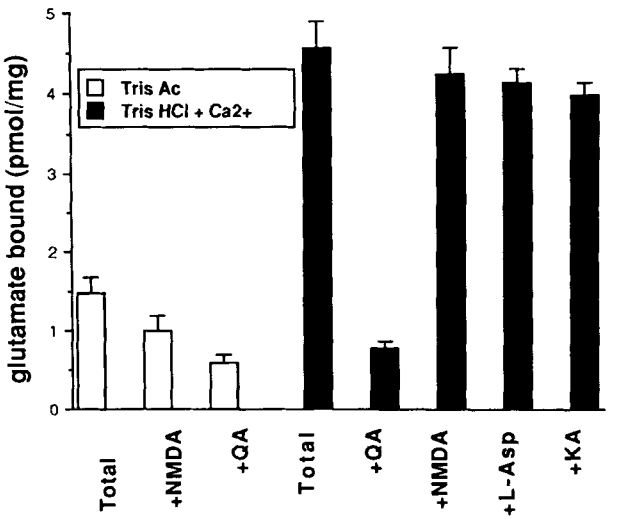

FIG. 6. Chloride and calcium stimulation of L-[^{3}H]glutamate binding sites in the molecular layer of the cerebellum. Data are mean ± SEM (bars) values from four individual animals. KA, kainic acid; QA, quisqualic acid.

TABLE 1. *Pharmacological profile of chloride/calcium-stimulated L-[^{3}H]glutamate binding*

Buffer condition	Cerebellar		Stratum radiatum of CA_1	Stratum moleculare of dentate gyrus
	Molecular layer	Granule cell layer		
Tris-Ac				
Total	1.47 ± 0.20	1.53 ± 0.16	6.54 ± 1.05	7.12 ± 0.57
+2.5 μ*M* QA	0.58 ± 0.11 (39.5%)	1.08 ± 0.07 (70.6%)	5.53 ± 0.60 (84.6%)	5.44 ± 0.40 (76.4%)
Tris-HCl + 2.5 m*M* $CaCl_2$				
Total	4.57 ± 0.31	2.19 ± 0.26	7.55 ± 0.29	8.93 ± 0.32
+2.5 μ*M* QA	0.78 ± 0.09 (17.1%)	0.99 ± 0.13 (45.2%)	5.10 ± 0.33 (67.5%)	6.05 ± 0.11 (67.7%)
+100 μ*M* NMDA	4.24 ± 0.33 (92.3%)	1.37 ± 0.09 (62.6%)	4.30 ± 0.16 (57.0%)	5.57 ± 0.47 (62.4%)
+10 μ*M* L-Asp	4.14 ± 0.17 (90.6%)	1.75 ± 0.06 (79.9%)	5.73 ± 0.22 (75.9%)	7.56 ± 0.37 (84.7%)
+10 μ*M* KA	3.98 ± 0.15 (87.1%)	1.98 ± 0.16 (90.4%)	6.62 ± 0.44 (87.7%)	7.85 ± 0.44 (87.9%)

Data are mean ± SEM values (n = 4), in pmol of glutamate bound/mg of protein. Percentages reflect the amount of binding remaining in the presence of the displacer in question relative to the total binding for the buffer condition used. KA, kainic aid; QA, quisqualic acid.

able by quisqualate, with a K_I value in the low nanomolar range. In the brains of neonatal rats, there exists a component of L-[^{3}H]glutamate binding that is displaceable by neither quisqualate nor NMDA (Greenamyre et al., 1987*b*). A similar quisqualate- and NMDA-insensitive chloride/calcium-stimulated L-[^{3}H]glutamate binding site may exist in human brain (Debowey et al., 1987). Several other chloride-dependent L-[^{3}H]glutamate binding sites have been reported (Table 2). In general, binding of L-[^{3}H]-glutamate to these sites is also enhanced by calcium ions (Table 2).

APB site. APB is believed to interact with a subpopulation of glutamate receptors that may be presynaptically localized (Cotman et al., 1986). Binding of [^{3}H]APB is increased by chloride and calcium in synaptic plasma membranes (Monaghan et al., 1983*b*; Robinson et al., 1985). The K_I of quisqualate in inhibiting [^{3}H]APB binding is 260 (Robinson et al., 1985) or 380 n*M* (Monaghan et al., 1983*b*), at least 10-fold higher than the K_I for quisqualate-sensitive binding in autoradiography studies. The APB site, however, has yet to be visualized in an autoradiographic binding assay, owing to its susceptibility to freezing (Fagg et al., 1983). The quisqualate-sensitive L-[^{3}H]glutamate binding site presented here is studied on brain sections that were previously frozen. Indeed, APB itself did not compete for L-[^{3}H]glutamate binding in the present preparation. Finally, the ability of [^{3}H]APB to bind to synaptic plasma membranes is greatly reduced at 4°C compared with binding at 37°C (Monaghan et al., 1983*b*), whereas in the present study, there is an increase in binding at 4°C. In spinal cord synaptic membranes, two populations of chloride-dependent glutamate binding sites are observed, one of which is sensitive to APB and a second of which is insensitive to APB (Mena et al., 1986). Despite similarities in dependence on chloride and calcium, the quisqualate-sensitive L-[^{3}H]-glutamate binding site differs from the APB site in affinity for quisqualate, sensitivity to temperature, sensitivity to displacement by APB, and susceptibility to freezing. Thus, they likely represent two separate binding sites.

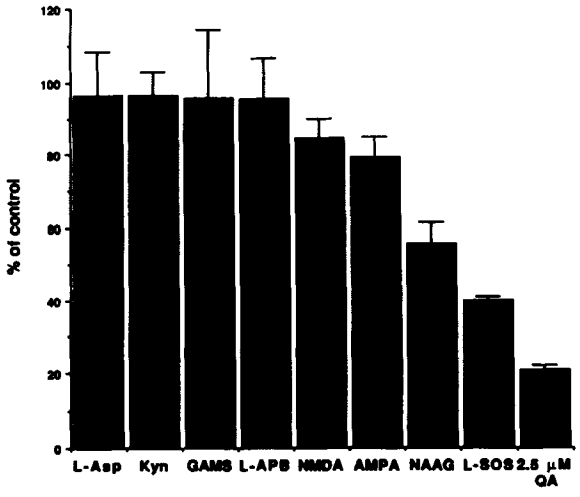

FIG. 7. Effects of glutamate compounds (100 μ*M*) on L-[^{3}H]-glutamate binding in the cerebellar molecular layer in the presence of chloride and calcium ions. Data are mean ± SEM (bars) values from four individual animals. Kyn, kynurenate; L-SOS, L-serine-*O*-sulfate; QA, quisqualic acid.

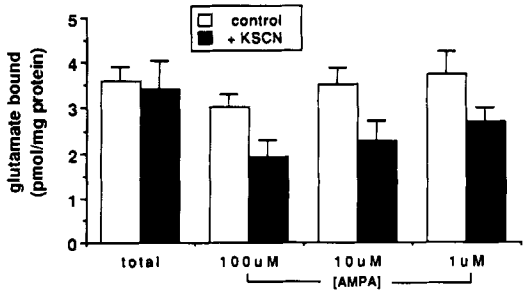

FIG. 8. Displacement of L-[^{3}H]glutamate by AMPA in the presence and absence of 100 m*M* KSCN. Data are mean ± SEM (bars) values from four individual animals.

Chloride-dependent uptake site. Sodium-independent, chloride-dependent uptake of L-[^{3}H]glutamate into resealed membrane vesicles formed during homogenization has been demonstrated in homogenate preparations (Pin et al., 1984; Kessler et al., 1987; Recasens et al., 1987; Zaczek et al., 1987). This uptake of L-[^{3}H]glutamate has been shown to be further enhanced by calcium ions. The K_I values for quisqualate, however, are in the micromolar range, in contradistinction to the nanomolar K_I values obtained in the present studies. Vesicular uptake is greatly reduced at 4°C vs. 37°C, unlike quisqualate-sensitive L-[^{3}H]glutamate binding. It is unlikely that the autoradiographic binding assay used here results in significant vesicle formation compared with a homogenization procedure used for preparing synaptic plasma membranes, although this was not directly examined. The anion channel blocker DIDS blocks chloride-dependent uptake with a K_I of 15 μM (Recasens et al., 1987), whereas 1 mM DIDS reduced quisqualate-sensitive binding by only 41%. Finally, unlike high-affinity quisqualate-sensitive binding, chloride-dependent L-[^{3}H]glutamate sequestration is sensitive to APB [and may, in fact, represent the same component of binding as does the APB site (Fagg and Lanthorn, 1985)].

Astrocytic membrane binding site. Bridges et al. (1987) have reported a L-[^{3}H]glutamate binding site in astrocyte membranes that is chloride dependent, insensitive to freezing, and not displaced by APB. Glutamate binding to this site is not enhanced by calcium. It is unlikely that this astrocytic site is the same as the quisqualate-sensitive site, because the K_I value of quisqualate for the astrocytic site is ~100 μM. Furthermore, the binding of L-[^{3}H]glutamate to astrocyte membranes is greatly reduced at 4°C compared with 37°C. The binding of L-[^{3}H]glutamate to astrocytic membranes is markedly more sensitive to SITS and DIDS (K_I = 15 μM) than is quisqualate-sensitive binding (K_I > 1 mM). Other findings argue against the possibility that quisqualate-sensitive L-[^{3}H]glutamate binding is due to astrocytic binding. Brain areas with gliosis demonstrate reduced, rather than enhanced, L-[^{3}H]glutamate binding in autoradiography (Greenamyre et al., 1985*b*; Olson et al., 1987). Experiments with cerebellar mutant mice have suggested that the quisqualate-sensitive L-[^{3}H]-glutamate binding sites are located on Purkinje neurons (Olson et al., 1987). Quisqualate produces neurotoxic lesions in cerebellar slices, an observation substantiating this proposed neuronal location of quisqualate receptors (Garthwaite and Garthwaite, 1984; Garthwaite et al., 1986).

Enzymatic site. Robinson et al. (1986, 1987) have reported an *N*-acetylated-α-linked acidic dipeptidase (NAALADase) activity in rat brain that is believed to mediate cleavage of the dipeptide NAAG. The activity of NAALADase is stimulated by chloride ions, enhanced by calcium ions, and survives freezing. The most potent inhibitor of NAALADase activity yet reported is quisqualate, with a K_I value of 480 nM. NAALADase is slightly inhibited by APB. Its activity is greatly reduced at 4°C, however, and the regional distribution of NAALADase activity is quite disparate from that of quisqualate-sensitive L-[^{3}H]-glutamate binding. In particular, of all tissues tested, kidney possesses the highest levels of NAALADase activity, whereas by autoradiographic assay, the kidney demonstrates miniscule levels of quisqualate-sensitive L-[^{3}H]glutamate binding.

Calcium-dependent protease-increased site. Calcium has been reported to activate a protease that, when activated, can increase the number of glutamate binding sites (Baudry and Lynch, 1980, 1984). The binding sites that are recruited after calpain activation appear to be different from the quisqualate-sensitive sites. The distribution of calpain activity in the brain is markedly different from that of quisqualate-sensitive L-[^{3}H]glutamate binding sites, with a notable absence of calpain activity in the cerebellum.

TABLE 2. *Characteristics of chloride-dependent glutamate binding processes*

	APB-sensitive binding[a–c]	Vesicle uptake[d–g]	Astrocytic membrane binding[h]	NAALADase activity[i,j]	QA-sensitive autoradiographic binding
Cl^- dependent?	Yes	Yes	Yes	Yes	Yes
Ca^{2+} enhanced?	Yes[b,c]	Yes	No	Yes	Yes
Sensitive to freezing?	Yes	?	No	No	No
APB sensitive?	Yes	Yes[e,f]	No	Slightly	No
Activity at 4 vs. 37°C	Greatly reduced[a]	Greatly reduced	Reduced to 17%	Reduced to <10%	Enhanced
K_I for QA	380 nM[a] 260 nM[c]	Micromolar	~100 μM	480 nM	14–30 nM

QA, quisqualic acid.
[a] Monaghan et al. (1983*b*).
[b] Fagg et al. (1983).
[c] Robinson et al. (1985).
[d] Pin et al. (1984).
[e] Bridges et al. (1986).
[f] Zaczek et al. (1987).
[g] Kessler et al. (1987).
[h] Bridges et al. (1987).
[i] Robinson et al. (1986).
[j] Robinson et al. (1987).

The EC_{50} for calcium ions in stimulating new receptor sites is 30 μM, compared with a millimolar concentration of calcium required to influence quisqualate-sensitive sites. Finally, the binding of L-[^{3}H]-glutamate to binding sites uncovered by calpain in the presence of calcium is markedly sensitive to low temperatures, and calcium itself has no stimulatory effect at 4°C.

In summary, the autoradiographic quisqualate-sensitive L-[^{3}H]glutamate binding site differs from all of the glutamate binding sites described above, in that it has the highest affinity for quisqualate. The binding detected by autoradiography occurs readily at 4°C, a temperature at which enzymatic and transport processes are likely to be retarded. This quisqualate-sensitive binding is not sensitive to freezing or displacement by APB and is displaced by AMPA in the presence of KSCN. The distribution of these binding sites coincides with hypothesized glutamatergic pathways (Fagg and Foster, 1983).

The most likely localization of these binding sites is on postsynaptic neuronal membranes (Olson et al., 1987). Quisqualate-sensitive glutamate binding has been localized to postsynaptic densities (Fagg and Matus, 1984) and synaptosomal membranes (Quinn and Spraguer, 1986). These findings are in agreement with the hypothesis that quisqualate receptors mediating neurotoxic events are located postsynaptically (Garthwaite and Garthwaite, 1984; Garthwaite et al., 1986). In short, quisqualate-sensitive L-[^{3}H]-glutamate binding most likely represents binding to the physiologically defined quisqualate receptor on postsynaptic neuronal membranes.

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REFERENCES

Baudry M. and Lynch G. (1980) Regulation of hippocampal glutamate receptors: evidence for the involvement of a calcium-activated protease. *Proc. Natl. Acad. Sci. USA* **77,** 2298–2302.

Baudry M. and Lynch G. (1984) The biochemistry of memory: a new and specific hypothesis. *Science* **224,** 1057–1063.

Beaumont K., Maurin Y., Reisine T., Fields J. Z., Spokes E., Bird E. D., and Yamamura H. I. (1979) Huntington's disease and its animal model: alterations in kainic acid binding. *Life Sci.* **24,** 809–816.

Beitz A. J., Larson A. A., Monaghan P. J., Altshuler R. A., Mullet M. A., and Madl J. E. (1986) Immunohistochemical localization of glutamate, glutaminase and aspartate aminotransferase in neurons of the pontine nuclei of the rat. *Neuroscience* **17,** 741–753.

Bridges R. J., Hearn T. J., Monaghan D. T., and Cotman C. W. (1986) A comparison of 2-amino-4-phosphonobutyric acid (AP4) receptors and [^{3}H]AP4 binding sites in the rat brain. *Brain Res.* **375,** 204–209.

Bridges R. J., Nieto-Sampedro M., Kadri M., and Cotman C. W. (1987) A novel chloride-dependent L-[^{3}H]glutamate binding site in astrocyte membranes. *J. Neurochem.* **48,** 1709–1715.

Cha J. J., Hollingsworth Z., Greenamyre J. T., Collins J., Penney J. B., and Young A. B. (1987) Possible contamination of quisqualic acid by naturally occurring glutamic acid detected by quantitative autoradiographic binding assay and HPLC analysis. (Abstr) *Soc. Neurosci. Abstr.* **13,** 1564.

Cotman C. W. and Iversen L. L. (1987) Excitatory amino acids in the brain—focus on NMDA receptors. *Trends Neurosci.* **10,** 263–265.

Cotman C. W., Flatman J. A., Ganong A. H., and Perkins M. N. (1986) Effects of excitatory amino acid antagonists on evoked and spontaneous excitatory potentials in guinea-pig hippocampus. *J. Physiol. (Lond.)* **378,** 403–415.

Croucher M. J., Meldrum B. S., Jones A. W., and Watkins J. C. (1984) γ-D-Glutamylaminomethylsulphonic acid (GAMS), a kainate and quisqualate antagonist, prevents sound-induced seizures in DBA/2 mice. *Brain Res.* **322,** 111–114.

Crunelli V., Forda S., and Kelly J. S. (1983) Blockade of amino acid-induced depolarizations and inhibition of excitatory post-synaptic potentials in rat dentate gyrus. *J. Physiol. (Lond.)* **341,** 627–640.

Cull-Candy S. G. and Usowicz M. M. (1987) Multiple-conductance channels activated by excitatory amino acids in cerebellar neurons. *Nature* **325,** 525–528.

Dauth G. W., Frey K. A., and Gilman S. (1984) A densitometer for quantitative autoradiography. *J. Neurosci. Methods* **9,** 241–253.

Davies J. and Watkins J. C. (1985) Depressant actions of γ-D-glutamylaminomethyl sulfonate (GAMS) on amino acid-induced and synaptic excitation in the cat spinal cord. *Brain Res.* **327,** 113–120.

Debowey D. L., Maragos W. F., Hollingsworth Z. H., Greenamyre J. T., Young A. B., and Penney J. B. (1987) Receptor changes in hippocampus of Alzheimer's disease. (Abstr) *Soc. Neurosci. Abstr.* **13,** 439.

Fagg G. E. and Foster A. C. (1983) Amino acid neurotransmitters and their pathways in the mammalian central nervous system. *Neuroscience* **9,** 701–719.

Fagg G. E. and Lanthorn T. H. (1985) Cl^-/Ca^{2+}-dependent L-glutamate binding sites do not correspond to 2-amino-4-phosphonobutanoate-sensitive excitatory amino acid receptors. *Br. J. Pharmacol.* **86,** 743–751.

Fagg G. E. and Matus A. (1984) Selective association of *N*-methyl aspartate and quisqualate types of L-glutamate receptor with brain postsynaptic densities. *Proc. Natl. Acad. Sci. USA* **81,** 6876–6880.

Fagg G. E., Mena E. E., Monaghan D. T., and Cotman C. W. (1983) Freezing eliminates a specific population of L-glutamate receptors in synaptic membranes. *Neurosci. Lett.* **38,** 157–162.

Foster A. C. and Fagg G. E. (1984) Acidic amino acid binding sites in mammalian neuronal membranes: their characteristics and relationship to synaptic receptors. *Brain Res. Rev.* **7,** 103–164.

Foster A. C. and Fagg G. E. (1987) Comparison of L-[^{3}H]-glutamate, D-[^{3}H]aspartate, DL-[^{3}H]AP5 and [^{3}H]NMDA as ligands for NMDA receptors in crude postsynaptic densities from rat brain. *Eur. J. Pharmacol.* **133,** 291–300.

Fukuda H., Tanaka T., Kajima M., Nakai H., and Yonemasu Y. (1985) Quisqualic acid-induced hippocampal seizures in unanesthetized cats. *Neurosci. Lett.* **59,** 53–59.

Garthwaite G. and Garthwaite J. (1984) Differential sensitivity of rat cerebellar cells in vitro to the neurotoxic effects of excitatory amino acid analogues. *Neurosci. Lett.* **48,** 361–367.

Garthwaite J., Garthwaite G., and Hajós F. (1986) Amino acid neurotoxicity: relationship to neuronal depolarization in rat cerebellar slices. *Neuroscience* **18,** 449–460.

Giuffrida R. and Rustioni A. (1987) Glutamate immunoreactivity in cortico-cortical and corticofugal neurons of rats. (Abstr) *Soc. Neurosci. Abstr.* **13,** 1562.

Greenamyre J. T., Young A. B., and Penney J. B. (1983) Quantitative autoradiography of L-[^{3}H]glutamate binding to rat brain. *Neurosci. Lett.* **37,** 155–160.

Greenamyre J. T., Young A. B., and Penney J. B. (1984) Quantitative autoradiographic distribution of L-[^{3}H]glutamate-binding sites in rat central nervous system. *J. Neurosci.* **4,** 2133–2144.

Greenamyre J. T., Olson J. M. M., Penney J. B., and Young A. B. (1985*a*) Autoradiographic characterization of *N*-methyl-D-aspartate-, quisqualate- and kainate-sensitive glutamate binding sites. *J. Pharmacol. Exp. Ther.* **233,** 254–263.

Greenamyre J. T., Penney J. B., Young A. B., D'Amato C. J., Hicks S. P., and Shoulson I. (1985*b*) Alterations in L-glutamate binding in Alzheimer's and Huntington's diseases. *Science* **227,** 1496–1499.

Greenamyre J. T., Penney J. B., D'Amato C. J., and Young A. B. (1987*a*) Dementia of the Alzheimer's type: changes in hippocampal L-[^{3}H]glutamate binding. *J. Neurochem.* **48,** 543–551.

Greenamyre T., Penney J. B., Young A. B., Hudson C., Silverstein F. S., and Johnston M. V. (1987*b*) Evidence for transient perinatal glutamatergic innervation of globus pallidus. *J. Neurosci.* **7,** 1022–1030.

Halpain S., Parsons B., and Rainbow T. C. (1983) Tritium-film autoradiography of sodium-independent glutamate binding sites in rat brain. *Eur. J. Pharmacol.* **86,** 313–314.

Honoré T. and Nielsen M. (1985) Complex structure of quisqualate-sensitive glutamate receptors in rat cortex. *Neurosci. Lett.* **54,** 27–32.

Jahr C. E. and Jessell T. M. (1985) Synaptic transmission between dorsal root ganglion and dorsal horn neurons in culture: antagonism of monosynaptic excitatory postsynaptic potentials and glutamate excitation by kynurenate. *J. Neurosci.* **5,** 2281–2289.

Jahr C. E. and Stevens C. F. (1987) Glutamate activates multiple single channel conductances in hippocampal neurons. *Nature* **325,** 522–525.

Kano M. and Kato M. (1987) Quisqualate receptors are specifically involved in cerebellar synaptic plasticity. *Nature* **325,** 276–279.

Kessler M., Petersen G., Vu H. M., Baudry M., and Lynch G. (1987) L-Phenylalanyl-L-glutamate-stimulated, chloride-dependent glutamate binding represents glutamate sequestration mediated by an exchange system. *J. Neurochem.* **48,** 1191–1200.

Krogsgaard-Larsen P., Honoré T., Hansen J. J., Curtis D. R., and Lodge D. (1980) New class of glutamate agonist structurally related to ibotenic acid. *Nature* **285,** 64–66.

MacDonald J. F. and Porietis A. V. (1982) DL-Quisqualic and L-aspartic acids activate separate excitatory conductances in cultured spinal cord neurons. *Brain Res.* **245,** 175–178.

Maragos W. F., Chu D. C. M., Greenamyre J. T., Penney J. B., and Young A. B. (1986) High correlation between the localization of [^{3}H]TCP binding and NMDA receptors. *Eur. J. Pharmacol.* **123,** 173–174.

McCaslin P. P. and Morgan W. W. (1987) Cultured cerebellar cells as an in vitro model of excitatory amino acid receptor function. *Brain Res.* **417,** 380–384.

Mena E. E., Pagnozzi M. J., and Gullak M. F. (1986) Characterization of L-glutamate binding sites in rat spinal cord synaptic membranes: evidence for multiple chloride ion-dependent sites. *J. Neurochem.* **47,** 1052–1060.

Monaghan D. T., Holets V. R., Toy D. W., and Cotman C. W. (1983*a*) Anatomical distributions of four pharmacologically distinct ^{3}H-L-glutamate binding sites. *Nature* **306,** 176–179.

Monaghan D. T., McMills M. C., Chamberlin A. R., and Cotman C. W. (1983*b*) Synthesis of [^{3}H]2-amino-4-phosphonobutyric acid and characterization of its binding to rat brain membranes: a selective ligand for the chloride/calcium-dependent class of L-glutamate binding sites. *Brain Res.* **278,** 137–144.

Monaghan D. T., Yao D., and Cotman C. W. (1985) L-[^{3}H]-Glutamate binds to kainate-, NMDA-, and AMPA-sensitive binding sites: an autoradiographic analysis. *Brain Res.* **340,** 378–383.

Munson P. J. and Rodbard D. (1980) LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107,** 220–239.

Murphy D. E., Snowhill E. W., and Williams M. (1987) Characterization of quisqualate recognition sites in rat brain tissue using DL-[^{3}H]α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and a filtration assay. *Neurochem. Res.* **12,** 775–782.

Nelson P. G., Pun R. Y. K., and Westbrook G. L. (1986) Synaptic excitation in cultures of mouse spinal cord neurones: receptor pharmacology and behaviour of synaptic currents. *J. Physiol. (Lond.)* **372,** 169–190.

Nicoletti F., Wroblewski J. T., Novelli A., Alho H., Guidotti A., and Costa E. (1986) The activation of inositol phospholipid metabolism as a signal-transducing system for excitatory amino acids in primary cultures of cerebellar granule cells. *J. Neurosci.* **6,** 1905–1911.

Olson J. M. M., Greenamyre J. T., Penney J. B., and Young A. B. (1987) Autoradiographic localization of cerebellar excitatory amino acid binding sites in the mouse. *Neuroscience* **22,** 913–923.

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

Paxinos G. and Watson C. (1986) *The Rat Brain in Stereotaxic Coordinates.* Academic Press, Orlando, Florida.

Pin J.-P., Bockaert J., and Recasens M. (1984) The Ca^{2+}/Cl^- dependent L-[^{3}H]glutamate binding: a new receptor or a particular transport process? *FEBS Lett.* **175,** 31–36.

Quinn M. R. and Spraguer P. A. (1986) Chloride-dependent binding sites for L-[^{3}H]glutamate on dendrodendritic synaptosomal membranes of rat olfactory bulb. *J. Neurosci. Res.* **16,** 409–419.

Recasens M., Pin J.-P., and Bockaert J. (1987) Chloride transport blockers inhibit the chloride-dependent glutamate binding to rat brain membranes. *Neurosci. Lett.* **74,** 211–216.

Robinson M. B., Crooks S. L., Johnson R. L. and Koerner J. F. (1985) Displacement of DL-[^{3}H]-2-amino-4-phosphonobutanoic acid ([^{3}H]APB) binding with methyl-substituted APB analogues and glutamate agonists. *Biochemistry* **24,** 2401–2405.

Robinson M. B., Blakely R. D., and Coyle J. T. (1986) Quisqualate selectively inhibits a brain peptidase which cleaves *N*-acetyl-L-aspartyl-L-glutamate in vitro. *Eur. J. Pharmacol.* **130,** 345–347.

Robinson M. B., Blakely R. D., Couto R., and Coyle J. T. (1987) Hydrolysis of the brain dipeptide *N*-acetyl-L-aspartyl-L-glutamate: identification and characterization of a novel *N*-acetylated α-linked acidic dipeptidase activity from rat brain. *J. Biol. Chem.* **262,** 14498–14506.

Silverstein F. S., Chen R., and Johnston M. V. (1986) The glutamate analogue quisqualic acid is neurotoxic in striatum and hippocampus of immature rats. *Neurosci. Lett.* **71,** 13–18.

Sladeczek F., Pin J.-P., Récasens M., Bockaert J., and Weiss S. (1985) Glutamate stimulates inositol phosphate formation in striatal neurones. *Nature* **317,** 717–719.

Watkins J. C. and Evans R. H. (1981) Excitatory amino acid transmitters. *Annu. Rev. Pharmacol. Toxicol.* **21,** 165–204.

Watkins J. C. and Olverman H. J. (1987) Agonists and antagonists for excitatory amino acid receptors. *Trends Neurosci.* **10,** 265–272.

Young A. B., Oster-Granite M., Herndon R. M., and Snyder S. H. (1974) Glutamic acid: selective depletion by viral induced granule cell loss. *Brain Res.* **72,** 1–13.

Young A. B., Bromberg M. B., and Penney J. B. (1981) Decreased glutamate uptake in subcortical areas deafferented by sensorimotor cortical ablation in the cat. *J. Neurosci.* **1,** 241–249.

Zaczek R., Arlis S., Markl A., Murphy T., Drucker H., and Coyle J. T. (1987) Characteristics of chloride-dependent incorporation of glutamate into brain membranes argue against a receptor binding site. *Neuropharmacology* **26,** 281–287.