Annu. Rev. Pharmacol. Toxicol. 1989. 29:365-402

THE EXCITATORY AMINO ACID RECEPTORS: Their Classes, Pharmacology, and Distinct Properties in the Function of the Central Nervous System

Daniel T. Monaghan

Division of Neurosurgery, University of California, Irvine California 92717

Richard J. Bridges

Department of Neurology, University of California, Irvine, California 92717

Carl W. Cotman

Department of Psychobiology, University of California, Irvine, California 92717

INTRODUCTION

The vast majority of synapses in the central nervous system appear to use excitatory amino acids (EAA)[1] as their neurotransmitters. Recent progress has greatly advanced our understanding of the properties of those receptors

[1]The following abbreviations have been used in the text; β-L-ODAP, β-N-oxalyl-L-α,β-diamino-propionic acid; ACPD, *Trans*-1-amino-cyclopentyl-1,3-dicarboxylate; AMPA, α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate; AP4, 2-amino-4-phosphonobutyrate; AP5, 2-amino-5-phophonovalerate; ASP, aspartate; CNQX, 6-cyano-7-nitro-quinoxaline-2,3-dione; CPP, 3-(2-carboxypiperazin-4-yl)propyl-1-phosphate; cyclo-Leu, cyclo-leucine; DAA, D-α-amino-adipate; DGG, γ-D-glutamylglycine; DNQX, 6,7-dinitro-quinoxaline-2,3dione; EAA, excitatory amino acids; GABA, gamma-amino-butyric acid; GDEE, glutamate diethyl ester; GLU, glutamate; GLY, glycine; HA-966, 3-amino-1-hydroxypyrrolidone-2; IBO, ibotenate; IP, inositol phosphate; KA, kainate; KYN, kynurenate; MK-801, dibenzocycloheptenеimine; NMDA, N-methyl-D-aspartate; PCP, phencyclidine; QA, quisqualate; SER, serine; SOP, serine-O-phosphate; TCP, 1-[1-(2-thienyl)-cyclohexyl]piperidine

0362-1642/89/0415-0365$02.00

serving synaptic transmission along these pathways. Perhaps not surprisingly, at least five receptors exist, all with significantly distinct functions. Three have been defined by the depolarizing actions of selective agonists (N-methyl-D-aspartate, NMDA; kainate; quisqualate, or α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, AMPA) and their blockade by selective antagonists. A fourth, the AP4 receptor (L-2-amino-4-phosphonobutyrate), appears to represent an inhibitory autoreceptor. The fifth receptor, activated by *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) modifies inositol phosphate (IP) metabolism. Excitatory transmission appears to involve actions mediated by one or more combinations of these receptors, even at single synapses. The definition, properties, and apparent interplay of these multiple receptors is the major theme of this review. Although recent attention has focused primarily on the NMDA receptor [for reviews see: 1–4], the development of new drugs and even the identification of new receptors (the AP4 and ACPD receptors) have significantly increased our understanding of the other receptor subtypes and their roles in synaptic transmission.

Excitatory amino acid receptors appear not only to mediate normal synaptic transmission along excitatory pathways, but also to participate in the modification of synaptic connections during development and changes in the efficacy of synaptic transmission throughout life. Ironically, however, overactivation of select receptors can also mediate neuronal degeneration and even cell death. Thus, there appears to be a fine line between normal function and toxic reactions. Recent research is beginning to provide some insight into the mechanisms underlying these aspects of plasticity and pathology. The properties, mechanisms, and interactions that set this delicate balance comprise the second major theme of this review.

In the first section current data on the properties of the receptor types is discussed, including: (a) the increasingly complicated pharmacological profile of the NMDA receptor-ionophore complex, (b) the pharmacological and physiological distinctions of kainate and quisqualate receptors, (c) the properties and functions of the less well understood, but increasingly important L-AP4 receptor, and (d) the identification of a novel receptor class (ACPD activated) that is clearly distinct from the other sites and unusual in its ability to access IP metabolism. In the second section, the role of the receptors in mediating normal neural transmission and synaptic plasticity is examined from the perspective of the better-known actions of NMDA receptors and their potential interaction with the other receptor types. In the third section, the neurotoxic actions of EAAs are discussed. It is evident that several of the receptor types are capable of mediating excitotoxicity. In the concluding section, we discuss possible mechanisms underlying the paradoxical balance between plasticity and pathology. We suggest that the capacity to modify or refine the efficacy of excitatory synaptic transmission is associated with a greater vulnerability to excitotoxicity.

EXCITATORY AMINO ACID RECEPTOR CLASSIFICATION

The Historical Emergence of Multiple Receptor Types

The elucidation of EAA receptor classes has evolved over the past thirty years. Several years after the initial suggestion of a single receptor type, accumulating evidence suggested the presence of at least two classes. This scheme was soon replaced by a three- (or four-) receptor system that is widely accepted today. Current electrophysiological, biochemical, and radioligand binding data indicate, however, that there are at least five distinct receptors.

In their initial descriptions of the excitatory activity of a wide variety of acidic amino acids, Curtis & Watkins (5, 6) identified the specific structural features required for excitatory activity as based on a "three point attachment" receptor model. Of the compounds tested, excitatory activity was found in those with an acidic group in a position α to an amino group (preferably primary) and two or three carbon atoms distant to another acidic group. Addition of side groups to the carbon chain generally reduced excitatory activity. Taken together, the dicarboxylic amino acids L-aspartate and L-glutamate thus appeared to be the prototypical compounds. The finding that essentially all neurons were excited by EAAs, initially led to the suggestion that the responses were the result of a general property of neuronal membranes, rather than specific transmitter receptors. This excitatory action appeared to be mediated by only one site of interaction.

Later, detailed studies indicated differing agonist activities in select brain regions (7). For example, L-glutamate was found to be a more potent agonist upon dorsal horn interneurons of the cat spinal cord, whereas L-aspartate was more potent upon ventral horn renshaw cells (8, 9). These findings raised the possibility that there were two populations of EAA receptors; one "glutamate-preferring" (later recognized as quisqualate receptors) and one "aspartate-preferring" (later recognized as NMDA receptors). Thus, each of these two putative neurotransmitters appeared to have its own respective receptors.

A major breakthrough in the identification of EAA receptor classes was the identification of D-α-aminoadipate (DAA) as a relatively selective blocker of NMDA-activated receptors, i.e. the aspartate-preferring receptor class. Consistent with the two-receptor classification, DAA blocked aspartate and NMDA responses but only weakly inhibited L-glutamate and quisqualate responses (10, 11). A new generation of more potent and selective aspartate-preferring (NMDA) receptor antagonists was obtained by replacing the ω-carboxy group of DAA with a phosphonate group (12). This compound, D-2-amino-5-phosphonopentanoate (D-AP5), then became the NMDA antagonist of choice for the next several years and directly led to many of the

discoveries of NMDA receptor function. The availability of potent and selective NMDA antagonists allowed researchers to demonstrate that glutamate also acted at the aspartate-preferring receptor (13, 14). Therefore, these receptors are termed NMDA receptors and the inference regarding the endogenous neurotransmitter was thereby avoided (13).

EARLY RADIOLIGAND BINDING STUDIES In parallel with electrophysiological data, various investigators began studying radioligand binding to membranes (15–21). Although initially inconsistent, these findings now appear to support current receptor classes. In retrospect, a major factor that contributed to the inability to demonstrate binding to the characterized receptors was attributable to the inclusion of chloride ions in the assay buffers. Cl^- ions were found to dramatically increase glutamate binding (22), but this stimulated binding displayed a pharmacological profile distinct from the EAA receptors (23). Subsequent investigations have shown that this Cl^-dependent binding represents the interaction of L-[^{3}H]glutamate with an uptake system (see the discussion below of the L-AP4 receptor.) Thus, the complications introduced by this system hindered the identification of the EAA receptors.

The binding of L-[^{3}H]glutamate to NMDA, kainate, and quisqualate receptors was initially described by quantitative autoradiographic techniques (24). By taking advantage of the anatomical segregation of EAA receptor populations and by avoiding Cl^- and Na^+-dependent glutamate uptake sites, researchers could demonstrate that L-[^{3}H]Glutamate bound to three anatomically and pharmacologically distinct binding sites as well as to a Cl^--dependent binding site. L-[^{3}H]glutamate binding sites in the hippocampal stratum radiatum displayed the characteristic NMDA receptor pharmacology; binding sites in the stratum lucidum showed kainate receptor pharmacology; and binding sites in the stratum pyrimidale displayed quisqualate receptor properties. The distributions and pharmacological profiles of each of the three L-[^{3}H]glutamate binding sites (24–27) corresponded to the distributions and pharmacological profiles obtained with the receptor-specific radioligands D-[^{3}H]AP5 for NMDA receptors (28, 29), [^{3}H]kainate for kainate receptors (18, 21, 30, 31), and [^{3}H]AMPA for quisqualate receptors (17, 28, 32–34). Thus, these results confirmed the three-receptor classification scheme and showed that data from the binding studies using D-[^{3}H]AP5, [^{3}H]kainate, [^{3}H]AMPA, and now L-[^{3}H]glutamate were consistent with one another and with results of electrophysiological studies (see Figure 1).

The NMDA Receptor-Ionophore Complex

In addition to the transmitter recognition site, evidence now indicates that there are other functional subcomponents of the NMDA receptor, each with discrete ligand binding domains. In this manner, the NMDA receptor is

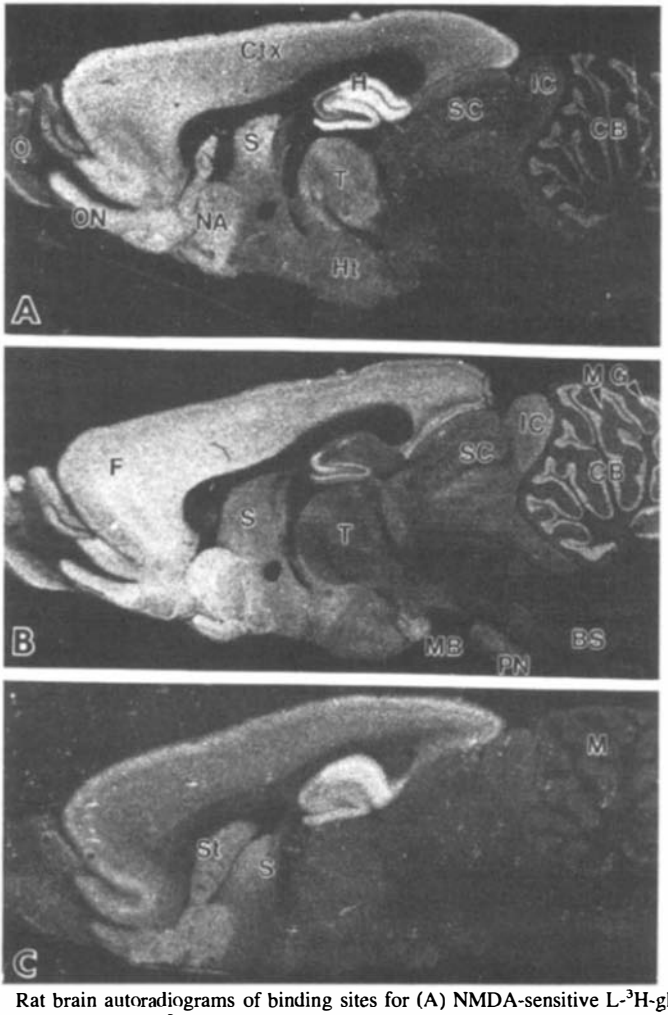

Figure 1 Rat brain autoradiograms of binding sites for (A) NMDA-sensitive L-^{3}H-glutamate, (B) ^{3}H-kainic acid, and (C) ^{3}H-AMPA. Abbreviations: O, olfactory bulb; Ctx, cerebral cortex; ON, anterior olfactory nuclei, NA, nucleus accumbens; S, septum; H, hippocampus; T, thalamus; SC, superior colliculus; IC, inferior colliculus; Ht, hypothalamus; CB, cerebellum; F, frontal cortex; MB, mammillary bodies; PN, pons; BS, brain stem; M, molecular layer; G, granule cell layer; and St, striatum.

analogous to the γ-aminobutyric acid (GABA)-benzodiazapine receptor complex. Currently there are at least five pharmacologically distinct sites through which compounds can alter the activity of this receptor. They include (a) a transmitter binding site, which binds L-glutamate, (b) a regulatory or coactivator site, which binds glycine, (c) a site within the channel that binds

phencyclidine and related compounds, (d) a voltage-dependent Mg^{++} binding site, and (e) an inhibitory divalent cation site that can bind Zn^{++}. In addition, ligand binding analysis indicates that there are two distinct binding sites (or states) associated with the transmitter recognition site, one that preferentially binds agonists and one that preferentially binds antagonists.

NMDA RECEPTOR RECOGNITION SITE; ANTAGONISTS AND RADIOLIGANDS The current generation of NMDA receptor antagonists incorporates the carbon backbone of D-AP5 and D-AP7 (D-2-amino-7-heptanoate) into heterocyclic ring derivatives (Figure 2). For example, modifications of a piperazine ring have produced 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), a derivative approximately five fold more potent than D-AP5 (35–37). A similar strategy led to the synthesis of *cis*-4-(phosphonomethyl)-2-piperidine-carboxylic acid, CGS-19755, a piperidine analog that is more potent than CPP (38). Yet more potent, orally active, competitive NMDA antagonists have recently been reported (39, 40).

Detailed characterization of the NMDA receptor L-glutamate recognition site is now possible with a variety of radioligands. D-[^{3}H]AP5 binding sites (41, 42), [^{3}H]CPP binding sites (43–46), and NMDA-sensitive L-[^{3}H] glutamate binding sites have been used to characterize the NMDA receptor's anatomical distribution (47, 48), pharmacological profile, subcellular localization, ligand binding requirements (49–53), and status in human diseases (54, 55).

THE NMDA-GLYCINE RECEPTOR In the course of identifying an endogenous potentiator of NMDA-induced responses, Johnson & Ascher (56) discovered that glycine greatly enhances the actions of NMDA agonists but has no action by itself. At concentrations as low as 0.1 μM, glycine greatly increased NMDA receptor responses in a manner not blocked by strychnine. Patch-clamp analysis of single channels indicated that glycine increased the frequency of channel opening and not the current amplitude. Since this effect was observed in outside-out patches, glycine's action probably does not require an intracellular second messenger. These findings have now been confirmed by others (57–59).

Recent studies in which glycine contamination was reduced suggest that there is an absolute requirement for glycine binding before the receptor can be activated by L-glutamate (57). Since NMDA receptors could be from the same genetically related superfamily as the glycine, GABA, and nicotinic receptors (60), the combined glutamate and glycine binding sites of the NMDA receptor are hypothesized to be analogous to the two acetylcholine or two GABA recognition sites found on nicotinic acetylcholine or GABA-A receptors, respectively (57).

Although the glycine-binding component of the NMDA receptor was discovered only recently, several glycine agonists and antagonists have already been identified. To date, glycine itself is the most potent agonist tested in electrophysiological preparations (56, 57) and in radioligand binding preparations, as assayed by stimulation of [^{3}H]1-[1-(2-thienyl) cyclohexyl]-piperidine ([^{3}H]TCP) binding (see further details below) (59, 61–63). Various glycine analogs exhibit a potency of glycine>D-serine>D-alanine>L-alanine and L-serine. The general EAA antagonist kynurenate competitively inhibits glycine stimulation of [^{3}H]TCP binding. Physiological studies report that kynurenate is not a simple, competitive antagonist at the NMDA receptor (58, 64). Furthermore, kynurenate antagonism of NMDA receptor activity can be reversed by glycine, which suggests that it acts as a competitive glycine antagonist (61). More recently, cycloleucine (65), 3-amino-1-hydroxypyrrolidine-2 (HA-966) (66), and 7-chlorokynurenate (67) have also been shown to be glycine antagonists. Addition of chlorine to carbon 7 of the kynurenate molecule increases its potency and selectivity at the glycine site relative to the glutamate site on the NMDA receptor. Similarly, addition of chloride to the 7 and 6 positions on the kainate antagonist (68) 3-hydroxy-quinoxaline-2-carboxylic acid molecule also increases its potency as a glycine antagonist (M. Kessler, G. Lynch, M. Baudry, unpublished observations). Interestingly, the structures of these molecules are quite similar to the potent quisqualate/kainate receptor antagonists 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Thus, not surprisingly these compounds also display glycine antagonist activity in radioligand binding assays (M. Kessler, G. Lynch, M. Baudry, unpublished observations) and act as noncompetitive NMDA receptor antagonists in electrophysiological preparations (69).

The glycine recognition site can be directly labelled in autoradiographic (70, 71) and membrane fraction preparations (61, 72, 73) with [^{3}H]glycine. This ligand's specificity corresponds to that seen for NMDA receptor activation and [^{3}H]TCP binding stimulation. In addition, the anatomical distribution of the [^{3}H]glycine binding site is essentially identical to that observed for NMDA receptors labelled by L-[^{3}H]glutamate (71).

THE NMDA RECEPTOR ION CHANNEL The very important role that NMDA receptors play in central nervous system function is a consequence of the special properties displayed by the ion channel gated by these receptors. A major advance in the understanding of the NMDA receptor was the demonstration by MacDonald et al (74) and Flatman et al (75) that NMDA-induced responses are voltage-dependent. The agonist-induced currents are greatest at moderately depolarized potentials (−30 to −20 mV) and are reduced at both more hyperpolarized and depolarized potentials. Consequently, NMDA

receptor action is suppressed at the normal resting potential. The work of Nowak et al (76) and Mayer et al (77) demonstrated that the voltage dependency of the NMDA receptor is attributable to extracellular Mg^{++} ions that block the ion channel only at potentials more negative than -20 or -30 mV.

Equally important to our understanding of NMDA receptor function is the observation that NMDA receptor channels are permeable to Ca^{++} as well as to Na^{+} and K^{+}. Voltage-clamp studies of cultured neurons injected with a calcium-sensitive dye directly indicate that Ca^{++} can enter cells after NMDA application in a manner independent of the voltage-dependent Ca^{++} channels (78, 79). Furthermore, the reversal potentials of NMDA-induced currents are appropriately altered by changes in the extracellular Ca^{++} concentration (80–82). The increase in intracellular Ca^{++} ions is thought to initiate the biochemical processes responsible for both NMDA receptor-induced plasticity observed in developing and adult animals (2, 83, 84) and NMDA receptor-mediated excitotoxic cell death (2, 85).

BLOCKADE OF NMDA RECEPTOR ION CHANNELS BY PHENCYCLIDINE Lodge and colleagues first reported (86, 87) that the dissociative anesthetic ketamine and phencyclidine (PCP) are selective antagonists of NMDA receptor function. Without altering responses to kainate or quisqualate, ketamine and PCP can completely block the actions of NMDA. Since those initial reports, PCP and related compounds have been found to inhibit NMDA receptor activity in every preparation thus far examined (e.g. 88–91). In more recent studies, the structurally related compounds TCP (1-(1-thienylcyclohexyl)piperidine) and MK-801 (dibenzocycloheptenimine) have been found to display greater potency and selectivity. Of these compounds, MK-801 appears to be more potent and selective (92).

PCP appears to attenuate NMDA receptor action by blocking the cation channel gated by the NMDA recognition site. PCP, ketamine, and MK-801 are noncompetitive antagonists of NMDA-induced responses (89, 93, 94). This property is consistent with results of ligand binding studies that demonstrate that ketamine and related compounds do not inhibit binding to the NMDA recognition site (45, 51, 52). Further studies suggest that the PCP-like compounds bind to a site within the open channel. PCP blockade of NMDA receptor activity is use dependent (58, 93, 95–97). Thus, an application of agonist is required before blockade can develop. The removal of the blockade (presumably PCP dissociation) has also been shown to be agonist-dependent.

These observations formed the basis of a proposal that the receptor must be in the agonist-bound, open-channel state for the antagonist (PCP) to bind or dissociate from a site within the receptor-gated channel. The partial voltage dependency of both the block and its removal suggests that the PCP binding

site is within the transmembrane electric field, i.e. within the ion channel itself. The ability of Mg^{++} to voltage-dependently prevent MK-801 blockade suggests that these two sites are very near to one another (93).

Radioligand binding studies support the conclusions derived from physiological experiments. [^{3}H]PCP binds to at least two distinct sites in rat brain, a PCP-preferring site and a σ-opiate site (98). Of these two, pharmacological (99–102) and anatomical evidence (43, 47, 48, 71, 103–105) indicates that the PCP-preferring site is the one that corresponds to the NMDA receptor complex. This PCP site can be selectively labelled by [^{3}H]TCP, (98, 106) and by the more selective and potent ligand [^{3}H]MK-801 (92).

Direct evidence that [^{3}H]MK-801 and [^{3}H]TCP label a site on the NMDA receptor complex comes from a large body of work that demonstrates that agents active at the NMDA receptor modify [^{3}H]MK-801 and [^{3}H]TCP binding properties. Following the initial observation by Loo et al (107) that glutamate enhances [^{3}H]TCP binding, both [^{3}H]TCP (61, 108–13) and [^{3}H]MK-801 binding (62, 114, 115) have been shown to be stimulated by NMDA agonists in a manner that is blocked by NMDA antagonists. In addition, the stimulation of [^{3}H]MK-801 and [^{3}H]TCP binding by glutamate is further enhanced by glycine (61, 62, 108, 111, 112, 116, 117). Analogous to the physiological result that glycine is ineffective by itself (56, 57), glycine stimulation of [^{3}H]TCP binding appears to require concurrent NMDA agonist binding, as AP5 can block glycine enhancement (61, 116). Whether the increased binding produced by glutamate and glycine is due to a change in binding site density, affinity, accessibility, or a combination of these has been a matter of disagreement. In well washed membranes, however, the association and dissociation rates of [^{3}H]MK-801 and [^{3}H]TCP are clearly greatly accelerated by glutamate and glycine (61, 112). This property is presumed to represent the greater access of these ligands to a site within the ion channel that becomes more exposed when agonist is bound.

Thus, as predicted from electrophysiological studies, the ability of ligands to bind to the PCP site largely depends upon the simultaneous binding of L-glutamate and glycine. The ability to observe changes in affinity (K_d), maximal binding site density (B_{max}), or time required to achieve equilibrium, might possibly depend upon the specific assay conditions, i.e. ionic strength, assay buffer, endogenous glutamate and glycine, and incubation time.

DIVALENT CATIONS Mg^{++} and certain other divalent cations were among the first pharmacological tools used to selectively inhibit the responses to NMDA (118). Initially, the significance of the observation that physiologically relevant levels of Mg^{++} inhibit NMDA-induced responses was only a matter of speculation. Then, in 1984, the recently described voltage dependency of NMDA-induced responses was discovered to be due to the

voltage-dependent block by Mg^{++} (76, 77). That is, Mg^{++} selectively blocks NMDA receptor activity at depolarized potentials. Since that time, additional studies have confirmed these results and further supported the concept that Mg^{++} acts by blocking the NMDA receptor–gated channel (e.g. 58).

Other divalent cations such as Zn^{++} are also effective NMDA antagonists (119, 120). In contrast to Mg^{++}, the inhibition by Zn^{++} is not voltage-dependent. Consequently, Zn^{++} has been proposed to interact at a site distinct from the Mg^{++} site. Results of [^{3}H]TCP and [^{3}H]MK-801 binding studies also suggest that divalent cations act upon two distinct sites within the NMDA receptor complex (113, 121, 122). Given the high concentrations of Zn^{++} in nerve terminals, this ion could conceivably modulate NMDA receptor function.

NMDA RECEPTOR HETEROGENEITY Quantitative comparisons of the distribution of NMDA receptors, as determined by NMDA-sensitive L-[^{3}H]glutamate and [^{3}H]CPP binding sites, indicate that these ligands display different distributions (44, 123). Pharmacological evaluation of L-[^{3}H]glutamate binding in different brain regions indicated that antagonists were more potent as displacers in the lateral thalamus and cerebral cortex, while agonists were better displacers in the medial striatum and cerebellum. These anatomical variations corresponded to the binding distributions displayed by L-[^{3}H]glutamate and [^{3}H]CPP, i.e. L-[^{3}H]glutamate binding density was relatively higher than [^{3}H]CPP in the medial striatum and cerebellum and lower in the lateral thalamus and cerebral cortex (44). Interestingly, these two distributions roughly correspond to the two differing distributions found for other NMDA receptor components. [^{3}H]Glycine binding at NMDA receptors generally has an agonist-preferring distribution, whereas [^{3}H]TCP binding generally has an antagonist-preferring distribution (71).

Differences between agonist and antagonist binding are also revealed by their differing responses to glycine (44, 123). Glycine causes a greater percentage increase in L-[^{3}H]glutamate binding to NMDA receptors in the thalamus and cerebral cortex than in the striatum, septum, and cerebellum. Radiolabelled NMDA antagonist binding, in contrast, is inhibited by glycine (44, 123). The difference between agonist and antagonist binding may represent two distinct receptors and/or two interconverting forms.

Thus, as observed for GABA-A receptors (124), NMDA receptors have an agonist-preferring binding site and an antagonist-preferring site that display different anatomical distributions(125). Since the GABA-A α subunit has been recently shown to be coded for by at least three distinct mRNAs with different brain distributions (126), the NMDA receptor quite plausibly also exhibits multiple mRNAs for distinct forms of the receptor. The distinction between the agonist-preferring and antagonist-preferring sites could also

represent a covalent modification (e.g. phosphorylation). Mody & MacDonald have demonstrated that NMDA receptor activity appears to be regulated by an adenosine triphosphate-dependent mechanism (127, 128). NMDA receptor heterogeneity has also been suggested by the differential actions of quinolinate and NMDA (129); see Stone & Burton (4) for a recent discussion of this hypothesis.

Kainate and Quisqualate Receptor Classes

Kainic acid and quisqualic acid were initially isolated during the purification of the anthelminthic activity found in the algae *Digenea simplex* (kainate) and seeds of the plant *Quisqualis fructus* (quisqualate). For a review of the discovery and isolation of these and related compounds, see Takemoto (130). In electrophysiological preparations, these compounds were potent excitants of presumed glutamate receptors in crayfish, frog, rat, and cat (9, 131–133). That these two compounds act at different receptors was initially suggested by the finding that glutamate diethyl ester (GDEE) inhibits quisqualate-induced responses but does not affect kainate responses (134, 135). Similarly, depolarizations induced by the selective quisqualate agonist, AMPA, are also selectively blocked by GDEE (136). Several compounds reportedly inhibit kainate activity but have less of an effect upon quisqualate responses. Among these are 2-amino-4-phosphonobutyrate (13), γ-D-glutamyl-glycine [DGG; (137, 138)], and kynurenic acid (139). Their use in identifying synaptic transmitter receptors has been hindered, however, by their inability to clearly separate kainate and quisqualate responses. This property has prompted many authors to refer to them as "non-NMDA" receptors.

The most potent kainate and quisqualate antagonists currently available are the quinoxaline derivatives DNQX and CNQX recently reported by Honore and colleagues (140). Low micromolar concentrations of these compounds block kainate and quisqualate responses but have minimal effects upon NMDA-induced responses (140–142). Schild analysis indicated that both kainate and quisqualate antagonism was competitive (69, 142). At higher concentrations, NMDA-evoked responses are antagonized by DNQX in a noncompetitive manner (69). The antagonist 6,7-dichloro-3-hydroxy-2-quinoxalinecarboxylic acid has been reported to selectively block kainate-induced responses relative to quisqualate responses (68). If this blockage is confirmed, this compound will represent a major pharmacological tool for studying EAA receptors.

KAINATE AND QUISQUALATE RECEPTORS DISPLAY DISTINCT PHARMACOLOGICAL AND ANATOMICAL PROPERTIES In both physiological and radioligand binding studies, kainate receptors display a pharmacological profile that differs from that of quisqualate receptors (for review see: 143–145).

The physiological potency of agonists at kainate receptors can be examined in spinal cord C-fiber afferents that appear to possess a relatively pure population of kainate receptors (13). In such preparations, kainate receptors display an agonist potency of: domoate>kainate>quisqualate>>L-glutamate (13, 64). The same pattern is observed for the displacement of [^{3}H]kainate binding to rat brain membranes (18, 21, 146). In contrast, quisqualate receptors, as defined by GDEE-sensitivity, display a different rank order of agonist potency (quisqualate≥AMPA>L-glutamate>kainate). This same pharmacological profile is also exhibited by the [^{3}H]AMPA binding site (17, 33, 147). Differing anatomical distributions of kainate and quisqualate receptors are dramatically illustrated by autoradiography of the respective radioligand binding sites. Both kainate and quisqualate sites have a preferential telencephalic localization, although their distributions are quite distinct. [^{3}H]Kainate binding sites show high relative densities in the hippocampal CA3 stratum lucidum, deep cerebral cortical layers, striatum, reticular nucleus of thalamus, and granule cell layer of the cerebellum (30, 31). [^{3}H]AMPA binding sites are more numerous in CA1 stratum radiatum, outer cortical layers, lateral septum, and molecular layer of the cerebellum (32–34).

Electrophysiological experiments have also shown different anatomical distributions for kainate and quisqualate receptors. Within the hippocampus, CA3 has been found to be exceptionally sensitive to kainic acid excitations (148, 149). Of the two binding site distributions, the kainate binding site population corresponds to the brain regions that are exceptionally sensitive to kainate receptor excitations and excitotoxicity (150, 151).

SYNAPTIC TRANSMISSION AT KAINATE OR QUISQUALATE RECEPTORS

Although NMDA receptors play a critical role in synaptic function, they do not mediate the excitatory postsynaptic potential resulting from a unitary synaptic activation. Pharmacological studies indicate that the kainate and/or the quisqualate receptors are responsible for the voltage-independent portion of the synaptic response in many neuronal pathways [(for reviews see: (144, 145)]. Although identifying the synaptic transmitter receptor is difficult, it is quite significant that the newly developed kainate/quisqualate antagonists, e.g. CNQX, block synaptic transmission at low concentrations (140, 141). Thus, with these new compounds and those previously identified [e.g. kynurenate and the piperazines; (139, 152, 153)], there is, for the first time, a characteristic pharmacological profile that should be sufficient to identify EAA-using synapses.

To resolve the identity of the fast-acting synaptic transmitter receptor, further studies are necessary to correlate the potency of antagonists at kainate and quisqualate receptors to antagonist potencies at the synaptic response. An important precaution for these studies, however, is the careful definition of

kainate and quisqualate receptors. Binding studies show that quisqualate and kainate display high or moderate affinity (respectively) for the other's binding site. Thus, in the absence of selective antagonists, the assumption of specificity is difficult to make when using high concentrations of kainate or quisqualate. Indeed, that kainate-induced depolarizations often require concentrations of kainate that are more comparable to kainate's affinity for [^{3}H]AMPA (quisqualate sites) than [^{3}H]kainate binding sites is noteworthy (144). These observations are not inconsistent with the findings that kainate and AMPA appear to activate the same receptor in mRNA-injected oocytes and that this receptor is most similar to the quisqualate (AMPA) receptor (154).

Some of the difficulty in studying kainate receptors in electrophysiological preparations could be due to a lack of ligand specificity. Thus, depolarizations induced by low micromolar concentrations of kainate, may, in some preparations, represent quisqualate receptor activity, while submicromolar effects of kainate observed in (a) the hippocampal CA3 upon burst activity (148), (b) CA1 upon the after-hyperpolarizing potential (155), and (c) other, possibly, presynaptic sites (156, 157) may represent the kainate receptor activity. Kainate excitotoxicity and [^{3}H]kainate binding are also likely to represent properties of kainate receptors. A more subtle physiological action by kainate receptors (e.g. presynaptic or second messenger mediated) however, is perhaps more consistent with the slow onset (144, 145) of kainate responses. Thus, kainate receptors could possibly have multiple actions, e.g. the opening of a voltage-independent cation channel as well as a modulatory action, possibly via calcium channels.

AP4 Receptors

L-AP4 BLOCKADE OF SYNAPTIC TRANSMISSION In contrast to the receptors characterized by the depolarizing actions of the selective agonists (i.e. NMDA, kainate, and AMPA), a fourth class of excitatory amino acid receptor was identified through the antagonistic action of L-AP4 upon certain presumed glutamate-using synapses. In this analog, the γ-carboxyl moeity of L-glutamate is replaced with a phosphonic acid group (see Figure 2). Superfusion of rat hippocampal slices with L-AP4 potently blocked lateral perforant path-evoked excitation of dentate gyrus granule cells in a stereospecific manner (158). Similarly, L-AP4 suppressed the mono-synaptic dorsal root-evoked ventral root potential in the spinal cords of both cat (159) and immature rat (160), lateral olfactory tract–evoked potentials in olfactory cortex slices (161–163), and the mossy fiber–CA3 synapse in guinea pig (but not rat) hippocampal slices (164, 165). Significantly, several of these studies demonstrated that although L-AP4 potently blocked synaptic transmission, it was unable to antagonize the effects of coapplied EAA agonists, such as

NMDA, kainate, quisqualate, L-aspartate, or L–glutamate. In contrast to the L-isomer, D-AP4 was identified as a nonselective moderate antagonist of NMDA, kainate, and quisqualate-induced depolarization (159, 160).

Another system in which AP4 has been carefully studied is within the retina (166, 167); [for review see (168)]. Although L-glutamate was found to both hyperpolarize ON- and depolarize OFF-bipolar cells, L-AP4 has been found to selectively hyperpolarize ON-bipolar cells. The analog has little or no effect on horizontal cells, OFF-bipolar cells, or photoreceptors. Thus, AP4 mimics the actions of the endogenous transmitter at ON-bipolar cells but not at OFF-bipolar cells.

PHARMACOLOGICAL PROFILE Relatively few compounds have been shown to be active at the L–AP4 receptor. Making the carbon chain of AP4 longer diminishes activity (158, 166) such that the six-carbon homolog is essentially inactive. L-Serine-O-phosphate (169) and certain cyclopentane analogs (170) have moderate activity. At present, no antagonist has been reported.

MECHANISM OF ACTION The finding that L-AP4 can potently block synaptic transmission in these systems yet is ineffective as an antagonist of the other well characterized excitatory amino acid agonists indicates that L-AP4 acts at receptors other than those identified by NMDA, kainate, or AMPA. This indication has led to the suggestion that L-AP4 may act at a postsynaptic site that represents only a small fraction of the receptor population activated by the

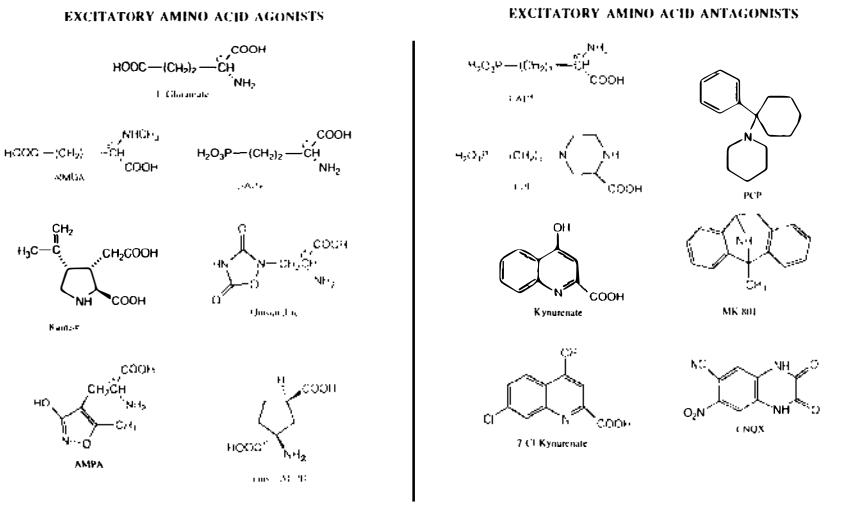

Figure 2 Structures of the more common excitatory amino acid receptor agonists and antagonists.

well-characterized agonists or act presynaptically (159, 164, 165). Quantal analysis (171) has demonstrated that in the case of the mossy fiber–CA3 synapse of guinea pig hippocampus, L-AP4 may act by a presynaptic mechanism. Analysis of spontaneous quantal synaptic events suggested that the antagonism of this synapse could be accomplished through two different mechanisms. The general EAA antagonists kynurenic acid and *N*-(*p*-bromobenzoyl)piperazine-2,3-dicarboxylate significantly shifted mean amplitudes of spontaneous miniature excitatory postsynaptic potentials (MEPSPs) to lower values, which suggested a postsynaptic site of action. L-AP4 and the related compound L-serine-O-phosphate, however, did not affect the amplitude of the MEPSPS when applied at concentrations that effectively blocked excitatory postsynaptic potentials (EPSPS), consistent with a presynaptic site of action. This mechanism has also been supported by other studies (172, 173). Recently, studies of cultured hippocampal neurons have demonstrated L-AP4 inhibition of synaptic responses by a presynaptic mechanism that is mimicked by L-glutamate (174). Thus, accumulating evidence indicates L-AP4 is a presynaptic receptor agonist.

CHLORIDE–DEPENDENT GLUTAMATE AND AP4 BINDING Although the physiological studies dramatically illustrate the presence of an AP4 class of EAA receptors, further biochemical characterization has proven elusive. Early studies suggested that the AP4 receptor could be identified in membrane preparations as a sodium-independent, chloride-dependent L-[^{3}H]glutamate binding site. Binding to this site was not inhibited by NMDA and kainate but was potently blocked by L-AP4 and quisqualate (175–177). Detailed analysis of pharmacology, binding density, temperature sensitivity, osmolar sensitivity, and dissociation kinetics showed that L-[^{3}H]glutamate and [^{3}H]DL-AP4 binding in the presence of chloride ions displayed properties more typical of transport phenomena than receptor binding (178–182). Subsequent studies have demonstrated the chloride-dependent transport of glutamate into synaptosomes and the presence of chloride-dependent glutamate binding and transport in glial cells (183–185).

A Novel EAA Receptor Class

A large body of evidence now indicates the presence of a novel EAA receptor coupled to phosphatidyl inositol metabolism. This receptor was first studied by observations of glutamate-induced oscillating Cl^- currents in frog oocytes following injection of rat brain mRNA (186). The response is thought to be mediated by inositol triphosphate (IP_3), since glutamate application "cross desensitizes" with IP_3 injection (187), and intracellularly injected IP_3 can evoke similar oscillating Cl^- currents (188). Consistent with this hypothesis, quisqualate and glutamate responses are blocked by intracellular injections of

ethyleneglycol-bis-(β-amino-ethyl ether) N, N^1-tetracetic acid, (EGTA), and pertussis toxin (187).

When the pharmacological properties of this receptor were examined, the acidic amino acid agonists quisqualate and glutamate, but not kainate, AP4, or NMDA, were found to elicit the oscillating Cl^- currents (154, 186, 189). Since the joro spider toxin (JSTX), which blocks quisqualate and kainate responses (190), did not block IP formation, the conclusion was that glutamate and quisqualate were activating a novel EAA receptor (187).

Recent investigations using mammalian brain slices agree with the oocyte experiments and indicate that this receptor is present in the mammalian brain and coupled to IP production (191–193). The pharmacological characteristics of quisqualate-induced IP turnover in mammalian brain are similar to those observed in the oocyte preparation. When neonatal hippocampal slices are incubated with ibotenate, quisqualate, or glutamate, there is a potent stimulation of IP formation (191, 192, 194, 195). Potent stimulation also occurs with the glutamate analog ACPD; (E. Palmer, D. T. Monaghan, C. W. Cotman, unpublished observations). NMDA and kainate are relatively weak stimulators of IP production. Consistent with the results from the oocyte/injected mRNA preparation, quisqualate activity in cultured cerebellar granule cells is inhibited by pertussis toxin (196).

This IP-coupled receptor is clearly not of either the kainate or NMDA classes, as it is insensitive to these and their related compounds. In rat brain slices, the quisqualate receptor agonist, AMPA, is also ineffective at eliciting IP formation (194, 195), and quisqualate-induced IP formation is not blocked by the quisqualate antagonist CNQX (194). Similarly, in mRNA-injected oocytes, AMPA is ineffective as an agonist (154, 189) and CNQX is ineffective as an antagonist (197). Since ACPD is the most selective agonist yet identified and the least likely to be confused with actions at the other receptor populations, reference to this new receptor class as the ACPD receptor seems appropriate.

Together, these two lines of evidence indicate that there is a discrete EAA receptor that is: (a) activated by L-glutamate, ibotenate, quisqualate, and ACPD; (b) not activated by NMDA, AMPA, or kainate; and (c) not blocked by the kainate/quisqualate antagonists CNQX or JSTX or by NMDA antagonists. ACPD receptor activation results in the formation of IP via a pertussis toxin–sensitive G protein.

AP4 reportedly antagonizes quisqualate-induced IP formation in hippocampal slices (192, 195), and the effect is stereospecific for the L isomer (195). These results prompted the suggestion that ACPD and AP4 receptors were the same (192). This conclusion seems unlikely, however, as AP4 and L-glutamate both act as AP4 receptor agonists in the retina (168) and in synapses of cultured hippocampal neurons (174). If AP4 does antagonize the stimula-

tion of IP formation (but see 189), then it is apparently acting at a site other than the previously identified AP4 receptor.

NMDA receptor activation blocks ACPD receptor–mediated IP production. In neonatal rat hippocampal slices, NMDA inhibited quisqualate-induced IP formation in a Ca^{++}-dependent fashion (194). Likewise, NMDA receptor activation has also been shown to inhibit muscarinic receptor–induced IP formation (191, 198). NMDA receptor activation could conceivably inhibit IP formation by an excitotoxic mechanism (198). The negative regulation of IP production by NMDA receptors would, however, account for the observations that NMDA antagonists enhance L-glutamate action at the ACPD receptor in mRNA-injected oocytes (199) and that quisqualate exhibits a bell-shaped dose-response curve (154). An excitotoxic action or a disruption of metabolic function on the oocyte preparation would appear unlikely, since facilitation of the oscillatory current (i.e. IP production) by NMDA antagonists occurred within several seconds of applying 10 μM glutamate.

Although the function of this receptor class is unknown, there is reason to speculate that this receptor plays a key role in synaptic growth and synaptic stabilization. In common with many growth factors, this receptor regulates IP formation. Furthermore, the activity of this receptor quickly diminishes following synaptogenesis (192) and reappears following deafferentation (200). Thus, this receptor is in a position to participate in activity-regulated synaptogenesis and synaptic stabilization/destabilization.

This receptor, together with its possible negative regulation by NMDA receptor activity, provides a potential mechanism much like that required by the theory that accounts for activity regulation of synaptic plasticity in the visual cortex (201). That is, the level of neuronal activity could determine the balance between two second messengers. At moderate stimulation levels, IP formation would predominate due to the lack of inhibition by NMDA receptor activation. At higher activity levels, NMDA inhibition of IP turnover, and the promotion of Ca^{++} entry, would lead to altered Ca^{++}/IP levels. Thus, increasing synaptic activation would result in a biphasic second messenger response that would be consistent with the biphasic properties required to account for activity-dependent neuronal plasticity.

RECEPTOR NOMENCLATURE IN RELATION TO RECENT DATA

In summary, pharmacological evidence provides a solid foundation for a receptor scheme based on five distinct receptors (Table 1). The currently used receptor nomenclature refers to four of these receptors as NMDA, kainate, quisqualate, and AP4. These receptors have been named, not defined, in terms of these relatively selective agonists. In light of currently available

information, as discussed above, two refinements to the present nomenclature appear appropriate: the use of *AMPA* to designate the receptor at which quisqualate potently depolarizes neurons and *ACPD* to indicate the receptor at which quisqualate activates IP formation. These refinements are consequences of the relatively nonspecific actions of quisqualic acid. This glutamate analog shows a broad spectrum of activity, interacting with AMPA receptors, kainate receptors, NMDA receptors, ACPD receptors, and the Cl^--dependent L-glutamate uptake sites on neurons and glia. Such a lack of specificity has led to confusion and the misassignment of receptor identity in several cases.

In contrast to quisqualate, AMPA and ACPD are more specific for their respective receptors. For the AMPA receptor, this specificity is demonstrated by (a) its inactivity at stimulating IP formation in mRNA-injected oocytes and brain slices, (b) much lower potency at kainate than quisqualate receptors, and (c) much lower potency at NMDA than quisqualate receptors. For the IP-coupled receptor, ACPD is the most selective of the agonists yet identified (L-glutamate, ibotenate, quisqualate, and ACPD) and does not exhibit the potent activity at the other EAA receptors that L-glutamate, ibotenate, or quisqualate display. Thus, this nomenclature is in keeping with current usage but avoids the problems of receptor misassignment associated with quisqualate. Importantly, though, none of the receptors is defined by the responses to a given agonist; rather, they are defined by the unique pharmacological profiles exhibited by each of the five currently known EAA receptors.

EAA SYNAPTIC TRANSMISSION AND PLASTICITY

It is becoming increasingly clear that EAA receptors play a role in plasticity mechanisms in the developing and mature brain. Recent evidence suggests common mechanisms may exist, though the exact regulatory properties may differ.

Developmental Plasticity

Apparently, NMDA receptors can play a critical role in early learning and synaptic specificity. The development of the visual system and probably other systems appears to depend on staged events; first, fibers are guided to their proper targets and second, their synaptic position is refined in an activity-dependent process. This fine-tuning appears to involve NMDA receptor activation. For example, in surgically produced three-eyed ***Rana pipien*** tadpoles, retinal ganglion cell fibers from normal and supernumerary eyes segregate in the optic tectum into stereotyped eye-specific stripes. If action potentials of retinal ganglion cells are blocked by tetrodotoxin (TTX),

Table 1 Excitatory amino acid receptors[1]

Receptor classes	Agonists	Antagonists	Radioligands
NMDA			
NMDA site	NMDA	D-AP5	L-[^{3}H]GLU
	L-GLU	CPP	D-[^{3}H]AP5
	L-ASP	D-α-AA	[^{3}H]CPP
	IBO	CGS-19755	[^{3}H]CGS-19755
GLY site	GLY	HA-966	[^{3}H]GLY
	D-SER	KYN	
		7-CL-KYN	
		cyclo-LEU	
Channel		PCP	[^{3}H]TCP
		TCP	[^{3}H]MK-801
		MK-801	
		Ketamine	
KA			
	KA	CNQX	[^{3}H]KA
	Domoate	DNQX	L-[^{3}H]GLU
	QA		
	L-GLU		
AMPA			
	AMPA	CNQX	[^{3}H]AMPA
	QA	DNQX	L-[^{3}H]GLU
	L-GLU		[^{3}H]CNQX
LAP4			
	L-AP4		
	L-SOP	?	?
	L-GLU		
ACPD			
	trans-ACPD		
	L-GLU	?	?
	QA		
	IBO		

[1] A summary of representative ligands for each of the excitatory amino acid receptor classes are shown above. This table is provided as an organizational aide to the text and is not intended to be all inclusive. Compounds are not necessarily ranked in order of pharmacological potency. Abbreviations: D-α-AA, D-α-amino-adipate; ACPD, 1-amino-cyclopentyl-1,3-dicarboxylate; AMPA, α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate; AP4, 2-amino-4-phosphonobutyrate; AP5, 2-amino-5-phophonovalerate; ASP, aspartate; CNQX, 6-cyano-7-nitro-quinoxaline-2,3-dione; CPP, 3-3(2-carboxypiperazine-4-yl) propyl-1-phosphate; cyclo-Leu, cyclo-leucine; DNQX, 6,7-dinitro-quinoxaline-2,3-dione, GLU, glutamate; GLY, glycine; IBO, ibotenate; KA; kainate; KYN, kynurenate; MK-801, dibenzocycloheptenemine; NMDA, N-methyl-D-aspartate; PCP, phencyclidine; QA, quisqualate; SER, serine; SOP, serine-O-phosphate; TCP, 1-(1-thienyl-cyclohexyl)piperidine.

segregation is prevented (202), which suggests that neural activity is a necessary step in establishing the retinal-tectal map. Chronic application of the NMDA antagonist AP5 to the optic tectum of three-eyed tadpoles also desegregates retinal ganglion terminals reversibly. Conversely, the application of the agonist NMDA appears to promote the development of sharper borders and of stripes that have fewer forks and turns (203). Similarly, the combination of neural activity and NMDA receptors appears to participate in the development of the mammalian visual cortex (204). In kittens, blocking impulse traffic prevents the formation of ocular dominance columns (205). Furthermore, chronic infusion of AP5 prevents the ocular dominance shift normally seen in response to monocular experience (206).

In the developing olfactory system, NMDA receptors also appear to participate in the mechanisms of early learning (207). In the same way neonatal rats learn to prefer the odor of their mother, they can develop a preference for an artificial odor if experienced at the proper developmental time with concurrent tactile stimulation. This learning process produces a large increase in the size of the glomeruli that are coding for the specific odor (208). The administration of the NMDA antagonist AP5 blocked both the enhanced response in focal areas of the glomerular layer (as monitored by 2-deoxyglucose uptake) and the specific behavioral response to early olfactory learning. Thus, the NMDA receptors may be involved in a general mechanism of developmental plasticity and learning.

Long Term Potentiation

The NMDA receptor is an essential component in the generation of long term potentiation (LTP). LTP represents an increase in synaptic efficacy that has been proposed as an underlying mechanism involved in learning and memory (209); [for recent review see (84)]. The activity-dependent increase in synaptic strength in LTP, which is induced by a train of high-frequency afferent stimulation, can occur in as rapidly as one second and has been shown to persist for several days.

The direct involvement of the NMDA receptor in this process was first demonstrated by the ability of AP5 to reversibly block the induction of LTP, as measured in the hippocampus by the potentiation of the population spike in the Schaffer/Commissural-Associational pathway (210). Subsequent studies have shown an excellent correlation between the ability of antagonists to block LTP and NMDA receptor activation (211, 36). The involvement of the NMDA receptor is further strengthened by the demonstration that noncompetitive inhibitors of the NMDA receptor, such as PCP and ketamine, also block LTP (212). These findings indicate that inhibiting NMDA receptor activation, whether competitively or noncompetitively, prevents the induction of LTP in the Schaffer pathway and suggests that these receptors normally

participate in LTP formation. The presence of NMDA receptors gives the synapse a voltage-dependent mechanism through which high frequency stimulation selectively activates NMDA receptors and permits enhanced Ca^{++} entry. Thus, NMDA receptors can account for the voltage-dependent (213) and Ca^{++}-dependent properties of LTP (83).

A considerable amount of evidence indicates that LTP is probably a critical component of learning (214). In support of this hypothesis, intraventricular administration of DL-AP5 (but not the inactive L-isomer of AP5) can prevent LTP of the perforant path input to the dentate gyrus and selectively impair learning of a spatial discrimination task (214a). Interestingly, LTP may also be associated with new synapse formation and/or growth (enlargement) of existing synapses (215, 216). This association suggests, in principle at least, that NMDA mediated LTP and developmental synaptic plasticity may be related processes that differ in degree but share the same mechanisms and end points.

NMDA receptors appear to be necessary for the initiation of LTP in most, but not all, pathways so far examined. Although the mossy fiber pathway in the rodent hippocampus displays LTP, the terminal field for this pathway has a relative paucity of NMDA receptors and quite high levels of kainate receptors (71). Direct examination of the pharmacological properties of LTP within this pathway indicates that NMDA receptor blockade does not block LTP formation (217). These results indicate that there are at least two distinct mechanisms for the generation of LTP and that these can be clearly separated by NMDA antagonists. Given the high density of kainate receptors in the mossy fiber pathway, it is tempting to speculate that these receptors may be involved in LTP formation in this pathway.

EAA and Cell Growth

In an attempt to define the possible mechanisms through which EAA receptors may be involved in plasticity, investigators have begun to directly examine the effect of EAAs on neural growth. Studies have demonstrated that increasing subtoxic concentrations of L-glutamate, quisqualate, and kainate could reduce the growth rates and lengths of dendrites without altering axonal growth in cultured hippocampal pyramidal cells (218). The ability to attenuate these effects with either DGG or calcium channel blockers, but not with D-AP5, suggests the effects on growth occurred as a result of the opening of voltage-gated calcium channels by quisqualate and kainate receptor activation. In cultures of dentate granule cells, Brewer and coworkers (219) have shown that NMDA and the noncompetitive NMDA antagonist MK-801 can modify the length and branching pattern of neural outgrowth. Although preliminary, these findings suggest that EAAs not only function in standard neurotransmission but potentially play a role in neural growth.

EXCITOTOXIC ACTIONS OF THE EAA RECEPTORS

Coincident with the early characterization of the excitatory action of L-glutamate by Curtis & Watkins (5, 6), Lucas & Newhouse (220) described the neurodegeneration in the inner layers of the retina produced by the administration of L-glutamate to immature mice. General interest in this area was later stimulated by the work of Olney and colleagues, who first demonstrated that systemic administration of L-glutamate to mice, rats, and monkeys produced substantial neuronal loss in those brain regions outside the protective influence of the blood-brain barrier (e.g. arcuate nucleus) (221), [for review see (222)]. Comparisons of the electrophysiological and toxicological action of these various compounds revealed that those L-glutamate analogs that most potently depolarize neurons were also the most potent toxins; these results led to the hypothesis of excitotoxicity (223). In light of the high concentrations of L-glutamate found in the brain, L-glutamate was suggested as a potential mechanism of neuronal loss in disease. Interest in and support for this hypothesis increased dramatically when the neuronal pathology induced by various excitotoxins was found to be similar to that associated with a wide spectrum of neurological insults. For example, the axon-sparing dendritic lesions produced by EAA agonists are similar to the neuronal damage found in anoxia, ischemia, hypoglycemia, and epilepsy (224–228). The probable involvement of the EAA receptors in an underlying mechanism in this pathological neurodegeneration strengthened by the dramatic illustration that EAA antagonists, particularly those of the NMDA receptor, could block the neuronal death in experimental models of these neuropathological conditions. Thus, administration of NMDA antagonists reduced the cell loss associated with ischemia, anoxia (229, 230) and hypoglycemia (231). For each of these conditions, studies are now determining the degree of insult that can be ameliorated by NMDA antagonists. Work by Meldrum and others has also demonstrated the anticonvulsive and antiepileptic action of NMDA antagonists (232). In light of these findings, EAAs and their receptors are, not surprisingly, a common focus of neurophysiologic and neuropathologic investigations.

The process of excitotoxicity is also readily demonstrable in vitro, where the external environment of the neuronal tissue is more amenable to manipulation. L-Glutamate, kainate, and NMDA produce substantial neurodegeneration in preparations of chick retina (233). Rat cortical slices were used (234) to demonstrate the neurotoxic action of kainate. Choi and collaborators have described the neurotoxic effects of L-homocysteic acid, quinolinic acid, L-glutamate, and kainate on mouse cortical cultures (235–237). Cultured hippocampal pyramidal cells have also been shown to be vulnerable to excitotoxicity mediated by NMDA (238). Consistent with the in vivo studies, NMDA

antagonists reportedly attenuate excitotoxic responses and permit the more selective investigation of the involvement of the NMDA receptor. Thus, the retinal degeneration produced by NMDA could be prevented by the inclusion of D-AP5 in the incubation media (233). Similarly, D-AP5 was found to protect cultured neurons from the toxic action of quinolinic acid and homocysteic acid (237).

NMDA vs non-NMDA Receptor Excitotoxicity

Although the protective effect of NMDA antagonists highlights the involvement of NMDA receptors in excitotoxic mechanisms, it is noteworthy that agonists at other receptor classes can also mediate excitotoxic cell death. This involvement has been well documented for kainate even prior to the discovery of the NMDA receptor class. In the hippocampus (150, 239), retina (233), and other brain regions, the various EAA agonists produce different patterns of excitotoxic damage. That multiple receptor classes could be involved in neuropathological conditions is suggested by the kainate-like pattern of cell death observed after excessive perforant path activation (240) and the NMDA-like lesions observed in the striatum in Huntington's disease (55).

Non–NMDA receptor mediated excitotoxicity is also indicated as a potential mechanism for the exogenous neurotoxin β-N-oxalyl-L-α,β-diaminopropionic acid (β-L-ODAP). This analog of L-glutamate has been identified as the causative agent in human neural lathyrisms [for review see (241)] and has been shown to preferentially interact with AMPA and kainate receptors (242). These results suggest that neuronal susceptibility to excitotoxic death by selective agonists is dependent upon the compliment of excitatory receptors that it possesses.

Excitotoxic Mechanisms

Of obvious importance is the elucidation of the biochemical events in the excitotoxic processes that occur after excitatory receptor activation. Although our knowledge of such mechanisms is far from complete, several experiments have identified an apparent ionic dependence of excitotoxicity. Particular attention has been paid to the possible involvement of Ca^{++}, which has long been believed to be involved in cellular degeneration (243). Jancso et al (244) reported increased intracellular accumulation of Ca^{++} in the areas damaged by glutamate administration to rats and suggested its role in excitotoxicity. Consistent with these findings, is the pronounced Ca^{++} dependence of the excitotoxicity elicited by kainate, quisqualate, and NMDA (234). Experiments in neuronal cultures have also demonstrated that by excluding the Ca^{++} from the extracellular media, the cell death produced by exposure to L-glutamate could be attenuated (245, 246). Further, the ion channel associ-

ated with the NMDA receptor was suggested to represent a major route of entry for this excessive Ca^{++} influx.

In contrast, other investigations have indicated that Ca^{++} may not be directly responsible for the neuronal death and have demonstrated that pretreatment with a calcium channel blocker (nimodipine) did not protect against glutamate toxicity (247). Studies by Rothman (248) and Olney et al (249) tended to support the Ca^{++} independence of the excitotoxicity and further proposed that the neuronal death is attributable to osmotic lysis that results from the passive influx of Cl^- ions. Current models, however, now include a contribution from both of these effects and divide the excitotoxic effect of L-glutamate into at least two components: those attributable to osmotic damage caused by ion influx and those attributable to Ca^{++}-related damage (233, 246).

EXCITATORY AMINO ACID TRANSPORT SITES

The process of the transport of L-glutamate into glial and neuronal compartments is a particularly important component of the EAA transmitter systems, as it is the mechanism responsible for the termination of the excitatory signal of L-glutamate [for review see: (250–251)]. Conditions that alter the efficacy of this removal, whether the result of decreased transport or excessive substrate, could potentially produce levels of L-glutamate or another EAA agonist sufficient to result in excitotoxic conditions and eventual cell death. Such a scheme of events is illustrated by the ability of transport inhibitors to potentiate the effects of agonists (252–254). Although the transport of the synaptically released glutamate has most often been attributed entirely to the action of high-affinity sodium-dependent uptake, recent evidence suggests that chloride-dependent systems may also be involved. The presence of this chloride-dependent transport process was brought to light in the course of studying chloride-dependent L-[^{3}H]glutamate binding (refer to above discussion on the AP4 receptor). Subsequently, chloride-dependent transport of L-glutamate into synaptosomes has been directly demonstrated (185). Kinetic analysis is still needed to assess the contribution of these systems to the total uptake of L-glutamate. The respective contributions may vary between brain regions; recent studies by Anderson et al (255) have shown a differential distribution of the sodium-dependent and chloride-dependent transport systems by radioligand autoradiography. The demonstration that astrocytes also possess chloride-dependent L-[^{3}H]glutamate binding sites and that glial cell lines are capable of transporting L-glutamate by chloride-dependent processes suggests that a similar chloride-dependent uptake system may also be present on astrocytes (183, 184).

Although speculative, the transport of L-glutamate may represent another

site at which excitatory transmission may be controlled. By altering the rate at which L-glutamate is removed from the synaptic cleft, the excitatory signal of L-glutamate can be modified. Thus, in the presence of abnormally high levels of L-glutamate, increased transport could serve as a protective mechanism to avoid excitoxic damage; conversely, in the instance of compromised transmission, decreased transport could augment the excitatory signal. That such mechanisms might be operative is suggested by the demonstration of a transient increase in the radioligand binding to sodium- and chloride-dependent transport sites in the dentate gyrus molecular layer of rats given entorhinal lesions (256). This apparent increased ability to transport L-glutamate may represent a mechanism by which the remaining neurons are protected from excitotoxic damage. On the other hand, the reported decrease in sodium-dependent L-glutamate transport in patients with Alzheimer's disease (257) may represent an attempt to compensate for decreased transmission attributable to neuronal loss. It is noteworthy, however, that the decreased transport of the excitatory compounds should also increase the vulnerability to excitotoxicity. Thus, what may have begun as a compensatory mechanism to increase transmission efficiency, could, in the long run, be responsible for the later death of the remaining cells by excitotoxic processes (see Figure 3).

CONCLUSION

It is now clear that there are at least five distinct receptors (NMDA, kainate, AMPA, AP4, and ACPD), each displaying distinct physiological characteristics. EAA synaptic transmission does not appear to follow the simple model of fast-acting synaptic transmission mediated by a single receptor class. Individual EAA-using synapses may use distinct receptors or combinations of

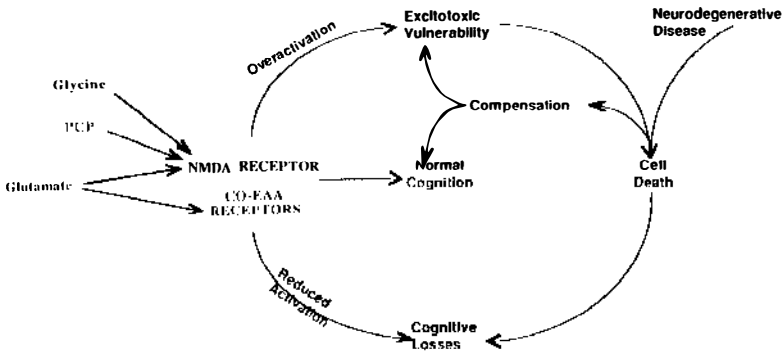

Figure 3 The NMDA receptor complex and co-Excitatory Amino Acid Receptors and the balance between normal function, compensation, and excitotoxic pathology.

receptors and thereby exhibit different input/output properties and second messenger responses. For example, the most commonly described EAA synapse contains NMDA receptors and either an AMPA or kainate receptor. At such a synapse, AMPA/kainate receptors would mediate the fast voltage-independent synaptic response and in turn promote the activation of the voltage-dependent NMDA receptors. The voltage-dependent calcium permeability of the NMDA receptor channel provides a mechanism for activity-dependent regulation of intracellular calcium. This mechanism satisfies the conjunctive requirements of Hebbian synapses and probably forms the basis for activity-dependent changes, such as refinement of synaptic position during development and the establishment of LTP.

The autoradiographic demonstration that NMDA receptors colocalize to a much greater degree with AMPA rather than kainate receptors suggests that the NMDA and AMPA receptors may represent a functional unit. The ratios of these two receptors also may vary between synapses, which would result in an AMPA-rich or NMDA-rich character. NMDA receptor activation displays an apparent requirement for glycine (or a related compound), which binds to a site on the NMDA receptor complex. In addition, the NMDA receptor may also exist in two forms, agonist- vs antagonist-preferring, similar to that found for GABA receptors. Protein phosphorylation mechanisms may also be involved in regulating NMDA receptor activity.

In principle, the newly described ACPD receptor also has the capacity to exert long-term control over various neuronal processes (e.g. synaptic efficacy) through its regulation of IP formation. Activation of IP metabolism via ACPD receptors is in turn inhibited by NMDA receptor activation. The combination of these two receptors provides a mechanism that can regulate calcium levels from either intracellular or extracellular stores, regulate IP formation, and regulate the overall activity of the neuron. Consistent with a special role in synaptic growth and network consolidation, the ACPD receptor is much more active during development and, possibly, regeneration.

In addition to the type of synapse described above (i.e. containing NMDA and AMPA/kainate receptors), others appear to use different EAA receptor combinations and consequently display differing physiological properties. In the retina, AP4 receptors mediate postsynaptic hyperpolarizations, while in select hippocampal pathways they appear to represent L-glutamate autoreceptors that provide for negative feedback regulation of synaptic activity. In the hippocampal mossy fiber pathway, different anatomical distributions suggest that kainate receptors probably function independently of NMDA receptors. This pathway, which has high concentrations of kainate receptors and low concentrations of NMDA receptors, shows a form of LTP not mediated by NMDA receptors. Thus, in addition to NMDA receptors, other EAA receptors clearly participate in plasticity mechanisms such as LTP and neuronal growth.

Current evidence also suggests that these receptors not only mediate plasticity but can trigger neuronal degeneration and cell death. The toxicity mediated by kainate and NMDA receptors is well documented in many systems. In addition, overactivation of the AMPA receptor may also lead to cell loss (e.g. β-L-ODAP toxicity). As the excitatory action of the EAA is terminated by transport, alterations in this process could also lead to excitotoxic damage. Thus, it may be important that the sodium-dependent and chloride-dependent transport systems appear to be affected by injury and may play a key role in the associated secondary cell loss in several diseases (e.g. Alzheimer's).

Given that EAA-using synapses comprise the vast majority of synapses, prevailing particularly in major cortical systems, new principles of synaptic transmission and integration should emerge. Indeed, research just over the past year is fulfilling this expectation and has led to many new avenues for multidisciplinary study not only in plasticity but also pathology.

Literature Cited

1. Multiple authors 1987. Excitatory amino acids in the brain—focus on NMDA receptors. *Trends Neurosci.* 10:263–302
2. Cotman, C. W., Monaghan, D. T., Ganong, A. H. 1988. Excitatory amino acid neurotransmission: NMDA receptors and Hebb-type synaptic plasticity. *Annu. Rev. Neurosci.* 11:61–80
3. Fagg, G. E., Foster, A. C., Ganong, A. H. 1986. Excitatory amino acid synaptic mechanisms and neurological function. *Trends Pharmacol. Sci.* 7:357–63
4. Stone, T. W., Burton, N. R. 1988. NMDA receptors and ligands in the vertebrate CNS. *Prog. Neurobiol.* 30:333–68
5. Curtis, D. R., Watkins, J. C. 1960. The excitation and depression of spinal neurons by structurally-related amino acids. *J. Neurochem.* 6:117–41
6. Curtis, D. R., Watkins, J. C. 1963. Acidic amino acids with strong excitatory actions on mammalian neurones. *J. Physiol.* 166:1–14
7. McLennan, H., Huffman, R. D., Marshall, K. C. 1968. Patterns of excitation of thalamic neurones by amino-acids and by acetylcholine. *Nature* 219:387–88
8. Duggan, A. W. 1974. The differential sensitivity to L-glutamate and L-aspartate of spinal interneurones and renshaw cells. *Exp. Brain Res.* 19:522–28
9. Johnston, G. A. R., Curtis, D. R., Davies, J., McCulloch, R. M. 1974. Spinal interneurone excitation by conformationally restricted analogues of L-glutamic acid. *Nature* 248:804–5
10. Biscoe, T. J., Evans, R. H., Francis, A. A., Martin, M. R., Watkins, J. C., Davies, J., Dray, A. 1977. D-α-aminoadipate as a selective antagonist of amino acid-induced and synaptic excitation of mammalian spinal neurones. *Nature* 270:743–45
11. Hall, J. G., McLennan, H., Wheal, H. V. 1977. The actions of certain amino acids as neuronal excitants. *J. Physiol.* 272:52–53
12. Davies, J. D., Francis, A. A., Jones, A. W., Watkins, J. C. 1981. 2-Amino-5-phosphonovalerate (2-APV), a potent and selective antagonist of amino acid-induced and synaptic excitation. *Neurosci. Lett.* 21:77–81
13. Davies, J. D., Evans, R. H., Francis, A. A., Watkins, J. C. 1979. Excitatory amino acid receptors and synaptic excitation in the mammalian central nervous system. *J. Physiol.* 75:641–54
14. Mayer, M. L., Westbrook, G. L. 1984. Mixed-agonist action of excitatory amino acids on mouse spinal cord neurons under voltage clamp. *J. Physiol.* 354: 29–53
15. Baudry, M., Lynch, G. 1979. Characterization of two L-[^{3}H]glutamate binding sites in rat hippocampal membranes. *Eur. J. Pharmacol.* 57:283–85
16. Foster, A. C., Roberts, P. J. 1978. High affinity L-[^{3}H]glutamate binding to postsynaptic receptor sites on rat cerebellar membranes. *J. Neurochem.* 31:1467–77
17. Honore, T., Lauridsen, J., Krogsgaard-Larsen, P. 1982. The binding of

[^{3}H]AMPA, a structural analogue of glutamic acid, to rat brain membranes. *J. Neurochem.* 38:173–78
18. London, E. D., Coyle, J. T. 1979. Specific binding of ^{3}H-kainic acid to receptor sites in rat brain. *Mol. Pharmacol.* 15:492–505
19. Michaelis, E. K., Michaelis, M. L., Boyarsky, L. L. 1974. High-affinity glutamate binding to brain synaptic membranes. *Biochim. Biophys. Acta* 367:338–48
20. Roberts, P. J. 1974. Glutamate receptors in the rat CNS. *Nature* 252:399–401
21. Simon, J. R., Contrera, J. F., Kuhar, M. J. 1976. Binding of [^{3}H]kainic acid, an analogue of L-glutamate to brain membranes. *J. Neurochem.* 26:141–47
22. Mena, E. E., Fagg, G. E., Cotman, C. W. 1982. Chloride ions enchance L-glutamate binding to rat synaptic membranes. *Brain Res.* 243:378–81
23. Fagg, G. E., Foster, A. C., Mena, E. E., Cotman, C. W. 1982. Chloride and calcium ions reveal a pharmacologically distinct population of L-glutamate binding sites in synaptic membranes: correspondence between biochemical and electrophysiological data. *J. Neurosci.* 2:958–65
24. Monaghan, D. T., Holets, V. R., Toy, D. W., Cotman, C. W. 1983. Anatomical distributions of four pharmacologically distinct ^{3}H-L-glutamate binding-sites. *Nature* 306:176–79
25. Fagg, G. E., 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* 81:6876–80
26. Greenamyre, J. T., Olson, J. M., Penney, J. B., Young, A. B. 1985. Autoradiographic characterization of NMDA-, quisqualate-, and kainate-sensitive glutamate binding sites. *J. Pharmacol. Exp. Ther.* 233:254–63
27. Monaghan, D. T., Yao, D., Cotman, C. W. 1985. L-^{3}H-glutamate binds to kainate-, NMDA-, and AMPA-sensitive binding sites: an autoradiographic analysis. *Brain Res.* 340:378–83
28. Monaghan, D. T., Yao, D., Olverman, H. J., Watkins, J. C., Cotman, C. W. 1984. Autoradiography of D-[^{3}H]2-amino-5-phosphonopentanoate binding-sites in rat-brain. *Neurosci. Lett.* 52:253–58
29. Olverman, H. J., Jones, A. W., Watkins, J. C. 1984. L-glutamate has higher affinity than other amino-acids for [^{3}H]D-AP5 binding-sites in rat-brain membranes. *Nature* 307:460–62
30. Monaghan, D. T., Cotman, C. W. 1982. Distribution of ^{3}H-kainic acid binding sites in rat CNS as determined by autoradiography. *Brain Res.* 252:91–100
31. Unnerstall, J. R., Wamsley, J. K. 1983. Autoradiographic localization of high-affinity ^{3}H-kainic acid binding sites in the rat forebrain. *Eur. J. Pharmacol.* 86:361–71
32. Monaghan, D. T., Yao, D., Cotman, C. W. 1984. Distribution of ^{3}H-AMPA binding sites in rat brain as determined by quantitative autoradiography. *Brain Res.* 324:160–64
33. Olsen, R. W., Szamraj, O., Houser, C. R. 1987. [^{3}H] AMPA binding to glutamate receptor subpopulations in rat brain. *Brain Res.* 402:243–54
34. Rainbow, T. C., Wieczorek, C. M., Halpain, S. 1984. Quantitatve autoradiography of binding-sites for ^{3}H-AMPA, a structural analogue of glutamic-acid. *Brain Res.* 309:173–77
35. Davies, J., Evans, R. H., Herrling, P. L., Jones, A. W., Olverman, H. J., et al. 1986. CPP, a new potent and selective NMDA antagonist. Depression of central neuron responses, affinity for D-[^{3}H]AP5 binding sites on brain membranes and anticonvulsant activity. *Brain Res.* 382:169–73
36. Harris, E. W., Ganong, A. H., Monaghan, D. T., Watkins, J. C., Cotman, C. W. 1986. Action of 3-(C±)-2-carboxypiperazin - 4 - yl)-propyl-1-phosphonic acid (CCP): a new and highly potent antagonist of N-methyl-D-aspartate receptors in the hippocampus. *Brain Res.* 382:174–77
37. Lehmann, J., Schneider, J., McPherson, S., Murphy, D. E., Bernard, P., et al. 1987. CPP, a selective N-methyl-D-aspartate (NMDA)-type receptor antagonist: characterization *in vitro* and *in vivo*. *J. Pharmacol. Exp. Ther.* 240: 737–46
38. Lehmann, J., Hutchson, A. J., McPherson, S. E., Mondadori, C., Schmutz, M., et al. 1988. CGS 19755, a selective and competitive N-methyl-D-aspartate-type excitatory amino acid receptor antagonist. *J. Pharmacol. Exp. Ther.* 246:65–72
39. Fagg, G. E., Olpe, H.-R., Bittiger, H., Schmutz, M., Angst, C., et al. 1988. Potent and selective competitive NMDA receptor antagonists with oral activity. *Soc. Neurosci.* 14:941 (Abstr.)
40. Schmutz, M., Klebs, K., Olpe, H.-R., Fagg, G. E., Allgeier, H., et al. 1988. Competitive NMDA receptor an-

tagonists with potent oral anticonvulsant activity. *Soc. Neurosci.* 14:864 (Abstr.)
41. Olverman, H. J., Jones, A. W., Mewett, K. N., Watkins, J. C. 1988. Structure/activity relations of N-methyl-D-aspartate receptor ligands as studied by their inhibition of [^{3}H]D-2-amino-5-phosphonopentanoic acid binding in rat brain membranes. *Neuroscience.* 26:17–31
42. Olverman, H. J., Jones, A. W., Watkins, J. C. [^{3}H]-2-Amino-5-phosphonopentanoate as a ligand for N-methyl-D-aspartate receptors in the mammalian central nervous system. *Neuroscience.* 26:1–15
43. Jarvis, M. F., Murphy, D. E., Williams, M. 1987. Quantitative autoradiographic localization of NMDA receptors in rat brain using [^{3}H]CPP: comparison with [^{3}H]TCP binding sites. *Eur. J. Pharmacol.* 141:149–52
44. Monaghan, D. T., Olverman, H. J., Nguyen, L., Watkins, J. C., Cotman, C. W. 1988. Two classes of NMDA recognition sites: Differential distribution and differential regulation by glycine. *Proc. Natl. Acad. Sci. USA* 85:9836–40
45. Murphy, D. E., Schneider, J., Boehm, C., Lehmann, J., Williams, M. 1987. Binding of [^{3}H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid to rat brain membranes: a selective, high-affinity ligand for N-methyl-D-aspartate receptors. *J. Pharmacol. Exp. Ther.* 240: 778–84
46. Olverman, H. J., Monaghan, D. T., Cotman, C. W., Watkins, J. C. 1986. [^{3}H]CPP, a new competitive ligand for NMDA receptors. *Eur. J. Pharmacol.* 131:161–62
47. Maragos, W. F., Chu, D. C. M., Greenamyre, J. T., Penney, J. B., Young, A. B. 1986. High correlation between localization of [^{3}H]TCP binding and NMDA receptors. *Eur. J. Pharmacol.* 123:173–74
48. Monaghan, D. T., Cotman, C. W. 1985. Distribution of NMDA-sensitive L-^{3}H-glutamate binding sites in rat brain as determined by quantitative autoradiography. *J. Neurosci.* 5:2909–19
49. Cowburn, R. F., Hardy, J. A., Roberts, P. J. 1988. Characterisation of Na^+-independent L-[^{3}H]glutamate binding sites in human temporal cortex. *J. Neurochem.* 50:1872–78
50. Foster, A. C., Fagg, G. E. 1987. Comparison of L-[^{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
51. Monaghan, D. T., Cotman, C. W. 1986. Identification and properties of NMDA receptors in rat brain synaptic plasma membranes. *Proc. Natl. Acad. Sci. USA* 83:7532–36
52. Monahan, J. B., Michel, J. 1987. Identification and characterization of an N-methyl-D-aspartate-specific L-[^{3}H] glutamate recognition site in synaptic plasma membranes. *J. Neurochem.* 48:1699–1708
53. Ogita, K., Yoneda, Y. 1988. Disclosure by triton x-100 of NMDA-sensitive [^{3}H]glutamate binding sites in brain synaptic membranes. *Biochem. Biophys. Res. Comm.* 153:510–17
54. Geddes, J. W., Chang-Chui, H., Cooper, S. M., Lott, I. T., Cotman, C. W. 1986. Density and distribution of NMDA receptors in the human hippocampus in Alzheimer's disease. *Brain Res.* 399:156–61
55. Young, A. B., Greenamyre, J. T., Hollingsworth, Z., Albin, R., D'Amato, C., et al. 1988. NMDA receptor losses in putamen with Huntington's disease. *Science* 241:981–84
56. Johnson, J. W., Ascher, P. 1987. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* 325:529–31
57. Kleckner, N. W., Dingledine, R. 1988. Requirement for glycine in activation of NMDA-receptors expressed in *Xenopus* oocytes. *Science* 241:835–37
58. Mayer, M. L., Westbrook, G. L., Vyklicky, L. 1988. Sites of antagonist action on N-methyl-D-aspartic acid receptors studied using fluctuation analysis and a rapid perfusion technique. *J. Neurophysiol.* 60:645–63
59. Reynolds, I. J., Murphy, S. N., Miller, R. J. 1987. ^{3}H-labeled MK-801 binding to the excitatory amino acid receptor complex from rat brain is enhanced by glycine. *Proc. Natl. Acad. Sci. USA* 84:7744–48
60. Barnard, E. A., Darlison, M. G., Seeburg, P. 1987. Molecular biology of the GABA receptor: the receptor/channel superfamily. *Trends Neurosci.* 10:502–9
61. Kessler, M., Tarramani, T., Lynch, G., Baudry, M. 1989. A glycine site associated with NMDA receptors: Characterization and identification of a new class of antagonists. *J. Neurochem.* In press
62. Wong, E. H. F., Knight, A. R., Ransom, R. 1987. Glycine modulates [^{3}H]MK801 binding to the NMDA

receptor in rat brain. *Eur. J. Pharmacol.* 142:487–88

63. Snell, L. D., Morter, R. S., Johnson, K. M. 1988. Structural requirements for activation of the glycine receptor that modulates the N-methyl-D-aspartate operated ion channel. *Eur. J. Pharmacol.* In press
64. Evans, R. H., Evans, S. J., Pook, P. C., Sunter, D. C. 1987. A comparison of excitatory amino acid antagonists acting at primary afferent C fibers and motoneurons of the isolated spinal cord of the rat. *Br. J. Pharmacol.* 91:531–37
65. Snell, L. D., Johnson, K. M. 1988. Cycloleucine competitively antagonizes the strychnine-insensitive glycine receptor. *Eur. J. Pharmacol.* 151:165–66
66. Fletcher, E. J., Lodge, D. 1988. Glycine modulation of NMDA antagonist action in rat neocortex slices. *J. Physiol.* In press
67. Kemp, J. A., Foster, A. C., Leeson, P. D., Priestley, T., Tridgett, R., Iversen, L. L. 1988. 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the N-methyl-D-aspartate receptor complex. *Proc. Natl. Acad. Sci. USA* 85:6547–50
68. Frey, P., Berney, D., Herrling, P., Werner, M., Urwyler, S. 1988. 6,7-Dichloro-3-hydroxy - 2 - quinoxalinecarboxylic acid is a relatively potent antagonist at NMDA and kainate receptors. *Neurosci. Lett.* 91:194–98
69. Birch, P. J., Grossman, C. J., Hayes, A. G. 1988. Kynurenate and FG9041 have both competitive and non-competitive antagonist actions at excitatory amino acid receptors. *Eur. J. Pharmacol.* 151:313–15
70. Bristow, D. R., Bowery, N. G., Woodruff, G. N. 1986. Light microscopic autoradiographic localisation of [^{3}H] strychnine binding sites in rat brain. *Eur. J. Pharmacol.* 126:303–7
71. Cotman, C. W., Monaghan, D. T., Ottersen, O. P., Storm-Mathisen, J. 1987. Anatomical organization of excitatory amino acid receptors and their pathways. *Trends Neurosci.* 10:273–79
72. Kishimoto, H., Simon, J. R., Aprison, M. H. 1981. Determination of the equilibrium dissociation constants and number of glycine binding sites in several areas of the rat central nervous system, using a Na-independent system. *J. Neurochem.* 37:1015–24
73. Johnson, K. M., Snell, L. D., Jones, S. M., Qi, H.-Q. 1988. Glycine antagonist activity of simple glycine analogues and N-methyl-D-aspartate receptor antagonists. In *Frontiers in Excitatory Amino Acid Research*, ed. E. A. Cavalheiro, J. Lehmann, L. Turski. New York: Liss. 745 pp.
74. MacDonald, J. F., Porietis, A. V., Wojtowicz, J. M. 1982. L-Aspartic acid induces a region of negative slope conductance in the current voltage relationship of cultured spinal cord neurons. *Brain Res.* 237:248–53
75. Flatman, J. A., Schwindt, P. C., Crill, W. E., Strafstrom, C. E. 1983. Multiple actions of N-methyl-D-aspartate on cat neocortical neurons *in vitro*. *Brain Res.* 266:169–73
76. Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., Prochiantz, A. 1984. Magnesium gates glutamate-activated channels in mouse central neurons. *Nature* 307:462–65
77. Mayer, M. L., Westbrook, G. L., Guthrie, P. B. 1984. Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* 309:261–63
78. MacDermott, A. B., Mayer, M. L., Westbrook, G. L., Smith, S. J., Barker, J. L. NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature* 321:519–22
79. Mayer, M. L., MacDermott, A. B., Westbrook, G. L., Smith, S. J., Barker, J. L. 1987. Agonist- and voltage-gated calcium entry in cultured mouse spinal-cord neurons under voltage clamp measured using arsenazo-III. *J. Neurosci.* 7:3230–44
80. Ascher, P., Nowak, L. 1986. Calcium permeability of the channels activated by N-methyl-D-aspartate (NMDA) in isolated mouse central neurons. *J. Physiol.* 377:P35
81. Jahr, C. E., Stevens, C. F. 1987. Glutamate activates multiple single channel conductances in hippocampal neurons. *Nature* 325:522–25
82. Mayer, M. L., Westbrook, G. L. 1985. Divalent cation permeability of N-methyl-D-aspartate channels. *Soc. Neurosci.* 11:785 *Abstr.*
83. Lynch, G., Larson, J., Kelso, S., Barrionuevo, G., Schottler, F. 1983. Intracelluar injections of EGTA block induction of hippocampal long-term potentiation. *Nature* 305:719–21
84. Collingridge, G. L., Bliss, T. V. P. 1987. NMDA receptors-their role in long term potentiation. *Trends Neurosci.* 10:288–94
85. Rothman, S. M., Olney, J. W. 1986. Glutamate and the patho-physiology of

hypoxic/ischemic brain-damage. *Ann. Neurol.* 19:105–11
86. Anis, N. A., Berry, S. C., Burton, N. R., Lodge, D. 1983. The dissociative anaesthetics ketamine and phencyclidine, selectively reduce excitation of central mammalian neurons by *N*-methyl-aspartate. *Br. J. Pharmacol.* 79:565–75
87. Lodge, D., Anis, N. A., Burton, N. R. 1982. Effects of optical isomers of ketamine on excitation of cat and rat spinal neurons by amino-acids and acetylcholine. *Neurosci. Lett.* 29:281–86
88. Duchen, M. R., Burton, N. R., Biscoe, T. J. 1985. An intracellular study of the interactions of N-methyl-DL-aspartate with ketamine in the mouse hippocampal slice. *Brain Res.* 342:149–53
89. Harrison, N. L., Simmons, M. A. 1985. Quantatative studies on some antagonists of *N*-methyl-D-aspartate in slices of rat cerebral cortex. *Br. J. Pharmacol.* 84:381–91
90. Lodge, D., Johnston, G. A. R. 1985. Effect of ketamine on amino acid-evoked release of acetylcholine from rat cerebral cortex *in vitro*. *Neurosci. Lett.* 56:371–75
91. Thomson, A. M., West, D. C., Lodge, D. 1985. An N-methylaspartate receptor-mediated synapse in rat cerebral cortex: a site of action of ketamine. *Nature* 313:479–81
92. Wong, E. H. F., Kemp, J. A., Priestley, T., Knight, A. R., Woodruff, G. N., Iverson, L. L. 1986. The anti-convulsant MK-801 is a potent *N*-methyl-D-aspartate antagonist. *Proc. Natl. Acad. Sci. USA* 83:7104–8
93. Huettner, J. E., Bean B. P. 1988. Block of N-methyl-D-aspartate-activated current by the anticonvulsant MK-801: Selective binding to open channels. *Proc. Natl. Acad. Sci. USA* 85:1307–11
94. Martin, D., Lodge, D. 1986. Ketamine acts as a non-competitive *N*-methyl-D-aspartate antagonist on frog spinal cord *in vitro*. *Neuropharmacology* 24:999–1003
95. Honey, C. R., Miljkovic, Z., MacDonald, J. F. 1985. Ketamine and phencyclidine cause a voltage-dependant block of responses to L-aspartic acid. *Neurosci. Lett.* 61:135–39
96. MacDonald, J. F., Miljkovic, Z., Pennefather, P. 1987. Use-dependent block of excitatory amino-acid currents in cultured neurons by ketamine. *J. Neurophysiol.* 58:251–65
97. Woodruff, G. N., Foster, A. C., Gill, R., Kemp, J. A., Wong, E. H. F., et al 1987. The interaction between MK-801 and receptors for N-methyl-D-aspartate: functional consequences. *Neuropharmacology* 26:903–9
98. Largent, B. L., Gundlach, A. L., Snyder, S. H. 1986. Pharmacological and autoradiographic discrimination of sigma and phencyclidine receptor binding sites in brain with (+)-[^{3}H] SKF 10,047, (+) - [^{3}H] - 3-[3-Hydroxyphenyl]-N-(1-propyl)piperidine and [^{3}H]-1-[1-(2-Thienyl) cyclohexyl]piperidine. *J. Pharmacol. Exp. Ther.* 238:739–48
99. Coan, E. J., Collingridge, G. L. 1987. Effects of phencyclidine, SKF 10,047 and related psychotomimetic agents on N-methyl-D-aspartate receptor mediated synaptic responses in rat hippocampal slices. *Br. J. Pharmacol.* 91:547–56
100. Jones, S. M., Snell, L. D., Johnson, K. M. 1987. Phencyclidine selectively inhibits N-methyl-D-aspartate-induced hippocampal [^{3}H]norepinephrine release. *J. Pharmacol. Exp. Ther.* 240:492–97
101. Lodge, D., Aram, J. A., Fletcher, E. J. 1988. Modulation of N-methylaspartate receptor-channel complexes: an overview. See Ref. 73, pp. 527–34
102. Kemp, J. A., Foster, A. C., Wong, E. H. F. 1987. Non-competitive antagonists of excitatory amino acid receptors. *Trends Neurosci.* 10:294–98
103. Bowery, N. G., Wong, E. H. F., Hudson, A. L. 1988. Quantitative autoradiography of [^{3}H]-MK-801 binding sites in mammalian brain. *Br. J. Pharmacol.* 93:944–54
104. Maragos, W. F., Penney, J. B., Young, A. B. 1988. Anatomic correlation of NMDA and [^{3}H]TCP-labelled receptors in rat brain. *J. Neurosci.* 8:493–501
105. Sircar, R., Zukin, S. R. 1985. Quantitative localization of [^{3}H]TCP binding in rat brain by light microscopic autoradiography. *Brain Res.* 344:142–45
106. Vignon, J., Chicheportiche, R., Chicheportiche, M., Kamenka, J.-M., Geneste, P., Lazdunski, M. 1983. [^{3}H]TCP: A new tool with high affinity for the PCP receptor in rat brain. *Brain. Res.* 280:194–97
107. Loo, P., Braunwalder, A., Lehmann, J., Williams, M. 1986. Radioligand binding to central phencyclidine recognition sites is dependant on excitatory amino-acid receptor tagonists. *Eur. J. Pharmacol.* 123:467–68
108. Benavides, J., Rivy, J.-P., Carter, C., Scatton, B. 1988. Differential modulation of [^{3}H]TCP binding to the NMDA

receptor by L-glutamate and glycine. *Eur. J. Pharmacol.* 149:67–72
109. Fagg, G. E. 1987. Phencyclidine and related drugs bind to the N-methyl-D-aspartate receptor-channel complex in rat brain membranes. *Neurosci. Lett.* 76:221–27
110. Javitt, D. C., Jotkowitz, A., Sircar, R., Zukin, S. R. 1987. Non-competitive regulation of phencyclidine/σ-receptors by the N-methyl-D-aspartate receptor antagonist D-(-)-2-amino-5-phosphonovaleric acid. *Neurosci. Lett.* 78:193–98
111. Johnson, K. M., Sacaan, A. I., Snell, L. D. 1988. Equilibrium analysis of [^{3}H]TCP binding: effects of glycine, magnesium and N-methyl-D-aspartate agonists. *Eur. J. Pharmacol.* 152:141–46
112. Kloog, Y., Haring, R., Sokolovsky, M. 1988. Kinetic characterisation of the phencyclidine-N-methyl-D-aspartate receptor interaction: evidence for a steric blockade of the channel. *Biochemistry* 27:843–48
113. Loo, P., Braunwalder, A., Lehmann, J., Williams, M., Sills, M. A. 1987. Interaction of L-glutamate and magnesium with phencyclidine recognition sites in rat-brain: evidence for multiple affinity states of the phencyclinine N-methyl-D-aspartate receptor complex. *Mol. Pharmacol.* 32:820–30
114. Foster, A. C., Wong, E. H. F. 1987. The novel anticonvulsant MK-801 binds to the activated state of the N-methyl-D-aspartate receptor in rat brain. *Br. J. Pharmacol.* 91:403–9
115. Kloog, Y., Nadler, V., Sokolovsky, M. 1988. Mode of binding of [3H]dibenzocycloalkenimine (MK-801) to the N-methyl-D-aspartate (NMDA) receptor and its therapeutic implication. *FEBS Lett.* 230:167–70
116. Snell, L. D., Morter, R. S., Johnson, K. M. 1987. Glycine potentiates N-Methyl-D-Aspartate-induced [^{3}H]TCP binding to rat cortical membranes. *Neurosci. Lett.* 83:313–17
117. Thomas, J. W., Hood, W. F., Monahan, J. B., Contreras, P. C., O'Donohue, T. L. 1988. Glycine modulation of the phencyclidine binding site in mammalian brain. *Brain Res.* 442:396–98
118. Ault, B., Evans, R. H., Francis, A. S., Oakes, D. J., Watkins, J. C. 1980. Selective depression of excitatory amino acid induced depolarizations by magnesium ions in isolated spinal cord preparations. *J. Physiol.* 307:413–28
119. Peters, S., Koh, J., Choi, D. W. 1987. Zinc selectively blocks the action of N-methyl-D-aspartate on cortical neurons. *Science* 236:589–92
120. Westbrook, G. L., Mayer, M. L. 1987. Micromolecular concentrations of Zn^{2+} antagonize NMDA and GABA responses of hippocampal neurons. *Nature* 328:640–43
121. Johnson, K. M., Snell, L. D., Morter, R. S. 1988. N-methyl-D-aspartate enhanced 3H-TCP binding to rat cortical membranes: effects of divalent cations and glycine. In *Sigma and Phencylidine-Like Compounds as Molecular Probes in Biology*, ed. E. F. Domino, J.-M. Kamenka, pp. 259–68. Ann Arbor, MI: NPP Books
122. Reynolds, I. J., Miller, R. J. 1988. [^{3}H]MK801 binding to the NMDA receptor/ionophore complex is regulated by divalent cations: evidence for multiple regulatory sites. *Eur. J. Pharmacol.* 151:103–12
123. Monaghan, D. T., Cotman, C. W., Olverman, H. J., Watkins, J. C. 1988. Two classes of NMDA recognition sites: Differential distribution and regulation by glycine. See Ref. 73, pp. 543–50
124. Olsen, R. W., Snowhill, E. W. 1983. [^{3}H]Bicuculline methochloride binding to low-affinity γ-aminobutyric acid receptor sites. *J. Neurochem.* 41:1653–63
125. Olsen, R. W., Snowhill, E. W., Wamsley, J. K. 1984. Autoradiographic localization of low affinity GABA receptors with [^{3}H]bicuculline methochloride. *Eur. J. Pharmacol.* 99:247–48
126. Levitan, E. S., Schofield, P. R., Burt, D. R., Rhee, L. M., Wisden, W., et al. 1988. Structural and functional basis for GABA A receptor heterogeneity. *Nature* 335:76–82
127. MacDonald, J. F., Salter, M. W., Mody, I. 1988. Intracellular regulation of the NMDA channel. See Ref. 73, pp. 159–66
128. Mody, I., Salter, M. W., MacDonald, J. F. 1988. Requirement of NMDA receptor/channels for intracellular high-energy phosphates and the extent of intraneuronal calcium buffering. *Neurosci. Lett.* In press
129. Perkins, M. N., Stone, T. W. 1983. Pharmacology and regional variations of quinolinic acid-evoked excitations in the rat central nervous system. *J. Pharmacol. Exp. Ther.* 226:551–57
130. Takemoto, T. 1978. Isolation and structural identification of naturally occurring excitatory amino-acids. In *Kainic Acid as a Tool in Neurobiology,*

ed. E. G. McGeer, J. W. Olney, P. L. McGeer, pp. 1–15. New York:Raven
131. Biscoe, T. J., Evans, R. H., Headley, P. M., Martin, M. R., Watkins, J. C. 1976. Structure-activity relationships of excitatory amino acids on frog and rat spinal neurones. *Br. J. Pharmacol.* 58:373–82
132. Shinozaki, H., Konishi, S. 1970. Actions of several anthelmintics and insecticides on rat cortical neurons. *Brain Res.* 24:368–71
133. Shinozaki, H., Shibuya, I. 1974. New potent excitant, quisqualic acid—effects on crayfish neuromuscular-junction. *Neuropharmacology* 13:665–72
134. Haldeman, S., McLennan, H. 1972. The antagonist action of glutamic aid diethyl ester towards amino acid-induced and synaptic excitations of central neurones. *Brain Res.* 45:393–400
135. McLennan, H., Liu, J. R. 1982. The action of six antagonists of the excitatory animo-acids on neurons of the rat spinal-cord. *Exp. Brain Res.* 45:151–56
136. Krogsgaard-Larsen, P., Honoré, T., Hansen, J. J., Curtis, D. R., Lodge, D. 1980. New class of glutamate agonist structurally related to ibotenic acid. *Nature* 284:64–66
137. Davies, J., Watkins, J. C. 1981. Differentiation of kainate and quisqualate receptors in the cat spinal cord by selective antagonism with D- (and L-)-glutamylglycine. *Brain Res.* 206:172–77
138. Teichberg, V. I., Goldberg, O., Tal, N., Luini, A. 1980. The interaction of excitatory amino acids with brain tissue: A study of amino acid-induced ion fluxes in rat striatal slices. In *Neurotransmitters and their receptors,* ed. U. Z. Littauer, Y. Dudai, I. Silman, V. Teichberg, Z. Vogel, New York: Wiley
139. Ganong, A. H., Lanthorn, T. H., Cotman, C. W. 1983. Kynurenic acid inhibits synaptic and acidic amino acid-induced responses in the rat hippocampus and spinal cord. *Brain Res.* 273:170–74
140. Honoré, T., Davies, S. N., Drejer, J., Fletcher, E. J., Jacobsen, P., et al. 1988. Quinoxalinediones: Potent competitive non-NMDA glutamate receptor antagonists. *Science* 241:701–3
141. Blake, J. F., Brown, M. W., Collingridge, G. L. 1988. CNQX blocks acidic amino acid induced depolarizations and synaptic components mediated by non-NMDA receptors in rat hippocampal slices. *Neurosci. Lett.* 89:182–86
142. Drejer, J., Honore, T. 1988. New quinoxalinediones show potent antagonism of quisqualate responses in cultured mouse cortical neurons. *Neurosci. Lett.* 87:104–8
143. Fagg, G. E. 1985. L-Glutamate, excitatory amino-acid receptors and brain-function. *Trends Neurosci.* 8:207–10
144. Mayer, M. L., Westbrook, G. L. 1987. The physiology of excitatory amino-acids in the vertebrate central nervous-system. *Prog. Neurobiol.* 28:197–276
145. Watkins, J. C., Evans, R. H. 1981. Excitatory amino acid transmitters. *Annu. Rev. Pharmacol. Toxicol.* 21:165–204
146. Slevin, J. T., Collins, J. F., Coyle, J. T. 1983. Analogue interactions with the brain receptor labelled by [3H]kainic acid. *Brain Res.* 265:169–72
147. Murphy, D. E., Snowhill, E. W., 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.* 12:775–82
148. Robertson, J. H., Deadwyler, S. A. 1981. Kainic acid produces depolarization of CA3 pyramidal cells in the *in vitro* hippocampal slice. *Brain Res.* 221:117–27
149. Westbrook, G. L., Lothman, E. W. 1983. Cellular and synaptic basis of kainic acid-induced hippocampal epileptiform activity. *Brain Res.* 273:97–109
150. Nadler, J. V., Perry, B. W., Cotman, C. W. 1978. Intraventricular kainic acid preferentially destroys hippocampal pyramidal cells. *Nature* 271:676–77
151. Schwob, J. E., Fuller, T., Price, J. L., Olney, J. W. 1980. Widespread patterns of neuronal damage following systemic or intracerebral injections of kainic acid: a histological study. *Neuroscience* 5:991–1014
152. Davies, J., Jones, A. W., Sheardown, M. J., Smith, D. A. S., Watkins, J. C. 1984. Phosphono dipeptides and piperazine derivatives as antagonists of amino acid-induced and synaptic excitation in mammalian and amphibian spinal-cord. *Neurosci. Lett.* 52:79–84
153. Ganong, A. H., Jones, A. W., Watkins, J. C., Cotman, C. W. 1986. Parallel antagonism of synaptic transmission and kainate/quisqualate responses in the hippocampus by piperazine-2,3-dicarboxylic acid analogs. *J. Neurosci.* 6:930–37
154. Verdoorn, T. A., Dingledine, R. 1988. Excitatory amino acid receptors expressed in *Xenopus* oocytes: Agonist

pharmacology. *Mol. Pharmacol.* 34:298–307
155. Cherubini, E., Rovira, C., Gho, M., Ben-Ari, Y. 1986. Effects of kainate on CA1 hippocampal neurons recorded *in vitro*. In *Excitatory Amino Acids and Epilepsy*, ed. R. Schwarcz, Y. Ben-Ari., pp. 475–84. New York: Plenum
156. Ben-Ari, Y., Gho, M. 1988. Long-lasting modification of the synaptic properties of rat CA3 hippocampal neurons induced by kainic acid. *J. Physiol.* 404:365–84
157. Fisher, R. S., Alger, B. E. 1984. Electrophysiological mechanisms of kainic acid-induced epileptiform activity in the rat hippocampal slice. *J. Neurosci.* 4:1312–23
158. Koerner, J. F., Cotman, C. W. 1981. Micromolar L-2-amino-4-phosphonobutyric acid selectively inhibits perforant path synapses from lateral entorhinal cortex. *Brain Res.* 216:192–98
159. Davies, J., Watkins, J. C. 1982. Actions of D- and L-forms of 2-amino-5-phosphonovalerate and 2-amino-4-phosphonobutyrate in the cat spinal-cord. *Brain Res.* 235:378–86
160. Evans, R. H., Francis, A. A., Jones, A. W., Smith D. A. S., Watkins, J. C. 1982. The effects of a series of -phosphonic -carboxylic amino acids on electrically evoked and amino acid induced responses in isolated spinal cord preparations. *Br. J. Pharmacol.* 75:65–75
161. Collins, G. G. S. 1982. Some effects of excitatory amino acid receptor antagonists on synaptic transmission in the rat olfactory cortex slice. *Brain Res.* 244:311–18
162. Hearns, T., Ganong, A. H., Cotman, C. W. 1986. Antagonism of lateral olfactory tract synaptic potentials in rat prepyriform cortex slices. *Brain Res.* 379:372–76
163. Hori, N., Auker, C. R., Braitman, D. J., Carpenter, D. O. 1981. Lateral olfactory tract transmitter: glutamate, aspatate, or neither? *Cell. Mol. Neurobiol.* 1:115–20
164. Lanthorn, T. H., Ganong, A. H., Cotman, C. W. 1984. 2-amino-4-phosphonobutyrate selectively blocks mossy fiber-CA3 responses in guinia pig but not rat hippocampus. *Brain Res.* 290:174–78
165. Yamamoto, C., Sawada, S., Takada, S. 1983. Suppressing action of 2-amino-4-phosphonobutyric acid on mossy fiber-induced excitation in the guinia pig hippocampus. *Exp. Brain Res.* 51:128–34
166. Neal, M. J., Cunningham, J. R., James, T. A., Joseph, M., Collins, J. F. 1981. The effect of 2-amino-4-phosphonobutyrate (APB) on acetylcholine release from the rat retina: evidence of on-channel input to cholinergic amacrine cells. *Neurosci. Lett.* 26:301–5
167. Slaughter, M. M., Miller, R. F. 1981. 2-Amino-4-phosphonobutyric acid: a new pharmacological tool for retina research. *Nature* 211:182–85
168. Miller, R., Slaughter, M. 1986. Excitatory amino acid receptors of the retina: diversity of subtypes and conductance mechanisms. *Trends Neurosci.* 9:211–18
169. Ganong, A. H., Cotman, C. W. 1982. Acidic amino acid antagonists of lateral perforant path synaptic transmission: agonist-antagonist interactions in the dentate gyrus. *Neurosci. Lett.* 34:195–200
170. Crooks, S. L., Robinson, M. B., Koerner, J. F., Johnson, R. L. 1986. Cyclic analogues of 2-amino-4-phosphonobutanoic acid (APB) and their inhibition of hippocampal excitatory transmission and displacement of [^{3}H]APB binding. *J. Med. Chem.* 29:1988–95
171. Cotman, C. W., Flatman, J. A., Ganong, A. H., Perkins, M. N. 1986. Effects of excitatory amino-acid antagonists on evoked and spontaneous excitatory potentials in guinia-pig hippocampus. *J. Physiol.* 378:403–15
172. Anson, J., Collins, G. G. S. 1987. Possible presynaptic actions of 2-amino-4-phosphonobutyrate in rat olfactory cortex. *Br. J. Pharmacol.* 91:753–61
173. Harris, E. W., Cotman, C. W. 1985. Effects of synaptic antagonists on perforant path paired pulse plasticity: differentiation of pre- and postsynaptic antagonism. *Brain Res.* 334:348–53
174. Forsythe, I. D., Clements, J. D. 1988. Glutamate autoreceptors reduce epsc's in cultured hippocampal neurons. *Soc. Neurosci.* 14:791 (Abstr.)
175. Butcher, S. P., Collins, J. F., Roberts, P. J. 1983. Characterization of the binding of DL-[^{3}H]2-amino-4-phosphonobutyrate to L-glutamate-sensitive sites on rat brain synaptic membranes. *Br. J. Pharmacol.* 80:355–64
176. Fagg, G. E., Foster, A. C., Mena, E. E., Cotman, C. W. 1983. Chloride and calcium ions separate L-glutamate receptor populations in synaptic membranes. *Eur. J. Pharmacol.* 88:105–10
177. Monaghan, D. T., McMills, M. C., Chamberlin, A. R., Cotman, C. W. 1983. Synthesis of [^{3}H]2-amino-4-phosphonobutyric acid and characteriza-

tion of its binding to rat brain membranes: a selective ligand for the chloride/calcium-dependent class of L-glutamate binding sites. *Brain Res.* 278:137–44

178. Bridges, R. J., Hearn, T. J., Monaghan, D. T., 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.* 375:204–9
179. Fagg, G., Lanthorn, T. 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.* 86:743–51
180. Kessler, M., Baudry, M., Lynch, G. 1987. Use of cystine to distinguish glutamate binding from glutamate sequestration. *Neurosci. Lett.* 81:221–26
181. Pin, J. P., Bockaert, J., Recasen, 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
182. Zaczek, R., Arlis, S., Markyl, A., Murphy, T., Drucker, H., Coyle, J. T. 1987. Characteristics of chloride-dependent incorporation of glutamate into brain membranes argue against a receptor binding site. *Neuropharmacology* 26:281–87
183. Bridges, R. J., Nieto-Sampedro, M., Kadri, M., Cotman, C. W. 1987. A novel 3H-L-glutamate binding-site in astrocyte membranes. *J. Neurochem.* 48:1709–15
184. Waniewski, R. A., Martin, D. L. 1984. Characterization of L-glutamic acid transport by glioma cells in culture, evidence for sodium-independent, chloride-dependent high affinity influx. *J. Neurosci.* 4:2237–46
185. Zaczek, R., Balm, M., Arlis, S., Drucker, H., Coyle, J. T. 1987. Quisqualate-sensitive, chloride-dependent transport of glutamate into rat brain synaptosomes. *J. Neurosci. Res.* 18:425–31
186. Gundersen, C. B., Miledi, R., Parker, I. 1984. Glutamate and kainate receptors induced by rat brain messenger RNA in *Xenopus* oocytes. *Proc. R. Soc. London Ser. B* 221:127–43
187. Sugiyama, H., Ito, I., Hirono, C. 1987. A new type of glutamate receptor linked to inositol phospholipid metabolism. *Nature.* 325:531–33
188. Parker, I., Miledi, R. 1987. Inositol trisphosphate activates a voltage-dependent calcium influx in *Xenopus* oocytes. *Proc. R. Soc. London Ser. B* 321:27–36
189. Sugiyama, H., Ito, I., Okada, D., Hirono, C., Ohmori, et al. 1988. Functional and pharmacological properties of glutamate receptors linked to inositol phospholipid metabolism. See Ref. 73, pp. 21–28
190. Jackson, H., Usherwood, P. N. R. 1988. Spider toxins as tools for dissecting elements of excitatory amino acid transmission. *Trends Neurosci.* 11:278–83
191. Baudry, M., Evans J., Lynch, G. 1986. Excitatory amino acids inhibit stimulation of phosphatidylinositol metabolism by aminergic agonists in hippocampus. *Nature* 319:329–31
192. Nicoletti, F., Iadarola, M. J., Wroblewski, J. T., Costa, E. 1986. Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism: Developmental changes and interaction with alpha$_1$-adrenoceptors. *Proc. Natl. Acad. Sci. USA* 83:1931–35
193. Sladeczek, F., Pin, J.-P., Recasens, M., Bockaert, J., Weiss, S. 1985. Glutamate stimulates inositol phosphate formation in striatal neurones. *Nature* 317:717–19
194. Palmer, E., Monaghan, D. T., Cotman, C. W. 1988. Glutamate receptors and phosphoinositide metabolism: stimulation via quisqualate receptors is inhibited by N-methyl-D-aspartate receptor activation. *Mol. Brain Res.* 4:161–65
195. Schoepp, D. D., Johnson, B. G. 1988. Excitatory amino acid agonist-antagonist interactions at 2-amino-4-phosphonobutyric acid-sensitive quisqualate receptors coupled to phoshoinositide hydrolysis in slices of rat hippocampus. *J. Neurochem.* 50:1605–13
196. Nicoletti, F., Wroblewski, J. T., Fadda, E., Costa, E. 1988. Pertussis toxin inhibits signal transduction at a specific metabolotropic glutamate receptor in primary cultures of cerebellar granule cells. *Neuropharmacology* 27:551–56
197. Verdoorn, T. A., Dingledine, R. 1988. Antagonist pharmacology of excitatory amino acid receptors expressed in *Xenopus* oocytes. *Soc. Neurosci.* (Abstr.) 14:1193
198. Schmidt, B. H., Weiss, S., Sebben, M., Kemp, D. E., Bockaert, J., Sladeczek, F. 1987. Dual action of excitatory amino acids on the metabolism of inositol phosphates in striatal neurons. *J. Pharmacol. Exp. Ther.* 32:364–68
199. Kushner, L., Lerma, J., Zukin, R. S., Bennett, M. V. L. 1988. Coexpression of N-methyl-D-aspartate and phencyclidine receptors in *Xenopus* oocytes injected with rat brain mRNA. *Proc. Natl. Acad. Sci. USA* 85:3250–54
200. Nicoletti, F., Wroblewski, J. T., Alho,

H., Eva, C., Fadda, E., et al. 1987. Lesions of putative glutamatergic pathways potentiate the increase of inositol phospholipid hydrolysis elicited by excitatory amino-acids. *Brain Res.* 436: 103–12

201. Bear, M. F., Cooper, L. N., Ebner, F. F. 1987. A physiological-basis for a theory of synapse modification. *Science* 237:42–48
202. Reh, T. A., Constantine-Paton, M. 1985. Eye specific segregation requires neural activity in three-eyed *Rana pipiens*. *J. Neurosci.* 5:1132–43
203. Cline, H. T., Debski, E., Constantine-Paton, M. 1987. NMDA receptor antagonist desegregates eye specific stripes. *Proc. Natl Acad. Sci. USA* 84:4342–45
204. Singer, W. 1987. Activity-dependant self-organization of synaptic connections as a substrate of learning. *Dahlem Workshop on Neural and Molecular Mechanisms of Learning*. Berlin: Springer-Verlag
205. Stryker, M. P., Harris, W. A. 1986. Binocular impulse blockade prevents the formation of ocular dominance columns in the cat visual cortex. *J. Neurosci.* 6:2117–33
206. Singer, W., Kleinschmidt, A., Bear, M. F. 1986. Infusion of an NMDA receptor antagonist disrupts ocular dominance plasticity in kitten striate cortex. *Soc. Neurosci.* 12:786 *Abstr.*
207. Lincoln, J., Coopersmith, R., Harris, E. W., Cotman, C. W., Leon, M. 1988. NMDA receptor activation and early olfactory learning. *Dev. Brain Res.* 39:309–12
208. Woo, C. C., Coopersmith, R., Leon, M. 1987. Localized changes in olfactory bulb morphology associated with early olfactory learning. *J. Comp. Neurol.* 263:113–25
209. Bliss, T. V. P., Lomo, T. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* 232:331–56
210. Collingridge, G. L., Kehl, S. J., McLennan, H. 1983. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J. Physiol.* 334:33–46
211. Harris, E. W., Ganong, A. H., Cotman, C. W. 1984. Long-term potentiation in the hippocampus involves activation in *N*-methyl-D-aspartate receptors. *Brain Res.* 323:132–37
212. Stringer, J. L., Greenfield, L. J., Hackett, J. T., Guyenet, P. G. 1983. Blockade of long-term potentiation by phencyclidine and σ opiates in the hippocampus in vivo and in vitro. *Brain Res.* 280:127–38
213. Malinow, R., Miller, J. P. 1986. Postsynaptic hyperpolarization during conditioning reversibly blocks induction of long-term potentiation. *Nature* 320: 529–30
214. Thompson, R. F. 1986. The neurobiology of learning and memory. *Science* 233:941–47

214a. Morris, R. G. M. 1988. Elements of a hypothesis concerning the participation of hippocampal NMDA receptors in learning. In *Excitatory Amino Acids in Health and Disease*, ed. D. Lodges, pp. 297–320. England: Wiley

215. Lee, K., Schottler, F., Oliver, M., Lynch, G. 1980. Brief bursts of high frequency stimulation produce two types of structural change in rat hippocampus. *J. Neurophysiol.* 44:247–58
216. Chang, F., Greenough, W. T. 1984. Transient and enduring morphological correlates of synaptic activity and efficacy change in the rat hippocampal slice. *Brain Res.* 309:35–46
217. Harris, E. W., Cotman, C. W. 1986. Long-term potentiation of guinea pig mossy fiber responses is not blocked by N-methyl-D-aspartate antagonists. *Neurosci. Lett.* 70:132–37
218. Mattson, M., Dou, P., Kater, S. B. 1988. Outgrowth-regulating actions of glutamate in isolated hippocampal pyramidal neurons. *J. Neurosci.* 8:2087–2100
219. Brewer, G., Cotman, C. W. 1988. NMDA promotes branching, MK-801 stimulates elongation of dentate gyrus granule neurons. *Soc. Neurosci.* Abstr. 14:115
220. Lucas, D. R., Newhouse, J. P. 1957. The toxic effect of sodium L-glutamate on the inner layers of the retina. *AMA Arch. Opthalmol.* 58:193–201
221. Olney, J. W. 1969. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* 164:719–21
222. Olney, J. W. 1988. Endogenous excitotoxins and neuropathological disorders. See Ref. 214a, pp. 337–52
223. Olney, J. W., Ho, O. C., Rhee, V. 1971. Cytotoxic effects of acidic and sulpher-containing amino acids on the infant mouse central nervous system. *Exp. Brain Res.* 14:61–76
224. Olney, S. 1969. Glutamate induced ne-

crosis in the infant mouse hypothalamus: an electron microscopic study. *J. Neuropathol. Exp. Neurol.* 30:75–90
225. Rothman, S. 1984. Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. *J. Neurosci.* 4:1884–91
226. Auer, R., Wieloch, T., Olsson, Y., Siesjo, B. K. 1984. The distribution of hypoglycemic brain damage. *Acta Neuropathol.* 64:177–91
227. Griffiths, T., Evans, M. C., Meldrum, B. S. 1983. Intracellular calcium accumulation in rat hippocampus during seizures induced by bicuculline or L-allylglycine. *Neuroscience* 10:385–95
228. Olney, J. W., Collins, R. C., Sloviter, R. S. 1986. Excitotoxic mechanisms of epileptic brain damage. *Adv. Neurol.* 44:857–77
229. Simon, R. P., Swan, S. H., Griffiths, T., Meldrum, B. S. 1984. Blockade of NMDA receptors may protect against ischemic damage in the brain. *Science* 226:850–52
230. Foster, A. C., Gill, R., Iverson, L. L., Woodruff, G. W. 1987. Systemic administration of MK-801 protects against ischemia-induced hippocampal neurodegeneration in the gerbil. *Br. J. Pharmacol. Proc. Suppl.* 90:9P
231. Wieloch, T. 1985. Hypoglycemia-induced neuronal damage prevented by an N-methyl-D-aspartate antagonist. *Science* 230:681–83
232. Patel, S., Chapman, A. G., Millan, M. H., Meldrum, B. S. 1988. Epilepsy and excitatory amino acid antagonists. See Ref. 214a, pp. 353–78
233. Rothman, S. M., Olney, J. W. 1987. Excitotoxicity and the NMDA receptor. *Trends Neurosci.* 10:299–302
234. Hori, N., Ffrench-Mullen, J. M. H., Carpenter, D. O. 1985. Kainic acid responses and toxicity show pronounced Ca2+ dependence. *Brain Res.* 358:380–84
235. Choi, D. W., Maulucci-Gedde, M., Kriegstein, A. R. 1987. Glutamate neurotoxicity in cortical cell culture. *J. Neurosci.* 7:357–68
236. Kim, J. P., Choi, D. W. 1987. Quinolinate neurotoxicity in cortical cell culture. *Neuroscience* 23:423–32
237. Kim, J. P., Koh, J., Choi, D. W. 1987. L-Homocysteate is a potent neurotoxin on cultured cortical neurons. *Brain Res.* 437:103–10
238. Peterson, C., Neal, J., Cotman, C. W. 1988. Glutamate neurotoxicity during development and aging. *Soc. Neurosci.* 14:1293 (Abstr.)
239. Schwarcz, R., Scholz, D., Coyle, J. T. 1978. Structure-activity relations for the neurotoxicity of kainic acid derivatives and glutamate analogues. *Neuropharmacology* 17:145–51
240. Sloviter, R. S. 1983. "Epileptic" brain damage in rats induced by sustained electrical stimulation of the perforant path. I. Acute electrophysiological and light microscopic studies. *Brain Res. Bull.* 10:675–97
241. Ludolph, A. C., Hugon, J., Dwivedi, M. P., Schaumburg, H. H., Spencer, P. S. 1987. Studies on the aetiology and pathogenesis of motor neuron disease. *Brain* 110:149–65
242. Bridges, R. J., Kadri, M., Monaghan, D. T., Nunn, P. B., Watkins, J. C., Cotman, C. W. 1988. Inhibition of 3H-AMPA binding by the excitotoxin β-N-oxalyl-L-α,β-diaminopropionic acid. *Eur. J. Pharmacol.* 145:357–60
243. Farber, J. L., Chicu, K. R., Mittnacht, S. 1981. The pathogenesis of irreversible cell injury in ischemia. *Am. J. Pathol.* 102:271–81
244. Jancso, G., Karscu, S., Kiraly, E., Szebeni, A., Toth, L., Bacsy, E., Joo, F., Parducz, A. 1984. Neurotoxin induced nerve-cell degeneration: possible involvement of calcium. *Brain Res.* 295:211–16
245. Choi, D. W. 1985. Glutamate neurotoxicity in cortical cell culture is calcium dependent. *Neurosci. Lett.* 58:293–97
246. Choi, D. W. 1987. Ionic dependence of glutamate neurotoxicity. *J. Neurosci.* 7:369–79
247. Price, M. T., Olney, J. W., Sampsom, L., Labruyere, J. 1985. Calcium influx accompanies but does not cause excitotoxin-induced neuronal necrosis. *Brain Res. Bull.* 14:369–76
248. Rothman, S. M. 1985. The neurotoxicity of excitatory amino acids is produced by passive chloride influx. *J. Neurosci.* 6:1483–89
249. Olney, J. W., Price, M. T., Samson, L., Labruyere, J. 1986. The role of specific ions in glutamate neurotoxicity. *Neurosci. Lett.* 65:65–71
250. Balcar, V. S., Johnston, G. A. R. 1972. Glutamate uptake by brain slices and its relation to the depolarization of neurons by acidic amino acids. *J. Neurobiol.* 3:295–301
251. Schousboe, A., Hertz, L. 1981. Role of astroglial cells in glutamate homeostasis. *Adv. Biochem. Psychopharmacol.* 27:103–113
252. Johnston, G. A. R., Lodge, D., Borstein, J. C., Curtis, D. R. 1980.

Potentiation of L-glutamate and L-aspartate excitation of cat spinal cord neurones by the stereoisomers of threo-3-hydroxyaspartate. *J. Neurochem.* 34:241–43

253. Sawada, S., Higashima, M., Yamamoto, C. 1985. Inhibitors of high-affinity uptake augment depolarizations of hippocampal neurons induced by glutamate, kainate, and related compounds. *Exp. Brain Res.* 60:323–29
254. Davies, J., Francis, A. A., Oakes, D. J., Sheardown, M. J., Watkins, J. C. 1985. Selective potentiating effect of β-p-chlorophenylglutamate on responses induced by certain sulphur-containing excitatory amino acids and quisqualate. *Neuropharmacology* 24:177–80
255. Anderson, K. A., Bridges, R. J., Tavoularis, A. L., Bhatt, D., Cotman, C. W. 1988. Autoradiographic comparison of Na^+ and Cl^- dependent excitatory amino acid uptake sites. *Soc. Neurosci.* 14:421 (Abstr.)
256. Bridges, R. J., Anderson, K. A., Tavoularis, A. L., Bhatt, D., Cotman, C. W. 1988. Lesion-induced changes in glutamate uptake sites. *Soc. Neurosci.* 14:421 (Abstr.)
257. Palmer, A. M., Proctor, A. W., Stratman, G. C., Bowen, D. M. 1986. Excitatory amino acid-releasing and cholinergic neurones in Alzheimer's disease. *Neurosci. Lett.* 60:199–204