A cDNA encoding a 100-kDa subunit (XenNR1) of the \(N\)-methyl-D-aspartate (NMDA) glutamate receptor type has been cloned from \textit{Xenopus} central nervous system. When XenNR1 is coexpressed in a mammalian cell line with a recently cloned 51-kDa non-NMDA receptor subunit (XenU1), also from \textit{Xenopus}, it forms a functional unitary receptor exhibiting the pharmacological properties characteristic of both NMDA and non-NMDA receptors. Firstly, XenU1 can replace NR2 subunits, in complementing XenNR1 to introduce the ligand binding properties of a complete NMDA receptor. Second, responses to both NMDA and non-NMDA receptor agonists and antagonists were obtained in patch-clamp recordings from the cotransfected cells, but no significant responses were recorded when the cells were singly transfected. Third, from solubilized cell membranes from the cotransfected cells, an antibody to the NR1 subunit coprecipitated the binding sites of the non-NMDA receptor subunit. The unitary glutamate receptor has a unique set of properties that denote intersubunit interaction, including a glycine requirement for the responses to non-NMDA as well as to NMDA receptor agonists and voltage-dependent block by Mg\(^{2+}\) of the non-NMDA agonist responses.

Few distinctions drawn in neuroscience are so universally accepted as that between the \(N\)-methyl-D-aspartate (NMDA)\(^1\) and the non-NMDA classes of vertebrate ionotropic glutamate receptors (1–3). This differentiation is now fundamental in the analysis of brain excitatory pathways. It was given a molecular basis through the discovery that the protein sequences of non-NMDA receptors (8, 9). However, this distinction was challenged recently when a protein purified from the central nervous system of the toad \textit{Xenopus laevis} was found to exhibit both NMDA and non-NMDA receptor properties (5, 6).

The central nervous system of \textit{X. laevis} is an exceptionally rich source (7) of both the AMPA and the kainate binding sites of non-NMDA receptors. These abundant sites and their unusual stability after detergent solubilization (7) permitted the purification on an affinity column containing immobilized domoate (a high affinity ligand for kainate receptors) of some of the ionotropic glutamate receptors. After reconstitution into bilayers two classes were found: (i) a non-NMDA (AMPA/kainate) receptor type, and (ii) a protein that exhibited both NMDA and non-NMDA binding and channel activation properties, \textit{i.e.} it behaved as a unitary ionotropic glutamate receptor (5, 6). Although the majority of the extracted NMDA receptors was not retained by that column and behaved as purely NMDA-type receptors, a significant minority of them was found to be of the unitary receptor type. These unexpected findings clearly required consolidation by the identification, through DNA cloning and expression, of the subunits constituting that novel type.

A cDNA (XenU1) encoding a non-NMDA receptor subunit has recently been cloned from \textit{Xenopus} central nervous system using nine peptide sequences obtained from the purified (type i) above) AMPA/kainate receptor (8). This 51-kDa protein corresponded in size and peptide sequence to the subunit isolated from the latter receptor. It has only 36–40\% sequence identity with rat non-NMDA receptors, despite the presence of four hydrophobic domains that have high sequence identity to four equivalent domains in the COOH-terminal half of mammalian non-NMDA receptor subunits (8, 9). However, XenU1 lacks most of the large NH\(_2\)-terminal domain that characterizes the latter proteins. Although similar in size to certain vertebrate kainate-binding proteins (10–12), XenU1 differs from them in its binding of AMPA (\(K_d = 62\) nM) as well as kainate (\(K_d = 9.1\) nM) when expressed in mammalian cells; NMDA is not a competitor for these on XenU1 (8). Expression of those AMPA/kainate binding sites was readily achieved by transfecting cells with XenU1 cDNA; however, extensive testing for functional expression of XenU1 in \textit{Xenopus} oocytes gave no significant response, suggesting that a native partner subunit is needed (8). We identify here such a partner: this is a \textit{Xenopus} NMDA receptor type of subunit, XenNR1, which we show reproduces, when (and only when) coexpressed with XenU1, the behavior of the above described native unitary glutamate receptor protein.

**EXPERIMENTAL PROCEDURES**

Methods and materials not specified were as in Refs. 6 and 8. \textit{l}-Glutamate, glycine, and NMDA were from Sigma Chemical Co. (purest...
grades), MK-801 was from Research Biochemicals International, and other noncrucial reagents for glutamate receptors were from Tatham and Cookson. AMPA and kainate were >99% pure and contained no glutamate by the manufacturer’s analysis. [3H]Kainate, [3H]DCKA, [3H]MK-801 were from DuPont NEN, and [3H]glutamate was from Amersham.

DNA Cloning for an Abundant Form of NR1 of Xenopus—Poly(A)− RNA was isolated from Xenopus central nervous system, and cDNA was synthesized as described previously (8). A Xenopus brain ZAP-II cDNA library (2.5 million primary plaques) (9) was screened with a fragment of the pN60 plasmid (4) containing the full coding region of rat NMDAR1A. The final washing of the filters was in 2 × SSC, 0.5% SDS at 40°C. A positive clone was identified with a ~3-kilobase cDNA insert which was found to be homologous to rat NMDAR1A. The library was rescreened twice under high stringency conditions, using synthetic oligonucleotides derived from the sequences found (5). PCR products were analyzed by electrophoresis in 1% agarose and 5% polyacrylamide gels and blotted onto the nylon membrane (10). The membranes were probed with the corresponding cDNA inserts. After prehybridization for 4 h at room temperature (25°C) and washing thoroughly with Tris-buffered saline (pH 7.6) at 50°C, membranes were hybridized with the cDNA insert at 40°C (except those used during transfections, which were washed twice at 50°C) five centrifugal washes with cold 50 mM Tris citrate (pH 7). The membranes were incubated at room temperature (25°C) with [3H]glutamate, [3H]kainate, or [3H]MK-801 (10–20 μM) and then twice with the solubilizing buffer (defined below). Membranes were solubilized in 50 mM Tris citrate buffer containing 1 mM EDTA, 1% n-octyl-β-p-glucopyranoside and protease inhibitors (6) for 1 h at 4°C followed by 1-h centrifugation at 100,000 × g. The immobilized antibody was added to the solubilized samples followed by an overnight incubation at 4°C with gentle rotary agitation. The beads were collected by centrifugation and washed thoroughly with the solubilizing buffer. The wash fractions were saved and combined with the rest of the supernatant to estimate the depletion of [3H]kainate binding sites therein. The amount of XenNR1 subunit precipitated onto the beads was measured by [3H]kainate binding (performed as above) to the washed Sepharose pellet.

RESULTS

A Lower Vertebrate NR1 Subunit—Poly(A)− RNA was isolated from Xenopus central nervous system and used in constructing a cDNA library (8). This was screened at low stringency with the rat NR1 subunit (4) cDNA. From the resulting clones a full-length cDNA was isolated which encodes an NR1-type subunit, XenNR1 (EMBL accession no. X94156). The translation start site is assigned to the first methionine residue of the largest open reading frame (Fig. 1). The sequence immediately following this is that of a signal peptide. The cleavage site was predicted (19) to be after the first 20 residues, equivalent to that for the rat NR1 subunit (4). The mature XenNR1 subunit has a predicted sequence (Fig. 1) of 884 amino acids and a calculated molecular mass of 99,600 Da. It shares high amino acid sequence similarity with mammalian NR1 subunits, almost all of the differences being concentrated in the first 448 residues of the 559-residue presumptive NH2-terminal domain. Identified NR1 subunits of demonstrated receptor functionality have hitherto been available for comparison only from mammals; it is interesting to see that there is absolute conservation in the NR1 sequence, from amphibian to man, of the entire region containing the four hydrophobic domains and the long loop between the third and the fourth hydrophobic domains (apart from two conservative changes), as well as of an adjacent 32-residue stretch of the COOH-terminal tail. A 91-residue stretch just prior to the assigned (4) first hydrophobic domain is also completely conserved; there is evidence that this segment contributes to the agonist binding site of glutamate receptors (20). The COOH terminus of XenNR1 also has the sequence motif (21) denoting interaction with the postsynaptic density protein PSD-95.

XenNR1 corresponds to the G isoform (Fig. 1), which in rat brain is the least abundant of the eight alternatively-spliced NR1 isoforms (22–25). That isoform is characterized by a 21-amino acid insertion within the NH2-terminal sequence region and a double peptide deletion that produces a COOH-terminal sequence different from that in all but one (the E form) of the other NR1 isoforms. Additional extensive screening of the Xenopus cDNA library was performed with oligonucleotides (as detailed under “Experimental Procedures”) which recognize the sequences around those potential splice junctional sites encoded by XenNR1 cDNA and that of two alternately-spliced forms of the XenNR1. The search was extended by analyzing the mRNA from Xenopus central nervous system using reverse transcriptase PCR. No product corresponding to
alternativesplicingintheCOOH-terminalpartoftheXenNR1 was detected. However, amplification of the cDNA correspond-
ing to a region around the potential alternative splicing site in the NH2-terminal part of XenNR1 generated two different products, which were separated, cloned, and sequenced. The nucleotide sequence of the major band exactly matched the previously found XenNR1 cDNA. The other, very minor band, was of a shorter product, with the same sequence but with the 63-nucleotide deletion, thus resembling the NH2-terminal re-
gion of the mammalian NR1E subunit (22, 24). When these PCR productswere subcloned (without a sizeseparation), 2 out of 20 randomly chosen clones represented the E isoform and 16 the G isoform. The predominance of the G isoform is the oppo-
site of what is found in the rat central nervous system (22, 25).

Expression was studied using subunit cDNA transfections into the HEK-293 cell line. In that system, expressed mammalian NR1 subunits do not form functional NMDA receptors unless combined with an NR2 subunit (15, 26–29). Likewise, XenNR1 expressed alone formed functional receptors with a very low probability (see below) and yielded very low binding of \[^{3}H\]MK-801 (Fig. 2A, left). \[^{3}H\]DCKA, a competitive antago-
nist at the glycine site of NMDA receptors (27), also gave little binding (Fig. 2A, right). That ligand, however, binds well (27, 30) to the rat NR1A subunit expressed alone in HEK-293 cells; the difference here may be because the rat G isoform has not been tested thus or because of its other sequence variations.
Complementation of XenNR1 by a Non-NMDA Receptor Subunit—When XenNR1 was coexpressed in HEK-293 cells with XenU1, the binding of ligands selective for NMDA receptors rose dramatically (Fig. 2B). In electrophysiological experiments on HEK-293 cells and Xenopus oocytes coexpressing XenNR1/NR2A (mouse) or XenNR1/NR2B (mouse), NMDA plus glycine (but not kainate or AMPA, with or without glycine) now activated abundant receptors. Hence XenNR1 is a subunit of a functional NMDA receptor.

Specificity for the Subunit Types in the Hybrid—Weak expression of a hybrid receptor resulted from cotransfection of XenU1 cDNA with each of two (rat and human) NR1A cDNAs. This conclusion is based upon the presence of a small amount of [3H]MK-801 binding (Fig. 2A, left). Expression was improved significantly when XenU1 cDNA was cotransfected with cDNA for the human NR1E subunit (14), but it was still less efficient than with XenNR1 (Fig. 2A, left). In contrast, NR1 subunit expression in 293 cells is not affected by other known functional non-NMDA receptor subunits. Thus, when either XenNR1 or human (14) NR1E or NR1A cDNA was coexpressed with a cDNA encoding the human (34) KA1, KA2, GluR6, or GluR7 kainate receptor subunits, no [3H]MK-801 or [3H]DCKA binding was introduced (data not shown).

Testing for Functional Expression of XenNR1 or XenU1 Alone—Receptor function was studied in whole cell current recordings obtained from transfected HEK-293 cells. Transfection with XenNR1 alone rarely led to the expression of functional homo-oligomeric receptors, and this with a consistently small (i.e., only 1 of 27 cells tested responded to application of 100 μM NMDA (plus 10 μM glycine) and this with a consistently small (~20 pA) inward current at \( V_h = -50 \) mV. Rat NR1 subunits, likewise, give little or no functional expression alone in HEK-293 cells (26, 27). Cells transfected with XenU1 alone also showed little functional expression; i.e., in response to 100 μM kainate, AMPA, or glutamate only 2 out of a total of 89 cells tested gave small currents, these being 20 pA or less at \( V_h = -50 \) mV. Addition of glycine (10 μM) did not increase the amplitude of these currents. Treatment with cyclothiazide, wheat germ agglutinin, or concanavalin A, which can suppress the desensitization

from XenNR1 (Fig. 1). However, when XenNR1 was coexpressed with a mouse NR2A subunit, binding of both MK-801 and DCKA was increased greatly (Fig. 2A). In electrophysiological experiments on HEK-293 cells and Xenopus oocytes coexpressing XenNR1/NR2A (mouse) or XenNR1/NR2B (mouse), NMDA plus glycine (but not kainate or AMPA, with or without glycine) now activated abundant receptors. Hence XenNR1 is a subunit of a functional NMDA receptor.

**Table I**

| Subunit composition | \[^{3}H\]DCKA | \[^{3}H\]kainate |
|---------------------|--------------|-----------------|
| XenNR1/XenU1        | ND           | 9.6 ± 1.3       |
| XenNR1/XenU1        | ND           | 9.4 ± 1.2 (with 20 μM glycine) |
| XenNR1/NR2A         | 147 ± 12     | ND              |
| (mouse)             |              |                 |
| Rat NR1A/NR2A       | 27 ± 4       |                 |
| (mouse)             |              |                 |
| Rat NR1A/NR2A       | 59 ± 8       |                 |

From Ref. 30.
of mammalian AMPA and kainate receptors (31, 32), neither increased the amplitude of these responses nor increased the proportion of responding cells.

**Functional Expression of XenNR1/XenU1 in Heteromeric Combination**—Consistent responses to NMDA and non-NMDA agonists were obtained when XenNR1 and XenU1 were cotransfected into HEK-293 cells. Inward currents of up to \(1,000\) pA at \(V_H = -50\) mV were elicited by \(100 \mu M\) NMDA. Inward currents of up to \(800\) pA at \(V_H = -50\) mV were also obtained from these cells in response to kainate or AMPA (see Fig. 3, A and B, for typical responses). The response was at a maximum at \(100 \mu M\) agonist, but it was elicited down to \(0.3 \mu M\) AMPA or less (Fig. 3C). The cells also responded to \(100 \mu M\) glutamate, with inward currents at \(V_H = -50\) mV which some- times exceeded \(1,000\) pA (see Fig. 3A for a typical response). The responses were seen in \(\sim 30\%\) of cells, which corresponds to the transfection efficiency, as estimated in parallel transfection methods with a reporter plasmid expressing \(\beta\)-galactosidase. Whenever tested, a cell responding to NMDA also responded to any of the non-NMDA agonists. The responses to kainate, but not those to AMPA, declined progressively in amplitude with repeated application of agonist at 30-s intervals (Fig. 3, D and E). This change could not be reversed by extensive washing of the preparation with agonist-free saline. This kainate-specific “run-down” was unaffected by coapplication of cyclothiazide or pretreatment of cells with lectins (31, 32). This run-down blocked a subsequent response to AMPA. AMPA and not kainase was used in further studies.

**A Glycine Requirement for Activation by non-NMDA Agonists**—Surprisingly, the responses of the heteromeric combination to NMDA and to non-NMDA agonists (Figs. 3B and 4A) were glycine-dependent, although glycine alone, even as high as \(1\) mM, never evoked a response. Dose-response relationships for this potentiation of the responses to glutamate, NMDA, and AMPA showed a high glycine sensitivity (Fig. 4A), different for the NMDA and the AMPA agonist sites. DCKA, an antagonist of the mammalian NMDA receptor glycine site, completely antagonized (at \(10 \mu M\)) the potentiation by glycine of the responses to non-NMDA agonists (Fig. 4B).

**Further Functional Properties of the Heteromeric Receptor**—The dose-response curve for NMDA (plus \(10 \mu M\) glycine) had an \(EC_{50}\) of \(74 \pm 16\) \(\mu M\) (Fig. 4C). If the non-NMDA agonists were acting at the same receptor where NMDA acts, the NMDA response should be less than additive with a response to coapplied AMPA. Indeed, even at the maximum response to NMDA the addition of AMPA produced no extra current but in fact decreased the NMDA response (Fig. 4D). The decrease observed could in theory be due to one of several causes, but to offer a detailed interpretation of it would not be justified here until we have data on the single channel conductances evoked by NMDA and by AMPA for the unusual case of the channel in this hybrid assembly.

Responses to NMDA (plus glycine) showed only slight desensitization and that was not \(Ca^{2+}\)-sensitive, although the shape of the current-voltage relationship for this agonist was \(Ca^{2+}\)-sensitive. When the \(Ca^{2+}\) concentration was raised from nominally \(0\) mM to \(1\) mM and then to \(10\) mM, the amplitude of the NMDA-induced current was reduced progressively at highly negative \(V_H\) (Fig. 5A). This change was accompanied by a shift of the current reversal potential to more positive values. These features indicate that the \(Ca^{2+}\)-permeability of the XenNR1/XenU1 heteromeric channel is similar to that of the mamman-
The NMDA-induced currents were (as expected) antagonized noncompetitively and voltage-dependently by Mg$^{2+}$ (100 µM to 1 mM) (Fig. 5B) and by MK-801 (Fig. 5C). As in mammalian NMDA receptors, the NMDA site ligand d,l-2-amino-5-phosphonopentanoic acid competitively antagonized the NMDA responses (data not shown). Interestingly, spermine, even at 1 mM, did not potentiate the responses to NMDA (plus glycine) (data not shown). In this respect the hybrid receptor behaves as the mammalian NMDA receptor when it has, as here, the NR1 subunit with the alternatively spliced NH$_2$-terminal insert (23).

In addition to its sensitivity to glycine, the responses of cotransfected cells to AMPA revealed other characteristics normally associated with NMDA receptors. They were antagonized noncompetitively by Mg$^{2+}$, but only at negative potentials (Fig. 5D). Further, the form of the current-voltage plot in that condition is just as found for the NMDA response (comparing the 1 mM Mg$^{2+}$ curves of Fig. 5B and D). This form reproduces exactly that which is characteristic for the rat NR1/NR2 receptors (with NMDA) in HEK-293 cells (26). In contrast, the responses to 100 µM AMPA (plus glycine), but not to NMDA (plus glycine), were completely blocked by 6,7-dinitroquinoxaline-2,3-dione (50 µM), a competitive antagonist (33) of mammalian AMPA receptors (data not shown). This confirms that the exceptional properties of the response to AMPA are due to its action at the AMPA site itself, which is present (8) on XenU1.

**Immunoprecipitation**—An ability of an antibody specific to one subunit of a receptor to precipitate also a second subunit or its binding sites has recently become a powerful tool for identifying pairs of subunit types coexisting in ionotropic receptor molecules. In NMDA receptors it has been applied, for example, to show that in rat cortex extracts NR1 can be variously associated with NR2A or with NR2B subunits or with both (35). To precipitate non-NMDA-specific binding sites we have used a commercially available monoclonal antibody against a rat amino acid sequence that is completely conserved in X. laevis, located in the loop between the third and the fourth hydrophilic domains of NR1. This antibody recognizes in Western blots a band (~ 110 kDa) derived from membranes from the X. laevis central nervous system or from cells transfected with XenNR1 cDNA, but not from cells transfected with XenU1 cDNA (Fig. 6, lanes A–C). Glutamate receptors from cotransfected 293 cells or from X. laevis central nervous system were extracted in n-octyl-$\beta$-glucopyranoside medium (6) and equilibrated with the anti-NR1 antibody immobilized on Sepharose beads (Table II). This treatment precipitated a majority of the solubilized XenNR1 subunits (Fig. 6, lanes D and E). It also precipitated a significant number of the kainate binding sites (Table II). An extract of 293 cells transfected with XenU1 cDNA alone showed no depletion by the anti-NR1 antibody, and none of the kainate binding sites there became attached to the beads. The cross-immunoprecipitation occurred both from the extract containing heteromeric recombinant receptors and from the native tissue extract (Table II).

**DISCUSSION**

Properties of XenNR1—Among the NR1 subunits whose amino acid sequences are known in the mammals, there is extremely high conservation, e.g. 99% between rat and man (14). In view of the much greater phylogenetic distance from these to the amphibian X. laevis, it is interesting to see that the entire COOH-terminal half of the subunit is almost completely identical to the mammalian NR1G sequence, whereas in the first 450 amino acids of the NH$_2$-terminal extracellular domain 13% are changed (Fig. 1). Although XenNR1 produces functional receptors when it is expressed in combination with rodent NR2 subunits, a fuller electrophysiological study is required to determine whether the sequence differences between...
the Xenopus and rodent NMDA receptor subunits are physiologically and/or pharmacologically significant.

Most of the NMDA receptors that can be extracted from Xenopus central nervous system do not contain the XenU1 subunit because about 70% of the total NMDA receptor binding sites present in an extract of this tissue could not be retained by the homote affinity column (6). In fact the true proportion of such conventional NMDA receptors is likely to be higher than 70% since the initial n-ctyl-β-d-glucopyranoside extraction of all of the AMPA/kainate sites failed to solubilize all the NMDA receptor binding sites (7). Functional expression of both the conventional and the unitary NMDA receptor activities was seen previously in oocytes injected with poly(A) RNA from Xenopus brain, and this gave a similar proportion (5). We presume that this majority fraction of conventional NMDA receptors in Xenopus contains NR1/NR2 hetero-oligomers as in mammals; Western blotting tests on Xenopus brain membrane extracts with an antibody raised to a peptide sequence conserved in all NR2 subunits shows the presence of the latter (data not shown), but they have not yet been cloned. It seems reasonable to conclude that XenNR1 is a component of a major fraction of conventional NMDA receptors in Xenopus central nervous system and also a component of a smaller fraction of there of unitary NMDA/non-NMDA receptors.

**XenU1 as a Partner Subunit to XenNR1**—In several respects, XenU1 is an exceptional non-NMDA receptor subunit. It is only about one-half the size of the mammalian non-NMDA receptors subunits (Glur1–7 and KA1–2), yet it has relatively low sequence similarity to the short kainate-binding proteins (10–12) of non-mammalian vertebrates; unlike them, it also binds AMPA with high affinity (8). For the following reasons we conclude that XenU1 forms functional hetero-oligomeric receptors in Xenopus central nervous system by combining with XenNR1. (i) Functional receptor activity is introduced by the coexpression of these two subunits in HEK-293 cells and is strong and robust. (ii) The receptors formed by XenNR1 plus XenU1 acquire specific binding sites and agonist or antagonist selectivity properties that are characteristic of both NMDA and non-NMDA receptors. (iii) The partial complementation of human NR1E indicates that XenU1 can combine even with a mammalian NR1 subunit in stabilizing an NMDA receptor assembly. In contrast, NR1 subunits in HEK-293 cells did not interact with previously known non-NMDA receptor subunit types, when coexpressed. (iv) If XenNR1 and XenU1 expressed only independent homomeric receptors after their cotransfection, then additive currents would be anticipated during coactivation of NMDA and non-NMDA receptor agonists. In fact, for the current seen when NMDA is present, not only is there no increase with AMPA, but a decrease is produced. (v) Interaction between the two subunits comprising the XenNR1/XenU1 receptor is evident. For example, a voltage-dependent Mg2+ block occurs when the receptor is activated by non-NMDA as well as by NMDA ligands. (vi) A unique feature of the XenNR1/XenU1 receptor is the glycine requirement for responses to AMPA and kainate. This is clearly dependent on the presence of the XenNR1 subunit, since it is DCKA-sensitive and since glycine failed to enhance the responsiveness to non-NMDA agonists in the few cells expressing homo-oligomeric XenU1 receptors. An allosteric effect of glycine on the open time of the channel gated by non-NMDA agonists appears to be involved, since in the binding studies the affinity of kainate was not increased by the glycine. (vii) The XenNR1 and the XenU1 subunits can be coprecipitated from extracts of cotransfected cells and from Xenopus central nervous system by an anti-NR1 antibody. A considerable amount of receptor (>2 pmol/mg initial protein) was cross-precipitated in the latter case. This, as with features (i) to (vi), is only explicable in terms of a unitary receptor. It should be noted that the combined evidence here for the hybrid is equivalent to the similar evidence from which NR1/NR2 combinations have been accepted (2, 3, 23, 35).

In addition to that cross-immunoprecipitation of the native receptor, further evidence for the in vivo occurrence of the unitary receptor is that, as noted in the Introduction, a native protein purified from Xenopus central nervous system and reconstituted to give glutamate-activated channels showed the same unitary behavior and interaction between NMDA and non-NMDA receptor sites (6). That protein contains the XenU1 subunit (8) and a subunit of XenNR1 size (6).

**Conclusions**—We conclude that XenNR1 and XenU1 form a unitary NMDA/non-NMDA receptor in vivo. Are unitary glutamate receptors peculiar to such amphibians? Evidence of direct interaction between native NMDA and non-NMDA subunits in other species, including mammals, has rarely been sought, but functional indications of this have in fact been detected in native preparations (31, 36, 37). The data presented herein suggest that a wider search is now merited.

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**REFERENCES**

1. Watkins, J. C., and Olverman, H. J. (1987) Trends Neurosci. 10, 265–272
2. Hollman, M., and Heinemann, S. (1994) Annu. Rev. Neurosci. 17, 31–108
3. Seeburg, P. H. (1993) Trends Pharmacol. Sci. 14, 297–302
4. Moriyski, K., Masu, M., Ishii, T., Shigemoto, R., Mizuno, N., and Nakaniishi, S. (1991) Nature 354, 31–37
5. Perry, C. J., Sudan, H. L., Abutidze, K., Meller, I. R., Barnard, E. A., and Usherwood, P. N. R. (1993) Mol. Pharmacol. 44, 142–152
6. Henley, J. H., Ambrosini, A., Rodriguez-Huirralde, D., Sultan, H., Brackley, P., Kerry, C., Meller, I., Abutidze, K., Usherwood, P. N. R., and Barnard, E. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4806–4810
7. Henley, J. M., and Barnard, E. A. (1989) J. Neurochem. 52, 31–37
8. Ishimaru, H., Kamboj, R., Ambrosini, A., Henley, J. M., Soloviev, M. M., Sudran, H., Abutidze, K., Rossier, J., Usherwood, P. N. R., Bateson, A. N., and Barnard, E. A. (1996) Recept. Channels 4, 33–52
9. Moguro, H., Mort, H., Araki, K., Kushiya, E., Kutsuwada, T., Yamazaki, M., Kumanishi, T., Arakawa, M., Sakimura, K., and Mishina, M. (1992) Nature 357, 70–74
10. Wada, K., Dechesne, C. J., Shimakawa, S., King, R. G., Kusano, K., Buonnano, A., Hampson, D. R., Banner, C., Wenthold, R. J., and Nakatani, Y. (1989) Nature 342, 684–689
11. Gregor, P., Mann, I., Mass, M., McKeown, M., and Teichberg, V. I. (1989)
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12. Wo, Z., and Oswald, R. E. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 7154–7158
13. Kutsuwada, T., Kashiwabuchi, N., Mori, H., Sakimura, K., Kushiya, E., Araki, K., Meguro, H., Masaki, H., Kumanishi, T., Arakawa, M., and Mishina, M. (1992) *Nature* 358, 36–41
14. Foldes, R. L., Rampersad, V., and Kamboj, R. K. (1993) *Genet. Mol. Biol.* 16, 293–298
15. Cin, M., Chazot, P. L., and Stephenson, F. A. (1993) *Biochem. J.* 296, 877–883
16. Mery, P. F., Lechene, P., and Fischmeister, R. (1992) *Pflugers Arch. Eur. J. Physiol.* 420, 529–535
17. Sigel, E., and Barnard, E. A. (1984) *J. Biol. Chem.* 259, 7219–7223
18. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* 76, 4350–4354
19. Von Heijne, G. (1986) *Nucleic Acids Res.* 14, 4683–4690
20. Stern, E., and Schenker, L. T., Kennedy, M. B., and Seeburg, P. H. (1995) *Nature* 329, 1345–1347
21. Kornau, H. C., Schenker, L. T., Kennedy, M. B., and Seeburg, P. H. (1995) *Science* 269, 1737–1740
22. Sugihara, H., Mortysishi, K., Ishii, T., Masu, M., and Nakanishi, S. (1992) *Biochem. Biophys. Res. Commun.* 185, 826–832
23. Durand, G. M., Bennett, M. V. L., and Zakin, R. S. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 6731–6735
24. Hollman, M., Bouler, J., Maron, C., Beasley, L., Sullivan, J., Pecht, G., and Heinemann, S. (1993) *Neuron* 10, 943–954
25. Durand, G. M., Gregor, P., Zheng, X., Bennett, M. V. L., Uhl, G. R., and Zakin, R. S. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 9359–9363
26. Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B., and Seeburg, P. H. (1992) *Science* 256, 1217–1221
27. Lynch, D. R., Anegawa, N. J., Verdoorn, T., and Pritchett, D. B. (1994) *Mol. Pharmacol.* 45, 540–545
28. Grimwood, S., Le Bourdelles, B., and Whiting, P. J. (1995) *J. Neurochem.* 64, 525–530
29. Wong, E. H. F., Kemp, J. A., Priestly, T., Knight, A. R., Woodruff, G. N., and Iversen, L. L. (1984) *Proc. Natl. Acad. Sci. U. S. A.* 81, 7014–7018
30. Laurie, D. J., and Seeburg, P. H. (1994) *Eur. J. Pharmacol.* 268, 335–344
31. Brackley, P. H., and Usherwood, P. N. R. (1994) *J. Pharmacol. Exp. Ther.* 265, 910–919
32. Weng, L. A., and Mayer, M. C. (1994) *J. Pharmacol. Exp. Ther.* 266, 337–337
33. Marin, P., Quignard, J. F., LafonCazal, M., and Bockaert, J. (1993) *Biomed. Pharmacol.* 35, 540–545
34. Honore, T., Daven, S. R., Depe, J., Fletcher, E. J., Jacobsen, P., Lodge, D., and Nielsen, E. E. (1988) *Science* 241, 701–703
35. Hoo, K. H., Nutt, S. L., Fletcher, E. J., Elliott, C. E., Korchak, B., Deverell, R. M., Rampersad, V., Fantaske, R. P., and Kamboj, R. K. (1994) *Recept. Channels* 2, 327–337
36. Cheng, M., Cummings, J., Roldan, L. A., Jan, Y. N., and Jan, L. Y. (1994) *Nature* 366, 144–147