

Nucleotide Sequence, Genomic Organization, and Chromosomal Localization of Genes Encoding the Human NMDA Receptor Subunits NR3A and NR3B

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The N-methyl-D-aspartate (NMDA) receptors are glutamate-regulated ion channels that are critically involved in important physiological and pathological functions of the mammalian central nervous system. We have identified and characterized the gene encoding the human NMDA receptor subunit NR3A (*GRIN3A*), as well as the gene (*GRIN3B*) encoding an entirely novel subunit that we named NR3B, as it is most closely related to NR3A (57.4% identity). *GRIN3A* localizes to chromosome 9q34, in the region 13–34, and consists of nine coding exons. The deduced protein contains 1115 amino acids and shows 92.7% identity to rat NR3A. *GRIN3B* localizes to chromosome 19p13.3 and contains, as does the mouse NR3B gene (*Grin3b*), eight coding exons. The deduced proteins of human and mouse NR3B contain 901 and 900 amino acid residues, respectively (81.6% identity). *In situ* hybridization shows a widespread distribution of *Grin3b* mRNA in the brain of the adult rat.

Key words: NMDA receptor, subunit, glutamate receptor, genomic organization, gene, human, mouse, rat, *in situ* hybridization

INTRODUCTION

The N-methyl-D-aspartate (NMDA) receptors belong to the superfamily of glutamate-regulated ion channels, and are present in neurons throughout the central nervous system (CNS). In addition to glutamate, NMDA receptors require the presence of glycine to become activated. Furthermore, depolarization is required to relieve Mg²⁺ from blocking the channel pore. The permeability of the ion channel to Ca²⁺, Na⁺, and K⁺ strongly depends on the pH. The influx of Ca²⁺ activates several Ca²⁺-regulated proteins, for example protein kinases that may regulate a variety of cellular events by the mechanism of protein phosphorylation. Hence, NMDA receptors are important for plastic changes in the CNS such as learning and memory, development, and synapse formation. An excess of Ca²⁺ influx (induced by for example ischemia) may lead to pathological changes and cell death [1].

NMDA receptors are made up by multiple subunits. Initially, five NMDA receptor subunits, NR1, NR2A, NR2B, NR2C, and NR2D, were cloned in rat [2–4], mouse [5–8], and human [9–16] (somewhat misleadingly, human NR2B was named hNR3 in the first description [17]). Functional NMDA receptors require the presence of both NR1 and NR2 subunits, as the NR1 subunit contains the glycine binding

site and the NR2 subunits contain the glutamate binding site [18].

Subsequently, an additional NMDA receptor subunit was identified in the rat [19,20]. It was initially named χ -1 [19] or NMDAR-L [20], but has since been renamed NR3 [21] or NR3A [22]. The NR3A subunit is expressed predominantly during development with only very low levels in the adult rat [19,20]. Rat NR3A does not generate agonist-induced currents when expressed alone, together with NR1, or with a NR2 subunit. However, when coexpressed with a NR1 and a NR2 subunit, NR3A decreases the agonist-induced current, and deletion of the NR3A gene (*Grin3a*) in mouse causes an aberrant neuronal organization of the visual cortex [22]. Recently, it has been shown that coexpression of rat NR1 is an absolute requirement for surface expression of rat NR3A [23].

Here, we have identified and characterized the gene encoding human NR3A (*GRIN3A*), as well as the human and mouse genes encoding a novel NMDA receptor subunit, NR3B (*GRIN3B* and *Grin3b*). We identified the nucleotide sequence and deduced amino acid sequence of the expressed subunits, and the genomic organizations and the chromosomal localizations of the genes. In addition, we characterized the distribution of *Grin3b* mRNA in the rat brain.

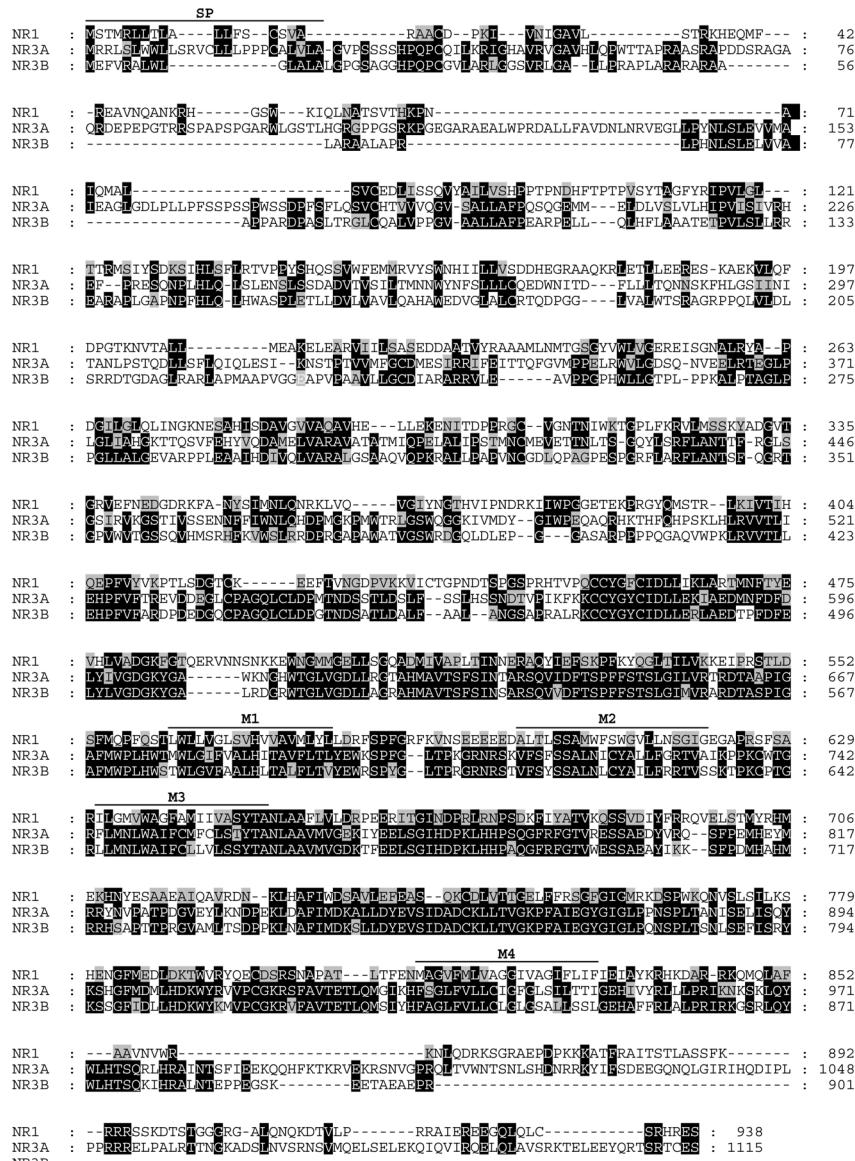


FIG. 1. Alignment of deduced amino acid sequences of the human NMDA receptor subunit NR1 and the novel human subunits NR3A and NR3B. Identical residues are enclosed in solid boxes, whereas conservatively related residues (within 4 distance units) are in shaded boxes. The bars show putative signal peptide (SP) and membrane-spanning regions (M1–M4) indicated by hydrophilicity analysis (data not shown).

RESULTS AND DISCUSSION

Identification of Human GRIN3A, Encoding the NMDA Receptor Subunit NR3A

GRIN3A is found on three genomic clones (bp ≤ 1304 on AL356516, bp ≥ 1305 on AL137023, and bp ≥ 700 on AL591377) on chromosome 9q34 in the region 13–34. The closely related gene encoding NR1 [24] is localized nearby, to 9q34.3 [25–27].

GRIN3A, as confirmed by sequencing cloned cDNA, contains 3345 bp, corresponding to 1115 amino acids (Fig. 1). Thus, the length of the subunit is identical to that of rat NR3A,

to which it shows 92.7% identity. Human NR3A shows 21.3% identity to human NR1, which is slightly less than the 27% identity between rat NR3A and NR1. A guanosine at position -3 (Fig. 2B), which is favorable for expression [28], precedes the initiation codon. The mature human NR3A subunit appears to contain 1089 amino acids, as the proposed signal peptide contains 26 amino acids. This is the same length as proposed in the rat [19], although a length of 32 amino acids also has been suggested [20].

An analysis of exon/intron boundaries suggested the presence of nine exons (Fig. 2). The exon/intron organization

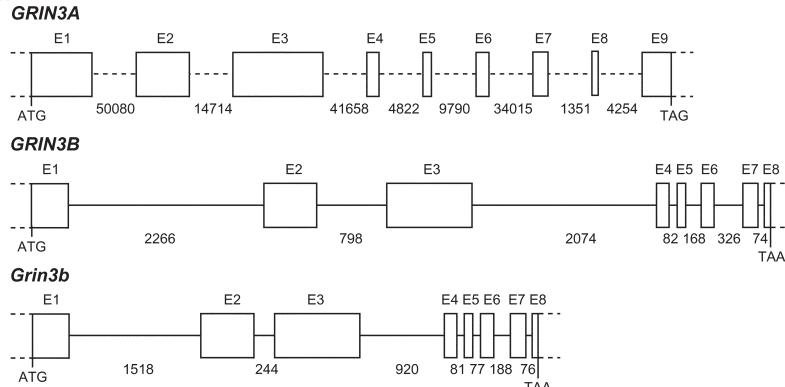
A

FIG. 2. Genomic organization of the genes for human NR3A (*GRIN3A*), human NR3B (*GRIN3B*), and mouse NR3B (*Grin3b*). (A) Exon/intron structures. Numbers indicate the length (bp) of introns. The introns of *GRIN3A* (164 kb) are not drawn to scale. (B) Lengths of exons and exon/intron boundaries. Gene sequence positions refer to clones AL35616.12 (exons 1, 2) and AL137023.9 (exons 3–9) for *GRIN3A*, AC004528.1 for *GRIN3B*, and AC087114.7 for *Grin3b*. Nucleotides in lowercase represent intronic sequences and untranslated sequences at the ends, whereas nucleotides in uppercase represent coding sequences. The single-letter code above the first nucleotide in the respective codon shows the deduced amino acids.

B

Gene	Exon	Length of exon (bp)	Position in gene clone	Sequence at exon-intron junction
<i>GRIN3A</i>	1	699	58428-57730	M R R L ... E S Q tca gta ATGAGGAGAC ... GGAGAGTCAGgt gagaggag
	2	605	7649-7045	N P L H ... L S R ttaaataac agAATCCCCTC ... ATTATCACAGgt taggatgc
	3	1048	8451-9498	F L A ... D P K tc tttcccg AGTTCTAGCC ... TGACCCCAAAGgt aataactc
	4	146	51157-51302	L H H P ... Y L K tccctcatag TTACATATC ... AGTATCTGAgt gagtgca
	5	116	56125-56240	N D P ... A I E G ttaaatata gGAATGATCCA ... GGCATAAGgt tattaaatca
	6	152	65942-66093	Y G M ... V T E tttcttctag GATACGGCAT ... TGTCA CGGAGgt tatggaaag
	7	165	100109-100273	T L Q M ... T S Q tctgtcacag ACTTGAAA ... CACCA GCGAGgt gagtgcca
	8	77	101625-101701	R L H ... E K R tctccatc agAGATTACACA ... TGAAAAGAGgt aagaagg
	9	337	105956-106292	S N V ... E S Stop tgtttcttag GTCTAATGTG ... TGAGTCCTag
<i>GRIN3B</i>	1	426	18665-19090	M E F V ... G A P tttgcg ATGGAGTTG ... CGGAGCCCCGt acgcggga
	2	593	21357-21949	N P F H ... L A R cctctcccg AACCCATTC ... CCTGGCACGgt gagtggggg
	3	1033	22748-23780	F L A ... D P K ctgcccctag GTTCTGGCC ... CGACCCCAAAGgt gggcgccc
	4	146	25855-26000	L H H P ... M L T ccccgcgcg CTGACCAACC ... CCATGCTCA Gt gagccccc
	5	116	26083-26198	S D P ... A I E G gccccacg GAGCGACCCC ... GCCATTGAGgt gagggca
	6	152	26367-26518	Y G I ... V T E gccccccacg GCATAGGAT ... GTTACAGAGgt gggggcagg
	7	165	26845-27009	T L Q M ... T S Q ttgccccacg ACCCTGAGA ... CACCA GCGAGgt gggggagcg
	8	72	27084-27155	K I H R ... P R Stop ggggcttag AAAATCCACC ... GCCCAGGtaa
<i>Grin3b</i>	1	426	127203-127628	M E C V ... G A P atccggg ATGGAGTTG ... CGGAGCCCCGt acgcgttag
	2	593	129147-129739	T P F H ... L A R cttctcccg AACCCGTTCC ... CCTGGCTA Gt gagtgtaggg
	3	1033	129984-131016	F L S ... D P K tattccacag GTTCTGGAC ... TGATCCCAAAGgt gaggtctg
	4	146	131937-132082	L H H P ... M L T tccctctcgac CTGACCAACC ... CCATGCTCA Gt gagtgctgt
	5	116	132164-132279	S D P ... A I E G tccccccacg GAGCGACCCC ... CGCATAGAGgt gggggcagg
	6	152	132357-132508	Y G I ... V T E atccctcgac GCTACGGCAT ... CGTCA GAGgt gggggcagg
	7	165	132697-132861	T L Q M ... T S Q ccattccacag ACGCTGAGA ... CACCA GCGAGgt gagggaggc
	8	69	132938-133006	K I H R ... C R Stop gtccccctag AAAGATCCACC ... GTGCAGGtaa

fitted well with the existence of the 60-bp insert of the splice variant termed NR3-1 [21] or NR3A-2 [29] described in the rat, except the insert should be located between bp 3008 and 3009 instead of between bp 3006 and 3007 as suggested previously [21]. However, we did not identify any homologous sequence to this insert in the human gene, suggesting that this splice variant is lacking in human.

There are preliminary reports of *GRIN3A* mRNA expression in the visual cortex of adult primates [30] and in fetal

human brain [31], but the regional and developmental distribution of human NR3A remains to be determined.

Identification of Human *GRIN3B*, Encoding the NMDA Receptor Subunit NR3B

Based on homology with NR3A, we identified a novel gene on clone AC004528 on chromosome 19p13.3, which we named *GRIN3B*. The gene encoding human NR2D (*GRIN2D*) is localized nearby, to 19q13.1 [32], whereas *GRIN2A*, *GRIN2B*, and *GRIN2C* are scattered on chromosomes 16p13.2, 12p12, and 17q25, respectively [27,32,33].

GRIN3B contains 2703 bp, corresponding to a protein, NR3B, of 901 amino acids (Fig. 1), and NR3B is therefore the shortest of all hitherto described NMDA receptor subunits. Human NR3B shows the highest homology to human NR3A (57.4% identity). Alignment of all seven known human NMDA receptor subtypes showed that NR3A and NR3B form a subgroup that shows the greatest similarity

to NR1 (Fig. 3). The sequence surrounding the initiation codon contains guanosines at positions -3 and +4, which is favorable for expression [28]. The proposed signal peptide contains 14 amino acids, suggesting that the mature human NR3B subunit contains 887 amino acids.

Analysis of exon/intron boundaries suggested the presence of eight exons in *GRIN3B* (Fig. 2). Hence, *GRIN3A* and *GRIN3B* contain fewer exons than what is known of other NMDA receptor subunit genes: *GRIN2C* contains 12 coding exons [34,35],

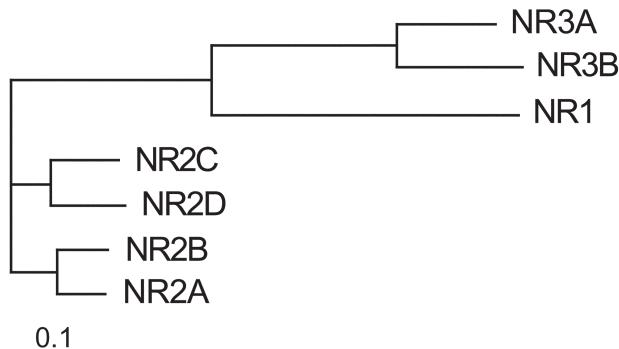


FIG. 3. Rooted phylogeny tree showing the relationship between the seven known human NMDA receptor subunits, based on the conserved lig_chan domain that corresponds to residues 559–834 in NR1. We observed the same relationships using the ANF receptor domain, corresponding to residues 27–382 in NR1 (data not shown).

whereas *GRIN1* contains 22 exons [36]. It has been suggested that NR1 is the oldest member of the NMDA receptor subunit family, and that the fewer exons in *GRIN2C* have been derived by exon fusion [34]. However, the alignments using the conserved domains (Fig. 3) suggest that NR1, NR3A, and NR3B are further differentiated from a putative common ancestor than the NR2A–D subunits.

Expression of *GRIN3B* mRNA is suggested by EST sequences AL040053 and AL359933 from clone DKFZp343P1912, but the distribution in the human brain remains to be determined.

Identification of Mouse *Grin3b*

We identified *Grin3b* on two clones from mouse chromosome 10 (AC087114 and AC073805). Based on homology with *GRIN3B*, as well as analysis of exon/intron boundaries, *Grin3b* consists of 2700 bp, which correspond to 900 amino acids (Fig. 4). This is one residue less than in human NR3B and results from *Grin3b* having three nucleotides less in the last exon (E8). This was confirmed by sequencing cloned cDNA containing the 3' end of NR3B. Mouse NR3B showed an 81.6% identity overall with human NR3B, which is far less than that between the human and rat counterparts of the other NMDA receptor subunits: 92.7% for NR3A (this study), 89.0% for NR2C, and 95.2–99.8% for the NR1, NR2A, NR2B, and NR2D subunits [37]. The lig_chan domain (residues 574–852, see below for domain definition) showed a marked homology (94.6%) between the human and mouse genes. In contrast, the amino- and carboxy-terminal regions surrounding the lig_chan domain displayed only 75.6% and 73.5% identity, respectively. This suggests that there are differences in the regulation by extracellular and intracellular factors of human and mouse NR3B. The C termini of human and rat NR2C show a similar low homology (71.5%) between human and rodent subunits [37].

Grin3b is identical to *GRIN3B* with regard to nucleotide sequence around the initiation codon and the exon/intron organization (Fig. 2). However, the length of the introns in the mouse is generally shorter than in the human. In mouse, there is an additional ATG sequence 240 bp upstream of the putative true initiation codon. This additional ATG is not likely to initiate transcription because it lacks an appropriate Kozak sequence (it has a C in -3 position and a T at +1) and *GRIN3B* lacks corresponding sequences.

Expression of Rat *Grin3b* mRNA

We identified parts of the rat *Grin3b* nucleotide sequence on EST sequences: AW525909 and BF563382 contain putative nucleotides 427–616 and 628–1019 in putative exon 2; and both BE108608 and BE112464 contain nucleotides 1818–2052 in putative exon 3. We used this sequence information to carry out *in situ* hybridization in the rat brain. *Grin3b* is expressed in all parts of the brain, suggesting that the encoded subunit is a common constituent of NMDA receptor complexes throughout the CNS. The highest levels are present in the substructures of the hippocampus (CA1, CA3, and dentate gyrus) and in the granule cell layer of the cerebellar cortex (Fig. 5A), possibly due to the high concentration of neurons in these areas (Fig. 5B). In addition, we observed labeling in the corpus callosum. This region is known to occasionally cause nonspecific labeling. However, evidence against this possibility is the fact that all four *Grin3b* probes labeled the corpus callosum but not white matter in the cerebellum. Furthermore, the nonsense probe did not label the corpus callosum (data not shown). Hence, the identity of the labeling in this area remains to be determined.

General Features of NR3 Subunits

All subunits described here contain the motif YTANLAA (Fig. 4), which is a common motif of all glutamate-regulated ionotropic receptor subunits [3,4]. Also, they contain the motif CC(Y/K)G(Y/F)CID(I/L)L, which has been predicted to be present in NMDA but not in non-NMDA ionotropic receptors, because only NMDA receptors are susceptible to redox modulation [38,39]. Both NR3A (residue 730) and NR3B (residue 630) contain an arginine in the Q/R site in M2, a site that is important for ion selectivity [40]. However, the Q/R site of these subunits is localized one residue further downstream than in the other five NMDA receptor subunits.

Human NR3A contains 10 putative sites for glycosylation (one less than in rat NR3A), 3 potential sites for Ca^{2+} /calmodulin-dependent protein kinase type II (one more than in rat NR3A), and 6 potential sites for protein kinase C (as in the rat; Fig. 4), of which one partly overlaps a potential site for tyrosine kinase [20]. NR3B contains only five putative glycosylation sites, suggesting that it is a less glycosylated subunit than NR3A [41]. The NR3B subunits also contain four potential sites for protein kinase C (three of which are in common with NR3A), but lack sites for tyrosine kinase. In addition, human (but not mouse) NR3B contains a potential site for Ca^{2+} /calmodulin-dependent protein kinase type II.

SP	
Rat NR3A :	MRLLSLWLLSRVCLLPPPCALVLAGVPSS--SSHOPCQILKRGHAVRVAHVLPWTAPRAASRAQEGG :
Human NR3A :	MRLLSLWLLSRVCLLPPPCALVLAGVPSS--SSHOPCQILKRGHAVRVAHVLPWTAPRAASRAPDD :
Human NR3B :	MEFVRALWLIG-----LALALGPGS--AGGHPQPCGVRLRGGSVRLGALLPR---APALARARA---- :
Mouse NR3B :	MBCVQTLWLS-----LALALARGSWVRGHPQPCGVTRAGSVRLAALLPR---APAARARV---- :
Rat NR3A :	RAGAQRDDPESGTWRPPAPSQARWLGSALHGRGPPGSRKLGEGAGAETLWPRDALLFAVENLNRLNRVEGLLPY-NLS :
Human NR3A :	RAGAQRDPESGTWRPPAPSQARWLGSALHGRGPPGSRKLGEGAGAETLWPRDALLFAVENLNRLNRVEGLLPY-NLS :
Human NR3B :	-----RALARAL----APRLPHNLS :
Mouse NR3B :	-----LAALATP----SPRLPHNLS :
Rat NR3A :	LEVVMAIEAGLDLPLMPFSSPSSPWSSDPFSFLQSVCVHTVVVQGSALLAFAFPQSQGEMMELDLVSSVLHIVPLS :
Human NR3A :	LEVVMAIEAGLDLPLMPFSSPSSPWSSDPFSFLQSVCVHTVVVQGSALLAFAFPQSQGEMMELDLVSSVLHIVPLS :
Human NR3B :	LELVVAAPP-----ARDPASLTRLGCOALVPGVVAALLFPEARPELLOLQLHFLAAATETPVLS :
Mouse NR3B :	LELVAVASP-----TRDPASLARGLCQLAPPGVVASITFPEARPELRLLQFLAAATETPVLS :
Rat NR3A :	IVRHED--PRESQNPLHLQLSLENSLSSADTVSILTMMNWYNFSLLLCQEDWNITDPLLTEENNSKFHLESVI :
Human NR3A :	IVRHED--PRESQNPLHLQLSLENSLSSADTVSILTMMNWYNFSLLLCQEDWNITDPLLTEENNSKFHLESII :
Human NR3B :	LLRREARAPLGAPNPFPHLQLHWASPLETILDVLAVALQAHAWEDVGLALCRTODPGLVALWTSRAGRPPQ-LVL :
Mouse NR3B :	VLRRREVRAPLGAPTPFHQLDWASPLETILDVLSVLVRHAWEIDLALVLCVRDPSGLVTLWTSRASQPK-FVL :
Rat NR3A :	NITANLSSTKDLLSFLQVQMDNIRNSTPT--MVMFGCDMSIRQIFEMSTQFGLSPPELHWVLDQSNVEELRTE :
Human NR3A :	NITANLSPQDNLQIQLIESIKNSTP--MVMFGCDMSIRQIFEMSTQFGLSPPELHWVLDQSNVEELRTE :
Human NR3B :	DLSRRTDGAGLRARLAPMAVPVGGEAPVPAVLLGCDIARARRVLEA-----VPPGPHWLLGTPLPKALPTA :
Mouse NR3B :	DLSQLDGSNDSLRATLALLGTLLEGGTPVSAVLLGCSTAHAHEVLEA-----APPGPQWLLGTPLPKALPT :
Rat NR3A :	GLPLGLIAHKGTQTQSVEFYVQDAMEVARAVATATMIPPELALLPSTMNCMDVKTTN-LTSGQYLSRFLANTTF :
Human NR3A :	GLPLGLIAHKGTQTQSVEFYVQDAMEVARAVATATMIPPELALLPSTMNCMDVKTTN-LTSGQYLSRFLANTTF :
Human NR3B :	GLPPGLLALGEVARPPLAEAAIHDTQLVARALGSAAQVQPKRALPAPVNCGDLQPGPESPGFLARFLPLNTSF :
Mouse NR3B :	GLPPGVLVLLGETQPSLEAAVHDMLVVELARALSMALMHPERALLPAVNCEDLKTGGSESTARFLARPLSNTSF :
Rat NR3A :	RGLSGSIKVKGSTIISSENNFFIWNLQHDPGMGKPMWTRLGWSWQGRIVMDSGIWIPEQAOQRKHTHFQHPNKLHLRV :
Human NR3A :	RGLSGSIKVKGSTIISSENNFFIWNLQHDPGMGKPMWTRLGWSWQGRIVMDSGIWIPEQAOQRKHTHFQHPNKLHLRV :
Human NR3B :	QGRTPGVWVTGSSOVMHSRHFVWSSLRDPGAPAWATVGSWRDQQLDEPGASARPSSQGAQWP--KLRV :
Mouse NR3B :	QGRTPGVWVAGSSQVHVSRFVWSSLRDPGAPAWATVGSWQDQQLDFQPGAAALRVPSPSGTQARP--KLRV :
Rat NR3A :	VTLIEHPFVFTREVDEGLCPAGQLCLDPMTNDSSMLDRLFSSLHSSNDTVPINKFKKCCYGYCIDLQLAEDMN :
Human NR3A :	VTLIEHPFVFTREVDEGLCPAGQLCLDPMTNDSSMLDRLFSSLHSSNDTVPINKFKKCCYGYCIDLQLAEDMN :
Human NR3B :	VTLIEHPFVFARPDDEDGQCPCAGLCLDPGTNDSATLDLFAALA--NGSAPRLRKCCYGYCIDLQLAEDTP :
Mouse NR3B :	VTLIEHPFVFTRSEDDEDGQCPCAGLCLDPGTNDSSRLDALFTALE--NGSVPRTLRRCCYGYCIDLQLAEDTP :
Rat NR3A :	FDFDLYIVGDGKYGAWKNGHWTGLVGDLSSGTANMAVTSFSINTARSQVIDFTSPFFSTSLGILVTRTDAAPIG :
Human NR3A :	FDFDLYIVGDGKYGAWKNGHWTGLVGDLSSGTANMAVTSFSINTARSQVIDFTSPFFSTSLGILVTRTDAAPIG :
Human NR3B :	FDFFELYIVGDGKYGALRDGRWTGLVGDLSSGTANMAVTSFSINTARSQVIDFTSPFFSTSLGIMVRARDTASPIG :
Mouse NR3B :	FDFFELYIVGDGKYGALRDGRWTGLVGDLSSGTANMAVTSFSINTARSQVIDFTSPFFSTSLGIMVRARDTASPIG :
M1	
Rat NR3A :	AFMWPLHWTMWLGIFVALHITAIFIYLIEWKSPFGMTPKGRNRNKVFSFSSALNCVYALLFGRTAIKPPKCWTG :
Human NR3A :	AFMWPLHWTMWLGIFVALHITAIFIYLIEWKSPFGMTPKGRNRNKVFSFSSALNCVYALLFGRTAIKPPKCWTG :
Human NR3B :	AFMWPLHWTMWLGIFVALHITAIFIYLIEWKSPFGMTPKGRNRNKVFSFSSALNCVYALLFGRTAIKPPKCWTG :
Mouse NR3B :	AFMWPLHWTMWLGIFVALHITAIFIYLIEWKSPFGMTPKGRNRNKVFSFSSALNCVYALLFGRTAIKPPKCWTG :
M2	
Rat NR3A :	FLMLNWLAIFCMPCLSTYTANLAAMVGEKIFYEELSGIHDPKLHHPSQGFRFTGVRESSAEDYVRQSFPEMHEY :
Human NR3A :	FLMLNWLAIFCMPCLSTYTANLAAMVGEKIFYEELSGIHDPKLHHPSQGFRFTGVRESSAEDYVRQSFPEMHEY :
Human NR3B :	FLMLNWLAIFCMPCLSTYTANLAAMVGEKIFYEELSGIHDPKLHHPSQGFRFTGVRESSAEDYVRQSFPEMHEY :
Mouse NR3B :	FLMLNWLAIFCMPCLSTYTANLAAMVGEKIFYEELSGIHDPKLHHPSQGFRFTGVRESSAEDYVRQSFPEMHEY :
M3	
Rat NR3A :	FLMLNWLAIFCMPCLSTYTANLAAMVGEKIFYEELSGIHDPKLHHPSQGFRFTGVRESSAEDYVRQSFPEMHEY :
Human NR3A :	FLMLNWLAIFCMPCLSTYTANLAAMVGEKIFYEELSGIHDPKLHHPSQGFRFTGVRESSAEDYVRQSFPEMHEY :
Human NR3B :	FLMLNWLAIFCMPCLSTYTANLAAMVGEKIFYEELSGIHDPKLHHPSQGFRFTGVRESSAEDYVRQSFPEMHEY :
Mouse NR3B :	FLMLNWLAIFCMPCLSTYTANLAAMVGEKIFYEELSGIHDPKLHHPSQGFRFTGVRESSAEDYVRQSFPEMHEY :
Rat NR3A :	RRYNVPATPGVQYLNPDKEKLNDFKDALAFIMDKALLDYEVSIDACKLTVGKPFIAIEGYGIGLGPNSPLTSNISELIS :
Human NR3A :	RRYNVPATPGVQYLNPDKEKLNDFKDALAFIMDKALLDYEVSIDACKLTVGKPFIAIEGYGIGLGPNSPLTSNISELIS :
Human NR3B :	RRHSAPTTPRGVAMLTSDDPKLNADFMDKSLLDYEVSIDACKLTVGKPFIAIEGYGIGLGPNSPLTSNISELFIS :
Mouse NR3B :	RRHSAPTTPRGVAMLTSDDPKLNADFMDKSLLDYEVSIDACKLTVGKPFIAIEGYGIGLGPNSPLTSNISELFIS :
M4	
Rat NR3A :	QYKSHGFMDVLHKWYKVVPCKGKRSFAVETETLQMGKHFGLFVLLCIGFGLSILTTTGEIHIVRLLLPRIKNKS :
Human NR3A :	QYKSHGFMDVLHKWYKVVPCKGKRSFAVETETLQMGKHFGLFVLLCIGFGLSILTTTGEIHIVRLLLPRIKNKS :
Human NR3B :	RYKSSGFIDLLHKWYKVVPCKGKRVFAVETETLQMGKHFGLFVLLCIGFGLSILTTTGEIHIVRLLLPRIKNKS :
Mouse NR3B :	RYKSSGFIDLLHKWYKVVPCKGKRVFAVETETLQMGKHFGLFVLLCIGFGLSILTTTGEIHIVRLLLPRIKNKS :
Rat NR3A :	KLQYWLHTSQRFHRAINTSFVVEKQPRSKTKEVKEKRSNLPQQLMVWNTSNLSHNDQRKYIFNDEEGQNLGTQA :
Human NR3A :	KLQYWLHTSQRFHRAINTSFVVEKQPRSKTKEVKEKRSNLPQQLMVWNTSNLSHNDQRKYIFNDEEGQNLGTQA :
Human NR3B :	RLQYWLHTSQRFHRAINTSFVVEKQPRSKTKEVKEKRSNLPQQLMVWNTSNLSHNDQRKYIFNDEEGQNLGTQA :
Mouse NR3B :	RLQYWLHTSQRFHRAINTSFVVEKQPRSKTKEVKEKRSNLPQQLMVWNTSNLSHNDQRKYIFNDEEGQNLGTQA :
Rat NR3A :	HQDIPLPQRRLPELPAASLTNTNGKADSLNVTRSSVIELSELEKQIQVIRQELQLAVSRKTELEYOKTNECTCES :
Human NR3A :	HQDIPLPQRRLPELPAASLTNTNGKADSLNVTRSSVIELSELEKQIQVIRQELQLAVSRKTELEYOKTNECTCES :
Human NR3B :	-----KEETAAEAPR-----:
Mouse NR3B :	-----QPERAEQECCR-----:

FIG. 4. Deduced amino acid sequences of the four described NR3 subunits: rat NR3A, human NR3A, human NR3B, and mouse NR3B. Putative sites are shown for glycosylation (yellow boxes) and possible sites for protein kinase C (red boxes), Ca^{2+} /calmodulin-dependent protein kinase II (green boxes), and tyrosine kinase (blue boxes), as analyzed by PROSITE. Bars show putative signal peptide (SP) and membrane-spanning regions (M1-M4).

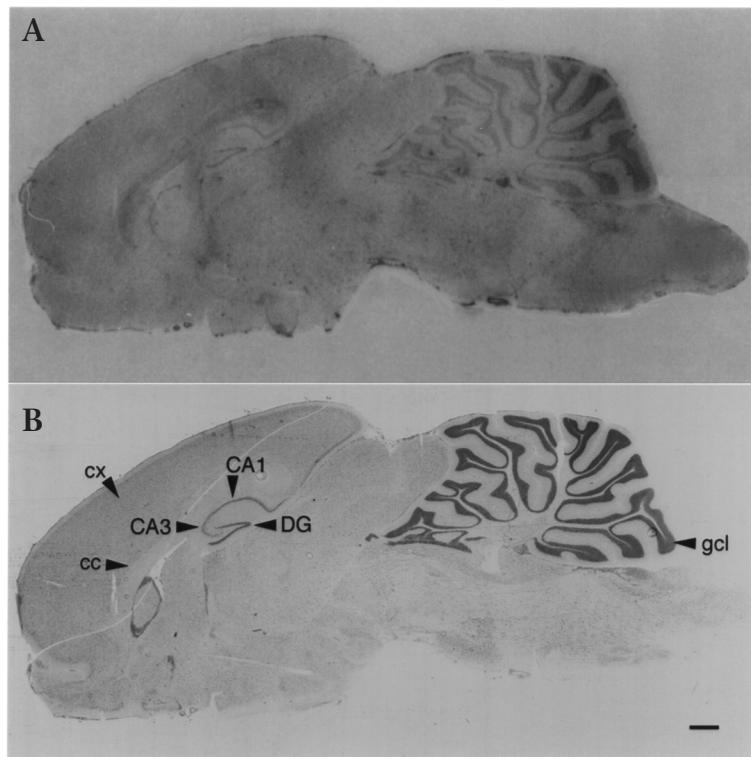


FIG. 5. Distribution of *Grin3b* mRNA in the adult rat brain. *In situ* hybridization of *Grin3b* mRNA (A) and cresyl violet staining (B) of the same sagittal section are shown. The autoradiogram was produced using probe E3-2. The three other probes used showed a similar distribution pattern, whereas the nonsense probe produced blank autoradiograms at the same exposure times (data not shown). CA1, cornus ammonis 1; CA3, cornus ammonis 3; dg, dentate gyrus of the hippocampus; cx, cerebral cortex; cc, corpus callosum; gcl, granular cell layer of the cerebellum. Bar, 1 mm.

Cloning. PCR reactions (Clontech's Advantage -2 and -GC2) were carried out using Marathon-Ready cDNA from human fetal brain or BALB/c mouse brain (Clontech), and primers designed on the basis of database information. We ligated PCR products into pT-Adv vectors which were transfected into TOP10F' *Escherichia coli* (Clontech's AdvanTAge PCR cloning kit). Clones were isolated and sequenced using ABI Prism BigDye terminator cycle sequencing (PE Biosystems), followed by analysis at Karolinska Institute's sequencing facility KISeq.

Analysis of deduced amino acid sequences. We performed Clustal W alignments and hydrophilicity analysis [45] of the deduced amino acid sequences using Megalign in the DNASTar package. We generated rooted phylogenograms using Treeview [46] (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>). We performed a Pfam search (<http://www.sanger.ac.uk/Software/Pfam>), using profile hidden Markov models, on the NMDA receptor subunits to identify consensus domains [47]. Also, we ran the sequences through the PROSITE database (<http://www.expasy.ch/prosite>) to detect potential sites for glycosylation and protein phosphorylation [48].

The conserved ligand-channel domain *lig_chan* [42] (acc. no. PF00060) is present both in NR3A (residues 674–952) and in NR3B (residues 574–852). One additional domain, the extracellular ligand binding domain *ANF_receptor* [43] (acc. no. PF01094), is detected in human NR3A (residues 114–487) and in mouse NR3B (residues 30–392), but not in rat NR3A or human NR3B. This may be because this site is nonfunctional and has undergone random deterioration, in view of the lack of known NR3A and NR3B ligands, or because the domain definition requires refinement.

Our study suggests that NMDA receptors may be even more diverse than hitherto believed. Hence, understanding the role of NR3A and NR3B subunits in physiological and pathological conditions will require a thorough characterization of their interactions with other NMDA receptor subunits, as well as identification of possible agonists and modulatory substances.

MATERIALS AND METHODS

Database searches and gene analyses. We identified genes and gene fragments using sequence information for human NR1 (the long splice variant NR1-3; GenBank/EMBL acc. no. NM_007327), human NR2A (NM_000833), human NR2B (NM_000834), human NR2C (NM_000835), human NR2D (NM_000836), and rat NR3A (L34938 and U29873), by search at the National Center for Biotechnology Information (NCBI) Internet site (<http://www.ncbi.nlm.nih.gov>) using BLAST [44]. We assembled gene fragments using homologous sequence information from rat NR3A and by identifying sequences for initiation, splicing, and termination.

In situ hybridization. *In situ* hybridization was performed basically as described [49]. We obtained a brain from a 300-g Sprague-Dawley rat (B&K, Sollentuna, Sweden), which was quickly frozen in isopentane and stored at –80°C until sectioning. The brain was cut in 14-μm sagittal sections using a Leica CM3000 cryostat. Sections were thaw-mounted onto slides (SuperFrost Plus from Menzel-GLäser) and stored at –20°C until hybridization.

We synthesized four *in situ* hybridization probes (Medprobe) using sequence information from rat ESTs (AW52590, BF563382, BE108608, and BE112464). We designed E2-1 (5'-CGGCAGAGTACTAGAGCAATGTC-CTCCCAGGCATGTGCCGTAC-3'; antisense bp 495–539) and E2-2 (5'-CTGTTTCAGGTCAATCACAGTTACCACACAGCTGGAAGCAGTGCCC-3'; antisense bp 941–985) to detect exon 2, and E3-1 (5'-GGCATAG-CACAGGTTGAGCGGGAGGAGTAAGAGAACAGTGCC-3'; antisense bp 1831–1875) and E3-2 (5'-TCCTCAAAGGTTTGCCCCAACAT-GACAGCAGCCAGGTGGCC-3'; antisense bp 1983–2027) to detect exon 3. The base pair annotations refer to the corresponding positions in the human and mouse sequences. In addition, we used a nonsense probe (5'-CCAACG-GTAGTGACAGAAGTACAAGCTATGAAGTCCGGACAGTC-3') to detect possible nonspecific labeling.

Oligonucleotides were labeled at the 3' end with α[35S]dATP (NEN) using terminal deoxyribonucleotidyl transferase (Amersham-Pharmacia Biotech). Probes were purified (Mini QuickSpin Oligo Columns from Boehringer-Mannheim) and hybridized (200 μl/slides) in a 20 mM phosphate buffer (pH 7.0) containing 50% (v/v) formamide, 4× SSC, 1× Denhardt's solution (Sigma), dextran 10% (w/v; Amersham-Pharmacia Biotech), and 1% *N*-lauroylsarcosine, which was diluted 1:10 (v/v) with a solution containing sheared salmon sperm DNA (0.05 mg/ml final concentration), dithiothreitol (200 mM f.c.), and hybridization probe (1 × 10⁷ cpm/ml f.c.).

Sections were hybridized overnight at 42°C in a sealed chamber humidified with 50% formamide and 2× SSC. After hybridization, we washed the slides in 1× SSC for four times for 15 min at 55°C, followed by rinsing for 1 min in 1× SSC and 1 min in water at room temperature. Sections were dehydrated in a graded series of ethanol and air-dried before exposure to a β-max hyperfilm (Amersham) for 2 weeks. We developed and fixed the film in Kodak LX24 and AL4, and counterstained the sections with cresyl violet.

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Sequence data from this article have been deposited with the DDBJ/EMBL/GenBank Data Libraries under accession numbers AF416558 (GRIN3A) and AF373861 (Grin3b).