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# Long-lasting enhanced expression in the rat hippocampus of NMDAR1 splice variants in a kainate model of epilepsy

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## Abstract

Chronic epilepsy is associated with increased excitability which may result from abnormal glutamatergic synaptic transmission involving altered properties of *N*-methyl-D-aspartate (NMDA) receptors. To date two gene families encoding NMDA receptor subunits have been cloned, NR1 and NR2. Eight NR1 mRNAs are generated by alternative splicing of exons 5, 21 and 22; the NR1–1 to NR1–4 C-terminal variants exist in the a or b version depending on the presence or absence of the domain encoded by exon 5. Epilepsy was induced in rats by unilateral intra-amygdalar injection of kainate and animals were killed from 6 h to 4 months following the injection. Increased NR1 mRNA levels were observed during status epilepticus (6–24 h after the injection), both ipsilateral and contralateral, while a second wave of NMDAR1 mRNA increase occurred in chronic epileptic animals, between 21 days and 4 months following kainate injection. Our data show: (i) a permanent increase of the NR1–2a and NR1–2b mRNA species (containing exon 22) in all hippocampal fields, both ipsilateral and contralateral, and (ii) an increase of the NR1–3 (a and b) mRNAs (containing exon 21) in the ipsilateral CA1, and NR1–3a mRNA in the ipsilateral dentate gyrus. No long-term changes were observed for the NR1–1 and NR1–4 splice variants. In the ipsilateral CA3 area a globally decreased mRNA expression was associated with neuronal loss. A possible contribution to the maintenance of the epileptic state by an increased expression of NMDA receptors is discussed.

## Introduction

Epilepsy is a chronic condition characterized by the presence of spontaneous episodes of neuronal discharges. Several reports suggest that this increased excitability may result, at least in part, from an abnormal glutamatergic synaptic transmission that may involve a modification of the properties of glutamate receptors. In the central nervous system (CNS), glutamate activates *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-kainate (KA) ionotropic receptors. The contribution of both types of receptors to epileptiform activity has been investigated in various models of epilepsy: while glutamate receptor antagonists act as anticonvulsants, AMPA-KA and NMDA mediated responses are increased in hippocampal slices of epileptic animals (Peterson *et al*., 1983; Mody & Heinemann, 1987; Gilbert, 1988; McNamara *et al*., 1988; Wheal, 1989; Simpson *et al*., 1991; Turner & Wheal, 1991; Martin *et al*., 1992) or in acute hippocampal slices (Johnston & Brown, 1984; Ben-Ari & Gho, 1988; Neuman *et al*., 1988a,b). Binding and autoradiographic studies show that NMDA (Yeh *et al*., 1989; Kraus *et al*., 1994) and KA (Represa *et al*., 1987, 1989a) receptor binding increases in the hippocampus of chronic epileptic animals and in hippocampal tissue from epileptic patients (Represa *et al*., 1989b; McDonald *et al*., 1991; Hosford *et al*., 1991). Changes in KA binding are likely related to synaptic remodelling of the hippocampal mossy fibres (Tauck & Nadler, 1985; Represa *et al*., 1987, 1989a; Ben-Ari & Represa, 1990; Represa *et al*., 1993). It is also possible that changes in AMPA-KA receptors are induced by molecular alterations of these receptors (Gall *et al*., 1990; Kamphuis *et al*., 1992; Pollard *et al*., 1993; Friedman *et al*., 1994). To date, persistent changes in NMDA binding have been observed in kindled animals but they are difficult to interpret. First, because the kindling model of epilepsy is not associated with the expression of spontaneous recurrent seizures nor with hippocampal lesions, characteristic of temporal lobe epilepsy. Second, although novel binding properties of NMDA receptors have been observed following kindling (Kraus *et al*., 1994), long-term maintenance of the kindled state seems to occur in the absence of long-term changes in the transcript levels of NMDA receptor genes (Pratt *et al*., 1993; Kraus *et al*., 1994; Vezzani *et al*., 1995).

Two types of NMDA receptor subunits have been cloned, NR1 and the NR2 family (Hollmann & Heinemann, 1994 for review). Eight splice variants have been reported for NR1 (Anantharam *et al*., 1992; Durand *et al*., 1992; Nakanishi *et al*., 1992; Sugihara *et al*., 1992; Yamazaki *et al*., 1992; Hollmann *et al*., 1993) generated by a combination of three alternative splicings involving exon 5 (N-terminal domain) and exons 21 and 22 (C-terminal domains). NMDA receptors containing NR1 subunits with the N-terminal insertion (NR1-b type, according to the nomenclature defined by Hollmann *et al*., 1993) display different agonist and antagonist responses, are less sensitive to polyamine potentiation,

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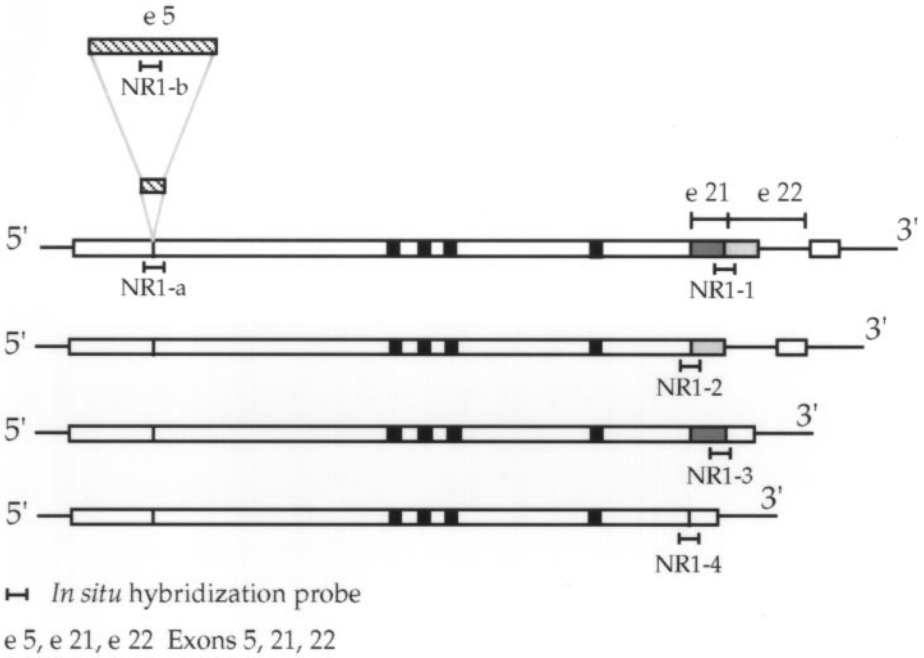


FIG. 1. Schematic representation of the NR1 splice variants and probes used in this study. Thin line, 5′ and 3′ non coding regions; boxes, coding region; cross-hatched bars, spliced exon in 5′; dark grey and light grey boxes, coding domains in 3′ exons (exon 21 and exon 22); closed bars, putative transmembrane domains TMI-IV; segments, antisense oligonucleotide probes.

and produce larger currents than do receptors lacking this insertion (NR1-a) (Nakanishi *et al*., 1992; Durand *et al*., 1992; Hollmann *et al*., 1993). NR1-a containing receptors exhibit a large sensitivity to proton inhibition and are potentiated by micromolar concentrations of $Zn^{2+}$ (Hollmann *et al*., 1993; Hollmann & Heinemann, 1994). Splice variants with C-terminal deletions (the C1 and C2 exon cassettes) show no differences in their basic responses, but exhibit a higher susceptibility to modulation by PKC. The C1 cassette allows NR1 subunit localization in receptor rich domains in the plasma membrane and PKC phosphorylation of this cassette disrupts the receptor-rich domains (Ehlers *et al*., 1995). A high-affinity binding site for calmodulin has been identified in the C1 exon cassette (Ehlers *et al*., 1996). Calmodulin binding regulates NMDA receptors via an activity-dependent inhibition and $Ca^{2+}$-dependent inactivation, and we have shown recently that the transient inactivation of NMDA receptors by $Ca^{2+}$ (Medina *et al*., 1994, 1995, 1996) is not mediated by the C1 cassette (Rafiki *et al*., 1997). These various elements suggest that variants of the NR1 subunit play a critical part in regulating NMDA receptor function and that in pathology, differential splicing may alter NMDA receptor function.

Using semiquantitative *in situ* hybridization, we have analysed in the adult rat hippocampus the expression of mRNAs encoding the N-terminal and C-terminal variants of the NR1 subunit following unilateral intra-amygdalar injections of KA. The KA model of epilepsy is characterized by the presence of spontaneous seizures, hippocampal lesions (CA3 pyramidal cells and hilar neurones), and reactive synaptogenesis. Therefore, it provides a suitable model of temporal lobe epilepsy. Expression levels of the NR1-a, NR1-b, NR1–1, NR1–2, NR1–3 and NR1–4 splice forms were analysed in this study at various time points following KA-induced seizures.

## Materials and methods

### *Animals*

Adult male Wistar rats (180–200 g) were anaesthetized with chloralhydrate. KA (1.2 μg; Sigma) was dissolved in 0.3 μL of phosphate buffer (PB; pH 7.4) and injected into the right amygdala as previously described (Represa *et al*., 1987). Animals showing typical KA-induced limbic motor seizures for at least 2 h upon recovery from anaesthesia were kept for the experiments. These animals were killed under anaesthesia hours (6 and 24), days (3, 4, 7, 12, 21, 30) and 4 months after PB (SHAM animals) or KA treatment.

### *In situ hybridization*

The NR1-a and NR1-b oligonucleotide probes used to analyse the corresponding mRNAs are the following: NR1-a, CAGCACCTTCTC-TGCCTTGGACTCCCGTTCCTCC; NR1-b, CTGCCTTGGGTCCG-CGCTTGTTGTCATAGGACAGTTGGTCGAGGT. Their respective positions are 816–849 and 857–901 in the rat NR1 sequence by Sugihara *et al*. (1992). NR1–1, NR1–2, NR1–3 and NR1–4 oligonucleotides are identical to those previously used by Laurie & Seeburg (1994).

Rats were killed at various time intervals following the onset of behavioural seizures (at least $n = 3$ for each time point). Whole brains were frozen in isopentane (–40 °C) and subsequently stored at –80 °C until used. Frontal brain sections (15 μm) of the whole brains were cut in a cryostat (–15 °C) and mounted on to gelatin-coated slides and kept at –80 °C. The NR1 antisense oligonucleotide probes used in this study (see Fig. 1) were 3′ end labelled and sections were processed as described previously (Pollard *et al*., 1993). Following hybridization, slices were washed twice 15 min at room temperature in 2× and 1× SSC. High stringency wash was in 0.5 × SSC for 30 min at 55 °C. SSC buffer contained 1 mM DTT.

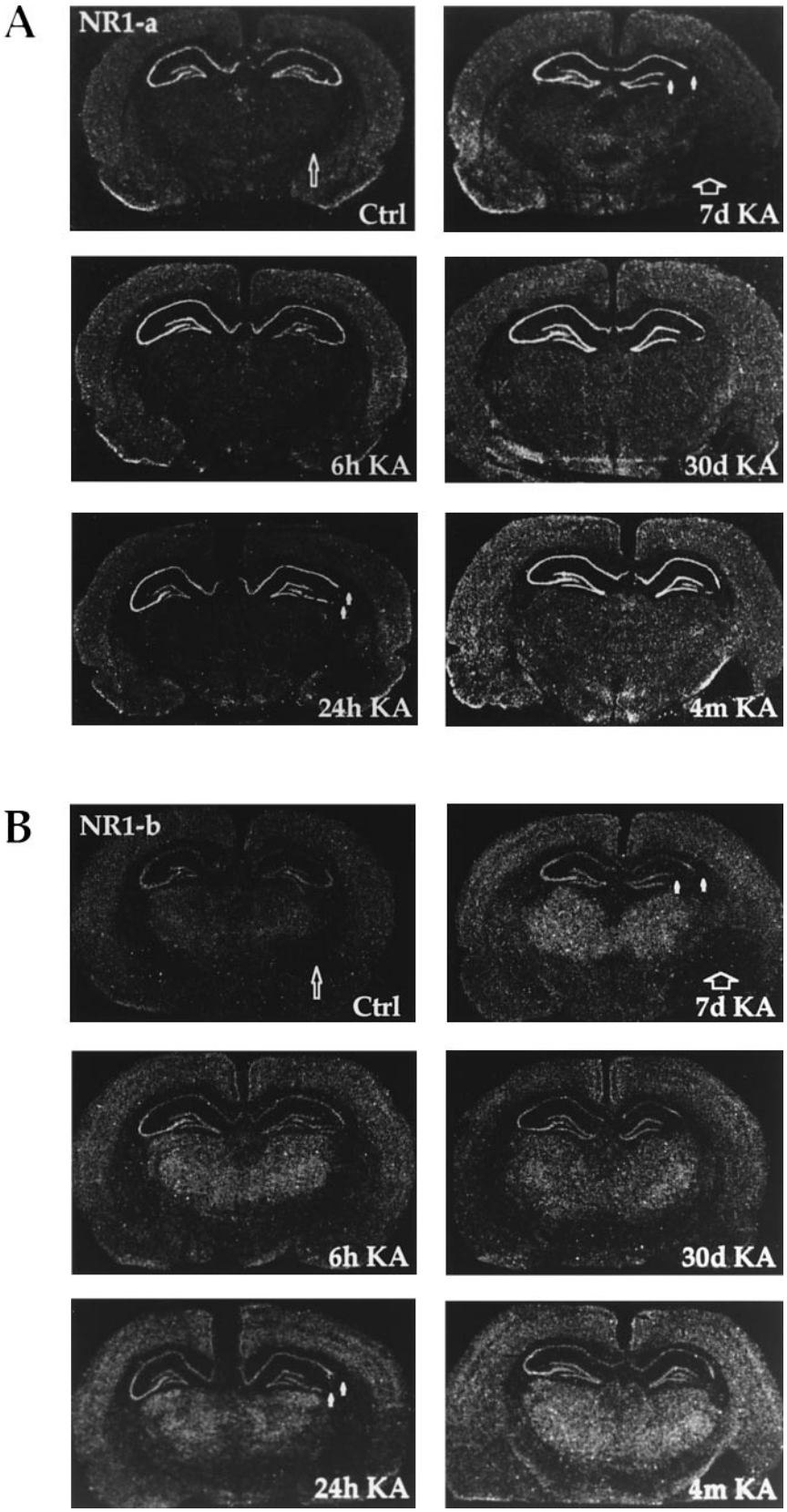


FIG. 2. Effects of kainate (KA) -induced seizures on NR1 mRNA expression. Dark-field photomicrographs obtained from representative autoradiograms from control animals injected with phosphate buffer (Ctrl) or epileptic animals (injected with KA and killed 6 h, 24 h, 7 days, 30 days and 4 months thereafter) hybridized with the NR1-a probe (A) or NR1-b probe (B). Injections were performed into the right amygdala (small open arrows depict the side ipsilateral to the injection and the large open arrows show the amygdalar lesion). Note the lack of hybridization in CA3 ipsilateral to the injection, due to the loss of pyramidal cells that may be observed from 24 h after KA (small closed arrows).

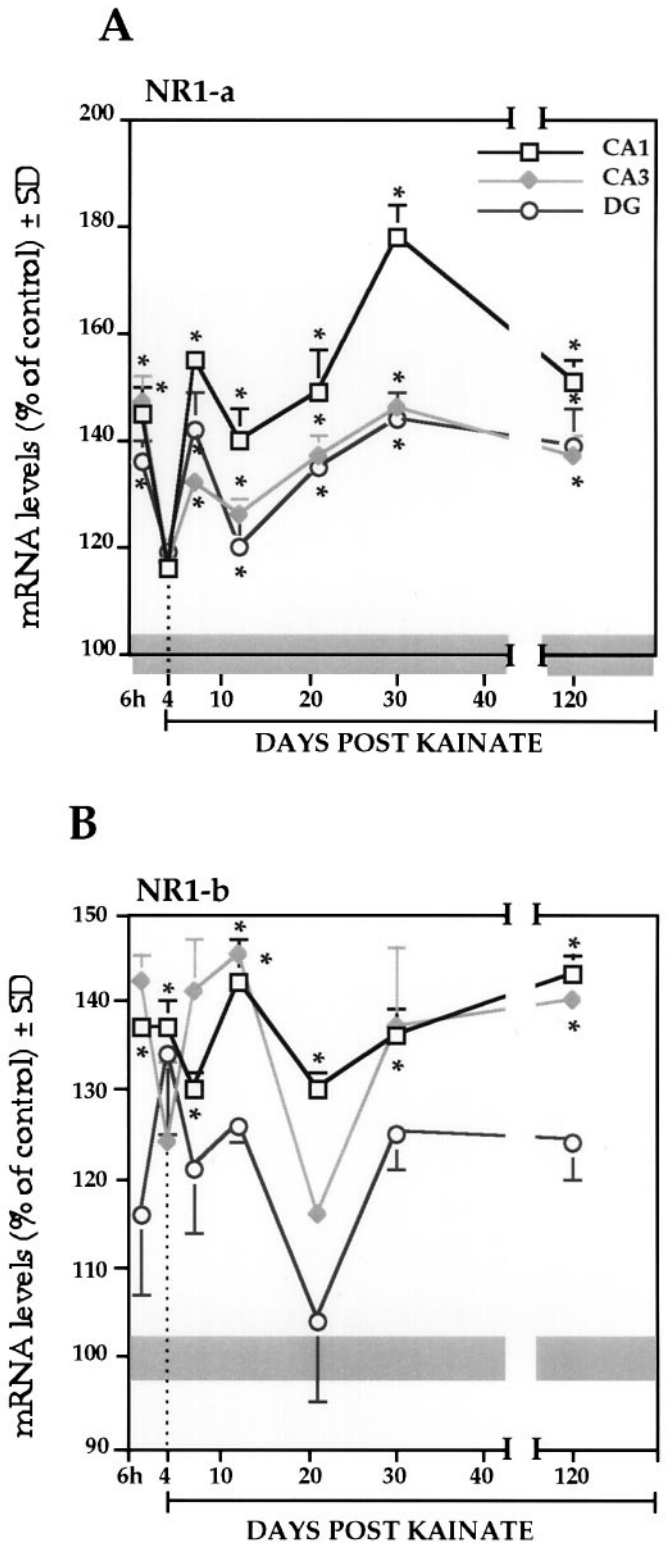


FIG. 3. Quantifications of the NR1-a (A) and NR1-b (B) mRNA levels in the contralateral CA1, CA3 and dentate gyrus as a function of time after kainate. Values, measured on autoradiograms, were plotted as percentage (± SD) of control values. Data were combined from three independent experiments. $P < 0.05$ (Fisher test) when compared with control values. Abbreviations: DG, dentate gyrus; CA1, area CA1; CA3, area CA3.

The slices were then dehydrated, air dried and exposed to Biomax MR films (Kodak) for 4–8 days. Some sections were further dipped in nuclear emulsion (NTB-2 from Kodak) and exposed at 4 °C for 20 days. After development, sections were counterstained with Cresyl Violet.

The specificity of the hybridization reaction for each probe was determined in previous studies by competition experiments in which excess (200-fold) of unlabelled probes was added to the hybridization mixture. The resulting autoradiographic images showed no signal (data not shown). Semiquantitative analysis of hybridization signals was realized as previously described by us (Pollard *et al*., 1993) and other groups (Friedman *et al*., 1994; Sussman *et al*., 1994; Paupard *et al*., 1997). Briefly, two rat brain sections sampled at various times after KA and two brain sections from control and SHAM operated rats, were incubated with the same hybridization mixtures and apposed to the same film. *In situ* hybridizations and quantifications were repeated three times. Several exposures were obtained for all brain sections in order to guarantee that hybridization signal was not saturated. All quantifications were realized in the linear range of the films as double exposure times lead to twofold increases in labelling. Sections hybridized with the different probes (some of which were selected for illustration) were not necessarily exposed for the same time (i.e. sections hybridized with the NR1–3 probes were exposed three times longer than those hybridized with the other probes) in order to obtain sufficient signal on the autoradiograms. These were quantified using the Samba/2005, TITN software (Alcatel, France). Optical densities of pixels overlying regions of interest were corrected for background (optical density of the film). Differences between control and KA-treated rats were calculated for each film and values were expressed as percentage of control. ANOVA followed by Fisher's PLSD test were used as statistical tests to compare changes obtained from different experiments and films. In most cases, results were highly reproducible for the same group of animals and differences between control and KA-treated rats were identical when using different films.

## Results

KA was injected into the right amygdala of 75 animals. Eight rats died succumbing to severe convulsions and were discarded. We have also excluded those rats ($n = 4$) that did not develop status epilepticus. CA3-CA4 pyramidal cells (Figs 2 and 5) and most hilar interneurones degenerated within the first 3 days following seizures as previously described (Pollard *et al*., 1994a,b; Represa *et al*., 1995). Animals that did not present these lesions ($n = 3$) were excluded from our *in situ* analysis. In the contralateral hippocampus lesions were not detected. Dentate gyrus granule cells and CA1 pyramidal cells were well preserved in both ipsilateral and contralateral hippocampi.

### *NR1 mRNA levels in the hippocampi of epileptic rats assessed by* in situ *hybridization*

*In situ* hybridization was performed in order to analyse in each of the hippocampal neuronal layers, the levels of expression of mRNAs encoding the various NR1 splice variants NR1-a and NR1-b, NR1–1 to NR1–4 in the epileptic rat hippocampus. SHAM treatment did not modify NR1 mRNA expression as compared with untreated animals. Old SHAM rats (6 months of age) presented similar values than 2-month-old rats (calculated from sections incubated with the same hybridization mixes and apposed to the same film) (data not shown). KA treatment induced rapid, significant and in some cases, permanent changes in the expression of the different NR1 mRNAs

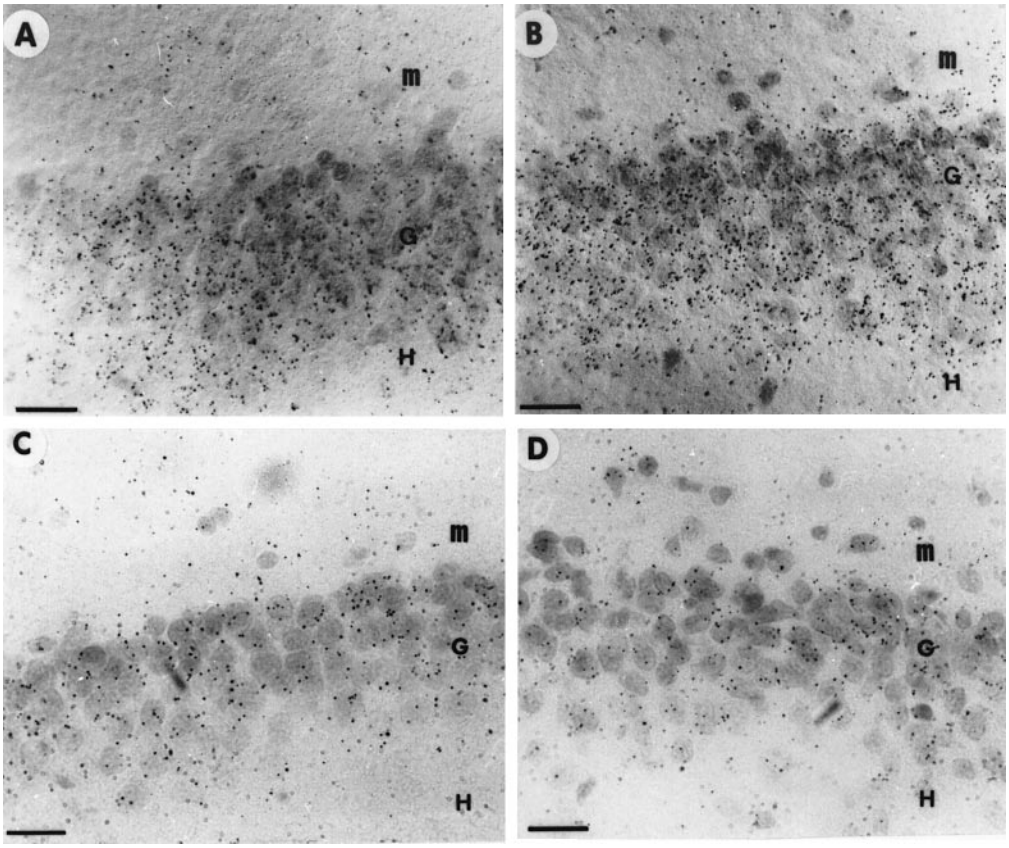


FIG. 4. Bright-field photomicrographs of emulsioned slides showing NR1-a (A,B) and NR1-b (C,D) mRNA expression in granule cells (contralateral to the injection) from a control rat (A, C) and a rat killed 4 months after kainate injection (B, D). Abbreviations: G, granule cell layer; H, hilus; m, molecular layer. Scale bar = 25 μm.

studied here. These changes were observed in both contralateral and ipsilateral hippocampi (Figs 2–7) and in other fields, including cerebral cortex and thalamic nuclei (quantifications not shown). The intra-amygdalar KA model of epilepsy is associated with lesions in the ipsilateral amygdala due to the injecting needle, and with KA-induced and/or seizure-induced lesions in the ipsilateral amygdalar nuclei and in the ipsilateral CA3 area of the hippocampus. The short-term (hours, days) quantifications for all splice variants were performed in the contralateral hippocampus while quantifications in chronic animals (4 months) were performed in both the contralateral and ipsilateral hippocampi.

### *NR1-a and NR1-b mRNA levels in the contralateral rat hippocampus following kainate-induced epilepsy*

In order to analyse the expression of the NR1-a and NR1-b splice variants, we performed *in situ* hybridization (Fig. 2) using the NR1-a and NR1-b oligonucleotide probes (Fig. 1). Detailed analysis and densitometric quantifications in the hippocampal fields contralateral to the site of injection (Fig. 3A) revealed variations in the NR1-a mRNA levels. In all hippocampal fields from which quantifications were performed (CA1, CA3 and dentate gyrus), the NR1-a levels are significantly increased at 6 and 24 h following KA, followed at 4 days by a severe but transient decrease. Subsequently, NR1-a levels are significantly increased from 7 to 21 days and are increased close to twofold at 30 days post-KA in CA1. NR1-a levels remain significantly higher than control at 4 months post-KA in all three layers (Figs 2 and 3A). Changes in the NR1-b mRNA levels differed in their amplitude in the hippocampal neuronal layers in the contralateral hippocampus. We observed an early phase (6–24 h, during status epilepticus) of increased levels which were significant in the CA1 and CA3 area but not in the dentate gyrus (Fig. 3B). This increase lasted 1–2 weeks and was followed by a slight drop at 21 days post-KA. At this point, NR1-b mRNA levels were still significantly higher than controls (40% increase) in CA1 while in the dentate gyrus they were increased but not significantly. At 30 days post-KA, NR1-b increases were significant only in the CA1 area. At 4 months, NR1-b levels were significantly increased in the CA1 and CA3 layers (40–50%, Fig. 3B).

Analysis of the expression of the NR1-a and NR1-b splice variants at the cellular level (from slides dipped in emulsion, Fig. 4), 4 months after KA confirms that in the dentate gyrus, changes in mRNA levels are most striking for the NR1-a mRNAs. In the contralateral dentate gyrus of the chronic animals, the NR1-a labelling is stronger (Fig. 4B) compared with the control (Fig. 4A). In contrast no obvious changes were observed between the controls and the treated animals for the NR1-b mRNA variants (Fig. 4C,D), in agreement with our autoradiogram quantifications (Fig. 3B).

### *NR1–1–NR1–4 mRNA expression in the contralateral rat hippocampus following kainate-induced epilepsy*

All C-terminal splice variants show increased levels of expression at 6 and 24 h following KA in all neuronal layers of the hippocampus (Figs 5 and 6). Increases (30–40%) were significant in area CA3 (NR1–1) and CA1 (NR1–3, NR1–4). At 4 days post-KA, expression

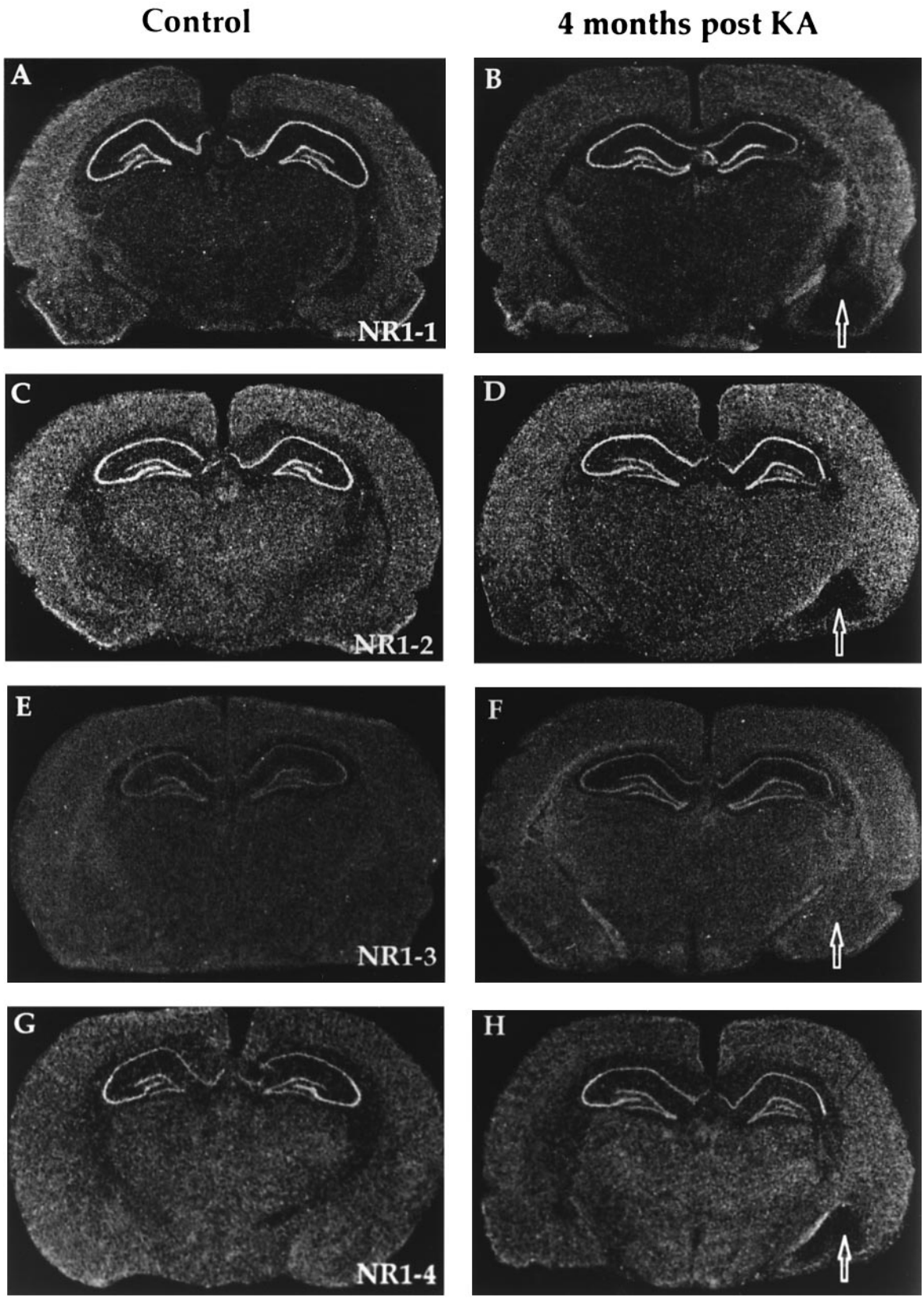


FIG. 5. Dark-field photomicrographs depicting NR1–1 to NR1–4 mRNA expression in chronic epileptic animals. (A, C, E, G) SHAM animals killed 24 h after intra-amygdalar injection of phosphate buffer. (B, D, F, H) epileptic animals killed 4 months after KA injection into the right amygdala (open arrows). Sections were hybridized with the NR1–1 (A, B), NR1–2 (C, D), NR1–3 (E,F) and NR1–4 (G,H) probes.

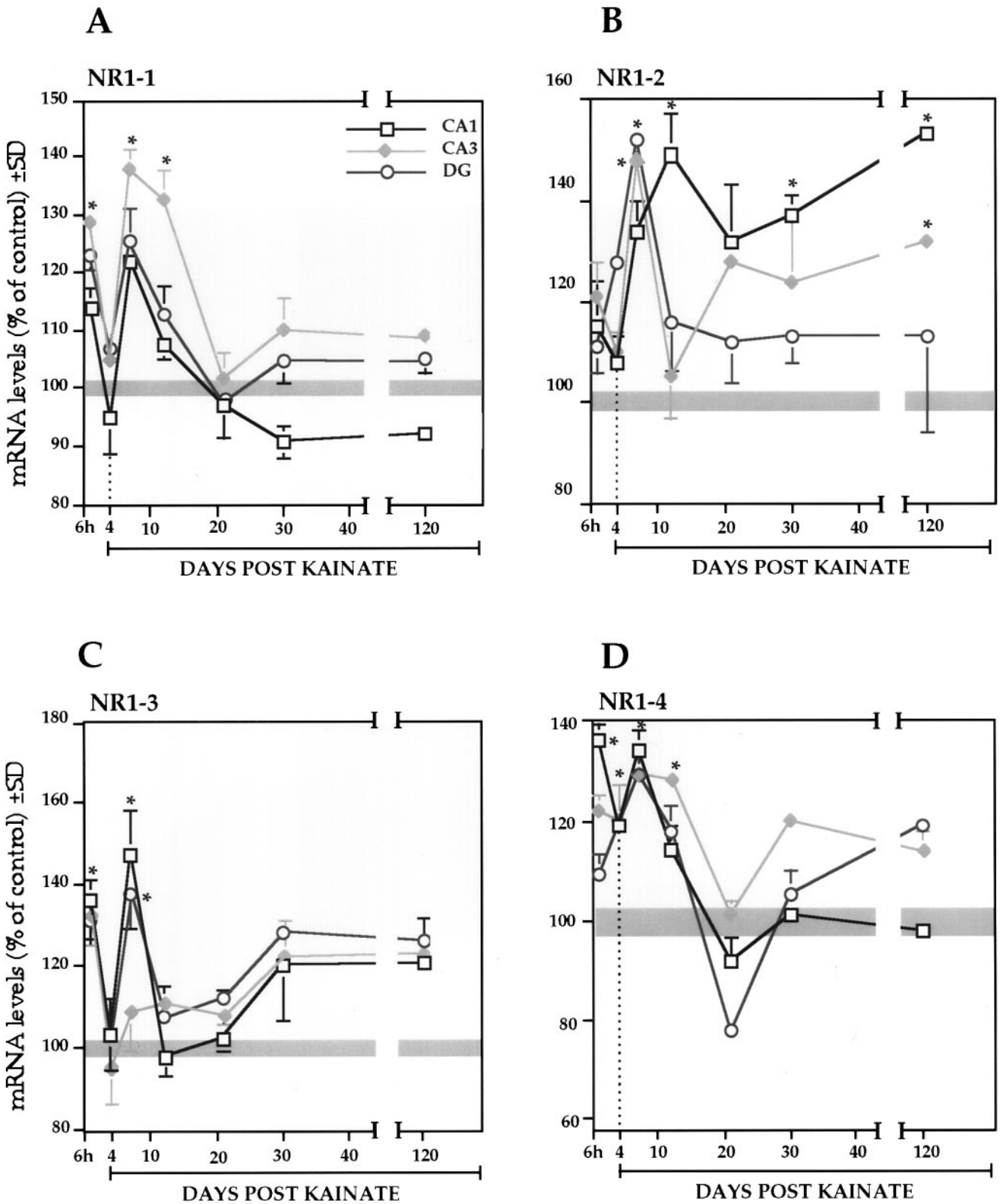


FIG. 6. Quantifications of the NR1 mRNA levels in the contralateral CA1, CA3 and dentate gyrus as a function of time after kainate. A–D correspond, respectively, to the NR1–1, NR1–2, NR1–3 and NR1–4 mRNA levels. Values, measured on autoradiograms, were plotted as percentage (± SD) of control values. Data were combined from three independent experiments. $P < 0.05$ (Fisher test) when compared with control values. Abbreviations: DG, dentate gyrus; CA1, area CA1; CA3, area CA3.

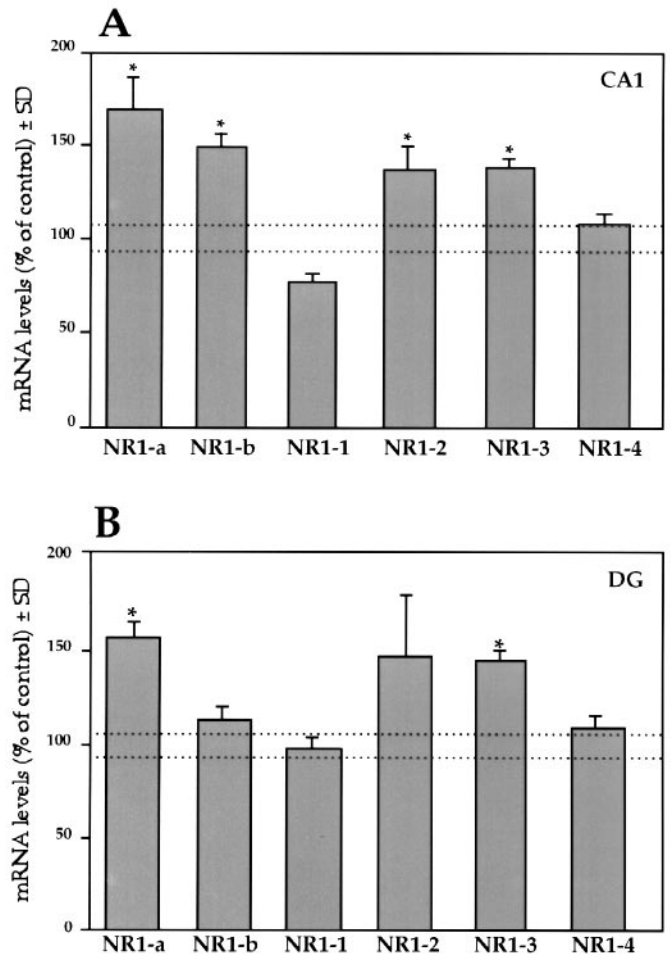


FIG. 7. Quantifications of the NR1 mRNA levels in the ipsilateral CA1 area (A) and dentate gyrus (B) of chronic animals, 4 months after kainate. Values, measured on autoradiograms, were plotted as percentage (± SD) of control values. Data were combined from three independent experiments. $P < 0.05$ (Fisher test) when compared with control values. Abbreviations: DG, dentate gyrus; CA1, area CA1.

levels of the C-terminal splice variants had returned to control values in all hippocampal fields except for the NR1–4 variant whose expression remained significantly elevated in the CA3 area. This global downregulation was followed at 7 days by a novel, significant raise (40–50%) in the levels of the mRNAs encoding the C-terminal variants in the CA3 area (NR1–1, NR1–2, NR1–4), in the dentate gyrus (NR1–2, NR1–3, NR1–4) and in area CA1 (NR1–3, NR1–4). The mRNA levels of all these subunits decrease to control values more or less rapidly, 14 days (NR1–3) to 21 days (NR1–1, NR1–4) after KA, with the exception of the NR1–2 splice variant whose expression remains significantly elevated in areas CA3 and CA1 (30–55%, respectively), 4 months after KA.

### *NR1 mRNA expression in the ipsilateral rat hippocampus following kainate-induced epilepsy*

We have quantified the levels of the different NR1 mRNA splice variants in the ipsilateral hippocampus 4 months after the onset of seizures, in the resistant neuronal layers of area CA1 (Fig. 7A) and in the dentate gyrus (Fig. 7B). For all NR1 mRNA variants, mean values remained decreased (data not shown) in the ipsilateral CA3 area in agreement with the loss of CA3 pyramidal cells (Figs 2 and 5).

In the CA1 pyramidal cell layer (Fig. 7A), NR1-a and NR1-b are significantly increased 70% and 50%, respectively. Among the mRNAs encoding the C-terminal variants, only NR1–2 and NR1–3 were significantly increased (⊤ 40%).

In the dentate gyrus (Fig. 7B), the NR1-a and NR1–3 mRNA levels were significantly increased 50% and 40%, respectively, relative to controls. The NR1–2 levels were also increased but increases were not significant due to individual variability. Levels of the NR1-b, NR1–1 and NR1–4 mRNAs were not different from controls.

## Discussion

The main conclusion of the present report is that the KA model of temporal lobe epilepsy is associated with modest but permanent changes of NR1 mRNA expression levels in the rat hippocampus. These are observed in Ammon's horn, particularly in CA1 and CA3 pyramidal cells and in the granule cells of the dentate gyrus, in the non-lesioned areas of the hippocampus (contralateral to the site of injection) as well as in the lesioned hippocampus (ipsilateral to the KA injection). On the side ipsilateral to the KA injection, an absence of NR1 labelling is observed in CA3, due to an important loss of the pyramidal cells. Our results suggest that in the rat hippocampus, NR1-b is poorly expressed compared with NR1-a and we estimate a nine- to 10-fold excess of NR1-a over NR1-b in this brain structure by reverse transcriptase–polymerase chain reaction (RT-PCR) (A. Rafiki *et al*. unpublished observations), in the range of that previously estimated in the rat brain (4 : 1; Anantharam *et al*., 1992) and in the rat forebrain (5 : 1; 6 : 1, Nakanishi *et al*., 1992; Sugihara *et al*., 1992, respectively). Visual estimates of the relative ratios of the NR1–1 to NR1–4 variants from our *in situ* hybridizations indicate that the NR1–2 is most abundantly expressed followed by NR1–1 and NR1–4. The NR1–3 mRNA is poorly expressed in comparison. These estimates are in good agreement with those reported in the adult rat brain by Laurie & Seeburg (1994).

### *Status epilepticus triggers rapid changes in NR1 mRNA expression*

At 6 h following KA treatment, mRNA levels of the different NR1 splice forms are increased in the hippocampus. These changes precede any lesion as the first signs of neuronal death are observed 12 h following treatment (Pollard *et al*., 1994b). They also precede synaptic reorganization, since the first signs of mossy fibre sprouting are detected about 12 days after KA (Represa *et al*., 1987). Therefore, it is likely that the early changes in NR1 mRNA levels are a direct consequence of the paroxysmal activity occurring during status epilepticus. Numerous reports demonstrated rapid changes in the levels of hippocampal mRNAs following seizures. These include the mRNAs encoding immediate–early genes and trophic factors for which changes are detected within 3 h following KA injection (reviewed in Khrestchatisky *et al*., 1995). In general, these changes are particularly important in the dentate gyrus where the expression of 500–1000 genes is increased 6 h after KA (Nedivi *et al*., 1993).

### *Chronic epilepsy is associated with increased levels of various NR1 splice forms*

A second wave of NR1 mRNA increase occurred in chronic epileptic animals, between 21 days and 4 months following KA injection: quantifications from the autoradiograms following *in situ* hybridization suggest (i) a permanent increase of the NR1–2a and NR1–2b mRNA species (containing exon 22) in all hippocampal fields, both ipsilateral and contralateral, and (ii) an increase of the NR1–3 (a and b) mRNAs

(containing exon 21) in the ipsilateral CA1, and of the NR1–3a mRNA in the ipsilateral dentate gyrus. However, if the increased mRNA levels translate into increased levels of receptors, the contribution of the NR1–2 subunits should be far greater than the NR1–3 subunits considering the important difference in their respective levels of expression. Seizures have been shown to induce delayed and long-lasting changes in the levels of a variety of mRNAs, including those encoding AMPA and $GABA_A$ receptors (Kamphuis *et al*., 1992; Pollard *et al*., 1993; Pellegrini-Giampetro *et al.* 1994), cytoskeletal proteins (Pollard *et al*., 1994c) as well as adhesion and extracellular matrix proteins (see Represa *et al*., 1995 for a review; Rivera *et al*., 1997).

### *Kainate- and kindling-induced seizures elicit distinct changes in NR1 mRNA expression*

To date, the molecular basis of persistent changes in NMDA binding have been studied in kindled animals. Novel binding properties of NMDA receptors have been observed 24 h and 28 days after the last evoked kindled-seizure (Kraus *et al*., 1994). However, these occurred in the absence of changes in the transcript levels of the NMDA receptor genes NMDAR1, NR2A, NR2B, NR2C and NR2D. In other studies addressing the expression of NMDAR subunit mRNAs, short-term alterations were described for the levels of NR2A and NR2B mRNAs, but long-term maintenance of the kindled state was not associated with altered NMDAR mRNA levels (Pratt *et al*., 1993). Expression of the NR1 splice variants has also been studied; using RT–PCR, Vezzani *et al*. (1995) demonstrated that kindling was associated with a rise of NR1–2 isoforms, in agreement with our results. However, this rise was detected 1 week after the acquisition of stage 5 seizures, but the changes were no longer detected 4 weeks thereafter, suggesting that in the kindling paradigm NR1 mRNA changes are transient. In the same paradigm, reports conclude to either slightly increased levels of exon 5 containing mRNAs (Kraus *et al*., 1994) or to an absence of changes (Vezzani *et al*., 1995). The parallel increase of the NR1–2 mRNA observed in both the kindling and KA models of epilepsy suggest that the NR1–2 mRNA, the most abundantly and most broadly expressed 3′ NR1 variant, is prone to upregulation in various situations of neuronal hyperactivity. In contrast, differences observed in the duration of the NR1–2 increase and in the expression of the other splice forms when comparing both epilepsy models, underline the important differences that characterize these models. Indeed, the kindling model of epilepsy is not associated with the expression of spontaneous recurrent seizures, nor with CA3 hippocampal lesions, nor with neo-synaptogenesis in CA1.

### *Persistent NMDA mRNA receptor changes may be associated with synaptic remodelling*

Previous reports demonstrated that in the hippocampi of KA-treated rats there are two phases of synaptic reorganization. The first one occurs during the first week following KA. The decreased number of axon terminals and synaptic contacts (Nadler *et al*., 1980a,b; Represa *et al*., 1987; Phelps *et al*., 1991; Magloczky & Freun, 1995), could explain the decreased levels of all NR1 mRNA variants we report in the days following the initial increase (6 h). A second phase has been described between 12 and 30 days after KA, during which the number of synapses increases as a result of sprouting, synaptic remodelling and neosynaptogenesis. These are most obvious in the mossy fibres and have been described in both the ipsilateral and contralateral hippocampi (Nadler *et al*., 1980a,b; Tauck & Nadler, 1985; Represa *et al*., 1987, 1993; Phelps *et al*., 1991; Sloviter, 1992; Sundstrom *et al*., 1993; Represa & Ben-Ari, 1994; Okazaki *et al*., 1995; La Caille *et al*., 1996) and could account for the increased levels of the NR1 splice variants. The overall NR1-a increase, that is more substantial than that of NR1-b is interesting as NR1-a is the main isoform expressed in the early phases of development while NR1-b appears late in the development of most CNS structures. Similarly, NR1–2 a major NR1 splice form in the adult, is also the major NR1 variant during early (E14) CNS development (Laurie & Seeburg, 1994).

### *Changes in NR1 RNA levels may lead to increased NMDA receptor density and may alter subunit combinations*

One may hypothesize that the increase in the steady-state levels of the NR1 mRNAs that we observe leads to an increased synthesis of NR1 receptor subunits, increased density of NMDA receptors and increased excitability of postsynaptic cells in the hippocampus. However, at this point, it is elusive to predict the functional consequences of the changes we observed as the stoichiometry of NMDA receptors in the various cell types is uncertain. It is likely that neurones express heteromeric receptors with NR2 subunits which modify substantially the properties of NMDA receptors expressed in oocytes and transfected cells as they promote for instance an increase of the current amplitudes when compared with homomeric receptors (Hollmann & Heinemann, 1994). Further understanding of the altered NMDA receptor properties in epileptic tissue will stem from a complete molecular analysis of all NMDA receptor subunits and their variants at the protein level when the appropriate tools become available, and from a detailed study of the functional alterations of receptors resulting from potentially novel subunit combinations in the pathological tissue.

In conclusion, we suggest that the increased excitability in epileptic tissue may be due, at least in part, to an increased expression of subtypes of NMDA receptors. It is possible that the changes we have found constitute, in addition to diminished GABAergic inhibition (Cornish & Wheal, 1989; Nakajima *et al*., 1991), a molecular basis for the maintenance of epilepsy. Our results are in agreement with previous reports demonstrating in epileptic animals increased responses to NMDA, not only after kindling (Mody & Heinemann, 1987; Martin *et al*., 1992) but also after KA (Simpson *et al*., 1991; Turner & Wheal, 1991; Wheal *et al*., 1991).

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## Abbreviations

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|---|---|
| NMDA | *N*-methyl-D-aspartate |
| CNS | central nervous system |
| AMPA | α-amino-3-hydroxy-5-methyl-4-isoxazol propionate |
| KA | kainate |
| PKC | protein kinase C |
| RT–PCR | reverse transcriptase coupled polymerase chain reaction |
| CA1, CA3 | Ammon's horn |
| RNA | ribonucleic acid |
| DNA | deoxyribonucleic acid |
| PB | phosphate buffer |

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