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Kamphuis, W.; Lopes da Silva, F.H.

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Research report

Editing status at the Q/R site of glutamate receptor-A, -B, -5 and -6 subunit mRNA in the hippocampal kindling model of epilepsy

Willem Kamphuis^{*}, Fernando H. Lopes da Silva*Graduate School of Neuroscience, Institute of Neurobiology, University of Amsterdam, Kruislaan 320, 1098 SM Amsterdam, The Netherlands*

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Abstract

The editing status of mRNA at the Q/R site of the glutamate receptor subtypes -A, -B, -5 and -6 modulates channel conductivity and ion selectivity of glutamate operated ion channels [4,15,26,30]. In order to investigate whether a modification of this editing process may be involved in kindling epileptogenesis, the percentage of edited variant was determined in the hippocampus of kindled rats and compared to the percentage in control animals. In the latter, GluR-A mRNA was detected only in the unedited form (with detection threshold for edited form < 0.7%), whereas GluR-B was completely edited (> 99%). For GluR-5, the mean edited fraction of transcripts was in controls 36% and for GluR-6 the edited fraction was 92%. These editing percentages were not significantly changed in Schaffer collateral/commissural pathway kindled animals that were sacrificed 24 h after the last generalized seizure. It is concluded that the increased sensitivity for the induction of seizures characteristic for Schaffer collateral kindled animals is not related to a less selective or less efficient mRNA editing process of the different glutamate receptor subunits in the hippocampus.

Keywords: Kindling; Epileptogenesis; Seizure; Hippocampus; Glutamate receptor subunit; RNA; Editing

1. Introduction

Repeated low-intensity electrical stimulation of brain sites results in the progressive development of electroencephalographic and behavioral seizures. This phenomenon is known as kindling [11].

Recently, it was reported that seizures elicited by kindling stimulations or kainate application induced an increase in the expression of the genes that encode for the AMPA-type glutamate receptor (GluR) subunits [14] in the granular neurons of the fascia dentata [16,17,23]. Moreover, in the same neurons the splicing route of these transcripts is profoundly changed, leading to an increased ratio of flip to flop splice variants [16,17] which may contribute to the potentiation of the local evoked field potential amplitude and may slow down the GluR desensitization during paroxysmal activity [21,25,29,33]. To address the question whether other alterations in the processing of GluR mRNA are triggered as a result of kindling, we investigated the

editing of the Q/R (glutamine/arginine) site which is located in the putative ion channel forming transmembrane II region of ionotropic GluRs [14]. The presence of a GluR-B subunit containing an arginine residue at the Q/R site imposes the typical voltage-current relationship and a low permeability for divalent ions on heteromeric AMPA-type GluRs expressed in *Xenopus* oocytes [4,13,15,28,30]. The other AMPA-type GluR subunits (GluR-A, -C and -D) carry a glutamine instead of an arginine at this position and combinations of these subtypes, without GluR-B, are associated with channels with a larger Ca²⁺ permeability. It has been inferred that the arginine in GluR-B transcripts is introduced by a highly selective and efficient editing process executed by a deaminase guided by an intron-exon RNA duplex of the primary GluR-B gene transcript [12]. This exchange of a single nucleotide causes the alteration of the gene encoded glutamine codon (CAG) into an arginine codon (CGG), thereby losing a *Bbv*I restriction site [1,7,22]. In the adult rat brain, as well as in the human brain, all GluR-B transcripts are edited whereas GluR-A, -C and -D are not modified in this way [7,26]. For the kainate-type GluR subunits -5

^{*} Corresponding author. Fax: (31) (20) 5257709.

and -6, a partial editing at the Q/R site accounts for the limited Ca^{2+} permeability, however, depending on the editing status of two other edit sites located in TMI [1,10,20,22,26,28].

It is conceivable that under extreme conditions, like during seizure activity, neurons could fail to maintain the editing of GluR-B, -5 and -6, resulting in the formation of glutamate receptor complexes that are permeable to Ca^{2+} , thereby creating an aberrant route of Ca^{2+} influx during synaptic activation and seizure activity [4,24,32]. To investigate whether a modified RNA editing regulation is involved in the process of kindling epileptogenesis, we analysed the percentage of edited variants in TMII region of the glutamate receptor subtypes GluR-A, -B, -5 and -6 mRNA in the hippocampus of kindled animals in comparison to controls.

2. Materials and methods

2.1 Electrode implantation and kindling

Male Wistar rats (200–250 g) were used. Chronic indwelling electrodes were placed in the CA1 area of the left dorsal hippocampus to enable kindling stimulations. The details of this procedure were described previously [18]. In short: under pentobarbital anesthesia, two bundles of stainless steel wire electrodes were implanted. The stimulation bundle was placed on the Schaffer-collateral/commissural fiber pathway and the recording bundle was placed in stratum radiatum of CA1. Local evoked field potentials, elicited by test stimuli, were used to optimize the position of the electrodes. After two weeks of recovery, the rats were connected to a stimulation/recording device for recording field potentials in the freely moving animal. The stimulation electrode that elicited the optimal evoked field potential in response to single pulse stimulation of the Schaffer collateral/commissural fiber pathway was selected and used as the electrode through which the kindling stimulations were delivered.

After one week of recovery from the implantation procedure, a group of 13 animals was divided into a control group ($n = 6$) and a group ($n = 7$) that received, twice daily, kindling stimulations at an intensity supra-threshold for the induction of an afterdischarge (200–300 μA , 50 Hz, 1–2 s) [11]. The animals of the control group were handled throughout the experimental period in a way comparable to the kindled rats but did not receive tetanic stimulations. Kindled animals received 33 kindling stimulations, resulting in a mean of 6 generalized tonic-clonic seizures (stage 5, fully kindled state). The kindled group of animals was sacrificed 24 h after the last class 5 seizure, at the same time as the control rats. Rats were deeply anesthetized with ether and decapitated. The left, stimulated, hippocampus was rapidly isolated, weighted, frozen on dry-ice and stored at -70°C .

2.2 PCR amplification of GluR subunits

Total hippocampal RNA was isolated using the Chomczynski and Sacchi method [8]. Total RNA (1 μg) served as template for cDNA synthesis with 200 U of M-MLV Reverse Transcriptase (Life Technologies, Inc) in the presence of 1 mM dNTPS and 0.5 U RNase inhibitor (Inhibit-ACE, 5 Prime $\rightarrow$ 3 Prime, Inc) in a total volume of 20 μl . As primer for the cDNA synthesis, we used an oligonucleotide

with the sequence GT(G/A)TA(C/G)GA(G/T/A) GA(G/A) AT(G/T)ATGAT, complementary to the residues IIISSYT located downstream of the Q/R site in the putative transmembrane spanning region TMIII of the GluR-A, -B, -5 and -6 subunits [2,3,9,19]. The reaction was terminated after 60 min by raising the temperature to 90°C and by adding 80 μl TE. The cDNA was directly used for PCR amplification of specific GluR subunit fragments containing the Q/R site. The following oligonucleotides served as specific PCR primers: 5'-GCTGGAGGGGACAACCTCAAG-3', encoding amino acid residues AGGDNSS of GluR-A [19]; 5'-TCAGGAAATGACACGTCTGGGC-3', encoding SGNDTSG of GluR-B [3]; 5'-TC-CAACAATATCACGGACTCGC-3', encoding SNNITDS of GluR-5 [2]; 5'-CGGACAAATGGAATGGAATGGTTTCG-3', encoding GQWNGMV of GluR-6 [9]; in combination with the oligonucleotide that was used for the cDNA synthesis. All PCRs were carried out in the presence of 0.3 mM dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl_2 , 0.01% Tween 20, 100 ng of each primer pair, 1 μl cDNA (GluR-A, -B and -6) or 3 μl cDNA (GluR-5) and 0.5 U of EuroTaq (Eurogentec) in 50 μl . Reactions for the amplification of GluR-A or -B were run for 30 cycles at 95°C , 1 min; 52°C , 2 min; 72°C , 1 min, with a final extension of 7 min. The amplification reactions for GluR-5 and -6 required an annealing temperature of 44°C and were run for 30 and 40 cycles, respectively. The amplified DNAs were resolved on a 1.2% agarose gel and stained with ethidium bromide. Only bands of the correct size were found (Figs. 2, 4, 5 and 6).

The specificity of the amplified DNA in the PCR samples was further checked by digesting the PCR samples with a panel of restriction endonucleases at 37°C for 60 min in the appropriate buffers. The restriction fragments were separated on agarose gels along with a DNA size marker.

2.3 Ratio of edited to unedited form of GluR subunits

To study the ratio of the edited to non-edited cDNA forms of GluR-A, -B, -5 and -6, the cDNA synthesis and subsequent PCRs were carried out simultaneously on all the hippocampal RNA preparations obtained from control and kindled rats along with a non-template control. For each GluR subunit two identical 1.2% agarose gels, marked Q or R were prepared using 15 μl of the PCR samples. Lanes were alternating loaded with kindled and control samples, together with size markers (1 μg EcoRI/HindIII cleaved λ DNA and a 100 bp ladder; Pharmacia). After electrophoretic separation of the samples, the two gels were stained with ethidium bromide and photographed to check the success of the PCR amplifications in the different lanes. Gels were put in 0.5 M NaOH + 1.5 M NaCl for 30 min and neutralized with 1 M Tris-HCl + 1.5 M NaCl (pH 7.0) and subsequently blotted to nylon filter (Hybond-N; Amersham) with $10\times\text{SSC}$ (1.5 M sodium chloride, 0.15 M sodium citrate) overnight. The DNA was UV-crosslinked to the filters. The gel was re-stained with ethidium bromide and inspected for any anomalies of the DNA transfer.

The Q and R blots were probed with a [^{32}P]ATP 5'-end labeled oligo specific for respectively the unedited (glutamine = CAG) and the edited (arginine = CGG) variant of the GluR subunits as described in Sommer et al. [26]. The following Q-R pairs were used; for both GluR-A and GluR-B, 5'-(A/G)CATCC(C/T)TG(C/T)TG(C/T)CATG(C/A)AAGGC-3' (2Q) and 5'-(A/G)CATCC(C/T)TG(C/T)CGCATG(C/A)AAGGC-3' (2R); for GluR-5, 5'-GCTCTCATG(C/A)CAAGGATCA-3' (5Q) and 5'-GCTCTCATG(C/A)CAAGGATCA-3' (5R); for GluR-6, 5'-AGAACCTTGCTGCATGAGAGC-3' (6Q) and 5'-AGAACCTTGCTGCATGAGAGC-3' (6R). The two filters were pre-hybridized for 2 h in $5\times\text{SSC}$, 30% formamide, 20 $\mu\text{g}/\text{ml}$ tRNA, 0.5% SDS at 37°C . The Q and R selective oligo's were labeled and the specific activity was checked and differed not more than 15% (0.8×10^9 cpm/ μg).

Fig. 1. Relation between the activity of a ^{32}P -labeled oligonucleotide probe, immobilized on nylon filter, and the resulting film extinction corrected for film background after a 4 h exposure ($r^2 = 0.995$). Exposures of various durations revealed a similar linear relationship at extinction values < 60 .

Labeled oligo was added to the hybridization buffer to a final concentration of 2 ng/ml and hybridization was for 3 h. The filters were then washed under low stringent conditions ($5\times\text{SSC}$, 0.1% SDS, RT, 1 min) and exposed to Fuji-RX film. This allowed us to determine possible systematic differences between the amount of DNA immobilized on the filter between the different lanes. Following this initial exposure, the filters were submitted to a high stringent wash ($0.4\times\text{SSC}$, 0.1% SDS, 60°C , 2 min) which, based on prelimi-

nary experiments, discriminated optimally between the Q and R forms. After several exposures of various durations, the filters were stripped with 0.4 M NaOH at 45°C for 30 min and neutralized with $0.1\times\text{SSC}$, 0.2 M Tris-HCl (pH 7.5) at 45°C for 15 min. The Q-filter was then hybridized with the R-probe and vice versa following the same protocol as described above.

2.4. Quantification

Bands of the autoradiograms were scanned and digitized using a CCD camera. A background image was obtained from a blank film region and correction was carried out. The corrected image was stored on disk for later analysis of the extinction of the bands.

In order to obtain information over the precise relation between the amount of activity on the filter and the extinction values, a labeled oligonucleotide probe was blotted and UV-crosslinked to nylon filter in a broad range of activities using a slot blot apparatus (Bio-Rad) making two identical series. The filter was apposed to a film and subsequently, the slots were cut out from the filter and the activity was determined by liquid scintillation counting. The linear relation between the activity immobilized on the filter and the extinction, corrected for blank film background, is presented in Fig. 1 ($r^2 = 0.995$). This relation deviated from linearity for extinction values above 60, and all quantifications were done within the linear range of the film.

Based on the results of Sommer et al. [26], we expected that, in control animals, GluR-A would be present completely in the unedited form while GluR-B would be totally edited. Anticipating the possibility that the kindling induced changes of the editing process may be small, we developed a method that allowed us to detect a small percentage of the edited or unedited variants within the total amount

Fig. 2. Analysis of the Q to R ratio of GluR-A in kindled and control animals. The top photographs show the resolved PCR samples from fully kindled (FK) and control (CTR) animals on the two agarose gels along with the size markers and a GluR-B sample. The second row shows the autoradiograms obtained from the GluR-A Q (left side) and the GluR-A R (right side) filters after the low stringent wash to check the equal loading of the kindled and control group on the filters. The third row is the pattern after the high stringent wash revealing the strong prevalence of the unedited (Q) form of GluR-A over the edited (R) variant in both kindled and control preparations. The lane loaded with the GluR-B sample shows the opposite pattern. The bottom row shows the same filters after probing with the reverse oligonucleotide.

of amplified GluR DNA. The amount of DNA loaded on the gels was, therefore, more than conventionally used for Southern blotting. To determine the percentage of edited GluR-A and unedited GluR-B that could be detected using the PCR-based analysis employed in this study, a range of dilutions of a GluR-B PCR sample from a control animal was put on a separate agarose gel. After blotting, the filter was probed with the 2R oligo together with the filter containing the control-kindled samples, washed and exposed to film under identical conditions. A similar dilution range was prepared for GluR-A and was probed with 2Q.

3. Results

3.1. Specificity of the PCR fragments

The amplifications of GluR-A, -B, and -5 resulted in single bands of approximately 700 bp as was expected based on the published cDNA sequences: 705 bp for GluR-A [19], 705 bp for GluR-B [3] and 693 bp for GluR-5 [2]. For GluR-6 the PCR resulted in single band of about 500 bp corresponding with the anticipated size of 473 bp [9]. GluR-A, -B and -6 bands were always detected after PCR amplification of hippocampal cDNA. GluR-5 was more difficult to amplify from hippocampal cDNA resulting in rather weak bands even when using 3 μ l cDNA and 40 PCR cycles. Amplification from cerebellar cDNA always resulted in strong bands. This result suggests low mRNA levels of GluR-5 in the hippocampus and a higher abundance in the cerebellum, which is in agreement with in situ hybridization patterns [34].

The endonuclease patterns of the amplified fragments were determined using a panel of restriction enzymes; *Bam*HI, *Eco*RI, *Hind*III, *Pst*I, *Sac*I, *Sma*I. The patterns were in complete agreement with the predictions that were made from the published se-

Table 1

Extinction (mean $\pm$ S.E.M.) of the GluR fragments obtained by PCR of cDNA preparations of control ($n = 6$) and fully kindled animals ($n = 7$)

	Q		R	
	Control	Kindled	Control	Kindled
GluR-A	40 $\pm$ 9	51 $\pm$ 10	1.2 $\pm$ 0.1	1.4 $\pm$ 0.1
GluR-B	nd (< 0.3)	nd (< 0.3)	39 $\pm$ 6	39 $\pm$ 5
GluR-5	29 $\pm$ 8	27 $\pm$ 6	16 $\pm$ 5	16 $\pm$ 3
GluR-6	5.1 $\pm$ 0.7	5.2 $\pm$ 0.5	56 $\pm$ 4	54 $\pm$ 5

Filters were probed by oligonucleotides specific for the unedited (Q) and the edited (R) form and washed under high stringent conditions. Densitometric analysis after discriminative washing ($0.4 \times$ SSC) revealed no significant differences between kindled and control samples. Note that the autoradiograms of the GluR-A fragments probed with 2R were exposed 3 times longer than the corresponding 2Q probed fragments and probably derive from some non-specific hybridization.

nd, not detectable.

Fig. 3. a: a dilution range of a GluR-B sample, with approximately the same concentration as the GluR-A DNA samples that were loaded on the test gels, was separated and blotted. The blot was processed along with the GluR-A 2R filters. A dilution of 1:145 (0.7%) was still detectable on the autoradiogram and had a higher density than a control lane loaded with GluR-A (100%). b: a dilution range of a GluR-A sample was processed in an identical way as the GluR-B samples on the 2Q test gels. The 1:110 (0.9%) dilution was still detectable as unedited after the same exposure as the 2Q probed GluR-B fragments, whereas a control lane with GluR-B (100%) was not detectable.

quences showing the specificity of the amplified GluR fragments [2,3,9,19].

3.2. GluR-A

The photographs of the GluR-A Q and R gels revealed no clear systematic differences between the amount of DNA applied to the different lanes of kindled and control animals, as illustrated in Fig. 2. No visible DNA bands were left in the gel after transfer to the nylon filters. After the initial non-stringent wash at $5 \times$ SSC the extinction values of the autoradiogram of the 2Q probed filter were 11 ± 1 (mean $\pm$ S.E.M) for controls ($n = 6$) and 12 ± 2 for kindled ($n = 7$); for the 2R probed filters the values were 6.7 ± 0.6 vs 6.9 ± 0.2 .

To determine the percentage of edited GluR-A that could minimally be detected using the PCR-based analysis employed in this study, a dilution series with a

3.3. *GluR-B*

The analysis of GluR-B was done in the same way as described for GluR-A and the results are illustrated in Fig. 4. The extinction of the bands after the initial, low-stringency wash was 14 ± 1 (controls) and 13 ± 1 (kindled) for 2Q and 20 ± 2 vs. 22 ± 2 for 2R, showing no significant differences between kindled and controls. After the wash under high stringent conditions, no detectable activity was retained on the GluR-B Q blot except for the lane loaded with a GluR-A sample. Densitometric analysis of the exposed GluR-B R filter revealed no significant difference between the kindled and control group (Table 1). The re-probing of the

Fig. 4. The analysis of the Q to R ratio of GluR-B in kindled and control animals. The top photographs show the resolved PCR samples from fully kindled (FK) and control (CTR) animals. The second row shows the autoradiograms obtained from the 2Q and 2R probes filters after the low stringent wash. The third row is the pattern after the high stringent wash showing the strong prevalence of the edited (R) form of GluR-B over the unedited (Q) variant in both kindled and control preparations. The lane loaded with the GluR-A sample shows the opposite pattern as the GluR-B samples. The bottom row shows the same filters but probed with the reverse oligo.

Fig. 5. Photographs of the agarose gels and autoradiograms obtained from the blots after high stringent wash to discriminate between the edited (5R) and unedited (5Q) form of GluR-5. In two lanes of amplified cDNA obtained from kindled animals no bands were found and these two animals, indicated within parentheses, were excluded from further analysis.

filters resulted again in the complete failure to detect hybridization of the 2Q probe with any of the GluR-B fragments.

A dilution series with a GluR-A sample with approximately the same concentration of DNA as that loaded on the test gels, was processed along with the 2Q filters. A dilution of 1:110, corresponding with 0.9% of the total applied amount of DNA on the Q-R test gels, was well detectable as unedited product after the same exposure as the 2Q probed GluR-B fragments (Fig. 3b).

3.4. GluR-5

In one kindled animal we were unable to amplify detectable GluR-5 band while in another animal only a

faint amplification product was found (Fig. 5). These two lanes were excluded from the densitometric analysis. After probing the two filters with the 5Q and 5R oligo's and washing at $5 \times \text{SSC}$, the extinction values of the autoradiogram of the 5Q probed filter were 36 ± 6 for controls ($n = 6$) and 35 ± 5 for kindled ($n = 5$) and for the 5R probed filters 19 ± 3 vs 19 ± 2 . Detectable bands were present on both the Q and the R filter after the selective wash and co-exposure the filters to the same film sheet (Fig. 5, Table 1). For each individual animal an editing percentage was calculated on basis of the extinction values ($R/Q + R \times 100\%$). From this, a mean editing percentage $\pm$ S.E.M. for the control and the kindled group was obtained. The percentage of control (35.5 ± 3.5) and kindled group (36.7 ± 2.9) were not significantly different (Students *t*-test).

Fig. 6. Photographs of the agarose gels and autoradiograms obtained from the blots after high stringent wash to discriminate between the edited (6R) and unedited (6Q) form of GluR-6. Note the predominance of the edited form although the unedited form is still present.

3.5. GluR-6

The density of the lanes after the first, low stringency, wash was not different between the control and kindled groups 6Q: 5.3 ± 0.6 vs. 5.4 ± 0.4 and for 6R: 15 ± 1 vs. 14 ± 2 . After the high-stringent wash step no significant differences between the two groups were encountered (Fig. 6, Table 1). The calculated mean edited fraction was 91.6 ± 1.1 for controls and 90.7 ± 1.2 for the kindled group (n.s.). In a second experiment, using new PCR samples and freshly labeled 6Q and 6R probes, the edited fraction was almost the same as that found in the first series: 91.6 ± 0.4 for controls and 92.1 ± 0.8 for kindled animals.

4. Discussion

The main result of this study on the editing of the Q/R site in TMII coding region of GluR-A, -B, -5 and -6 mRNA is that we can reject the hypothesis that kindling epileptogenesis affects this editing process.

The relative editing levels of the GluR subunits in control animals obtained by the PCR based analysis of hippocampal cDNA are in good agreement with the results described by Sommer et al. [26] and Köhler et al. [20]. The GluR-A transcript was present in the rat hippocampus in the unedited form. Only trace amounts of hybridization product were detected on GluR-A filters incubated with the probe specific for the edited form but this product was effectively removed under slightly more stringent wash conditions, indicating that the trace amounts of filter signal may be derived from non-specific hybridization. In contrast to GluR-A, GluR-B was completely edited ($> 99\%$) and in fact we were unable to detect even trace amounts of unedited GluR-B on the blots. The GluR-B subunit is likely to be present in most natural AMPA receptors since the AMPA selective ion channels in hippocampal slice preparation share most characteristics of the expressed recombinant heteromeric glutamate receptors containing a GluR-B subunit. Moreover, GluR-B mRNA is expressed widely in the central nervous system [19,25,33]. After high stringent washing of the GluR-5 and -6 blots a hybridization product was present on both the Q and the R probed filters. Thus, in contrast to GluR-A and -B, the edited and non-edited form of GluR-5 and -6 co-exist in cellular mRNA. Based on the densitometric analysis of the autoradiograms in controls a mean 36% of GluR-5 mRNA was edited. This percentage of edited GluR-5 transcript is in good agreement with an editing percentage of 30–40% in 3-week-old rat brain as reported by the group of Seeburg [26,28] and is somewhat less than the 50–55% GluR-5 editing in the hippocampus [1,22]. For GluR-6, we found that 92% of the hippocampal transcript was

edited, which is slightly higher than the 70–80% reported in literature for whole brain [20,26,28] and hippocampus [1]. The differences in editing percentage may be due to methodological differences (Q/R specific DNA probes versus image analysis of *Bbv*I restriction digests) or to differences in age since both GluR-5 and -6 show changes during brain development [1,22].

The finding that the editing of the GluR transcripts is not significantly modified in the hippocampus of kindled animals 24 h after the last generalized convulsion implies that changes in the calcium permeability of AMPA and kainate receptors are not involved in the process of epileptogenesis. Therefore, the enhanced change in calcium concentration, induced by electrical stimulation or iontophoretic application of excitatory amino acids found in kindled tissue cannot be attributed to an enhanced AMPA/kainate receptor mediated influx [32]. An increased Ca^{2+} influx through voltage dependent calcium channels is a more likely explanation for this observation [31].

Despite the fact that we were unable to find significant changes in the editing ratios of hippocampal RNA obtained from kindled animals, we cannot exclude that a perturbation of the editing machinery may occur in a small population of neurons, that could lead to the functional impairment of a select population and even to a limited cell loss of principal neurons [5,6,27]. The possibility that a transient flaw in the editing process may occur immediately after a kindling seizure remains to be investigated.

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References

- [1] Bernard, A. and Khrestchatsky, M., Assessing the extent of RNA editing in the TMII regions of GluR5 and GluR6 kainate receptors, *J. Neurochem.*, 62 (1994) 2057–2060.
- [2] Bettler, B., Boulter, J., Hermans-Borgmeyer, I., O'Shea-Greenfield, A., Deneris, E.S., Moll, C., Borgmeyer, U., Hollmann, M. and Heinemann, S., Cloning of a novel glutamate receptor subunit: GluR5: expression in the nervous system during development, *Neuron*, 5 (1990) 583–595.
- [3] Boulter, J., Hollmann, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C. and Heinemann, S., Molecular cloning

- and functional expression of glutamate subunit genes, *Science*, 249 (1990) 1033–1037.
- [4] Burnashev, N., Monyer, H., Seeburg, P.H. and Sakmann, B., Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit, *Neuron*, 8 (1992) 189–198.
 - [5] Cavazos, J.E., Das, I. and Sutula, T.P., Neuronal loss induced in limbic pathways by kindling: evidence for induction of hippocampal sclerosis by repeated brief seizures, *J. Neurosci.*, 14 (1994) 3106–3121.
 - [6] Cavazos, J.E. and Sutula, T.P., Progressive neuronal loss induced by kindling: a possible mechanism for mossy fiber synaptic reorganization and hippocampal sclerosis, *Brain Res.*, 527 (1990) 1–6.
 - [7] Cha, J.-H.J., Kinsman, S.L. and Johnston, M.V., RNA editing of a human glutamate receptor subunit, *Mol. Brain Res.*, 22 (1994) 323–328.
 - [8] Chomczynski, P. and Sacchi, N., Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, *Anal. Biochem.*, 162 (1987) 156–159.
 - [9] Egebjerg, J., Bettler, B., Hermans-Borgmeyer, I. and Heinemann, S., Cloning of a cDNA for a glutamate receptor subunit activated by kainate but not AMPA, *Nature*, 351 (1991) 745–748.
 - [10] Egebjerg, J. and Heinemann, S.F., Ca^{2+} permeability of unedited and edited versions of the kainate selective glutamate receptor GluR6, *Proc. Natl. Acad. Sci. USA*, 90 (1993) 755–759.
 - [11] Goddard, G.V., McIntyre, D.C. and Leech, C.K., A permanent change in brain function resulting from daily electrical stimulation, *Exp. Neurol.*, 25 (1969) 295–330.
 - [12] Higuchi, M., Single, F.N., Köhler, M., Sommer, B., Sprengel, R. and Seeburg, P.H., RNA editing of AMPA receptor subunit GluR-B: A base-paired intron-exon structure determines position and efficiency, *Cell*, 75 (1993) 1361–1370.
 - [13] Hollmann, M., Hartley, M. and Heinemann, S., Ca^{2+} permeability of KA-AMPA gated glutamate receptor channels depends on subunit composition, *Science*, 252 (1991) 851–853.
 - [14] Hollmann, M. and Heinemann, S., Cloned glutamate receptors, *Annu. Rev. Neurosci.*, 17 (1994) 31–108.
 - [15] Hume, R.I., Dingledine, R. and Heinemann, S., Identification of a site in glutamate receptor subunits that controls calcium permeability, *Science*, 253 (1991) 1028–1031.
 - [16] Kamphuis, W., De Rijk, T.C., Talamini, L.M. and Lopes da Silva, F.H., Hippocampal kindling induces changes in the glutamate receptor mRNA expression patterns in dentate granule neurons, *Eur. J. Neurosci.*, 6 (1994) 1119–1127.
 - [17] Kamphuis, W., Monyer, H., De Rijk, T.C. and Lopes da Silva, F.H., Hippocampal kindling increases the expression of glutamate receptor-A Flip and -B Flip mRNA in dentate granule cells, *Neurosci. Lett.*, 148 (1992) 51–54.
 - [18] Kamphuis, W., Wadman, W.J. and Lopes da Silva, F.H., Changes in local evoked potentials in the rat hippocampus (CA1) during kindling epileptogenesis, *Brain Res.*, 440 (1988) 205–215.
 - [19] Keinänen, K., Wisden, W., Sommer, B., Werner, P., Herb, A., Verdoorn, T.A., Sakmann, B. and Seeburg, P.H., A family of AMPA-selective glutamate receptors, *Science*, 249 (1990) 556–560.
 - [20] Köhler, M., Burnashev, N., Sakmann, B. and Seeburg, P.H., Determinants of Ca^{2+} permeability in both TM1 and TM2 of high affinity kainate receptor channels: diversity by RNA editing, *Neuron*, 10 (1993) 491–500.
 - [21] Maru, E. and Goddard, G.V., Alteration in dentate neuronal activities associated with perforant path kindling. I. Long-term potentiation of excitatory synaptic transmission, *Exp. Neurol.*, 96 (1987) 19–32.
 - [22] Paschen, W., Dux, E. and Djuricic, B., Developmental changes in the extent of RNA editing of glutamate receptor subunit GluR5 in rat brain, *Neurosci. Lett.*, 174 (1994) 106–112.
 - [23] Pollard, H., Héron, A., Ben-Ari, Y. and Khrestchatsky, M., Alteration of the GluR-B AMPA receptor subunit Flip/Flop expression in kainate-induced epilepsy and ischemia, *Neuroscience*, 57 (1993) 545–554.
 - [24] Regehr, W.G. and Tank, D.W., Calcium concentration dynamics produced by synaptic activation of CA1 hippocampal pyramidal cells, *J. Neurosci.*, 12 (1992) 4202–4223.
 - [25] Sommer, B., Keinänen, K., Verdoorn, T.A., Wisden, W., Burnashev, N., Herb, A., Köhler, M., Takagi, T., Sakmann, B. and Seeburg, P.H., Flip and flop: a cell-specific functional switch in glutamate-operated channels of the CNS, *Science*, 249 (1990) 1580–1585.
 - [26] Sommer, B., Köhler, M., Sprengel, R. and Seeburg, P.H., RNA editing in brain controls a determinant of ion flow in glutamate-gated channels, *Cell*, 67 (1991) 11–19.
 - [27] Spiller, A.E. and Racine, R.J., The effect of kindling beyond the 'stage 5' criterion on paired-pulse depression and hilar cell counts in the dentate gyrus, *Brain Res.*, 635 (1994) 139–147.
 - [28] Sprengel, R. and Seeburg, P.H., The unique properties of glutamate receptor channels, *FEBS Lett.*, 325 (1993) 90–94.
 - [29] Sutula, T. and Steward, O., Quantitative analysis of synaptic potentiation during kindling of the perforant path, *J. Neurophysiol.*, 56 (1986) 732–746.
 - [30] Verdoorn, T.A., Burnashev, N., Monyer, H., Seeburg, P.H. and Sakmann, B., Structural determinants of ion flow through recombinant glutamate receptor channels, *Science*, 252 (1991) 1715–1718.
 - [31] Vreugdenhil, M. and Wadman, W.J., Kindling-induced long-lasting enhancement of calcium in hippocampal CA1 area of the rat: relation to calcium-dependent inactivation, *Neuroscience*, 59 (1994) 105–114.
 - [32] Wadman, W.J., Heinemann, U., Konnerth, A. and Neuhaus, S., Hippocampal slices of kindled rats reveal calcium involvement in epileptogenesis, *Exp. Brain Res.*, 57 (1985) 404–407.
 - [33] Wisden, W. and Seeburg, P.H., Mammalian ionotropic glutamate receptors, *Curr. Opin. Neurobiol.*, 3 (1993) 291–298.
 - [34] Wisden, W. and Seeburg, P.H., A complex mosaic of high-affinity kainate receptors in rat brain, *J. Neurosci.*, 13 (1993) 3582–3598.