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Publication date
1995

Published in
Journal of neurochemistry

[Link to publication](#)

Citation for published version (APA):

Kamphuis, W., Smirnova, T., Hicks, A. A., Hendriksen, H., Mallet, L., & Lopes da Silva, F. H. (1995). The expression of syntaxin 1B/GR33 mRNA is enhanced in the hippocampal kindling model of epileptogenesis. *Journal of neurochemistry*, 65, 1974-1980.

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The Expression of Syntaxin1B/GR33 mRNA Is Enhanced in the Hippocampal Kindling Model of Epileptogenesis

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Abstract: Syntaxin, a protein required for the docking of synaptic vesicles, may be involved in the manifestation of synaptic plasticity. The possible involvement of syntaxin in epileptogenesis was investigated by assessing the expression levels of syntaxin1B/GR33 mRNA by in situ hybridization at different stages of hippocampal kindling epileptogenesis and after the induction of generalized seizures. Densitometric analysis of the autoradiograms revealed that the expression was not changed in pyramidal and granular neurons of the hippocampal formation 24 h after the first kindling stimulation. However, the mRNA levels in CA1, CA3, and fascia dentata neurons were bilaterally enhanced after six afterdischarges and remained at this elevated level during the whole period along which afterdischarges were elicited. An immunoassay was unable to reveal a clear significant increase of syntaxin1B/GR33 protein levels in hippocampus homogenates of fully kindled animals. The use of syntaxin1B-specific antibodies is necessary to draw definite conclusions on the changes at the protein level. At long term, 4 weeks after the last kindling-elicited generalized seizure, no significant alterations in transcript levels could be detected. The results suggest that the induction of kindling epileptogenesis is associated with an enhanced expression of syntaxin1B/GR33, but this enhanced expression is not necessary for persistence of kindling-induced synaptic plasticity. **Key Words:** Kindling—GR33—Syntaxin1B—Hippocampus—In situ hybridization—Epilepsy—Plasticity.
J. Neurochem. **65**, 1974–1980 (1995).

Synaptic plasticity plays a key role in the neurobiological mechanisms underlying learning and memory but is also involved in changes leading to pathophysiological conditions of the CNS such as epilepsy. The kindling model is frequently used to study the basic mechanisms of epileptogenesis at the synaptic level. Kindling epileptogenesis is provoked by the repeated application, typically at a rate of once or twice daily, of high-frequency electrical stimulations to a brain site.

Initially, the stimulation results in only short-lasting local epileptiform activity (afterdischarge), but subsequent stimulations result in a progressive spread, duration, and severity of the afterdischarge accompanied by an intensification of seizure behavior, culminating in generalized seizures. This state of enhanced excitability is persistent and can be maintained without need for further stimulations (Goddard et al., 1969).

The precise nature of the alterations that underly the enhanced seizure response have not yet been fully clarified. At the presynaptic level, kindling is associated with an enhanced release of different neurotransmitters. In the hippocampus, the release of glutamate (Kamphuis et al., 1991; Zhang et al., 1991; Minamoto et al., 1992; Ueda and Tsuru, 1994), GABA (Kamphuis et al., 1991; Minamoto et al., 1992), noradrenaline (Koikaia et al., 1989), somatostatin (Vezzani et al., 1992), and neuropeptide Y (Rizzi et al., 1993), as measured with various techniques, was increased during kindling, which suggests that an up-regulation of the mechanism of exocytosis for several neurotransmitters may be involved in the establishment and/or expression of a kindled focus.

The synaptic protein syntaxin is involved in the Ca^{2+} influx-triggered processes of neurotransmitter release, including the docking and fusion of vesicles with the presynaptic plasma membrane (Bennett and Scheller, 1993). Syntaxin is associated in a molecular complex with a number of proteins, which include synaptotagmin and a voltage-sensitive calcium channel, thus forming an integral component of the calcium-sensi-

Received January 12, 1995; revised manuscript received April 28, 1995; accepted May 31, 1995.

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Abbreviations used: LTP, long-term potentiation; PMSF, phenylmethylsulfonyl fluoride; RT, room temperature; SDS, sodium dodecyl sulfate; SSC, saline-sodium citrate; syn1B, syntaxin1B.

tive vesicle-docking machinery (Bennett et al., 1992, 1993; Yoshida et al., 1992; O'Connor et al., 1993; Lvque et al., 1994; Monck and Fernandez, 1994). Recently, the expression of syntaxin1B (syn1B) (Bennett et al., 1993), also named GR33 (Smirnova et al., 1993a), was found to be transiently enhanced after induction of long-term potentiation (LTP), indicating that synaptic plasticity may be associated with changes of proteins involved in the mechanism of exocytosis (Smirnova et al., 1993b). Here, we report that the expression of syn1B/GR33 is increased by kindling stimulations in a pattern different from that observed after LTP induced in the hippocampus.

MATERIALS AND METHODS

Materials

Unless otherwise stated, all chemicals were of analytical grade obtained from Sigma (St. Louis, MO, U.S.A.).

Kindling

Male Wistar rats (200–225 g, Harlan-Netherlands) were used. Stainless-steel, trimel-insulated electrodes were placed in the CA1 area of the left dorsal hippocampus of rats that were under pentobarbital anesthesia. The stimulation bundle was placed in the Schaffer collateral/commissural fiber pathway and the recording bundle was positioned in the stratum radiatum of CA1. The details of this procedure were described previously (Kamphuis et al., 1988). After 2 weeks of recovery, the rats were connected to a stimulation/recording device to enable the delivery of kindling stimulations consisting of a train of 50-Hz pulses of 1–2-s duration at an intensity of 200–300 μ A and to perform local electroencephalographic recordings.

All experimental procedures were controlled and approved by the Animal Experiments Committee of the Faculties of Biology and Chemistry, University of Amsterdam.

Experimental design and kindling

A group of 60 implanted animals was divided into a non-stimulated control group ($n = 24$) and a group ($n = 36$) that received, twice daily, kindling stimulations. The animals of the control group were handled throughout the experimental period in a way comparable with the kindled rats but did not receive tetanic stimulations.

The expression of syn1B/GR33 mRNA was studied in the following four groups of kindling-stimulated animals: (1) a 6-AD group ($n = 8$) killed after the sixth tetanic stimulation; (2) a 14-AD group ($n = 7$) killed after the 14th afterdischarge; (3) a fully kindled group ($n = 8$) killed after 26–34 afterdischarges when rats experienced generalized tonic-clonic convulsions [total number of class 5 seizures: 10 ± 1 , according to the scale of Racine (1972)]; (iv) a long-term group ($n = 8$), kindled to the same stage as the fully kindled animals (total number of 31–36 afterdischarges resulting in 9 ± 1 class 5 seizures). Animals of the 6-AD, 14-AD, and fully kindled groups were killed 24 h after the last seizure. Furthermore, three fully kindled animals were studied 2 h after the last class 5 seizure and two fully kindled animals after 72 h. Animals of the long-term group were killed 28 days after the last seizure. The rats of the control group were divided over the different stimulated groups and their brains were fixed at the same time.

In a second series to study the effects of one single after-

discharge on syn1B/GR33 expression, 23 animals were implanted. A single stimulus train of pulses, identical to those used in the kindling protocol, was applied to the Schaffer collateral/commissural fiber bundle in CA1 of 11 animals. All animals responded to the stimulation with an afterdischarge (25 ± 3 s; mean $\pm$ SEM). Control animals ($n = 12$) received the same number of pulses but at low frequency (0.25 Hz) without triggering an afterdischarge. The rats were divided into two groups and killed at two different times after the stimulation, 0.5 h ($n = 6$) and 24 h ($n = 5$). Along with each experimental group six controls were killed.

A third series of fully kindled rats was used to investigate the protein levels of syn/GR33 by using antibodies raised against a GR33 cDNA-encoded protein (Smirnova et al., 1993a). After 1 week of recovery from the implantation procedure, a group of 10 animals was divided into a control group ($n = 5$) and a group ($n = 5$) that was kindled in an identical manner as described above. Kindled animals received a mean of 33 (range, 28–36) kindling stimulations, resulting in a mean of seven (range, 6–9) generalized tonic-clonic seizures (stage 5, fully kindled state). The kindled group of animals was killed 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 hippocampus was rapidly isolated, frozen on dry ice, and stored at -70°C for later analysis.

In situ hybridization

Animals were deeply anesthetized with ether and killed by decapitation. The brain was rapidly removed and frozen on powdered dry ice and stored at -70°C . Coronal cryosections (12 μm) were cut, thaw-mounted onto poly-L-lysine-coated slides, and dried at room temperature (RT). Sections were fixed for 5 min in 4% paraformaldehyde (RT), washed in phosphate-buffered saline, dehydrated in 70% ethanol, and stored in 95% ethanol at 4°C until use. Before hybridization, sections were removed from the ethanol storage boxes and air dried. For this study, sections at a level of about -2.9 mm caudal to the bregma were selected, a region that is ~ 0.4 mm caudal with respect to the position of the stimulation electrode. In situ hybridization was performed as described in detail previously (Wisden et al., 1991; Kamphuis et al., 1994). In brief, a syn1B/GR33-specific 45-mer antisense oligonucleotide was 3' end-labeled with ^{35}S -dATP (NEN, 1,200–1,500 Ci/mmol) using terminal deoxynucleotidyl transferase (GIBCO-BRL Life Technologies). The labeled probe was purified and diluted to $1\text{--}2$ pg/ μl ($\pm 1,000$ cpm/pg) in a hybridization solution containing 50% formamide, 0.6 M NaCl, 0.06 M sodium citrate ($4\times$ SSC), 100 $\mu\text{g}/\text{ml}$ polyadenylic acid, 25 mM sodium phosphate, 1 mM pyrophosphate, $5\times$ Denhardt's, 200 $\mu\text{g}/\text{ml}$ sheared salmon sperm DNA, and 10% dextran sulfate. Sections were incubated with this hybridization solution overnight at 42°C . Sections were rinsed several times in $1\times$ SSC at 20°C and subsequently washed for 20 min at a stringency of $1\times$ SSC at 60°C . Sections were dehydrated and exposed to Kodak XAR-5 film for 5 weeks.

The sequence (5'-CCGTGAGAGTGTGGAGTGCTGGGTCTTACGGATACGCAGGTCTGC-3') of the oligonucleotide that was used here to examine the expression of syn1B/GR33 in kindled rats is identical to the probe used by Smirnova et al. (1993a). The specificity of the probe was checked by northern blot analysis of 1.5 μg poly(A)⁺ mRNA from forebrains of two adult rats. Hybridization was performed with the [^{32}P]ATP-tailed oligonucleotide probe.

FIG. 1. Northern blot of poly(A)⁺ RNA from the forebrain of two different rats. Each lane contained 1.5 μ g of the poly(A)⁺ mRNA. Hybridization was performed with the [³²P]ATP-tailed syn1B/GR33 mRNA-specific oligonucleotide probe. The positions of the ribosomal RNA 28S and 18S bands are indicated.

Hybridization conditions were 50% formamide, 4 $\times$ SSC, 1% sodium dodecyl sulfate (SDS), at 42°C. After hybridization, the blots were washed at a final stringency of 2 $\times$ SSC/0.1% SDS at 65°C (Fig. 1).

Densitometric analysis

The densitometric analysis of the obtained autoradiograms was described previously in detail (Kamphuis et al., 1994). In short, the ipsilateral (left, electrode implanted hemisphere) and contralateral hippocampal and cortical areas within the autoradiogram were scanned and digitized. The stored images were assigned a code and quantification was performed by an observer who had no knowledge of the treatment to which the animals under study had been subjected. The codes were broken only after completion of the quantification. The mean extinction value of the area overlying the CA1, CA3, fascia dentata granular layer, neocortical areas, and thalamus was calculated. Previously, it was shown that the extinction values, within the range of the *in situ* hybridization autoradiograms, increased linearly with labeled probe concentration blotted to nylon filter (Kamphuis et al., 1994). This demonstrated that relative changes in extinction value provide quantitative information on changes in the amount of probe bound to the tissue and consequently on changes in the amount of specifically hybridized target. Of the three mounted sections only one was used for analysis because the variation between animals was found to be larger than the negligible variation between different sections of the same animal (data not shown).

Immunodetection of syn1A-1B/GR33 levels

The hippocampus of fully kindled or control animals was homogenized in buffer containing 0.32 M sucrose, 1.0 M EDTA, 0.25 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride (PMSF). After centrifugation (15,000 g, 30 min at 4°C), the membranes were solubilized in 20 mM Tris-HCl, pH 8.0, 1% Triton X-100, 0.2 mM PMSF for 1 h at 4°C. Unsolubilized proteins were removed by centrifugation and the protein content of the samples was measured (Bio-Rad protein assay).

Slot-blot series containing 400, 200, 100, and 50 ng of total protein in Tris-buffered saline were prepared for each sample on Immobilon P filters (Millipore) using a Bio-Rad SF apparatus. Immunodetection was performed using anti-GR33 antibodies (1:5,000; Smirnova et al., 1993a), followed by chemiluminescence detection using an ECL kit (Amersham, The Netherlands) according to the manufacturer's instructions. A series of different exposures to Fuji-RX film were made (1–6 min) and the films were analyzed by densitometry. Preliminary tests had shown a linear relation between the detected chemiluminescence signal and the amount of protein applied on the slot over a range of 10 ng to 2.5 μ g. For each animal a graph was plotted of extinction

against blotted amount of protein. The correlation coefficient of the linear fit, r^2 , was for all samples > 0.95; the extinction value corresponding to 200 ng of blotted protein was used for statistical analysis (Student's *t* test).

Western blot analysis of the homogenates was performed after separation of 10 μ g of total protein on a 10% SDS-polyacrylamide gel and a transfer to Immobilon membrane.

Statistical analysis

Statistical comparison of the densitometric values of the autoradiograms was performed for each analyzed hippocampal region (CA1, CA3, fascia dentata, cortex, and thalamus) independently. The paired Student's *t* test revealed no statistical differences between ipsi- and contralateral hemispheres and further analysis was, therefore, performed on the mean extinction value of the two hemispheres. The variation in measured extinction values was comparable in control and kindled groups; SEM typically represented 4–6% of the mean extinction value. The statistical comparison of the extinction value from kindled with that obtained from the control groups fixed along with the experimental groups was done using the Student's *t* test. For the statistical analysis of the second series (single stimulation), the controls were pooled into one control group.

The percent change of the extinction values in the kindled group in comparison with the control groups was calculated by dividing the mean extinction of the kindled group by that of the control group and multiplying the outcome by 100. The difference from this 100% (corresponding to the expression levels in the control group) value and the outcome of the statistical analysis is presented in Table 1 for the pyramidal cell layer of CA1, CA3, and the granular cell layer of the fascia dentata.

RESULTS

Kindling

In the course of kindling, the afterdischarge duration increased from a duration of 20 s at the first session to 60–100 s at session 16. Thereafter, the afterdischarges stabilized around a length of ~90 s and were most often accompanied by the occurrence of class 5 behavioral seizures. The expression of syn1B/GR33 was studied in the following four groups of animals that were killed at different stages of kindling: (1) a 6-AD group ($n = 8$) killed after the sixth tetanic stimulation, which triggered afterdischarges lasting 36 ± 8 s (mean $\pm$ SEM); (2) a 14-AD group ($n = 7$) after the 14th afterdischarge, lasting 60 ± 12 s; (3) a fully kindled group ($n = 8$) killed 24 h after the 31st afterdischarge, lasting 111 ± 6 s, when rats exhibited generalized tonic-clonic convulsions (total number of class 5 seizures, 10 ± 1). The animals of these three groups were all fixed 24 h after the last session. Two small additional groups were killed at 2 h ($n = 3$) and 72 h ($n = 2$) after the last convulsion. The persistent changes in kindled tissue were studied in a (4) long-term group ($n = 8$). These animals were kindled to the same degree as the fully kindled animals (total number of 31 afterdischarges, resulting in 9 ± 1 class

FIG. 2. In situ hybridization of syn1B/GR33 mRNA-specific oligonucleotide probe in a coronal section of the rat brain. Note the widespread distribution of the transcripts in neocortex, hippocampus, thalamus, and hypothalamus. Bar = 2 mm.

5 seizures, lasting 91 ± 13 s), but they were killed 28 days after the last seizure.

Expression of syn1B/GR33 in kindled animals

In the rats of the control groups, hybridization signals were observed in all principal neurons of the hippocampus, and furthermore in the neocortex, amygdala, thalamic, and hypothalamic regions similar to the description of Smirnova et al. (1993a). An example of the expression pattern in a coronal section is given in Fig. 2. When a 50-fold excess of unlabeled probe was added to the in situ hybridization mix, no hybridization signal was detected. In animals of the different kindled groups, the qualitative distribution of the transcript was not changed. However, the quantitative densitometric analysis of the autoradiograms revealed significant bilateral changes in the hippocampus as illustrated in Fig. 3.

We determined the percentual change of the extinction values in the kindled groups in comparison with the control groups by normalizing the mean extinction of the kindled group using the mean of the control group, set at 100%, as reference. The estimated differences in the kindled groups from the control value and the outcome of the statistical analysis are presented in Table 1 for the pyramidal cell layer of CA1 and CA3 region and the granular cell layer of the fascia dentata. After six afterdischarges the mRNA level was significantly enhanced in CA1 and CA3. In the fascia dentata, the expression was also increased, but this was only significant using a one-sided test. After 14 afterdischarges, syn1B/GR33 expression was elevated in all hippocampal areas. In the fully kindled group the expression was still higher in comparison with controls although the difference diminished slightly.

The animals studied 2 h ($n = 3$) after the last generalized seizure showed a comparable (15–20%) increase as the fully kindled animals after an interval of 24 h. However, analysis of the group of fully kindled animals studied 4 weeks after the last seizure, the long-

Control

6-AD

14-AD

Fully-Kindled

Long-Term

FIG. 3. Photomicrographs of in situ hybridization autoradiograms illustrating the changes in the expression in the hippocampus of syn1B/GR33 at different stages during kindling development: (a) control; (b) 24 h after six afterdischarges; (c) 24 h after 14 afterdischarges; (d) 24 h after a generalized seizure in a fully kindled animal; (e) long-term, i.e., 4 weeks after a generalized seizure in a fully kindled animal. Bar = 5 mm.

TABLE 1. Relative changes in mean extinction values of the *in situ* hybridization autoradiograms of GR33/syn1B in the kindled groups

	6-AD (n = 8)	14-AD (n = 7)	FK (n = 8)	LT (n = 8)
CA1	20.6 ± 3.8 ^a	23.4 ± 4.7 ^a	11.6 ± 4.5 ^b	-1.2 ± 5.5
CA3	24.1 ± 4.9 ^a	22.5 ± 5.0 ^a	18.5 ± 5.0 ^c	-1.1 ± 3.6
FD	15.2 ± 5.4 ^b	25.5 ± 6.4 ^a	20.5 ± 4.4 ^d	-4.1 ± 3.4

The mean extinction value obtained in the control group was used as reference and set as 100%; the difference from this value is presented (% ± SEM). The animals of the 6-AD, 14-AD, and the fully kindled (FK) groups were killed 24 h after the last kindling stimulation. The long-term (LT) group was studied 28 days after the last stimulation. Statistical comparisons with respect to controls were performed on the determined extinction values using the Student's *t* test. FD, fascia dentata.

^a*p* < 0.01; ^b*p* < 0.05 (one tailed); ^c*p* < 0.025; ^d*p* < 0.001.

term group, revealed no significant differences in expression from controls that were fixed at the same time. The two fully kindled rats analyzed at a 72-h interval were comparable with controls. These observations suggest that in fully kindled animals, the rise in expression is relatively stable after the stimulation for a period of ~24 h.

The expression of syn1B/GR33 in the neocortex was increased after six afterdischarges, in the temporal cortex by 13% and in the occipital cortex area 18%. Significantly elevated levels were observed in the 14-AD group, 22% (*p* < 0.05) and 26% (*p* < 0.025) for the temporal and occipital areas, respectively. In fully kindled animals the expression was still enhanced, but the differences were no longer significant. No long-term changes were observed. In thalamic nuclei no changes in expression were encountered.

Expression of syn1B/GR33 mRNA after a single afterdischarge

The enhanced expression levels after six afterdischarges prompted us to study the effects of a single afterdischarge. All animals responded to the kindling stimulation, applied to the Schaffer collateral/commissural fiber pathway in CA1, with a short afterdischarge (25 ± 3 s; mean ± SEM) without overt signs of epileptiform behavior. The relative changes of the transcript levels after one afterdischarge are presented in Table 2. At an interval of 0.5 h after the afterdischarge no significant changes were observed, and also 1 day after the stimulation no alterations were detectable.

The same animals of this second series were previously investigated for mRNA levels of immediate early genes *c-fos* and *zif-268* (Kamphuis et al., 1994). All rats of the 0.5 h group showed an enhanced *c-fos* and *zif-268* expression, illustrating that the afterdischarge activity, induced with the stimulus paradigm used here, was sufficient to trigger changes in gene expression in all hippocampal areas (Kamphuis et al., 1994).

Immunodetection of syn1A-1B/GR33 level in fully kindled rats

Despite the relatively small increases in the level of syn1B/GR33 mRNA, we attempted to investigate whether the enhanced expression was accompanied by an increase in the levels of the encoded protein by direct slot blotting and western blot analysis. For this purpose, we made use of an antibody directed against a fusion protein that was generated by subcloning the GR33 cDNA into an *Escherichia coli* pGEX expression vector (Smirnova et al., 1993a). The antiserum recognizes both syntaxin1A and syn1B (T. Smirnova, unpublished observations). The film extinction values of the chemiluminescence-based immunodetection of syn1A-1B/GR33 in 50–400 ng of total slot-blotted protein were all within the linear range. Significant differences between fully kindled rats and control implanted animals were not found by slot-blot analysis; i.e., controls 32.3 ± 3.3 vs. kindled 32.7 ± 3.0 (mean extinction values corresponding to 200 ng of protein ± SEM; *n* = 5 for both groups). Western blot analysis of the hippocampal homogenates of the kindled and control rats revealed in all animals a major band of immunoreactivity corresponding to a molecular mass size of 33–37 kDa. The extinction values of the immunosignal of the major band showed a mean increase of 24% in the kindled group, although this difference was not statistically significant; i.e., controls 17.5 ± 1.8 vs. kindled 21.8 ± 3.7 (mean ± SEM; *n* = 5 for both groups).

DISCUSSION

Kindling stimulation-induced epileptogenesis is accompanied by an increased level of syn1B/GR33 mRNA in the hippocampal formation. The very first kindling stimulation induced no changes in the expression that were detectable at 24 h after the stimulus. A significant increase was observed after the application of six stimulations when the process of epileptogenesis is still in its initial stages. After 14 afterdischarges the enhanced expression was comparable with the changes found after six afterdischarges, and at this stage the changes had spread to neocortical regions. The levels

TABLE 2. Relative changes in mean extinction values of the *in situ* hybridization autoradiograms of GR33/syn1B at 0.5 and 24 h after a single afterdischarge (% ± SEM)

	0.5 h (n = 6)	24 h (n = 5)
CA1	-7.6 ± 4.2	-2.5 ± 3.4
CA1	-6.6 ± 4.4	-6.3 ± 3.0
FD	1.4 ± 4.4	7.7 ± 4.9

Figures represent the difference from the mean extinction value obtained in the control group set at 100%. Statistical comparisons with respect to pooled controls (*n* = 12) were performed on the determined extinction values using the Student's *t* test. FD, fascia dentata.

of syn1B/GR33 mRNA were still found to be enhanced in fully kindled animals that had experienced generalized seizures at regular intervals over a period of 5 days. However, 4 weeks after the establishment of a kindled focus, when the animals are still characterized by an enhanced sensitivity for the induction of seizures, no detectable alterations in the expression of syn1B/GR33 were found. These results indicate that changes in syn1B/GR33 gene expression do not underlie the persistent alterations in kindled animals but appear to be involved in the development of kindling epileptogenesis caused by the repeated occurrence of short periods of paroxysmal activity.

Recently, syn1B/GR33 expression was found to be enhanced in the fascia dentata granular neurons of animals that had received a high-frequency electrical stimulation leading to the induction of LTP (Smirnova et al., 1993b). At 2–5 h after the stimulus syn1B/GR33 transcript levels were enhanced 2–3-fold, but after 24 h no alterations were detectable. The stimulus used to induce LTP produced no detectable afterdischarges, and the observed changes in gene expression, including those bilateral at 5 h, were input specific. In contrast to these changes observed after induction of LTP, the kindling-related alterations were enhanced at a relatively invariable level, at least in fully kindled animals, over a period of 2–24 h after the stimulus. Another difference with LTP is that the changes are not restricted to the stimulated fascia dentata only, but are bilateral and include the CA1 and CA3 regions. This suggests that there may in fact be two different modulations of the syn1B/GR33 gene expression, i.e., a strong, short-lasting, LTP-associated type, which lasts at least 5 h, and a less dramatic but more sustained response associated with epileptiform activity.

The widespread distribution in the brain of the mRNA is in accordance with the proposed function of syn1B/GR33 in neurotransmitter release (Bennett et al., 1992, 1993; Yoshida et al., 1992; O'Connor et al., 1993; Lévesque et al., 1994; Monck and Fernandez, 1994). Because kindling is associated with an increase in the release of diverse neurotransmitters (Koikaia et al., 1989; Kamphuis et al., 1991; Zhang et al., 1991; Minamoto et al., 1992; Rizzi et al., 1993; Ueda and Tsuru, 1994), it opens the possibility that modifications of proteins involved in the docking and fusion process of transmitter vesicles may underlie these changes at the presynaptic level. Whether other synaptic proteins are also modified during epileptogenesis or whether the changes in syn1B/GR33 are restricted to certain neurotransmitter systems remains to be investigated.

Alterations occurring at the level of syn1B/GR33 mRNA do not necessarily mean specific and parallel changes in the levels of the protein but may also be the consequence of an overall change in mRNA stability, affecting all transcripts in a similar way. However, in previous experiments many different, transcript-specific patterns of kindling-induced changes were found,

supporting the hypothesis that a tight control of mRNA transcripts subserves specific changes at the protein levels leading to synaptic plasticity (Kamphuis et al., 1994, 1995). We made a first attempt to investigate whether the enhanced syn1B/GR33 expression was indeed accompanied by a change in the amount of encoded protein in the tissue. Yet, in fully kindled rat hippocampus, we were unable to detect clear significant changes on slot blots. On western blots, where the antiserum detected a protein band corresponding to a molecular mass of 33–37 kDa, kindled animals showed a trend for enhanced levels. However, it must be noted that the antiserum used here is not specific for syn1B/GR33 but also detects syntaxin1A. It may, therefore, be that alterations in syn1B/GR33 protein are masked by syntaxin1A. Clearly, generation of syn1B-specific antibodies and more experiments with different kindled groups are needed to clarify this issue.

Acknowledgment: W.K. was supported by the Dutch Organization for Scientific Research (NWO; grant 903-53-091), H.H. by the Committee on Epilepsy of the Division for Health Research of the National Epilepsy Fund (CLEO project number A-109). T.S. and A.H. were supported by Centre National de la Recherche Scientifique, Institut de la Santé et de la Recherche Médicale, Ministère de la Recherche et de la Technologie, Association de la Recherche contre le Cancer, the Bioavenir Program (Rhône-Poulenc Rorer, Ministère de la Recherche et de l'Espace, Ministère de l'Industrie et du Commerce Extérieur), and the HFSP. We thank S. van Mechelen for assistance with photography.

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