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Potential New Antiepileptogenic Targets Indicated by Microarray Analysis in a Rat Model for Temporal Lobe Epilepsy

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To get insight into the mechanisms that may lead to progression of temporal lobe epilepsy, we investigated gene expression during epileptogenesis in the rat. RNA was obtained from three different brain regions [CA3, entorhinal cortex (EC), and cerebellum (CB)] at three different time points after electrically induced status epilepticus (SE): acute phase [group D (1 d)], latent period [group W (1 week)], and chronic epileptic period [group M (3–4 months)]. A group that was stimulated but that had not experienced SE and later epilepsy was also included (group nS). Gene expression analysis was performed using the Affymetrix Gene Chip System (RAE230A). We used GENMAPP and Gene Ontology to identify global biological trends in gene expression data. The immune response was the most prominent process changed during all three phases of epileptogenesis. Synaptic transmission was a downregulated process during the acute and latent phases. GABA receptor subunits involved in tonic inhibition were persistently downregulated. These changes were observed mostly in both CA3 and EC but not in CB. Rats that were stimulated but that did not develop spontaneous seizures later on had also some changes in gene expression, but this was not reflected in a significant change of a biological process. These data suggest that the targeting of specific genes that are involved in these biological processes may be a promising strategy to slow down or prevent the progression of epilepsy. Especially genes related to the immune response, such as complement factors, interleukins, and genes related to prostaglandin synthesis and coagulation pathway may be interesting targets.

Key words: **epileptogenesis; gene ontology; immune response; CA3; entorhinal cortex; synaptic transmission; PCR; immunostaining; status epilepticus; seizure**

Introduction

Mesial temporal lobe epilepsy (MTLE) is a severe epilepsy syndrome that may evolve after an initial insult such as complex febrile seizures, stroke, brain infections, head trauma, or status epilepticus (SE) (Mathern et al., 1996; Hauser, 1997). The initial insult is usually followed by a latent period, after which spontaneous epileptic seizures occur that can become more and more frequent over time (Engel, 1996; Mathern et al., 1996). Epileptic patients are treated commonly with antiepileptic drugs that may suppress seizures. Antiepileptogenic drugs that retard or prevent epileptogenesis after an initial insult are not yet available (Temkin, 2001; Schmidt and Rogawski, 2002; Loscher and Schmidt, 2004).

The MTLE syndrome is mimicked in the so-called post-SE rodent models. The induced SE is followed, after a latent period, by the occurrence of spontaneous epileptic seizures. It is hypothesized that the initial insult triggers a range of molecular, structural, and functional changes leading to the occurrence of spontaneous seizures. Microarray and SAGE gene expression profiling have been used as tools to identify molecular processes that may be epileptogenic. In recent years, several experimental large-scale genomic studies have shown changes of hundreds of genes shortly after SE. Most of these studies identified genes that are commonly associated with neuronal plasticity, gliosis, neuronal death, neurogenesis, and structural reorganization (Tang et al., 2002; Becker et al., 2003; Elliott et al., 2003; Lukasiuk et al., 2003). Notwithstanding the valuable lists of "epilepsy candidate genes" yielded by these studies, little insight was obtained regarding specific biological pathways with potential epileptogenic properties. Moreover, the reproducibility of the results obtained in different studies was somewhat disappointing, which might be attributable to the different microarray platforms, the relatively small numbers of animals used, the pooling of data from individual

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animals, and the fact that different brain areas were selected for analysis (Lukasiuk and Pitkanen, 2004). With the rapid advance of the microarray technique, the expression of many more genes can be studied and the reproducibility improved, so that biological pathways can be identified in more detail (Bammler et al., 2005; Irizarry et al., 2005). Insight in the epileptogenic process can be greatly enhanced by (1) taking samples at sequential time points, particularly during the early stages of epileptogenesis, (2) choosing brain areas with different sensitivities to epileptogenesis, and (3) including a group of animals that is treated similarly but that has not developed epilepsy. The present study was designed to include all three features; in addition, we analyzed each animal separately instead of pooling the results from several animals. The construction of biological pathway maps at a sequence of epileptic stages can provide insight in the dynamics of the biological processes relevant to epileptogenesis (Doniger et al., 2003). Therefore, we performed an analysis according to well defined biological processes based on Gene Ontology (GO) categories (Bammler et al., 2005). Identification of specific biological processes and biochemical pathways during critical phases of epileptogenesis is of crucial importance to construct strategies that may prevent the development of epilepsy.

Materials and Methods

Experimental animals. Adult male Sprague Dawley rats (Harlan CPB, Zeist, The Netherlands) weighing 300–500 g were used in this study, which was approved by the University Animal Welfare committee. The rats were housed individually in a controlled environment (21 ± 1°C; humidity, 60%; lights on from 8:00 A.M. to 8:00 P.M.; food and water available *ad libitum*).

Electrode implantation and seizure induction. At 3–4 months of age, rats were anesthetized with an intramuscular injection of ketamine (57 mg/kg; Alfasan, Woerden, The Netherlands) and xylazine (9 mg/kg; Bayer, Leverkusen, Germany), and placed in a stereotactic apparatus. To record hippocampal EEG, a pair of insulated stainless-steel electrodes (70 μm wire diameter; tips were 80 μm apart) were implanted into the left dentate gyrus under electrophysiological control as described previously (Gorter et al., 2001). A pair of stimulation electrodes was implanted in the angular bundle. Several weeks after electrode implantation, rats underwent tetanic stimulation (50 Hz) of the hippocampus in the form of a succession of trains of pulses every 13 s. Each train had a duration of 10 s and consisted of biphasic pulses (pulse duration, 0.5 ms; maximal intensity, 500 μA). Stimulation was stopped when the rats displayed sustained forelimb clonus and salivation for minutes, which usually occurred within 1 h. However, stimulation never lasted longer than 90 min. EEG signals were amplified (10×) via a field effect transistor on the headstage and then led to a differential amplifier (CyberAmp; Molecular Devices, Burlingame, CA), amplified (20×), filtered (1–60 Hz), and sampled by a seizure detection program at a frequency of 200 Hz per channel (Harmonie; Stellate Systems, Montreal, Quebec, Canada). EEG recordings were visually monitored and screened for seizure activity. Behavior was observed during electrical stimulation and several hours thereafter. Immediately after termination of the stimulation, periodic epileptiform discharges occurred at a frequency of 1–2 Hz and were accompanied by behavioral and EEG seizures (SE). Rats were killed at three successive time points: (1) at 1 d after SE (group D; acute phase; $n = 3$); (2) at 1 week after SE (group W; $n = 6$) (the rats in this group did not exhibit spontaneous seizures during the first week) (i.e., they were in the latent period); (3) at 3–4 months after SE (group M; $n = 5$); for this latter group, we selected rats that had developed a progressive form of epilepsy and that exhibited daily seizures; and (4) also at 3–4 months after stimulation but including only rats that did not display the initial SE and later chronic seizures (group nS; $n = 5$). Age-matched controls (4–7 months of age) that were implanted but not stimulated except for field potential recordings, were also included (group C; $n = 6$). The EEG of all chronic epileptic rats was monitored for at least several weeks, to ensure that seizure progression had occurred. When they exhibited an increasing number of seizures (progressive form of epilepsy), they were disconnected (1–2 months after SE) and reconnected 1 week before killing, for quantification of their daily seizures. All non-SE rats were monitored until killing. The number of rats per group was chosen as a compromise between the requirements of sufficient statistical power and the wish to keep costs of the analysis as low as possible.

Tissue collection. After decapitation, the temporal lobe [which includes mainly the entorhinal cortex (EC) and parts of the perirhinal and posterior piriform cortex] was removed under RNase-free conditions by incision at the ventrocaudal part underneath the rhinal fissure until ~5 mm posterior to bregma, as well as the hippocampus. Both hippocampi were sliced into smaller parts (200–300 μm) and the CA3 region was cut out of the slices in 4°C saline solution under a dissection microscope. The whole cerebellum (CB) was also removed. All material was frozen on dry ice and stored at −80°C until use. Microarray analyses were performed on ipsilateral hippocampal CA3 and EC from each rat in each group (two chips per animal; total $n = 50$) and on cerebellum of a subset of these rats [control (C), $n = 5$; 1 week (W), $n = 5$; chronic (M), $n = 5$], yielding a data set of 65 microarrays.

RNA isolation and Affymetrix Genechip processing. After pottering the selected brain material in glass tubes, total RNA was isolated using TRIzol LS Reagent (Invitrogen, Groningen, The Netherlands) with Phase Lock Gel-Heavy tubes (Eppendorf, Hamburg, Germany), following the manufacturer's instructions. RNA was quantified using a nanodrop spectrophotometer (Ocean Optics, Dunedin, FL). Using the Superscript II cDNA kit (Invitrogen), total RNA was amplified and labeled according to the Affymetrix (Santa Clara, CA) Small Sample Labeling Protocol (vII). In short, in the first cycle, 100 ng of total RNA was reverse transcribed using an oligo-dT primer containing the T7 promotor (70°C; 6 min). The resultant double-strand cDNA was subjected to a first round of *in vitro* transcription (MEGAscript T7 kit; Ambion, Austin, TX). The cRNA product was purified using RNeasy Mini kit (Qiagen, Hilden, Germany). In the second cycle of amplification, cRNA was transcribed using an oligo-dT primer containing the T7 promotor (70°C; 6 min). Double-strand cDNA was subjected to *in vitro* transcription (EnzoBioarray High Yield RNA Transcript Labeling kit; Affymetrix) in the presence of biotinylated UTP and CTP. The cRNA product was purified using the sample cleanup module (RNeasy; Qiagen), quantified using a nanodrop spectrophotometer (Ocean Optics) and checked for the correct size distribution, using the Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA). Samples (15 μg) were hybridized to the Rat RAE 230A GeneChip. After 16 h of hybridization, the GeneChips were washed and stained on a fluidics station (Affymetrix) and scanned in a confocal scanner (Agilent Affymetrix GeneArray Scanner) according to the Affymetrix GeneChip Expression Analysis Manual. The RAE230A GeneChip oligonucleotide microarray (Affymetrix) comprises 681,012 distinct oligonucleotide features, which are combined into probe sets. In the original Affymetrix configuration, 15,866 probe sets were generated, which represent 4699 well annotated full-length genes, 10,467 expressed sequence tags (ESTs), and 700 non-ESTs (excluding full-lengths). The expression levels and present/absent calls were calculated for all 15,866 probe sets with the affy package from Bioconductor in the R program for statistical computing, using the MAS5 algorithm (Gautier et al., 2004). To incorporate newly described gene sequences and other knowledge described since the original Affymetrix annotation, we also incorporated the updated probe set definitions according to Dai et al. (2005). To compare with previous studies, genes that were eliminated by the Dai annotation are still presented within each process but they are marked as ($^{\text{Dai}}$). All probes set scaling was used to normalize overall intensities of the different arrays (MAS5.0).

Microarray data analysis. Data transformations ($\log_2$ conversion), selection, and statistical analyses were performed with either Excel (version 9.0; Microsoft, Redmond, WA) or custom-written software. All statistical tests were performed on the log transformed intensities, using a combination of Excel and SigmaStat (SPSS, version 2). The first step in the analysis for the study reported here was to determine which genes to consider "present" or "absent." The Affymetrix analysis provides a p value for the presence of each gene on each chip. A gene is considered present in an experimental group when $p < 0.05$ holds for all animals, or

Table 1. List of primers used for quantitative RT-PCR

Gene	Forward primer	Reverse primer
Ftl	5′-TCTCCTCAAGTTGCAGAAC-3′	5′-AGTGGCTTTCCAAGAAGT-3′
Kncd2	5′-AGACACTCTGCTGGGCAGTTC-3′	5′-TTGAGGATGTGGCGGAAGAT-3′
Gal	5′-CTGCTAGCCTGGCTCCTGTT-3′	5′-TGTTCAGGGTCCAGCCTCTCT-3′
Hmox1	5′-CAACCCCACCAAGTTCAAACA-3′	5′-AGGCGGTCTTAGCCTCCTCTG-3′
Viaat	5′-CCATCGGCATCATCGTGTT-3′	5′-CCAGTTCATCATGCAGTGGAA-3′
Gria2 (*Glur2*)	5′-AATCCTGGACTGTGAAAGGGATAA-3′	5′-CAATGATATAATGGTACCCTTTAACATGTT-3′
β-actin	5′-TGAAGATCAAGATCATTGCTCC-3′	5′-ACTCATCGTACTCCTGCTTGC-3′
CycA	5′-CCCACCGTGTTCTTCGACAT-3′	5′-AAACAGCTCGAAGCAGACGC-3′
Tbp1	5′-CACTGTTGGTCCAGTTTTCCTT-3′	5′-CACAGCCGTGGAGCAGAT-3′

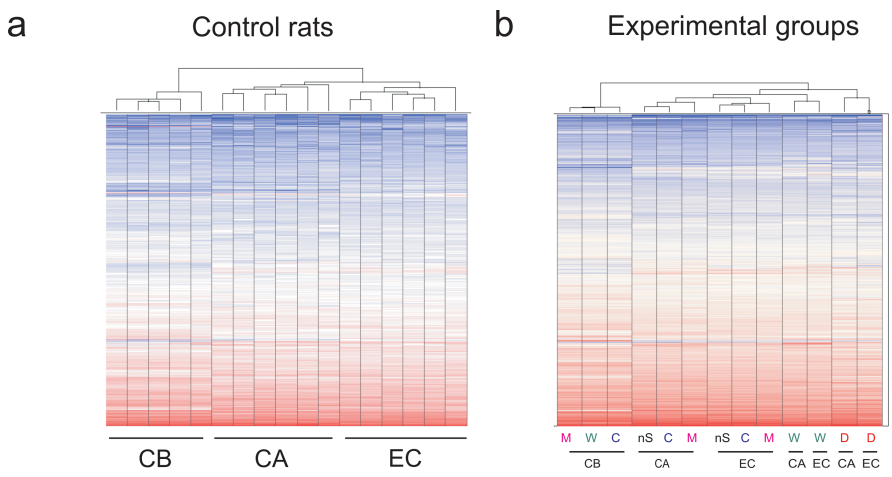

Figure 1. ***a***, Hierarchical clustering showed that the profiles of genetic expression of control rats cluster according to brain area. ***b***, Hierarchical clustering performed on the mean expression values of experimental animals showed that they cluster according to the stage of epileptogenesis, with the exception of the chronic phase. The analysis also showed that changes in gene expression were more similar in CA3 and EC in the early phases than in the chronic phase. The CB group completely separates from the other groups. Red depicts higher expression levels, and blue depicts lower; the deeper shade indicates a larger difference.

no more than one animal had a $p > 0.07$ or no more than two animals had $0.05 < p < 0.07$. When a gene was "present" in at least one region at one time point, we included the gene in the comparisons. In this way, we do not ignore "inducible" or strongly repressed genes. We also excluded outliers based on the expression intensity within a group (defined as a data point more than twice the SDs away from the mean); such values were extremely rare and did not affect the outcome of the analysis reported here. Comparisons were made between control and experimental samples, taken at the same time points, requiring at least $p < 0.05$ for a significant difference (ANOVA). Because array analysis deals with large numbers of multiple comparisons, it is necessary to take into consideration the false discovery rate (FDR); for an in-depth discussion, see Benjamini et al. (2001). We calculate FDR as the ratio "expected false positives" to "observed positives." There is no absolute level that FDR should obey; it depends on the subjective balance between missing genes that changed versus accepting genes that did not really change. In this study, we will consider changes in gene expression when $p < 0.05$ (but only if that gene belongs to a process that is significantly changed as a whole). For our data, this implies a FDR of <1% for genes that change similarly ($p < 0.05$) in the CA3 and EC region and a FDR of <15% for genes that change ($p < 0.05$) in only one of the two regions. If in a single region, the change of gene expression is significant at the $p < 0.01$ level, the FDR drops to a value <5% for our data. Fold changes in gene expression were calculated by dividing the mean intensity signal from a specific time or region by the mean intensity signal from the corresponding control samples. With the aim of obtaining a global impression of the "present" genes changed in this process, an unsupervised hierarchical clustering analysis was performed and scatter plots were constructed, using Spotfire Decision Site for Functional Genomics program. The dataset consisting of the significantly altered genes was entered into GenMAPP (Gene Map Annotator and Pathway Profiler), a computer program that is designed for viewing and analyzing genome-scale data on MAPPs representing biological pathways and any other grouping of genes. MAPPFinder is an accessory program that works with GenMAPP and uses the annotations from the GO Consortium to identify global biological trends in gene expression data. MAPPFinder relates microarray data meeting a user-defined criterion for a "significant" gene expression change to each term in the Gene Ontology hierarchy, and calculates the percentage of genes changed within each GO biological process, cellular component, and molecular function term. MAPPFinder then calculates the total number of genes changed within a "parent GO term" and all of its "children" (local MAPPs), and a statistical score (*z* score), giving a comprehensive picture of the gene expression changes associated with a particular GO term (Doniger et al., 2003).

Real-time quantitative PCR analysis. To validate some of the changes that were detected on the microarrays, real-time quantitative PCR analysis was performed using RNA obtained from the same tissue samples as used in the array experiment. Samples were run in triplicate. Because the amount of tissue was limited, RNA of the rats belonging to the same experimental group was pooled. The concentration and purity of RNA (isolated using the TRIzol LS Reagent) were determined spectrophotometrically at 260/280 nm. Five micrograms of total RNA were reverse-transcribed into cDNA using 125 pmol two-base anchored oligo-dT primers [5′-(dT)14-d(A/G/C)-d(A/G/C/T); Amersham Biosciences, Roosendaal, The Netherlands]. The reverse transcription was performed in 50 μl reactions. Five nanomole oligo-dT primers were annealed to 5 μg of total RNA in a total volume of 20 μl by incubation at 72°C for 10 min and cooled to 4°C. Reverse transcription was performed by the addition of 25 μl of RT-mix, containing the following: 50 mM Tris-HCl, 75 mM KCl, 3 mM $MgCl_2$, 20 mM DTT, 0.1 mM dNTPs (Amersham Biosciences), 30 U of RNase inhibitor (Roche Applied Science, Indianapolis, IN), and 400 U of Moloney murine leukemia virus reverse transcriptase (Invitrogen). This mixture was incubated at 37°C for 60 min, heated to 95°C for 10 min, and cooled to 4°C. Real-time monitoring of PCRs was performed using the LightCycler system (Roche Applied Science). PCR primers (Sigma-Genosys, Zwijndrecht, The Netherlands) were designed on the basis of the reported cDNA sequences and are listed in Table 1. For each PCR, a master mixture was prepared on ice, containing the following per sample: 1 μl of cDNA, 1 μl of FastStart Reaction Mix SYBR Green I (Roche Applied Science, Indianapolis, IN), 0.5 μl of 10 μM primers, and 1.6 μl of 25 mM $MgCl_2$. The final volume was adjusted with H_2O to 10 μl. After the reaction mixture was loaded into a glass capillary tube, the cycling conditions were performed as follows: initial denaturation at 95°C for 6 min, followed by 45 cycles of denaturation at 95°C for 15 s, annealing between 56 and 60°C for 5 s and extension at 72°C for 10–15 s. The temperature transition rate was set at 20°C/s. Fluorescent product was measured by a single acquisition mode at 72°C after each cycle. Separate calibration (standard) curves for the different primers and TBP1 (TATA box binding protein-like protein-1) (as reference) were constructed using serial dilutions of cDNA from rat hippocampus. The standard curve samples were included in each PCR. Standards were defined to contain an arbitrary starting concentration, because no primary calibrators exist. Hence, all calculated concentrations are relative to the concentration of the standard. For distinguishing specific from nonspecific products and primer dimers, a melting curve was obtained after amplification by holding the temperature at 65°C for

15 s followed by a gradual increase in temperature to 95°C at a rate of 0.1°C/s, with the signal acquisition mode set continuous. Quantification of data was performed using the LightCycler analysis software. Background fluorescence was removed by setting a noise band. The log-linear portion of the standard's amplification curve was selected. The crossing points were identified at the intersection of the best fit line through the log-linear region and the noise band. Using calibration curves, the concentration of a given product was calculated. The amount of each specific product was divided by the amount of a household protein (β-actin, cyclophilin, and TATA-Box-Protein-Like) for each sample and normalized to control values.

Tissue preparation and histology. To confirm whether changes in gene expression indicated changes in protein expression, we performed a number of immunocytochemical analyses. For this procedure, a separate group of 15 rats was used with $n = 3$ in each group. Rats were deeply anesthetized with pentobarbital (Nembutal, 60 mg/kg, i.p.). The animals were perfused through the ascending aorta with 300 ml of 0.37% Na_2S solution and 300 ml of 4% paraformaldehyde plus 0.2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were post-fixed *in situ* overnight at 4°C, dissected, and cryoprotected in 30% phosphate-buffered sucrose solution, pH 7.4. After overnight incubation at 4°C, the brain was frozen in isopentane (−25°C) and stored at −80°C until sectioning. The brain was cut on a sliding microtome, and 40 μm horizontal sections were collected in 0.1 M phosphate buffer for immunocytochemistry or histology. Horizontal sections of control and post-SE rats were washed in 0.05 M PBS, pH 7.4, and incubated for 30 min in 0.3% hydrogen peroxide in PBS to inactivate endogenous peroxidase. Sections were then washed (10 min, two times) in 0.05 M PBS, followed by washing (60 min, one time) in PBS plus 0.5% Triton X-100 plus 0.4% bovine serum albumin (BSA). Sections were incubated with CD11b/c (Ox-42; monoclonal mouse; BD Pharmingen, San Diego, CA; 1:100 as marker for microglia), Spp1 (Osteopontin; monoclonal mouse; 1:100; ITK Diagnostics, Uithoorn, The Netherlands), and Npy (polyclonal anti-rabbit; 1:3000; no. 7698 (7699/4); Swant, Bellinzona, Switzerland). After incubation in primary antibody for 24 h, sections were washed in PBS (10 min, three times) and incubated for 1.5 h in biotinylated sheep anti-rabbit or anti-mouse Ig (Amersham Biosciences; diluted 1:200 in PBS plus 0.1% Triton X-100 plus 0.4% BSA). Sections were washed in PBS (10 min, three times) and incubated for 1.5 h in streptavidin-horseradish peroxidase (Zymed Laboratories, San Francisco, CA), diluted 1:200 in PBS plus 0.1% Triton X-100 plus 0.4% BSA. After washing in 0.05 M Tris-HCl, pH 7.9, the sections were stained with 3,3′-diaminobenzidine tetrahydrochloride (30 mg; Sigma-Aldrich, Zwijndrecht, The Netherlands) and 750 μl of 1% hydrogen peroxide in a 100 ml solution of Tris-HCl. The staining reaction was monitored under the microscope and stopped by washing the sections in Tris-HCl. After mounting on gelatin-coated slides, the sections were air dried, dehydrated in alcohol and xylene, and coverslipped with Entellan (Merck, Darmstadt, Germany).

Western blot. The CA3 and EC (C, 5; D, 3; W, 3; M, 6) were homogenized in lysis buffer containing the following (per 20 ml): 200 μl of 1 M Tris, pH 8.0; 1 ml of 3 M NaCl; 2 ml of 10% NP-40; 4 ml of 50% glycerol; 800 μl of Na-orthovanadate (10 mg/ml); 200 μl of 0.5 M EDTA, pH 8.0; 400 μl of protease inhibitors; 200 μl of 0.5 M NaF; and 11.2 ml of H_2O. Fifty micrograms of total protein per lane, as determined using bicinchoninic acid method (Smith et al., 1985), were separated by SDS-PAGE, and transferred to nitrocellulose by electroblotting (Transblot SD; Bio-Rad, Hercules, CA). Blots were incubated with primary antibodies [goat anti-cox2 polyclonal antibody; catalog #100034; Cayman Chemical, Ann Arbor, MI; 1:500; mouse anti-Glur2 monoclonal antibody 6C4; 1:5000; gift from Dr. J. H. Morrison (Mount Sinai, New York, NY)] and the secondary antibody, anti-goat/mouse labeled with horseradish peroxidase (1:2500; DakoCytomation, High Wycombe, UK). Immunoreactivity was visualized with Lumi-Light Plus Western blotting substrate (Roche Diagnostics, Mannheim, Germany), and the blots were digitized using a Luminescent Image Analyzer (LAS-3000; Fuji Film, Tokyo, Japan). The optical density of each sample was measured using Scion Image (release β 3b; Scion Corporation, Frederick, MD) software. For each sample, the background was subtracted and optical density values were normalized with the amount of β-actin in each sample. Statistical analysis was performed using the Student's *t* test. Differences with $p < 0.05$ were considered significant.

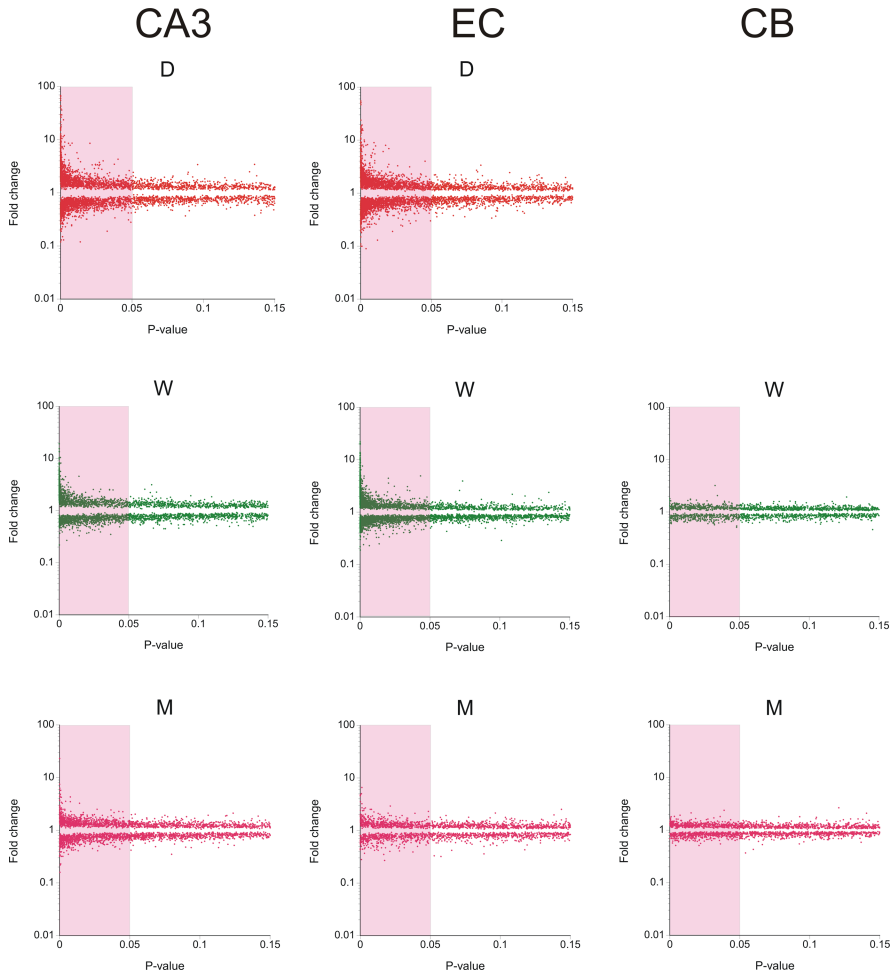

Figure 2. Scatter diagrams at the three time points after SE (acute, D; latent, W; chronic, M) in CA3, EC, and CB, showing fold change (with respect to control = 1) as a function of the *p* value of that change (up to 0.15). Significance ($p < 0.05$) is indicated with shaded area. The scatter is much larger during the acute and latent phases in both CA3 and EC than during chronic phase. Scatter is small in CB during both latent and chronic phases.

Results

Severity of the SE and subsequent epilepsy

We collected material of rats that were killed at three time points after SE. All groups (D, W, M) experienced an SE of ~10 h. The 1 d group (D) was killed 24 h after the electrical stimulation was stopped. The 1 week group (W) was killed at 1 week after SE in the latent period. The chronic epileptic rats (M) were killed 3–4 months after SE when they exhibited on average 8.3 ± 1.2 sei-

Table 2. The total numbers of upregulated and downregulated genes in EC, CA3, both in EC and CA3 and CB, at 1 d (D), 1 week (W), 3–4 months after SE (M), and in the non-SE group (nS), obtained using three different *p* values (0.05, 0.01, and 0.001)

	D	W	M	nS
EC				
$p < 0.05$	1241 up (13%)	1107 up (15%)	369 up (45%)	285 up (68%)
	1307 down (13%)	1133 down (15%)	313 down (53%)	326 down (64%)
$p < 0.01$	761 up (4%)	786 up (4%)	114 up (29%)	63 up (52%)
	740 down (4%)	564 down (6%)	95 down (35%)	85 down (39%)
$p < 0.001$	330 up (1%)	449 up (1%)	24 up (14%)	6 up (55%)
	289 down (1%)	194 down (2%)	18 down (18%)	13 down (25%)
CA3				
$p < 0.05$	1050 up (15%)	775 up (20%)	609 up (26%)	230 up (58%)
	1128 down (15%)	625 down (25%)	627 down (25%)	246 down (64%)
$p < 0.01$	610 up (5%)	464 up (7%)	262 up (12%)	53 up (59%)
	615 down (5%)	221 down (14%)	243 down (13%)	46 down (68%)
$p < 0.001$	285 up (1%)	244 up (1%)	77 up (4%)	6 up (52%)
	192 down (2%)	40 down (8%)	54 down (6%)	7 down (45%)
EC and CA3				
$p < 0.05$	726 up (1%)	559 up (2%)	87 up (10%)	16 up (54%)
	763 down (1%)	325 down (3%)	53 down (16%)	10 down (87%)
$p < 0.01$	391 up (0%)	356 up (0%)	25 up (1%)	0 up
	356 down (0%)	100 down (0%)	12 down (3%)	0 down
$p < 0.001$	162 up (0%)	186 up (0%)	15 up (1%)	0 up
	82 down (0%)	13 down (0%)	4 down (2%)	0 down
CB				
$p < 0.05$		254 up (61%)	334 up (46%)	
		232 down (67%)	359 down (43%)	
$p < 0.01$		53 up (55%)	99 up (31%)	
		50 down (62%)	83 down (37%)	
$p < 0.001$		8 up (39%)	16 up (19%)	
		5 down (62%)	10 down (31%)	

The FDR is indicated in parentheses.

zures (range, 5–12) per day. In addition, we analyzed the non-SE group (nS) of rats that experienced some seizures during the initial electrical stimulation but that did not develop SE or chronic seizures. During continuous EEG monitoring, we never detected a spontaneous seizure in the latter group.

Exploratory data analysis: general features

We organized our data according to the criteria proposed by Dai et al. (2005), using the most recent annotation of Unigene (January 2006; RN230A_RN_UG_6). After recalculation of the original Affymetrix probe set definitions, 10,179 Unigene-based probe sets were left (of the original 15,866 Affymetrix probe sets). Based on the criteria mentioned in Materials and Methods for "present" and "absent" calls, 6987 probe sets were finally accepted "present" in our experiment and used for additional analysis (69%).

Gene expression in CA3, temporal lobe, and cerebellum

In our experimental model of temporal lobe epilepsy (TLE), the epileptogenic area involves the hippocampus and the EC (Spencer and Spencer, 1994; Avoli et al., 2002; de Guzman et al., 2004), but most likely not in the CB (Bohnen et al., 1998), although some cerebellar abnormalities can be observed in human TLE patients (Niedermeyer, 2004; Hermann et al., 2005). We analyzed gene expression of the CB and not only of CA3 and EC, to incorporate in this study a region in the same animals that was most likely not involved in epileptogenesis. First, we performed a hierarchical clustering on the full probe sets of the three different brain areas of the control groups (C). This hierarchical clustering analysis shows that CB, CA3, and EC samples of the different rats form distinct clusters, which indicates that these regions have their own characteristic genetic profile. The analysis also suggests that the CA3 and EC region are more similar to each other than to the cerebellum (Fig. 1*a*).

Time course of epileptogenesis

The fact that we analyzed rats that were killed at three different time points after SE, enabled us to identify changes related to critical epileptic stages in the course of epileptogenesis: the acute phase (D), the latent phase (W), and the chronic epileptic phase (M). A chronic group that had received stimulation but that had not developed SE was also included (nS). We performed hierarchical clustering on the mean expression values (log-transformed) of the different groups: C, nS, D, W, and M rats of all three regions: CB, CA, EC (Fig. 1*b*). First of all, it can be seen that CB clusters separately for all available three time points (C, W, M). Figure 1*b* also illustrates the different behavior of the D and W groups on the one hand, and the C and M groups on the other. Whereas the former form clusters according to time sample (D: CA-D and EC-D; W: CA-W and EC-W), the latter (M and C) cluster according to brain area (CA: CA-M and CA-C; EC: EC-M and EC-C). This indicates that the epileptogenic process causes changes of gene expression that are common to the two areas directly involved, CA3 and EC, but in the chronic phase the specificities of the brain area prevail over the epileptogenic changes. Thus, the changes in gene expression were much more similar in CA3 and EC in the early phases than in the chronic phase (see also Fig. 3). In Figure 2, we show the "fold change" versus *p* value (up to 0.15) at the three specific time points after SE in the CA3, EC, and CB region. From these plots, it is easy to see that at 1 d many more genes had a more than twofold change than at later time points. We also see that most genes with on average more than twofold change were significantly different from controls at $p < 0.05$. In the cerebellum, the scatter was much smaller, indicating that no large changes in gene expression were evident in this region, although a considerable number of genes could still be significantly changed at 1 week or during the chronic phase.

The total numbers of upregulated and downregulated genes in the different areas are indicated in Table 2 and were obtained using three different *p* values (0.05, 0.01, and 0.001). They relate to the Dai defined probe set. A considerable fraction of the genes that showed changed expression was similarly regulated in CA3 and EC. The overlap was stage dependent and appeared to be primarily related to the biological processes that were affected in both regions (see below). In Figure 3, the average gene profile patterns are shown for the genes that had significantly ($p < 0.01$) changed expression in CA3, EC, and CB region at D (Fig. 3*a*), W (Fig. 3*b*), and M (Fig. 3*c*) in comparison with controls. A similar pattern was observed in CA3 and EC region but not in the CB region. The number of genes that changed expression after SE (both upregulated and downregulated) was initially larger in the EC (1501) than in the CA3 (1225). Remarkably, in the chronic

phase, however, the number of genes with altered expression was larger in the CA3 (505) than in the EC (209). The Venn diagrams (Fig. 3) represent, in addition to the number of genes that was significantly downregulated or upregulated in each region, also the number that changes similarly in any two or three regions. The largest overlap between CA3 and EC was found at 1 d after SE where 58% of the CA3 downregulated genes were also downregulated in EC and where 64% of the CA3 upregulated genes were also upregulated in EC. A similar calculation showed that, in the chronic phase (M), only 5% of the CA3 downregulated genes were also downregulated in EC, whereas ~10% of the CA3 upregulated genes were also upregulated in EC (the percentages with respect to the EC are indicated in Fig. 3 and Table 2). This difference in overlap percentages between the early phases of the epileptogenic process (D, W) and the chronic phase (M) is remarkable. The changes in gene expression in CB were much smaller. Genes that changed expression in the CB also hardly overlapped with the other two regions. At 1 week, there were 24 of 103 cerebellar genes changed (both upregulated and downregulated, 23%), that changed similarly with either CA3 and/or EC. During the chronic phase, 5 of 182 cerebellar genes had similarly changed as in either CA3 or EC. From this exploratory analysis (summarized in Figs. 1 and 3 and Table 2), we conclude that changes in gene expression were similar in regions CA3 and EC in the early phases, whereas in the chronic phase (M) gene expression changes appeared to be rather specific for each brain area.

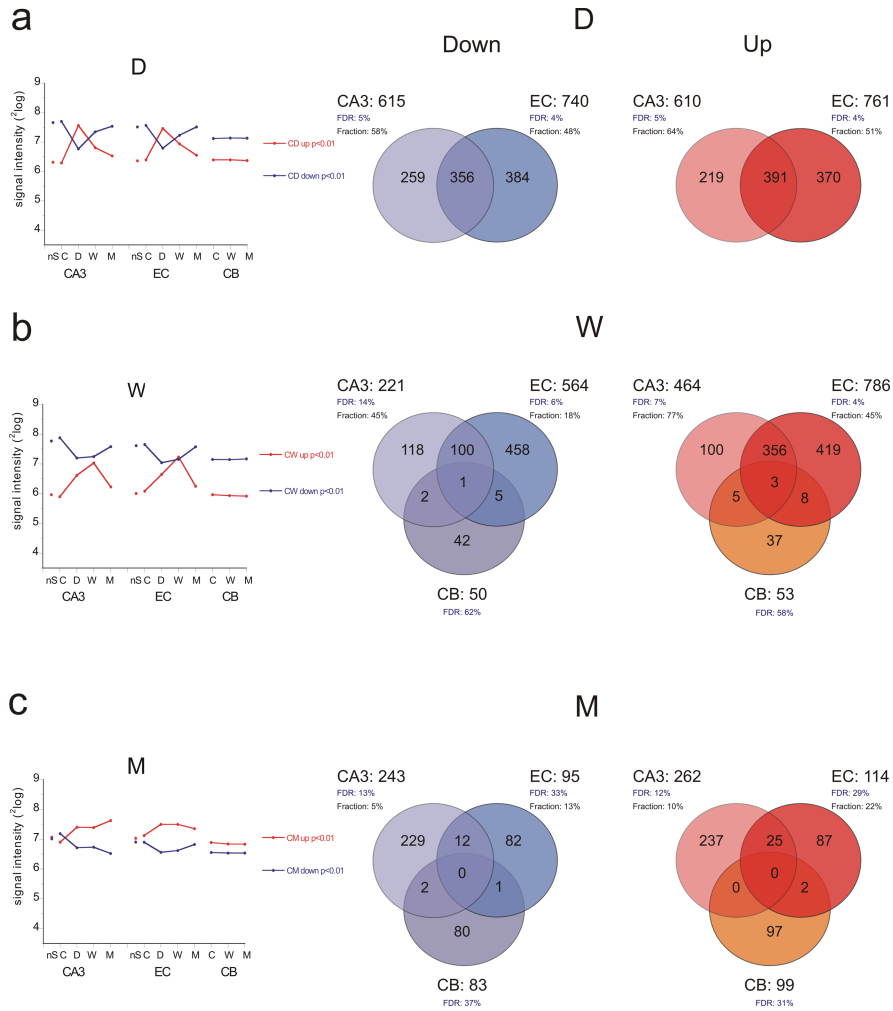

Figure 3. Number of genes changed (upregulated or downregulated) at $p < 0.01$ of rats killed at three post-SE stages, D (1 d), W (1 week), and M (3–4 months post-SE), for the CA3 and EC region and the CB. The panels show the average signal expression for the total set of changed genes and accompanying Venn diagrams in which the numbers of downregulated (blue) and upregulated (red) genes exclusively in each area and simultaneously in two or three areas are indicated. For example, in ***b***, 100 were upregulated only in CA3, and 419 only in EC; additionally, 356 were upregulated in both; thus, the fraction of genes that changed in both areas was 77% for CA3 (356 of 464), and for EC (356 of 786), the fraction was 45%. The FDR is indicated in blue (next to each circle).

Gene expression profiles

To go beyond the identification of series of individual genes that showed a changed expression associated with epileptogenesis, we determined groups of functionally related genes, based on the Gene Ontology system (GO). Three main classes of processes were distinguished according to the ordering of this ontology system: (1) *biological processes*, (2) *molecular functions*, and (3) *cellular components* (Doniger et al., 2003). In the following text, classified GO terms will be indicated in italics. In this analysis, we used GenMAPP and the associated MAPPFinder software. Because the hierarchical clustering shows that CA3 and EC cluster together at D and W, indicating a large similarity of the gene profile at these time points, we made profiles for the two regions combined. Because the hierarchical clustering separates CA3 from EC in the chronic phase (M), we also made profiles for CA3 and EC separately at this latter stage. Considering that we deal here with processes or functions that consist of combined sets of genes, we did not want to use a strict gene selection criterion and admitted any individual gene as long as its expression was changed at $p < 0.05$. The Z scores of the corresponding set of genes were calculated.

Biological processes during epileptogenesis

Acute phase (D)

We related the microarray data using D > C ($p < 0.05$) as criterion for a "significant" upregulation (or D < C for downregulation) for each term in the Gene Ontology hierarchy, and calculated the percentage of genes changed for each GO *biological process*, *cellular component*, and *molecular function* term. Table 3 shows the significant GO terms according to a Z score >1.96 (which corresponds to a p value of 0.05) according to the lists of ontology classes (*processes*, *functions*, and *components*); to limit the number of classes, we added the criterion that a class to be considered for additional analysis should comprise more than two genes. In Table 3, we present those that were *upregulated* at 1 d in both CA3 and EC in comparison with controls. A total of 726 probes met the criteria of upregulated in both regions. Of this collection of probes, 406 genes met the criterion and were linked to a GO term. A total of 763 probes met the criteria for downregulation in both regions. Of this collection of probes, 317 genes met the criterion and were linked to a GO term. In the second column, we show whether the process was also marked at one of the other time points (D, W, or M). The number of genes with a

Table 3. Significant GO terms according to a *Z* score > 1.96 (which corresponds to a *p* value of 0.05) according to the lists of ontology classes (*processes*, *functions*, and *components*) at 1 d (D), the latent period (W), or in the chronic phase (M) in CA3 and EC

GOID			No. genes up	% changed	*Z* score
Group D		**726 probes upregulated in both CA3 and EC (406 GO)**			
		Process			
6357		Regulation of transcription from RNA polymerase II promoter	3	60	4.73
6986		Response to unfolded protein	6	60	4.47
45944		Positive regulation of transcription from RNA polymerase II promoter	3	38	4.15
6950	**M**	Response to stress	3	50	4.08
6412		Protein biosynthesis	17	29	3.79
6413		Translational initiation	5	42	3.51
6355		Regulation of transcription, DNA-dependent	32	19	3.31
6953	**M**	Acute-phase response	4	50	3.15
6915	**W**	Apoptosis	8	24	3.02
6886	**M**	Intracellular protein transport	15	19	2.82
6260		DNA replication	6	33	2.75
6955	**WM**	Immune response	8	28	2.67
6350		Transcription	20	21	2.59
6457	**M**	Protein folding	15	26	2.57
6888	**M**	ER to Golgi transport	6	32	2.45
42981	**W**	Regulation of apoptosis	7	37	2.31
8152		Metabolism	5	5	2.20
30163		Protein catabolism	3	33	2.10
7517		Muscle development	3	21	2.05
		Function			
8243		Plasminogen activator activity	3	100	4.52
16563		Transcriptional activator activity	9	45	4.51
3743		Translation initiation factor activity	11	42	4.51
3676		Nucleic acid binding	18	14	3.93
17017		MAP kinase phosphatase activity	3	75	3.72
5544		Calcium-dependent phospholipid binding	4	50	3.15
3690	**W**	Double-stranded DNA binding	4	50	3.15
51082		Unfolded protein binding	11	28	2.89
4298		Threonine endopeptidase activity	4	40	2.57
3723		RNA binding	14	25	2.54
3677		DNA binding	20	16	2.48
31072	**M**	Heat shock protein binding	5	33	2.38
3700		Transcription factor activity	17	21	2.28
		Component			
5634		Nucleus	82	19	3.88
5622		Intracellular	12	15	2.78
5839		Proteasome core complex (sensu Eukaryota)	4	40	2.57
9986	**M**	Cell surface	3	33	2.34
5615	**W**	Extracellular space	10	23	2.19
Group W		**559 probes upregulated in both CA3 and EC (298 GO)**			
		Process			
6955	**DM**	Immune response	13	45	8.65
19886		Antigen processing, exogenous antigen via MHC class II	6	100	7.61
19884		Antigen presentation, exogenous antigen	5	100	6.94
9611		Response to wounding	4	67	4.82
6954		Inflammatory response	5	42	4.50
8283		Cell proliferation	4	33	4.25
7242	**M**	Intracellular signaling cascade	18	20	3.74
6508		Proteolysis	19	19	3.43
6935		Chemotaxis	3	43	3.32
7165		Signal transduction	15	12	3.04
6915	**D**	Apoptosis	4	12	2.56
6631		Fatty acid metabolism	5	22	2.53
42981	**D**	Regulation of apoptosis	4	21	2.51
6817	**M**	Phosphate transport	4	27	2.29
30154		Cell differentiation	4	9	2.07

(*Table continues*)

Table 3. *continued*

GOID			No. genes up	% changed	*Z* score
Group W		**559 probes upregulated in both CA3 and EC (298 GO)**			
		Function			
3690		MHC class II receptor activity	5	100	6.94
30106	**DM**	Double-stranded DNA binding	4	50	3.94
8233		MHC class I receptor activity	3	20	3.88
4871		Peptidase activity	6	14	3.57
16798		Signal transducer activity	5	31	3.15
4872	**M**	Hydrolase activity, acting on glycosyl bonds	4	13	2.95
16787		Receptor activity	23	10	2.92
16798		Signal transducer activity	5	31	3.15
4872	**M**	Hydrolase activity, acting on glycosyl bonds	4	13	2.95
16787		Receptor activity	23	10	2.92
5096		Hydrolase activity	17	25	2.54
4896		GTPase activator activity	3	100	2.29
		Component			
5764	**M**	Lysosome	16	67	10.23
16020		Membrane	55	12	3.02
5737	**M**	Cytoplasm	15	13	2.50
5615	**D**	Extracellular space	8	18	2.30
5886	**M**	Plasma membrane	4	11	2.02
	M	**609 probes upregulated in CA3 (307 GO)**			
		Process			
6817	**W**	Phosphate transport	6	40	3.98
6953	**D**	Acute-phase response	3	38	2.66
7242	**W**	Intracellular signaling cascade	11	15	2.55
6888	**D**	ER to Golgi transport	5	26	2.46
7264		Small GTPase mediated signal transduction	13	18	2.42
6886	**D**	Intracellular protein transport	13	16	2.36
6950	**D**	Response to stress	3	15	2.31
7155		Cell adhesion	7	18	2.24
6955	**DW**	Immune response	3	18	2.24
16567		Protein ubiquitination	11	17	2.10
		Function			
3690	**D**	Double-stranded DNA binding	3	38	2.66
31072	**D**	Heat shock protein binding	4	27	2.23
16874		Ligase activity	5	17	2.12
4842		Ubiquitin-protein ligase activity	12	17	2.08
Group M		**369 probes upregulated in EC (200 GO)**			
		Process			
15986		ATP synthesis coupled proton transport	4	24	2.93
7218		Neuropeptide signaling pathway	3	21	2.33
9058		Biosynthesis	4	33	2.32
5975		Carbohydrate metabolism	5	12	2.22
6865		Amino acid transport	3	23	2.18
6457	**D**	Protein folding	8	14	2.05
		Function			
5544	**D**	Calcium-dependent phospholipid binding	4	50	5.08
46933		Hydrogen-transporting ATP synthase activity, rotational mechanism	4	25	3.08
46961		Hydrogen-transporting ATPase activity, rotational mechanism	4	25	3.08
16798		Hydrolase activity, acting on glycosyl bonds	3	23	2.62
31072	**D**	Heat shock protein binding	3	20	2.18
		Component			
5764	**W**	Lysosome	6	25	4.21
9986	**D**	Cell surface	3	33	4.10
16469		Proton-transporting two-sector ATPase complex	4	25	2.93
5737	**W**	Cytoplasm	13	11	2.69
5886	**W**	Plasma membrane	3	9	2.19

(*Table continues*)

Table 3. *continued*

GOID			No. genes up	% changed	*Z* score
Group M		**369 probes upregulated in EC (200 GO)**			
		Process			
15986		ATP synthesis coupled proton transport	4	24	2.93
7218		Neuropeptide signaling pathway	3	21	2.33
9058		Biosynthesis	4	33	2.32
5975		Carbohydrate metabolism	5	12	2.22
6865		Amino acid transport	3	23	2.18
6457	**D**	Protein folding	8	14	2.05
		Function			
5544	**D**	Calcium-dependent phospholipid binding	4	50	5.08
46933		Hydrogen-transporting ATP synthase activity, rotational mechanism	4	25	3.08
46961		Hydrogen-transporting ATPase activity, rotational mechanism	4	25	3.08
16798		Hydrolase activity, acting on glycosyl bonds	3	23	2.62
31072	**D**	Heat shock protein binding	3	20	2.18
		Component			
5764	**W**	Lysosome	6	25	4.21
9986	**D**	Cell surface	3	33	4.10
16469		Proton-transporting two-sector ATPase complex	4	25	2.93
5737	**W**	Cytoplasm	13	11	2.69
5886	**W**	Plasma membrane	3	9	2.19
Group D		**763 probes downregulated in both CA3 and EC (317 GO)**			
		Process			
7268		Synaptic transmission	4	24	4.65
48167		Regulation of synaptic plasticity	3	50	3.92
7417		CNS development	3	60	2.92
6874		Calcium ion homeostasis	3	75	2.90
6816	**W**	Calcium ion transport	6	29	2.84
7399		Nervous system development	5	22	2.77
8654		Phospholipid biosynthesis	3	38	2.50
6811		Ion transport	15	18	2.20
7155		Cell adhesion	8	19	2.12
6468	**W**	Protein amino acid phosphorylation	21	15	1.98
		Function			
4685	**WM**	Calcium- and calmodulin-dependent protein kinase activity	5	100	6.71
4364		Glutathione transferase activity	3	60	3.73
30170		Pyridoxal phosphate binding	3	60	3.73
5516	**W**	Calmodulin binding	9	29	3.55
5262		Calcium channel activity	3	33	2.84
16740		Transferase activity	35	16	2.83
3779	**W**	Actin binding	10	23	2.75
5230		Extracellular ligand-gated ion channel activity	3	30	2.71
5216	**W**	Ion channel activity	8	22	2.70
3774		Motor activity	5	31	2.55
16301		Kinase activity	9	17	2.37
4890		$GABA_A$ receptor activity	3	33	2.33
19992		Diacylglycerol binding	4	29	2.32
5509		Calcium ion binding	26	15	2.23
4674	**W**	Protein serine/threonine kinase activity	16	16	2.22
5215		Transporter activity	14	17	2.19
5515	**W**	Protein binding	30	12	2.17
3924	**W**	GTPase activity	4	27	2.15
5234		Glutamate-gated ion channel activity	3	30	2.11
15297		Antiporter activity	3	43	2.11
16757		Transferase activity, transferring glycosyl groups	5	23	1.96
		Component			
5954		Calcium- and calmodulin-dependent protein kinase complex	3	100	5.19
5887		Integral to plasma membrane	4	21	2.80
5886	**W**	Plasma membrane	6	17	2.59
8021		Synaptic vesicle	3	23	2.52

(*Table continues*)

Table 3. *continued*

GOID			No. genes up	% changed	*Z* score
16020	**W**	Membrane	61	14	2.48
45211	**M**	Postsynaptic membrane	6	24	2.34
16021	**M**	Integral to membrane	71	12	2.19
Group W		**325 probes downregulated in both CA3 and EC (147 GO)**			
		Process			
46928		Regulation of neurotransmitter secretion	3	75	6.69
6695		Cholesterol biosynthesis	4	40	6.44
6836		Neurotransmitter transport	4	22	3.41
6629		Lipid metabolism	3	7	2.66
6468	**D**	Protein amino acid phosphorylation	13	9	2.64
6816	**D**	Calcium ion transport	3	14	2.11
		Function			
4685	**D**	Calcium- and calmodulin-dependent protein kinase activity	3	60	5.89
5516	**D**	Calmodulin binding	6	19	3.91
4674	**D**	Protein serine/threonine kinase activity	11	11	3.14
19992	**D**	Diacylglycerol binding	3	21	2.99
3924	**D**	GTPase activity	3	20	2.83
5216	**D**	Ion channel activity	3	8	2.34
20037		Heme binding	3	14	2.11
5515	**D**	Protein binding	13	5	2.10
3779	**D**	Actin binding	5	11	2.08
		Component			
8076		Voltage-gated potassium channel complex	4	20	3.27
5886	**D**	Plasma membrane	4	11	2.67
16020	**D**	Membrane	33	7	2.67
5856		Cytoskeleton	6	13	2.38
Group M		**627 probes downregulated in CA3 (279 GO)**			
		Function			
4685		Calcium- and calmodulin-dependent protein kinase activity	3	60	4.04
3899		DNA-directed RNA polymerase activity	4	44	3.78
3735		Structural constituent of ribosome	9	24	3.35
5198		Structural molecule activity	4	11	2.44
		Component			
5840		Ribosome	7	23	3.04
45211	**W**	Postsynaptic membrane	5	20	1.98
Group M		**313 probes downregulated in EC (110 GO)**			
		Process			
6814		Sodium ion transport	3	15	2.82
		Function			
5516	**DW**	Calmodulin binding	5	16	3.87
		Component			
45211	**W**	Postsynaptic membrane	3	12	2.34

The total number of genes upregulated or downregulated at a specific stage is indicated at the top; the number of GO-related terms is in parentheses. In the first column, the GO identity is shown; the second column indicates whether a process also occurred during another epileptogenic stage. The fourth column shows the number of genes that are upregulated or downregulated ("local term"); the fifth column shows the percentage of the genes in the specific GO term that is upregulated or downregulated; the sixth column indicates the *Z* score.

changed expression is indicated in the fourth column. The percentage of changed genes over the total number of genes that belong to a given GO term, is presented in the fifth column. We should remember that the same gene may belong to different GO processes. The main characteristic of the acute phase was that a large series of identified *biological processes* appeared to be related to *transcriptional and translational processes* and were involved in the short-term *stress response* to the SE. These changes were associated with the considerable amount of cell death and local tissue inflammatory reaction occurring immediately after the SE. At this stage, genes related to *apoptosis* and *immune response* were also enhanced. In contrast, processes associated with *synaptic transmission*, *synaptic plasticity*, *calcium ion homeostasis*, *ion transport and cell adhesion* were repressed at the same acute phase, which likely results in a disruption of normal neuronal and glial activity. Many upregulated processes are localized to "*cellular component—nucleus*" (*Z* score, 3.88), whereas downregulated processes belong mainly to the "*cellular components—(plasma) membrane*" or *synaptic vesicle*.

Latent period (W)

Several of the processes that were activated at 1 d were also activated at 1 week after SE. GO terms that were commonly upregulated at both time points included the following: *immune re-*

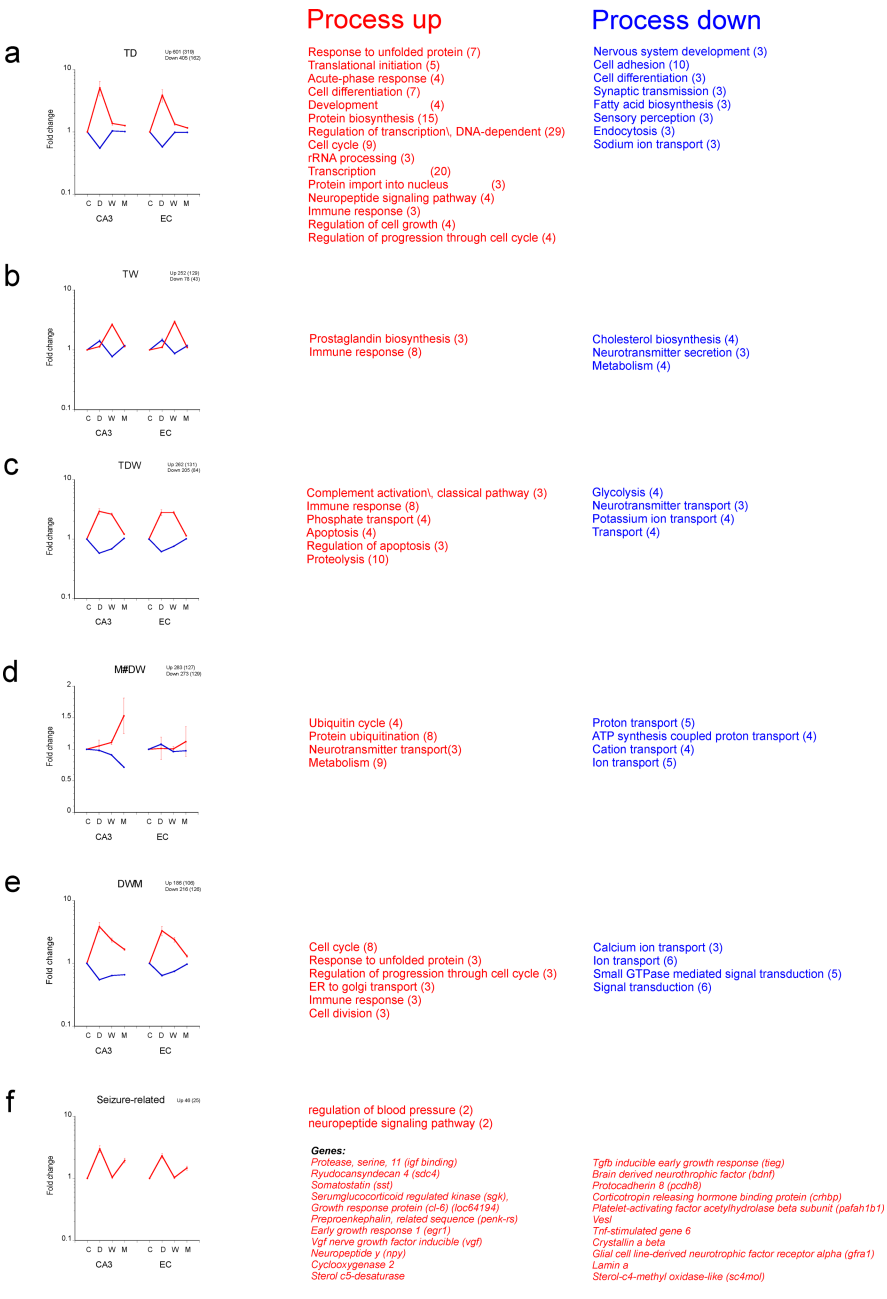

Figure 4. Different waves of gene expression in CA3 and EC. Average fold change (±SEM) of those genes that have significantly changed in CA3 ($p < 0.01$, with respect to control expression) transiently at 1 d only (TD) (***a***); at 1 week only (TW) (***b***); at both 1 d and 1 week (TDW) (***c***); at the chronic phase only (M#DW) (***d***); during all three time points (DWM) (***e***); and "seizure-related" (not changed during the latent period) (***f***). Except for the M#DW pattern, the average expression pattern is similar in EC. The number of genes involved in each pattern is indicated above each graph (GO-related genes in between brackets). Because there are only 25 genes identified with a seizure-related pattern, a list of these genes is presented. The processes that are significant with each specific pattern are mentioned next to each graph (number of genes involved between brackets).

sponse, *apoptosis and double strand DNA binding*. In the list of GO classes of upregulated genes ordered according to *Z* scores (Table 3), it is worth noting that at day 1 post-SE (D) the most prominent places are occupied by processes involved in *transcription and translation*, and *response to unfolded protein*, whereas at 1 week (W) the *immune and inflammatory response and antigen processing*, *the response to wounding*, and processes associated with the *cellular component*—"*lysosomes*" become prominent, and occupy the top positions of the list of *Z* scores. A total of 559 probes met the criteria of upregulated in both regions. Of this collection of probes, 298 genes met the criterion and were linked to a GO term. A total of 325 probes met the criteria of downregulated in both regions. Of this collection of probes, 147 genes met the criterion and were linked to a GO term. Some downregulated processes at 1 week (W) overlapped with those similarly changed at 1 d (D), namely *protein phosphorylation*, *calcium ion transport*, *calmodulin binding*, *protein and actin binding*, *protein serine/threonine kinase activity*, *GTPase activity*, *ion channel activity and plasma membrane* belong to this category. However, at this stage, processes involved in *regulation of neurotransmitter secretion* and *cholesterol biosynthesis* appeared at top places of downregulated processes according to *Z* scores. With respect to *cellular components*, there was a shift from the *nucleus* (at 1 d) to the *lysosome*, *cytoplasm (plasma) membrane*, and *extracellular space*. In particular, the *cytoskeleton*, *membrane and voltage gated potassium channel complex* were downregulated *cellular components*.

Chronic phase (M)

We found a much smaller number of GO processes that changed significantly in both regions in the chronic epileptic phase when rats have ~5–10 seizures per day (3–4 months after SE). The overlap between genes that change in CA3 and EC was remarkably large especially in the acute and latent phases and was much higher than previously reported when hippocampus and temporal lobe were compared (Lukasiuk et al., 2003). Differences between regions, however, became evident in the chronic phase when especially *immune* and *acute phase* responses were significantly activated in CA3 but not in EC, whereas in the latter, and not in the former, *neuropeptide signaling pathway* and *amino-acid transport* were upregulated processes. Because the hierarchical clustering showed that CA3 and EC did not cluster together in this phase (Fig. 1*b*), we analyzed CA3 and EC separately. There were several region-specific changes at M (Table 3). In the CA3, the main changed processes were related to *stress* and *immune response*, whereas in the EC these were related to *proton and amino acid transport*, *biosynthesis and neuropeptide signaling*. *Heat shock protein binding* was the only GO term that was significantly upregulated in both CA3 and EC. Several GO terms that appeared significantly upregulated in the chronic phase were also apparent in the acute but not in the latent phase of epileptogenesis; in CA3 these include *acute phase response*, *intracellular protein transport*, *response to stress and heat shock protein binding*; in EC: *protein folding*, *calcium dependent phospholipids binding*, *double stranded DNA binding*, *heat shock protein binding*. These processes belong mainly to the *cellular components: lysosome*, *cell surface*, *cytoplasm*

Table 4. List of gene names and accompanying GO term (*biological process*)

Gene symbol	Gene name	Biological process
A2m	α-2-Macroglobulin	Intracellular protein transport; acute-phase response; inflammatory response; response to wounding; negative regulation of calcium-mediated signaling
Abat	4-Aminobutyrate aminotransferase	Synaptic transmission; GABA metabolism; neurotransmitter catabolism
Ada	Adenosine deaminase	Purine nucleotide metabolism; immune response; nucleotide metabolism; purine ribonucleoside monophosphate biosynthesis; antimicrobial humoral response (sensu Vertebrata); regulation of circadian sleep/wake cycle, sleep; adenosine metabolism
Anxa1	Annexin A1	Lipid metabolism; cell motility; inflammatory response; cell cycle; signal transduction; cell surface receptor linked signal transduction; insulin secretion; regulation of cell proliferation; arachidonic acid secretion
Ap3m2	Adaptor-related protein complex 3, μ2 subunit	Transport; intracellular protein transport; neurotransmitter secretion
Apba2	Amyloid β (A4) precursor protein-binding, family A, member 2	Transport; intracellular protein transport; intracellular signaling cascade; neurogenesis; protein transport; synaptic vesicle exocytosis
Apoe	Apolipoprotein E	Response to reactive oxygen species; lipid transport; induction of apoptosis; cytoskeleton organization and biogenesis; synaptic transmission, cholinergic; synaptogenesis; learning and/or memory; circulation; regulation of axon extension; neurite regeneration; lipoprotein metabolism; cholesterol homeostasis; intracellular transport; regulation of neuronal synaptic plasticity
Atf3	Activating transcription factor 3	Gluconeogenesis; regulation of transcription, DNA-dependent
Atp1a2	ATPase, Na^+/K^+ transporting, α2 polypeptide	Neurotransmitter uptake; cation transport; potassium ion transport; sodium ion transport; metabolism; monovalent inorganic cation transport; ATP hydrolysis coupled proton transport; sperm motility; hydrogen ion homeostasis
B2m	β-2 Microglobulin	Immune response; cellular defense response; antigen presentation, endogenous antigen; antigen processing, endogenous antigen via MHC class I
Bdnf	Brain-derived neurotrophic factor	Antiapoptosis; neurogenesis; feeding behavior; dendrite morphogenesis; regulation of metabolism; regulation of long-term neuronal synaptic plasticity; regulation of short-term neuronal synaptic plasticity
Bhlhb2	Basic helix-loop-helix domain containing, class B2	Regulation of transcription, DNA-dependent; neurogenesis; entrainment of circadian clock; negative regulation of neuronal synaptic plasticity
C1qa_predicted	Complement component 1, q subcomponent, α polypeptide (predicted)	Phosphate transport; complement activation, classical pathway; cell–cell signaling
C1qb	Complement component 1, q subcomponent, β polypeptide	Phosphate transport; immune response; complement activation, classical pathway
C1qg_predicted	Complement component 1, q subcomponent, γ polypeptide (predicted)	Phosphate transport; immune response; complement activation, classical pathway
C3	Complement component 3	Positive regulation of type IIa hypersensitivity; inflammatory response; complement activation; complement activation, alternative pathway; complement activation, classical pathway; signal transduction; G-protein-coupled receptor protein signaling pathway; positive regulation of phagocytosis
C3ar1	Complement component 3a receptor 1	Chemotaxis; complement activation; G-protein-coupled receptor protein signaling pathway
C4a	Complement component 4a	Inflammatory response; complement activation; complement activation, classical pathway; humoral defense mechanism (sensu Vertebrata); positive regulation of smooth muscle contraction
C8b	Complement component 8, β polypeptide	Complement activation, alternative pathway; complement activation, classical pathway; cytolysis
Cacna1a	Calcium channel, voltage-dependent, P/Q type, α1A subunit	Calcium ion transport; elevation of cytoplasmic calcium ion concentration; synaptic transmission; neurogenesis
Cacna1c	Calcium channel, voltage-dependent, L type, α1C subunit	Calcium ion transport; regulation of heart contraction rate
Cacna2d1	Calcium channel, voltage-dependent, α2/δ subunit 1	Ion transport
Cacnb3	Calcium channel, voltage-dependent, β3 subunit	Calcium ion transport
Cacng3	Calcium channel, voltage-dependent, γ subunit 3	Calcium ion transport
Cacng4	Calcium channel, voltage-dependent, γ subunit 4	Calcium ion transport
Cacng7	Calcium channel, voltage-dependent, γ subunit 7	Calcium ion transport
Calb1	Calbindin 1	Learning and/or memory; locomotory behavior; vitamin D metabolism; regulation of synaptic plasticity
Calca	Calcitonin/calcitonin-related polypeptide, α	Skeletal development; inflammatory response; G-protein signaling, coupled to cAMP nucleotide second messenger; adenylate cyclase activation; phospholipase C activation; elevation of cytoplasmic calcium ion concentration; neuropeptide signaling pathway; cell–cell signaling; feeding behavior; regulation of blood pressure; negative regulation of smooth muscle contraction
Camk2a	Calcium/calmodulin-dependent protein kinase IIα subunit	G_1/S transition of mitotic cell cycle; protein amino acid phosphorylation; protein amino acid phosphorylation; calcium ion transport; autophosphorylation; regulation of neurotransmitter secretion; plasticity

(*Table continues*)

Table 4. *continued*

Gene symbol	Gene name	Biological process
Camk2b	Calcium/calmodulin-dependent protein kinase IIβ subunit	G_1/S transition of mitotic cell cycle; protein amino acid phosphorylation; calcium ion transport; signal transduction; autophosphorylation; plasticity
Camk2g	Calcium/calmodulin-dependent protein kinase IIγ	G_1/S transition of mitotic cell cycle; response to hypoxia; calcium ion transport response to oxidative stress; autophosphorylation; regulation of long-term neuronal synaptic plasticity
Casp1	Caspase 1	Response to hypoxia; proteolysis and peptidolysis; apoptosis; induction of apoptosis; signal transduction; protein processing; regulation of apoptosis; positive regulation of I-κB kinase/NF-κB cascade
Cck	Cholecystokinin	Apoptosis; signal transduction
Ccl2	Chemokine (C-C motif) ligand 2	Protein amino acid phosphorylation; calcium ion homeostasis; antiapoptosis; chemotaxis; inflammatory response; immune response; humoral immune response; cell adhesion; signal transduction; G-protein signaling, coupled to cyclic nucleotide second messenger; JAK-STAT cascade; cell–cell signaling; response to pathogenic bacteria; organogenesis; viral genome replication
Ccl3	Chemokine (C-C motif) ligand 3	Calcium ion homeostasis; exocytosis; cell motility; chemotaxis; inflammatory response; immune response; cytoskeleton organization and biogenesis; signal transduction; G-protein-coupled receptor protein signaling pathway; cell–cell signaling; antimicrobial humoral response (sensu Vertebrata); neutrophil chemotaxis; immune cell chemotaxis; regulation of viral genome replication
Cd14	CD14 antigen	Inflammatory response; immune response
Cd59	CD59 antigen	Immune response; cell surface receptor linked signal transduction; blood coagulation; regulation of complement activation
Cd74	CD74 antigen (invariant polpypeptide of major histocompatibility class II antigen-associated)	Protein folding; immune response
Cd81	CD81 antigen	Activation of MAPK activity; phosphatidylinositol biosynthesis; protein localization; positive regulation of cell proliferation; response to wounding; phosphoinositide metabolism; positive regulation of B cell proliferation; regulation of growth; regulation of cell proliferation; positive regulation of 1-phosphatidylinositol 4-kinase activity; viral entry into host cell; virion attachment, binding of host cell surface receptor; positive regulation of peptidyl-tyrosine phosphorylation
Cdk5	Cyclin-dependent kinase 5	Cytokinesis; protein amino acid phosphorylation; cell cycle; cell-matrix adhesion; axonogenesis; central nervous system development; cell migration; regulation of cell migration; regulation of synaptic plasticity; synaptic vesicle endocytosis
Cfh	Complement component factor H	Complement activation, alternative pathway; regulation of complement activation
Cltb	Clathrin, light polypeptide (Lcb)	Neurotransmitter transport; intracellular protein transport; neurotransmitter secretion; synaptic vesicle coating
Crcp	Calcitonin gene-related peptide-receptor component protein	Response to stress; inflammatory response
Crh	Corticotropin releasing hormone	Response to stress; inflammatory response; immune response; signal transduction; synaptic transmission; pregnancy; parturition; learning and/or memory; long-term memory; induction of apoptosis by hormones
Crry	Complement receptor related protein	Negative regulation of complement activation
Cryab	Crystallin, αB	Eye development (sensu Mammalia); protein folding; muscle contraction; response to stress; sensory organ development; muscle development; visual perception; response to heat
Cx3cl1	Chemokine (C-X3-C motif) ligand 1	Chemotaxis; immune response; cell adhesion; signal transduction; cytokine and chemokine mediated signaling pathway; immune cell chemotaxis; positive regulation of inflammatory response; leukocyte adhesive activation; positive regulation of calcium-independent cell–cell adhesion
Cxcl1	Chemokine (C-X-C motif) ligand 1	Regulation of progression through cell cycle; inflammatory response; immune response; neutrophil chemotaxis; cellular physiological process
Cxcl10	Chemokine (C-X-C motif) ligand 10	Cell motility; chemotaxis; inflammatory response; immune response; signal transduction; muscle development; positive regulation of cell proliferation; protein secretion; positive regulation of cell migration
Dbi	Diazepam binding inhibitor	Acyl-CoA metabolism; steroid biosynthesis; transport
Dnaja1	DnaJ-like protein	Protein folding; response to unfolded protein; DNA damage response, perception of DNA damage
Dnajb9	DnaJ (Hsp40) homolog, subfamily B, member 9	Protein folding; antiapoptosis; response to stress
Dnajc3	Protein kinase inhibitor p58	Protein folding; negative regulation of protein kinase activity
Dnajc5	Cysteine string protein	Protein folding; synaptic transmission
Ece1	Endothelin converting enzyme 1	Proteolysis and peptidolysis; cell–cell signaling; protein processing
Egr1	Early growth response 1	Regulation of transcription, DNA-dependent; learning and/or memory; T cell differentiation; positive regulation of transcription from RNA polymerase II promoter

(*Table continues*)

Table 4. *continued*

Gene symbol	Gene name	Biological process
F10	Coagulation factor X	Proteolysis and peptidolysis; blood coagulation
F2	Coagulation factor 2	Proteolysis and peptidolysis; acute-phase response; blood coagulation; wound healing
F2r	Coagulation factor II (thrombin) receptor	Regulation of progression through cell cycle; apoptosis; caspase activation; cell motility; signal transduction; G-protein-coupled receptor protein signaling pathway; tyrosine phosphorylation of STAT protein; STAT protein nuclear translocation; blood coagulation; response to wounding; morphogenesis; positive regulation of I-κB kinase/NF-κB cascade
F3	Coagulation factor 3	Immune response; blood coagulation
F5	Coagulation factor 5	Cell adhesion; blood coagulation
Faf1	Fas-associated factor 1	Apoptosis; induction of apoptosis; negative regulation of NF-κB-nucleus import
Fcgrt	Fc receptor, IgG, α chain transporter	Immune response; humoral immune response; pregnancy; antigen presentation
Fos	FBJ murine osteosarcoma viral oncogene homolog	NA
Ftl1	Ferritin light chain 1	Iron ion transport; iron ion homeostasis
Gabarap	GABA receptor associated protein	Microtubule cytoskeleton organization and biogenesis; protein targeting; transport; synaptic transmission; protein transport
Gabbr1	$GABA_B$ receptor 1	Osteoblast differentiation; G-protein-coupled receptor protein signaling pathway; negative regulation of adenylate cyclase activity; GABA signaling pathway; synaptic transmission
Gabra4	$GABA_A$ receptor, subunit α4	Transport; ion transport; chloride transport; GABA signaling pathway; synaptic transmission
Gabra5	$GABA_A$ receptor, α5	Behavioral fear response; transport; ion transport; chloride transport; signal transduction; GABA signaling pathway; synaptic transmission; brain development; associative learning
Gabra6	$GABA_A$ receptor, α6	Ion transport; chloride transport; signal transduction; GABA signaling pathway; synaptic transmission
Gabrb1	GABA receptor, subunit β1	Ion transport; chloride transport; chloride transport; signal transduction; GABA signaling pathway; synaptic transmission
Gabrd	$GABA_A$ receptor, δ	Ion transport; chloride transport; signal transduction; GABA signaling pathway; synaptic transmission
Gabrg1	$GABA_A$ receptor, γ1	Ion transport; chloride transport; GABA signaling pathway; synaptic transmission
Gabrg3	$GABA_A$ receptor, subunit γ3	Ion transport; chloride transport; GABA signaling pathway; synaptic transmission; response to drug
Gad1	Glutamate decarboxylase 1	Amino acid metabolism; glutamate decarboxylation to succinate; synaptic transmission; protein-pyridoxal-5-phosphate linkage; neurotransmitter biosynthesis
Gak	Cyclin G associated kinase	Regulation of progression through cell cycle; protein folding; protein amino acid phosphorylation; cell cycle
Gal	Galanin	Smooth muscle contraction; inflammatory response; neuropeptide signaling pathway; neurogenesis; negative regulation of cell proliferation; insulin secretion; growth hormone secretion; positive regulation of apoptosis; negative regulation of lymphocyte proliferation; regulation of immune response
Gclc	Glutamate-cysteine ligase, catalytic subunit	Cysteine metabolism; glutamate metabolism; glutathione biosynthesis; circulation
Gfra1	Glial cell line-derived neurotrophic factor family receptor α1	Cell surface receptor linked signal transduction; transmembrane receptor protein tyrosine kinase signaling pathway; neurogenesis; morphogenesis
Gpx1	Glutathione peroxidase 1	Response to reactive oxygen species; response to oxidative stress; induction of apoptosis by oxidative stress
Gria2	Glutamate receptor, ionotropic, 2	Transport; ion transport; potassium ion transport; signal transduction; synaptic transmission
Gria3	Glutamate receptor, ionotropic, AMPA3 (α3)	Lipid metabolism; transport; ion transport; potassium ion transport; glutamate signaling pathway; synaptic transmission
Gria4	Glutamate receptor, ionotropic, 4	Ion transport; potassium ion transport; glutamate signaling pathway; synaptic transmission
Grik2	Glutamate receptor, ionotropic, kainate 2	Ion transport; potassium ion transport; glutamate signaling pathway; synaptic transmission
Grik4	Glutamate receptor, ionotropic, kainate 4	Ion transport; potassium ion transport; glutamate signaling pathway; synaptic transmission
Grin1	Glutamate receptor, ionotropic, NMDA 1	Ion transport; cation transport; potassium ion transport; calcium ion homeostasis; glutamate signaling pathway; synaptic transmission; learning and/or memory; response to ethanol; regulation of synaptic plasticity
Grin2a	Glutamate receptor, ionotropic, NMDA 2A	Ion transport; glutamate signaling pathway; synaptic transmission; learning and/or memory
Grin2c	Glutamate receptor, ionotropic, NMDA 2C	Ion transport; glutamate signaling pathway; synaptic transmission
Grina	Glutamate receptor, ionotropic, NMDA-associated protein 1 (glutamate binding)	NA

(*Table continues*)

Table 4. *continued*

Gene symbol	Gene name	Biological process
Grm1	Glutamate receptor, metabotropic 1	G-protein-coupled receptor protein signaling pathway; synaptic transmission; perception of pain; calcium-mediated signaling
Grm3	Glutamate receptor, metabotropic 3	G-protein-coupled receptor protein signaling pathway; negative regulation of adenylate cyclase activity; synaptic transmission; regulation of synaptic transmission
Grm4	Glutamate receptor, metabotropic 4	G-protein-coupled receptor protein signaling pathway; negative regulation of adenylate cyclase activity; metabotropic glutamate receptor signaling pathway; synaptic transmission
Grm8	Glutamate receptor, metabotropic 8	G-protein-coupled receptor protein signaling pathway; synaptic transmission; perception of smell
Hcn1	Hyperpolarization-activated cyclic nucleotide-gated potassium channel 1	Potassium ion transport; sodium ion transport
Hcn2	Hyperpolarization activated cyclic nucleotide-gated potassium channel 2	Potassium ion transport; sodium ion transport
Hmox1	Heme oxygenase (decycling) 1	Phospholipid metabolism; heme oxidation; intracellular signaling cascade; small GTPase mediated signal transduction; DNA damage response, signal transduction resulting in induction of apoptosis; positive regulation of I-κB kinase/NF-κB cascade; response to stimulus
Homer1	Homer homolog 1 (*Drosophila*)	Metabotropic glutamate receptor signaling pathway
Hspa1a	Heat shock 70 kDa protein 1A	Telomere maintenance; inhibition of caspase activation; DNA repair; mRNA catabolism; protein folding; antiapoptosis; defense response; response to unfolded protein; response to heat
Hspb1	Heat shock 27 kDa protein 1	Regulation of translational initiation; protein folding; response to unfolded protein
Il18	Interleukin 18	Angiogenesis; immune response; cell–cell signaling; induction of apoptosis via death domain receptors; regulation of cell adhesion; sleep; chemokine biosynthesis; T helper 1 type immune response; T helper 2 type immune response; interleukin-2 biosynthesis; interferon-γ biosynthesis; positive regulation of activated T cell proliferation; interleukin-13 biosynthesis; granulocyte macrophage colony-stimulating factor biosynthesis; regulation of circadian sleep/wake cycle, non-REM sleep
Il1a	Interleukin 1α	Regulation of progression through cell cycle; apoptosis; antiapoptosis; chemotaxis; inflammatory response; immune response; cell–cell signaling; cell proliferation; cytokine and chemokine mediated signaling pathway
Il1b	Interleukin 1β	Regulation of progression through cell cycle; angiogenesis; apoptosis; inflammatory response; immune response; signal transduction; cell–cell signaling; cell proliferation; cytokine and chemokine mediated signaling pathway; antimicrobial humoral response (sensu Vertebrata); neutrophil chemotaxis; positive regulation of chemokine biosynthesis; positive regulation of interleukin-6 biosynthesis
Il2rg	Interleukin 2 receptor, γ (severe combined immunodeficiency)	Protein complex assembly; immune response; signal transduction; cell surface receptor linked signal transduction; cell proliferation
Il6	Interleukin 6	Programmed cell death, neutrophils; acute-phase response; immune response; negative regulation of chemokine biosynthesis; regulation of circadian sleep/wake cycle, non-REM sleep
Il6r	Interleukin 6 receptor	Immune response; cell surface receptor linked signal transduction; development; cell proliferation
Junb	Jun-B oncogene	Regulation of progression through cell cycle; regulation of transcription, DNA-dependent; positive regulation of transcription from RNA polymerase II promoter, mitotic; positive cellular physiological process
Kcna4	Potassium voltage-gated channel, shaker-related subfamily, member 4	Potassium ion transport
Kcna5	Potassium voltage-gated channel, shaker-related subfamily, member 5	Potassium ion transport
Kcnb1	Potassium voltage-gated channel, Shab-related subfamily, member 1	Potassium ion transport
Kcnc1	Potassium voltage-gated channel, Shaw-related subfamily, member 1	Potassium ion transport
Kcnc2	Potassium voltage-gated channel, Shaw-related subfamily, member 2	Potassium ion transport
Kcnc3	Potassium voltage-gated channel, Shaw-related subfamily, member 3	Potassium ion transport
Kcnd2	Potassium voltage-gated channel, Shal-related family, member 2	Regulation of action potential; transport; ion transport; cation transport; potassium ion transport; synaptic transmission
Kcnd3	Potassium voltage-gated channel, Shal-related family, member 3	Potassium ion transport
Kcnj11	Potassium inwardly rectifying channel, subfamily J, member 11	Potassium ion transport

(*Table continues*)

Table 4. *continued*

Gene symbol	Gene name	Biological process
Kcnj12	Potassium inwardly rectifying channel, subfamily J, member 12	Potassium ion transport; muscle contraction; regulation of heart contraction rate
Kcnj3	Potassium inwardly rectifying channel, subfamily J, member 3	Potassium ion transport
Kcnj8	Potassium inwardly rectifying channel, subfamily J, member 8	Potassium ion transport
Kcnk15	Potassium channel, subfamily K, member 15	Potassium ion transport
Lmna	Lamin A	Spermatogenesis; muscle development
Mgst1	Microsomal glutathione *S*-transferase 1	Glutathione metabolism
Nfix	Nuclear factor I/X	DNA replication; transcription; regulation of transcription, DNA-dependent; transcription from RNA polymerase II promoter; regulation of transcription
Nfkb1	Nuclear factor of κ light chain gene enhancer in B-cells 1, p105	Regulation of transcription, DNA-dependent; transcription from RNA polymerase II promoter; apoptosis; antiapoptosis; inflammatory response; response to oxidative stress; signal transduction; response to pathogenic bacteria; antibacterial humoral response (sensu Vertebrata); negative regulation of interleukin-12 biosynthesis
Nfkbia	Nuclear factor of κ light chain gene enhancer in B-cells inhibitor, α	Protein-nucleus import, translocation; apoptosis; cytoplasmic sequestering of NF-κB; response to pathogenic bacteria; regulation of cell proliferation
Nlgn1	Neuroligin 1	Protein targeting; cell adhesion; neurogenesis; synaptogenesis; synaptic vesicle targeting; calcium-dependent cell– cell adhesion; ion channel clustering; regulation of neuron differentiation
Npy	Neuropeptide Y	Calcium ion transport; cell motility; G-protein signaling, coupled to cyclic nucleotide second messenger; neuropeptide signaling pathway; synaptic transmission; digestion; feeding behavior; feeding behavior; circulation; regulation of blood pressure; cell proliferation
Npy5r	Neuropeptide Y receptor Y5	G-protein-coupled receptor protein signaling pathway; synaptic transmission
Nqo1	NAD(P)H dehydrogenase, quinone 1	Electron transport; xenobiotic metabolism; nitric oxide biosynthesis; synaptic transmission, cholinergic; response to toxin
Opcml	Opioid-binding protein/cell adhesion molecule-like	Cell adhesion; neuron recognition
Pcdh8	Protocadherin 8	Somitogenesis; cell adhesion; homophilic cell adhesion; cell– cell signaling; synaptic transmission; long-term memory; morphogenesis of embryonic epithelium
Penk-rs	Preproenkephalin, related sequence	Immune response; neuropeptide signaling pathway
Pla2g4a	Phospholipase A2, group IVA (cytosolic, calcium-dependent)	Proteolysis and peptidolysis; platelet activating factor biosynthesis; icosanoid metabolism; phospholipid catabolism; lipid catabolism
Plat	Plasminogen activator, tissue	Protein modification; proteolysis and peptidolysis; blood coagulation; platelet-derived growth factor receptor signaling pathway
Plau	Plasminogen activator, urokinase	Proteolysis and peptidolysis; chemotaxis; signal transduction; blood coagulation; negative regulation of blood coagulation
Ppt	Palmitoyl-protein thioesterase	Protein modification; neurogenesis; visual perception; grooming behavior; adult locomotory behavior; regulation of synaptic plasticity
Prkcd	Protein kinase C, δ	Protein amino acid phosphorylation; induction of apoptosis; intracellular signaling cascade; humoral defense mechanism (sensu Vertebrata); B cell proliferation
Psmb9	Proteosome (prosome, macropain) subunit, β type 9	Proteolysis and peptidolysis; ubiquitin-dependent protein catabolism; immune response
Ptgds	Prostaglandin D2 synthase	Prostaglandin biosynthesis; prostaglandin metabolism; transport; regulation of circadian sleep/wake cycle, sleep
Ptgds2	Prostaglandin D2 synthase 2	Prostaglandin biosynthesis; prostaglandin metabolism; signal transduction; cell– cell signaling; locomotory behavior
Ptgs1	Prostaglandin-endoperoxide synthase 1	Prostaglandin biosynthesis; lipid metabolism; response to oxidative stress; physiological process; regulation of blood pressure; keratinocyte differentiation
Ptgs2	Prostaglandin-endoperoxide synthase 2	Regulation of progression through cell cycle; prostaglandin biosynthesis; cell motility; response to oxidative stress; physiological process; regulation of blood pressure; positive regulation of cell proliferation; negative regulation of cell proliferation; cyclooxygenase pathway; keratinocyte differentiation; regulation of inflammatory response
Scn2a1	Sodium channel, voltage-gated, type 2, α1 polypeptide	Sodium ion transport; apoptosis; neurogenesis; generation of action potential
Scn6a	Sodium channel, voltage-gated, type 6, α polypeptide	Sodium ion transport; muscle contraction
S100b	S100 protein, β polypeptide	Energy reserve metabolism; regulation of protein biosynthesis; calcium ion homeostasis; induction of apoptosis; axonogenesis; central nervous system development; learning and/or memory; memory; cell proliferation; regulation of cytokine biosynthesis; positive regulation of complement activation; astrocyte activation; hyperphosphorylation; regulation of neuronal synaptic plasticity
Sc4 mol	Sterol-C4-methyl oxidase-like	Fatty acid metabolism; metabolism; steroid metabolism; sterol biosynthesis
Scn1a	Sodium channel, voltage-gated, type 1, α polypeptide	Sodium ion transport; generation of action potential
Scn1b	Sodium channel, voltage-gated, type I, β polypeptide	Sodium ion transport
Scn3a	Sodium channel, voltage-gated, type III, α polypeptide	Sodium ion transport; response to wounding; perception of pain
Scn3b	Sodium channel, voltage-gated, type III, β	Sodium ion transport

(*Table continues*)

Table 4. *continued*

Gene symbol	Gene name	Biological process
Sdc4	Syndecan 4	Cell adhesion; cell–cell signaling
Sgk	Serum/glucocorticoid regulated kinase	Protein amino acid phosphorylation; sodium ion transport; sodium ion homeostasis; apoptosis; response to stress
Slc6a1	GABA transporter protein	Transport; neurotransmitter transport; synaptic transmission
Snap25	Synaptosomal-associated protein 25	Neurotransmitter uptake; exocytosis; synaptic transmission; neurotransmitter secretion; synaptic vesicle docking during exocytosis
Sod1	Superoxide dismutase 1	Activation of MAPK activity; DNA fragmentation during apoptosis; superoxide metabolism; response to oxidative stress; neurogenesis; removal of superoxide radicals
Spnb3	β-Spectrin 3	Vesicle-mediated transport
Spp1	Secreted phosphoprotein 1	Ossification; antiapoptosis; inflammatory response; cell adhesion; cell-matrix adhesion; cell–cell signaling; negative regulation of bone mineralization; immune cell chemotaxis; T helper 1 type immune response; positive regulation of T cell proliferation; regulation of myeloid cell differentiation
Sst	Somatostatin	Regulation of cell migration
Sstr1	Somatostatin receptor 1	G-protein-coupled receptor protein signaling pathway; G-protein signaling, coupled to cyclic nucleotide second messenger; glutamate signaling pathway; neuropeptide signaling pathway; cell–cell signaling; response to nutrients; digestion; negative regulation of cell proliferation; T cell differentiation
Stat3	Signal transducer and activator of transcription 3	Negative regulation of transcription from RNA polymerase II promoter; eye photoreceptor cell differentiation; regulation of transcription, DNA-dependent; regulation of transcription from RNA polymerase II promoter; cell motility; acute-phase response; signal transduction; intracellular signaling cascade; JAK-STAT cascade; neurogenesis
Stx4a	Syntaxin 4A (placental)	Neurotransmitter transport; intracellular protein transport; synaptic vesicle docking during exocytosis
Sv2a	Synaptic vesicle glycoprotein 2a	Transport; neurotransmitter transport; calcium ion homeostasis; synaptic transmission
Syn2	Synapsin II	Synaptic transmission; regulation of neurotransmitter secretion
Syp	Synaptophysin	Transport; endocytosis; synaptic transmission; synaptic vesicle maturation; regulation of neuronal synaptic plasticity; synaptic vesicle membrane organization and biogenesis
Syt12	Synaptotagmin 12	Transport; regulation of neurotransmitter secretion
Syt4	Synaptotagmin 4	Transport; neurotransmitter secretion; regulation of calcium ion-dependent exocytosis; synaptic vesicle transport
Tbxas1	Thromboxane A synthase 1	Prostaglandin biosynthesis; electron transport; blood coagulation
Thbd	Thrombomodulin	Blood coagulation
Tieg	TGFB inducible early growth response	Induction of apoptosis; regulation of cell proliferation
Timp1	Tissue inhibitor of metalloproteinase 1	C21-steroid hormone biosynthesis
Tlr4	Toll-like receptor 4	Inflammatory response; immune response; signal transduction; I-κB kinase/NF-κB cascade; activation of NF-κB-inducing kinase; Toll signaling pathway; metabolism; detection of pathogenic bacteria; detection of fungi; T helper 1 type immune response; macrophage activation; positive regulation of interleukin-12 biosynthesis; innate immune response; positive regulation of interleukin-1 biosynthesis; positive regulation of interleukin-13 biosynthesis; positive regulation of interleukin-6 biosynthesis; mast cell activation; negative regulation of osteoclast differentiation
Tnfsf4	Tumor necrosis factor (ligand) superfamily, member 4	Immune response; T cell proliferation
Trh	Thyrotropin releasing hormone	Signal transduction; cell–cell signaling; hormone-mediated signaling
Trim9	Tripartite motif-containing 9	Synaptic vesicle exocytosis; protein ubiquitination; negative regulation of calcium ion-dependent exocytosis
Txnrd1	Thioredoxin reductase 1	Electron transport; thioredoxin pathway; response to oxidative stress; signal transduction
Ugt1a6	UDP glycosyltransferase 1 family, polypeptide A6	Glucuronate metabolism
Unc13c	unc-13 homolog C (*C. elegans*)	Synaptic transmission; regulation of neurotransmitter secretion
Vgf	VGF nerve growth factor inducible	Ovarian follicle development; generation of precursor metabolites and energy; sexual reproduction; neuron differentiation
Viaat	Vesicular inhibitory amino acid transporter	Transport; neurotransmitter transport; amino acid transport; glycine transport
Vip	Vasoactive intestinal polypeptide	Vasodilation
Znf179	Zinc finger protein 179	Immune response; protein ubiquitination

Abbreviations are listed in alphabetical order.

and plasma membrane. The *postsynaptic membrane* was a GO term that belonged to the downregulated structural components in acute and chronic phases (Table 3). GO terms that appeared in the latent as well as in the chronic phase included in CA3: *immune response*, *intracellular signaling cascade and phosphate transport*; in EC several *cellular components* appeared significantly affected: *lysosome*, *cytoplasm, and plasma membrane*.

Waves of gene induction

Genes that belong to a specific process were often induced or repressed at a specific time point during epileptogenesis. Examples of these different waves of induction or repression of genes are presented in Figure 4*a–f*. Here, we display the average response of the selected genes ($p < 0.05$) that were transiently (T) and significantly regulated in CA3 only at 1 d (TD) or at 1 week

(TW) or both (TDW), genes that were only regulated during the chronic phase and not in the acute and latent phases (M#DW), genes that were induced or repressed during all phases (DWM), and genes that had a biphasic response with regulation in acute and chronic phases ("seizure related"). The figure indicates that regulation of expression of these genes was mostly similar in the EC except for the M#DW and DWM patterns where EC responded somewhat differently. We also analyzed the processes that were prominent in each pattern. These are indicated next to each specific pattern. Not surprisingly, many of the processes during the early period (TD, TW, and TDW) were mostly similar to what was observed in the CD and CW comparisons (Table 3). These show a prominent position for the immune response including antigen presentation, prostaglandin biosynthesis, and complement activation. Neurotransmitter secretion and transport were downregulated processes during these specific phases.

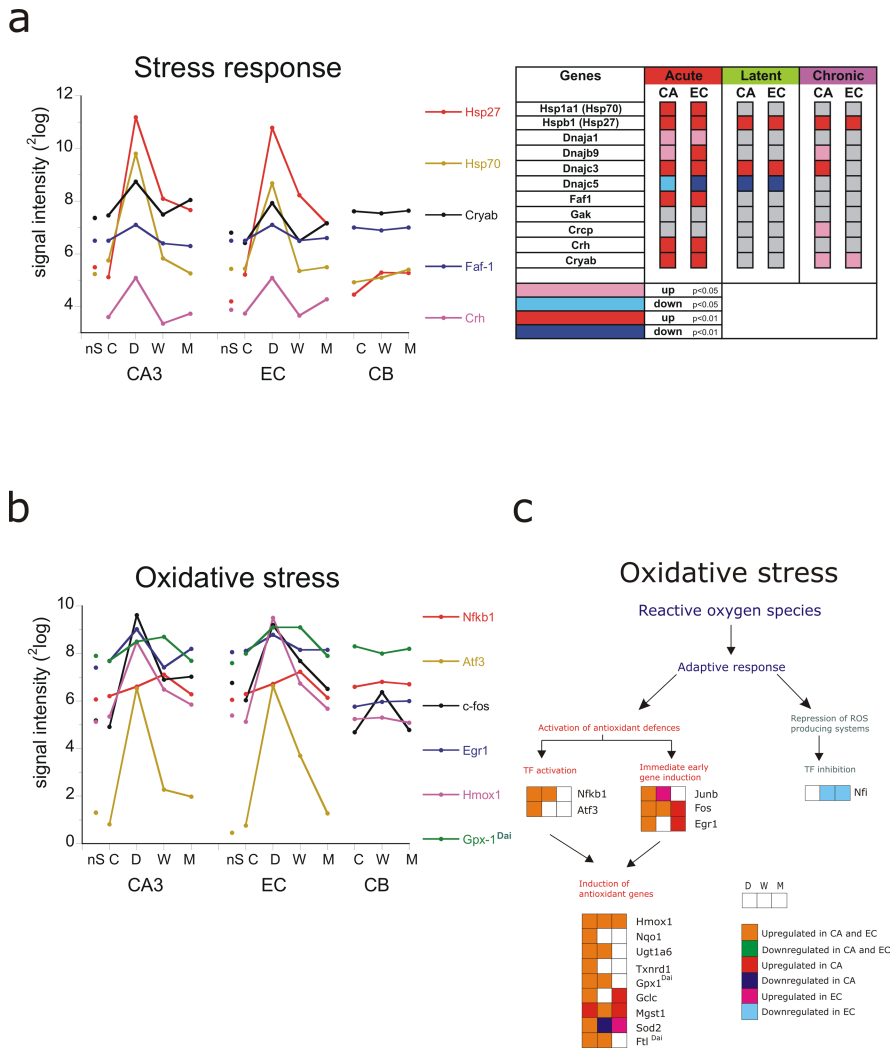

Figure 5. Changes in gene expression after SE related to stress response (***a***) and oxidative stress (***b***). Graphs show the signal intensity of six to eight genes in CA3, EC, and CB in the non-SE group (nS), the control group (C), 1 d after SE (D), 1 week after SE (W), and during the chronic phase (M). The statistics of the genes within the GO process are displayed in a color-coded map next to the graph. Genes with a significant change in CA3 or EC of <0.01 are coded in red (up) or dark blue (down), and genes that changed with $p < 0.05$ are coded in pink (up) and light blue (down). ***c***, Scheme adapted from Morel et al. (1999) that shows the adaptive response during each phase (DWM) on reactive oxygen species. Orange indicates upregulated in CA3 and EC, and green is downregulated in CA3 and EC. Red is upregulated in CA3 only. Pink is upregulated in EC only. Blue is downregulated in CA3 only. Light blue is downregulated in EC only. Gene abbreviations are presented in Table 4.

Dynamic changes in GO processes at the gene level

To identify how genes belonging to a given GO process changed in the course of epileptogenesis, we determined the corresponding signal intensities for the three phases of the process as well as for the controls and the nS animals. Furthermore, we modified GENMAPP maps to be able to examine more precisely whether the changes in signal intensity, either in the "up" or the "down" sense, were statistically different from controls. Each gene is indicated in abbreviation and the full name plus associated GO term can be found in Table 4. We identified the most conspicuous genes that were involved in the *stress response* and that appeared not only prominently in the acute phase but also in the chronic phase. Some of the genes involved were *heat shock proteins*. Several of these genes are displayed on the left side of Figure 5*a*. For clarity, only a limited number of genes (usually six to eight) that changed most dramatically after SE are shown in each graph. The statistics of the genes within the GO process are displayed in color-coded maps next to the graph. Genes with a significant change of <0.01 are coded in red (up) or dark blue (down), and genes that changed with $p < 0.05$ are coded in pink (up) and light blue (down). In the lists, only the genes are shown that were present in the process and that were not ESTs. Most of the *stress* related genes displayed were acutely and manifold activated and restored in the latent phase. The genes encoding the heat shock proteins *Hspb1* (*Hsp27*), *Dnajb9* and *Dnajc3*, however, were still activated in the chronic phase; *Crystalline a β* (*Cryab*) showed a biphasic response with an upregulation during the acute phase and at the chronic phase in both EC and CA (and not in CB). The expression level of these genes can determine the fate of a cell in response to an injury; particularly the apoptosis-inhibitory *Hspb1* and *Hsp1a1* (*Hsp70*) genes (Didelot et al., 2006). *Corticotropin-releasing hormone* (*Crh*) was also significantly upregulated in the acute phase and tended to increase again in the chronic phase. Activation of this hormone, which by itself has some depolarizing effects (Hollrigel et al., 1998), leads to the systemic secretion of glucocorticoids, which in turn have immunosuppressant effects. Genes involved in *oxidative stress* are shown in Figure 5*b*. The *nuclear factor kB1* (*Nfkb1*), which regulates genes encoding *cytokines*, *cytokine receptors*, *cell adhesion molecules*, *proteins involved in coagulation*, and genes involved in *cell growth control*, was acutely upregulated and reached an almost twofold increase at 1 week after SE (Fig. 5*c*). Among the *stress-activated* genes, *fos oncogene* (*Fos*) was among the most activated genes during the acute phase (~30-fold in CA3) and was still significantly activated in CA3 in the chronic phase. This gene showed also activation in the cerebellum in the latent phase ($p < 0.05$). Apart from activation of *transcription factors* (*Nfkb1* and *Atf3*) and *immediate early genes*

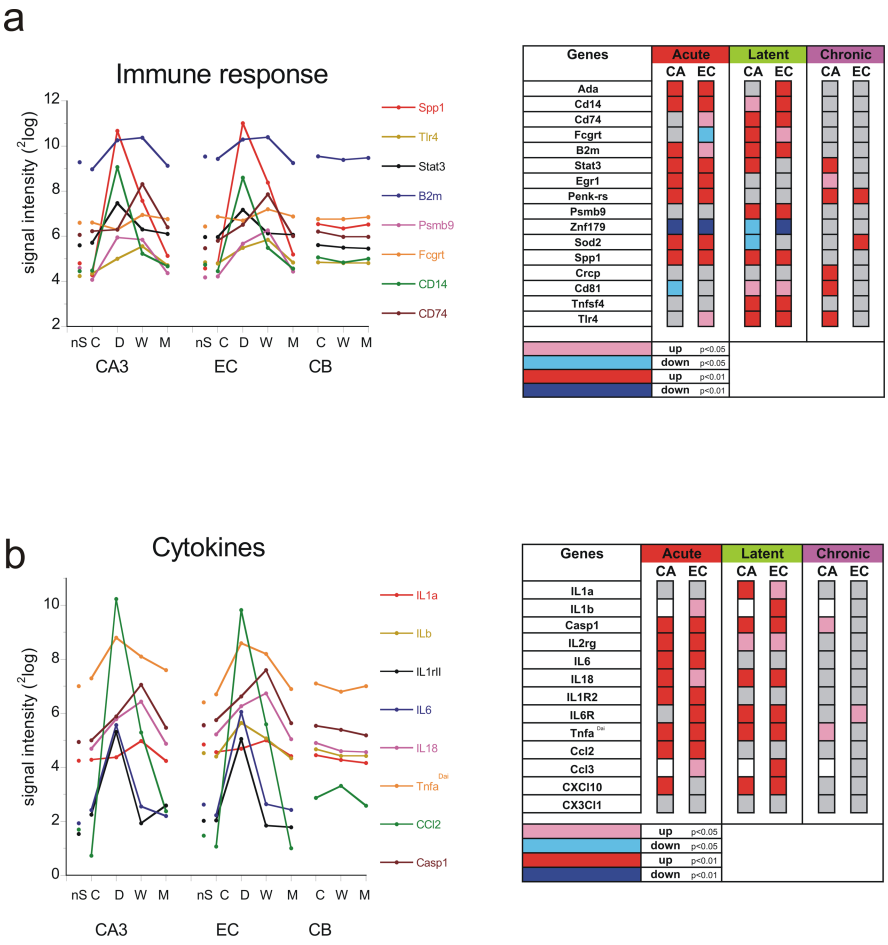

Figure 6. Changes in gene expression after SE related to immune response (***a***) and cytokine production (***b***). Graphs show the signal intensity of eight genes in CA3, EC, and CB in the non-SE group (nS), the control group (C), 1 d after SE (D), 1 week after SE (W), and during the chronic phase (M). The statistics of the genes within the GO process are displayed in a color-coded map next to each graph. Genes with a significant change in CA3 or EC of <0.01 are coded in red (up) or dark blue (down), and genes that changed with $p < 0.05$ are coded in pink (up) and light blue (down). White indicates "not present." Gene abbreviations are presented in Table 4.

(*Fos* and *Jun*), genes that were activated to prevent further production of reactive oxygen species (ROS) included the following: *glutathione peroxidase* (*Gpx*), *thioredoxin reductase* (*Txnrd*), *superoxide dismutase* (*Sod*), *heme oxygenase 1* (*Hmox1*), and *ferritin* (*Ftl*). Most of these genes were activated in both acute and latent phases. *Hmox1* was upregulated ~10-fold in both CA3 and EC and was still activated in the chronic phase. *Sod2* was acutely and chronically upregulated. *Nuclear factor X* (*NFI*), a gene that can contribute to ROS, was repressed in the latent and chronic phase (in EC). This gene has been previously shown to be repressed by oxidative stress (Morel et al., 1999). Oxidative stress changes the *immune response*, which is another major process that was activated not only shortly after SE but also during the latent and chronic phases (Table 3 and below). Apoptotic mechanisms via activation of *caspases and proteases* further contribute to cell death during the acute and latent phases (for more detailed description of gene regulation, see Gorter et al., 2006).

Genes associated with immune response

The *immune response* was not only induced during both acute and latent phases of epileptogenesis but also during the chronic phase (in CA3) (Table 3). The *immune response* was most prominent at 1 week after SE (*Z* score, 8.65), but some genes peaked at 1 d after SE as shown in Figure 6*a* [e.g., *Stat3*, *CD14 antigen*, and *secreted phosphoprotein 1* (*Spp1* or *osteopontin*), a glycoprotein that promotes macrophage migration]. *Hg2a* (*or CD74; H-2 class II histocompatibility antigen*), *proteosome* (*Psmb9*, *macropain*), *toll-like receptor 4* (*Tlr4*), and *tumor necrosis factor superfamily 4* (*Tnsf4*) peaked at 1 week. *Proenkephalin related sequence* (*Penk-rs*) and *crystalline a β* (*Cryab*) showed a biphasic response with an upregulation during the acute phase and chronic phase. *Zinc finger protein 179* and *S100β* were downregulated during the acute and latent phases. Regarding the *interleukin* and *chemokines* (Fig. 6*b*), we also observed waves of expression. *IL6* and *IL1b* and the receptors *IL1rII* and *Il2rg* clearly peaked at 1 d after SE, whereas *IL1a*, *IL18*, and *IL6 receptor* peaked at 1 week after SE. Caspase 1, which cleaves the inactive pro-IL1b and inactive IL18 in the biologically active IL1b and active IL18 peaked at 1 week after SE and was still upregulated in the chronic phase (CA3). *Tumor necrosis factor α* (*Tnfa*) showed upregulation both during 1 d and 1 week after SE. Similarly to *Tnfa*, *IL1* acts as an amplifier of the inflammatory and wound healing response. It induces expression of multiple inflammatory genes and neurotrophic factors in glial cells (Benveniste, 1998). IL6, which was also dramatically induced at 1 d after SE, has also antiinflammatory properties via inhibition of proinflammatory cytokines and induction of IL1ra. The small inducible *chemokine Ccl2* (also *Scya2* or *MCP1*, *monocyte secretory protein JE*), a chemokine that is involved in macrophage recruitment (Mahad et al., 2006), was the most activated gene of all genes present on the array and peaked at 1 d after SE.

Inflammation, complement pathway, and coagulation pathway

Release and subsequent receptor binding of proinflammatory cytokines initiates the prostaglandin pathway and activates both the complement and the coagulation pathways, which are involved in the acute phase response. The *acute phase response* was not only activated immediately after SE but also during the chronic phase (CA3) (Table 3). In Figure 7, we illustrate the most relevant genes in these processes.

Prostaglandin pathway (Fig. 7a). Phospholipase A2 (Pla2g4a) activation and subsequent prostaglandin synthesis is considered to be a pivotal event in inflammation. *Phospholipase A2*, which hydrolyzes phospholipids, was strongly upregulated in the acute and latent phases. This protein has been previously shown to be upregulated during kindling epileptogenesis (Tu and Bazan, 2003). *Cox-2* [*prostaglandin–endoperoxide synthase 2* (*Ptgs2*)] showed a biphasic responses with upregulation in the acute and chronic phases, but not significant in the latent phase. *Thromboxane A synthase 1* (*Tbxas1*) was upregulated in the acute and latent phases in both EC and CA3. *Annexin I* (*Lipocortin*; *Anxa1*) that regulates prostaglandin metabolism via inhibition of *Pla2*, was also acutely upregulated after SE.

The complement pathway (Fig. 7b). Genes involved in the complement pathway (e.g., *C1qa*, *C1qc*, *C3*, *C4a*, *Cfh*) were also induced, and many of them remained elevated in the chronic phase. C1qa and C1qc were already induced in the acute phase. C3 and C4a were induced in the latent phase and showed the highest expression at the end of the latent period. Crry, an inhibitor of complement activation was also upregulated at 1 week, whereas CD59, another inhibitor of complement activation, was upregulated in CA3 at 1 week and during the chronic phase (CA3). *α-2 macroglobulin* (*A2m*), a member of the same protein family as the complement protein *C3* and an inhibitor of metalloproteinases (Mmps), remained upregulated at the same level as during the latent period in both CA3 and EC region, indicating a continued challenge to counteract the proteolytic activities of Mmps (Cucullo et al., 2003).

The coagulation cascade (Fig. 7c). Several *coagulation factors* (*F2*, *F3*, and *F5*) were acutely upregulated. *Coagulation factor X* (*F10*) was strongly induced in the latent phase. *Tissue and urokinase plasminogen activators* (*Plat* and *Plau*), which facilitate anticoagulant activity, were induced in the acute and latent phases, whereas *Plat* was still increased compared with control in the chronic phase (CA3). Inhibition of tissue plasminogen activity with neuroserpin has been shown to have antiseizure activity (Yepes et al., 2002).

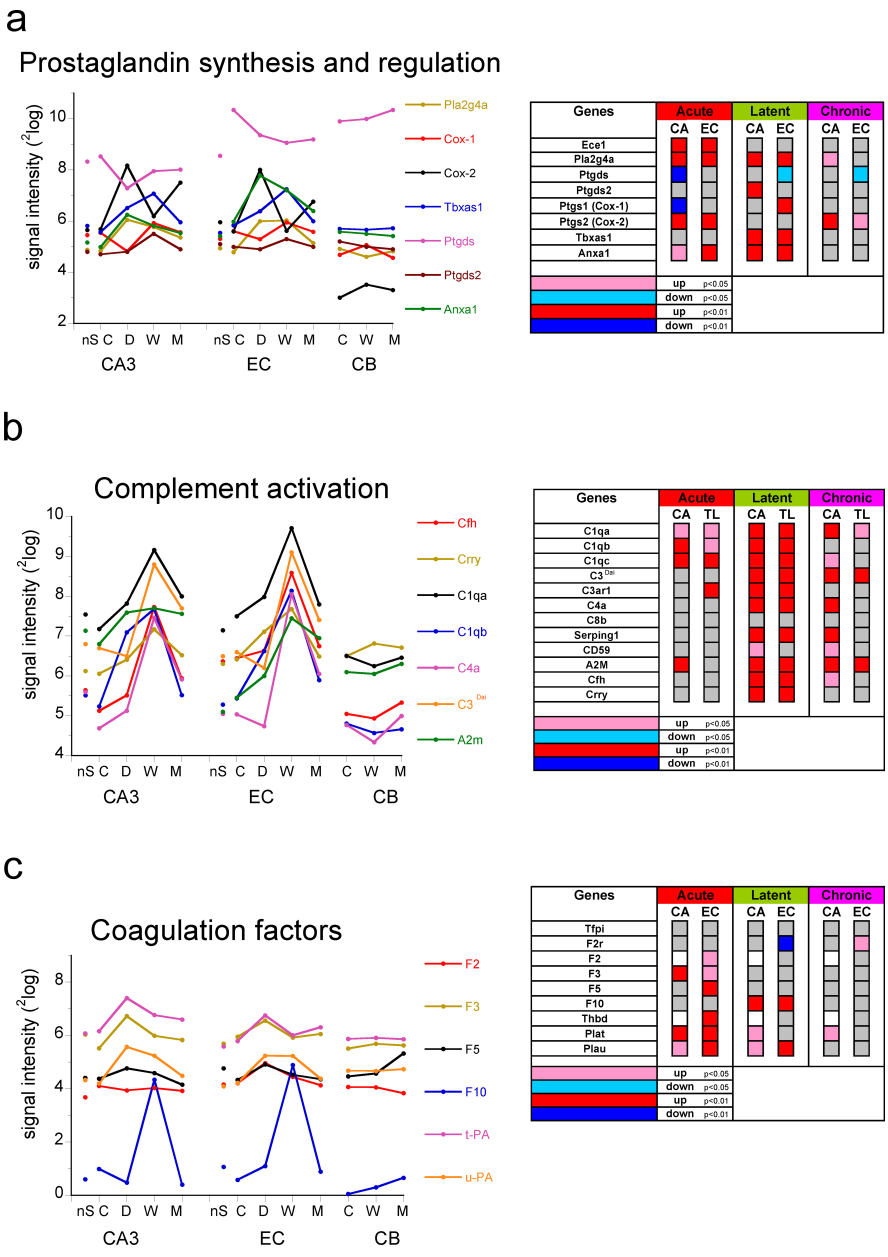

Figure 7. Changes in gene expression after SE related to prostaglandin regulation (***a***), complement activation (***b***), and coagulation factors (***c***). Graphs show the signal intensity of six to eight genes in CA3, EC, and CB in the non-SE group (nS), the control group (C), 1 d after SE (D), 1 week after SE (W), and during the chronic phase (M). The statistics of the genes within the GO process are displayed in a color-coded map next to each graph. Notice the seizure-related regulation of Cox-2, the strong activation of coagulation factors (F10), and the strong activation of complement factors in the latent period, which can persist into the chronic phase [complement component 3 (C3)]. Gene abbreviations are presented in Table 4.

Genes associated with synaptic transmission

The process of synaptic transmission was most significantly downregulated in the acute phase (*Z* score, 4.65). Figure 8*a* displays the list related to genes that code for proteins related to the synaptic release machinery. Although a recovery of expression to control levels was apparent along the epileptogenic process, most genes were still downregulated in the latent phase and some were even downregulated during the chronic phase such as in CA3: an amyloid β precursor protein-binding (*Apba2*), a putative vesicular trafficking protein, and *β-spectrin* (*Spnb3*), a gene involved in secretion; in EC: *Unc13h3* (*Munc13–3*), *neuroligin 1* (*Nlgn1*), which is a postsynaptic cell adhesion molecule, and *Atp12*, a Na^+/K^+ transporting ATPase. In contrast, Syntaxin 4 (*Stx4*) and synaptotagmin 4 (*Syt4*) were genes that were transiently upregulated during the acute phase. *Syt4*, an immediate early gene present in presynaptic terminals, is also upregulated after kainate seizures (Tocco et al., 1996). Synaptic vesicle protein *Sv2a*, the binding site for levetiracetam (Lynch et al., 2004), was only transiently downregulated in EC. Other genes involved in synaptic transmission were related to neurotransmitter receptors, the expression of which is shown below.

Genes associated with synaptic plasticity

Epileptogenesis reflects a strong form of plasticity, and there are indications that it is related to memory impairments (Stefan and Pauli, 2002). Therefore, we also looked to plasticity-related genes and in particular to genes linked to learning and memory. The profiles are shown in Figure 8*b*. This process emerged as significantly downregulated during the acute phase (Table 3). CaMK-associated genes were downregulated in the early phase, and *CaMkIIα* and *CaMkIIβ* (CA3) were still downregulated in the chronic phase. The *calcium- and calmodulin-dependent protein kinase activity* emerged as a downregulated function during all three phases. CaMKII is one of the best candidates for being a molecular component of the learning and memory machinery in

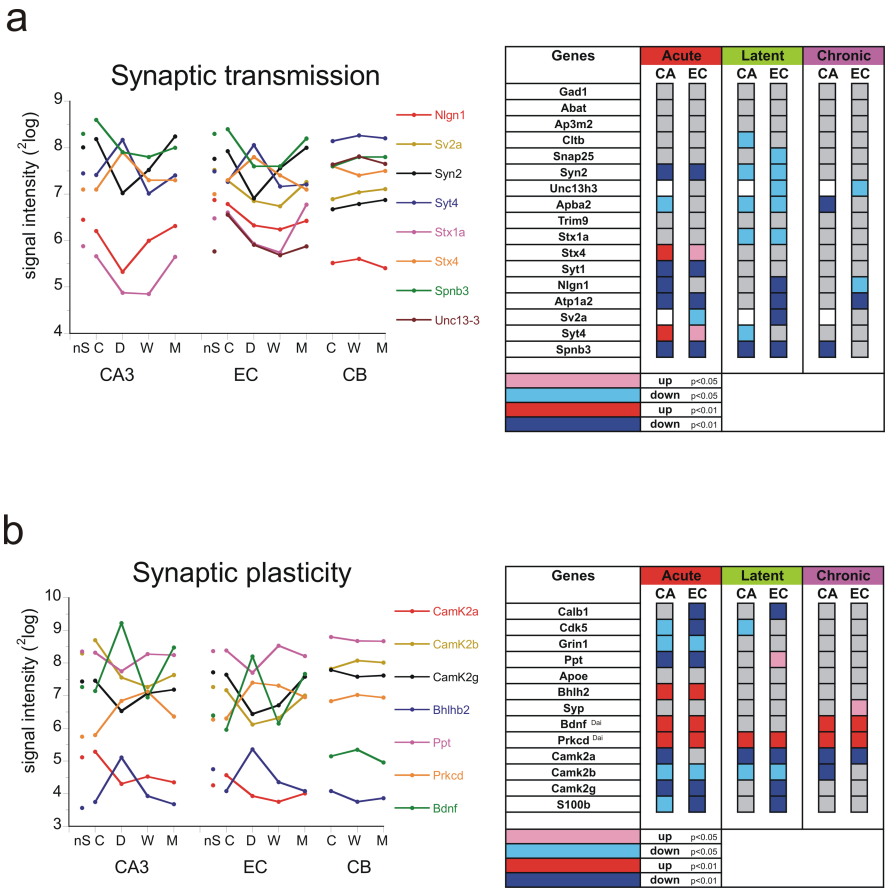

Figure 8. Expression of genes related to synaptic transmission and plasticity. Changes in gene expression in the three regions after SE related to synaptic transmission (***a***) and plasticity (***b***). Graphs show the signal intensity of six to eight genes in CA3, EC, and CB in the non-SE group (nS), the control group (C), 1 d after SE (D), 1 week after SE (W) and during the chronic phase (M). The statistics of the genes within the GO process are displayed in a color-coded map next to each graph. Notice that most but not all of the synaptic proteins are downregulated after SE and recover to a large extent during epileptogenesis. ***b***, Plasticity-related genes such as the CaMKIIa are downregulated after SE and remain so during all three phases. Protein kinase C δ (Prkcd) and brain-derived neurotrophic factor (Bdnf) are still upregulated during the chronic epileptic phase in CA3 and EC. Gene abbreviations are presented in Table 4.

the mammalian brain (Silva et al., 1992; Mayford et al., 1996). Absence or a downregulation of CaMKIIα has previously been reported to be related to hyperexcitability and epilepsy (Butler et al., 1995; Churn et al., 2000; Simonato et al., 2002). *Protein kinase δ* (*Prkcd*) and *brain-derived neurotrophic factor* (*Bdnf*) were upregulated genes. Prkcd was upregulated during all three phases, whereas Bdnf displayed a biphasic pattern with upregulation in the acute and chronic phases but not in the latent phase. Prkcd can be an activator of the Ras/Raf/MEK/MAP kinase signaling pathway (Jackson and Foster, 2004). Interestingly the Ras signaling pathway has been shown to be involved in different kinds of learning and memory formation in the hippocampus (Giese et al., 2001) and amygdala (Merino and Maren, 2006).

Genes associated with ionic channels and receptor processes

As indicated above, *synaptic transmission* was a downregulated process during the early phase. This process consists of genes that encode presynaptic and postsynaptic proteins. Because there is ample evidence that epileptogenesis is associated with changes in voltage-gated ion channels and GABA and glutamate receptor expression, we focus here especially on these channel and receptor functions. Moreover *ion channel activity* and *GABA* and *glutamate-gated receptor activities* emerged as significant repressed functions during the acute phase (Table 3).

Ion channels

Figure 9 show the profiles of the changes in signal intensity of the voltage-gated channels during epileptogenesis and in the nS group in CA3, EC, and CB region, next to the gene maps. The maps representing *sodium* channel subunits shows that *Scn1a*, *Scn2a*, and *Scn3b* were downregulated during the early phase but had recovered in the chronic phase (except *Scnb3*). *Scn6a* was upregulated in the acute phase (CA3) and latent period and even more increased in the chronic phase in both regions (Fig. 9*a*).

The *voltage-gated potassium channel complex* emerged as repressed component during the latent phase (Table 3). Several *potassium* channel genes that were present on the array were already downregulated during the acute phase, except for the inward rectifier gene [*Kcnj8* (=*Kir6.1*)]. The expression recovered for most downregulated genes in the chronic epileptic phase, although some potassium channel genes in CA3 were still downregulated compared with control levels [e.g., *Kcnc1* (=*Kv3.1*) and *Kcnd2* (=*Kv4.2*, which represents an A-type potassium channel)] (Fig. 9*b*). *Kcnmb4*, a large conductance, calcium-activated potassium (BK) channel was notably downregulated in CA3 and EC in the acute and latent periods.

Calcium channel activity was repressed during the acute phase (Table 3). Several voltage-gated *calcium* channels were responsible for this downregulated activity. However, they appeared to be mostly recovered in the chronic phase, except for *Cacnb3* in the EC (Fig. 9*c*).

Glutamate signaling

As shown in Figure 10*a*, most glutamate signaling-related genes were acutely downregulated (approximately twofold) but recovered to control levels after the latent period [except *Gria1* (Glur1) and *Gria2* (Glur2) and *Grm1* (metabotropic GluR1) in EC, which remained below control levels]. NMDA receptor glutamate-binding chain (*Grina*) was upregulated, but only in the EC during the chronic phase. The only glutamate signaling molecule that was acutely upregulated was *Vesl/homer*, which appears to play a role in synaptic potentiation (Hennou et al., 2003). Increased expression of homer1a, however, has some antiepileptogenic effects as shown in kindled mice with overexpression of homer1a (Potschka et al., 2002).

GABA signaling

Figure 10*b* shows various genes that are related to GABA signaling. Most GABA receptor subunits were downregulated during the acute phase including α4 and α5, β1 subunit, and δ subunit. The genes encoding the GABA-B1 (Gabbr1; CA3) and GABA-B2 (Gabbr2; EC) receptor subunit were also downregulated. The genes that encode α5 and the δ GABA receptor subunit were most dramatically downregulated (around twofold). Still a significant downregulation of the α5 and δ was observed in the chronic phase in CA3 or EC, respectively. A GABA transporter (*Slc6a1* or

Gabt1) was also transiently downregulated in the acute phase. In contrast, a significant upregulation of GABA receptor-associated protein (*GABArap*) that is involved in postsynaptic clustering of synaptic GABAa receptor subunits (Coyle and Nikolov, 2003) was present during the acute and latent phases in EC. The vesicular inhibitory amino acid transporter *Viaat* was initially downregulated, but significantly upregulated in the chronic epileptic phase in EC (see below).

Neuropeptide signaling

Because several neuropeptides have strong antiseizure actions, we also looked in more detail to changes in gene expression of several neuropeptides. Moreover, the *neuropeptide signaling* pathway became an activated process in the chronic phase (EC). As shown in Figure 10*c*, these genes were almost all acutely and many fold upregulated. Various neuropeptides showed a "biphasic" response with an upregulation at 1 d after SE and in the chronic epileptic phase but not in the latent period in both CA3 and EC, although this only reached statistical significance for *Npy*, *Penk-rs*, and *Trh* and *somatostatin* (*Sst*). *Npy5* receptor was transiently downregulated and *somatostatin receptor 1* (*Sstr1*) was downregulated in EC during the latent phase. Diazepam binding inhibitor (*Dbi*), an endogenous neuropeptide for benzodiazepine receptors, is upregulated during the latent period in the EC. Interestingly, Dbi-derived peptide fragments induce a typical pattern of limbic seizures in rats (Vezzani et al., 1991).

Gene expression in the chronic epileptic phase

The animals in the chronic group (M group) exhibited a relatively high seizure incidence, on average 8.3 ± 1.2 seizures (range, 5–12) per day. This study was designed in such a way that we tried to compose groups of animals that could function as replicates. Because the M group has the longest experimental history, they carry the highest risk of being nonuniform. This could be related to their seizure history, seizure severity, recency of last seizure before decapitation, or eventually other environmental factors. A cluster analysis based on the 280 (GO-related) upregulated genes in the CA3 region of the 11 animals (M group and controls), however, gave a clear separation into two highly consistent groups (M and C; Cophenet correlation coefficient, 0.94) (data not shown). We also found that the variance in gene expression of the M group was not different from that of the C group (the *F* test comparing the variances in the two groups for each gene was not significant for 95% of the 280 genes). Because the experiments exhibited little variation within groups, it is not surprising that a correlation between gene expression and, for example, seizure recency before decapitation (range, 1–5 h) could not be detected in the M group. In addition, we found that none of the possible rankings of the recency values (or any other seizure-related parameter associated with an animal) showed a significant correlation with the expression of this set of 280 genes.

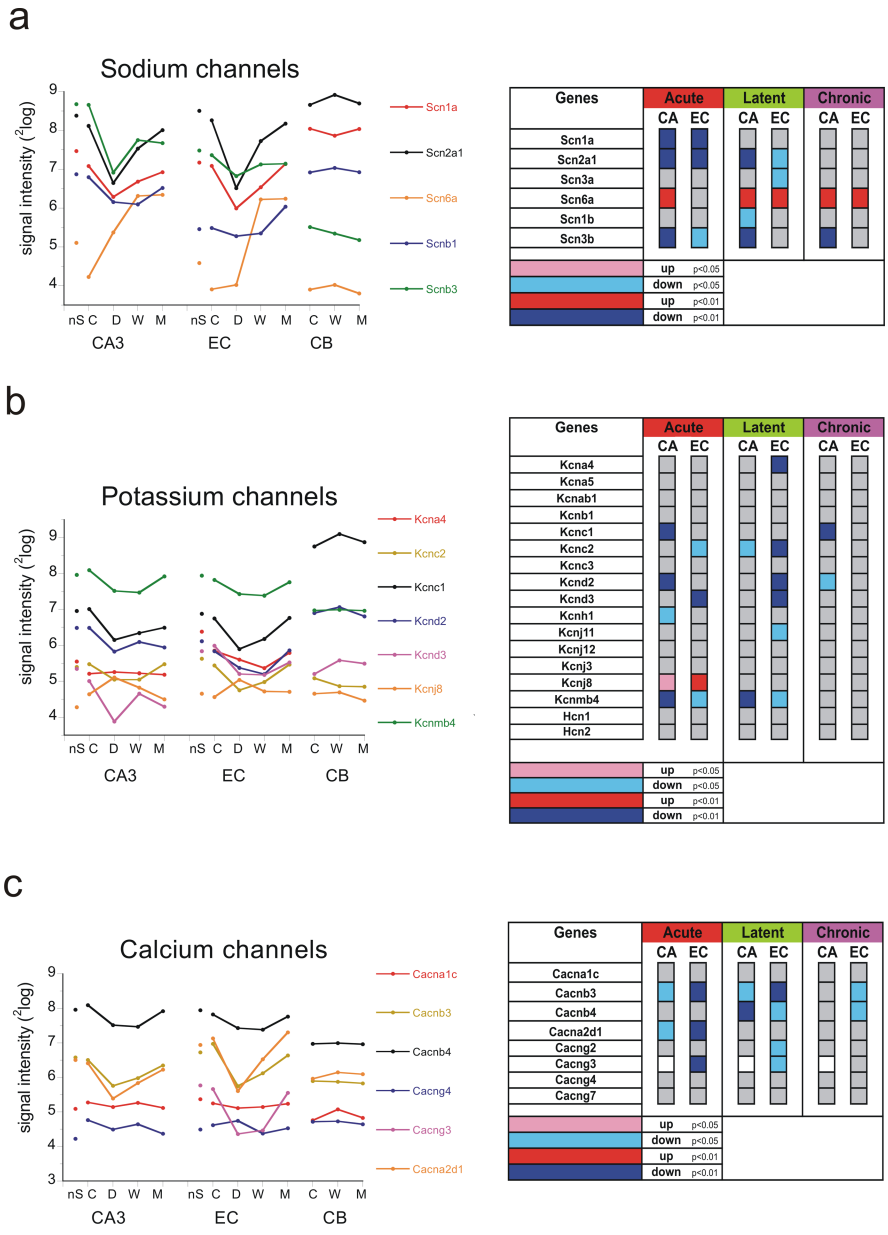

Figure 9. Expression of genes related to ion channels. Changes in gene expression in the three regions after SE related to sodium channels (***a***), potassium channels (***b***), and calcium channels (***c***). Graphs show the signal intensity of six to eight genes in CA3, EC, and CB in the non-SE group (nS), the control group (C), 1 d after SE (D), 1 week after SE (W), and during the chronic phase (M). The statistics of the genes within the GO process are displayed in a color-coded map next to each graph. Notice that different subunits of the ion channels are downregulated after SE but recover to a large extent. The exception is the Scn6a channel, which is persistently upregulated in CA3 and EC but not in CB. Gene abbreviations are presented in Table 4.

Replication of gene pattern profiles by PCR

To validate the microarray results, we investigated gene expression profiles of six different genes and three household genes (*β-actin*, *TBP like protein*, and *cyclophilin-A*) using quantitative PCR. Material from the same rats that was used for the array was used in the PCR experiment, but the material was pooled per time point. We chose genes that, according to the microarray data, were expressed with a characteristic time pattern. To avoid the possible influence of a single household gene that may also

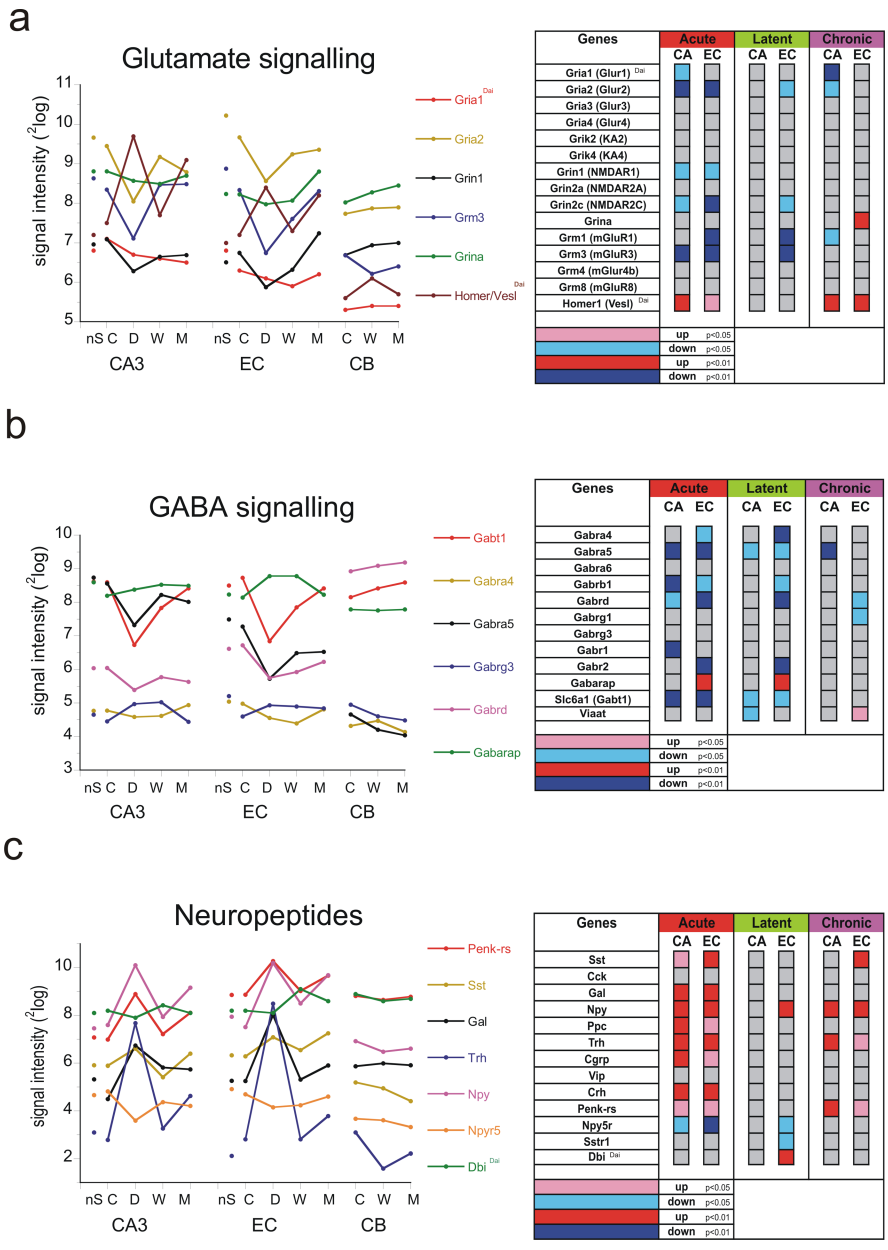

Figure 10. Expression of genes related to neurotransmitter receptors. Changes in gene expression in the three regions after SE related to glutamate signaling (***a***), GABA signaling (***b***), and neuropeptides (***c***). Graphs show the signal intensity of six to eight genes in CA3, EC, and CB in the non-SE group (nS), the control group (C), 1 d after SE (D), 1 week after SE (W), and during the chronic phase (M). The statistics of the genes within the GO process are displayed in a color-coded map next to each graph. Notice that several subunits of the glutamate receptor channels were significantly downregulated after SE and have recovered to a large extent during the chronic phase. ***a***, Homer/Vesl was the exception with a biphasic, seizure-related upregulation. ***b***, GABA receptor subunit $\alpha 5$ (CA3) and δ (EC), two subunits involved in tonic inhibition, were permanently downregulated. ***c***, Several neuropeptides had a biphasic upregulation. Gene abbreviations are presented in Table 4.

change expression because of the SE, we normalized over the average of all three household genes obtained at each time point. In most cases, gene profile patterns were nicely reproduced (Fig. 11), although the fold change could differ. *Heme oxygenase* (*Hmox1*) (see above) was significantly upregulated during all three phases, peaking at 1 d after SE. *Ferritin-L* (*Ftl*), a protein involved in iron ion homeostasis and previously shown to be upregulated during epileptogenesis (Gorter et al., 2005), was significantly upregulated at 1 d and at 1 week after SE. *Viaat* (*Slc32a1*), a vesicular GABA transporter, was significantly downregulated in the latent phase (CA3) but upregulated in the chronic phase (in EC). An upregulation of *Viaat* has been previously shown in rats with spontaneous seizures (Lamas et al., 2001). *Kncd2*, a potassium channel, and *Gria2* (the Glur2 glutamate receptor) were mainly downregulated during the acute and latent phases. *Gria2* or *Glur2* also has been previously shown to be downregulated after kainate seizures and ischemic insult (Friedman et al., 1994; Gorter et al., 1997). *Galanin* (*Gal*), a neuropeptide with antiseizure properties (Mazarati et al., 1998; Haberman et al., 2003), was mostly activated at 1 d after SE and appeared to be slightly upregulated again in the chronic phase in EC.

Replication of gene profiles by immunostaining and Western blot

To determine whether the transcript regulation corresponded with protein expression, we performed immunohistochemistry (IHC) and Western blotting (WB) on tissue samples of rats that were killed at the same time point. The selected proteins included the following: *CD11b/c* (*OX42*), *osteopontin* (*Spp1*) and *Npy* (*IHC*) and *Cox-2* and *Glur2* (*WB*). CD11b/c, a marker for microglial activation (Fig. 12*a*), was clearly induced at 1 d after SE, peaked at 1 week after SE, and was mostly recovered in the chronic phase. Osteopontin protein expression was induced in glial cells with astrocyte and microglial morphology with highest expression at 1 d after SE (Fig. 12*b*). Npy expression was clearly induced in mossy fiber endings with a similar seizure-related time course as gene expression (Fig. 12*c*). Figure 12*d* shows the optical densities for Glur2 and Cox-2 protein expression in CA3 and EC, respectively. Glur2 was mainly downregulated at 1 d after SE and recovered during epileptogenesis to some extent. The biphasic pattern of Cox-2 observed in the array was reproduced by the Western blot.

Discussion

This microarray analysis of the process of epileptogenesis in this post-SE model of temporal lobe epilepsy in the rat, revealed dynamic changes in gene expression. Microarray results for selected genes were consistent with PCR results and immunostaining. Similarly to previous studies that looked at the acute effects in SE rat models (Becker et al., 2003; Lukasiuk et al., 2003; Hunsberger et al., 2005), we also found a dramatic upregulation of death and inflammation-related genes soon after electrically induced SE. However, our study yields novel information by including measurements at later time points and by identifying many more genes and biological processes involved at each phase.

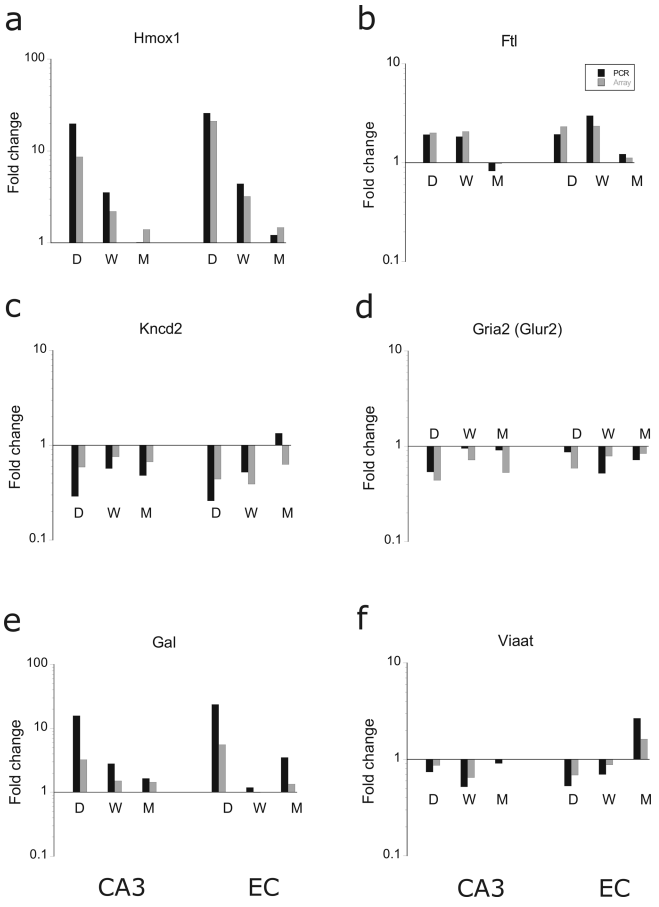

Figure 11. PCR comparison with array expression. Graphs showing the fold change (with respect to control) during the post-SE time points in CA3 and EC. PCR analysis shows a large correspondence with the array expression patterns of the different genes. Hmox1, Heme oxygenase 1; Ftl, Ferritin light chain 1; Kncd2, potassium voltage-gated channel, Shal-related family 2; Gria2, ionotropic glutamate receptor subunit 2; Gal, galanin; Viaat, vesicular inhibitory amino acid transporter.

The immune response is the most prominent process changed during all three phases of epileptogenesis

Within the immune response, prostaglandin synthesis and complement activation were prominent processes. Prostaglandin synthesis, illustrated by Cox-2 induction, was activated in the acute and chronic phases but not in the latent period, indicating that this process is related to the occurrence of seizure activity. Activation of prostaglandin receptors could increase intracellular calcium and subsequent glutamate release, which would increase excitability in the surrounding networks (Bezzi et al., 1998). An important component of the immune response is the activation of the complement pathway. Although complement factors might invade the brain via a leaky blood–brain barrier (BBB), part of the increased expression is likely to originate from activated glial cells (Gasque et al., 1997; Hosokawa et al., 2003). The complement system may be useful in eliminating aggregated and toxic proteins. However, an overactivation of the complement system can also have damaging effects through the activation of microglia and proinflammatory cytokines. Interestingly, sequential infusion of individual proteins of the membrane attack pathway into the hippocampus of freely moving rats induces seizures as well as cytotoxicity (Xiong et al., 2003). A recent microarray study on entorhinal cortex material of MTLE patients also has pointed to the involvement of complement activation (Jamali et al., 2006). The changes in the immune response, the activation of proinflammatory cytokines and associated processes found in our model may be related to the breakdown of the BBB that occurs directly after SE (van Vliet et al., 2006). This leads to intrusion of monocytes, neutrophils, and blood components into the neuropil, which might also destabilize the surrounding networks (Newman et al., 2005). The activation of coagulation factors genes, upregulation of plasminogen activators, and the induction of matrix metalloproteinases (Mmps) (Gorter et al., 2006) are likely associated with BBB damage. Many of these acute changes are also found after other excitotoxic insults such as ischemia (Tang et al., 2002; Lu et al., 2004), hemorrhage and hypoglycemia (Tang et al., 2002), and after brain trauma (Raghavendra Rao et al., 2003). Although seizures are observed within the first day of the postischemic period (Hartings et al., 2003), it is not known whether some of these animals later become epileptic. Thus, we assume that the dynamics of the changes and the localization of the changed gene expression changes are a crucial factor that determines whether epilepsy develops or not. This issue needs a more detailed analysis.

Can we observe waves of gene expression in the course of post-SE epileptogenesis?

Insight in the timing of the molecular changes is of crucial importance when we want to develop drugs that can be applied to prevent epilepsy or to stop the progression of epilepsy. We can identify a series of transient changes of gene expression peaking at different times. A typical time pattern is the seizure-related pattern, which consists of genes that are upregulated during the acute and the chronic phases, when seizures occur, but have a relatively normal expression during the latent period. This was the case of genes related to neuropeptide signaling and also with growth-related factors (*Bdnf*, *Tieg*, *Vgf*, *Gfra1*) and *Cox-2*. Activation of these genes could contribute to aggravate the epileptic condition, because increased excitability has been observed when the expression of some of these proteins is increased (Kokaia et al., 1995; Bezzi et al., 1998; Scharfman et al., 1999).

Similarly to what has been observed after brain ischemia (Raghavendra Rao et al., 2002; Lu et al., 2004), synaptic transmission was one of the processes that were repressed after SE. The most extensive downregulation was observed during the acute phase and might account for the temporary absence of seizures after the acute phase (i.e., in the latent period). This downregulation could reflect a homeostatic protective, rather than an epileptogenic response. It does not simply reflect neuronal loss, but it consists of a transient repression of gene expression because channel and receptor gene expression mostly recovers. The downregulation of glutamate receptor activity can help to limit additional excitotoxic damage. The seizure-related increase of neuropeptides can be seen as a compensatory response because several neuropeptides have antiepileptic properties (Baraban et al., 1997; Tallent and Siggins, 1999; Mazarati et al., 2001; Vezzani et al., 2002). Nevertheless, this increased gene expression is not very effective because rats continue to have frequent daily seizures, but we should note that Npy5 receptors are downregulated, which may be proepileptogenic (Nanobashvili et al., 2004).

Glia and ion homeostasis

The role of astrocytes in epileptogenesis is being recognized as contributing greatly to the origin of epileptic seizures (Tian et al., 2005). In our animal model, gliosis peaks at the end of the latent period (Aronica et al., 2000). A large number of the glial genes

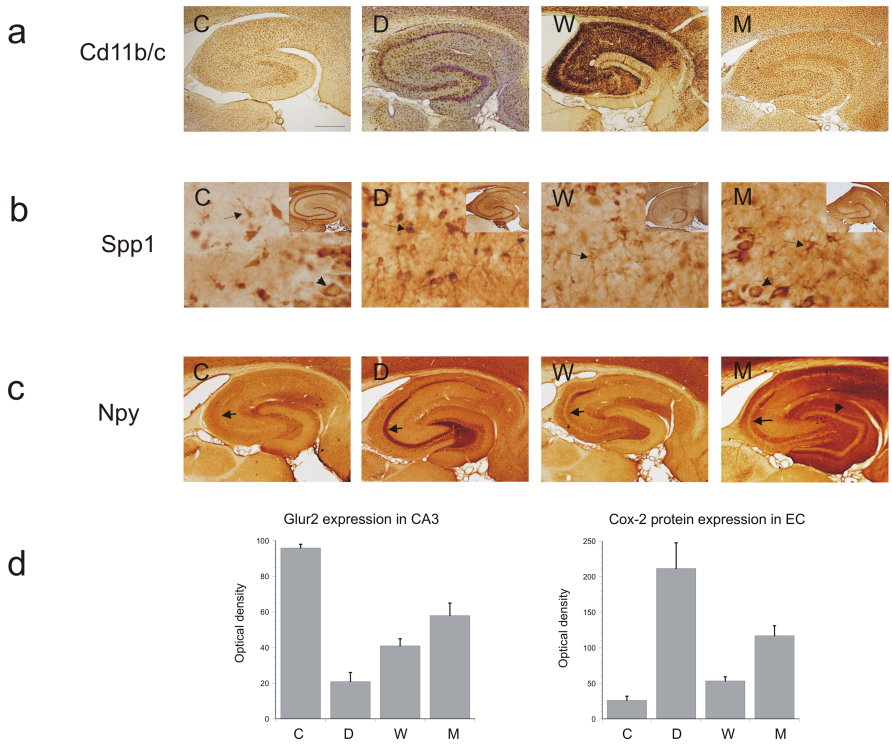

Figure 12. Immunohistochemistry and Western blot. Immunocytochemistry of CD11b/c (Ox42) (***a***), Spp1 (Osteopontin) (***b***), and Neuropeptide Y (Npy) (***c***). ***a***, Microglial activation that was induced at 1 d, peaked at 1 week after SE, and recovers to large extent in the chronic phase. ***b***, Spp1 expression in control (C) is present in neurons and glial cells with microglial morphology; at 1 d (D) is strongly induced in microglial cells with ameboid morphology and still increased in glial cells with microglial and astroglial morphology at 1 week (W). In the chronic phase, some microglial cell still show increased Spp1 expression. ***c***, In control (C), the strongest Npy expression was found in individual neurons scattered throughout hippocampus; Npy expression was strongly induced at 1 d (D) in the mossy fiber endings in hilus and CA3, but also in CA1 neurons and granule cells and in cortical regions. In the latent period (W), Npy expression was much lower than at 1 d after SE. In the chronic epileptic phase (M), Npy expression increased again in mossy fibers in CA3 (arrows) but also in the mossy fibers that had sprouted to the inner molecular layer of the dentate gyrus (arrowheads). Scale bar: ***a***, ***c***, 400 μm; ***b***, 80 μm. ***d***, Two Western blots of ionotropic AMPA2 receptor Gria2 (also Glur2) taken from CA3 material and Cox-2 taken from the EC. Both patterns were similar to the pattern observed with the microarray gene expression.

that have maximum induced expression at 1 week are genes linked to the immune and inflammatory response (Aloisi, 1999; Dong and Benveniste, 2001; Bonifati and Kishore, 2007). Other genes that are expressed in glial cells are related to water and ion homeostasis (Simard and Nedergaard, 2004). A gene that was persistently upregulated was the *Scn6a*, an atypical sodium channel gene that serves as a sodium-level sensor of the body fluid (Hiyama et al., 2002; Watanabe et al., 2006). This gene was originally considered to be a glial sodium channel (Gautron et al., 1992), although neuronal localization has also been observed (Grob et al., 2004). The persistent increased expression was the more remarkable because expression of other sodium channel subunits was reduced. Together with a repression of ion transport via the downregulation of several sodium, potassium, and calcium channels, transporters and glial redistribution of water channels (*Aqp4*) involved in water homeostasis (de Lanerolle and Lee, 2005), these changes point to a persistent and disturbed ion homeostasis and transport that could lead to increased excitability (Schwartzkroin et al., 1998; Bonan et al., 2000).

Genes and neuronal network dynamics

The most salient gene changes that can contribute to a change of the dynamics of the local networks are those involving *GABA* signaling. The most consistent downregulated genes were those encoding for the subunits $\alpha 5$ (CA3) and δ (EC). Both are extrasynaptic receptors that control tonic inhibition (Houser and Esclapez, 2003; Dibbens et al., 2004; Peng et al., 2004; Glykys and Mody, 2006). This leads us to assume that in our epileptic rats GABAergic tonic inhibition is likely impaired, which can lead to a disturbance of the dynamics of neuronal networks leading to epileptiform oscillations. GABA-α5 also emerged as downregulated gene in a number of clinical studies (de Lanerolle and Lee, 2005; Arion et al., 2006; Ozbas-Gerceker et al., 2006).

Suggested candidate targets

We may suggest a number of targets that could have antiepileptogenic or antiepileptic actions. In addition to possible targets involved in apoptosis and cell death including the cysteine and serine proteases and Mmps (discussed in Gorter et al., 2006), other targets are related to the immune response, in particular aiming at inhibition of microglial activation. However, because many proteins involved in the immune response do not only have proinflammatory and harmful effects but also antiinflammatory and beneficial effects, more knowledge concerning cytokine (inter)actions and the timing of such a treatment is crucial. Most studies show effects of different antiinflammatory treatments on acute seizure activity in experimental models (Vezzani and Granata, 2005), but antiepileptogenic effects of these treatments have not been reported. Similarly, although inhibition of complement activation has successfully reduced ischemic damage (De Simoni et al., 2003), no studies have been performed concerning epileptogenesis. Other strategies could consist in inhibiting proteins that are induced by seizures and that have proepileptogenic actions by amplifying the actions of glutamate, as Bdnf (Kokaia et al., 1995; Scharfman et al., 1999, 2002; He et al., 2004) and Cox-2 (Bezzi et al., 1998). GABA receptor subunits related to tonic inhibition are among the most persistent downregulated receptors and could be a target in any phase during epileptogenesis. Similarly, an intervention to counterbalance the atypical ion sodium channel Scn6a could be an interesting target worth exploring.

Conclusions

This microarray investigation yields a large number of genes related to important biological processes and functions, the expression of which changes significantly in the course of epileptogenesis. Some suggestions for the possible functional consequences of these changes are advanced. Furthermore, a few suggestions regarding possible targets that may be explored to counteract the epileptogenic process, or may be novel targets for antiepileptic drugs, are put forward.

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