The circadian hippocampus and its reprogramming in epilepsy: impact for chronotherapeutics

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Gene and protein expression displays circadian oscillations in numerous body organs. These oscillations can be disrupted in diseases, thus contributing to the disease pathology. Whether the molecular architecture of cortical brain regions oscillates daily and whether these oscillations are modified in brain disorders is less understood. We identified 1200 daily oscillating transcripts in the hippocampus of control mice. More transcripts (1600) were oscillating in experimental epilepsy, with only one fourth oscillating in both conditions. Proteomics confirmed these results. Metabolic activity and targets of antiepileptic drugs displayed different circadian regulation in control and epilepsy. Hence, the hippocampus, and perhaps other cortical regions, shows a daily remapping of its molecular landscape, which would enable different functioning modes during the night/day cycle. The impact of this remapping in brain pathologies needs to be taken into account not only to study their mechanisms, but also to design drug treatments and time their delivery.

Circadian rhythms regulate numerous brain functions (e.g. wakefulness, feeding, etc.) with 24-hour oscillations in most species. The core time keeping machinery is the suprachiasmatic nucleus (SCN). Its molecular architecture undergoes a daily remapping driven by transcriptional and translational feedback loops, thus allowing switching between different functional states [1,2]. Several studies suggest the existence of additional (ancillary) clocks in the brain outside the SCN [3]. Indeed, clock genes, including Per1, Per2, Bmal1, Cry1 and Cry2, exhibit rhythmic expression in a brain region- and gender-dependent manner [4,5]. Since these genes are involved in the regulation of transcription/translation of many other genes, brain regions outside the SCN may also undergo a complex daily remapping of their molecular architecture [6,7]. Such circadian regulation of neuronal circuits may enable appropriate

coordination between physiology and behavior, since learning and memory processes are regulated in a circadian manner [8,9]. In the hippocampus, a region critical for numerous learning and memory processes, synaptic responses [10], long term synaptic plasticity [11,12] and the memory-associated MAPK pathway [13] display circadian rhythmicity.

The first goal of this study is to provide the community with a resource listing of the genes and proteins undergoing circadian regulation in the mouse hippocampus. Such information is crucial not only for our understanding of hippocampal function, but also for a proper interpretation of already published data and for resolving discrepancies, since experiments performed at different times may occur within different molecular landscapes. The second goal of this study is to provide a similar resource in a mouse model of temporal lobe epilepsy (TLE), the most frequent form of epilepsy in adults. Several reasons motivate this choice. Alterations of circadian rhythms can lead to pathologies [14], and conversely, disruption of the circadian clock can have direct deleterious consequences [15,16], e.g. on sleep patterns [17]. In the liver, the circadian clock is reprogrammed by nutritional challenge, resulting in a different cycling of genes and proteins [18]. Since clock genes display different cycling behavior in experimental epilepsy [19], the circadian regulation of downstream genes may follow different rules in control and TLE conditions. This information is essential for a proper interpretation of studies comparing the levels of expression of genes and proteins, a standard experimental procedure to assess the reorganization of neuronal circuits in TLE (and in neurological disorders in general). It is also essential for chronotherapy (the proper timing of drug delivery) [20], as many pharmaceutical molecules target gene products showing circadian rhythmicity [21].

RESULTS

Circadian regulation of genes and proteins in the mouse hippocampus

Since the distribution of transcripts is heterogeneous along the septo-temporal axis of the hippocampus [22], we focused on its ventral part [23], which is most epileptogenic in human TLE and experimental models [24]. Using Affymetrix microarrays and cluster analysis, we identified 1256 transcripts oscillating in the ventral hippocampus of control mice (**Fig. 1a** and **Supplementary Table 1**). Hence, 10% of hippocampal genes are regulated in a circadian manner, as compared to 19% in the SCN [5]. Data from post mortem human hippocampus suggests that less genes (n=659) are oscillating [7]. Comparison with the latter study is difficult because investigating circadian rhythms requires a strict control of circadian conditions (e.g. no light exposure during the night), which could not be achieved in the human study.

Using cluster analysis and a false discovery rate < 0.01, we found 197 transcripts differentially expressed in at least one time point, which could be identified based on biological functions (**Fig. 1b**). When compared to subcortical structures (**Table 1**), we found a clear overlap of core clock genes involved in transcriptional and translational feedback loops in the SCN across all investigated brain regions (highlighted in green in **Table 1**). Oscillating core clock genes in the hippocampus included Per1-3, Cry1, Arnt1, Nr1d1-2, and Dbp (**Fig. 1c**, **Table 1**). Transcriptome analysis was confirmed with qPCR (**Supplementary Fig. 1**). Interestingly, a large set of oscillating genes was specific to the hippocampus (**Table 1**), such as Creb1 (**Fig. 2a**), which is involved in both synaptic plasticity and synchronization of circadian rhythms [25], demonstrating that circadian rhythmicity of genes is brain region-dependent.

The analysis of the heatmaps (**Supplementary Fig. 2**) revealed that gene oscillations were distributed in a bimodal manner in control mice, with either a very low or very high

oscillation amplitude (**Fig. 2b**). The majority of genes with high amplitude oscillations were phase advanced in comparison to the rest of the oscillatory transcripts (**Fig. 2b**).

Proteomic data enabled the analysis of 1,500 peptides. We found 170 peptides oscillating in controls (**Fig. 1e** and **Supplementary Table 2**), i.e. the same percentage (11%) as for gene transcripts. Nine proteins were oscillating in both proteomic and transcriptome datasets, which partly originates from the fact that 3% of peptides have a correspondence in the transcriptome dataset.

Together these results demonstrate that 10% of the gene/protein map in the hippocampus of control mice undergoes a continuous remapping during the night/day cycle. We then performed the same analysis in age-matched TLE mice in order to determine whether such circadian regulation was maintained in pathological conditions.

Reprogramming of the circadian regulation of genes and proteins in experimental TLE

In experimental TLE, the number of oscillating transcripts was increased by 30% as compared to control (1650 versus 1256, **Fig. 1a** and **Supplementary Table 3**). Remarkably, only 29% of the oscillating transcripts were common to both control and TLE conditions, demonstrating a reprogramming of the daily remapping of hippocampal genes. Cluster analysis clearly separated the different groups by time and function (**Fig. 1b**). The core clock genes all gained in oscillation amplitudes in TLE as compared to control (**Fig. 1c,d**). Many important circadian-related genes gained oscillatory activity in TLE, from which we highlighted Bhlhe40 and Bhlhe41 (**Fig. 1d, 2a**). These genes encode proteins that interact with Arnlt and compete for E-box binding sites upstream of Per1 and repress Clock/Bmal activation of Per1, hence controlling circadian rhythms

[25]. Increased oscillations of Bhlhe40 and Bhlhe41 may play a key role in the larger recruitment of oscillating transcripts in TLE. Although the mechanism of the reprogramming in TLE was outside the scope of this study, we identified a possible epigenetic mechanism. Runx1, a DNA binding regulator, gained statistical significance in TLE (**Fig. 2a**). Runx1 regulates Creb1, Cry1, Per1 and Per3, and has protein-protein interaction with two key histone deacetylases, Hdac1 and Hdac2. In keeping with this, proteomics revealed oscillatory activity for Hdac1 and Hdac2 in TLE (**Supplementary Table 4**). Finally, Hdac8 also gained oscillatory activity in TLE. Together, these results suggest the involvement of multiple epigenetic mechanisms in the reprogramming of the circadian regulation of hippocampal genes in TLE. The amplitudes of the oscillations were increased in TLE as compared to control, and genes with high amplitude oscillations were phase advanced as compared to the rest of the transcripts (**Fig. 2b** and **Supplementary Fig. 2**).

As for transcripts, the number of oscillating peptides was increased by 40% in TLE as compared to control (237 versus 170 peptides, Fig. 1E and table S4), with only 5% common to both experimental groups.

We conclude that the cycling of genes and proteins is very different in experimental TLE as compared to control, which makes comparing levels of expression, even at the same time point, more difficult.

Functional analysis

The enriched terms reported from DAVID bioinformatics for oscillating genes found in control condition alone, epileptic condition alone, and oscillating in both conditions for three different p-

values are listed in **Supplementary Tables 5-13**. Many biological pathways, including those linked to metabolism, showed circadian regulation in control and TLE, and specific alterations in TLE as revealed by KEGG pathways gene sets, Gene Ontology biological process, Gene Ontology metabolic function and REACTOME pathway database (**Supplementary Fig 3-14**, **Supplementary Table 14-25**).

In order to show how the resource we provide can be used to predict/test functional consequences, we focus, in this example, on metabolic pathways, which were altered in TLE. Metabolic activity was assessed *in vitro* in the ventral CA1 region of the hippocampus using NAD(P)H imaging in stratum radiatum during stimulation of Schaffer collaterals (**Fig. 3a**). Activation of hippocampal cells by Schaffer collateral stimulation results in characteristic changes in NAD(P)H fluorescence intensity: the initial 'dip' is followed by the 'overshoot' component both of which reflect enhanced oxidation and reduction of the pyridine nucleotides, respectively [26]. In similar experimental conditions, the NAD(P)H fluorescence overshoot amplitude is mainly related to the intensity of cytosolic glycolysis in both astrocytes and neurons [26]. In slices prepared at ZT4, glycolysis was considerably decreased in TLE as compared to control (**Fig. 3a**), as previously reported [27]. However, it was considerably increased in TLE in slices prepared at ZT8 (**Fig. 3a**), as compared to TLE at ZT4 and as compared to control at ZT8 (**Fig. 3a**). Interestingly, glycolysis was also different between ZT4 and ZT8 in control slices, suggesting a circadian control of metabolism in hippocampal neurons (**Fig. 3a**). In keeping with these results, there was a marked increase in the expression of the type 3 glucose transporter specifically at ZT8 in TLE (**Fig. 3b**). Hence, for a cellular process as fundamental as metabolism, different circadian functional modes characterize control and TLE conditions. Whether this is due to a reprogramming of the hippocampal clock remains to be determined.

The circadian rhythmicity can also be appreciated at the system level. Seizures are regulated in a circadian manner in human and experimental mesial TLE [28], a result we confirmed in our mouse model (**Fig. 3c**). One explanation could be that the daily remapping of the molecular landscape brings neuronal networks close to seizure threshold at specific time points [29]. In line with this scenario, we found different seizure susceptibility at different time points between control and TLE animals (**Supplementary Fig. 15**). This further exemplifies the fact that control and TLE animals are subject to different operating modes.

Consequences for antiepileptic drug targets and chronotherapy

Another possible use of such resource is for drug target design and chronotherapy. Many common drugs (e.g. for gastritis, chronic pain, asthma, and rheumatoid arthritis) target products of genes characterized by circadian rhythmicity [21]. We obtained information on drugs and genes from DrugBank database version 5.0.6 [30], which contains information about 8283 drugs including 62 drugs that exert antiepileptic and anticonvulsant effects. In the database, we found mammalian genes encoding 2230 drug targets, 35 drug carriers, 238 drug enzymes and 130 drug transporters. For the 62 antiepileptic/anticonvulsant drugs, we found genes encoding 102 drug targets, 1 drug carrier, 32 drug enzymes and 18 drug transporters.

Among the genes that oscillate in control and TLE animals (JTK_CYCLE adjusted $p < 0.05$), we found 24 and 71 drug targets, 2 and 2 drug carriers, 2 and 7 drug enzymes, and 2 and 9 drug transporters, respectively (**Table 2**). There was little overlap between control and experimental TLE datasets, further highlighting the necessity to take into account the reorganization of gene variations that is specific to experimental TLE. We obtained similar conclusions when looking at genes which expression changes (one-way ANOVA, FDR < 0.05)

in at least one time point in control and TLE animals. We found 20 and 62 drug targets, 2 and 0 drug carriers, 2 and 11 drug enzymes, and 2 and 7 drug transporters, respectively (**Table 2**). **Table 3** provides examples of oscillating and differentially expressed gene products controlled by antiepileptic and anticonvulsant drugs.

Finally, new classes of antiepileptic drugs targeting AMPA and NMDA receptors are being developed [31]. Remarkably, many of the corresponding genes of AMPA and NMDA subunits present in the database showed different expression patterns at specific time points in TLE as compared to control (**Fig. 3d, e**).

Together these results suggest that the efficacy of antiepileptic and anticonvulsant drugs is time-dependent. It is important to stress that drug efficacy testing must be performed in experimental epilepsy models as the time-dependent regulation of the drug targets are different in TLE and control conditions. The resource we provide can be used to assess such factor.

DISCUSSION

We conclude that hippocampal networks undergo a complex daily remapping at the gene and protein level. The number of oscillating genes and proteins is likely to be an underestimate as hippocampal samples were collected every four hours. The remapping may drive hippocampal networks into different functioning modes in a circadian-dependent manner to optimize information processing at specific time points. For example, the remapping may contribute to the circadian regulation of theta oscillations and place cell firing in the hippocampus [32,33], or, if a remapping also occurs in the cortex, to the circadian regulation of cortical excitability in humans [34]. Such oscillations could be a downstream consequence of those occurring in the SCN, and the

daily activation of various hormonal and neuromodulator pathways [8]. Alternatively, the clock could be intrinsic since some hippocampal genes continue to oscillate *ex vivo* [35]. Yet, the daily molecular remapping is important to consider for understanding hippocampal function. Since multiple other damped (secondary) circadian oscillators exist in the brain, each with specific phase properties [36], it is likely that a remapping also exists in other brain regions, each with a specific set of regulated genes and proteins. In addition, a similar resource should be provided for other hippocampal regions (like the dorsal hippocampus), since the molecular architecture of the hippocampus is not homogenous along its septo-temporal axis [22].

One major limitation of our approach is to lump all cell types from the different hippocampal subfields. Future studies should focus on cell-type specific analysis [37], a complex endeavor since it requires instantaneous "freezing" of cells and networks to stop the cycling process at specific times during the night and day cycle.

In epilepsy, we report a large reprogramming of the cycling behavior, with the possible involvement of epigenetic mechanisms [38,39]. It is not yet possible to determine whether this is a cause or consequence of seizures, altered sleep/wake cycles, or just a homeostatic mechanism. However, the important result is that the hippocampus uses a different functional regime in TLE as compared to control. This makes comparing genes and proteins from control and TLE tissues very difficult, as down or up-regulation could just sign different oscillating properties in both conditions (e.g. a phase shift). In fact, if two systems are under two different functioning modes, one can argue that they will have to be studied for themselves, rather than being compared to each other. Based on this novel information, it will be now important to indicate the circadian time a given animal was used in any experimental condition, and to take into consideration a possible reprogramming when studying neurological disorders. Indeed, we can propose that such

reprogramming may occur in most (if not all) neurological disorders [40]. This argument is even more crucial for chronotherapeutics [20], highlighting the necessity to take into account the fact that the expression of drug targets may display different expression time-dependent changes in pathologies as compared to controls.

The time of the day is thus a critical parameter to consider for the proper interpretation of molecular, physiological and behavioral studies, as the functional architecture of the hippocampus may be dynamically regulated in a time-dependent manner. Overall, the present findings have important implications for disease pathogenesis and therapy.

Figure 1. Circadian regulation of genes and proteins in the hippocampus and their reprogramming in epilepsy. (**a**) In control mice (n=4 per Zeitgeber Time – ZT), 1256 transcripts are oscillating. In TLE (n=4 per ZT), more transcripts are oscillating (1650). Only 474 are common to both conditions, showing a remodeling of the landscape of oscillating genes in TLE. (**b**) Right panel. Heatmap of 1105 mRNAs showing circadian regulation. 124 mRNAs were differentially expressed in at least one time point only in control animals, 908 mRNAs were differentially expressed only in TLE and 73 mRNAs were differentially expressed in both groups. Each column per time window represents an individual animal and each row represents an individual mRNA. Colors on the heatmap represent Z-score: higher – red, lower – blue. The hour of tissue collection is indicated below (ZT). The dendrogram obtained from hierarchical clustering is shown on the left side of the heatmap panel. Genes are ordered by clustering complete-linkage method together with Pearson correlation distance measure. Colors in the bar on the left side of the heatmap panel represent clusters obtained by cutting dendrogram at selected level heights to obtain nine groups. Black lines in the bar on the right side of the heatmap panel mark genes showing differences in expression between control or TLE animals based on one-way ANOVA (cut-off FDR < 0.1). Left top panel. Biological functions for each gene cluster, according to Gene Ontology vocabulary using DAVID. Only terms, which are represented by more than 5% of genes in a given cluster, are presented in the Table. Left bottom panel. Transcription factors with binding sites overrepresented in different gene clusters. (**c** and **d**) Oscillation patterns and amplitudes of core clock genes. Note the phase change and general increase in oscillation amplitude in TLE as compared to controls. (**e**) In control mice 170 peptides are oscillating, whilst 337 are oscillating in TLE. Only 11 peptides oscillate in both

conditions, indicating a reprogramming of the molecular daily remapping of the hippocampus in TLE.

Figure 2. Different oscillatory patterns of gene transcripts in control mice and their alterations in TLE. (**a**) Oscillatory patterns of genes, which may contribute to the reprogramming of the circadian hippocampal remapping in TLE. *Creb1* oscillates in both conditions. Key controllers of circadian rhythms, *Bhlhe40* and *Bhlhe41*, show increased oscillation amplitudes in TLE, which may contribute to the large recruitment of oscillating genes in TLE. *Runx1*, a DNA binding regulator, and *Hdac8*, a chromatin remodeler, gain statistically significant oscillation in TLE. *JunD*, which interacts with the AP-1 transcription factor complex and *Creb1* shows a 180° phase shift in TLE as compared to control. (**b**) Phase-Amplitude matrices. Most of the transcripts show high amplitude oscillations in TLE, whilst they are distributed in a bimodal manner in controls between low and high amplitude. The majority of high amplitude oscillatory transcripts are phase advanced as compared to the rest of the oscillatory transcripts, a property more pronounced in TLE.

Figure 3. Alteration of circadian regulation of metabolism and drug targets in TLE. (**A**) (**a**) Averaged NAD(P)H fluorescence changes induced by 10 Hz 30s electrical pulse train stimulation of Schafer collaterals (rectangle) imaged in *Stratum Radiatum* of the hippocampal slices of control and TLE mice. (**a**) **I.** Averaged NAD(P)H fluorescence recorded in TLE (yellow trace) and control (dark gray trace) slices prepared at ZT4. The mean amplitude of the NAD(P)H transient overshoot was significantly smaller in TLE than in control mice (TLE: 1.3±0.17% n=9, control: 3.5±0.5%, n=10, $p<0.01$, dashed arrows) suggesting reduced activity of

cytosolic glycolysis in TLE as compared with control. The decrease in NAD(P)H fluorescence dip amplitude in TLE did not reach statistical significance (TLE: -1.3±0.2%, control: -1.9±0.20%, p=0.2, dotted double-head arrows). **II.** There was no difference in NAD(P)H overshoot in slices prepared at ZT8 in TLE (red trace) and control (grey trace) mice (TLE: 4.1±0.4%, n=6, control: 4.6±0.9%, n=6, p=0.3). However, dip amplitudes were significantly different (TLE: -0.6±0.1%, control: -1.7±0.3%, $p<0.05$). A smaller dip indicates a reduced activity of oxidative phosphorylation or/and an increased activity of cytosolic glycolysis, which rapid onset produces a massive reduction of NAD^+ and masks NADH oxidation in mitochondria. Tthe time of slice preparation had also a significant impact on metabolic activity in both controls (**III**) and TLE (**IV**) conditions. In slices made at ZT8 (grey trace) from control animals, NAD(P)H overshoot amplitude was slightly but significantly larger than in slice made at ZT4 (4.6±0.9%, n=6 vs 3.5±0.5%, n=10, $p<0.05$). The mean amplitudes of the initial dips were similar at both time points (-1.9±0.2% vs -1.7±0,4, p=0.7, n=6). In TLE slices made at ZT8 (red trace) NAD(P)H fluorescence rise started 8±2 s earlier ($n>6$, $p<0.05$) and reached greater levels than in slice made at ZT4 (yellow trace). (**b**) The type 3 glucose transporter shows a different circadian regulation in control and TLE, with a specific significant upregulation at ZT15 in TLE. (**c**) Top. Circadian regulation of seizure incidence during the night and day cycle. The highest seizure probability is found around ZT15. Bottom. Two days after shifting the light/dark cycle by 8 hours in the animal facility, the temporal pattern of seizure incidence shifted accordingly. (**d** and **e)** Alterations of the temporal expression of genes encoding for NMDA and AMPA receptors subunits, respectively.

Table 1: List of genes showing a circadian regulation in control mice, in the hippocampus (Hip), suprachiasmatic nucleus (SCN), Cerebellum (Cer), Brain Stem (Bstm) and Hypothalamus (Hyp). If most core clock genes display circadian regulation in most structures, numerous genes oscillate specifically in the hippocampus.

Table 2: List of genes that oscillate (JTK_CYCLE adjusted $p < 0.05$) or that show changes in expression in at least one time point (one-way ANOVA, FDR < 0.05) in control animals and experimental TLE. Genes are separated in drug targets, carriers, enzymes and transporters. There is little overlap between the control and TLE conditions (common ones are underlined). The ABCC1 is not only transporter for *Phenobarbital* but also a drug target for Sulfinpyrazone and Biricodar dicitrate (in italics). Genes in Bold are shown in table 3.

Table 3: Genes that are drug target (T) or transporters (Tr), and that oscillate (JTK_CYCLE, adjusted p value < 0.05; OSC) or that are differentially expressed (one-way-ANOVA, adjusted p value < 0.05; DE) in control (CTR) or TLE animals. Information on drugs can be found in the Supplemental Material.

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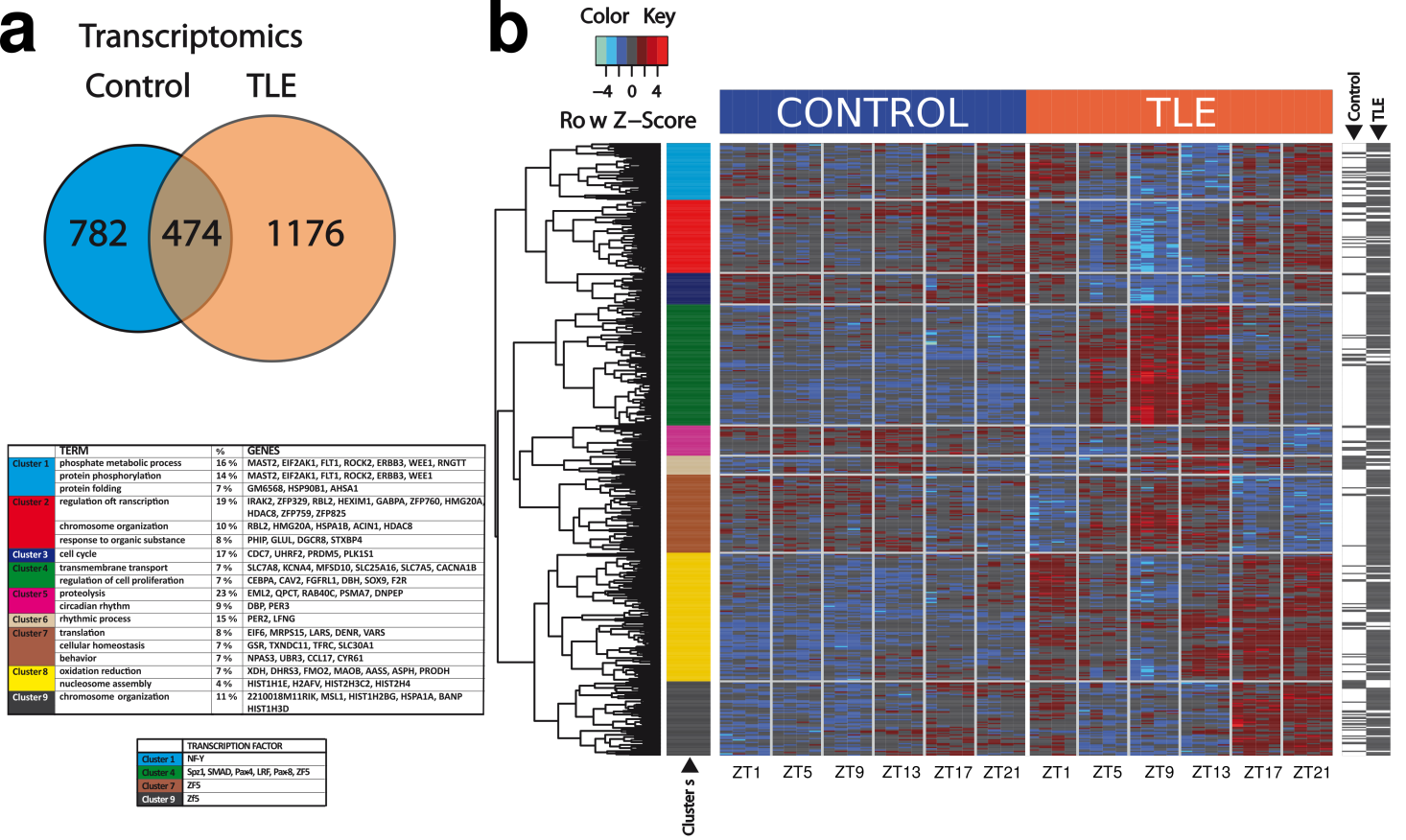
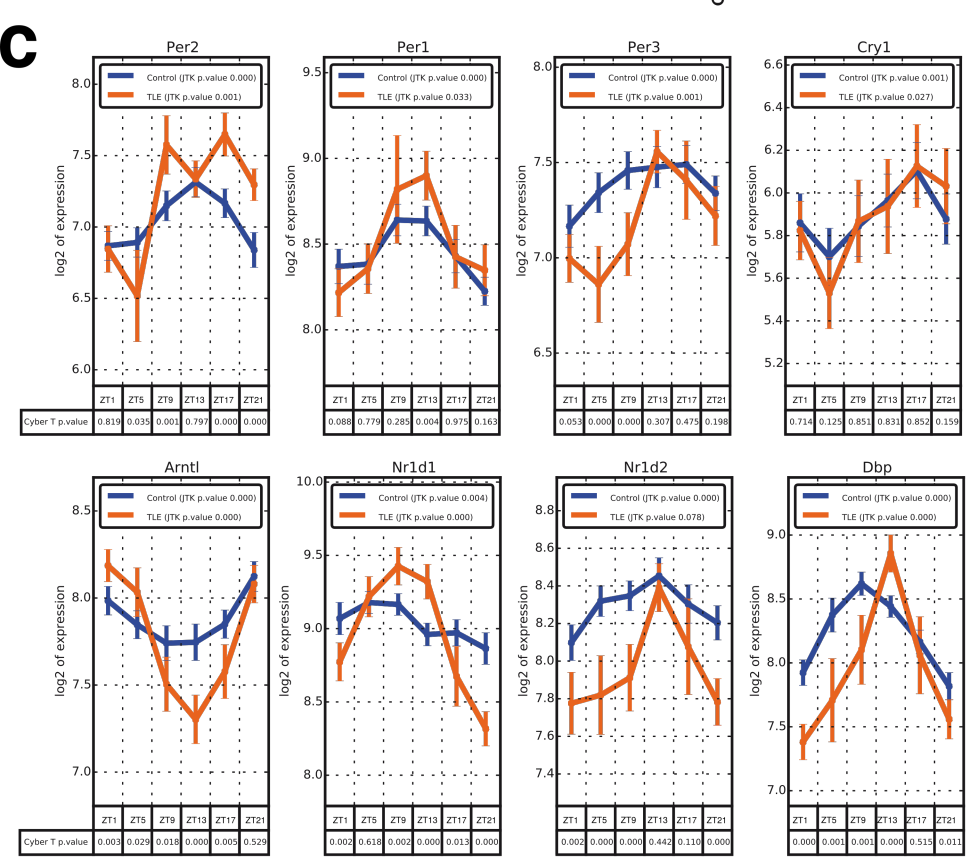
Acknowledgments:

This work was supported by Inserm, and the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no602102 (EPITARGET). WL and SB are supported by the Niedersachsen-Research Network on Neuroinfectiology (N-RENNT) of the Ministry of Science and Culture of Lower Saxony in Germany. KJD and KL are supported by the Polish Ministry of Science and Higher Education grants 888/N-ESF-EuroEPINOMICS/10/2011/0 and W19/7.PR/2014.



	TERM	%	GENES
Cluster 1	phosphate metabolic process	16 %	MAST2, EIF2AK1, FLT1, ROCK2, ERBB3, WEE1, RNGTT
	protein phosphorylation	14 %	MAST2, EIF2AK1, FLT1, ROCK2, ERBB3, WEE1
	protein folding	7 %	GM6568, HSP90B1, AHSA1
Cluster 2	regulation oft rancription	19 %	IRAK2, ZFP329, RBL2, HEXIM1, GABPA, ZFP760, HMG20A, HDAC8, ZFP759, ZFP825
	chromosome organization	10 %	RBL2, HMG20A, HSPA1B, ACIN1, HDAC8
	response to organic substance	8 %	PHIP, GLUL, DGCR8, STXBP4
Cluster 3	cell cycle	17 %	CDC7, UHRF2, PRDM5, PLK1S1
Cluster 4	transmembrane transport	7 %	SLC7A8, KCNA4, MFSD10, SLC25A16, SLC7A5, CACNA1B
	regulation of cell proliferation	7 %	CEBPA, CAV2, FGFRL1, DBH, SOX9, F2R
Cluster 5	proteolysis	23 %	EML2, QPCT, RAB40C, PSMA7, DNPEP
	circadian rhythm	9 %	DBP, PER3
Cluster 6	rhythmic process	15 %	PER2, LFNG
Cluster 7	translation	8 %	EIF6, MRPS15, LARS, DENR, VARS
	cellular homeostasis	7 %	GSR, TXNDC11, TFRC, SLC30A1
	behavior	7 %	NPAS3, UBR3, CCL17, CYR61
Cluster 8	oxidation reduction	7 %	XDH, DHRS3, FMO2, MAOB, AASS, ASPH, PRODH
	nucleosome assembly	4 %	HIST1H1E, H2AFV, HIST2H3C2, HIST2H4
Cluster 9	chromosome organization	11 %	2210018M11RIK, MSL1, HIST1H2BG, HSPA1A, BANP HIST1H3D

	TRANSCRIPTION FACTOR
Cluster 1	NF-Y
Cluster 4	Spz1, SMAD, Pax4, LRF, Pax8, ZF5
Cluster 7	ZF5
Cluster 9	ZF5



d

Gene	Control Amp	Epileptic Amp
Bhlhe40	0.2375081574	0.4140875868
Bhlhe41	0.3839730036	0.8064160334
Arntl	0.3834664913	0.8833480509
Hdac8	0.1094111846	0.4769890427
Runx1	0.1811503567	0.7019302037
Per2	0.4750034544	1.130855182
Cry1	0.4035340666	0.5957495958

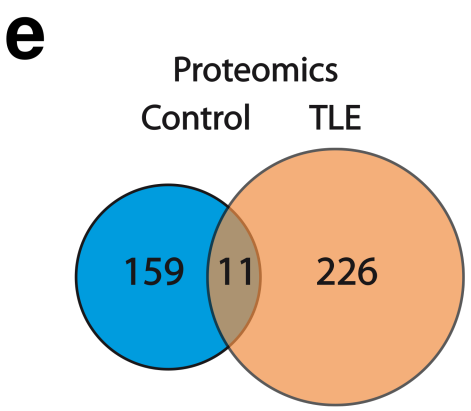
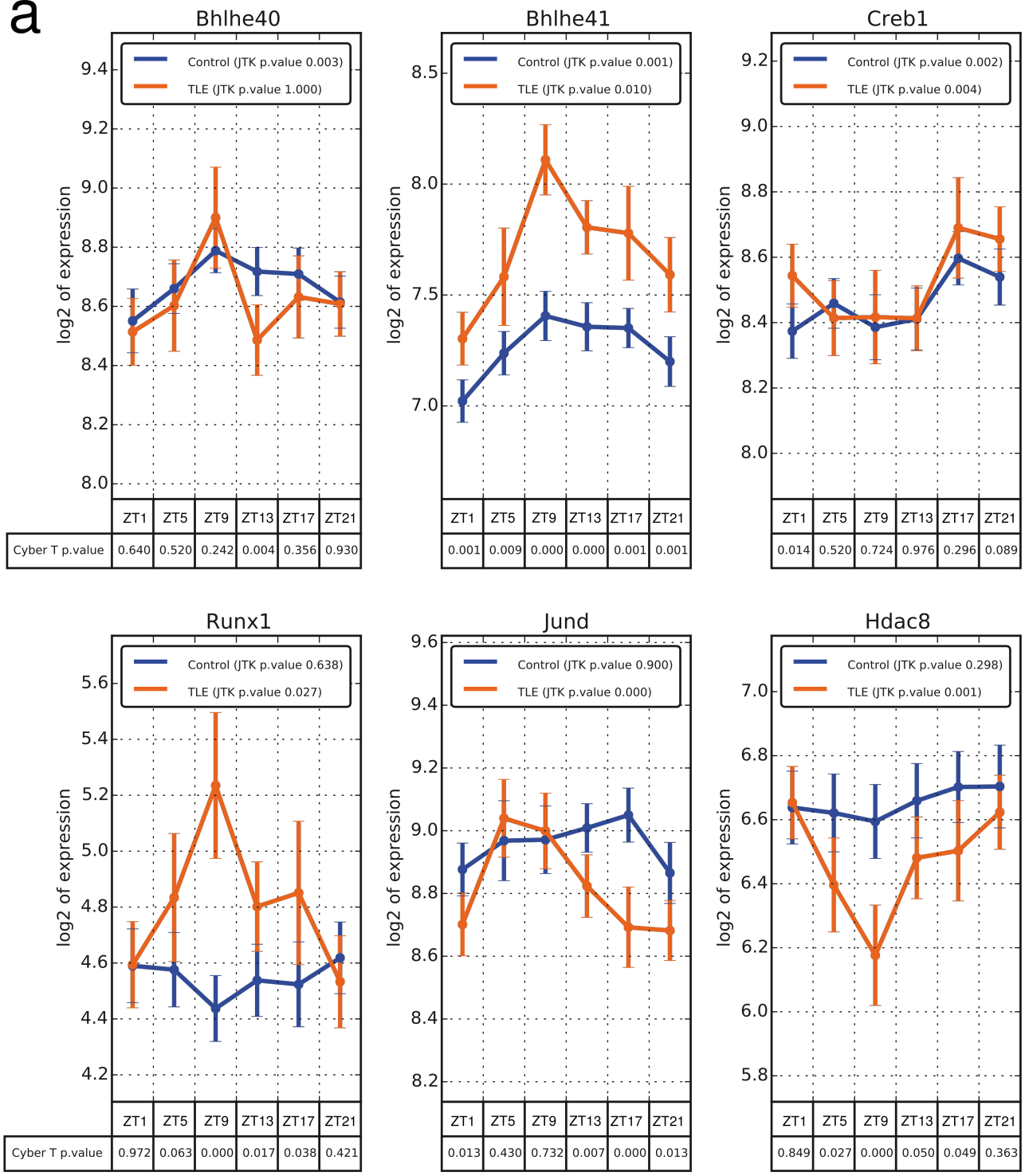


Figure 1

a


Bhlhe40
Control (JTK p.value 0.003)
TLE (JTK p.value 1.000)
log2 of expression
ZT1 ZT5 ZT9 ZT13 ZT17 ZT21
Cyber T p.value 0.640 0.520 0.242 0.004 0.356 0.930
Bhlhe41
Control (JTK p.value 0.001)
TLE (JTK p.value 0.010)
0.001 0.009 0.000 0.000 0.001 0.001
Creb1
Control (JTK p.value 0.002)
TLE (JTK p.value 0.004)
0.014 0.520 0.724 0.976 0.296 0.089
Runx1
Control (JTK p.value 0.638)
TLE (JTK p.value 0.027)
Cyber T p.value 0.972 0.063 0.000 0.017 0.038 0.421
Jund
Control (JTK p.value 0.900)
TLE (JTK p.value 0.000)
0.013 0.430 0.732 0.007 0.000 0.013
Hdac8
Control (JTK p.value 0.298)
TLE (JTK p.value 0.001)
0.849 0.027 0.000 0.050 0.049 0.363

b

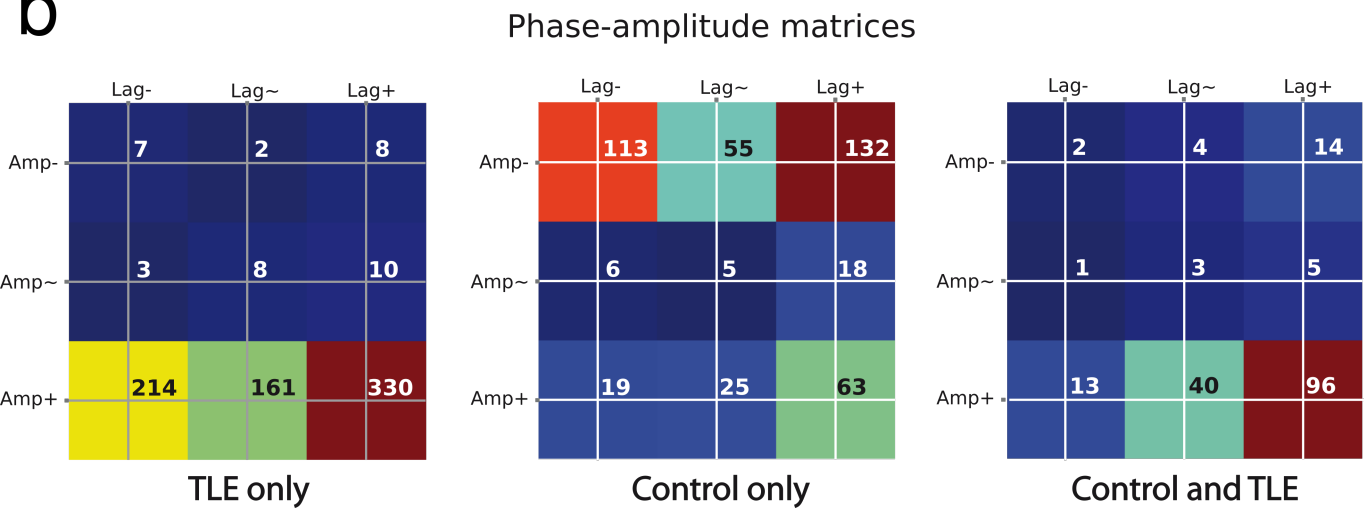

Phase-amplitude matrices
Lag- Lag~ Lag+
Amp- Amp~ Amp+
7 2 8
3 8 10
214 161 330
TLE only
113 55 132
6 5 18
19 25 63
Control only
2 4 14
1 3 5
13 40 96
Control and TLE

Figure 2

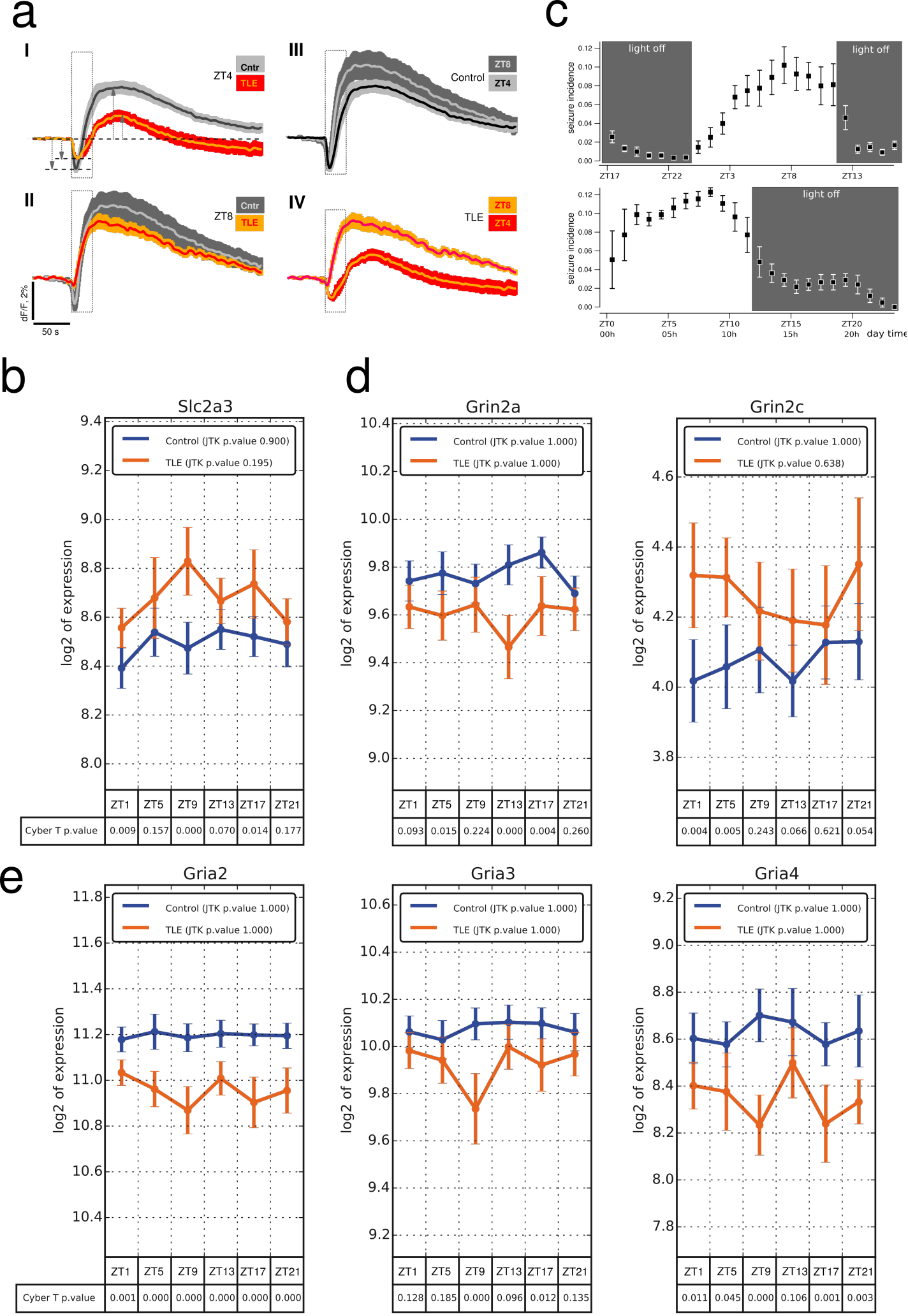

a
I
ZT4
Cntr
TLE
II
ZT8
Cntr
TLE
dF/F, 2%
50 s
III
Control
ZT8
ZT4
IV
TLE
ZT8
ZT4
c
light off
seizure incidence
ZT17
ZT22
ZT3
ZT8
ZT13
ZT0
00h
ZT5
05h
ZT10
10h
ZT15
15h
ZT20
20h
day time
b
Slc2a3
Control (JTK p.value 0.900)
TLE (JTK p.value 0.195)
log2 of expression
ZT1 ZT5 ZT9 ZT13 ZT17 ZT21
Cyber T p.value 0.009 0.157 0.000 0.070 0.014 0.177
d
Grin2a
Control (JTK p.value 1.000)
TLE (JTK p.value 1.000)
ZT1 ZT5 ZT9 ZT13 ZT17 ZT21
0.093 0.015 0.224 0.000 0.004 0.260
Grin2c
Control (JTK p.value 1.000)
TLE (JTK p.value 0.638)
ZT1 ZT5 ZT9 ZT13 ZT17 ZT21
0.004 0.005 0.243 0.066 0.621 0.054
e
Gria2
Control (JTK p.value 1.000)
TLE (JTK p.value 1.000)
ZT1 ZT5 ZT9 ZT13 ZT17 ZT21
Cyber T p.value 0.001 0.000 0.000 0.000 0.000 0.000
Gria3
Control (JTK p.value 1.000)
TLE (JTK p.value 1.000)
ZT1 ZT5 ZT9 ZT13 ZT17 ZT21
0.128 0.185 0.000 0.096 0.012 0.135
Gria4
Control (JTK p.value 1.000)
TLE (JTK p.value 1.000)
ZT1 ZT5 ZT9 ZT13 ZT17 ZT21
0.011 0.045 0.000 0.106 0.001 0.003

Figure 3

Gene	Hip	SCN	Cer	Bstm	Hyp
Dbp	0.0000	NA	0.0000	0.0000	0.0000
Hif3a	0.0000	0.1914	0.0001	0.0028	0.0057
1700084c01rik	0.0000	NA	1.0000	0.0002	0.0028
Folh1	0.0000	1.0000	0.0002	0.0001	0.0013
P4ha1	0.0000	0.0023	0.0112	0.0028	0.0080
Zbtb40	0.0000	NA	0.1654	0.0261	0.0001
Mfsd2a	0.0000	NA	0.0001	0.0035	0.0003
Cirbp	0.0000	0.0000	0.0139	0.0017	0.0004
Arntl	0.0000	0.0006	0.0000	0.0000	0.0000
0610040b10rik	0.0000	NA	0.0261	1.0000	1.0000
Prr5	0.0000	1.0000	0.0090	0.3962	1.0000
Per2	0.0000	0.0000	0.0000	0.0000	0.0000
Mertk	0.0000	1.0000	0.0570	0.0112	0.0003
Fabp7	0.0000	0.0000	0.0000	0.0000	0.0000
Fmo2	0.0000	NA	0.0000	0.0000	0.0000
Smox	0.0000	NA	0.0319	1.0000	1.0000
Sdf2l1	0.0000	NA	0.0687	0.0002	0.0000
Plekho2	0.0000	NA	1.0000	1.0000	1.0000
Rrp8	0.0000	NA	0.2692	0.0000	0.0071
Mmaa	0.0000	NA	0.2494	0.0004	0.0004
Sdc4	0.0000	0.0063	0.0045	0.0007	0.0002
1110002b05rik	0.0000	1.0000	1.0000	1.0000	1.0000
Gm129	0.0000	NA	0.0000	0.0000	0.0002
Per3	0.0000	0.7163	0.0000	0.0000	0.0000
Gpr139	0.0000	NA	1.0000	1.0000	1.0000
4933437f05rik	0.0000	NA	0.0521	0.0005	0.0015
H1f0	0.0000	0.0371	0.0035	0.0319	0.4932
Mrps6	0.0000	NA	NA	NA	NA
Kcnv1	0.0000	NA	0.2494	1.0000	0.3666
Mpzl1	0.0000	NA	0.1397	0.5695	0.0019
Rftn2	0.0000	NA	0.0139	0.3962	0.0057
Flt1	0.0000	1.0000	0.0035	0.0354	0.0010
Rtp1	0.0000	NA	1.0000	1.0000	1.0000
Lfng	0.0000	NA	0.0017	0.0002	0.0021
Hsp90b1	0.0000	NA	0.0001	0.0028	0.0004
Scg2	0.0000	1.0000	1.0000	0.6556	1.0000
Nmnat1	0.0000	NA	0.0319	1.0000	0.1654
Oaf	0.0000	NA	0.5695	1.0000	1.0000
Tsc22d3	0.0000	0.3071	0.0000	0.0000	0.0002
9430083a17rik	0.0000	NA	NA	NA	NA
9230110c19rik	0.0000	NA	0.6556	0.0987	0.9836
Sox2	0.0000	0.0964	0.0319	1.0000	0.4258
Loc100862610	0.0000	NA	NA	NA	NA
Pdia6	0.0000	0.0127	0.0472	0.0002	0.0000
Lpcat3	0.0000	0.9843	0.0570	0.0212	0.0009
Calr	0.0001	0.0551	0.0002	0.0000	0.0000
Slc2a1	0.0001	0.0063	0.0002	0.0017	0.1654
Ago3	0.0001	NA	0.3406	0.0000	0.0064
Zfp410	0.0001	NA	1.0000	0.5695	0.0004
Dio2	0.0001	NA	NA	NA	NA
Eid2	0.0001	NA	1.0000	0.1526	1.0000
Sft2d2	0.0001	NA	0.8616	1.0000	1.0000
Gabpa	0.0001	1.0000	0.0172	0.1397	0.0057
Bphl	0.0001	1.0000	1.0000	1.0000	1.0000
Ankrd13a	0.0001	NA	0.0071	0.0139	0.0001
Hyou1	0.0001	1.0000	0.0472	0.0028	0.4932
Rtp3	0.0001	NA	NA	NA	NA
Wdr78	0.0001	0.1914	0.9836	0.4595	0.0354
Slc38a2	0.0001	0.1622	0.3146	0.0000	0.0000
Loc100505262	0.0001	NA	NA	NA	NA
Tsga10	0.0001	NA	1.0000	0.0035	0.0521
Rasl10a	0.0001	NA	1.0000	0.5313	1.0000
Spry3	0.0001	NA	1.0000	1.0000	1.0000
Kirrel2	0.0001	NA	0.1526	0.0906	0.0319
Gm15453	0.0001	NA	NA	NA	NA
Per1	0.0001	0.0001	0.0007	0.0000	0.0000
Hspa5	0.0001	0.0007	0.0319	0.0002	0.0000
Xdh	0.0001	1.0000	0.0021	0.0687	0.1397
Hist2h4	0.0001	NA	1.0000	0.0172	0.2494
Traf4	0.0001	0.0246	1.0000	0.4932	1.0000
Tmem55a	0.0001	0.2249	0.4595	0.0002	0.0570
Hnrpdl	0.0001	0.0090	0.0987	0.0001	0.4932
Mmp14	0.0001	NA	0.0001	0.0000	0.0000
Hmg20a	0.0001	NA	0.9836	1.0000	0.5695
Cst3	0.0001	0.0667	0.0001	1.0000	0.0825
Kdr	0.0001	0.3568	0.0101	0.0011	0.0035
Atp10a	0.0001	NA	0.0001	0.0006	0.0035
Nop10	0.0001	0.0000	1.0000	1.0000	1.0000
Ormdl1	0.0001	0.0004	0.0388	1.0000	0.0290
Uba6	0.0002	NA	0.1654	0.0001	0.0172
Xbp1	0.0002	0.1622	0.0388	0.0045	0.1176
L3mbtl3	0.0002	NA	1.0000	0.0006	0.0090
Pllp	0.0002	NA	0.0003	0.0000	0.0000
Slco1c1	0.0002	NA	0.0139	1.0000	1.0000
Fam84a	0.0002	NA	1.0000	0.0172	0.0002
Gm10069	0.0002	NA	0.0354	0.2296	0.0051
Pid1	0.0002	NA	0.0388	0.1952	1.0000
Angptl4	0.0002	0.0303	1.0000	0.0430	0.0906
Prkab2	0.0002	NA	1.0000	1.0000	1.0000
Rasal2	0.0002	NA	1.0000	0.0057	1.0000
Opalin	0.0002	NA	0.0000	0.0000	0.0004
Upf2	0.0002	0.1622	1.0000	0.0045	0.1654
Tmc7	0.0002	NA	1.0000	0.6126	0.0987
Jhdm1d	0.0002	NA	0.0035	0.4932	0.0261
Cdk2ap2	0.0002	1.0000	1.0000	1.0000	0.2494
Arxes1	0.0002	NA	0.1397	1.0000	1.0000
Tbc1d4	0.0002	NA	0.9836	0.0629	1.0000
Ndufa1	0.0002	1.0000	1.0000	1.0000	1.0000
Zfp429	0.0002	NA	1.0000	1.0000	1.0000
Glul	0.0002	0.1914	0.4595	1.0000	0.0388
Gm14025	0.0003	NA	NA	NA	NA
Srrt	0.0003	0.0101	0.0261	0.0000	0.0017
Spats1	0.0003	NA	1.0000	1.0000	1.0000
Prtg	0.0003	NA	0.6556	1.0000	1.0000
Cables1	0.0003	NA	0.0001	0.0000	0.0687
B230206f22rik	0.0003	NA	NA	NA	NA
Osbp2	0.0003	NA	1.0000	1.0000	1.0000
Rit1	0.0003	0.0246	1.0000	1.0000	1.0000
Cacna1i	0.0003	NA	1.0000	0.3666	1.0000

Type of action	Oscillating genes Control	Oscillating genes TLE	Expression change Control	Expression change TLE
Target	ADAM10, BPHL, **CACNA1I**, CALR, CRBN, FLT1, FOLH1, GLUL, HSP90B1, HSPA5, IGF1R, KDR, MERTK, MMAA, MMP14, NDUFA1, NDUFA6, NMNAT1, NPEPPS, P4HA1, PRKAB2, RAB8B, SMOX, XDH	***ABCC1***, ACAN, ADRBK1, ADSSL1, AFG3L2, AKT2, ALAS1, ALDH6A1, ALKBH3, ANO1, APRT, BCL2, CD52, CTSK, CYB5R3, CYSLTR2, DDAH2, DHPS, DNPEP, EDNRB, F2R, FASN, FPGS, GARS, GLS, GLUL, GPT2, GRIK5, GRM7, GSR, HCN2, HDAC6, HDAC8, HIF1AN, HRH3, IMPG2, LARS, MAPT, MERTK, MLLT4, MMAA, MMP14, MTRR, NDUFA10, NFKBIA, PDE7A, PFN1, PIK3CA, PRKAB1, PSMA4, PSMA7, PSMB7, PTPRS, QPCT, RAB8B, RNGTT, RPL19, RPS6KA4, RYR2, SGPL1, SHMT1, SLC15A2, SLC16A3, SLC6A9, SLC7A8, STMN4, SULT2B1, TSTA3, TUBD1, VARS, WEE1	ADAM10, BRAF, CALR, CXCR4, FLT1, FOLH1, GLUL, HSP90B1, HSPA5, MERTK, MMAA, NDUFA1, NDUFA6, NFKBIA, P4HA1, PRKAB2, SLC16A1, SMOX, TOP1, XDH	AASS, ***ABCC1***, ADSSL1, AGT, AKT2, ALDH6A1, ALDH7A1, AMT, APRT, ASPH, CACNA1B, CALR, CD44, CYB5R3, CYSLTR2, DBH, DDAH2, DDX6, DHPS, DHRS3, DNPEP, F2R, **GABRA3**, GLUL, GPT2, GSR, GSTM5, HDAC6, HDAC8, HIF1AN, HSP90B1, HSPA5, HSPB1, IMPG2, KCNA4, LARS, **MAOB**, MERTK, MLLT4, MMAA, NCOA5, NDUFA10, NFKBIA, P4HA1, PAICS, PDE7A, PDK4, PRKAB1, PRODH, PSMA7, PTPRS, QPCT, RB1, RNGTT, ROCK2, SERPINF2, SHMT1, SLC7A8, SULT2B1, TUBD1, VARS, WEE1
Carrier	FABP7, SLC2A1	FABP5, SLC2A1	FABP7, SLC2A1	
Enzyme	GLUG, XDH	APRT, FPGS, GLS, GLUL, GSR, HIF1AN, VARS	GLUL, XDH	APRT, CHKA, DBH, GLUL, GSR, HIF1AN, MAOB, PRODH, SRM, TGM1, VARS
Transporter	SLC2A1, **SLCO1C1**	**ABCC1**, ABCC5, SLC15A2, SLC16A3, SLC2A1, SLC2A9, SLC7A5, SLC7A8, TFRC	**SLC16A1**, SLC2A1	**ABCC1**, ABCC5, SLC14A1, SLC2A9, SLC7A5, SLC7A8, TFRC

Table 2

Gene symbol	Function	OSC	DE	Animals	Anticonvulsant drug names
ABCC1	Tr	+	+	TLE	Phenobarbital
CACNA1B	T	-	+	TLE	Gabapentin, levetiracetam
CACNA1I	T	+	-	CTR	Paramethadione, zonisamide, flunarizine
GABRA3	T	-	+	TLE	Meprobamate, metharbital, thiopental, primidone, diazepam, methylphenobarbital, estazolam, fludiazepam, nitrazepam
MAOB	T	-	+	TLE	Zonisamide
SLC16A1	Tr	-	+	CTR	Valproic Acid
SLCO1C1	Tr	+	-	CTR	Phenytoin

Table 3