Pgc-1α and *Nr4a1* Are Target Genes of Circadian Melatonin and Dopamine Release in Murine Retina

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Purpose. The neurohormones melatonin and dopamine mediate clock-dependent/circadian regulation of inner retinal neurons and photoreceptor cells and in this way promote their functional adaptation to time of day and their survival. To fulfill this function they act on melatonin receptor type 1 (MT_1 receptors) and dopamine D4 receptors (D_4 receptors), respectively. The aim of the present study was to screen transcriptional regulators important for retinal physiology and/or pathology (*Dbp*, *Egr-1*, *Fos*, *Nr1d1*, *Nr2e3*, *Nr4a1*, *Pgc-1α*, *Rorβ*) for circadian regulation and dependence on melatonin signaling/MT_1 receptors or dopamine signaling/D_4 receptors.

Methods. This was done by gene profiling using quantitative polymerase chain reaction in mice deficient in MT_1 or D_4 receptors.

Results. The data obtained determined *Pgc-1α* and *Nr4a1* as transcriptional targets of circadian melatonin and dopamine signaling, respectively.

Conclusions. The results suggest that *Pgc-1α* and *Nr4a1* represent candidate genes for linking circadian neurohormone release with functional adaptation and healthiness of retina and photoreceptor cells.

Keywords: retina, circadian regulation, melatonin receptor type 1, dopamine D4 receptor, *Nr4a1*, *Pgc-1α*

The mammalian retina has the ability to adapt to the marked daily changes in the environment. This facilitates the retina, and in particular photoreceptor cells (PRCs), to comply with daily changes in metabolic and functional demands and on a long-term basis may contribute to their survival. Daily adaptation of retinal physiology[1–3] is driven by retinal clocks[4] partly through the rhythmic release of the hormones melatonin from PRCs and dopamine from amacrine cells (for review, see Ref. 5). Melatonin and dopamine play opposing roles in the control of retinal adaptation (for reviews, see Refs. 6, 7). Melatonin release occurs at night and mediates dark adaptation of retina through G-protein receptors named melatonin type 1 (MT_1) and type 2 (MT_2) receptors (for review, see Ref. 8) that appear to function as MT_1/MT_2 heteromers.[9] In this process it modulates visual processing and viability of PRCs.[10–12] On the other hand, dopamine release is higher during daytime[13,14] and promotes adaptation of visual function to light (for reviews, see Refs. 6, 15, 16). Dopamine exerts its effects on various types of dopamine receptors widespread through the retina, but in particular on dopamine D4 (D_4) receptors expressed primarily on PRCs to entrain light-adapted vision including contrast sensitivity function,[12,16,17] the photoreceptor circadian clock in terms of protein phosphorylation,[18] melatonin release,[19] and adenylyl cyclase 1/cAMP signaling.[20]

In the mammalian retina, transcriptional regulators are critical for visual function,[21,22] cell fate determination (for reviews, see Refs. 23, 24), and homeostasis.[25–28] They are also important for retinal cell health and viability. Thus, transcriptional regulators (1) underlie different forms of inherited retinal degeneration in humans, such as *Nr2e3*[29–31] and *Rorβ*,[32] and (2) are protective against retinal dystrophy, such as *Nr1d1*[33] and *Pgc-1α*,[27] or activate immune defense pathways in response to damage of the retina, such as *Egr-1* (*Krox24*; *Ngfi-a*; *Zif268*; Zenk[28,34,35]; *Fos*[34,36] and *Nr4a1* (*Ngfi-b*, *Nur77*[34]). Remarkably, numerous transcriptional regulators display a daily rhythm of expression in the retina (*Dbp*[37]; *Egr-1*[38]; *Fos*[39]; *Nr1d1*[40]; *Nr2e3*[41]; *Nr4a1*[42]; *Pgc-1α*[43]; *Rorβ*[44]). Therefore, their positive role in retinal health may be based on their ability to promote daily adjustment of the retina to comply with ambient demands.

The aim of the present study was to determine transcriptional regulators that may mediate retinal responses to circadian neurotransmitter release in retina of melatonin-proficient mice not carrying *rd* mutations. For this, daily regulation of the transcriptional regulators *Dbp*, *Egr-1*, *Fos*, *Nr1d1*, *Nr2e3*, *Nr4a1*, *Pgc-1α*, *Rorβ* was seen to be driven by a circadian clock, to take place in PRCs, and, most importantly, to depend on melatonin signaling/MT_1 receptors or dopamine signaling/D_4 receptors. As a result, *Pgc-1α* and *Nr4a1* were identified as

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Table 1. Primer Sequences Used for qPCR

Gene	Accession Number	Primer Sequence 5′ to 3′		Length of PCR Product, bp
Dbp	NM_016974.3	Forward	GGAGGTGCTAATGACCTTTG	146
		Reverse	GGACTTTCCTTGCCTTCTTC	
Egr-1	NM_007913.5	Forward	CCCTTCCAGTGTCGAATC	127
		Reverse	CACTCCTGGCAAACTTCC	
Fos	NM_010234	Forward	CTTGGAGCCAGTCAAGAG	141
		Reverse	TAGAAGGAACCGGACAGG	
Gapdh	BC082592	Forward	GTCATCCCAGAGCTGAAC	144
		Reverse	CTCAGATGCCTGCTTCAC	
Nr1d1	NM_145434	Forward	CTGCATCATCGTCATCCTCTTC	150
		Reverse	ACAGTAGCACCATGCCATTC	
Nr2e3	NM_013708	Forward	GCCCTGGTCCTCTTCAAAC	112
		Reverse	GGTGGTGAGCCTTGCTATG	
Nr4a1	NM_010444	Forward	TGATGTTCCCGCCTTTGC	126
		Reverse	GCCATGTGCTCCTTCAGAC	
Nrl	NM_008736	Forward	GTGGAGGAACGGTCCAGATG	149
		Reverse	GAACTGGAGGGCTGGGTTAC	
Pgc-1α	NM_008904	Forward	GTGTTCCCGATCACCATATTCC	101
		Reverse	GGTGTCTGTAGTGGCTTGATTC	
Rorβ	NM_00146095.3	Forward	CCTGGCTGATCGAACCAAG	144
		Reverse	TGCAGACTGCCGTGATAG	
Th	NM_009377.1	Forward	CAGCCCTACCAAGATCAAAC	129
		Reverse	GTACGGGTCAAACTTCACAG	

putative transcriptional targets of the circadian melatonin/dopamine system in murine retina.

Materials and Methods

Animals

Adult male and female mice with intact PRCs not carrying *rd* mutation were used in this study. As a rule the mice used were melatonin proficient (C3H/He (*rd*$^{+/+}$), C3H/f$^{+/+}$MT1$^{+/+}$, C3H/f$^{+/+}$MT1$^{-/-}$, C3H/f$^{+/+}$Drd4$^{+/+}$, and C3H/f$^{+/+}$Drd4$^{-/-}$) and where indicated melatonin deficient (C57BL/6Jb). Mice were genotyped by PCR analysis of genomic DNA. The mice were kept under standard laboratory conditions (illumination with fluorescent strip lights, 200 lux at cage level during the day and dim red light during the night; 20 ± 1°C; water and food ad libitum) under light/dark 12:12 (LD 12:12) for 3 weeks. When indicated, the animals were then kept for one cycle under dim red light and killed during the next cycle. Animals (two for each time point) were killed at the indicated time points by decapitation following anesthesia with carbon dioxide. All dissections during the dark phase were carried out under dim red light. Retinas (four for each time point) were quickly removed, pooled, and immediately frozen or processed for laser microdissection and pressure catapulting (LMPC). Each experiment was carried out four times independently. Animal experimentation was carried out in accordance with the National Institutes of Health Guide on the Care and Use of Laboratory Animals and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and approved by the Institutional Animal Care and Use Committees of Morehouse School of Medicine, Emory University, and the European Communities Council Directive (86/609/EEC).

Laser Microdissection and Pressure Catapulting

To prepare the retinas for LMPC, the HOPE technique (HOPE, Hepes-glutamic acid buffer-mediated organic solvent protection effect; DCS, Hamburg, Germany) was applied for the fixation.[45] Photoreceptor cells were isolated from the stained sections in a contact- and contamination-free manner by using the LMPC technique as described previously.[46] The purity grades of the preparations obtained were verified by using a specific gene marker of PRCs, namely, *Nrl* (as markers for rods[47]), and of inner retinal neurons, namely, *Th* (as a marker for amacrine cells[48]). In comparison to whole-retina preparations, in PRCs collected by LMPC, the ratio of *Nrl* to *Th* was increased 84-fold.

RNA Extraction, Reverse Transcription (RT), and Quantitative Polymerase Chain Reaction (qPCR)

RNA was isolated and reverse transcribed as described previously.[43] In brief, PCR amplification and quantification were performed in an i-Cycler (BioRad, Munich, Germany) according to the following protocol: denaturation for 3 minutes at 95°C, followed by 40 cycles of 30 seconds at 95°C, 20 seconds at 60°C, and 20 seconds at 72°C. All amplifications were carried out in duplicate. By using agarose gel electrophoresis, the generated amplicons for all genes under examination were shown to possess the predicted sizes (Table 1). The amount of RNA was calculated from the measured threshold cycles (C_t) using an internal standard curve with 10-fold serial dilutions (10^1–10^8 copies/μL). The values were normalized with respect to the amount of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA present.

Statistical Analysis

All PCR data are expressed as the mean ± standard error of the mean (SEM) of four independent experiments including eight time points. Transcript levels were calculated relative to average expression of each dataset throughout 24 hours to plot temporal expression. Significance of daily regulation was defined by showing a $P < 0.05$ in ANOVA (one-way analysis of variance). Cosinor analysis was used to fit sine wave curves to the circadian data to mathematically estimate the time of peak gene expression (acrophase) and to assess the amplitude.[49,50] The model can be expressed according to the equation $f(t) = A$

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$+ B \cos [2\pi (t + C) / T]$ with the $f(t)$ indicating relative expression levels of target genes, t specifying the time of sampling (h), A representing the mean value of the cosine curve (MESOR; midline estimating statistic of rhythm), B indicating the amplitude of the curve (half of the sinusoid), and C indicating the acrophase (point of time when the function $f(t)$ is maximum). T gives the time of the period, which is 24 hours for this experimental setting.

Results

Daily Profiling of the Transcriptional Regulators in Whole Retina of Melatonin-Proficient Mouse

With the exception of a recently published study,[37] daily profiling of gene expression in murine retina has so far been performed in mice strains that either are deficient for melatonin or carry *rd* mutation (resulting in damaged PRCs).[51–55] The objective of this study—to investigate melatonin-dependent regulation of genes in the retina including PRCs—requires melatonin-proficient mice with intact PRCs (C3H/He ($rd^{+/+}$)). It was seen that in these mice all transcriptional regulators under investigation display daily changes with peak expression during the day (*Dbp*, *Nr1d1*, *Nr2e3*, *Pgc-1α*) or at night (*Egr-1*, *Fos*, *Nr4a1*, *Rorβ*) (Fig. 1, blue lines; for statistical analysis, see Table 2).

Daily Profiling of the Transcriptional Regulators in PRCs of Melatonin-Proficient Mouse

In order to compare daily regulation of the transcriptional regulators between preparations of the whole retina and PRCs, the LMPC technique was applied.[46] The transcript levels of the genes were observed to exhibit daily rhythms in PRCs (Fig. 1, red lines; for statistical analysis, see Table 2) with profiles resembling those obtained from preparations of the whole retina (Fig. 1, blue lines; for statistical analysis, see Table 2). The findings obtained suggest that daily rhythmicity of the transcriptional regulators also occurs in PRCs. Interestingly, the relative strength of cycling of the genes (indicated in terms of the height of the amplitude) was different in PRCs (*Nr4a1* > *Fos* > *Dbp* > *Nr2e3* > *Pgc-1α* > *Rorβ*) and whole retina (*Nr4a1* > *Fos* > *Rorβ* > *Dbp* > *Nr2e3* > *Pgc-1α* > *Rorβ*). This might reflect different circadian output in outer and inner retina.

Daily Profiling of the Transcriptional Regulators in Retina of Melatonin-Proficient Mice in Constant Darkness

To gain a view of transcriptional control of the retina by a circadian clock, light input, or both, mice (C3H/He ($rd^{+/+}$)) adapted to LD 12:12 were kept in constant darkness (DD) for one cycle and monitored during the subsequent cycle (Fig. 1, black lines; for statistical analysis, see Table 2). Under these conditions, the daily rhythm in mRNA amount continued for all transcriptional regulators investigated (*Dbp*, *Egr-1*, *Fos*, *Nr1d1*, *Nr2e3*, *Nr4a1*, *Pgc-1α*, *Rorβ*). This observation suggests that daily rhythmicity of the transcriptional regulators investigated requires a circadian clock. As far as assessable ($P < 0.05$ in cosinor analysis), the retained rhythmicity of the transcriptional regulators occurred with reduced amplitude under DD conditions when compared with LD conditions with the exception of *Nr1d1*. Therefore clock-dependent rhythmicity of the transcriptional regulators appears to be enhanced by light/dark transitions.

Daily Profiling of the Transcriptional Regulators in Melatonin-Deficient Mice

The retinal clock system—probably in terms of a clock within PRCs[40,46]—evokes a daily rhythm in melatonin release (for review, see Ref. 5). Therefore clock-driven rhythmicity of the transcriptional regulators may be conveyed by melatonin signaling. To test this assumption, daily profiling of the transcriptional regulators in melatonin-proficient mice C3H/He ($rd^{+/+}$) was compared with that of melatonin-deficient C57BL/6Jb mice (Fig. 2, blue versus red lines; for statistical analysis, see Table 2). This revealed that *Pgc-1α* rhythmicity was prevented in melatonin-deficient mice. Conversely, the other transcriptional regulators under investigation (*Dbp*, *Egr1*, *Fos*, *Nr1d1*, *Nr2e3*, *Nr4a1*, *Rorβ*) were found to display daily patterns similar to those being observed in melatonin-proficient mice. These observations suggest that melatonin signaling is a prerequisite for the *Pgc-1α* rhythmicity.

Daily Profiling of the Transcriptional Regulators in Melatonin-Proficient $MT_1^{-/-}$ Mice

To verify and specify the response of the transcriptional regulators to melatonin signaling, their 24-hour profiles were compared in wild-type (WT) and MT_1 receptor-deficient retina (Fig. 3, blue versus red lines; for statistical analysis, see Table 3), with both genotypes deriving from a melatonin-proficient mice strain with intact PRCs (C3H/$f^{+/+11}$). Consistent with the results obtained from melatonin-deficient mice, the loss of MT_1 receptors prevented the cycling of *Pgc-1α*. As far as assessable ($P < 0.05$ in cosinor analysis), it was seen to slightly dampen the rhythmicity of the other transcriptional regulators under investigation (*Dbp*, *Nr1d1*, *Nr2e3*, *Nr4a1*, *Rorβ*). This observation suggests that MT_1 receptors couple the pulsatile melatonin signal to the expression of *Pgc-1α* and possibly play a general role in amplifying circadian regulation of retinal transcription.

Daily Profiling of the Transcriptional Regulators in $Drd4^{-/-}$ Mice

Clock-dependent regulation of the transcriptional regulators may also involve dopamine signaling via dopamine D_4 receptors (for review, see Ref. 5). To investigate this possibility, 24-hour profiling of the transcriptional regulators was performed in *Drd4*-deficient mice (C3H/$f^{+/+}$, melatonin-proficient mice with intact PRCs) (Fig. 4, blue versus red lines; for statistical analysis, see Table 3). The rhythmicity of *Nr4a1* was seen to be dampened in $Drd4^{-/-}$ mice, with a decrease in amplitude of 84%, whereas the other transcriptional regulators under investigation were seen to display similar daily profiles in both the genotypes. This finding suggests that *Nr4a1* links the clock-driven dopamine signaling with retinal gene expression.

Discussion

The retinal clocks exert their influence on visual processing and survival of the retina through the neurohormones melatonin and dopamine and their respective action on MT_1 and D_4 receptors (for review, see Ref. 5). The main objective of this study was to identify candidate genes for linking clock-dependent neurohormone release with daily adaptation and healthiness of the retina. For this purpose, transcriptional regulators were screened for dependence on melatonin signaling/MT_1 receptors or dopamine signaling/D_4 receptors

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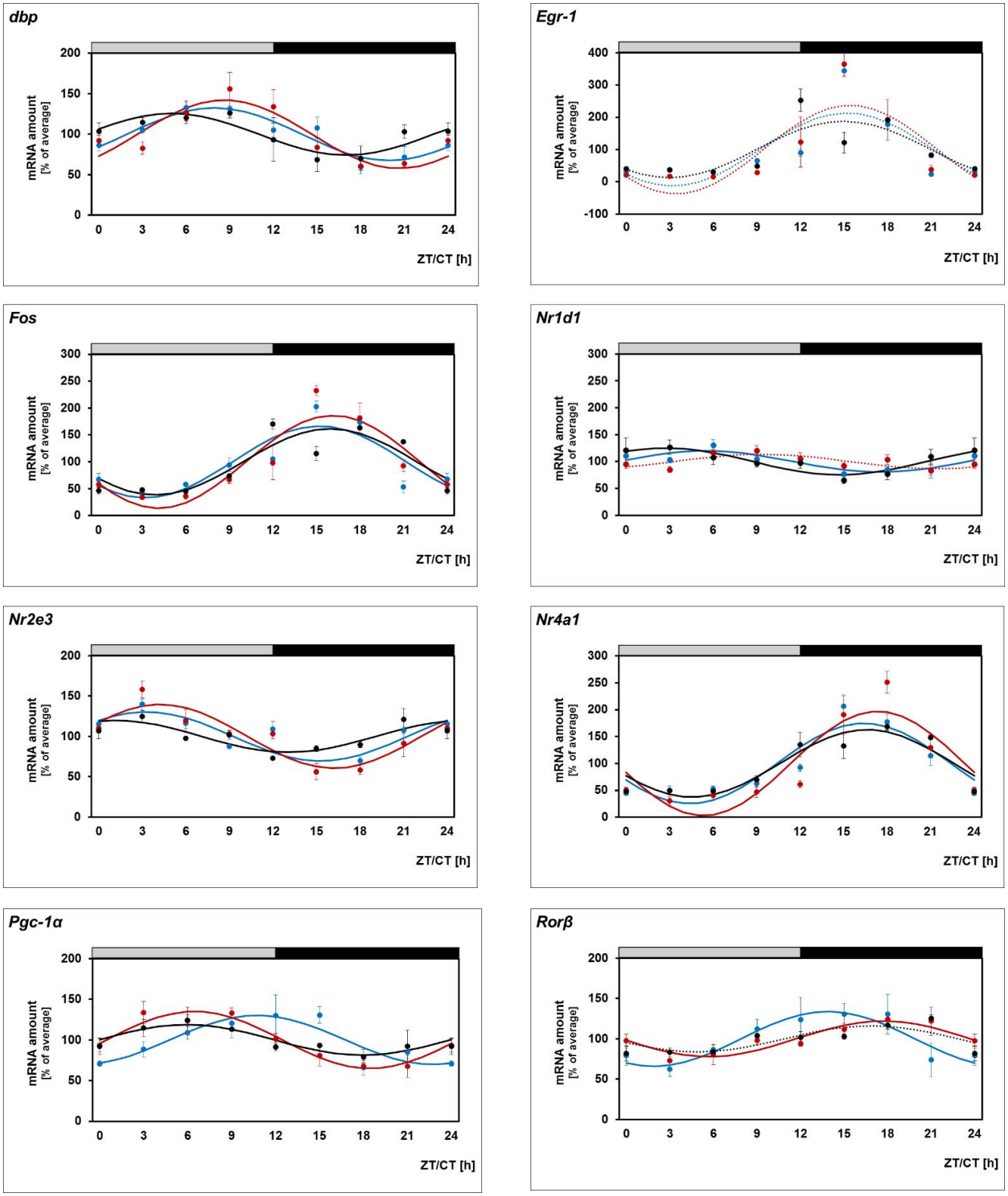

Figure 1. Twenty-four-hour profiling in whole retina and photoreceptor cells. 24-hour profiling of the transcriptional regulators *Dbp*, *Egr-1*, *Fos*, *Nr1d1*, *Nr2e3*, *Nr4a1*, *Pgc-1α*, *Rorβ* in preparations of the whole retina under light/dark (LD) 12:12 (*blue lines*), in microdissected photoreceptor cells under light/dark (LD) 12:12 (*red lines*), and in preparations of the whole retina of mice under constant darkness (DD) (*black lines*). The mRNA levels are plotted as a function of Zeitgeber time (ZT), and the *lines* represent the periodic sinusoidal functions (*solid* and *dotted lines* for $P < 0.05$ and $P > 0.05$ in cosinor analysis). The *solid bars* indicate the dark period. Data represent a percentage of the average value of transcript amount during the 24-hour period. The value of ZT0 was plotted twice at both ZT0 and ZT24. Each value represents mean ± SEM ($n = 4$). Statistical analysis of transcriptional profiling is provided in Table 2.

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Table 2. Statistical Analysis of Transcriptional Profiling Illustrated in Figures 1 and 2

	Retina of Mouse, C3H/He (rd^{++})				PRCs of Mouse, C3H/He (rd^{++})				Retina of Mouse, C3H/He (rd^{++}); DD				WT C57BL/6Jb; LD			
	1-Way ANOVA	Cosinor Analysis			1-Way ANOVA	Cosinor Analysis			1-Way ANOVA	Cosinor Analysis			1-Way ANOVA	Cosinor Analysis		
Gene	*P* Value	*P* Value	Acrophase, h	Amplitude, %	*P* Value	*P* Value	Acrophase, h	Amplitude, %	*P* Value	*P* Value	Acrophase, h	Amplitude, %	*P* Value	*P* Value	Acrophase, h	Amplitude, %
Dbp	< 0.001	< 0.05	7.9	32.4	< 0.001	< 0.05	8.6	42.1	= 0.039	< 0.05	5.0	25.7	< 0.001	< 0.05	8.5	40.5
Egr1	< 0.001	> 0.05	-	-	< 0.001	> 0.05	-	-	< 0.001	> 0.05	-	-	= 0.041	> 0.05	-	-
Fos	= 0.041	< 0.05	15.2	66.5	< 0.001	< 0.05	16.1	86.1	< 0.001	< 0.05	15.9	58.5	= 0.001	< 0.05	17.2	44.4
Nr1d1	= 0.041	< 0.05	5.5	19.6	= 0.032	> 0.05	-	-	= 0.043	< 0.05	2.8	24.8	= 0.047	< 0.05	4.6	26.2
Nr2e3	< 0.001	< 0.05	3.3	30.4	< 0.001	< 0.05	4.2	40.0	< 0.001	< 0.05	1.1	19.7	= 0.036	< 0.05	5.3	23.9
Nr4a1	< 0.001	< 0.05	16.4	77.6	< 0.001	< 0.05	17.4	105.3	< 0.001	< 0.05	16.6	62.7	< 0.001	< 0.05	18.2	55.5
Pgc-1α	= 0.005	< 0.05	10.8	30.0	< 0.001	< 0.05	6.5	35.1	= 0.047	< 0.05	5.9	18.7	= 0.477	> 0.05	-	-
Rorb	= 0.041	< 0.05	13.9	34.0	= 0.020	< 0.05	17.7	22.1	< 0.001	> 0.05	-	-	= 0.006	> 0.05	-	-

Significance of daily regulation was tested by ANOVA. The acrophase (time of peak gene expression) and the amplitude of daily profiles were obtained from cosinor analysis.

Table 3. Statistical Analysis of Transcriptional Profiling Illustrated in Figures 3 and 4

	$C3H/f^{+/+}MT_1^{+/+}$				$C3H/f^{+/+}MT_1^{-/-}$				$C3H/f^{+/+}Drd_4^{+/+}$				$C3H/f^{+/+}Drd_4^{-/-}$			
	1-Way ANOVA	Cosinor Analysis			1-Way ANOVA	Cosinor Analysis			1-Way ANOVA	Cosinor Analysis			1-Way ANOVA	Cosinor Analysis		
Gene	*P* Value	*P* Value	Acrophase, h	Amplitude, %	*P* Value	*P* Value	Acrophase, h	Amplitude, %	*P* Value	*P* Value	Acrophase, h	Amplitude, %	*P* Value	*P* Value	Acrophase, h	Amplitude, %
Dbp	= 0.014	< 0.05	6.7	32.1	< 0.001	< 0.05	6.7	28.8	= 0.013	< 0.05	4.4	43.8	= 0.010	> 0.05	-	-
Egr1	< 0.001	> 0.05	-	-	< 0.001	> 0.05	-	-	< 0.001	> 0.05	-	-	< 0.001	> 0.05	-	-
Fos	< 0.001	> 0.05	-	-	< 0.001	> 0.05	-	-	< 0.001	> 0.05	-	-	< 0.001	< 0.05	14.4	20.5
Nr1d1	< 0.001	< 0.05	7.0	25.0	< 0.001	< 0.05	5.1	21.9	< 0.001	< 0.05	4.6	22.5	< 0.001	< 0.05	3.3	16.5
Nr2e3	< 0.001	< 0.05	3.4	23.5	< 0.001	< 0.05	2.0	21.9	= 0.005	> 0.05	-	-	< 0.001	> 0.05	-	-
Nr4a1	< 0.001	< 0.05	14.1	61.8	< 0.001	< 0.05	14.3	48.7	= 0.040	< 0.05	15.3	46.9	= 0.040	< 0.05	16.5	9.9
Pgc-1α	= 0.001	< 0.05	7.3	25.8	= 0.066	> 0.05	-	-	= 0.001	< 0.05	6.7	16.4	= 0.003	< 0.05	10.6	16.6
Rorb	= 0.017	< 0.05	15.9	23.9	< 0.001	< 0.05	15.3	22.2	= 0.011	> 0.05	-	-	= 0.041	< 0.05	18.0	27.4

Significance of daily regulation was tested by ANOVA. The acrophase (the time of peak gene expression) and the amplitude of daily patterns were obtained from cosinor analysis.

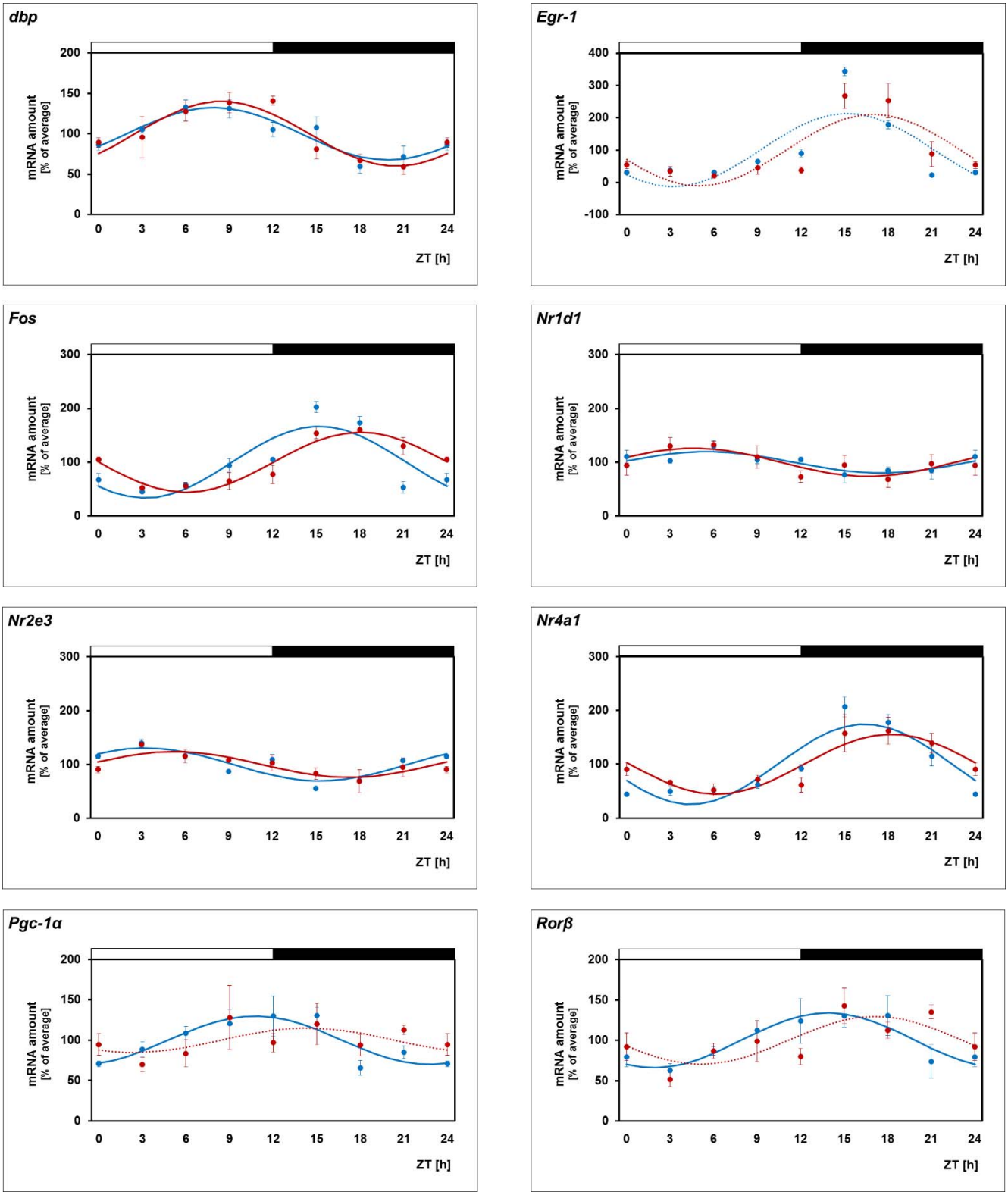

FIGURE 2. Daily profiling in melatonin-proficient and -deficient mice. Daily profiling of the transcriptional regulators *Dbp*, *Egr-1*, *Fos*, *Nr1d1*, *Nr2e3*, *Nr4a1*, *Pgc-1α*, *Rorβ* in retina of melatonin-proficient C3H/He (rd^{++}) mice (*blue lines*, corresponding to those in Fig. 1) and melatonin-deficient C57BL/6Jb mice (*red lines*). The mRNA levels are plotted as a function of Zeitgeber time (ZT), and the *lines* represent the periodic sinusoidal functions (*solid* and *dotted lines* for $P < 0.05$ and $P > 0.05$ in cosinor analysis). The *solid bars* indicate the dark period. Data represent a percentage of the average value of transcript amount during the 24-hour period. The value of ZT0 was plotted twice at both ZT0 and ZT24. Each value represents mean ± SEM ($n = 4$). Statistical analysis of transcriptional profiling is provided in Table 2.

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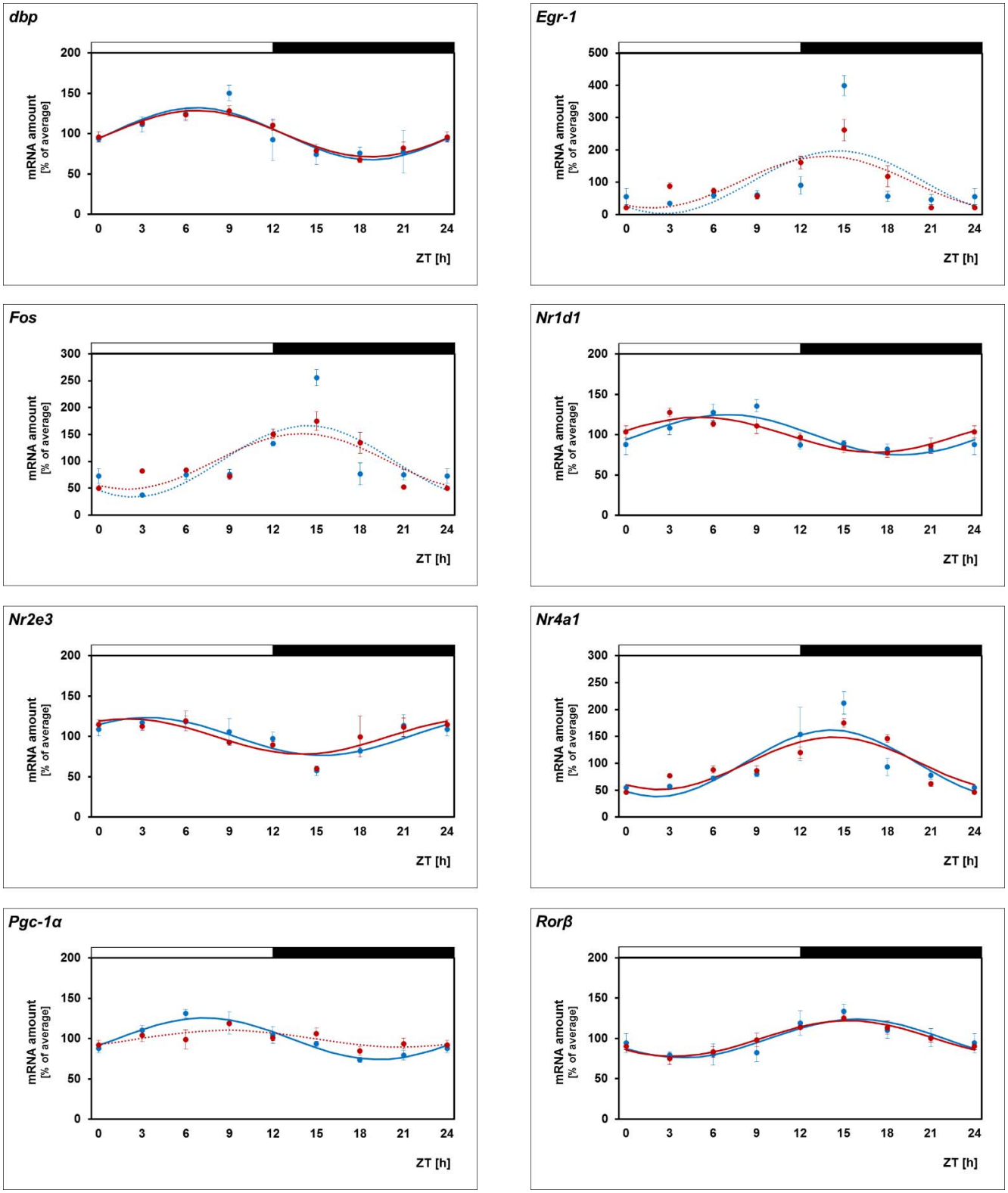

FIGURE 3. Twenty-four-hour profiling in WT and $MT_1^{-/-}$ mice. 24-hour profiling of the transcriptional regulators *Dbp*, *Egr-1*, *Fos*, *Nr1d1*, *Nr2e3*, *Nr4a1*, *Pgc-1α*, *Rorβ* in retina of WT (*blue lines*) and $MT_1^{-/-}$ mice (*red lines*). The mRNA levels are plotted as a function of Zeitgeber time (ZT), and the *lines* represent the periodic sinusoidal functions (*solid* and *dotted lines* for $P < 0.05$ and $P > 0.05$ in cosinor analysis). The *solid bars* indicate the dark period. Data represent a percentage of the average value of transcript amount during the 24-hour period. The value of ZT0 was plotted twice at both ZT0 and ZT24. Each value represents mean ± SEM ($n = 4$). Statistical analysis of transcriptional profiling is provided in Table 3.

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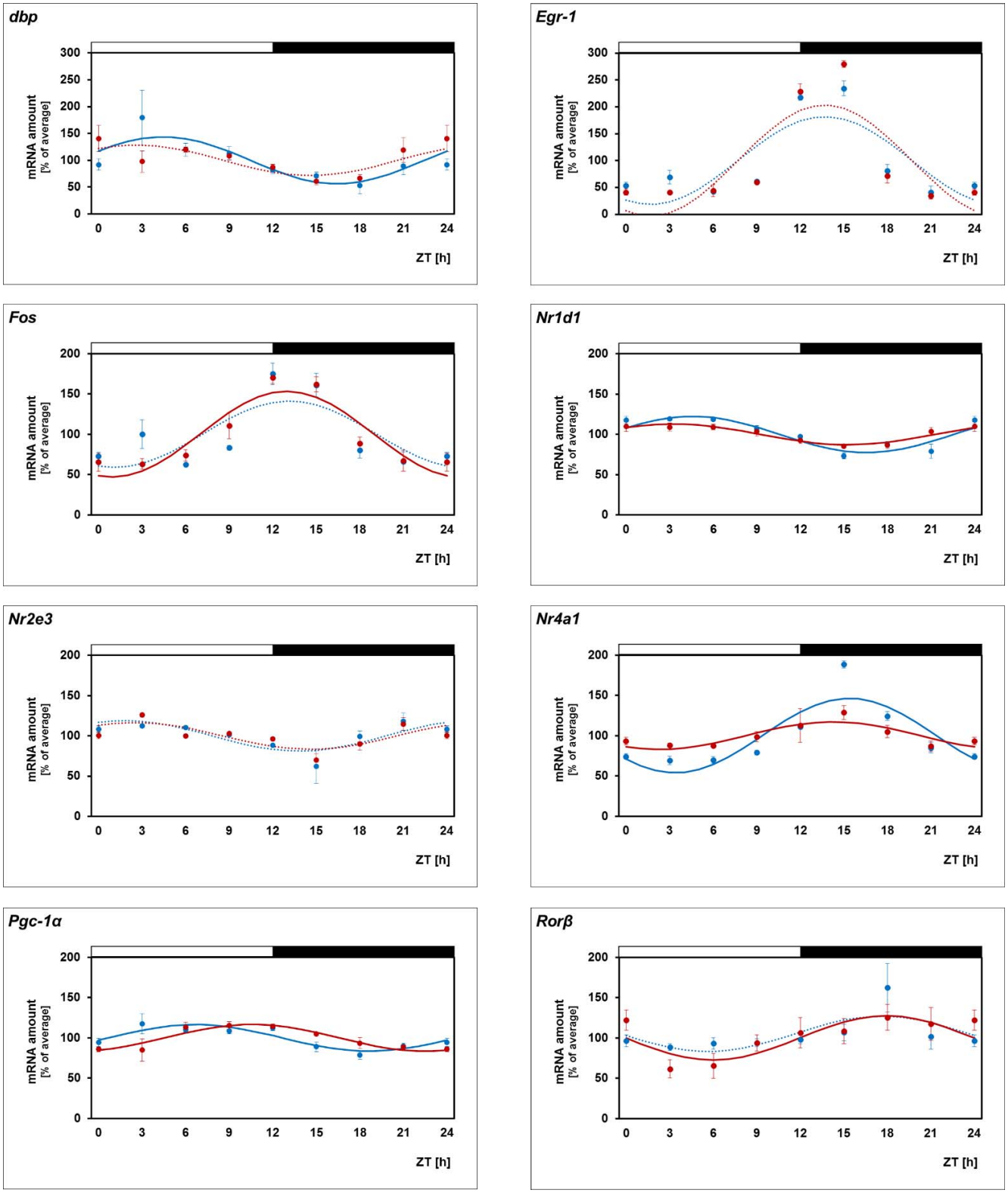

FIGURE 4. Twenty-four-hour profiling in WT and Drd4$^{-/-}$ mice. 24-hour profiling of the transcriptional regulators *Dbp*, *Egr-1*, *Fos*, *Nr1d1*, *Nr2e3*, *Nr4a1*, *Pgc-1α*, *Rorβ* in retina of WT (*blue lines*) and Drd4$^{-/-}$ mice (*red lines*). The mRNA levels are plotted as a function of Zeitgeber time (ZT), and the *lines* represent the periodic sinusoidal functions (*solid* and *dotted lines* for $P < 0.05$ and $P > 0.05$ in cosinor analysis). The *solid bars* indicate the dark period. Data represent a percentage of the average value of transcript amount during the 24-hour period. The value of ZT0 was plotted twice at both ZT0 and ZT24. Each value represents mean ± SEM ($n = 4$). Statistical analysis of transcriptional profiling is provided in Table 3.

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that fulfill the requirements to (1) cycle in PCRs (*Dbp*[37]: Fig. 1; *Egr-1*: Fig. 1; *Fos*, *Nr1d1*[40]: Fig. 1; *Nr2e3*: Fig. 1; *Nr4a1*: Fig. 1; *Pgc-1α*[43]: Fig. 1; *Rorβ*[40]: Fig. 1), (2) be driven by a circadian clock (*Dbp*[37]: Fig. 1; *Egr-1*[39]: Fig. 1; *Fos*[39]: Fig. 1; *Nr1d1*[40]: Fig. 1; *Nr2e3*: Fig. 1; *Nr4a1*: Fig. 1; *Pgc-1α*: Fig. 1; *Rorβ*[32]: Fig. 1), and (3) influence retinal physiology or pathology (*Egr-1*, *Fos*[34,36]; *Nr1d1*[33]; *Nr2e3*[29–31]; *Nr4a1*[34]; *Nr1d1*: *Pgc-1α*[27]; *Rorβ*[32]). As our experimental system we used mice with targeted deletion of MT_1 receptors and D_4 receptors, respectively, that we have crossed onto the $C3Hf^{+/+}$ background.[56,57] Thus the mouse model used in this study combines physiological melatonin formation and intact photoreceptors.

The data obtained determine the transcriptional coactivator *Pgc-1α* as a transcriptional target of circadian melatonin/MT_1 receptor signaling. This becomes evident from the finding that the daily rhythmicity of *Pgc-1α* mRNA amount was dampened in both melatonin-deficient (C57BL/6Jb) and $MT_1^{-/-}$ mice.

Melatonin/MT_1 receptor-dependent control of *Pgc-1α* may take place in PRCs. This follows from the observation that PRCs combine a prominent rhythmicity of *Pgc-1α* (this study) with high density of MT_1 receptors.[11,12] Of interest is that, in PCRs, melatonin appears to act as an autocrine signal.[11] Therefore PRCs may regulate their own *Pgc-1α* expression by melatonin release and its subsequent action on MT_1 receptors located at their cell membrane. Recent studies have shown that the action of melatonin in the PRCs is mediated by MT_1/MT_2 heteromers.[9] Therefore, MT_1 receptor deficiency may result in nonfunctional MT_1/MT_2 heterodimers, and MT_2 knockout mice would have produced similar results from those obtained in MT_1.

Pgc-1α is a master regulator of glucose and energy metabolism in various tissues (for review, see Ref. 58) including retina.[27] Therefore the observed melatonin/MT_1 receptor-dependent regulation of *Pgc-1α* may contribute to daily changes in the energy metabolism of the retina and thus to its ability to comply with 24-hour changes in metabolic demands.[59]

Remarkably, *Pgc-1α* knockout mice reportedly suffer from increased light damage susceptibility, overexpression of *Pgc-1α* has antiapoptotic effects, and *Pgc-1α* expression is reduced in different mouse models of retinitis pigmentosa.[27] This suggests that *Pgc-1α* provides protection against light damage and is therefore a promising candidate gene for transferring the positive effect of melatonin/MT_1 receptors on the viability of retinal PRCs and ganglion cells.[11] Accordingly, melatonin/MT_1 receptor-dependent control of *Pgc-1α* could contribute to the protection of retinal cells against light damage and on a long-term basis to the survival of retinal cells during aging.

Outside the retina, MT_1 receptor-dependent expression of *Pgc-1α* could play a role in the pathogenesis of type 2 diabetes. This becomes evident from the observations that the removal of MT_1 receptors[60] or *Pgc-1α* (for review, see Ref. 61) equally leads to increased insulin resistance in peripheral tissues.

As with *Pgc-1α* in the melatonergic system, the nuclear orphan receptor *Nr4a1* (Nur77, Ngfi-b) was identified to respond to the dopaminergic system. This is evident from the finding that the daily rhythmicity of *Nr4a1* is reduced in $Drd4^{-/-}$ mice. Dopaminergic regulation of retinal *Nr4a1* probably occurs in PRC since this cell type combines circadian regulation of *Nr4a1* (this study) with the abundance of D_4 receptors.[62,63] In the retina, clock-dependent dopamine release occurs from the unique population of cells in the inner nuclear layer that are either amacrine or interplexiform neurons.[15] The dopamine neurons in retina express a full complement of core circadian clock genes.[64,65] Therefore circadian/dopaminergic control of *Nr4a1* in PRCs appears to be driven by these cell types. Furthermore, *Drd4*, the gene that encodes the D_4 receptor, is itself a circadian clock-controlled gene in retina,[3,20,42,66] and this too may contribute to circadian regulation of *Nr4a1*.

Dopaminergic regulation of *Nr4a1* was until now believed to be confined to the motor/motivation system (for reviews, see Refs. 67, 68). Therefore its concurrent occurrence in the visual (this study) and the motor/motivation system suggests that it appears to be a more general feature of different functional brain systems affected by dopamine.

Furthermore, *Nr4a1* is a promising candidate gene for being responsible for dysfunction of the retinal dopaminergic system and its subsequent visual defects in type I diabetes mellitus.[69] This becomes evident from the observations that *Nr4a1* expression on one hand influences the functioning of the dopaminergic system (for reviews, see Refs. 67, 68) and on the other hand depends on glucose metabolism.[70–72]

Compelling experimental evidence indicates that *Nr4a1* not only contributes to dopaminergic responses but also is a decisive factor in dopamine-related neuroadaptation (for reviews, see Refs. 67, 68). If this is also valid in retina, circadian/dopaminergic regulation of *Nr4a1* expression (this study) may feed back to the dopaminergic system of the retina to promote its reported circadian plasticity.[42]

It is important to note that—at variance with previous studies dealing with the role of melatonin in retina[51,52]—the present investigation was performed in mouse strains/genotypes that combine melatonin proficiency with intact PRCs and thus in mouse models that reflect retinal physiology as closely as possible. On the other hand, a general limitation of the present study is that the observed phenotypes of the mouse models used may reflect not only a direct action of the neurotransmitters/receptors, but also the secondary effects downstream of them. Since melatonin signaling[73–75] and dopamine signaling[76–78] make a cellular feedback loop in which melatonin inhibits the release of dopamine through melatonin receptors,[73–75] whereas dopamine downregulates melatonin formation, probably through dopamine D_4 receptors,[76,77] this includes a possibility that genetic disturbance of melatonin signaling affects dopamine signaling and vice versa.

In conclusion, the data of the present investigation determined candidate transcriptional regulators for linking the circadian neurohormone systems to adaptation, function, and healthiness of the retina. They could provide a reliable basis for further investigations that may achieve a better understanding of how clock-dependent neurotransmitter release influences visual processing and survival of the retinal neurons.

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