Circadian Rhythms in Mice: Entrainment of Peripheral Serotonin and Platelets

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Abstract

Most organisms are known to possess biological clocks, which control and coordinate numerous physiological processes over each 24-hour day. Circadian oscillators play a role in generating biological rhythms and coordinating numerous processes with environmental stimuli, such as timing of a meal or exposure to light. The indolamine molecule, serotonin, is an important peripheral hormone produced by the intestinal mucosa of mammals, but its regulation as an output of the circadian clock is not well understood. Recent studies in our lab have investigated circadian rhythmicity of serotonin and its entrainment to light stimuli or food availability in various tissue compartments in mice, including blood serum, stools, and the intestinal wall. Because most serotonin is released into the blood and taken up by platelets, we investigated regulation of circulating platelet levels as well. In these experiments, mice were fed ad libitum (AL) or placed on a gradual daytime restricted feeding regimen (DRF) while maintained in a 12:12 light-dark cycle (LD) or constant darkness (DD). We assessed serotonin levels in duodenum, colon, and stool and demonstrated a high-amplitude circadian rhythm of serotonin in stool samples that persisted in constant conditions and entrained to both light and food availability, with a peak occurring close to the day-night transition under LD conditions. In contrast to some published findings, no circadian rhythm of serotonin was detected in blood serum. Serotonin levels from duodenum and colon also exhibited food-entrainable circadian rhythms, peaking in the early morning under LDRF. mRNA levels of tph 1, the rate-limiting enzyme for non-neuronal serotonin biosynthesis, was not shown to be rhythmic or food-entrainable in the duodenum, although levels peaked approximately 16 hours prior to the peak in serotonin. The delayed peak in serotonin may reflect the kinetics of protein synthesis and turnover, as well as rising levels of serotonin transporter (SERT) measured in the late evening under RF conditions. Interestingly, a circadian rhythm in total platelet number was strongly entrained to cycles of food availability, but not to light.

Methods and Objectives

- Mice were entrained to 12:12 LD cycle and either 1) maintained under ad libitum feeding (AL) or 2) switched to a progressive DRF regime over a 12 day period
- After 1 week under AL or 4-hour DRF regime, stool and tissue samples (duodenum, liver and blood) were collected every 4 hours for a 24-hour period under 1) LD conditions or 2) after 3 days under DD conditions
- Serum 5HT levels were measured using a commercial ELISA kit (*Enzo Life Sciences*)
- Duodenal and colonic 5HT levels were measured using LC/MS/MS
- Platelets were counted manually with hemocytometer
- *mper1*, *tph1*, and *sert* gene expression was measured in liver or duodenal tissue samples using qPCR
- Data were subjected to cosinor analysis (*CircWave v1.4*) or one-way ANOVA to test for statistical significance of rhythmicity and to assess circadian rhythm parameters

Progressive DRF protocol

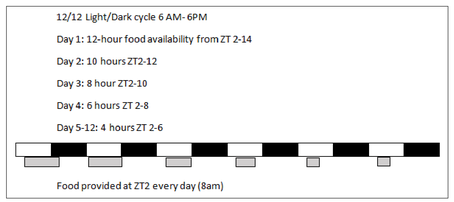


Fig. 1 Model of rodent circadian clock

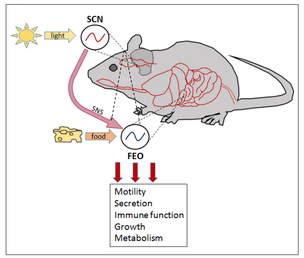


Fig. 2 Model of circadian oscillator transcriptional feedback loop

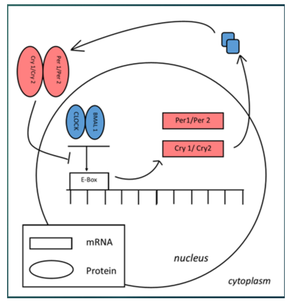


Fig. 3 Peripheral oscillators (e.g. in intestinal mucosa)

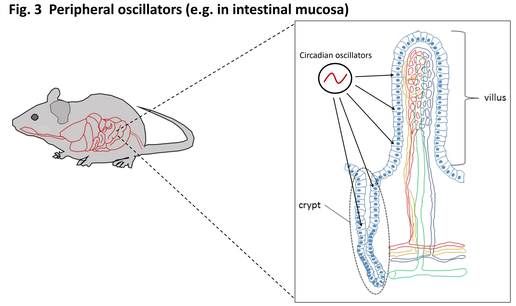
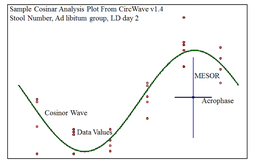


Fig. 4 Cosinor statistical analysis



Platelet Counts

(n = 8/timepoint)

Figure 8. Total platelet levels

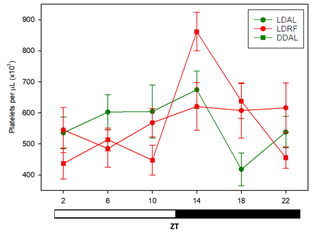


Table 4. Cosinor analysis (platelets)

Treatment	Cosinor p	ANOVA p
LD AL	0.112	0.088
LD RF	0.322	0.753
DD RF	<0.001	<0.001

Food consumption and Motility

(n = 5/timepoint)

Figure 5. Food Consumption

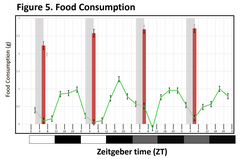


Table 1. Cosinor analysis (food consumption)

Feeding Group	Light Cycle	Acrophase	p-value
AL	LD 1	21.285 (±2.373)	p < 0.001
AL	LD 2	18.284 (±1.538)	p < 0.001
AL	DD 1	20.356 (±2.59)	p < 0.001
AL	DD 2	18.907 (±2.779)	p < 0.001

Figure 6. GI Motility (Stool Weights)

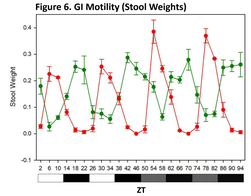


Table 2. Cosinor analysis (stool weights)

Feeding Group	Light Cycle	Acrophase	p-value
AL	LD 1	19.293 (±2.545)	p < 0.001
AL	LD 2	19.024 (±2.004)	p < 0.001
AL	DD 1	20.306 (±2.749)	p < 0.001
AL	DD 2	18.657 (±2.502)	p < 0.001
RF	LD 1	8.744 (±1.457)	p < 0.001
RF	LD 2	9.519 (±1.630)	p < 0.001
RF	DD 1	9.406 (±1.413)	p < 0.001
RF	DD 2	8.793 (±1.308)	p < 0.001

Figure Legend

- Ad Libitum
- Restricted Feeding
- Day
- Night
- Subjective Day
- Subjective Night
- Period of feeding in RF group

Figure 7. GI Motility (Stool Number)

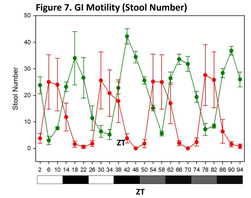
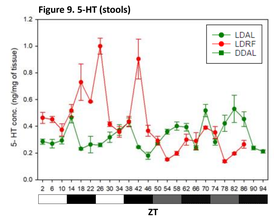


Table 3. Cosinor analysis (stool number)

Feeding Group	Light Cycle	Acrophase	p-value
AL	LD 1	20.248 (±2.551)	p < 0.001
AL	LD 2	19.162 (±2.088)	p < 0.001
AL	DD 1	21.629 (±3.055)	p < 0.001
AL	DD 2	18.829 (±2.602)	p < 0.001
RF	LD 1	8.510 (±1.293)	p < 0.001
RF	LD 2	9.012 (±1.464)	p < 0.001
RF	DD 1	8.418 (±1.238)	p < 0.001
RF	DD 2	8.170 (±1.157)	p < 0.001

Figure 9. 5-HT (stools)



5-HT

(n = 8/timepoint)

Table 5. Cosinor analysis (5-HT, stools))

Treatment	Cosinor p	ANOVA p
LD AL	<0.001	<0.001
LD RF	<0.001	<0.001
DD AL	0.014	0.049
DD RF	<0.001	<0.001

Figure 10. 5-HT (duodenum, LDRF)

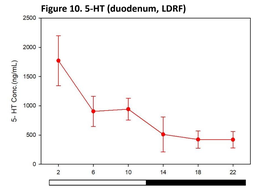
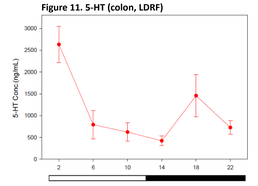


Table 6. Cosinor analysis (5-HT, stools))

Tissue	Cosinor p	ANOVA p
duodenum	0.012	0.006
colon	0.010	<0.001

Figure 11. 5-HT (colon, LDRF)



Gene Expression

(n = 4/timepoint)

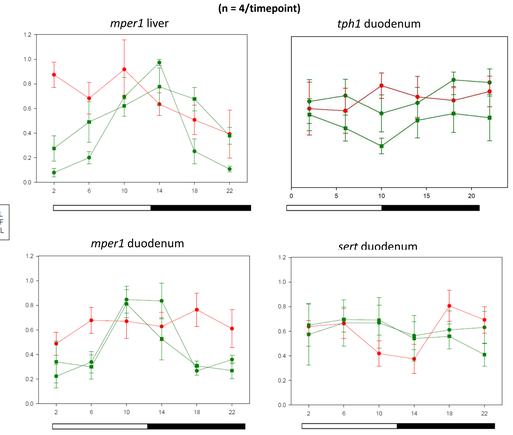


Table 7. Cosinor analysis (gene expression)

Tissue	Gene target	Treatment	Cosinor p	ANOVA p
Liver	mper1	LD AL	<0.001	<0.001
Liver	mper1	LD RF	0.105	0.181
Liver	mper1	DD AL	0.004	0.049
Duodenum	mper1	LD AL	<0.001	<0.001
Duodenum	mper1	LD RF	0.604	0.752
Duodenum	mper1	DD AL	0.020	0.029
Duodenum	tph1	LD AL	0.321	0.681
Duodenum	tph1	LD RF	0.850	0.931
Duodenum	tph1	DD AL	0.386	0.750
Duodenum	sert	LD AL	0.999	0.997
Duodenum	sert	LD RF	0.066	0.075
Duodenum	sert	DD AL	0.282	0.646

Conclusions

- High amplitude, food entrainable circadian rhythms in 5-HT were observed in stools, duodenum, and colon, with peak levels during the early morning (ZT2) under LD.
- No 5-HT rhythm detected in blood serum (data not shown).
- A food-entrainable rhythm of *sert*, but not *tph1*, was detected in duodenum, suggesting circadian regulation of transport rather than biosynthesis of serotonin.
- An apparent antiphasic pattern in *tph1* expression between light-entrained/free running and DRF conditions suggests tph1 and serotonin synthesis may be weakly regulated by the clock.
- Circulating platelet levels were rhythmic and strongly entrained by food, whereas lower amplitude rhythm was apparent under LD conditions; chronodisruptive effect was also observed in LD/RF mice.
- We conclude that food availability is a stronger zeitgeber for entrainment of rhythms in peripheral serotonin and abundance of total circulating platelets .

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