Circadian Rhythms in Mice: Entrainment of Peripheral Serotonin and Platelets

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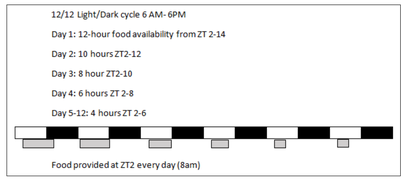
Abstract

Most organisms 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 my lab have investigated circadian rhythmicity of serotonin and its entrainment to light stimuli or food availability in various tissues or compartments in mice, including blood serum, stools, and the intestinal wall. 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). Using a repeated measures design, we 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. Preliminary data suggest that duodenal serotonin is rhythmic in LD and peaks later during the late night. This is consistent with our measurements of tph1 mRNA rhythms, which peaked during the late night in LD or DD, respectively. Taken together, these data suggest that peripheral serotonin is differentially regulated by the circadian clock in different compartments, and the rhythm of serotonin in stools is likely contributed to by oscillators outside the duodenum.

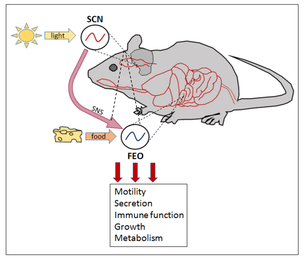
Methods and Objectives

- 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 5HT levels were measured by LC/MS/MS
- Platelets were counted manually with hemocytometer
- *mper1*, *tph1*, and *sert* gene expression was measured in liver and duodenal tissue samples from using qPCR
- Data were subjected to cosinor analysis using *CircWave v1.4* to test for statistical significance of rhythmicity and to assess circadian rhythm parameters

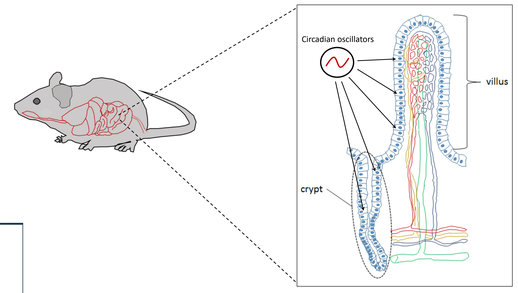
Progressive DRF protocol



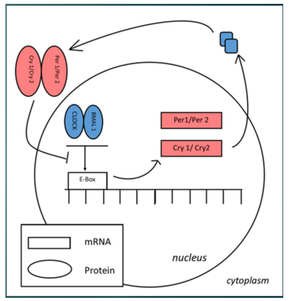
Model of rodent circadian clock



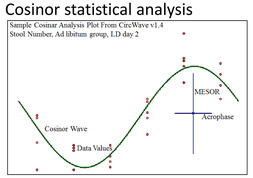
Oscillators in intestinal mucosa



Model of circadian oscillator transcriptional feedback loop

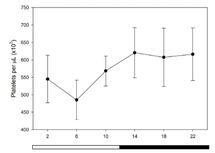


Cosinor statistical analysis



Platelet Counts

(n = 8/timepoint)



	Cosinor	ANOVA
Mean P	0.322	0.753

Food consumption and Motility

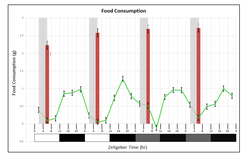
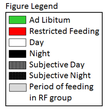


Table 1. Circadian Parameters of Food Consumption

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	20.358 (±2.59)	p < 0.001
AL	DD 2	18.907 (±2.779)	p < 0.001

Figure Legend



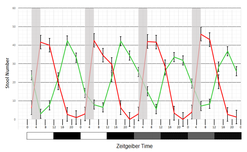


Table 2. Circadian Parameters of Stool Number

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

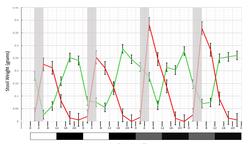
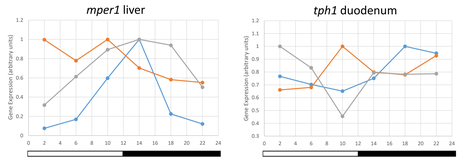


Table 3. Circadian Parameters of Stool Weight

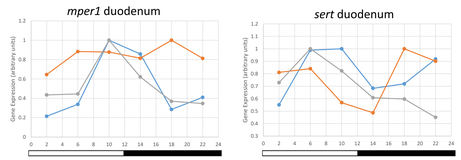
Feeding Group	Light Cycle	Acrophase	p-value
AL	LD 1	20.248 (±2.551)	p < 0.001
AL	LD 2	19.162 (±2.088)	p < 0.001
AL	DD 1	21.629 (±3.055)	p < 0.001
AL	DD 2	18.829 (±2.602)	p < 0.001
RF	LD 1	8.510 (±1.293)	p < 0.001
RF	LD 2	9.012 (±1.464)	p < 0.001
RF	DD 1	8.418 (±1.238)	p < 0.001
RF	DD 2	8.170 (±1.157)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

(n = 5/timepoint)

Gene Expression

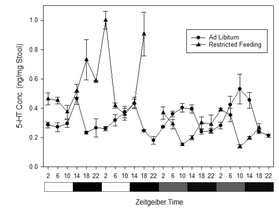


(n = 4-5/timepoint)



5-HT data

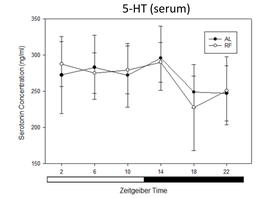
5-HT (stools)



AL

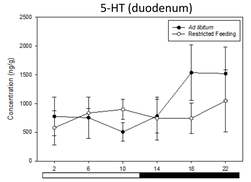
Light Cycle	Cosinor p
LD	0.002
DD	<0.0001

5-HT (serum)



	Ad libitum	Restricted Feeding
Acrophase	9.75	7.63
Mesor	309 ng/ml	268
Mean p (cosinor)	0.668	0.599

5-HT (duodenum)



	Ad libitum	Restricted Feeding
Acrophase	10.53	18.64
Mesor	2043	2221
Mean p (cosinor)	0.123	0.667

(n = 3-5/timepoint)

Conclusions

- High amplitude circadian rhythm of 5-HT in stools, with peak near ZT 12; possible duodenal rhythm with peak at ZT 18-22.
- Stool 5-HT entrainable to light and food, uncoupled from LD cycle under RF, with apparent chronodisruptive effects
- No light or food entrainable 5-HT rhythm detected in serum
- Duodenal tph1 and clock gene expression uncoupled from LD cycle under RF
- Smaller phase shift of *sert* gene expression under LD+RF
- Platelet rhythm not detected under LD+RF, but apparent nadir at ZT 6
- Chonodisruption in motility not observed, suggesting tighter coupling of motility oscillators with FEO

Future Directions

- Investigate duodenal and colonic 5-HT rhythms under LD, DD, LD+RF with increased replication
- Extension of platelet study under LD+AL and DD+RF conditions
- Characterization of microbiome role in generation or response to stool 5-HT rhythms

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