Time-Series Characterization of Circadian Temperature Rhythms During Pregnancy

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Background

Pregnancy represents a profound physiological state in which endocrine, metabolic, and neural systems are dynamically reorganized to support fetal development and prepare for parturition and lactation. Among these adaptations, changes in thermoregulation are well documented in humans and other mammals, yet the impact of pregnancy on the daily rhythms of core body temperature (CBT) has not been systematically characterized in mice. The medial preoptic area (MPOA) of the hypothalamus is a critical integrative center for both thermoregulation and the neuroendocrine control of reproduction, and its function is strongly modulated by estrogen signaling.[1,2] Estrogen receptors, particularly ERα, within the MPOA have been implicated in the modulation of circadian and homeostatic processes that shape CBT rhythms.[3,4] We therefore hypothesize that pregnancy disrupts the temporal organization of CBT in mice, and that this effect is mediated, at least in part, by estrogen receptor–dependent signaling in the MPOA.

Hypotheses

(1) Pregnancy disrupts rhythms in core body temperature (CBT) in mice.
(2) This impact of pregnancy on CBT is partially dependent upon estrogen receptors in the medial preoptic area (MPOA).

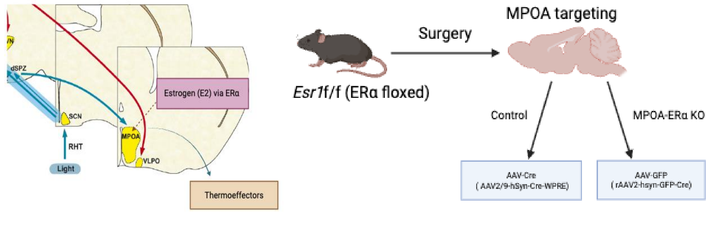
Methods

Experiment 1: Effect of Pregnancy on CBT Rhythms

- Subjects: Adult female C57BL/6J mice (9-10 weeks of age).
  - Pregnant (n=7) vs. non-pregnant (n=7).
- Surgery: Intraperitoneal telemetry implants (G2-emitters) ≥7 days pre-breeding.
- Housing: 12:12 LD cycle (lights on 07:00, ZT0); food and water *ad libitum.*
- Recording: CBT sampled every 5 minutes for 24 days; Pregnancy day 0 was confirmed by presence of copulatory plug. Pregnancy was from days 1-19 and postpartum was days 20-24.
- Data processing included collapsing the 5 minute bins into hourly bins and using daily averages.
- Data analysis involved observing circadian metrics: amplitude, rhythmicity, intradaily variability (IV), and peak phase, using ClockLab (v6.1.02) and Nitecap. Rhythm robustness was measured with Chi-Square Periodogram, JTK_Cycle, and RAIN; ANOVA and t-tests were used to perform statistical analysis.

Experiment 2: MPOA ERα Knockout and CBT Rhythms

- Subjects: Adult *Esr1*f/f (ERα floxed) on a mixed B6;129 background.
- Surgery: MPOA-ERα KO received rAAV2-hsyn-GFP-Cre (UNC Vector Core, Chapel Hill, NC, USA) into the MPOA to knock out *Esr1* expression. Control mice received rAAV2-hsyn-eGFP Bilateral injections were targeted to the MPOA using preliminary coordinates (AP +0.4 mm, ML ±0.4, DV −5.2 (150–250 nL/site).
- Recording: CBT sampling like Experiment 1
- Histology: The viral spread (mCherry in AAV-Cre, GFP in controls) and ERα loss within the MPOA were confirmed by immunohistochemistry ; mice with off-target expression or incomplete knockout were excluded from the analysis.
- Data analysis was similar to Experiment 1



Results

Figure 1

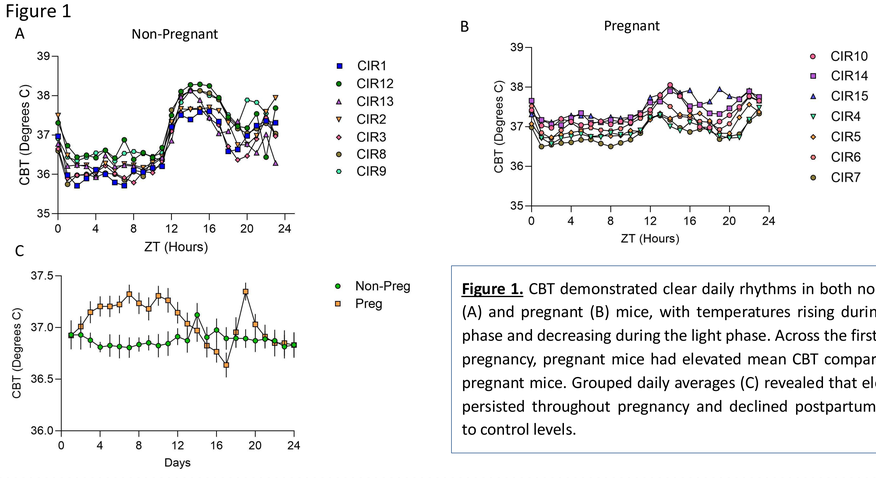


Figure 1. CBT demonstrated clear daily rhythms in both non-pregnant (A) and pregnant (B) mice, with temperatures rising during the dark phase and decreasing during the light phase. Across the first 13 days of pregnancy, pregnant mice had elevated mean CBT compared to non-pregnant mice. Grouped daily averages (C) revealed that elevated CBT persisted throughout pregnancy and declined postpartum, returning to control levels.

Figure 2

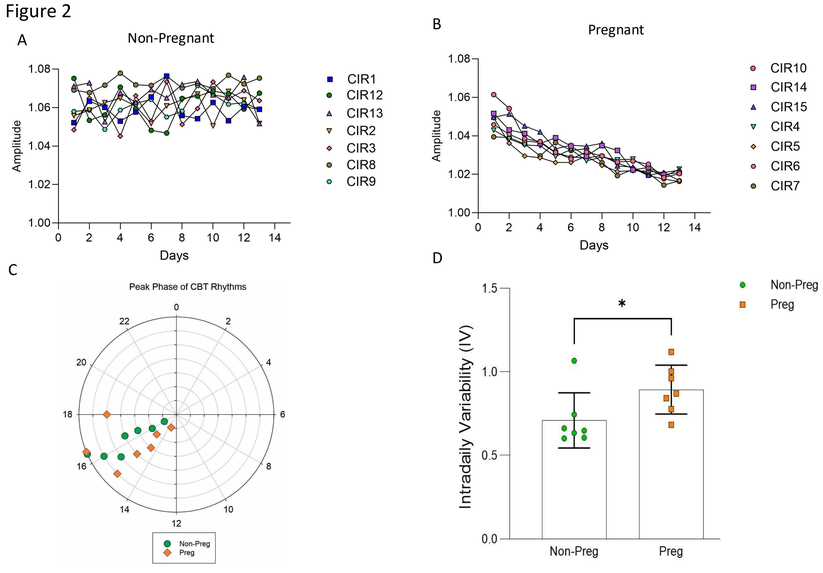


Figure 2. (A-B) Non-pregnant mice maintained stable CBT rhythm amplitude throughout the first 13 days, but pregnant mice had a progressive decline in amplitude during the same period of time - early to mid-pregnancy. (C) Peak phase remained relatively similar between the two groups, demonstrating no significant difference in the time CBT peaked. No consistent phase shifts over the first 13 days. (D) Pregnant mice had significantly higher IV compared to non-pregnant mice, which indicated less stable in rhythms during pregnancy. (*=p<0.05, **=p<0.005, ***=p<0.0005)

Figure 3

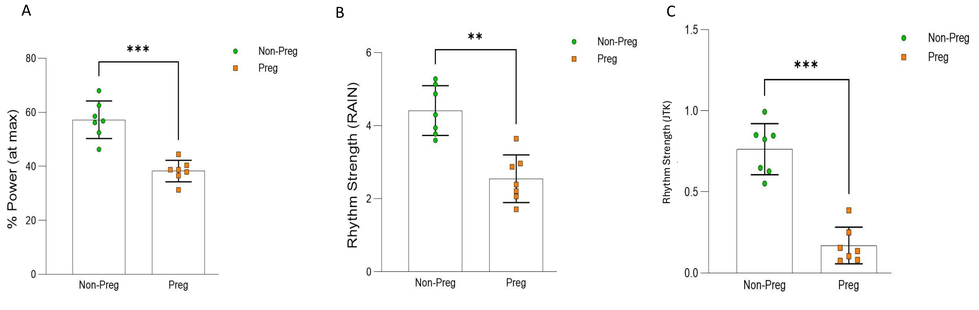


Figure 3. Pregnancy reduced rhythm strength as measured by 3 different time-series analysis methods. (A) Pregnant mice had significantly lower % variance or maximum power compared to non-pregnant mice. (B-C) Both RAIN and JTK demonstrated that rhythm strength was significantly reduced in pregnant mice compared to non-pregnant mice. (*=p<0.05, **=p<0.005, ***=p<0.0005).

Figure 4

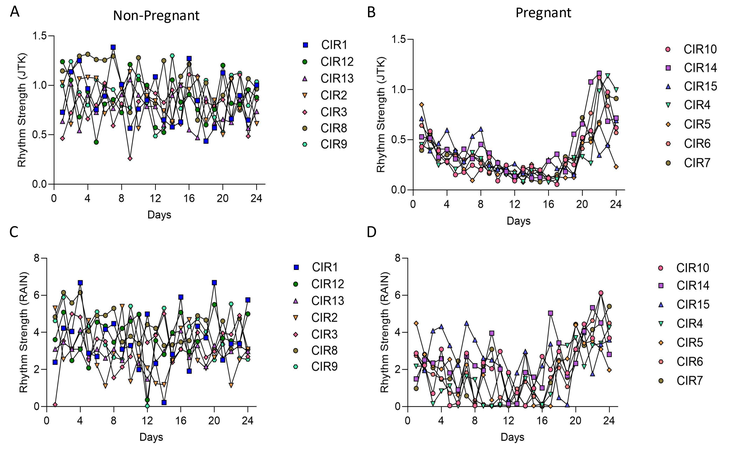


Figure 4. JTK cycle and RAIN analysis tools allowed measurement of rhythm strength per day over the 24 days of recording. (A, C) Non-pregnant mice maintained stable rhythm strength throughout the whole recording period. (B, D) Pregnant mice a reduction in rhythm strength during the same time period. Rhythm strengths rapidly recovered postpartum (days 20-24).

Figure 5: Comparison of the three methods for time-series analysis used in this study

Feature	Chi-Square Periodogram	JTK_CYCLE	RAIN
Assumes sinusoidal waveform	Yes	No	No
Handles asymmetric rhythms	No	No	Yes
Handles uneven sampling	Yes	No	Yes (slightly uneven)
Handles missing data	Yes	No	Limited
Statistical p-value output	Yes	Yes	Yes
Detects multiple periods	Yes (automatic scan)	Yes (user-defined)	No (user must loop)
Estimates amplitude/phase	Yes	Yes (limited)	No
Computational efficiency	High	High	Moderate
Sensitivity to noise	High	Moderate	High
Ease of interpretation	Moderate	High	Moderate
Software availability	R, Python, MATLAB, BioDare2	R (JTK_CYCLE, MetaCycle)	R (RAIN, MetaCycle, BioDare2)

Conclusion and Future Directions

Conclusion for Experiment 1:

- Pregnancy elevated average CBT.
- Pregnancy altered the amplitude and cycle-to-cycle stability of daily rhythms in CBT.
- Pregnancy reduced CBT rhythm robustness and strength as measured by 3 analytical tools.
- Each analysis tool had distinct strengths and weakness.

Future Directions:

- We are presently applying to same data analysis to test the hypothesis that the impact of pregnancy on CBT is partially dependent upon estrogen receptors in the MPOA.
- Expanding on data analysis to observe potential different parameters (such as amount of chaos in system)

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