

Entrainment of the Fetal Circadian Clock by Temperature Cycles

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Abstract:

Circadian rhythms are 24-hour rhythms in behavior, physiology, and biochemistry affecting processes from gene expression, to the timing of sleep and wakefulness. In the cells of humans, and other animals, circadian rhythms are generated by interactions among a few key genes and their protein products. While these molecular "clocks" are in every cell, a specialized region of the brain, the Suprachiasmatic Nucleus (SCN), is essential for coordinating rhythms in cells and tissues throughout the body. This coordinated timing is often referred to as "the Circadian System." Disruptions of the Circadian System, as a result of shift work, for example, are correlated with a range of detrimental conditions, from sleep disorders, to an increased risk of cancer, and certain metabolic diseases.

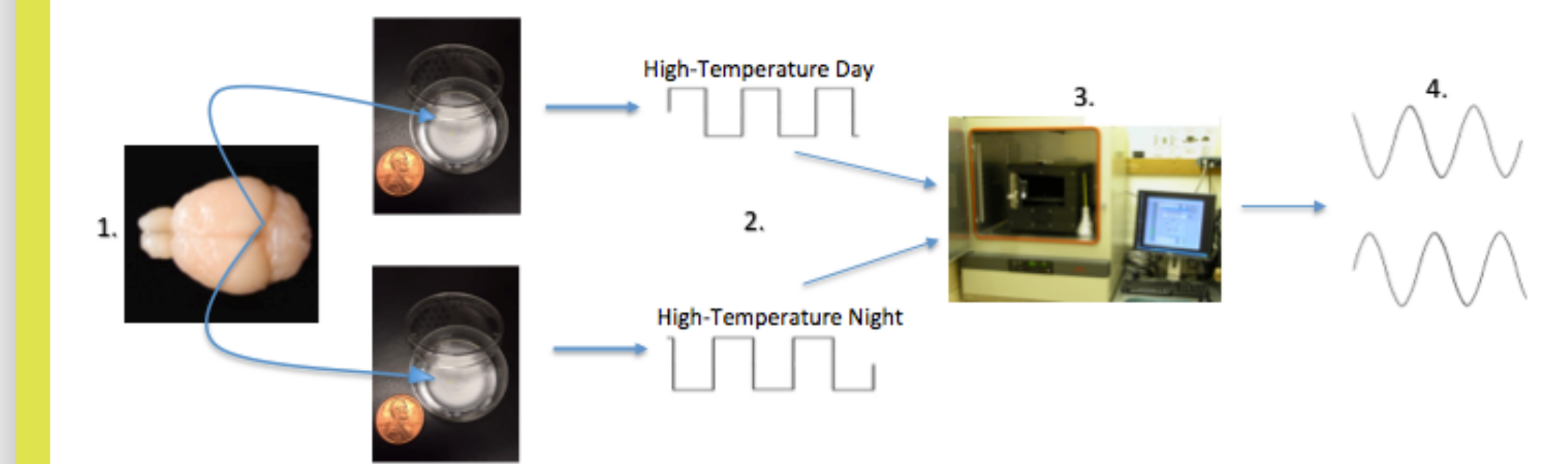
Our project is related to the general hypothesis that during development, a mammal, such as the human fetus, is a component of the mother's circadian system. More specifically, we tested the hypothesis that the fetal circadian clock is entrained by temperature cycles, a cue thought to be important for maintaining circadian organization in adults. We isolated the SCN of fetal mice and monitored molecular rhythms, while exposing the SCN to different temperature cycles. The results showed that the fetal circadian clock, at an age equivalent to mid-gestation in humans, is strongly influenced by temperature cycles similar to human body temperature rhythms. Our results support the hypothesis that fetal circadian clocks are normally entrained by maternal rhythms. Disruption of maternal circadian rhythms could have negative consequences for normal development and pregnancy.

Goal:

Our goal is to test the hypothesis that 24-hour temperature cycles entrain the fetal circadian clock (the SCN). Our longer-term goal is to determine if the development of circadian rhythms is affected by prenatal conditions, including rhythms in maternal physiology, such as body temperature rhythms.

Methods:

Suprachiasmatic nucleus (SCN) were removed from fetal knock-in *per2::luc* mice. *Per2::luc* mice express a reporter gene for the biological clock. This cycling can be detected (with a LumiCycle) by quantification of the expression of the bioluminescent reporter protein (Luciferin). Luciferase presence is required for protein expression. This bioluminescence was quantified and further analyzed. All tissue samples were kept in nutrient rich media for the entirety of the experiment and replaced with media with Luciferase before input into the LumiCycle.



1. The SCN Fetal mice sacrificed day 18 of gestation were dissected and placed in two different incubators of pre-set temperatures, high and low, with a one-degree difference.
2. The SCN were subjected to each condition, alternating every 12 hours for three subsequent days after dissection. One set of SCN were placed in high temperature during the day, while the other at high temperature during the night. This allowed for temperature entrainment of the biological clock.
3. Following entrainment, the samples were placed in the Lumi-Cycle for bioluminescence quantification, data collection, and further analysis.
4. Successful entrainment of the fetal SCN results in opposite cycles in the two groups (high-temperature day, high temperature night). Control SCN were placed in the machine (time zero) to monitor rhythms pre-temperature entrainment.

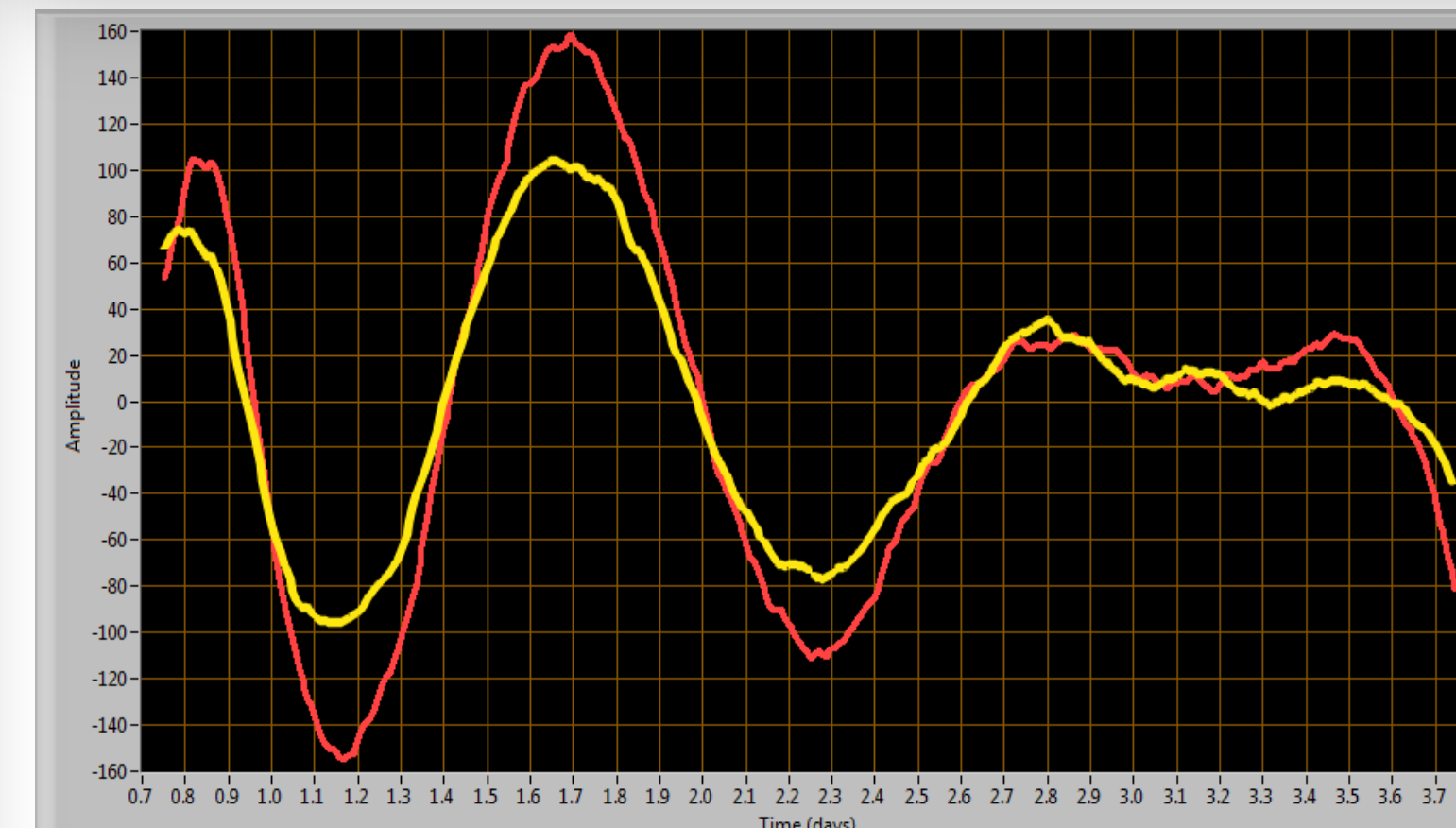


Fig. 3. Each fetus has 2 SCN (one from each side of the brain) that are in synchrony when exposed to the same conditions. The rhythms below were expressed by SCN from the same fetus and exposed to the same constant temperature conditions immediately after dissection.

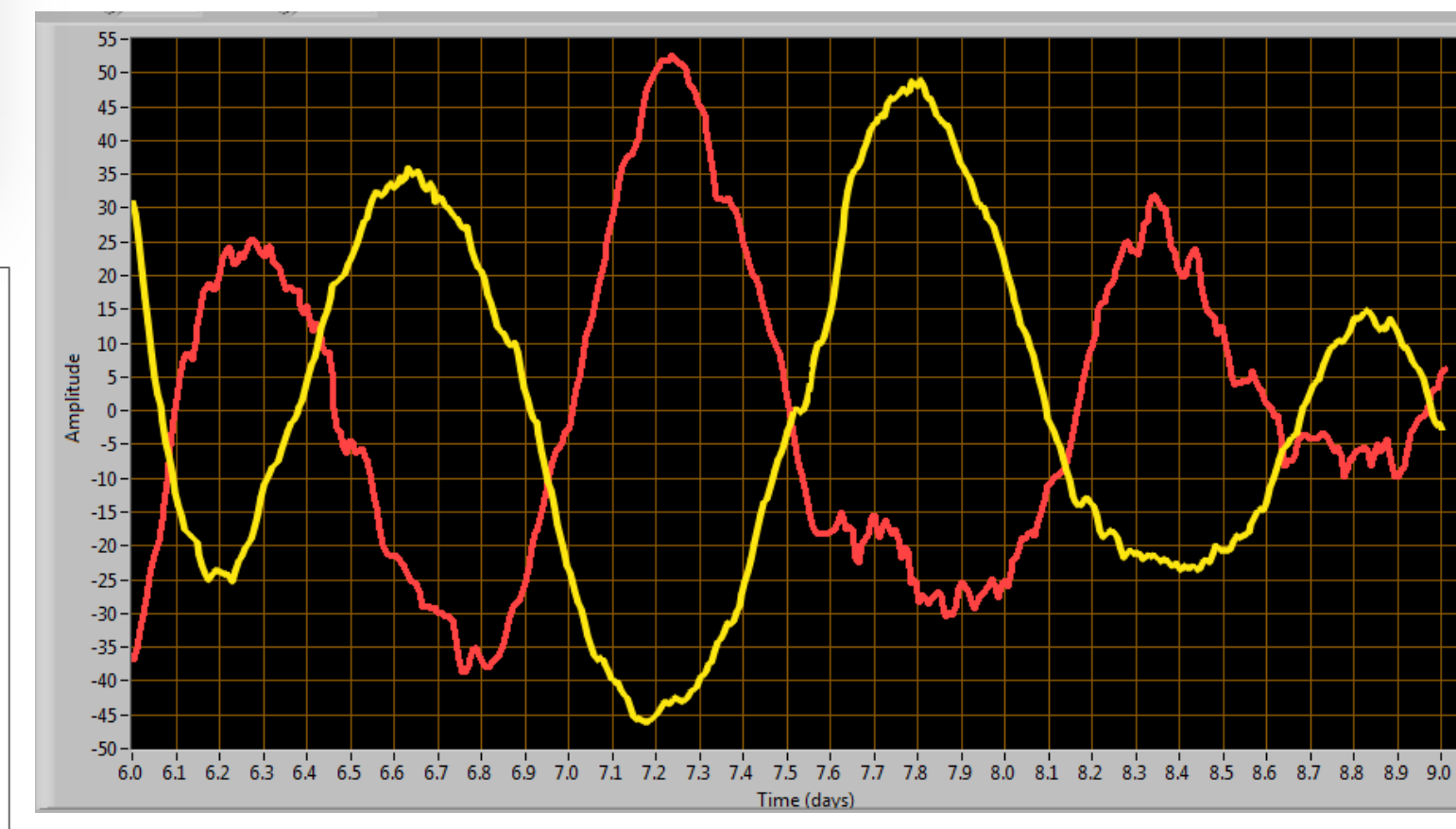


Fig. 4. Fetal SCN are entrained by 24-hour temperature cycles (12 hours at 35.5°C and 12 hours at 39.5°C). The rhythms below were expressed by two SCN from the same fetus exposed to different temperature cycles for 5 days before recording. The rhythms show opposite phases similar to the 12-hour difference between temperature cycles.

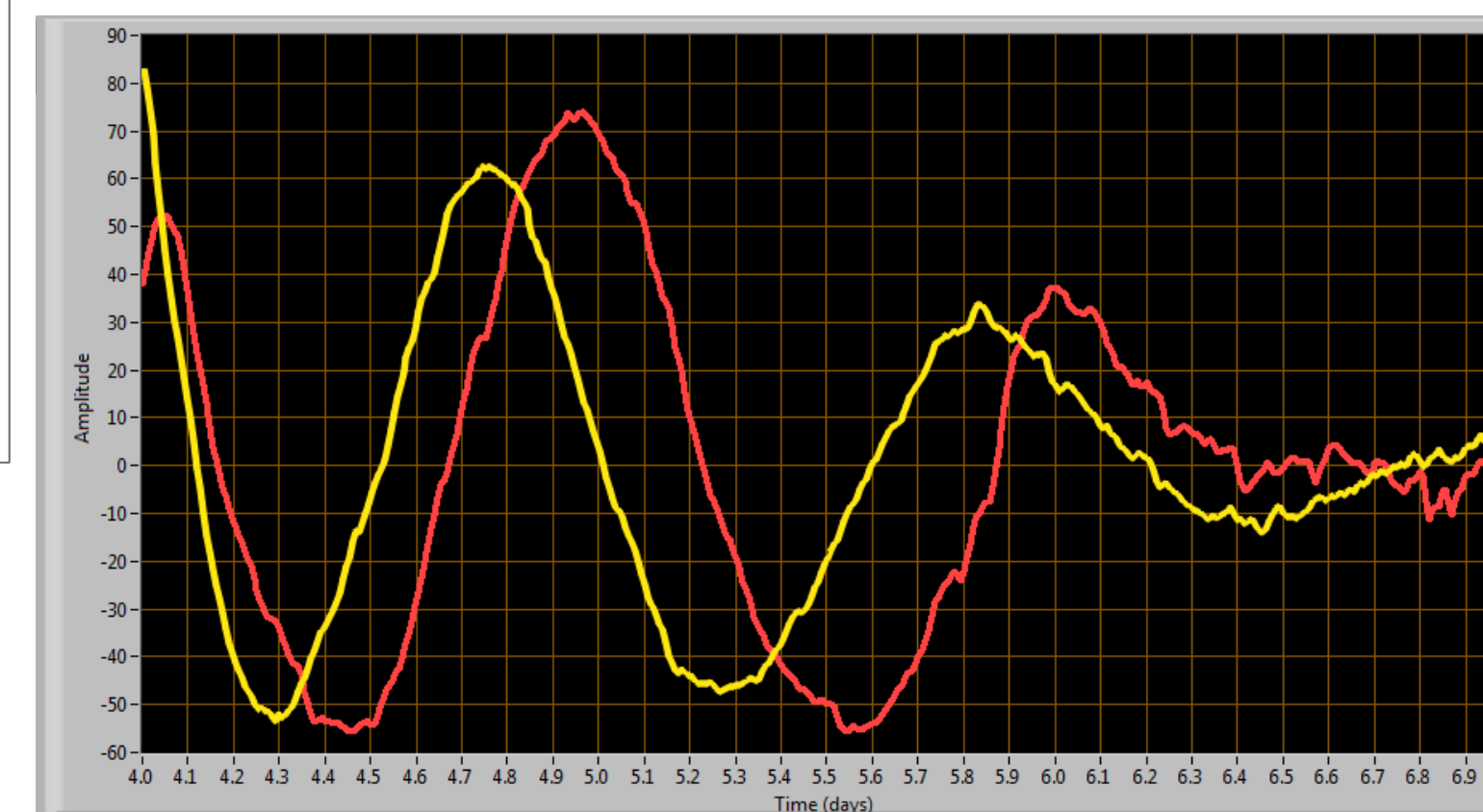


Fig. 5. Fetal SCN are partially entrained by weak 24-hour temperature cycles (12 hours at 35.75°C and 12 hours at 36.75°C). The rhythms below were expressed by two SCN from the same fetus exposed to different temperature cycles for 3 days before recording. The rhythms had not yet shifted to match the 12-hour difference between temperature cycles.

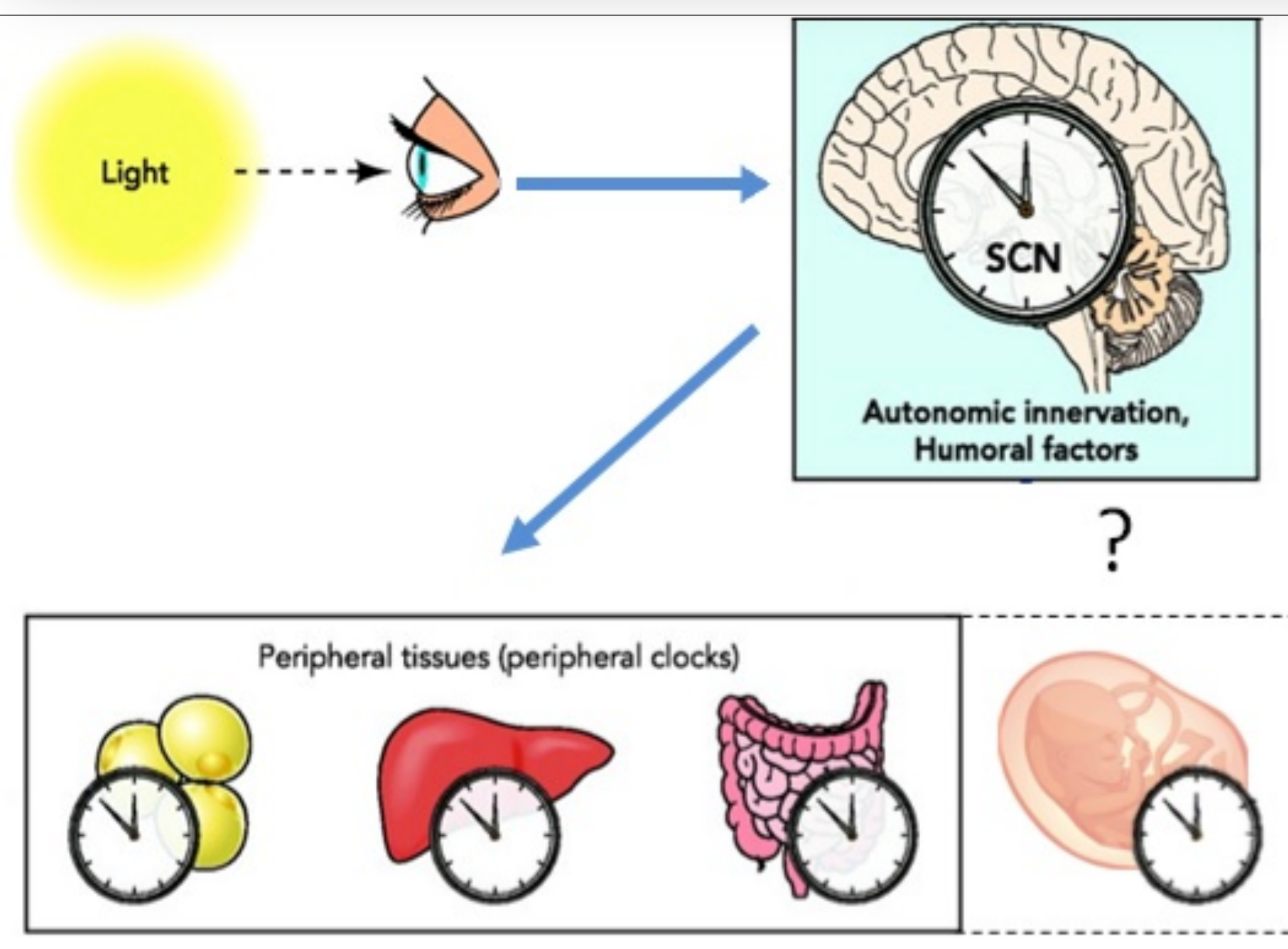


Fig. 1. The Suprachiasmatic Nucleus (SCN).

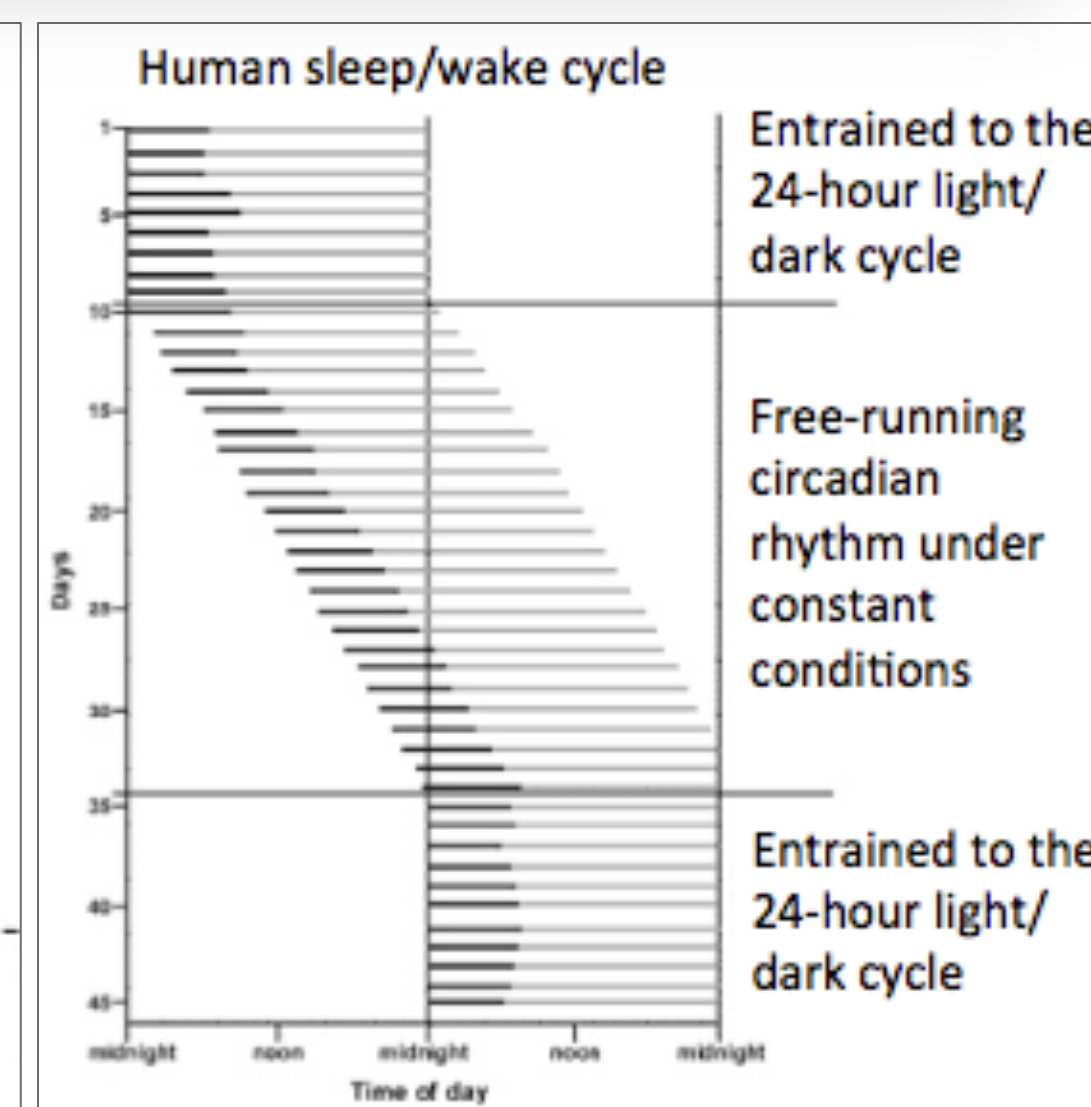


Fig. 2. Circadian rhythms in the absence of a light-dark cycle, expressing their innate "circa" 24-hour period. Also known as "free-running" rhythms.

Background:

The Circadian System

Circadian rhythms are 24-hour rhythms in behavior, physiology, and biochemistry which manifest in functions from gene expression to the timing of sleep and wakefulness. In humans and other animals, circadian rhythms are generated through interactions among several key genes, and their protein products, in cells and tissues throughout the body. Circadian timing is now recognized as a major regulator of normal cellular homeostasis.

Although molecular clocks are in every cell, a specialized region of the brain, the Suprachiasmatic nucleus (SCN), is essential for the coordination among rhythms in other tissues and organs (Fig. 1). This coordinated timing is often referred to as "the Circadian System." Disruptions of the Circadian System, as a result of shift work for example, are correlated with a range of detrimental effects from sleep disorders to increased risk of cancer and metabolic disease.

The light/dark cycle is the predominant external entraining signal for behavioral rhythms, acting through the retina and direct neural connections to the SCN. In the absence of light/dark cycles, circadian rhythms "free-run", expressing the innate circa-24-hour period of the clock within the SCN (Fig. 2). Less is known about the internal signals that mediate entrainment of the circadian system. The nervous and endocrine systems are probably involved, but the body temperature rhythm is also thought to be important.

Development

Much less is known about the circadian system during development. When, for example, do rhythms develop and when, or how, are they first entrained? Evidence indicates that the SCN, and possibly other clocks, begin to function before birth. If so, then it may be appropriate to think of the mother and fetus as part of the same circadian system. We have been studying the development of the SCN in mice to determine when it begins to generate rhythms and if, after they appear, they are entrained by the mother's circadian system. In particular, we are testing the hypothesis that temperature cycles (mimicking the body temperature rhythm) can entrain the fetal SCN. If fetal circadian rhythms are part of the maternal circadian system, then disruptions of the circadian system and/or the signals that keep it coordinated could have detrimental effects on pregnancy and development, as they do for adults.

Peak PER2 expression on strong (●) and weak (▲) temperature cycles.

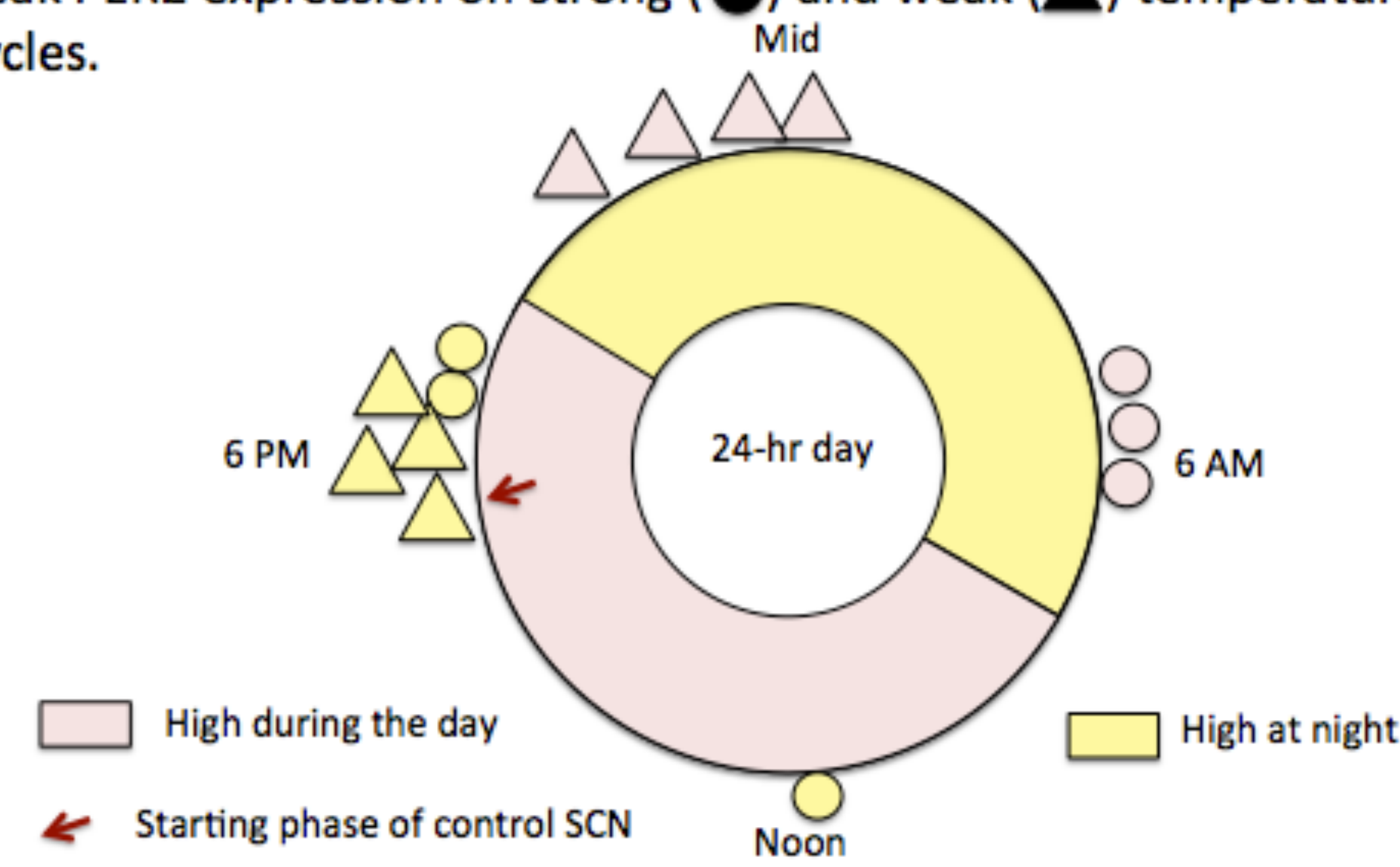


Fig. 6. The small circles and triangles indicate the timing of fetal SCN rhythms relative to temperature cycles experienced *in vitro*. During the cycles, temperature was high at night (8PM to 8AM) or during the day (8AM to 8PM). Each symbol represents the timing for a single fetal SCN. A strong temperature cycle caused complete separation of rhythms, while a weak cycle only partially reset the rhythms.

Conclusions:

- The dominant circadian clock in the brain, the Suprachiasmatic nucleus (SCN), begins to generate rhythms during prenatal development
- Development of the circadian clock in mice suggests that the circadian clock also develops several weeks before birth in humans
- The SCN from each side of the fetal brain have similar properties
- The fetal SCN of mice are entrained by temperature cycles *in vitro*
- Entrainment by temperature cycles *in vitro* indicate that the body temperature rhythm of the mother would be sufficient to entrain the fetal clock
- Entrainment of the fetal clock *in vitro* indicates that the maternal and fetal circadian would be in synchrony during development (see Fig. 7)
- The human fetus is likely to have functioning circadian clocks that are integrated (entrained) into the maternal circadian system

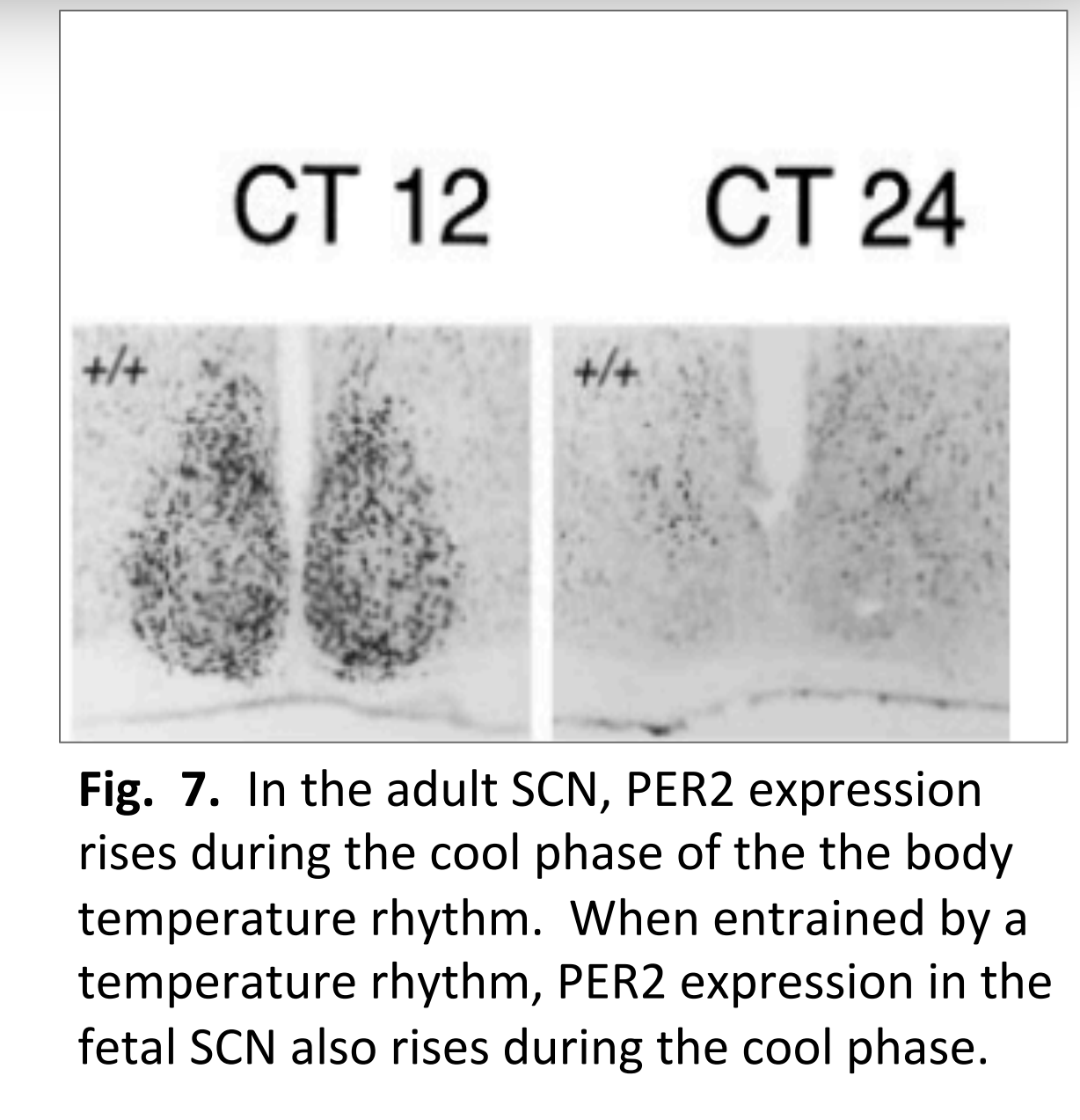
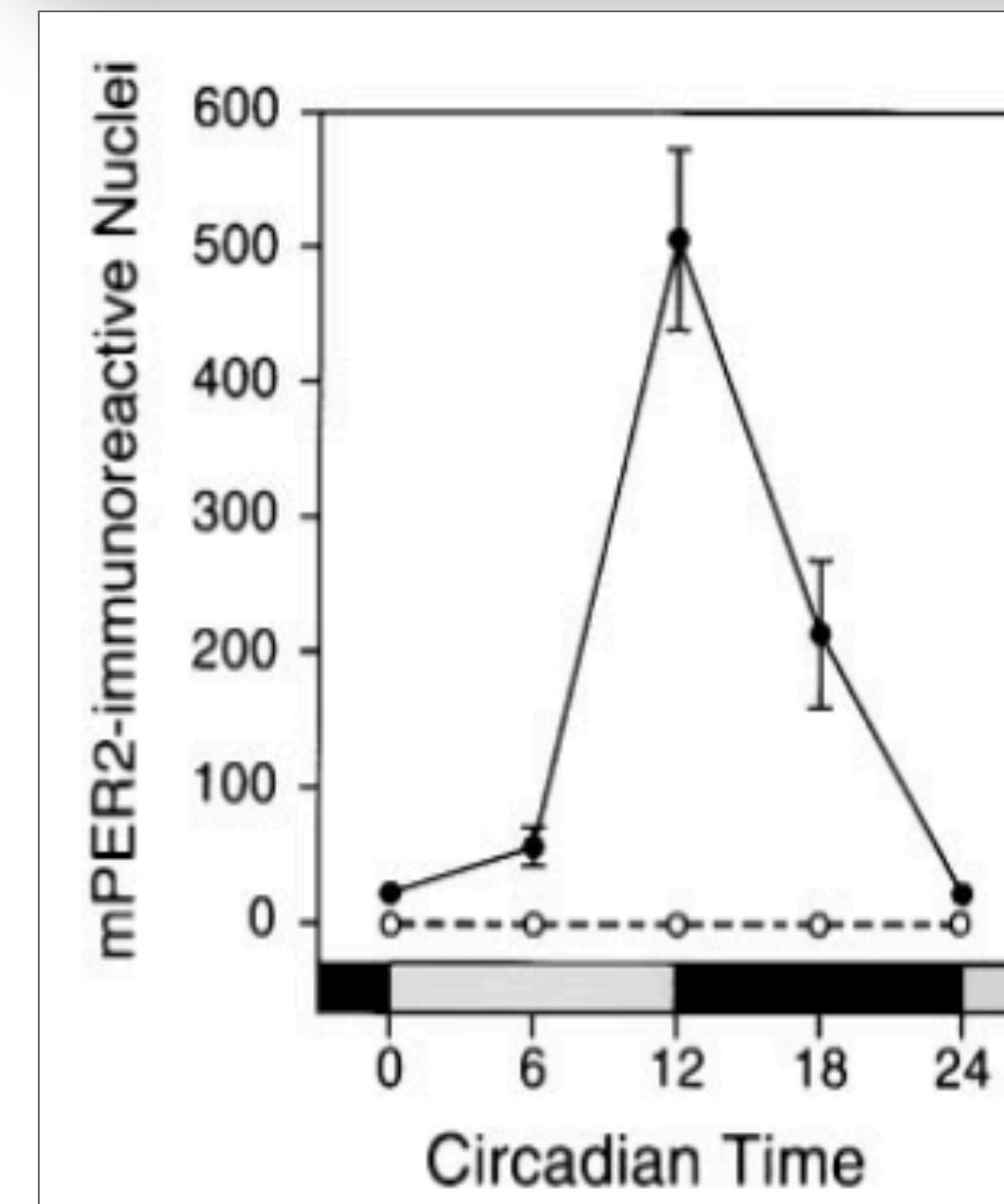


Fig. 7. In the adult SCN, PER2 expression rises during the cool phase of the body temperature rhythm. When entrained by a temperature rhythm, PER2 expression in the fetal SCN also rises during the cool phase.

References:

Bae, K., et al. (2001). "Differential functions of mPer1, mPer2, and mPer3 in the SCN circadian clock." *Neuron* 30(2): 525-536.