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ONTOGENY OF CLOCK GENE EXPRESSION IN THE RAT KIDNEY

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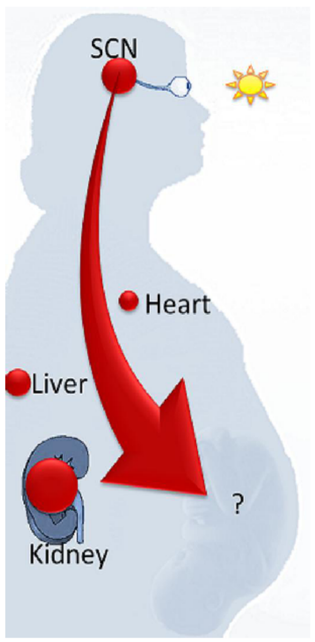
Introduction and objectives



Most physiologic processes, including kidney functions exhibit day/night rhythms driven by the circadian clockwork. The central circadian clock located in the suprachiasmatic nucleus (**SCN**) receives photic cues from the retina and synchronizes peripheral clock systems operative in various tissues.

At the cellular level, the mRNA and resultant protein oscillations of the clock genes are based on an interlocked transcription-translation feedback loop. (Fig. 1) The canonical clock genes ***Clock, Bmal1, Rev-Erbα, Per1, Per2, Cry1, Cry2*** have been identified in almost every peripheral tissue. Recent studies have revealed circadian expression of numerous genes critical for kidney functions (e.g. ***α ENaC, NHE3, AVPR2*** **and** ***SGK1***).

Study aim: We sought to describe the development of the circadian gene expression patterns in the kidneys.

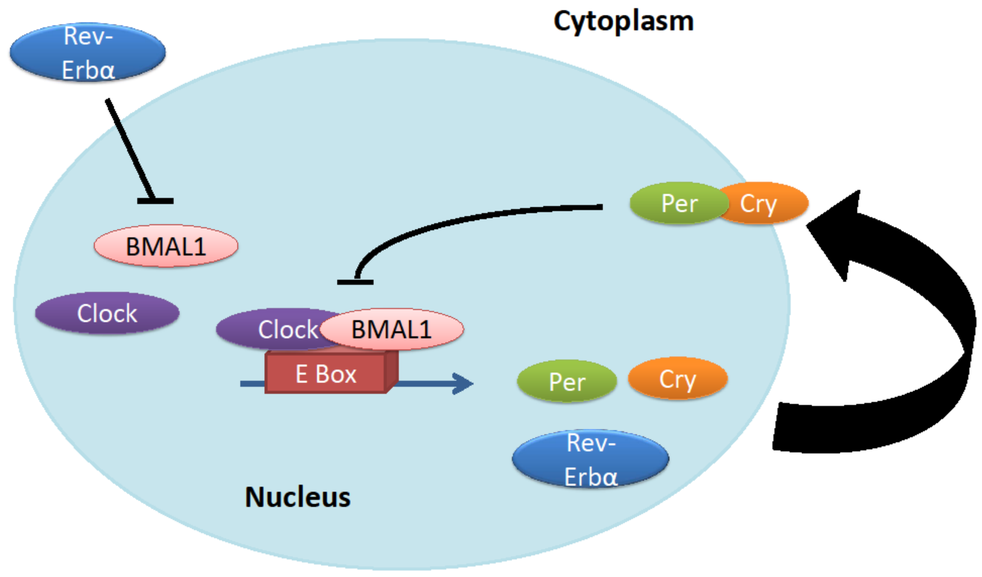


Fig.1 The molecular clockwork involves core circadian transcription factors (CLOCK and BMAL1) and E-box–mediated transcription of Clock Genes, including activators (e.g. Per, Cry) and repressors (e.g. Rev-Erbα) of the circadian system.

Methods

Pregnant SD rats and their pups were housed under 12/12h light-dark cycles and constant temperature with free access to food and water. (Light 05:00-17:00)

At specific ages offspring (7/group) were sacrificed at 4 hour intervals (Fig.2).

The daily gene expression patterns were profiled by real-time PCR for the canonical clock genes and the kidney specific clock-controlled genes αENaC, SGK1, NHE3 and AVPR2.

One-way ANOVA was used for exploratory analyses. For the analysis of time effects, circadian rhythms were analyzed by the single cosinor procedure including the fit of a cosine wave to the data by least-squares linear regression. P<0.05 was required for significance.

Embryonic day 20 ~Newborns (**NB**)

1 week old (**1W**)

4 weeks old (**4W**)

12 weeks old (**12W**)

Fig.2 Ages of sacrificing

NB
Bmal1 p=0,417
Clock p=0,009
Rev-erbα p=0,012
Per1/2 p=0,342/0,022
Cry1/2 p=0,705/0,218
α ENaC p<0,001
SGK1 p=0,108
NHE3 p=0,003

1W
BMAL1 p=0,039
Clock p=0,029
Per1/2 p=0,047/0,005
Cry1/2 p=0,002/0,004
Rev-erb α p=0,003
α ENaC p<0,001
SGK1 p=0,002
NHE3 p<0,001

4W
BMAL1 p=0,013
Clock p=0,629
Rev-erb α p=0,017
Per1/2 p=0,044/0,047
Cry1/2 p<0,001/0,506
α ENaC p=0,006
SGK1 p=0,003
NHE3 p=0,011

12W
BMAL1 p<0,01
Clock p=0,196
Rev-erb α p<0,001
Per1 /2 p<0,001
Cry1/2 p<0,001
α ENaC p<0,324
SGK1 p=0,004
NHE3 p=0,17

Results

- Fig.3 shows the postnatal development of BMAL1, Per1, Cry1 and SGK1 gene oscillation. Gene expression showed no circadian rhythmicity in NB but significant circadian rhythmicity had developed during early postnatal life by 1W. Rhythmic expression increased in amplitude and showed a significant phase shift at 4W and at 12W.
- Rev-Erbα and Per2 exhibited significant circadian rhythms throughout development.
- Rhythms of Clock α ENaC, NHE3 were observed at neonatal age in NB, phase-shifted by 1W. Circadian variation of α ENaC and NHE3 were lost at 12W. The daily rhythm of Clock gene expression was not significant after 1W.
- Cry2 showed transiently circadian oscillation at 1W then displayed distinct circadian rhythms at 12W.

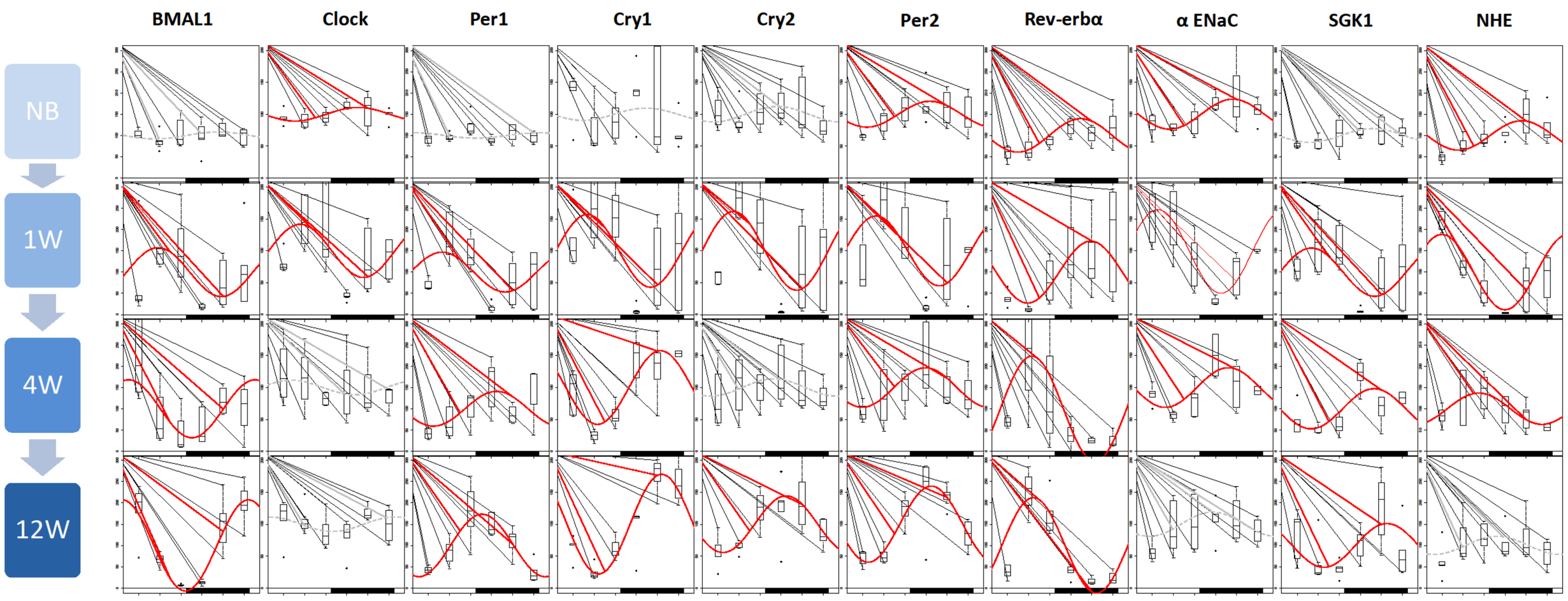


Fig.3 Daily pattern of gene expression of clock genes and clock controlled genes in the kidneys at different ages in 12 /12h LD (7 animals per time point).

Discussion

Our findings demonstrate a complex time course of development of the canonical clock genes BMAL1, Per1, Cry1 during pre- and early postnatal life in rats. Light is known as the most important cue to entail the circadian system. It is assumed that with those circadian genes which showed rhythms transiently or lost, the oscillation during development might respond to other enviromental factors affecting the maturation of peripheral clocks.

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