PH.D. THESIS (SUMMARY)

Investigation of the embryonic chicken pineal gland as a model for experimental jet lag

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2013.

INTRODUCTION:

Biological Rhythms:

Most biological rhythms are periodically recurring, with a typical period length, and are rhythmic in nature. They can be classified according to their duration/length:

- Ultradian rhythms last less than a day; examples being breathing and heart rate regulation
- Infradian rhythms last for longer than a day, such as the menstural cycle
- Circadian (circa = approximately, diem =day) rhythms last roughly 24 hours and include rhythms such as sleep/wake cycle, and regulation of hormone levels such as cortisol

Circadian Rhythms:

Circadian rhythms are amongst others regulated by the light dark cycle (LD). To be classified as circadian a rhythm:

- Must last roughly 24 hours
- Be endogenous, meaning it persists even under constant conditions such as constant darkness (DD)
- Have a phase which can be shifted by rhythmic environmental stimuli called Zeitgebers (ZT) (time-givers) such as light.

Circadian clock:

Circadian rhythms are driven by so called biological clocks which contain all the components of the pacemaker oscillator, allowing them to control the circadian time with amazing accuracy. The oscillating system relies on a combination of positive and negative feed-back loops which are composed of (fig 1):

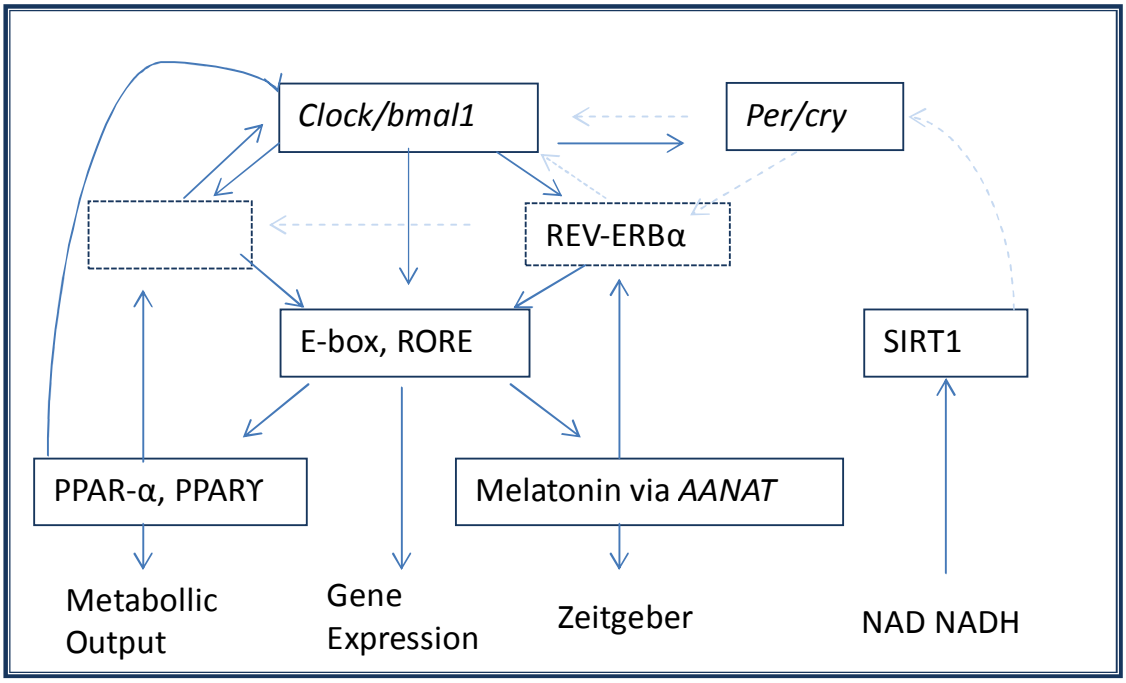

fig 1. Schematic outline of the molecular oscillator in mammals. *Clock/bmal1* activates the *cry* and *per* promoters. Next there is transcription/translation of *per* and *cry*, before they enter the nucleus and in turn inhibit the *clock/bmal1* activity. The key output is an E-box mediated transcriptional activation of hundreds of target genes on a daily basis such as *ΔANAT*.

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- Activation complex: consists of genes that act as positive regulators and activate the expression of other genes. In the circadian clock of mammals this most

frequently consist of *Clock/Bmal* heterodimer which binds an E-box promoter in the nucleus and thereby induces transcription.

- Inhibitory complex: consists of genes that act as negative regulators by counteracting the function of the activation complex. In mammals this is most commonly a *per/cry* heterodimer which can interact with *clock/bmal* and inhibit their activity.

A fundamental part of circadian rhythms is that they follow changes in environmental periodic stimuli by a shift in the phase of the rhythm. Thus a change in the light dark schedule will trigger a change in the circadian rhythmic expression of clock genes and clock controlled genes. The time at which light is applied is important with regards to which change is seen. Light in the early night causes phase delay of the rhythm, and light at late night causes a phase advance of the rhythm. The phase advance and phase delay of circadian rhythms in response to changes in the light schedule are a popular area of study. Incongruence between the internal clock time and the rhythmic environmental stimuli is called chronodisruption and this can lead to a variety of symptoms such as those associated with jet-lag. People that are constantly exposed to chronodisruption, e.g. shift workers, have an increased risk of a variety of disorders such as metabolic disease and certain cancers.

Circadian rhythms play an integral part in physiology and disease. Amongst others circadian clocks are involved in maintaining normal homeostasis and they have been shown to be involved in regulation of the cell cycle. Diseases such as cardiovascular emergency cases, asthma attacks and rise in blood glucose in diabetic patients all show predominance in the early morning hours when there is a rise in hormonal levels, which is regulated by the circadian system. Chronotherapy focuses on administering drugs to patients at appropriate time of neuro-humoral conditions to minimize the dose and side-effects of the drugs.

Models of the circadian clock:

Classical laboratory models to study the inner clock mechanisms and its rhythms include (fig. 2): the study of growth dynamics of fungi, the metabolism of unicellular eukaryotic organisms, the growth and flowering of plants, timing of metamorphosis of insects and the locomotor and endocrine functions of vertebrates. Common features in all of these models are that there is a roughly 24 hour rhythm found also under DD, and that the rhythms are regulated by positive and negative feedback loops. Differences include the exact period length of the rhythm, diurnal or nocturnal zenith, factors which entrain the rhythm, clock genes and the localization and interaction of the components of the clock mechanisms.

In mammals the master pacemaker is found within the suprachiasmatic nucleus (SCN) in the hypothalamus. Information about the light dark schedule arrives here via the retinohypothalamic tract from the retina. The SCN can entrain to light dark conditions, and SCN neurons contain oscillators that stay synchronized to each other even without a Zeitgeber. The SCN in turn sends information to a variety of nuclei in the hypothalamus which regulate a variety of physiological process such as hormonal release and locomotion. Fibers from the SCN also go the pineal gland where they secrete norepinephrine in a rhythmic manner which in turn leads to a rhythmic release of melatonin.

In most birds the master pacemaker is the pineal gland itself. The pineal gland of birds has several advantages to the mammalian system when it comes to circadian research which makes it a favored model for many. These advantages include:

1) It is directly light sensitive.
2) It oscillates *in vitro* under DD conditions for more than five cycles
3) It provides an *in vitro* model which can be completely isolated from the surrounding neuroendocrine and paracrine environment.

4) Embryonic chicken are "isolated" in eggs and are thus not affected by maternal hormonal rhythms during ontogenesis.

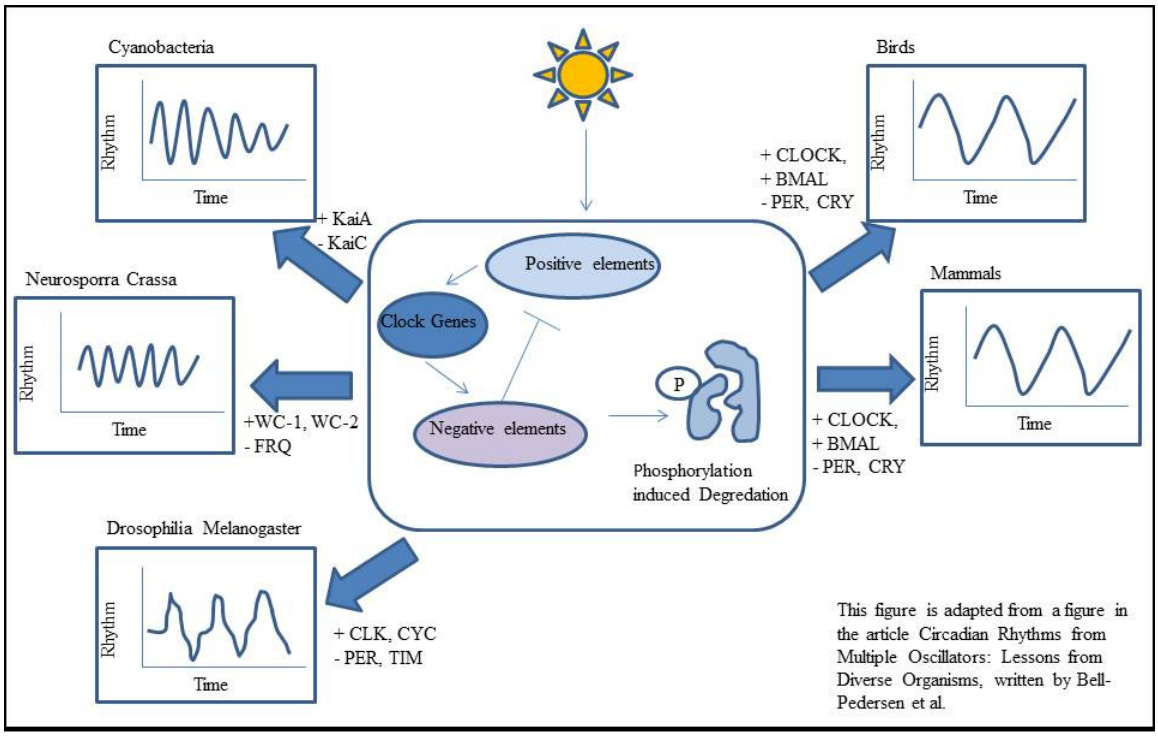

Fig) Although the underlying circadian mechanisms are the same across species the exact clock genes involved vary from species to species. The rhythms which we study may also be different across species. In mammals we frequently study locomotion as an output of the rhythm. In birds melatonin is sometimes studied. In Cyanobacteria the rhythm of bioilluminecencse may be studied. In Neurosporra Crassa the rhythm of growth. In Drosophilia Melanogaster locotmotion is also frequently studied. Across species we can directly study both the clock genes and the output of the clock.

Melatonin:

Melatonin is the so called "darkness" hormone in vertebrates as it peaks during the night phase both within the pineal gland and the plasma in diurnal and nocturnal species. It is the key messenger hormone of the circadian system. It functions to spread the photoperiodic signal giving the organism information about the day/night cycle as well as reflecting the seasonal changes in daily length. Rhythmic changes in the serum level of melatonin are known to play a key role in the sleep wake cycle. Melatonin has also been shown to be a part of the entrainment of the circadian system to phase advance and phase delay.

The avian pineal gland can generate rhythmic expression of melatonin both under *in vivo* and *in vitro* conditions. In birds the expression of melatonin can be detected from the second week of embryonic development. In birds the production of melatonin corresponds directly to changes in mRNA expression of *AANAT* (arylalkylamine-N-acetyltranseferase), therefore measuring *AANAT* levels is sufficient to study the rhythmic production of melatonin in this species.

Development of the embryonic clock in chicken:

The study of the circadian clock under development has been done in a variety of species. Previous experiments have shown the presences of clock genes whose rhythm is responsive to changes in light already from the 1st day in the developing Senegalese sole. In rats *HIOMT, AANAT* and melatonin have been shown to be rhythmic already from an early stage of development and the rhythm of these are also light responsive. In zebra fish studies have

shown that already 22 h after fertilization the master pacemaker, the pineal gland, is developed, and already 2 days after fertilization melatonin is rhythmic in expression as long as the fish are exposed to light and dark. Earlier we have shown that the embryonic chicken pineal gland has a working clock mechanism which is synchronized to environmental stimuli by embryonic day (ED) 17. However as of yet the effect of phase advance on the expression of *clock* and clock controlled genes within the embryonic chicken pineal glands have not been studied.

Aims:

- Determine if the transcriptional regulation of the circadian clock gene *clock* shows any response to acute changes in the environmental light-dark conditions both *in vivo* and *in vitro*
- To prove that the embryonic chicken pineal gland is a suitable model for studying circadian rhythm-related disorders at the molecular level by investigating the expression of genes involved in melatonin synthesis such as *AANAT* and *HIOMT* (hydroxyindole-O-methyltransferase) and circadian clock gene *clock* under DD, LD and LD+4

MATERIALS AND METHODS:

1) Animals:
   a. Experiments with adult chicken
      Newly hatched white leghorn chickens were kept under 14/10 hour light dark schedule for 6 weeks.
      For the *in vivo* experiments chickens were placed in a DL 10/14 schedule at 20:00. The chickens were sacrificed by decapitation every four hours and the pineal glands were collected from 18:00.
      For the *in vitro* experiments adult chicken were decapitated at 12:00 and the pineal glands were placed in a multichannel perifusion system. The light schedule was changed from LD to DL at 20:00 on the second day *in* vitro. Pineal glands were removed from the perifusion chambers every four hours starting from 18:00.
   b. Experiments with embryonic chicken
      Fertilized eggs of white leghorn chickens were incubated from embryonic day 1 at constant temperature (37.5C) and humidity (70%) with mechanical rocking. Control eggs were kept under LD conditions. Experimental eggs were subjected to DD or LD+4 conditions from ED19, prior to this they had been housed under normal LD conditions. The embryos were sacrificed by decapitation every four hours between ED19-21.
2) Semi quantitative RT-PCR:
   Total RNA was extracted from pineal glands of post-hatch chicken with Sigma's TRI Reagent following the manufacturer's protocol (T9424, Sigma–Aldrich, and St. Louis, Missouri, USA). Absorbance was measured at 260nm with Ultrospec 2100 instrument. Total RNA extracts (200 ng) of each pineal specimen were analyzed with primers for clock mRNA (forward: TCCCAGTCTCTTGGACAACC, reverse: GCTGTTGCTGGATCATGTGT) and b-actin mRNA (forward: GATGGACTCTGGTGATGGTG, reverse: AGGGCTGTGATCTCCTTCTG) as an internal standard. The one-step semi-quantitative RT-PCR was run with 5 U MMLV Reverse Transcriptase (Life Technologies, Carlsbad, California, USA) and 0.2 U RedTaq DNA polymerase (Sigma); this protocol was optimized and standardized previously (Nagy A.D., 2007). The conditions were as follows: RT – 42 ^{o}C 15 min, 94

^{o} C 5 min; and PCR – 26 cycles of 94 ^{o} C 30 min, 60 ^{o}C 30min, and 72 ^{o}C 1min. End products were separated on agarose gels stained with SYBR Green I. Gels were trans-illuminated with blue light. The fluorescence of PCR products was measured as the mean pixel intensities of the DNA bands on gel pictures (ImageJ software, National Institutes of Health, USA).

3) Real-time quantitative RT-PCR:
Total RNA of embryonic chicken pineal glands was extracted as mentioned above. cDNA was generated by reverse transcription using 500 ng of total RNA in a total of 50 µL reaction volumes following the instructions of the manufacturer (Applied Biosystems, SuperScript RT enzyme, 4374966). Real-time PCR was run using 3 µL cDNA solution per 15 µL reaction volume with Applied Biosystems_ TaqMan gene expression assays for *AANAT, HIOMT* and *clock* and also for internal reference gene β-*actin*.
Composition of reaction mixture was made based on the instructions of the manufacturer (Applied Biosystems, 4370048). StepOne Real-Time PCR instrument (Applied Biosystems) was used with default cycling parameters (50 ^{o}C 2 min, 95 ^{o} C 10 min, 40 cycles of 95 ^{o}C 15 sec and 60 ^{o}C 1 min). For quantitation calculations the delta-delta Ct method was used, after control measurements for reaction efficiency normalizations.

4) Data Analysis:
Group differences were evaluated using two-way ANOVA followed by Tukey's post hoc test. $P < 0.05$ was considered as statistically significant difference.

RESULTS:

1. *In vivo* effect of DD compared to normal LD on the expression of *AANAT* in the embryonic chicken pineal gland:

On ED 19 the eggs were placed under DD. Glands were collected starting from zeitgeber time (ZT) 12, 12 h prior to the switch in the lighting conditions, every four hours until ED 21. The control group was kept under LD conditions. *AANAT* showed a robust daily rhythm, even under DD conditions (fig. 3A). Lack of light at ZT 4:00 does not cause a significant change in the expression of *AANAT*. In fact there is no significant difference in the expression of *AANAT* between the LD and DD groups throughout the duration of the experiment.

2. *In vivo* effect of DD compared to normal LD on the expression of *HIOMT* in the embryonic chicken pineal gland

Eggs of ED19 chicken were placed under DD. Pineal glands were collected every four hours until ED21. For the LD group *HIOMT* has a high amplitude rhythm with a peak during the light phase at ZT4 (fig. 3B). Under DD conditions at ZT4 the absence of light causes a shift in the expression of *HIOMT* compared to that seen under LD (fig. 1B). Then in the second cycle of DD there is a lower expression of *HIOMT* at ZT4, compared that seen under LD conditions.

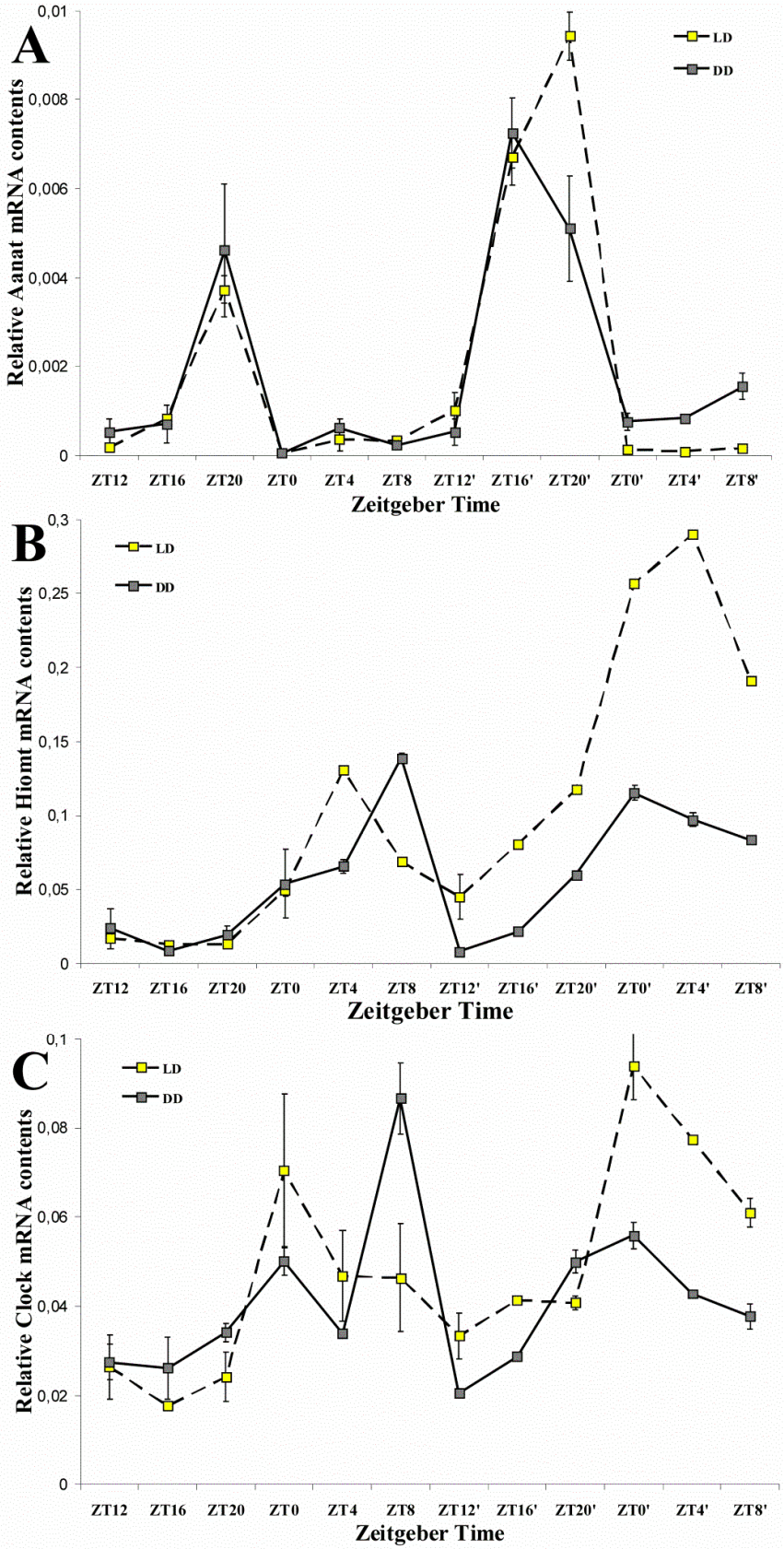

fig. 3. In vivo effect of DD compared to LD on the expression of Aanat, Hiomt and clock. in the embryonic chicken pineal gland. Pineal glands were collected every four hours starting from ED 19 at ZT12. Graphs represent the relative mRNA level of the pineal glands at each time point (mean of n = 3, ±SEM) collected from chicken exposed to DD (–) and the control group LD (- -)

3. *In vivo* effect of DD compared to normal LD on the expression of *clock* in the embryonic chicken pineal gland

The eggs were placed under DD at ED 19, and the pineal glands were collected every four hours until ED21. In the control group, which was kept under normal LD conditions, *clock* mRNA shows a peak during the light phase (fig. 3C). In the DD group at ZT 4, four hours after a change in the light schedule, there was no significant difference in the level of *clock* mRNA compared to the LD group. Then at ZT8 there is an increase in the expression of *clock* mRNA compared to that seen under LD conditions. In the second cycle of DD the expression of clock mRNA is suppressed (fig. 1C).

4. *In vivo* effect of four hour phase delay on the expression of *AANAT* in the embryonic chicken pineal gland compared to normal LD conditions

On ED19 the light schedule was changed in such a way that lights were left on for 4 h longer (LD + 4) than in the control LD group, effectively causing a four hour phase delay. Pineal glands were collected every four hours starting from ZT12 which is prior to the phase delay. Samples were collected over a 48 h period. In the LD group *AANAT* shows a clear robust 24 h rhythm (fig. 4A). In the LD + 4 group during the first four hours of phase delay, at ZT16, there was no acute change in the expression of *AANAT* when compared to the LD group. However, looking at the second cycle of LD + 4 conditions a clear phase delay of the expression of *AANAT* is observed.

5. *In vivo* effect of four hour phase delay on the expression of *HIOMT* in the embryonic chicken pineal gland compared to normal LD conditions

ED19 eggs were subjected to a four phase delay (LD + 4). Pineal glands were collected every four hours, with the first time point being at ZT12, prior to the change in the light schedule. In the LD group *HIOMT* shows a high amplitude rhythm with a peak during the light phase (fig. 4B). In the LD + 4 group there is no acute change seen in *HIOMT* expression within the first four hours of prolonged light exposure (ZT16) when compared to the LD group. At ZT8 the peak for *HIOMT* expression shows phase delay in the LD + 4 group compared with the LD group (fig. 4B).

6. *In vivo* effect of four hour phase delay on the expression of *clock* in the embryonic chicken pineal gland compared to normal LD conditions

On ED19 eggs were subjected to a 4 h phase delay (LD + 4) of the light schedule. Pineal glands were collected every four hours from ED19-ED21, and the first collection occurred prior to the phase delay. Under LD conditions *clock* shows a clear 24 h pattern with a peak during the light phase (fig. 4C). Within the first four hours of phase delay at ZT16, *clock* mRNA expression shows acute change with a clear peak in its expression. Furthermore the phase delay of the *clock* mRNA expression is maintained until the end of the experiment, seen clearly by a shift of the rhythm compared to that of the LD control group (fig. 4C).

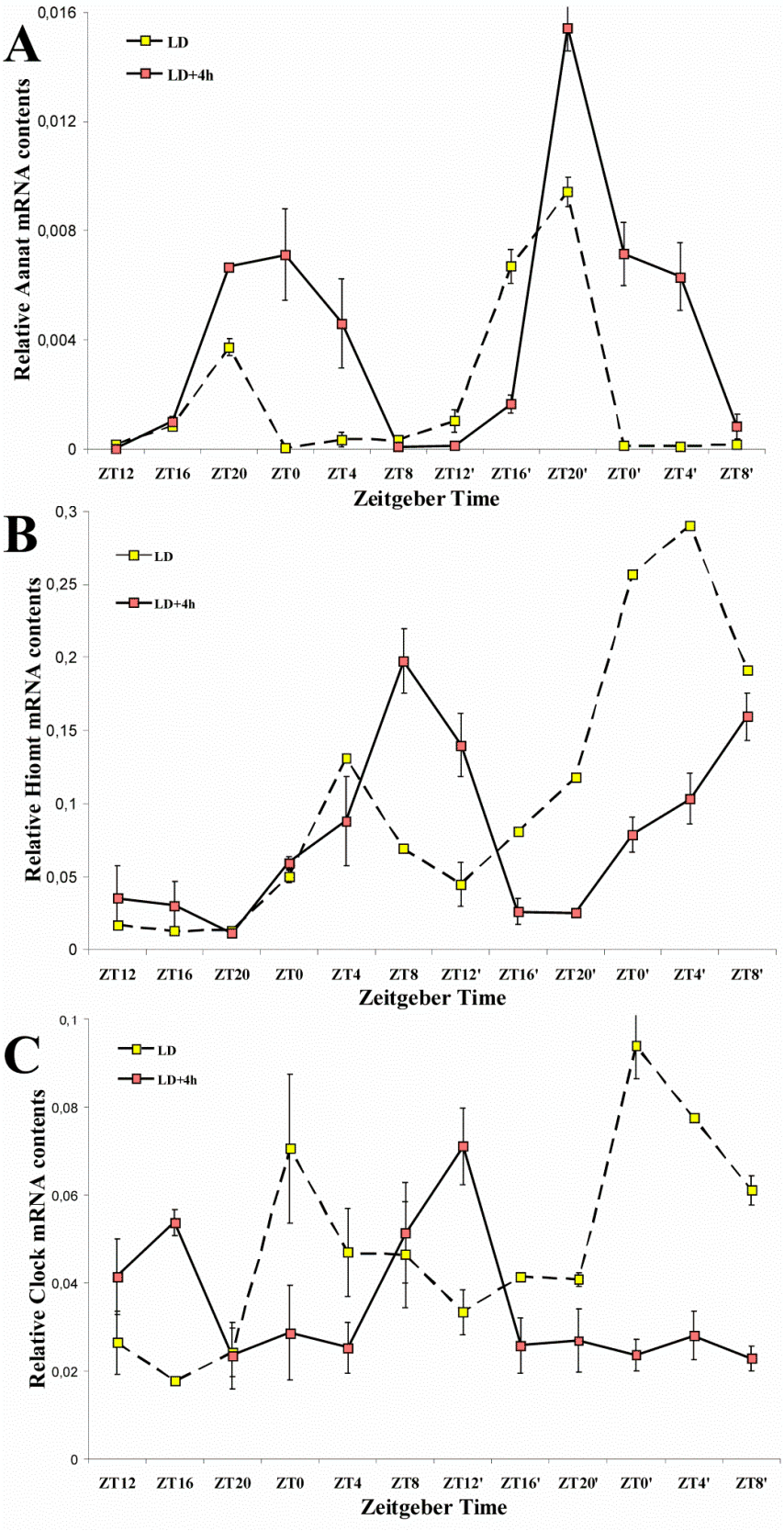

fig. 4. In vivo effect of 4 h phase delay (LD + 4) on the expression of Aanat, Hiomt and clock in the embryonic chicken pineal gland compared to LD. Pineal glands were collected every four hours starting from ED 19 at ZT12, prior to the change in light schedule. Graphs represent the relative mRNA level of the pineal glands at each time point (mean of n = 3, ±SEM) collected from chicken exposed to LD + 4 (–) and the control group LD (- -).

7) *In vivo* effects of acute inversion of the light/dark schedule on the 24 hour pattern of *clock* expression in the chicken pineal gland in post-hatch chicken:

The pineal glands were collected over a 30 hour period every four hours. The first sample was taken 2 hours prior to the shift in the light/dark schedule at 20:00. In the LD group *clock* mRNA levels increased during the dark phase with a peak at 2:00 which corresponds to ZT 20 (ZT 20 indicates the time 20 hours after the lights have been switched on).
The DL group showed a significant increase in the *clock* mRNA levels already at 22:00, merely two hours after the unexpected light exposure at night, which is assumed to be a response to the acute phase delay of the normal LD cycle (fig .5). However during the second DL cycle the levels of *clock* mRNA is significantly decreased at 2:00 during the "new" light phase compared to the control group (fig 5).

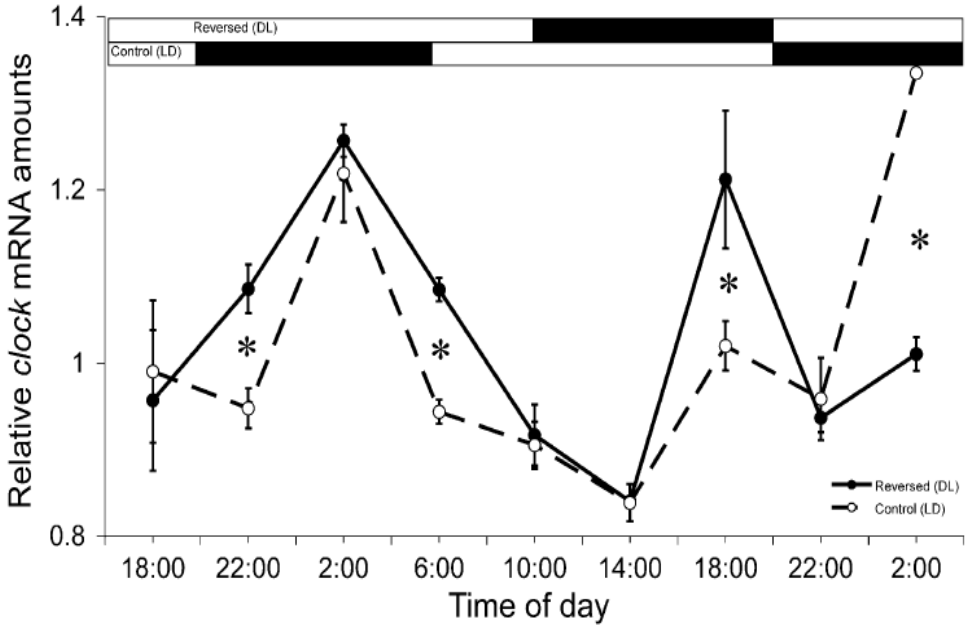

fig.5) *In vivo* effects of a phase-delayed light/dark schedule on the 24 h pattern of clock mRNA expression in the chicken pineal gland. Graphs represent relative *clock* mRNA levels of pineal glands at each time point (mean of n = 3, ±SEM) collected from chickens exposed to a reversed LD cycle (DL) from 20:00 (—) or kept under LD conditions (- - -) as a control group. Horizontal bars show the light/dark conditions for each group (black indicates darkness). Significant differences ($p < 0.05$) between groups are indicated with *.

8.) *In vitro* effects of acute inversion of the light/dark schedule on the 24 hour pattern of *clock* expression in the chicken pineal gland in post hatch chicken:

Pineal glands were collected over a 20 hour period every four hours. The first sample was taken 2 hours prior to switching the LD conditions to DL at 20:00. In the LD group the peak of *clock* mRNA was seen at subjective night at 22:00/ZT6 (fig. 6).
In the DL group the levels of *clock* mRNA remained stable during the first cycle, without any significant peaks. , There was no significant change in the *clock* mRNA levels between the LD and the DL groups within the first 12 hours of the new DL schedule (fig. 6).

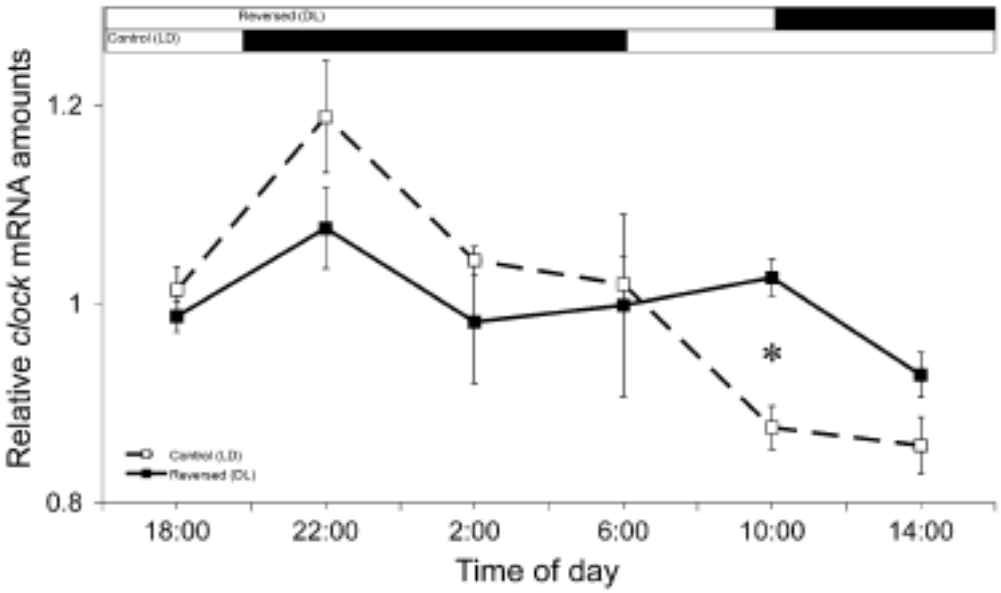

fig. 6) *In vitro* effects of a phase-delayed light/dark schedule on the 24 h pattern of *clock* mRNA expression in the chicken pineal gland. Graphs represent the relative *clock* mRNA levels of pineal specimens (mean of n= 3, ±SEM) that have been subject to a reversed LD cycle (DL) from 20:00 (-) or kept under normal LD conditions (- -) as a control group. The keys are similar to those in fig. 5. Pineal samples were collected *in vitro* with four hour intervals, with the first sample being taken 2 hours prior to a switch from LD to DL. Significant differences ($p < 0.05$) between groups are indicated with *.ROR α

DISCUSSION:

In post-hatch chickens kept under normal LD cycles, the *in vivo* (control group) *clock* shows a peak in expression during night time (fig.5). Our data supports the idea, that under normal entrained conditions, the transcriptional activity of *clock* in the chicken pineal gland shows daily oscillations, with a maximum during the dark phase under *in vivo* conditions. The oscillation in *clock* mRNA expression is known to have a small amplitude (it was around 1.5 fold in this study), which may explain why others have suggested earlier that *clock* is constitutively expressed within the chicken pineal gland.

When the chickens were exposed to a reversed light cycle (DL), *clock* mRNA expression showed an acute change within the second hour of unexpected light exposure *in vivo* (fig. 5). This indicates that there is a rapid change in the transcriptional control of *clock* in response to the acute phase-delay of the periodic environmental stimuli. Further studies should be carried out to determine the underlying mechanism of this sudden change to determine, which complexes are involved in the light dependent response of *clock* transcription *in vivo* in the chicken pineal model.

When the chicken pineal glands were exposed to prolonged DL conditions, *clock* mRNA levels decreased during the light phase (fig. 5). The difference between the mRNA expression of *clock* during the first cycle of DL and the second cycle of DL conditions may indicate that unexpected light exposure and expected light exposure trigger different transcriptional mechanisms in the chicken pineal gland; similar mechanisms have earlier been described for *cry1*.

In vitro under normal LD conditions *clock* mRNA levels peak during dark phase (fig. 6). After placing the pineal glands under DL condition *in vitro,* the expression of *clock* mRNA

showed no significant peak in the first cycle, and likewise in the second cycle, there was no significant difference, when compared to the control LD group. This suggests that *clock* is not directly light inducible.

A clear difference is seen in the expression of *clock* mRNA under DL *in vivo* (fig. 5), where there was a clear difference between the control and experimental group, and *in vitro* (fig. 6), where there was no similar difference between the DL and LD group. This may indicate that there are also neuroendocrine signals involved in the regulation of *clock* in response to acute changes in the environmental lighting conditions.

In our experiments done on embryonic chicken pineal glands, *AANAT,* a key enzyme involved in the synthesis of melatonin, shows a robust rhythm with a peak during the dark phase (fig. 3A). The change of the light schedule from LD to DD did not cause a significant change in the expression of *AANAT* (fig. 3A), as expected. There was a lack of both acute changes to sudden darkness and a lack of a delayed response seen in the second cycle of DD. This supports the idea that *AANAT* in the embryonic chicken pineal model is regulated primarily by the pineal clock itself as opposed to being a light sensitive gene.

Exposing the embryonic chicken to a phase-delay of 4 hours (LD+4), essentially prolonging lights on by four additional hours, did not cause an acute change in the expression of *AANAT* (fig.4A). The second cycle of LD+4 caused a phase delay of the expression of *AANAT*; the whole rhythm of *AANAT* mRNA expression was shifted compared to that of the control LD group (fig.4A). This again suggests that *AANAT* is not directly light sensitive, as it did not respond acutely to prolonged light exposure, but that in fact it is regulated by the circadian clock itself.

HIOMT is the other key enzyme of melatonin production. In our experiments on embryonic chicken pineal gland under normal LD conditions, the expression of *HIOMT* mRNA shows a high amplitude rhythm with a peak during the light phase (fig. 3B). Once the chicken were placed under DD the expression of *HIOMT* mRNA was shifted compared to that seen in the control group (fig. 3B). During the second cycle of DD there is a significant difference seen in the expression of *HIOMT* when compared with the LD group. There is a reduction of the rhythm amplitude of *HIOMT* under DD, but it still shows a peak during the light phase and a general 24 hour pattern.

The exposure of the embryonic chicken to a 4-hour phase-delay did not cause any acute changes in the expression of *HIOMT* mRNA (fig. 4B). However, during the second cycle of LD+4, there is a clear phase delay of the expression of *HIOMT*, indicating that also this gene is regulated by the circadian clock.

Rhythmic expression of both *AANAT* and *HIOMT* under LD, DD and LD+4 in this experimental protocol proves that the chicken embryonic clock at ED19-21 has a functioning clock mechanism, and that it is a good model for phase shifting experiments as well as experiments on the ontogenesis of the circadian clock in the chicken pineal gland.

Clock is a clock gene acting as a positive regulator together with *Bmal1* and *Npas2*. Amongst its functions, it acts together with *Bmal1* as a heterodimer to regulate the expression of *AANAT*. Knockdown of *clock* has been shown to cause a decrease of *AANAT* activity, as well as *Npas2* and *Per2* demonstrating that this is an essential component of the circadian clock.

Placing the embryonic chicken under DD did not cause any acute changes in the expression of *clock* mRNA during the first four hours (fig. 3C), when compared to the LD group. However 8 hours later, there is a peak in the expression of *clock,* which indicates some role for *clock* in light-dependent synchronization *in vivo*. In the second cycle of DD, the expression of *clock* was reduced, but it still maintained a rhythmic pattern. This correlates well with the results seen for *AANAT,* which did not show a change in its rhythm under DD compared to LD.

In the embryonic pineal glands exposed to a four hour phase delay (LD+4) there was a two-fold change in the rhythm of *clock* mRNA expression. First of all, there was an acute change seen within the first four hours of prolonged light exposure. *Clock* expression showed a clear peak in at ZT16, which was absent under LD conditions (fig. 4C). Secondly *clock* mRNA expression showed a clear phase delay of its rhythm in response to LD+4 conditions which persisted until the end of the experiment. This again indicates a role for *clock* in light-dependent synchronization *in vivo*.

To summarize the embryonic chicken experiments we can clearly see that *AANAT*, *HIOMT* and *clock* all show clear 24 hour rhythms within the embryonic pineal gland, and rhythms which in fact resemble those seen in entrained adult animals making this an excellent model for further circadian experiments.

Furthermore it is clear from the LD+4 experiments that one cycle of altered lighting conditions is sufficient to alter the expression of clock genes themselves as well as genes involved in melatonin synthesis making this a good model to carry out future experiments.

CONCLUSION: Our studies have demonstrated that the effects of change of the light dark schedule on the clock genes and clock controlled genes are varied depending on the type of change and the duration of the change. An acute change in the lighting schedule may lead to an acute change in the clock genes which are light sensitive, such as *clock*, and only a delayed change in genes which are clock controlled such as *AANAT* and *HIOMT*. Additionally the embryonic chicken pineal gland is light sensitive and responds to changes in the light dark schedule in a similar manner to the adult chicken pineal gland.

In addition we have proven that the embryonic chicken pineal gland is an adequate model to do circadian experiments, including phase shift experiments. The embryonic chicken pineal gland clearly shows a rhythmic expression of certain clock genes suck as *clock* and also of clock controlled genes such as *AANAT* and *HIOMT*. Additionally the embryonic chicken pineal gland is light sensitive and responds to changes in the light dark schedule in a similar manner to the adult chicken pineal gland.

Further experiments should be done to determine the underlying mechanisms of the different transcriptional regulation of clock genes and clock controlled genes that operate during the first and second cycle of altered light dark conditions.

NEW FINDINGS:

- In adult chicken *clock* shows an acute change to a reversed light cycle (DL) already within two hours of unexpected light exposure *in vivo*
- Under prolonged DL conditions the expression of *clock* in adult chicken is decreases. This suggests that there are different transcriptional regulatory mechanisms during the first and second cycle of DL exposure.
- *In vitro* DL conditions had no effects on the expression of *clock* in adult chicken.
- *AANAT* expression was not affected by DD conditions in the embryonic chicken pineal gland.
- The first cycle of LD+4 had no effect on the expression of *AANAT,* however during the second cycle there was a phase delay in the expression of *AANAT*
- The expression of *HIOMT* was damped under the DD conditions in the embryonic chicken.
- *HIOMT* expression was not affected by LD+4 during the first cycle of LD+4. During the second cycle there was a clear phase delay of HIOMT.
- *Clock* showed no acute changes to DD conditions in the embryonic chicken pineal gland
- In response to LD+4 *clock* showed a new peak at ZT16, and additionally a phase delay of its rhythm

PUBLICATIONS:

Peer reviewed articles related to this thesis:

1. S. Kommedal, G. Bódis, A. Matkovits, V. Csernus, A.D. Nagy: Expression pattern of clock under acute phase-delay of the light/dark cycle in the chicken pineal model. – *Gen. Comp. Endocrinol.* 172: 170-172. 2011. [IF: **3.267**]

2. S. Kommedal, V Csernus, AD Nagy.: The embryonic pineal gland of the chicken as a model for experimental jet lag - *Gen Comp Endocrinol* 188: 226-231. 2013. [IF: **2.823**]

Other peer reviewed articles:

3. A. D. Nagy, S. Kommedal, K. Seomangal and V. Csernus: Circadian Expression of clock genes clock and cry1 in the embryonic chicken pineal gland. - Annals N.Y. Acad. Sci. 1163: 484-487. 2009. [**IF: 2.303**]

4. A. D. Nagy, K. Seomangal, S. Kommedal and V. Csernus: Expression of cry2 in the chicken pineal gland: effects of changes in the light/dark conditions. - Annals N.Y. Acad. Sci. 1163: 488-490. 2009. [**IF: 2.303**]

5. Lengyel Zs., Lovig Cs, Kommedal S, Keszthelyi R, Szekeres Gy, Battyáni Z, Csernus V, Nagy AD. Altered expression patterns of clock gene mRNAs and clock proteins in human skin tumors. Tumour Biol. 2013 Apr;34(2):811-9. [IF: **2.568**]

Conference Abstracts

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