Development and photosensitivity of the rhythmic melatonin secretion of the chicken pineal gland

PhD Thesis

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Contents

1. Introduction

Major part of the biological processes undergoes in a rhythmic manner, for example the sleep-wake cycle, daily variations of certain hormone levels, the menstruation cycle or the yearly leaves falling. As most of the processes from time to time need regeneration and refilling of energy and/or stocks, cyclic regulation is almost unavoidable in living organisms.

One of the basic attributes and also criteria of biological rhythms, generated by so called oscillator mechanisms, is that the periodic phenomenon continues to work even in the complete lack of rhythmic environmental stimuli. A biological oscillator has two major features: the period time, aka the length of one period, and the phase, that shows the actual position of the cycle. The period time depends on the length of the physico-chemical processes maintaining the oscillation. That is why this feature cannot be significantly changed. At the same time, it is relatively easy to change the phase of the rhythm (phase-shift). The possibility of shifting the phase of the cycle is used by the so called synchronizing factors that help to harmonize the organism's different rhythms to each other and to the environment. According to their period time, rhythms can be divided into three main groups. Those with period time longer than one day are called „infradian" (e.g. the menstruation cycle). Others that have periods shorter than a day are named as „ultradian" (e.g. the heart cycle, EEG waves, etc.). And the ones with approximately 24 hours long cycles, for example the sleep-wake cycle or the alterations of blood levels of kortizol, are the „circadian" rhythms. With only very few exceptions, the presence of circadian rhythms is the common attribute of the eukaryotes (Dunlap, 1999).

In non-mammalian species the pineal gland and it's hormone, the melatonin (MT) is responsible for the control of the organism's circadian rhythmic processes and for the synchronization of these to the environment (Binkley et al. 1978; Korf and Wicht 1992). In mammals, this organ has already lost its autonomy and is not able to produce rhythmic activity on its own. The only remaining function of the gland is the MT production. The removed pineal gland of the rat maintained the MT production even for several days *in vitro* but no variations or changes in the hormone levels throughout these days were found (Rékasi et al. 1991). The explanation is that the circadian oscillator has been translocated into the *suprachiasmatic nucleus* (SCN) that is synchronized to the environment by photic stimuli percepted by the retina (Binkley et al. 1978; Collin et al. 1984; Korf 1994; van Veen et al.

1986). And the SCN controls the hormone production of the pineal gland through sympathetic fibres. In contrast, in birds (and in non-mammalian vertebrates) the pineal glands still includes a complete fully functional biological oscillator and at the same time some photosensitive elements that can influence the function of the oscillator. Just like in mammals, the SCN has connections with the pineal body in these species but the role of this nucleus is only to modify and not to control the MT rhythm (Binkley et al. 1978; Takahashi et al. 1980).

As the avian pineal gland, in accordance to the facts above, maintains its rhythmic hormone production even *in vitro,* and this *in vitro* rhythm can be influenced by both environmental illumination and bioactive agents (Noradrenalin, VIP, PACAP, etc.) (Csernus et al. 1998; Mess et al. 1996; Takahashi et al. 1980), the chicken pineal gland was chosen as a model organ for our experiments.

Photosensitivity of the chicken pineal gland

The most important and at the same time the most simple signal of the environment's cycle is light. The avian pineal gland is located directly beneath the calvaria and contains photosensitive elements. These elements (e.g. pinealopsin), that are also linked to the MT synthesizing apparatus, are histological identified and proven to be fully functional (Korf and Vígh-Teichmann 1984; Vígh-Teichmann and Vígh 1992; Vígh and Vígh-Teichmann 1988). Our research team previously showed that the phase of the rhythmic MT secretion could be completely inverted (shifted with 12 hours) if kept under reversed illumination (light phase at night, and dark phase at daytime). The completion of the phase inversion took about 36 hours (Csernus et al. 1998; Mess et al. 1996). It was also shown, that an only 3 hours long daily illumination, if applied in the proper phase, has the same effects as the reversed illumination has (Csernus 2003).

Gwinner (1997) published that illumination of starling eggs with the light at the intensity of 10 lux was enough to synchronize the MT secretion of the embryos. However, in this *in vivo* experiment it remained unclear whether the effects of light were transmitted through the retina or were percepted directly by the pineal gland or even both ways took a part. In another study, the activity of arylalkylamine N-acetyltransferase (AANAT), the rate-limiting enzyme of MT production, was shown to change in the chicken pineal gland following illumination of

the animal's retina (Zawilska et al. 2004). The question emerges whether light with low intensity has any direct effect on the avian pineal gland or not.

Development of circadian MT secretion

The circadian MT secretion from the pineal gland of mammals starts only somewhat after birth, so the rhythmic functions of the foetus are simply following the maternal rhythm (Zeman et al. 1992). In contrast, the circadian MT rhythm of birds develops already in the embryonic life. Zeman (1992 and 1999) sacrificed chicken embryos, kept under normal illumination (12 hours light and 12 hours dark), in every 2 hours at the age of 18^{th} and 19^{th} embryonic days (E18 and E19 respectively) and showed that at nighttimes the MT contents were higher than that at daytime. The *in vivo* experiments of Herichová (2001) proved that MT secretion and mRNA levels of certain clock genes of E19 chicken embryos were altered by environmental illumination. Akasaka (1995) also described that the environmental illumination modified the MT secretion of cultured chicken pineal gland already at the age of E13-E14. Lamosová (1995) found that after E17 the embryonic pineal glands secreted MT in a rhythmic manner even *in vitro* if were kept under normal illumination cycles, but the rhythm was abolished if kept in constant darkness. However, all the above mentioned *in vitro* experiments were undertaken in two days long static tissue cultures and the changes of MT levels were detected from only two (Akasaka et al. 1995), or from only four samples (Lamosova at al. 1995) daily.

In case of the *in vivo* experiments the possible role of the retina in the transduction of the light information cannot be ruled out. It is also unclear, in both the *in vitro* and the *in vivo* investigations, that the detected differences between daytime and night-time MT contents are corresponding to a real circadian rhythm. The exact timing of the development of the circadian rhythm and the possible role of the environmental illumination in this ontogenesis has still not been revealed. Further investigations are required to clarify these questions.

Effects of Neuropeptides on the embryonic pineal gland

The pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide that was found in several tissues of living organisms. Amongst its widespread effects, PACAP plays a role in the regulation of circadian activities of mammals (Chen et al. 1999; Harrington and

Hoque 1997; Harrington et al. 1999). PACAP has a highly conservated molecular structure, the difference between mammalian and avian PACAP is only one amino acid (Arimura, 1998). The presence and even circadian changes of PACAP levels were shown in the chicken brain (Józsa and tsai., 2001). Other studies described that PACAP stimulates the secretion of MT from the adult chicken pineal gland (Csernus and tsai., 2004; Nakahara and tsai., 2002). The possible effects of the inhibition of PACAP, by administering a PACAP antagonist agent (PACAP6-38), in the development of the avian nervous system were also investigated (Hollósy et al. 2004). In this study, it was found that the *in ovo* PACAP6-38 adminstration on E8 caused a temporary alteration in the motoric behaviour and a more durative alteration in the social behaviour after hatching. From the data listed above, the possibility of PACAP playing a role in the development of the pineal gland's circadian rhythm can be suggested.

Vasoactive intestinal peptide (VIP), a neuropeptide that (just like PACAP) acts through the VPAC receptors, was also shown to stimulate MT secretion from the adult chicken pineal gland even *in vitro* (Csernus and tsai. 2004; Nakahara and tsai. 2002; Zatz and tsai. 1990). Since no previous data was found about the possible roles of VIP in the development and/or regulation of the embryonic pineal gland's hormone rhythm, we planed to investigate the effects of this neuropeptide in our experimental setup as well.

2. **Aims**

To gain information on the questions raised in the introduction, we planned to monitor MT secretion from (both adult and embryonic) chicken pineal glands in our dynamic *in vitro* tissue culture system.

Main objectives:

1. Examination of the effects of periodic illumination with low light intensities to describe the direct photosensitivity of the chicken pineal gland *in vitro*.
2. Investigation of the *in vitro* MT secretion of embryonic pineal gland under different environmental circumstances to understand the time-course and conditions of the development of the circadian rhythm.
3. Examination of the effects of PACAP and VIP on the development of the circadian MT secretion from embryonic pineal glands

3. Materials and methods

Animals

White Leghorn chickens of both sexes were used. Animals were kept at 25 °C and fed with maize. Water and food was accessible *ad libitum*. The chickens were kept under constant, standardized light cycles; 14 hours light (L; from 06.00 to 20.00) and 10 hours dark (D) for at least two weeks before our experiments.

Fertilized eggs of domestic chicken were incubated at 37.5 °C, with a relative humidity of 60% and being turned manually 2 times a day in one part of the experiments. In other experiments the eggs were turned in every half hour by the automatic incubator (Hemel Brutgerät). The animals were decapitated between 13.00 and 14.00. The removed pineal glands were cut in four to six pieces with a sterile blade. Sephadex G10 and pineal pieces mixed from more than one animal were placed into one glass column of the perifusion system. The in vitro experiments were usually four days long.

In ovo treatments

Chicken embryos were injected *in ovo* with 50µg PACAP6-38 dissolved in 25µl physiological saline or only with saline in the same volume (controls) once on E15. The solution was injected to under the chorioallantoic membrane with a Hamilton syringe. Afterwards the whole was covered with sterile tape.

The perifusion system

A tissue culture medium, saturated with a gas mixture of 5% CO_2 and 95% air, was drained through Teflon tubing to the columns containing the pineal fragments. By turning a valve, test sample could be administered to the columns instead of the medium. Thirty minute fractions of the effluent medium were collected by a fractioncollector. The continous flow, at the rate of 0.1 ml/min was maintained by a peristaltic pump located between the columns and the fractioncollector.

Melatonin assay and analysis

Melatonin content of the collected perifusion fluid fractions was determined with radioimmunoassay (RIA) developed in our laboratory (Rekasi and tsai. 1991). The results of RIA of duplicate samples were performed with the aid of a computer program, written in our

laboratory (RIA32 - Csernus). Perifusion results were analyzed and ploted on graphs by our computer program (SUP 32 3.10 - Csernus).

4. Results and discussion

Examination of the photosensitivity of the pineal gland

In contrast to mammals, the non-mammalian pineal gland has direct photosensitivity and functional autonomy as it contains both a complete biological oscillator and the MT synthesizing apparat. The avian pineal gland maintains its circadian MT rhythm even if kept under constant darkness *in vitro*. Just like under normal illumination, the maximal levels of hormone secretion (peaks) appear around midnight and the minimal levels around noon as well (Csernus and tsai. 1998).

The phase of the *in vitro* MT rhythm can be modified by environmental illumination (Binkley and tsai. 1978; Csernus and tsai. 1998; Takahashi and tsai. 1980). In accordance to these data, we found that if the pineal glands were kept in the perifusion system under reversed illumination (20.00-08.00: light phase, 08.00-20.00: darkness), where the light intensity was 600 lux, the phase of the MT rhythm became complete reverted (peaks at noon) as well. The phaseshift was complete under 24 hours, so it took only one cycle for the gland to adopt to the new environment. Similar results were observed if the intensity of light was as low as 10 lux. However, in this case the MT level of the first shifted peak, that appeared at noon, was visibly lower than that of the same peak in the previous experiment This result can lead to the conclusion that the speed of the completion of the phaseshift is slower if the light intensity is smaller (Faluhelyi and Csernus 2007).

If the length of the light phase was decreased to only 3 hours daily (20.00-23.00) and the intensity of the light was only 10 lux, the phaseshift was even more delayed. In this case, the MT secretion was still minimal on the first day's noon under darkness. The first MT peak under the dark hours was shown only on the second day and even that was slightly early, appearing between 9.00 and 10.00. All the peaks in this experiment were wider than usual and the amplitude of the rhythm was decreased. These changes can be due to a partial desynchronization caused by the difference in the reactions of the different oscillator units to

this illumination. Most probably some units follow the new light-dark cycle immediately while other ones, being less sensitive to this light, hesitate and start to follow the same rhythm a bit later. In conclusion, light stimulus at the intensity of 10 lux, applied for only 3 hours, is already very near the limit of the chicken pineal gland's direct photosensitivity (Faluhelyi and Csernus 2007).

It is unclear whether the effects of low intensity illumination on the starling embryos in the experiments of Gwinner (1997) were the consequence of a direct photosensation of the pineal gland or the retina participated in this process as well. Our experiments showed that MT secretion of the explanted pineal glands of E17 chicken embryos could be controlled by environmental illumination with light intensity of only 10 lux. These results proved that the embryonic pineal gland is directly (and independently from the retina) sensitive to illumination. As the pineal photosensitivity of E17 embryos was the same as that of adults, it was concluded that the intracellular apparat responsible for light detection is already fully developed and functional by this age (Faluhelyi and Csernus 2007).

Examination of the embryonic MT secretion

Although in mammals the maternal rhythm determines and controlles all the periodic functions of the foetus, in birds, the autonomous MT rhythm develops already in the embryonic life (Zeman and tsai. 1999). Previous studies showed *in vivo* and *in vitro* differences between daytime and night-time MT secretion and/or AANAT activity (Herichova and tsai. 2001; Zeman and Illnerova 1990; Zeman and tsai. 1992; Zeman and tsai. 1999). However, no further information was found on the development of the circadian MT rhythm and on its conditions. In our preliminary experiments we found out that the thirteenth day of the embryonic life (E13) is the earliest age of the chicken when quick and safe explantation of the pineal gland is possible. With a series of experiments, we showed that without any periodic environmental stimuli (illumination, temperature changes, mechanical stress, etc.) the MT secretion does not become rhythmic throughout the embryonic life at all. This finding is in accordance with the data of Akasaka (1995) who did not find any difference between daytime and nighttime MT concentrations if the embryos were kept under constant darkness.

It was also observed that if the eggs were turned twice daily (always in the same timepoints of the day) during the incubation, pineal glands explanted after E17 secreted MT in a clear,

circadian manner. No similar results were seen in case of pineal glands younger than E17. From these data we can conclude that even mechanical (and/or gravitational) stimuli of the eggs induce the development of the MT rhythm if the egg is exposed to them periodically. Since no rhythmic hormone production appeared before E17 under the same stimuli, we suggest that the oscillator mechanism, or the connection pathways between this and the MT synthesis develops between E16 and E17 (Faluhelyi and tsai. 2004 and 2005). Lamosova (1995) came to the same conclusion based on their experiments on static tissue cultures.

As most authors, who described daily changes in the embryonic MT levels, kept the animals or the tissue cultures under normal illumination (Lamosova and tsai. 1995; Zeman and tsai. 1999), we supposed that periodic light stimuli can also induce the development of the rhythm. In our experiments, where embryonic pineals at different ages were keptunder periodic illumination *in vitro*, similar results emerged as in case of the periodic egg turnings; MT rhythm appeared only after E17. This finding also supports the conclusion, that the pineal gland becomes capable of rhythm formation only around E16 and E17 (Faluhelyi and Csernus 2007; Csernus et al. 2007). In addition, in these experiments some changes in the MT secretion under the beginning of the "light hours" were also recognized, showing that photosensation is possible already at this age and even *in vitro* (Csernus et al. 2007).

The possible effects of egg incubation under rhythmic light conditions were also investigated. We found that if the *in ovo* light-dark cycles, started from E8, were also continued in the perifusion system after the explantation of the glands, circadian MT secretion was observed already from E13 *in vitro*. In other words, the *in ovo* periodic illumination induced an earlier appearance of the circadian MT rhythm than observed in any other experiment (Faluhelyi and tsai. 2009). As the eggs are exposed to rhythmic environmental stimuli during their natural incubation as well, our conclusion can be inverted; the lack of periodic stimuli leads to a delay in the development of the circadian rhythm or in the synchronization of the oscillator units of the pineal gland (**1. table**). Other experiments on zebrafish embryos ended up with a similar conclusion (Kazimi and Cahill, 1999; Vuilleumier 2006). In these studies, the authors showed that changes in the environmental light conditions were essential to start the function of the circadian clock.

	in ovo	*in vitro*	E13	E17	
rhythmic illumination	-	-	-	-	**rhythmic** MT secretion
	-	+	-	+	
	+	-	-	+	
	+	+	+	+	

1. table

Relation of the circadian rhythm's development and the environmental illumination

The + (positive) sign refers to the circadian rhythm of the experimental illumination and/or MT secretion. The – (negative) signs means experiment under constant darkness and/or the abscence of rhythmic hormone secretion. E13 and E17 stand for the embryonic age when the glands were explanted and the perifusion was started. MT rhythm appeared from already E13 if both the egg incubation and the *in vitro* experiment were undertaken in periodic illumination. In contrast, circadian hormone production after E17 appears in any case where the pineals were exposed to rhythmic illumination at some point of their life.

Table 1 shows that circadian rhythm can be observed from E13 only if the eggs were already exposed to periodic illumination that continued even *in vitro*. However, if we compare this early MT rhythm with the circadian hormone production of older embryos (after E17), the amplitude of the E13 rhythm is visibly smaller. In addition, the MT secretion of E13 embryos does not show the signs of the autonomous oscillator function; there is no decrease before the the light phase or increase before the dark phase in the hormone levels. All these observations indicate that the MT "rhythm" appearing before E17 is not a genuine oscillation synchronized by the light, but it is rather a continous hormone production temporarly inhibited by the light.

In contrast, from E17 the MT rhythm is clear, continues even in constant darkness and appears in any case if the pineal gland were exposed to periodic illumination either *in ovo* or *in vitro* or if the eggs met rhythmic mechanical stimuli. In conclusion, by this age the oscillator mechanism is already mature and to start its function only some sort of rhythmic environmental stimulus is required. Probably this induction from the environment is necessary to synchronize the function of different oscillator units of the pineal gland to each other.

In summary, we can conclude that the maturity of the pineal gland's clock mechanisms and a sign of the environment's periodicity are both necessary for the onset of the circadian rhythm. As daily rhythms are tools of adaptation to the day-night cycle, it is understandable that periodic signs from the environment participate in the formation of circadian rhythms even at the level of the individual animal.

Examination of the effects of PACAP and VIP on the embryonic MT secretion

The presence and activity of PACAP was shown from neuroblasts of chicken embryos already at the age of E4 (Erhardt et al. 2001). Although it is known that PACAP stimulates the MT secretion from adult chicken pineal glands (Csernus et al. 2004; Nakahara et al. 2002), no data on its effects on the embryonic gland was found. Our experiments presented that MT production was increased after *in vitro* PACAP administrations from the perifused pineal glands from the age of E14. However, apart of the repeated two to three hours long PACAP responses, the pattern of MT secretion was similar than that of untreated control pineals (Faluhelyi et al. 2004 and 2005). This result suggested that daily PACAP exposures did not change the development of the circadian MT rhythm.

Since we previously concluded that the development of the oscillator mechanism is fulfilled sometimes between E16 and E17, the effects of *in ovo* anti-PACAP treatment on E15 was investigated. Although changes in the post-hatch motor and social behaviour are known after the same treatment (Hollósy et al. 2004),the MT secretion of the treated and the that of the control pineal glands were found to be similar in our experiment. We concluded that, in this experimental setup, inhibitaion of PACAP did not affect the main function of the pineal gland (Faluhelyi et al. 2006).

Another neuropeptide, VIP was also described to induce an increase in the MT synthesis in adult chicken (Nakahara et al. 2002; Zatz et al. 1990). After testing the effects of periodic VIP administration in our perifusion system on embryonic chicken pineal glands, similar results were obtained as in case of PACAP injections; although the cells were sensitive to VIP from E14, no major changes in the pattern of MT secretion could be found (Faluhelyi et al. 2006).

According to our findings we can conclude that chicken pineal glands are sensitive to both PACAP and VIP and both neuropeptides can induse a temporary increase in the hormone production, but neither of them plays a significant role in the development of the circadian MT rhythm. This conclusion agrees with the findings in adult animals, where both PACAP and VIP were shown to stimulate MT secretion without changing the phase of the rhythm (Nakahara et al. 2002; Zatz et al. 1990). However, to come to a final conclusion about the relation of these neuropeptides and the development and synchronization of the rhythmic MT secretion, further investigations are necessary.

Summary of our new findings

1. We confirmed several data that were supposed by other authors based on *in vivo* experiments or "snapshot-like" results of studies undertaken in static *in vitro* cultures with only two or four sampling a day.
2. We showed that the photoreceptors of chicken pineal glands are sensitive to dim light (with the intensity of only a few lux), and even the *in vitro* MT secretion can be synchronized by such an illumination.
3. The photoreceptors mediate the light information to the MT synthesis already from E14 and the photosensitivity of the E17 gland is similar to that of adult pineal glands.
4. We also proved that the presence of periodic environmental stimuli is crucial for the development of the circadian MT rhythm.
5. These possible environmental stimuli range on a broad spectrum: from mechanical inducments (like for example egg turnings) to illumination.
6. We concluded that the development of the oscillator mechanism and/or the signal transduction between this and the MT synthetizing apparat is completed between E16 and E17, but is influenced by the enviroment.
7. Neither PACAP nor VIP seems to play significant role in the development of the circadian rhythm.

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5. **Publications**

Publications related to the thesis:

Faluhelyi N, Reglődi D, Lengvári I and Csernus V (2004): Development of the circadian melatonin rhythm and the effect of PACAP on melatonin production in the embryonic chicken pineal gland. An *in vitro* study. - *Regulatory Peptides, 123, 23-28*.
Impact factor: 2.531

Faluhelyi N, Reglődi D and Csernus V (2005): Development of the circadian thythm and it's responsiveness to PACAP in the embryonic chicken pineal gland. - *Annals N.Y. Acad. Sci., 1040, 305-309*.
Impact factor: 1.971

Faluhelyi N, Reglődi D and Csernus V (2006): The effects of PACAP and VIP on the *in vitro* melatonin secretion from the embryonic chicken pineal gland. - *Annals N Y Acad Sci., 1070, 271-275.*
Impact factor: 1.93

Faluhelyi N and Csernus V (2007): The effects of environmental illumination on the *in vitro* melatonin secretion from the embryonic and adult chicken pineal gland. - *General and Comparative Endocrinology, 152, 154-158.*
Impact factor: 2.562

Csernus VJ, Nagy AD and **Faluhelyi N** (2007): Development of the rhythmic melatonin secretion in the embryonic chicken pineal gland. - *Gen Comp Endocrinol. 152, 148-153.* Impact factor: 2.562

Faluhelyi N, Matkovits A, Párniczky A and Csernus V (2009): The *in vitro* and *in ovo* effects of environmental illumination and temperature on the melatonin secretion from the embryonic chicken pineal gland. - *Trends in Comparative Endocrinology and Neurobiology: Ann. N.Y. Acad. Sci., 1163, 383–385.*
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Publications not related to the thesis:

Csernus V, Faluhelyi N and Nagy AD (2005): Features of the circadian clock in the avian pineals. - *Annals N.Y. Acad. Sci., 1040, 281-287.*
Impact factor: 1.971

Faluhelyi N and Csernus V (2005): The effects of periodic alteration of the temperature on the rhythmic melatonin release of explanted chicken pineals. - *Neuro Endocrinol Lett., 26, 503-510.*
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6. References

Akasaka K, Nasu T, Katayama T and Murakami N **(1995)**. *Brain Research 692, 283-286.*

Arimura A **(1998).** *Jpn.J.Physiol 48, 301-331.*

Binkley SA, Riebman JB and Reilly KB **(1978)**. *Science 202, 1198-1200.*

Chen D, Buchanan GF, Ding JM, Hannibal J and Gillette MU **(1999)**. *Proceedings of the National Academy of Sciences of the United States of America 96, 13468-13473.*

Collin JP, Juillard MT and Voisin P **(1984)**. *Ophthalmic Research 16, 107-113.*

Csernus V, Ghosh M and Mess B **(1998)**. *General & Comparative Endocrinology 110, 19-28.*

Csernus V **(2003)**. In *Rhythmic biological processes. The role of the biological clocks.*, pp 119-133. Eds V Csernus & B Mess. Budapest: Dialog Campus Inc.

Csernus V, Józsa R, Reglődi D, Hollósy T, Somogyvári-Vígh A and Arimura A **(2004)**. *General & Comparative Endocrinology 135, 62-69.*

Dunlap JC **(1999)**. *Cell 96, 271-290.*

Erhardt NM, Fradinger EA, Cervini LA, Rivier JE and Sherwood NM **(2001)**. *Endocrinology 142, 1616-1625.*

Gwinner E, Zeman M and Klaassen M **(1997)**. *Journal of Pineal Research 23, 176-181.*

Harrington ME and Hoque S **(1997)**. *Neuroreport 8, 2677-2680.*

Harrington ME, Hoque S, Hall A, Golombek D and Biello S **(1999)**. *Journal of Neuroscience 19, 6637-6642.*

Herichova I, Zeman M, Mackova M and Griac P **(2001)**. *Neuroscience Letters 298, 123-126.*

Hollósy T, Józsa R, Jakab B, Németh J, Lengvári I and Reglődi D **(2004)**. *Regulatory Peptides 123, 99-106.*

Józsa R, Somogyvari-Vígh A, Reglődi D, Hollósy T and Arimura A **(2001)**. *Peptides 22, 1371-1377.*

Kazimi N and Cahill GM. **(1999)**. *Brain Res Dev Brain Res.117 (1), 47-52.*

Korf HW and Vígh-Teichmann I **(1984)**. *Ophthalmic Research 16, 96-101.*

Korf HW and Wicht H. **(1992)**. *Prog Brain Res. 91, 285-97.*

Korf HW **(1994)**. *Annals of the New York Academy of Sciences 719, 13-42.*

Lamosova D, Zeman M, Mackova M and Gwinner E **(1995)**. *Experientia 51, 970-975.*

Mess B, Rékasi Z, Ghosh M and Csernus V **(1996)**. *Acta Biologica Academiae Scientiarum Hungaricae 47, 313-322.*

Nakahara K, Abe Y, Murakami T, Shiota K and Murakami N **(2002)**. *Brain Research 939, 19-25.*

Rékasi Z, Csernus V, Horváth J, Vígh S and Mess B **(1991)**. *Journal of Neuroendocrinology 3, 563-568.*

Takahashi JS, Hamm H and Menaker M **(1980)**. *Proceedings of the National Academy of Sciences of the USA 77, 2319-2322.*

van Veen T, Elofsson R, Hartwig HG, Gery I, Mochizuki M, Cena V and Klein DC **(1986)**. *Experimental Biology 45, 15-25.*

Vígh B and Vígh-Teichmann I **(1988)**. *Pineal.Res.Rev. 6, 1-65.*

Vígh-Teichmann I and Vígh B **(1992)**. *Microscopy Research & Technique 21, 227-241.*

Vuilleumier R, Besseau L, Boeuf G, Piparelli A, Gothilf Y, Gehring WG, Klein DC and Falcón J. **(2006)**. *Endocrinology. 147 (5), 2273-2279.*

Zatz M, Kasper G and Marquez CR **(1990)**. *Journal of Neurochemistry 55, 1149-1153.*

Zawilska JB, Berezinska M, Rosiak J, Skene DJ, Vivien-Roels B and Nowak JZ **(2004)**. *J.Pineal Res. 36, 80-86.*

Zeman M and Illnerova H **(1990).** *Comparative Biochemistry and Physiology A - Comparative Physiology 97, 175-178.*

Zeman M, Gwinner E and Somogyiova E **(1992)**. *Experientia 48, 765-768.*
Zeman M, Gwinner E, Herichova I, Lamosova D and Kostal L **(1999)**. *Journal of Pineal Research 26, 28-34.*