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Rhythmic Pineal Functions in Birds

Introduction

As one of the most fruitful discovery of biology in the past few decades, we realized that much higher percentage of biological functions are periodically repeating, rhythmic in nature than we earlier thought. Although many rhythmic functions have been described, the underlying mechanisms, the structures of the "biological clocks" mostly remain to be clarified. The period length of the biological rhythms ranges from millisecond, like nervous trains, to months, like the seasonal rhythms, or even longer. From this wide variety of phase length, the one day long, **circadian** rhythms have outstanding importance in various life forms on the Earth. From the large number of bioactive materials that is involved in or controls circadian activities, this study primarily focuses on the role of the pineal melatonin.

Melatonin (MT) is one of the most ancient bioactive molecule during **phylogenesis**. This conservative molecule, a tryptophane derivative, has been detected even in higher plants and in algae (Hardeland, R. *et al.* 1995). It is present in nearly all invertebrate species and in every vertebrate (Korf, H.W. 1994). Since pineal gland, as a defined organ, first appears in the cyclostomes (lamprey, for references see Vigh, B. and Vigh-Teichmann, I. 1999), MT is produced in lower species by extra-pineal sources. Extra-pineal MT production is well known also in the vertebrates (Bubenik, G.A. *et al.* 1974; Bubenik, G.A. *et al.* 1976; Bubenik, G.A. *et al.* 1977; Bubenik, G.A. *et al.* 1978; Zawilska, J.B. and Nowak, J.Z. 1992; Bubenik, G.A. and Pang, S.F. 1997).

Pineal gland of higher vertebrates is a derivative of the so called third eye or parietal eye. Studies on the transformation of the retinal light-sensory cells of the parietal eye to secretory (glandular) cells of the pineal gland is a special chapter of pineal research. Many outstanding laboratories in different countries were dealing with this comparative aspect using morphological (light and electron microscopy, immunocytochemistry [ICC]), biochemical and electrophysiologic techniques (Oksche, A. and Vaupel-von Harnack, M. 1965; Korf, H.W. and Vigh-Teichmann, I. 1984; Collin, J.P. *et al.* 1984; Vigh, B. and Vigh-Teichmann, I. 1988; Collin, J.P. *et al.* 1989). The rhythmic and rhythm-regulating functions of the pineal gland in lower vertebrates have also been widely studied (Vivien-Roels, B. and Arendt, J. 1983; Underwood, H. and Harless, M. 1985; Mendonca, M.T. *et al.* 1995).

The basic functions of the pineal gland are related to the control of daily and seasonal rhythmic functions. It participates in the regulation of daily sleep-wakefulness cycles (Redfern, P. *et al.* 1994; Arendt, J. *et al.* 1997), daily cycles of motoric activity (Morita, Y. *et al.* 1987), circadian endocrine changes (see for references Mess, B. 1983), or the regulation of reproductive processes (Mess, B. *et al.* 1978; Reiter, R.J. 1981a; Trentini, G.P. *et al.* 1982; Vivien-Roels, B. and Arendt, J. 1983). It also controls seasonal reproductive activity (Reiter, R.J. 1974), circannual changes in thyroid function (Peschke, D. *et al.* 1989) or hibernation (Saarela, S. and Reiter, R.J. 1994; Kocsard-Varo, G. 2000).

The main hormone of the pineal gland, MT was introduced also in the medical practice to cure insomnia (Armstrong, S.M. 1999; Zisapel, N. 1999), to prevent jag lag or sleep disorders in shift-workers (Arendt, J. *et al.* 1992; Arendt, J. 1999), to protect against free radical damage (Reiter, R.J. 1997; Reiter, R.J. *et al.* 1994; Bromme, H.J. *et al.*

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1999) or to reduce tumor growth (Blask, D.E. *et al.* 1992; Bartsch, C. and Bartsch, H. 1999).

Pineal MT secretion shows well defined circadian and circannual (seasonal) rhythms both in mammals and birds. Both rhythms were most throughly studied in different mammalian species. Based on *in vivo* experiments, Axelrod, J. *et al.* (1965) showed first that in mammals the circadian MT rhythm is characterized by a nocturnal high peak and a diurnal nadir. The circadian rhythm of serotonin blood level (the precursor of MT) shows an inverse pattern (Snyder, S.H. *et al.* 1967). Based on these data the pineal gland was called a "biological clock" (Axelrod, J. and Wurtman, R.J. 1966). Similar daily rhythm of pineal MT secretion was also shown in birds under *in vivo* circumstances (Cassone, V.M. and Lu, J. 1994). MT seems to be one of the primary messenger controlling othe circadian and seasonal biological rhytms.

Daily rhythm of pineal MT production is regulated by the environmental light-dark changes in *mammals* (Wurtman, R.J. *et al.* 1964). Light impulses received by the retinal receptors and transmitted by the retino-hypothalamic pathways play key role in maintaining this circadian rhythmicity. Moore *et al.* (1967) described the neuronal circuit through which light regulates pineal MT secretion. As it is evident from the character of the circadian MT cycle (night peak), light seems to play an inhibitory role. It inhibits one of the key-enzymes of MT biosynthesis, hydroxy-indole-O-methyl-transferase (HIOMT), in the pineal gland (Axelrod, J. *et al.* 1965).

Most of the physiologists deny direct light-sensitivity of the *mammalian* pineal (Wurtman, R.J. *et al.* 1964; Reiter, R.J. 1981b; Karasek, M. *et al.* 1988; Pevet, P. 1988; Reiter, R.J. 1993). At the same time, photopigment and rod- and cone-like pinealocytes were shown in mammalian pineal (McClung, R. and Dafny, N. 1975; Reiter, R.J. 1981b; Luo, Z.R. *et al.* 1984; Korf, H.W. *et al.* 1985; Vigh, B. and Vigh-Teichmann, I. 1999) and some electrophysiological data also indicated direct pineal light sensitivity in rats (Taylor, A.N. and Wilson, R.W. 1970; Barajas-Lopez, C. *et al.* 1987; Bromme, H.J. *et al.* 1999). However, rat pineal explanted into an *in vitro* perifusion system, failed to show circadian MT rhythm. These pineals, deprived of neuronal connections, had a steady basal secretion rate of MT, independently of environmental light changes (Simonneaux, V. *et al.* 1989; Rekasi, Z. *et al.* 1991; Mess, B. *et al.* 1991a). Apparently the light perception of mammalian pineal seem to be decoupled from the MT synthesizing machinery.

In contrast, explanted *chicken* pineal gland had preserved its circadian rhythmicity also under *in vitro* circumstances (Wurtman, R.J. *et al.* 1964; Takahashi, J.S. *et al.* 1980; Mess, B. *et al.* 1991a). Avian pineal, like that in most species of non-mammal vertebrates, preserved functioning photoreceptors (Collin, J.P. *et al.* 1976; Moller, W. and Moller, G. 1990; Araki, M. *et al.* 1992; Vigh, B. and Vigh-Teichmann, I. 1999). The light-sensor pigment of the avian pineal, named pinopsin is similar in chemical structure to retial opsins (Okano, T. *et al.* 1994; Max, M. *et al.* 1995; Fejer, Z. *et al.* 1997; Takanaka, Y. *et al.* 1998). Biochemical (Okano, T. *et al.* 1994) and *in vitro* physiological (Csernus, V. *et al.* 1999) data indicate that this photopigment shows blue light sensitivity.

Circadian MT rhythm in chicken pineal gland develops also in lack of rhythmic changes of the environmental illumination indicating that avian pineal gland has a genetically coded autonomy of rhythmic MT producing ability (Csernus, V. *et al.* 1998).

Thus, avian pineal gland has a direct sensitivity to environmental illumination and it includes a complete circadian biological clock which controls rhythmic MT secretion. Therefore *in vitro* chicken pineal seems to be an especially appropriate model for studying mechanism of circadian biological rhythms.

I. Morphology of the Chicken Pineal Gland (ChPG)

A. The structure of the gland

The histology, ultrastructure and cytochemistry of the pineal gland have been throughly studied in different vertebrates, especially mammalian species (Collin, J.P. and Kappers, J.A. 1968; Collin, J.P. and Kappers, J.A. 1971; Vollrath, L. 1979; Reiter, R.J. 1981b; Mennenga, K. *et al.* 1991; Karasek, M. and Reiter, R.J. 1992; Shedpure, M. and Pati, A.K. 1995; Vigh, B. *et al.* 1998; Vigh, B. and

Vigh-Teichmann, I. 1999). However, only few detailed descriptions were found in the literature dealing with the histological character of the avian pineal. (For further references see Vigh, B. and Vigh-Teichmann, I. 1999; Haldar, C. and Guchhait, P. 2000.)

The chicken pineal body is surrounded by a thin connective tissue capsule from which tunnel-shaped, thin connective tissue septa penetrate into the parenchyma separating it into convoluted parenchymal trabecules. According to our light microscopic studies (Csernus, V. and Schwarcz, A., unpublished yet), two main cell types can be distinguished in the parenchyma of the ChPG. The majority of cells, called follicular cells, formed small follicle-like structures with very narrow lumen, while other cells were located between these follicles. These parafollicular cells show deeper basophilic staining property. In the connective tissue of the interlobular septa, few specific cells with bigger, ovoid nuclei of looser chromatin substance are distributed (Fig. 1.).

Interestingly enough, avian pineal is also a lymphatic organ in young chicken, but lymphatic follicles disappear from the pineal in a few weeks after posthatching life (Cogburn, L.A. and Glick, B. 1981; Cogburn, L.A. and Glick, B. 1983; Olah, I. and Glick, B. 1984; Olah, I. and

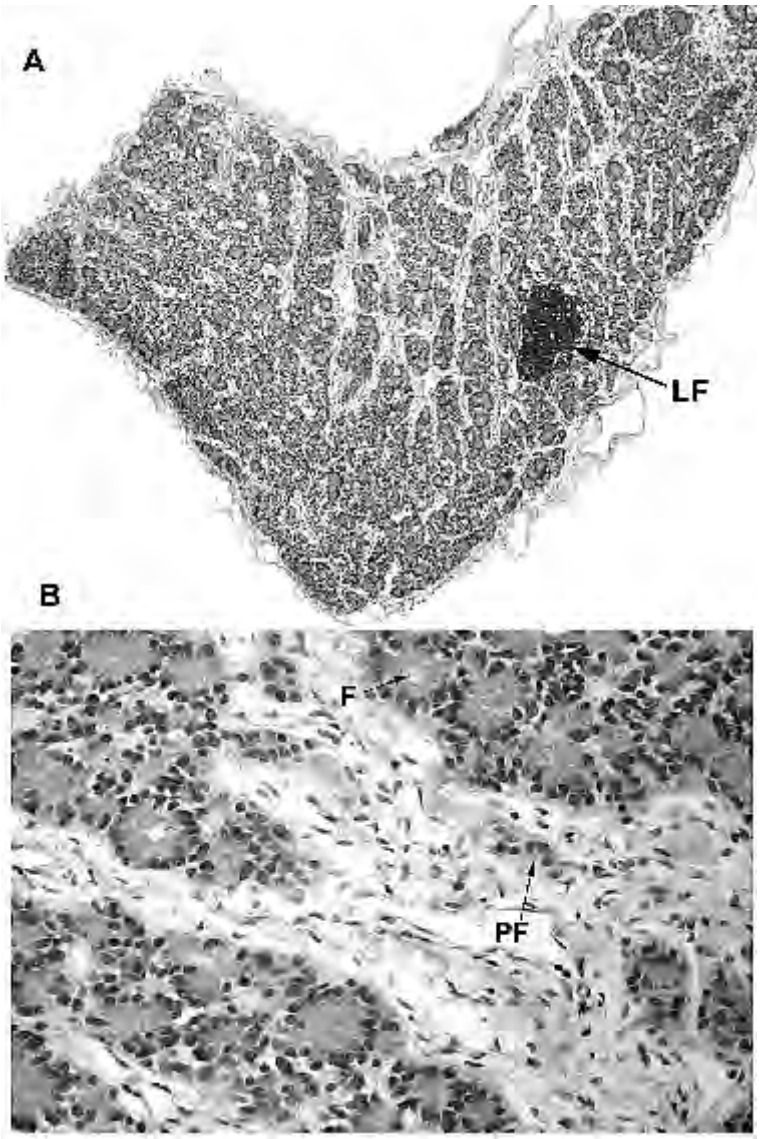

Fig. 1. Light microscopic pictures of the pineal gland of a young chicken. Haematoxylin-eosin staining, "A" = 40×, "B" = 400× magnification. F = follicular cells, PF = parafollicular cells, LF = lymphatic follicle.

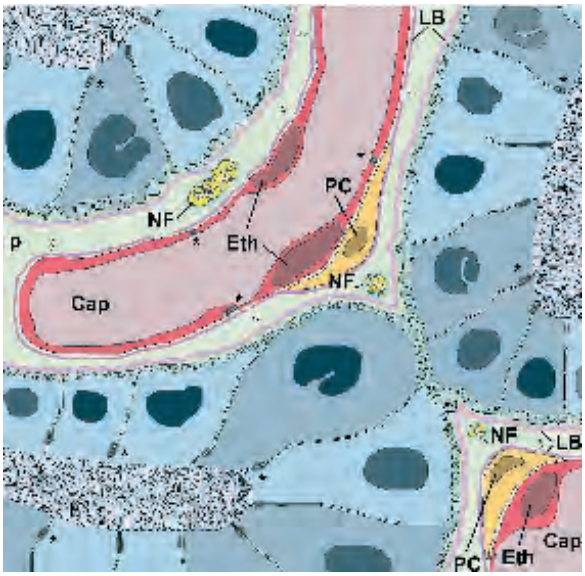

Fig. 2. Fine structure of the chicken pineal gland—schematic illustration. Cap = capillary, Eth = endothelial cell, PC = perycite, LB = lamina basalis, NF = nerve fiber, p = pericapillary space. "≫" label incontinuities on the basal lamina of the capillaries, "*" indicates tight junctions (zonula occludens) between the apical parts of the pineal cells and capillaries. For further details see text. Picture courtesy of P. Toth.

Magyar, A. 1996) (Fig. 1.A). The significance of this phenomenon and its possible relation to the development of the rhythmic MT secretion in the avian pineal gland remains to be clarified.

Based on electronmicroscopical studies, connective tissue septa in the ChPG are to be considered as enlarged perivascular and pericapillary spaces through which, in addition to blood vessels of different sizes, unmyelinated nerve fibers run. The parenchymal trabecules consist of groups of pinealocytes, resembling to "acini". A summary of our EM observations on the ChPG (Toth, P. and Csernus, V., yet unpublished) is summarized in Fig. 2. Regarding the 3D appearance of pinealocytes, we confirmed the original findings of Hortega, who described golf club-like processes extending to various distances in almost any directions from the cell bodies (Hortega, R.P. 1932). The pinealocytes seem to have three different surfaces: 1.) The *contact surfaces* are fairly regular and strait, and are always kept together by circular junctional complexes (zonulae occludentes) near to their apical ends (Fig. 3.A). 2.) The *basal surface* contacting the perivascular- or pericapillary spaces presents relatively short, sparsely branching protoplasmic processes, thus commonly forming an irregular border towards the trabecules. No matter how irregular this border is, we always found a strictly continuous, relatively thick basal lamina, which separates the cells from the perivascular- or pericapillary spaces (Fig. 3.B). 3.) The *apical surface* facing the "lumen of acini" is always studded with profusely branching protoplasmic processes of various length and thickness. Our

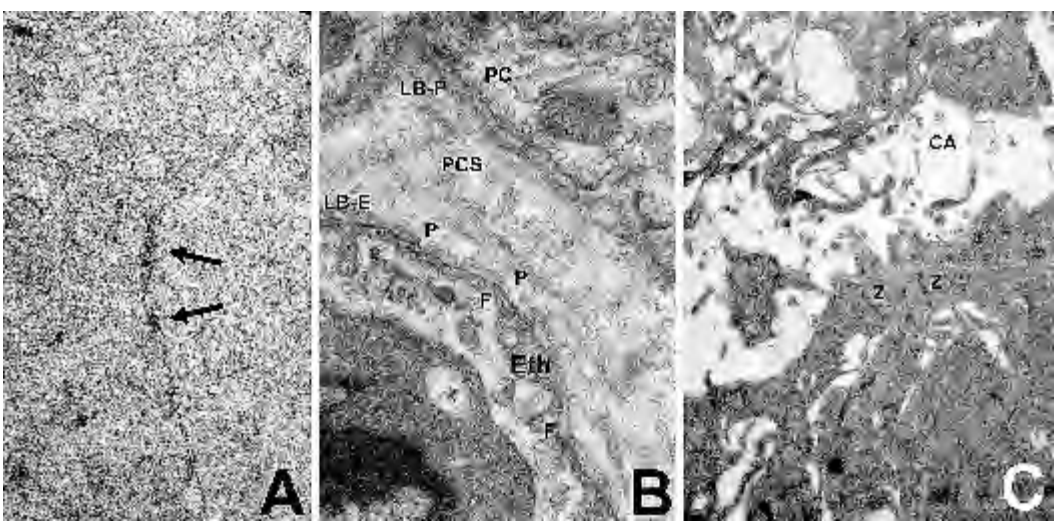

Fig. 3. Electronmicroscopic structure of the chicken pineal gland in 30 000× (A and B) and 10 000× (C) magnification. (A) Tight junctions (arrows) between the contact surfaces of the pineal cells near to their apical surface. (B) The structure of the pericapillary space (PCS). Below the irregular basal border of pinealocytes (P) thick, continuous basal lamina (LB-P) can be seen. The basal lamina (LB-E) of the fenestrated (F) endothelial cells (Eth) is thinner, and has multiple pores (P). (C) The area of the center of an "acinus" (CA) is hermetically sealed by zonulae occludentes (Z).

studies revealed that these processes are intermingled in the "center of the acinus", a space that is hermetically separated from any other spaces of the pineal body by systems of zonulae occludentes (Fig. 3.C).

Another novel observation of us is that the capillaries within the trabecules are frequently lined with fenestrated endothelial cells and surrounded by incontinuous and relatively thin basal lamina (Fig. 3.B, not shown on Fig. 2.). In the pericapillary spaces, occasionally groups of unmyelinated nerve fibers were seen (Fig. 4.). In spite of the presence of round, clear synaptic vesicles and a few "dense core" vesicles, synaptic contacts were not found.

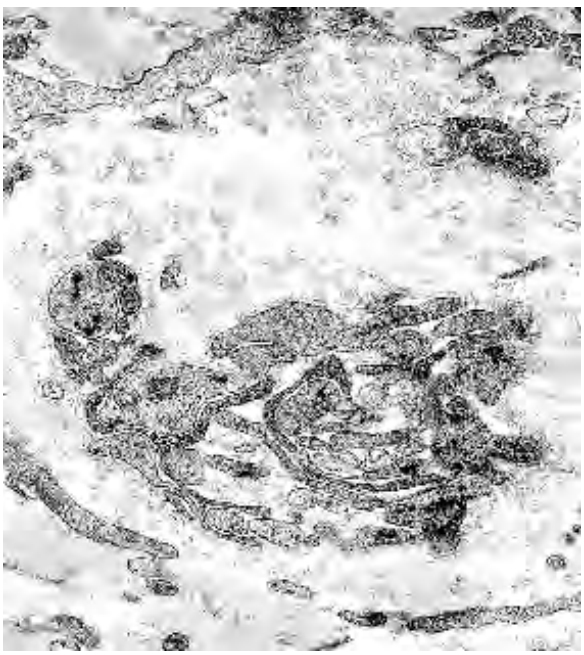

Fig. 4. Unmyelinated nerve fibers in the pericapillary space of the chicken pineal gland. Note "dense core vesicles" in some of them. 20 000 × magnification.

B. Neural connections

The innervation of the pineal gland has also been intensively studied in different *mammalian* species (Kenny, G.C. 1965; Kappers, J.A. 1967; Korf, H.W. and Möller, M. 1984). Beside the classic, sympathetic innervation, different pinealopetal (cholinergic, NPY, VIP) and even pinelofugal fibers were described with light and electronmicroscopic, as well as with various ICC methods (see for references Korf, H.W. and Möller, M. 1984). However, no direct data were found dealing with the innervation of the *chicken* pineal gland. In our studies aiming at this topic, retrograde fibre-tracing technique with biotinilated amino-dextran (BAD) tracer was utilized (Csernus, V. and Kovács, A., unpublished yet). This tracer was introduced *in vivo* into the pineal gland by iontoforetic technique. After a 48 hour incubation period, chickens were killed and the tracer was visualized applying avidin-peroxidase-diaminobenzidine technique. Beside the pineal gland and most of the brain, different cephalic and cervical ganglia, and the cervical and thoracic segments of the spinal chord were studied. BAD labeled fibers, but no cell bodies were found in the pineal gland especially in the pineal stalk (Fig. 5.A). The most pronounced fibre system were present in the walls of the carotid artery and marked cells were present in the upper sympathetic cervical ganglion (Fig. 5.B and C). Labeled fibers and a few perikarya were found in the pterygopalatine ganglion (Fig. 5.D). Longitudinal fibers were also found in the sagittal section of the pons and medulla around the Edinger-Westphal nucleus and in the caudal and gigantocellular parts of the reticular nucleus. A few, slightly immunopositive perikarya were also located in the near vicinity of these fibers (Fig. 5.G and H). Similar marked fibers were detected in the anterior horn of the thoracic spinal cord corresponding the fibers of the anterior root (Fig. 5.E). Markedly stained immunopositive cells were located in these segments in the intermediate horn (Fig. 5.F). At the same time, no labeled fibers or cells could be seen in the ggl. trigeminale, ggl. geniculi or ggl. superius of the vagus. These findings indicate that in the birds pineal glands receives nerve fibers from the sympathetic ganglia and various regions of the brain stem and spinal chord. The functional significance of these fibers are mostly remains to be clarified.

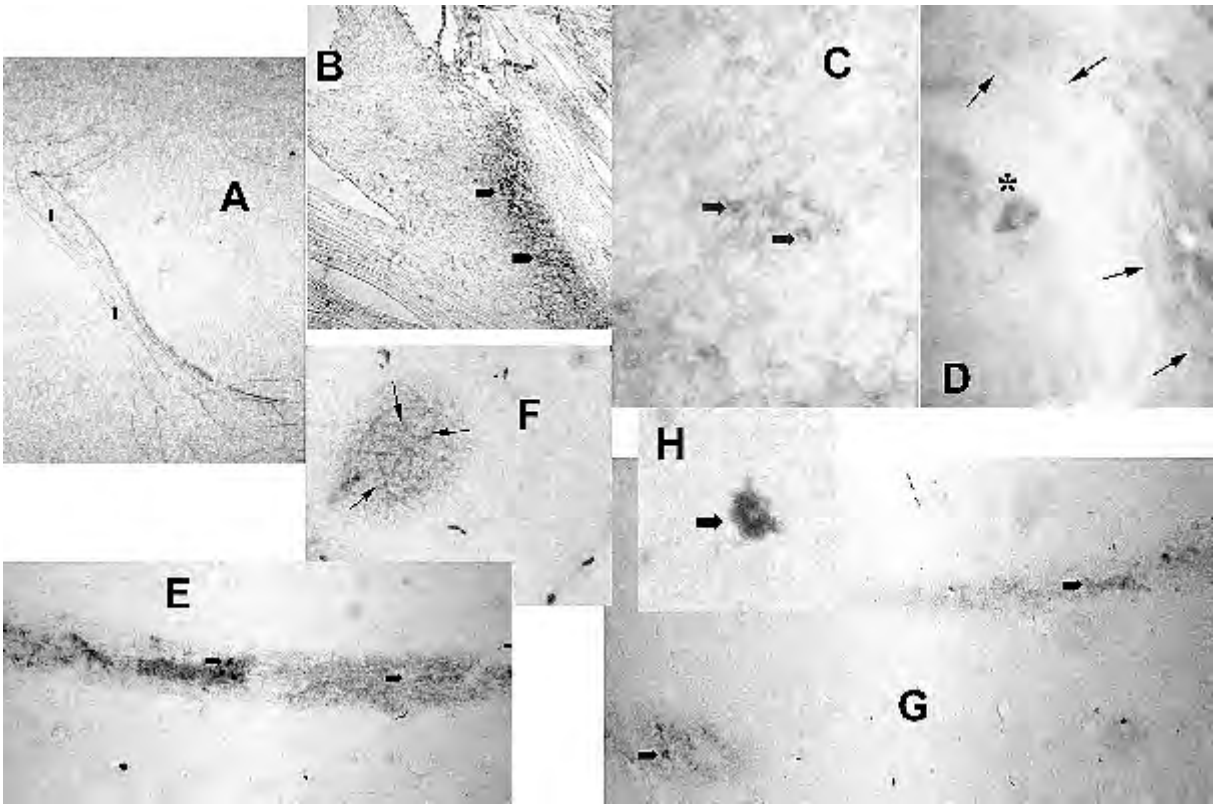

Fig. 5. Retrograde axon tracing from chicken pineal gland using biotinilated amino-dextran (BAD). BAD labeled nerve fibers and perikarya in the pineal gland and various areas of the nervous tissue in chicken. "A" fibers in the stem of the pineal gland. "B" fibers in the walls of the carotid artery close to the bifurcation, "C" labeled perikarya in the superior cervical ganglion, "D" pericaryon (*) and fibers (arrows) in the pterygopalatine ganglion, "E" BAD labeled fibers and perikarya (arrows) in the lower cervical segment of the spinal chord, cross section, anterior horn, "F" perikarya in the lateral horn of the upper thoracic spinal chord, "G" fiber and perikarya (arows) in the pons, "H" pericaryon in the same area with higher magnification.

II. Chicken pineal gland as circadian rhythm generator

The pineal gland of most non-mammalian vertebrates, including that of chicken, contains fully functioning circadian biological clock. This clock is controlled directly by the environmental illumination utilizing functioning pineal light receptors and by several bioactive substances, like norepinephrine (NE) or VIP. The MT release in these species are controlled primarily by the biological clock in the gland. In contrast, *mammalian* pineal gland lost both its own rhythm generator and light sensitivity. It is "degraded" to a "slave" endocrine gland controlled by an extra-pineal circadian clock (suprachiasmatic nucleus) via the sympathetic nervous system. Mammalian circadian clock is synchronized to the environmental illumination through the retina.

The main functional components of the chicken pineal gland are: (1) input channels: receptors to various physical (light, temperature, magnetic field) and chemical (NE, VIP, PACAP etc.) factors, (2) a circadian clock, which is able to run even deprived of its neural and humoral connections (being explanted into an *in vitro* system), (3) an output channel: MT synthesis and release and (4) the intracellular signal transduction system controlling and connecting these units (Fig. 6.). The fact that these functions seem to be fully functional even *in vitro* suggesting that explanted chicken pineal is an especially suitable model for studying mechanisms of a circadian biological clock.

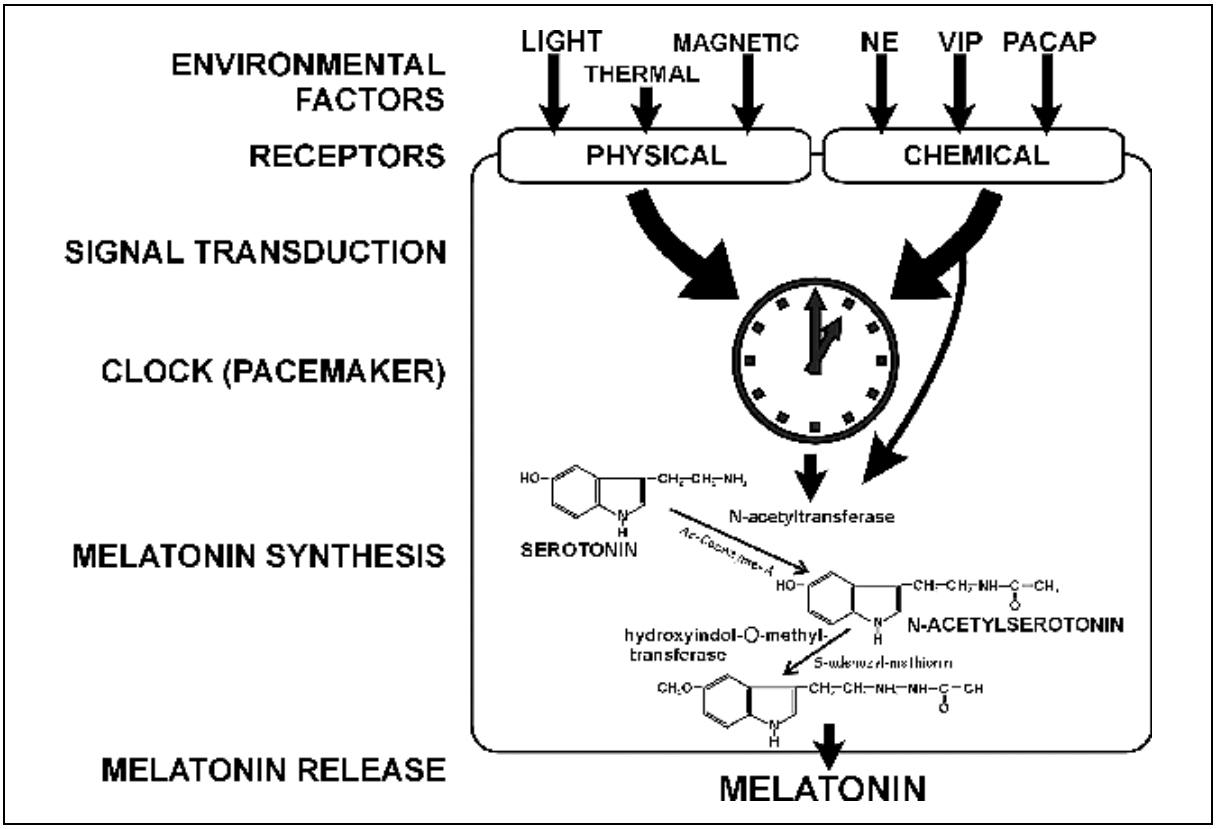

Fig. 6. Summary of the functions of the avian pineal gland. For more details see text.

A. Input channels of the chicken pineal

1. Light

Among the physical factors directly controlling pineal functions, light is the best known and most thoroughly studied (Wurtman, R.J. *et al.* 1964; Klein, D.C. and Weller, J.E. 1972; Zawilska, J. and Nowak, J.Z. 1991). Direct effect of the light on the enzyme activity of the explanted chicken pineal were first described in the sixties (Axelrod, J. *et al.* 1965; Lauber, J.K. *et al.* 1968; Bischoff, M.B. 1969). Continuous monitoring of the direct effect of the environmental illumination on the rhythmic MT release from explanted avian pineal was made possible by introduction of dynamic *in vitro* methods, like perifusion (Takahashi, J.S. *et al.* 1980; Csernus, V.J. and Schally, A.V. 1991).

In perifusion systems, no rhythmic MT secretion from *rat* pineal gland could be found (Simonneaux, V. *et al.* 1989; Rekasi, Z. *et al.* 1991; Mess, B. *et al.* 1991b; Csernus, V. *et al.* 1992). A rapid, light-induced decrease in rat pineal AANAT activity has been described following *in vivo* illumination during the dark phase (Klein, D.C. and Weller, J.E. 1972). *In vitro*, however, changes in environmental illumination did not influence either basal or NE induces increase of MT secretion from explanted rat pineal (Rekasi, Z. *et al.* 1991; Mess, B. *et al.* 1991b).

In contrast, MT released from *chicken* pineals in a circadian fashion also *in vitro* as revealed by tissue culture (Robertson, L.M. and Takahashi, J.S. 1988; Leung, F.C. 1991) and perifusion experiments (Simonneaux, V. *et al.* 1989; Csernus, V.J. *et al.* 1994). The daily rhythm of MT secretion of the perifused pineal was independent whether chickens were sacrificed in the light or in the dark phase of the day. Furthermore, neither the phase nor the amplitude of the MT rhythm-curve was altered by keeping chickens either in continuous light or dark environment 2 weeks *before* sacrifice (Ghosh, M. *et al.* 1994). These data indicate that the endogenous circadian rhythm generator shows significant autonomy in the avian pineal.

Rhythmic MT secretion from *explanted chicken pineal* could be influenced efficiently by changes

in environmental illumination. However, *short light impulses* (1–15 minutes), applied in the dark phase, failed to influence the elevated night level of pineal MT production. Similarly, *constant darkness* throughout 4–5 days also did not abolish the circadian rhythmicity of the explanted pineal gland (Ghosh, M. *et al.* 1994; Csernus, V. *et al.* 1998) (Fig. 7.A). In contrast, continuous illumination for the same duration results in a gradual attenuation of the amplitude of daily rhythm; MT production tends to stabilize in a high level, close to that at the night-peak by the 3rd day (Fig. 7.B) (Ghosh, M. *et al.* 1994; Mess, B. *et al.* 1996; Csernus, V. *et al.* 1998).

In a series of *in vitro* experiments, using the perifusion system, we studied how the environmental illumination influences rhythmic MT secretion from explanted chicken pineals. The

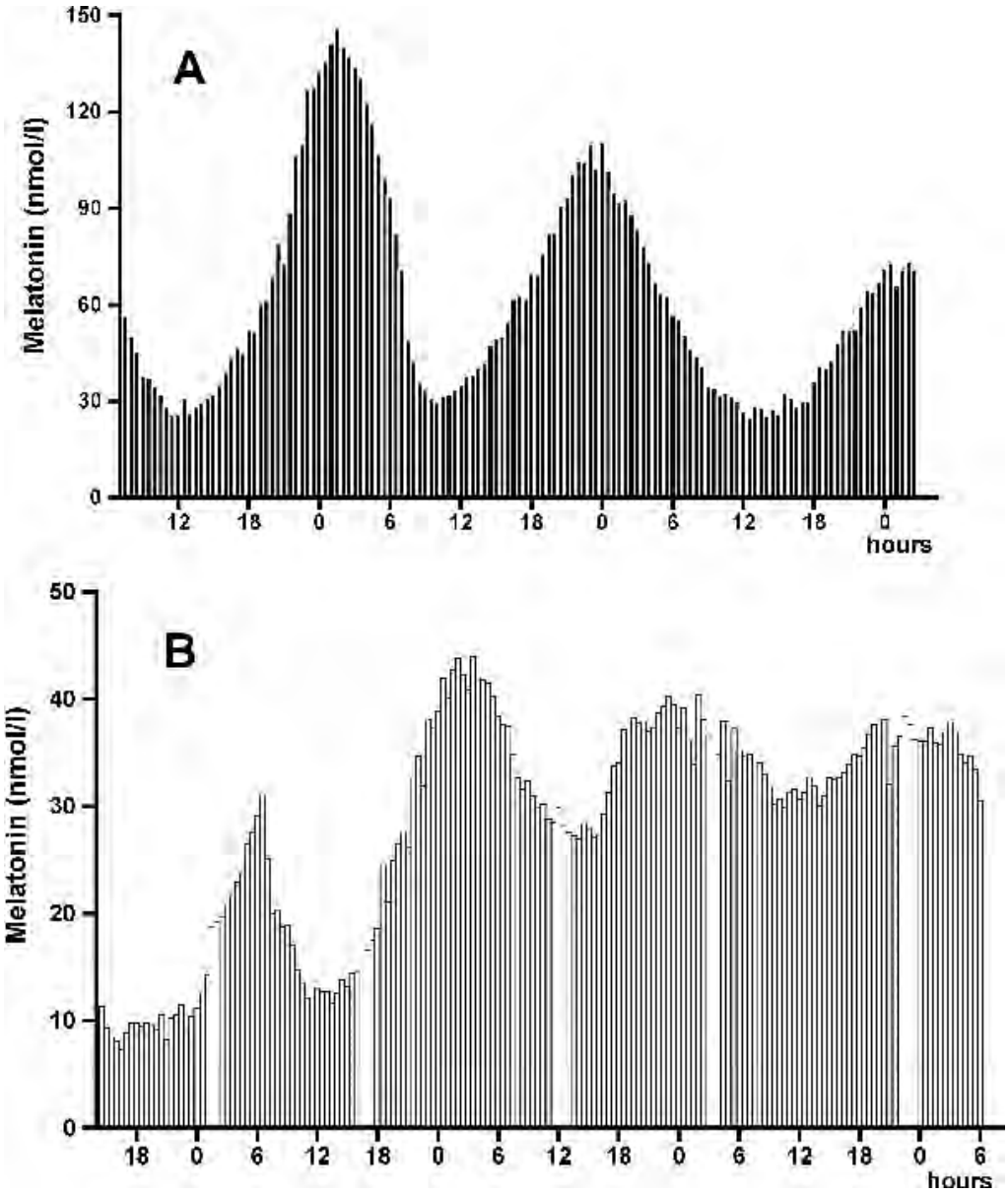

Fig. 7. MT secretion from explanted chicken pineal cells kept under continuous darkness ("A") or continuous illumination ("B") *in vitro*. MT content of consecutive 30 min fractions were collected in 3 or 4 day perifusion experiments.

chicken were kept under normal light-dark cycle *in vivo* prior to the experiment. Illumination of various duration (1 to 24 hours), cycle length (6 to 48 hours) and phase were applied (Ghosh, M. *et al.* 1994; Ghosh, M. *et al.* 1996; Csernus, V. *et al.* 1998). From the data collected this way we concluded: (1) significant modification of the circadian MT release pattern was experienced if the illumination lasted for at least 2 hours. (2) Rapid alteration of the light-dark cycle (e.g. 3 : 3 of 4 : 4 hour L : D) has an effect similar to that of continuous illumination (3 : 3 h, Fig. 8.A). (3) Any cycle length that significantly differed from 24 hours just modified the shape or induced phase shift but did not alter the frequency of the 24 hour MT cycle (6 : 6 h, Fig. 8.B). (4) Repeated "out of phase" but of 24 hour cycle length illumination patterns induced a rapid phase-shift in the circadian MT rhythm. Reversed illumination induces complete reversal of the MT cycle in two days (Fig. 8.C). Periodic light "pulses" (4 hour light 20 hour dark) applied on the onset of the original dark period (20 to 24 hours) also rapidly reverses the MT cycle (Fig. 8.D, yet unpublished observations of Csernus, V.).

Reiter (1983) have shown that the quality of light (wavelength) is an important factor in the regulation of pineal MT secretion in mammals under *in vivo* circumstances. Since in mammals light affects pineal function through the retina, the question arose, whether wavelength of the light is also influential in the regulation of rhythmic MT secretion also in the perifused avian pineal gland. In our experiments, blue, green, and red lights were used for the illumination of the perifused chicken pineals in an inversed phase, and the speed of the phase shift of MT production was observed (Csernus, V. *et al.* 1999). Although all three used wavelengths resulted finally in complete reversal of the phase of MT peak, there was a considerable difference in the speed of this phase-reversing effect. Blue light was found to reverse MT

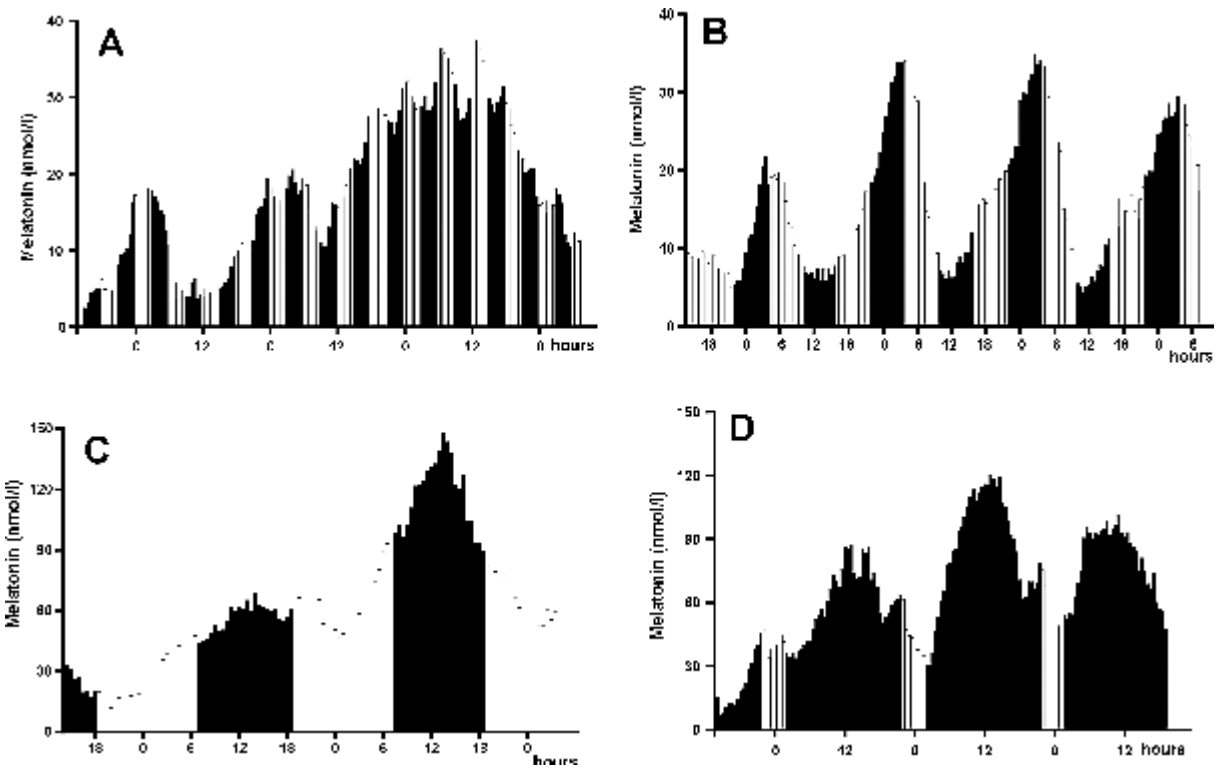

Fig. 8. The effect of different light regime on the rhythmic MT secretion from explanted chicken pineals in perifusion experiments (30 min. fractions). The tissues were exposed *in vitro* to different light patterns "A" L : D 3 : 3 hours, "B" L : D 6 : 6 hours "C" L : D 12 : 12 hours, reversed illumination, "D" L : D 4 : 20 hours. Black columns indicate the dark periods.

rhythm most rapidly, less effective were green then red lights; i.e. the shorter the wavelength the more effective the reversed illumination is in the reversal of the phase of circadian pineal MT secretion was. These data are in accord with findings of others who showed that the absorption maximum of the photopigment of the pineal gland, pinopsin, is in the blue range (Okano, T. *et al.* 1994; Max, M. *et al.* 1995).

2. Effect of environmental physical factors other than light (magnetic fields, temperature, moisture)

Earth magnetic field is one of the most important factors to induce seasonal migratory behavior and orientation during migration in the migratory birds (Gwinner, E. 1996). It was proposed that pineal gland also plays an integrative role in inducing migration behavior in the same species (Wiltschko, W. and Wiltschko, R. 1991). Pied flycatchers pinealectomized in early age, lost their orientation capacity, while birds pinealectomized in a later period (experienced birds) have kept this property (Schneider, T. *et al.* 1994). Also, alterations of pineal morphology and serum MT level have been described following *in vivo* exposure to altered magnetic field (Martinez, S.F. *et al.* 1992; Bartsch, H. *et al.* 1994).

On the ground of the above data, the question arose, whether circadian rhythmicity of the avian pineal gland can be *directly* influenced by magnetic fields of the Earth. Large number of parallel membranes, present in the photoreceptors of the avian pineal, are possible candidates for a magnetic sensor. To collect data on this field, we investigated the effects of artificial magnetic fields on phase shift of the circadian MT rhythm from perifused pineal. In our system, vertical or horizontal vector of the magnetic field of the Earth was reversed or increased for 4 to 12 hours daily. The coils were driven with DC or AC (50 Hz frequency) current (Csernus, V. and Faluhelyi, N., yet unpublished observations). In our experiments, artificial magnetic field, in any combination we tried so far, failed to shift the phases of daily MT peak. These negative results might indicate that (1) *in vivo* applied magnetic field might be perceived by extrapineal organs, or (2) the parameters of the magnetic field we applied were not optimal for inducing alterations in MT release, or (3) our biological model, pineal from adult chicken was not the optimal target to answer the question. Based on data of Semm (1990), newly hatched chicken might be more appropriate for these studies. We plan to extend our investigations on this field using different magnetic parameters and biological models.

Another factor which might influence pineal activity is the ambient temperature (Zatz, M. *et al.* 1994). Daily MT rhythm of reptiles (turtle, Vivien-Roels, B. and Arendt, J. 1983; lizard, Underwood, H. and Harless, M. 1985) can be altered by changes in environmental temperature. In contrast, low temperature failed to influence diurnal variations of pineal MT concentrations in mammals (golden hamster, Pevet, P. *et al.* 1989). Low temperature, however, reinforces or accelerates the gonad inhibiting effect of short photoperiod in seasonal breeders (circannual rhythm, Pevet, P. *et al.* 1991). Alteration of the environmental temperature can modify the amplitude or entrain the avian pineal circadian clock *in vitro* (Zatz, M. *et al.* 1994; Barrett, R.K. and Takahashi, J.S. 1995; Barrett, R.K. and Takahashi, J.S. 1997). Elevation of the temperature also enhances TPH mRNA rhythm (Green, C.B. *et al.* 1996).

A series of other environmental factors, like moisture, sound and possibly unknown components of meteorological conditions could also influence rhythmic pineal functions. On these fields, however, even less data are available.

3. The effect of bioactive materials on rhythmic MT release from avian pineal

MT release from *mammalian* pineal is primarily controlled by the sympathetic nervous system with NE as transmitter. In mammals, removal of superior cervical ganglion is considered as “functional pinealectomy” (Axelrod, J. 1971). Vasoactive intestinal polypeptide (VIP), peptide histidine isoleucine (PHI), neuropeptide Y (NPY), substance P (SP), calcitonin gene-related peptide (CGRP), arginine vasopressin (AVP) and oxytocin (OXT) containing axons have been also described in the pineal gland (Moller, M. *et al.* 1985; Cozzi, B. *et al.* 1992; Reuss, S. *et al.* 1992; Mikkelsen, J.D. *et al.* 1994; Matsushima, S. *et al.* 1994; Shinohara, K. and Inouye, S.T. 1994; Hernandez, G. *et al.* 1994). NE, VIP and PACAP were shown to stim-

ulate MT, HIOMT and AANAT synthesis in rat pineal (Buda, M. and Klein, D.C. 1978; Kaufman, C.M. and Menaker, M. 1991; Gupta, B.B.P. *et al.* 1992; Simonneaux, V. *et al.* 1993; Yuwiler, A. *et al.* 1995). *In vitro* dynamic bioassays revealed stimulatory effect of NE, VIP and PACAP on MT release from explanted rat pineal, while NPY inhibited NE induced MT release (Rekasi, Z. *et al.* 1991; Mess, B. *et al.* 1991a; Mess, B. *et al.* 1991b; Csernus, V.J. *et al.* 1994; Mess, B. *et al.* 1996; Rekasi, Z. *et al.* 1998).

In avian pineal, NE, VIP and PACAP containing nerve fibers and effect of these peptides on the elements of the MT synthesizing pineal mechanism were also described (Pratt, B.L. and Takahashi, J.S. 1987; Voisin, P. *et al.* 1987; Zatz, M. *et al.* 1990; Zatz, M. 1991; Nowak, J.Z. *et al.* 1999). Perifusion experiments were also carried out on explanted chicken pineal to study how momentary MT release of the phase of the circadian MT rhythm can be altered with bioactive compounds. Unlike in mammals, NE showed inhibitory effect on MT release from chicken pineal in every phase of the circadian rhythm. The phase of the MT cycle was not altered independent of the dose (0.1 to 100 μM[†]), time and duration (20 minutes to 4 hours) of the exposure to NE (Mess, B. *et al.* 1991a; Mess, B. *et al.* 1996). These data support the view that in non-mammalian species, pineal gland possesses high degree of self-control; NE is not the primary controller just modifies the function of the gland. Similar to its effect on mammals, VIP stimulates MT release also from avian pineal in a dose dependent manner between 1 and 100 nM concentrations. PACAP also showed similar stimulatory effect on MT release and cAMP synthesis in a dose similar to that of VIP (Csernus V. and Reglõdi D., yet unpublished observation). In our perifusion experiment, other neuropeptides shown to be present in the pineal gland (NPY, SP) were ineffective in influencing either the level or the rhythm of pineal MT secretion in chicken (our, yet unpublished data).

B. The construction of the biological clock in the avian pineal. Intracellular signal transduction pathways influencing rhythmic MT release

We have very limited knowledge on the events what happen in the "black box" between the physical or chemical stimulation of the pineal cells (activation of an "input channel") and activation of the MT synthesizing intracellular machinery (see below). The central element in the "middle of the black box" is a complete, circadian biological clock. Our knowledge on the mechanism and composition of this clock is very limited, however, some fundamental data on the functional features on this biological oscillator have been discovered. Its period time, due to the fixed speed of the underlying biochemical processes, is "circadian". It can not be forced to deviate from the 24 hours cycle significantly. At the same time, the phase of the clock, the actual amplitude of the activity can be relatively easily changed. As detailed above, with reversed illumination, MT release from explanted chicken pineal gland could be completely reversed in two days—in just two periods.

The biochemical processes involved in the machinery of the circadian clock in the avian pineal are mostly unknown. Based on the time-scale—a day long period—pineal clock consists of a series of complex biochemical processes, probably including a cascade of genomis regulatory processes (Rachidi, M. *et al.* 1997; Sawyer, L.A. *et al.* 1997; Sun, Z.S. *et al.* 1997; Foulkes, N.S. *et al.* 1997; Price, J.L. *et al.* 1998; Yoshimura, T. *et al.* 2000).

The core mechanism for the master circadian clock consists of interacting positive and negative transcription and translation feedback loops involving a series of genes with an approximately 24-hr oscillatory transcription period, which were identified in several organisms from procaryotes to eucaryotes (Iwasaki, K. and Thomas, J.H. 1997; Bae, K. *et al.* 1998; Andretic, R. *et al.* 1999; Ceriani, M.F. *et al.* 1999). The genes primarily involved in maintaining the biological rhythmicity, the "machinery of the biological clocks", are named *clock genes*. Several clock genes have been described in various species. Among them, Per/Cry and Clock/Bmal1 dimers seem to be the most fundamental elements. Clock/Bmal1 dimers regu-

† μM = micromole/liter

late the synthesis and release of melatonin in several species by activating of the NAT enzyme expression through the E-box of its promoter (Chong, N.W. *et al.* 2000). The structures and functions of the clock genes seem to be rather conservative during the phylogenesis.

Since no significant data on the role of the clock genes in the birds have been published yet, we tried to identify and clarify the role of the clock genes involved in the rhythmic activity of the ChPG (Nagy, A.D. and Csernus, V., yet unpublished observations). Based on our earlier data indicating that avian pineals possess direct light-sensitivity influencing its thythmic functions, first we started studying a unique light-responsive gen: cryptochrome (CRY). CRY was proposed as a "cryptic"-extraretinal blue-light receptor, hence comes its name (Kasemir, H. 1979; Ahmad, M. and Cashmore, A.R. 1996). CRY is a vitamin B2-based pigment evolved from photolyases, which catalyze light-dependent repair of UV-induced DNA damage. It also has a radical-pair-based magneto-receptor property suggesting to be the site of a neurobiochemical reaction that lets birds utilize magnetic field to orient (Ritz, T. *et al.* 2000). CRY may control the phase of the circadian pacemaker by its light and magnetic receptor property being an essential component of the negative segment of the transcriptional feedback loop involving Per/Cry and Clock/Bmal1 dimers. By comparing known mRNA sequences of CRY of various species with the chicken genenomic library, we were able to design primers and detect CRY-mRNA from chicken pineal gland. In our experiment utilizing semi quantitative RT-PCR, expression of chicken pineal CRY mRNA showed a clear circadian rhythm with highest level at 5 p.m. and nadir at 5 a.m. (Fig. 10.). These data suggest that CRY is involved in the "biological clock" of the ChPG and may mediate the influence of light to the phase of the clock. Currently, we are carrying out further experiments to clarify the role of

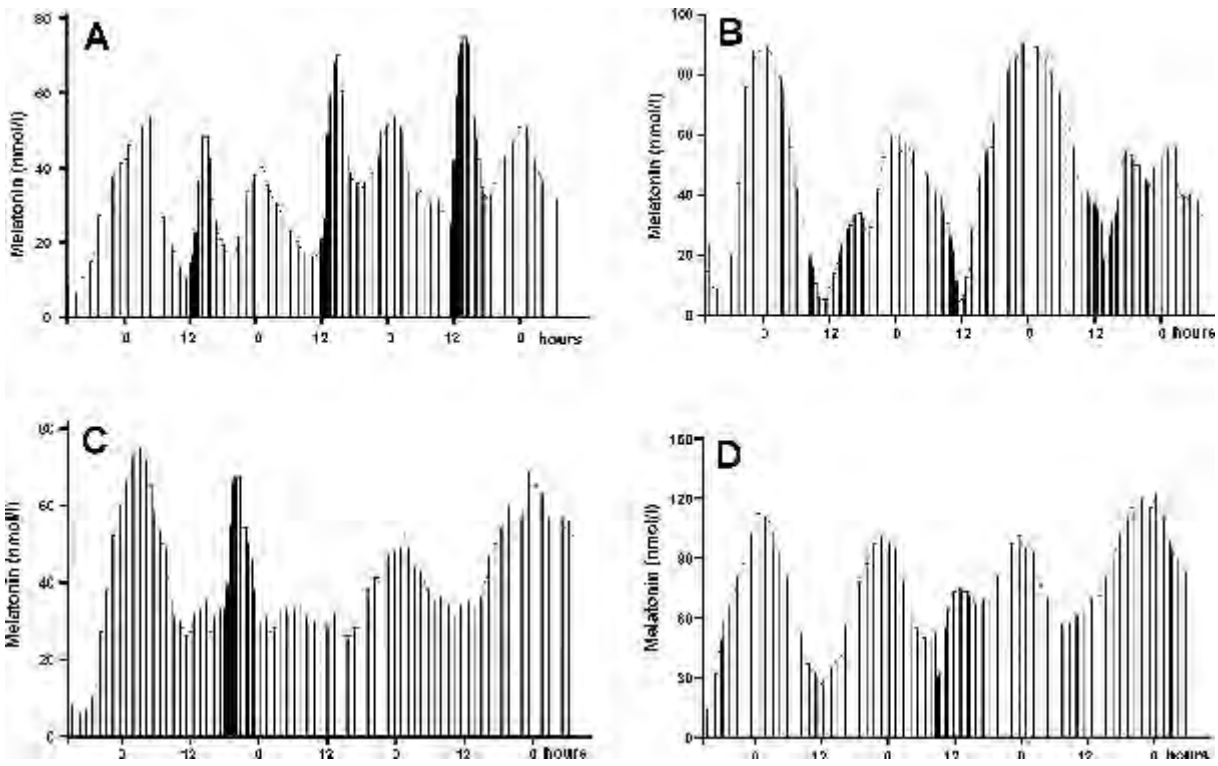

Fig. 9. The effects of drugs, affecting various elements of the intracellular signal transduction system, on rhythmic MT secretion from explanted chicken pineal gland. MT contents of 30 minute fractions in perifusion experiments are plotted. The drugs used: "A" 2 μM Forskolin for 2 hours on three consecutive days at 12 p.m., "B" 1 μM NS-398 (a cyclooxygenase inhibitor) for 1 hour on three consecutive days at 12 p.m., "C" 500 μM nitroprussid-sodium (a NO donor) for 150 min, and "D" 10 μM Fluphenazine (a calmodulin inhibitor) for 1 hour. Black columns indicate the time of the drug exposure, thick-wall columns label the post-exposure response fractions.

the CRY and other clock genes in maintaining and control of the rhythmic activity of the ChPG.

Another interesting, still not fully answered question: how signals from the "input channels" reach the clock and how the "output signal" of the clock controls the MT synthesizing enzymes.

Existence of functioning intracellular receptor for light in ChPG is generally accepted. Avian pineal cells are very similar in structure to the rods and cones of the retina. The—not fully developed—outer segments of the cells containing parallel membranes and a light sensitive pigment pinopsin. Signal transduction between the light receptors and the clock probably involves cAMP. As our experiments suggest (see above), light control of the pineal clock by CRY can be an alternative.

Similarly, signal transduction pathways between the clock and the MT synthesizing enzymes are not fully revealed. Apparently, AANAT is the key enzyme responsible for the circadian MT rhythm. The control of the circadian MT synthesis is fundamentally based on the regulation of the activity of AANAT and that of expression of AANAT-mRNA.

In our perifusion system, explanted chicken pineal cells were exposed to various drugs which activate or inhibit known elements of the intracellular signal transduction cascade in 5-day perifusion experiments. The test compounds, targeting specific elements of the intracellular signal transduction system, included forskolin, fluphenazin, H89, suramin, D-609, NS-398, TPA, thapsigargin, U73122, SQ22536, nipridin and nitro-arginine. From data on alterations of the MT release pattern we concluded that cAMP-protein-kinase-A, calmodulin, nitric oxide synthase and arachidonic acid-prostaglandin systems are participating in the control of the MT release from avian pineal. At the same time, the phospholipase-C-protein-kinase-C system and modifiers of the intracellular calcium ion concentration are not involved in this process (Fig. 9.; Csernus, V., yet unpublished data). Non of the signal transduction lines, found active above, modified the phase of the circadian clock only temporarily affected the MT release. The data indicate that these signal transduction pathways are either carrying message between the clock and MT synthesis or not related to the clock.

Nitric oxide synthase (NOS) were found in some neurons of the chicken brain primarily in the cerebellum using ICC or NADPH-diaphorase cytochemistry (Bruning, G. 1993; Bruning, G. *et al.* 1994; Yamakawa, Y. *et al.* 1997). NOS is considered to be involved in the learning process of chicken (Ambalavanar, R. *et al.* 1994; von Bartheld, C.S. and Schober, A. 1997). We also found NADPH-diaphorase positive neurons in the area where we found perikarya of neurons that projected to the pineal gland (see above and in Fig. 5.). A circadian change in the NOS-positivity was also detected in these neurons indicating that these neurons may be correlated to rhythmic pineal functions (Fig. 11.).

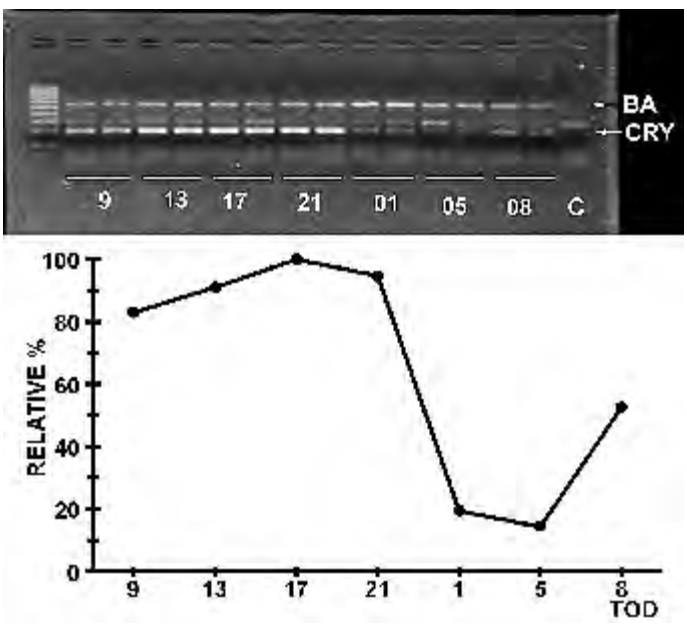

Fig. 10. Semi-quantitative RT-PCR for Cryptochrome (CRY) mRNA form chicken pineal gland. Groups of chicken were sacrificed in 4 hour intervals. On the upper part of the figure, ethidium bromide labeled agarose gel is seen. For each time periods (numbers, hours of the day) two parallel experiments are shown. "CRY" labels cryptochrome mRNA product, "BA" is beta actin mRNA product used as internal standard. "C" indicates control (no RT enzyme). On the bottom, changes of relative CRY-mRNA content of the pineals are plotted against the time of the day (TOD). Clear circadian rhythmicity is seen.

We have limited data not only on the components of the biological clock in the avian pineal, but also on whether all elements of the clock are located within one cell or a clock works as "collaboration" of a group of cells. It seems plausible that several, synchronized clocks work in a gland. Another interesting question is, what synchronizes these clocks? The recently discovered gap junctions between the pineal cells might be one of the synchronizing elements (Berthoud, V.M. *et al.* 2000). In experiments targeting this problem, we performed pCREB ICC on pineals of chicken sa-

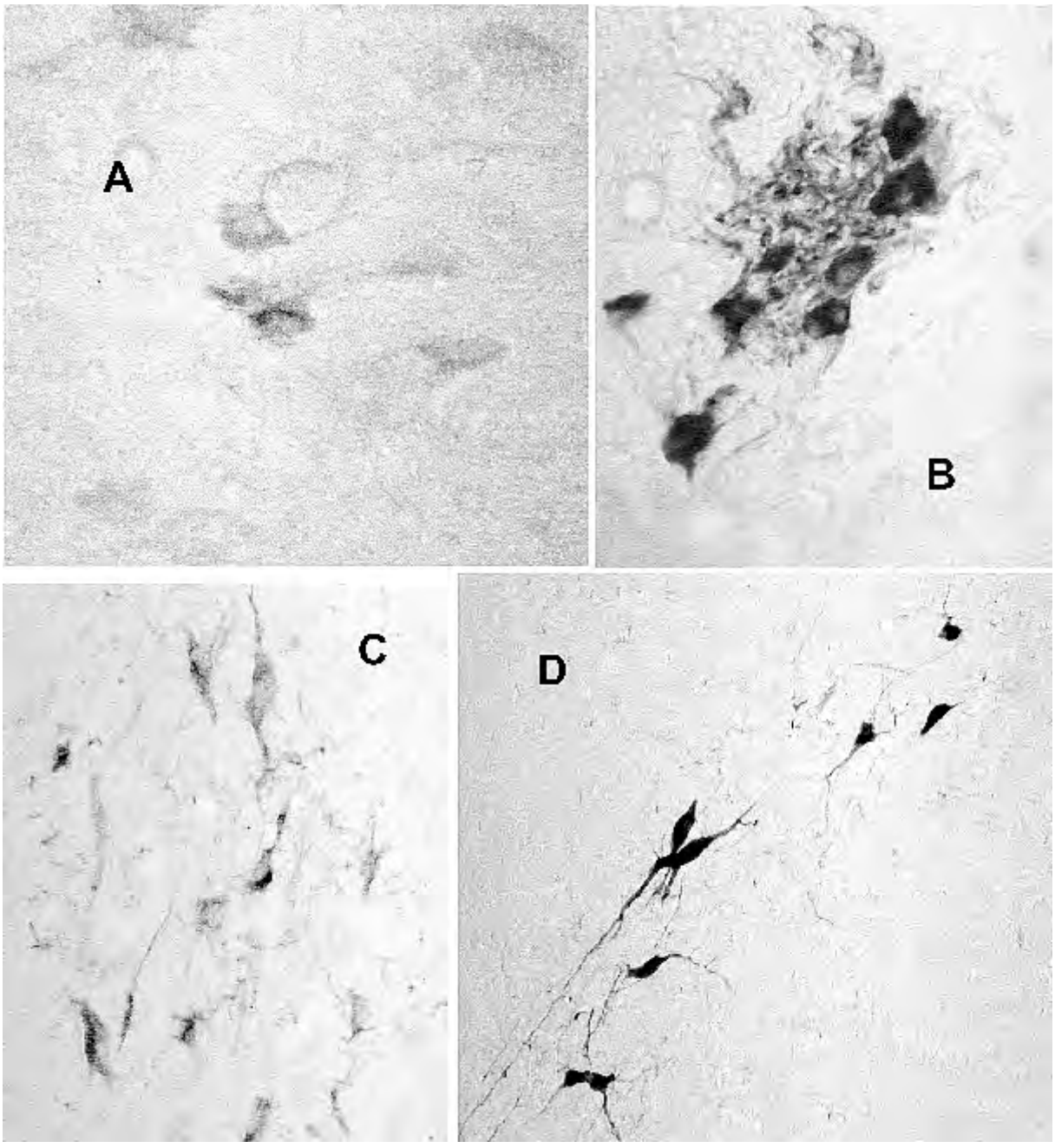

Fig. 11. Rhythmic changes in nitric oxide synthase (NOS) activity. NADPH-diaphorase histochemical reaction. Labeled neurons and nerve fibers in the superior cervical ganglion (A and B) and the brainstem (C and D). The chicken were sacrificed at 10 a.m. (A and C) or 10 p.m. (B and D) and the tissues were processed simultaneously. In the morning samples the reactions are clearly weaker.

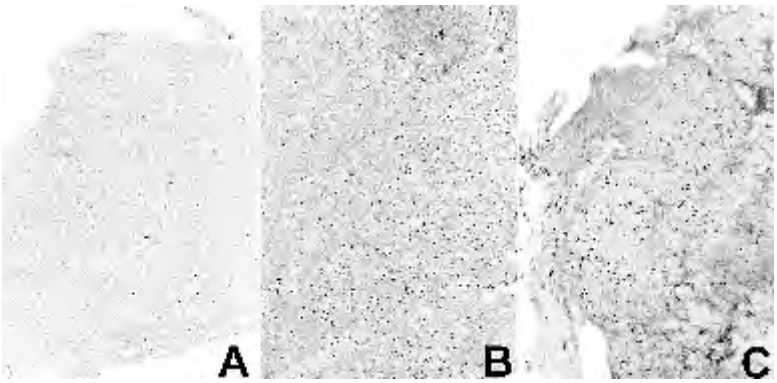

Fig. 12. pCREB immunohistochemistry (IHK) of chicken pineal gland. When the chicken were sacrificed at noon (A), only very week immunoreactivity was found. However, nuclei of most pineal cells of chicken showed strong immunoreaction when sacrificed at night (B). Pineals of chicken, kept under continuous illumination *in vitro* for 5 days in a perifusion system, (C) showed both positive and negative pCREB immunostained nuclei. 400× magnification.

crificed at daytime (at 11 a.m.), nighttime (at 11 p.m.) or at the end of a 5 day perifusion experiment carried out under continuous *in vitro* illumination (Csernus, V. and Schwarcz, A., yet unpublished observations). In chicken sacrificed in the middle of the light period, minimal pCREB immunoreactivity was found (Fig. 12.A) while in those sacrificed in the night, most of the pineal cells showed high pCREB immunoreactivity (Fig. 12.B). Pineal gland of the chicken kept under continuous illumination in a 5-day perifusion experiment showed mosaic-like picture: some cells showed strong pCREB immunoreactivity, other stained just faintly (Fig. 12.C). Effluent medium from the same pineals showed characteristic gradually diminishing MT cycle stabilizing at high MT level (see also above). From these results we concluded that for the diminishing the MT rhythm during continuous illumination, desynchronization and not a complete stop of the circadian clock is responsible.

C. The mechanism of MT synthesis and release in avian pineal

Biochemical machinery of MT biosynthesis is a relatively simple, well studied process. From the amino acid, tryptophane (Trp), first serotonin, a widely present neurotransmitter is made in two steps. Serotonin is then acetylated by arylalkylamine-N-acetyltransferase (AANAT) forming N-acetyl-serotonin then this compound is further methylated by HIOMT resulting MT.

The mRNA-s encoding three enzymes of the MT synthesis pathway, tryptophan hydroxylase (TPH), AANAT and HIOMT, are expressed with a day/night rhythm in the chicken pineal gland and retina (Klein, D.C. *et al.* 1997; Iuvone, P.M. *et al.* 1997; Chong, N.W. *et al.* 1998; Grechez-Cassiau, A. *et al.* 1998; Grechez-Cassiau, A. *et al.* 1999; Bernard, M. *et al.* 1999; Kato, H. *et al.* 1999). TPH and AANAT mRNA levels reach their peak at night. HIOMT mRNA levels peak at night in the retina, but during the day in the pineal gland (Bernard, M. *et al.* 1999). Similarly, daily changes in the activities of these enzymes are also shown (Yochim, J.M. and Wallen, E.P. 1974; Binkley, S. 1976; Deguchi, T. 1979a; Deguchi, T. 1979b; Doi, O. *et al.* 1983; Binkley, S.A. *et al.* 1987; Zeman, M. and Illnerova, H. 1990). From the enzymes involved in MT synthesis, AANAT is considered to be the rate limiting.

MT is a small hydrophobic molecule. It apparently easily penetrates cell membranes. This is why in contrast to peptide hormone-producing endocrine glands in which the hormone concentration is very high, MT concentration within the pineal cells is similar to that in its environment. This explains why extracts of pineal contain relatively low level of MT (Csernus, V.J. *et al.* 1994) and why ICC detection of MT is relatively difficult and does not result in a high contrast picture. This feature of the molecule also explains the ob-

servation, that following a specific or non-specific pulsatile stimulus, both the onset and the duration of the MT secretion is much more prolonged than those of hormone secretion from peptide hormone-producing cells. After pulsatile stimulus with NE, MT secretion from both mammalian and avian pineal becomes apparent only after an about 60–90 minute lag, the secretion reaches the top in an another hour and returns back to the baseline only about five hours after the stimulation has been stopped. Not having significant intracellular reserve, after a stimulus, newly synthesized MT is released. This process requires expression of AANAT mRNA, synthesis and activation of the enzymes and the synthesis of new MT. In contrast, following a short, pulsatile stimulus, an apparently immediate (measurable within seconds) onset of hormone release from peptide hormone producing cells is experienced from the usually immense, membrane enwrapped intracellular reserves. Since in this case the synthesis and the release mechanisms are separated, hormone discharge peaks then falls back to baseline value within a few minutes after the stimulation (Csernus, V.J. *et al.* 1994).

III. Development of the rhythmic MT synthesis in the avian pineals

The chick pineal primordium appears first as a small evagination in the diencephalic roof at 60 h of incubation. Pinealocytes and supporting cells are first distinguishable at 7–8 days, and parafollicular cells are appear at 12 days of incubation. During embryonic development, the chick pineal gland has both photosensory and secretory elements. Photosensory elements undergo further maturation in the first 6 weeks of post-hatching life (Ohshima, K. and Matsuo, S. 1988; Ohshima, K. and Hiramatsu, K. 1993; Voisin, P. *et al.* 1994). Data suggested that chick pineal cells from embryonic day 16 onwards are photosensitive but that the endogenous component of the MT rhythm is not completely developed at that age (Lamosova, D. *et al.* 1995).

In contrast to the situation in mammals, in which circadian MT production by the pineal gland does not begin until some time after birth, the development of pineal gland rhythmicity is an embryonic event in birds. Ueck (1973) reported that MT rhythm was observed under a light-dark regime already in the pineal of chick embryos (see for further references Vigh, B. and Vigh-Teichmann, I. 1999). A distinct MT rhythm was found in 13–14-day-old chick embryos maintained under cyclic illumination but not under continuous darkness. When the light-dark cycle was reversed, the pattern of MT release in the culture also reversed (Zeman, M. *et al.* 1992; Akasaka, K. *et al.* 1995; Zeman, M. *et al.* 1999). Rhythmic changes of HIOMT mRNA (Voisin, P. *et al.* 1994) and AANAT mRNA (Grechez-Cassiau, A. *et al.* 1995; Bernard, M. *et al.* 1997) have also been described in chicken embryo. In contrast, high, but illumination-insensitive HIOMT and AANAT activity in the *retina* of the chicken embryo (from day 7) has been reported (Iuvone, P.M. 1990; Espinar, A. *et al.* 1994).

These data seemed to indicate that rhythmic environmental illumination is necessary for development of the circadian clock in the chicken embryo. In a series of our experiment, targeting this question, chickens were hatched and bred under continuous illumination from the very first day of incubation. To reduce the effects of the environmental rhythm, eggs were turned and the chickens were fed in counterbalanced times. The chickens were sacrificed at ages of 8 to 18 post-hatch weeks and MT release of their explanted pineals was studied in perifusion system. Pineal glands from these animals that never experienced rhythmic light-dark changes from the very first day of embryonic development revealed fully normal circadian MT rhythm in the perifusion system with midnight peaks. Similarly, when perifused pineals of these "illuminated-chickens" were kept under continuous illumination also *in vitro* reacted with an attenuated amplitude of the circadian MT cycle stabilizing in a high level of MT like normal chicken pineal under the same conditions (Csernus, V. *et al.* 1998) (see also Fig. 7.B). These data indicate that for development and daily synchronization of the circadian MT pacemaker in the chicken pineal gland, periodic changes in the environmental illumination are not necessary. The "biological clock" in the chicken pineals seems to be genetically coded and not light-dependent. Environmental factor(s), other than light, might be responsible for its synchronization to the day.

References

Ahmad, M., A.R. Cashmore (1996) Seeing blue: the discovery of cryptochrome. Plant Mol. Biol. 30: 851–861.

Akasaka, K., T. Nasu, T. Katayama, N. Murakami (1995) Development of regulation of melatonin release in pineal cells in chick embryo. Brain Res. 692: 283–286.

Ambalavanar, R., B.J. McCabe, G. Horn (1994) NADPH diaphorase (nitric oxide synthase) in a part of the chick brain involved in imprinting. Brain Res. 644: 160–163.

Andretic, R., S. Chaney, J. Hirsh (1999) Requirement of circadian genes for cocaine sensitization in Drosophila. Science 285: 1066–1068.

Araki, M., Y. Fukada, Y. Shichida, T. Yoshizawa, F. Tokunaga (1992) Differentiation of both rod and cone types of photoreceptors in the in vivo and in vitro developing pineal glands of the quail. Brain Res. Develop. Brain Res. 65: 85–92.

Arendt, J. (1999) Jet-lag and shift work: (2). Therapeutic use of melatonin. J. Royal Soc. Med. 92: 402–405.

Arendt, J., S. Deveson, S. Folkard, P. Totterdell, J. English (1992) Use of Melatonin in circadian rhythm disturbance associated with jet-lag and shift work. J. Interdisc. Cycle Res. 23: 156–158.

Arendt, J., D.J. Skene, B. Middleton, S.W. Lockley, S. Deacon (1997) Efficacy of melatonin treatment in jet lag, shift work, and blindness. J. Biol. Rhythms 12: 604–617.

Armstrong, S.M. (1999) Melatonin as a chronobiotic for circadian insomnia. Clinical observations and animal models. Adv. Exp. Med. Biol. 460: 283–297.

Axelrod, J. (1971) Noradrenaline: fate and control of its biosynthesis. Science 173: 598–606.

Axelrod, J., R.J. Wurtman (1966) The pineal gland: a biological clock. Probl. Act. Endocr. Nutr. 10: 201–212.

Axelrod, J., R.J. Wurtman, S.H. Snyder (1965) Control of hydroxyindole-O-methytransferase activity in the rat pineal by environmental lighting. J. Biol. Chem. 240: 949–954.

Bae, K., C. Lee, D. Sidote, K.Y. Chuang, I. Edery (1998) Circadian regulation of a Drosophila homolog of the mammalian Clock gene: PER and TIM function as positive regulators. Mol. Cell. Biol. 18: 6142–6151.

Barajas-Lopez, C., M.A. Barrientos-Martinez, C. Reyes-Vazquez (1987) Persistence of photic evoked responses in pineal gland after its pedunculotomy and superior cervical ganglionectomy. J. Pineal Res. 4: 287–294.

Barrett, R.K., J.S. Takahashi (1995) Temperature compensation and temperature entrainment of the chick pineal cell circadian clock. J. Neurosci. 15: 5681–5692.

Barrett, R.K., J.S. Takahashi (1997) Lability of circadian pacemaker amplitude in chick pineal cells: a temperature-dependent process. J. Biol. Rhythms 12: 309–318.

Bartsch, C., H. Bartsch (1999) Melatonin in cancer patients and in tumor-bearing animals. Adv. Exp. Med. Biol. 467: 247–264.

Bartsch, H., C. Bartsch, D. Mecke, T.H. Lippert (1994) Seasonality of pineal melatonin production in the rat—Possible synchronization by the geomagnetic field. Chronobiol. Internat. 11: 21–26.

Bernard, M., J. Guerlotte, P. Greve, C.A. Grechez, P.M. Iuvone, M. Zatz, N.W. Chong, D.C. Klein, P. Voisin (1999) Melatonin synthesis pathway: circadian regulation of the genes encoding the key enzymes in the chicken pineal gland and retina. Reprod. Nutr. Develop. 39: 325–334.

Bernard, M., D.C. Klein, M. Zatz (1997) Chick pineal clock regulates serotonin N-acetyltransferase mRNA rhythm in culture. Proc. Natl. Acad. Sci. 94: 304–309.

Berthoud, V.M., D.H. Hall, E. Strahsburger, E.C. Beyer, J.C. Saez (2000) Gap junctions in the chicken pineal gland. Brain Res. 861: 257–270.

Binkley, S. (1976) Pineal gland biorhythms: N-acetyltransferase in chickens and rats. Fed. Proc. 35: 2347–2352.

Binkley, S.A., K. Mosher, B.H. White (1987) Circadian rhythm in pineal N-acetyltransferase activity: rapid phase reversal and response to shorter than 24-hour cycles (IV). J. Neurochem. 49: 828–833.

Bischoff, M.B. (1969) Photoreceptoral and secretory structures in the avian pineal organ. J. Ultrastruct. Res. 28: 16–26.

Blask, D.E., A.M. Lemuswilson, S.T. Wilson (1992) Breast cancer—a model system for studying the neuroendocrine role of pineal melatonin in oncology. Biochem. Soc. Trans. 20: 309–311.

Brömme, H.J., H. Ebelt, D. Peschke, E. Peschke (1999) Alloxan acts as a prooxidant only under reducing conditions: influence of melatonin. Cell. Mol. Life Sci. 55: 487–493.

Bruning, G. (1993) Localization of NADPH-diaphorase in the brain of the chicken. J. Comp Neurol. 334: 192–208.

Bruning, G., U. Funk, B. Mayer (1994) Immunocytochemical localization of nitric oxide synthase in the brain of the chicken. Neuroreport 5: 2425–2428.

Bubenik, G.A., G.M. Brown, L.J. Grota (1976) Immunohistochemical localization of melatonin in the rat Harderian gland. J. Histochem. Cytochem. 24: 1173–1177.

Bubenik, G.A., G.M. Brown, L.J. Grota (1977) Immunohistological localization of melatonin in the rat digestive system. Experientia 33: 662–663.

Bubenik, G.A., G.M. Brown, I. Uhlir, L.J. Grota (1974) Immunohistological localization of N-acetylindolealkylamines in pineal gland, retina and cerebellum. Brain Res. 81: 233–242.

Bubenik, G.A., S.F. Pang (1997) Melatonin levels in the gastrointestinal tissues of fish, amphibians, and a reptile. Gen. Comp. Endocrinol. 106: 415–419.

Bubenik, G.A., R.A. Purtill, G.M. Brown, L.J. Grota (1978) Melatonin in the retina and the Harderian gland. Ontogeny, diurnal variations and melatonin treatment. Exp. Eye Res. 27: 323–333.

Buda, M., D.C. Klein (1978) A suspension culture of pinealocytes; regulation of N-acetyltransferase activity. Endocrinology 103: 1483–1493.

Cassone, V.M., J. Lu (1994) The pineal gland and avian circadian organization: the neuroendocrine loop. In: Adv. Pineal Res., M. Moller, P. Pevet, eds., John Libbey, London, pp. 31–40.

Ceriani, M.F., T.K. Darlington, D. Staknis, P. Mas, A.A. Petti, C.J. Weitz, S.A. Kay (1999) Light-dependent sequestration of timeless by cryptochrome. Science 285: 553–556.

Chong, N.W., M. Bernard, D.C. Klein (2000) Characterization of the chicken serotonin N-acetyltransferase gene. Activation via clock gene heterodimere/E box interaction. J. Biol. Chem. 275: 32991–32998.

Chong, N.W., V.M. Cassone, M. Bernard, D.C. Klein, P.M. Iuvone (1998) Circadian expression of tryptophan hydroxylase mRNA in the chicken retina. Brain Res. Mol. Brain Res. 61: 243–250.

Cogburn, L.A., B. Glick (1981) Lymphopoiesis in the chicken pineal gland. Am. J. Anat. 162: 131–142.

Cogburn, L.A., B. Glick (1983) Functional lymphocytes in the chicken pineal gland. J. Immunol. 130: 2109–2112.

Collin, J.P., A. Calas, M.T. Juillard (1976) The avian pineal organ. Distribution of exogenous indoleamines: a qualitative study of the rudimentary photoreceptor cells by electron microscopic radioautography. Exp. Brain Res. 25: 15–33.

Collin, J.P., M.T. Juillard, P. Voisin (1984) Circadian information and messages in the modified photoreceptor cells of the avian pineal organ: retrospect and prospect. Ophthal. Res. 16: 107–113.

Collin, J.P., J.A. Kappers (1968) Electron microscopic study of pineal innervation in lacertilians. Brain Res. 11: 65–106.

Collin, J.P., J.A. Kappers (1971) Synapses of the ribbon type in the pineal organ of Lacerta vivipara (Reptiles, Lacertilians). Experientia 27: 1456–1457.

Collin, J.P., P. Voisin, J. Falcon, J.P. Faure, P. Brisson, J.R. Defaye (1989) Pineal transducers in the course of evolution: molecular organization, rhythmic metabolic activity and role. Arch. Histol. Cytol. 52 Suppl.: 441–449.

Cozzi, B., J.D. Mikkelsen, J.P. Ravault, M. Moller (1992) Neuropeptide Y (NPY) and C-flanking peptide of NPY in the pineal gland of normal and ganglionectomized sheep. J. Comp. Neurol. 316: 238–250.

Csernus, V., P. Becher, B. Mess (1999) Wavelength dependency of light-induced changes in rhythmic melatonin secretion from chicken pineal gland in vitro. Neuroendocrinol. Lett. 20: 299–304.

Csernus, V., M. Ghosh, B. Mess (1998) Development and control of the circadian pacemaker for melatonin release in the chicken pineal gland. Gen. Comp. Endocrinol. 110: 19–28.

Csernus, V., Z. Rekasi, B. Mess (1992) Differences in dynamic hormone release from pineal gland and anterior pituitary cells in vitro. In: Melatonin and the Pineal Gland, Satellite symposium of the IXth Internatl. Congress of Endocrinology, Paris, 1992. Abstract p. 19.

Csernus, V.J., Z. Rekasi, B. Mess (1994) Differences in hormone release patterns from the anterior pituitary and the pineal gland. Acta Biol. Acad. Sci. Hung. 45: 207–221.

Csernus, V.J., A.V. Schally (1991) The dispersed cell superfusion system. In: Neuroendocrine Research Methods, B.D. Greenstein, ed., Harwood Acad. Publ., London, pp. 71–109.

Deguchi, T. (1979a) Circadian rhythm of serotonin N-acetyltransferase activity in organ culture of chicken pineal gland. Science 203: 1245–1247.

Deguchi, T. (1979b) Circadian rhythms of indoleamines and serotonin N-acetyltransferase activity in the pineal gland. Mol. Cell. Biochem. 27: 57–66.

Doi, O., E. Koyama, T. Nakamura, Y. Tanabe (1983) Photoperiodic regulation of serotonin N-acetyltransferase activity in the pineal gland of the chicken. Comp. Biochem. Physiol. A74: 195–198.

Espinar, A., C. Osuna, C. Feliu, J.M. Guerrero (1994) High activity of retinal N-acetyltransferase in the early development of the chick embryo: independence of lighting conditions. Neurosci. Lett. 179: 103–106.

Fejer, Z., A. Szel, P. Rohlich, T. Gorcs, M.J. Manzanoe-Silva, B. Vigh (1997) Immunoreactive pinopsin in pineal and retinal photoreceptors of various vertebrates. Acta Biol. Acad. Sci. Hung. 48: 463–471.

Foulkes, N.S., D. Whitmore, P. Sassone-Corsi (1997) Rhythmic transcription: the molecular basis of circadian melatonin synthesis. Biol. Cell 89: 487–494.

Ghosh, M., V. Csernus, B. Mess (1994) In vitro melatonin secretion pattern of the avian pineal gland with special reference to light reactivity. Neuroendocrinol. Lett. 3: 195–200.

Ghosh, M., V. Csernus, Z. Rekasi, B. Mess (1996) Comparative studies on the regulation of pineal melatonin

secretion in mammals and birds. In: The pineal gland: its molecular signals, C. Haldar, ed., Hindustan Publ. Corp., New Delhi, pp. 5–15.

Grechez-Cassiau, A., M. Bernard, K. Ladjali, I.R. Rodriguez, P. Voisin (1998) Structural analysis of the chicken hydroxyindole-O-methyltransferase gene. Eur. J. Biochem. 258: 44–52.

Grechez-Cassiau, A., P. Greve, J. Guerlotte, J.P. Collin, P. Voisin (1995) Hydroxyindole-O-methyltransferase gene expression in the pineal gland of chicken embryo: development of messenger RNA levels and regulation by serum. Brain Res. Develop. Brain Res. 88: 204–211.

Grechez-Cassiau, A., P. Greve, J. Guerlotte, P. Voisin (1999) Cyclic AMP increases hydroxyindole-O-methyltransferase mRNA levels in the chicken pineal gland, but is not required for circadian rhythmicity of this transcript. Brain Res. 835: 97–103.

Green, C.B., J.C. Besharse, M. Zatz (1996) Tryptophan hydroxylase mRNA levels are regulated by the circadian clock, temperature, and cAMP in chick pineal cells. Brain Res. 738: 1–7.

Gupta, B.B.P., A. Seidel, R. Spessert, W. Buttner, N. Klauke, J. Spanier, A. Weber, D. Ziemer, L. Vollrath (1992) In vitro effects of putative neurotransmitters on synaptic ribbon numbers and N-acetyltransferase activity in the rat pineal gland. J. Neural. Transm. Gen. Sect. 89: 167–178.

Gwinner, E. (1996) Circadian and circannual programmes in avian migration. J. Exp. Biol. 199: 39–48.

Haldar, C., P. Guchhait (2000) Pineal gland of a nocturnal bird, Indian spotted owlet, Athene brama: morphological and endocrine observations. J. Exp. Zool. 287: 145–150.

Hardeland, R., I. Balzer, B. Poeggeler, B. Fuhrberg, H. Uria, G. Behrmann, R. Wolf, T.J. Meyer, R.J. Reiter (1995) On the primary functions of melatonin in evolution: mediation of photoperiodic signals in a unicell, photooxidation, and scavenging of free radicals. J. Pineal Res. 18: 104–111.

Hernandez, G., A.R. Bello, I. Lopezcoviella, P. Abreu, N. Fajardo, R.J. Reiter, A. Hernandez, R. Alonso (1994) Tyrosine hydroxylase activity in peripherally denervated rat pineal gland. Neurosci. Lett. 177: 131–134.

Hortega, R.P. (1932) Pineal gland. In: Cytology and Cellular Pathology of the Nervous System, W. Penfield, ed., Hoeber Inc., New York, Vol. 2, pp. 637–703.

Iuvone, P.M. (1990) Development of melatonin synthesis in chicken retina: regulation of serotonin N-acetyltransferase activity by light, circadian oscillators, and cyclic AMP. J. Neurochem. 54: 1562–1568.

Iuvone, P.M., M. Bernard, A. Alonso-Gomez, P. Greve, V.M. Cassone, D.C. Klein (1997) Cellular and molecular regulation of serotonin N-acetyltransferase activity in chicken retinal photoreceptors. Biol. Signals 6: 217–224.

Iwasaki, K., J.H. Thomas (1997) Genetics in rhythm. Trends in Genetics 13: 111–115.

Kappers, J.A. (1967) The sensory innervation of the pineal organ in the lizard, Lacerta viridis, with remarks on its position in the trend of pineal phylogenetic structural and functional evolution. Z. Zellforsch. Mikroskop. Anat. 81: 581–618.

Karasek, M., K. Marek, P. Pevet (1988) Influence of a short light pulse at night on the ultrastructure of the rat pinealocyte: a quantitative study. Cell Tiss. Res. 254: 247–249.

Karasek, M., R.J. Reiter (1992) Morphofunctional aspects of the mammalian pineal gland. Microscop. Res. Tech. 21: 136–157.

Kasemir, H. (1979) Control of chloroplast formation by light. Cell Biol. Int. Rep. 3: 197–214.

Kato, H., Z. Fu, N. Kotera, K. Sugahara, T. Kubo (1999) Regulation of the expression of serotonin N-acetyltransferase gene in Japanese quail (Coturnix japonica): I. Rhythmic pattern and effect of light. J. Pineal Res. 27: 24–33.

Kaufman, C.M., M. Menaker (1991) Ontogeny of the pineal response to norepinephrine. J. Pineal Res. 11: 173–178.

Kenny, G.C. (1965) The innervation of the mammalian pineal body (A comparative study). Proc. Aust. Assoc. Neurol. 3: 133–140.

Klein, D.C., S.L. Coon, P.H. Roseboom, J.L. Weller, M. Bernard, J.A. Gastel, M. Zatz, P.M. Iuvone, I.R. Rodriguez, V. Begay, J. Falcon, G.M. Cahill, V.M. Cassone, R. Baler (1997) The melatonin rhythm-generating enzyme: molecular regulation of serotonin N-acetyltransferase in the pineal gland. Rec. Progr. Horm. Res. 52: 307–357.

Klein, D.C., J.E. Weller (1972) Rapid light-induced decrease in rat pineal serotonin N- acetyltransferase activity. Science 179: 532–533.

Kocsard-Varo, G. (2000) Role of the pineal gland in hibernators: a concept proposed to clarify why hibernators have to leave torpor and sleep. Med. Hypoth. 54: 645–647.

Korf, H.W. (1994) The pineal organ as a component of the biological clock. Phylogenetic and ontogenetic considerations. Ann. N.Y. Acad. Sci. 719: 13–42.

Korf, H.W., R.G. Foster, P. Ekstrom, J.J. Schalken (1985) Opsin-like immunoreaction in the retinae and pineal organs of four mammalian species. Cell Tiss. Res. 242: 645–648.

Korf, H.W., M. Moller (1984) The innervation of the mammalian pineal gland with special reference to central pinealopetal projections. In: Pineal Res. Rev., R.J. Reiter, ed., Alan R. Liss, New York, Vol. 2, pp. 41–86.

Korf, H.W., I. Vigh-Teichmann (1984) Sensory and central nervous elements in the avian pineal organ. Ophthal. Res. 16: 96–101.

Lamosova, D., M. Zeman, M. Mackova, E. Gwinner (1995) Development of rhythmic melatonin synthesis in cultured pineal glands and pineal cells isolated from chick embryo. Experientia 51: 970–975.

Lauber, J.K., J.E. Boyd, J. Axelrod (1968) Enzymatic synthesis of melatonin in avian pineal body: extraretinal response to light. Science 161: 489–490.

Leung, F.C. (1991) Circadian rhythms of melatonin release from chicken pineal in vitro: modified melatonin radioimmunoassay. Proc. Soc. Exp. Biol. Med. 198: 826–832.

Luo, Z.R., R.L. Schultz, E.F. Whitter, L. Vollrath (1984) Ultrastructural characterization of glial cells in the rat pineal gland with special reference to the pineal stalk. Anat. Rec. 210: 663–674.

Martinez, S.F., G.M. Gimenez, E. Armanazas, T.A. Ruiz (1992) Pineal 'synaptic ribbons' and serum melatonin levels in the rat following the pulse action of 52-Gs (50-Hz) magnetic fields: an evolutive analysis over 21 days. Acta Anat. (Basel) 143: 289–293.

Matsushima, S., Y. Sakai, Y. Hira, Y. Oomori, S. Daikoku (1994) Immunohistochemical studies on sympathetic and non-sympathetic nerve fibers and neuronal cell bodies in the pineal gland of Cotton rats, Sigmodon Hispidus. Arch. Histol. Cytol. 57: 47–58.

Max, M., P.J. McKinnon, K.J. Seidenman, R.K. Barrett, M.L. Applebury, J.S. Takahashi, R.F. Margolskee (1995) Pineal opsin: a nonvisual opsin expressed in chick pineal. Science 267: 1502–1506.

McClung, R., N. Dafny (1975) Neurophysiological properties of the pineal body. II. Single unit recording. Life Sci. 16: 621–627.

Mendonca, M.T., A.J. Tousignant, D. Crews (1995) Seasonal changes and annual variability in daily plasma melatonin in the red-sided garter snake (Thamnophis sirtalis parietalis). Gen. Comp. Endocrinol. 100: 226–237.

Mennenga, K., M. Ueck, R. J. Reiter (1991) Immunohistological localization of melatonin in the pineal gland and retina of the rat. J. Pineal Res. 10: 159–164.

Mess, B. (1983) Interactions between pineal and nonreproductive endocrine glands. In: The pineal gland and its endocrine role, J. Axelrod, F. Fraschini, P. Velo, eds., Plenum Press, New York, London, pp. 477–58.

Mess, B., V. Csernus, Z. Rekasi (1991a) The role of different neurotransmitters and neuropeptides in the regulation of pineal hormone secretion. A dynamic in vitro study. In: Adv. Pineal Res., A. Foldes, R.J. Reiter, eds., John Libbey, London, Vol. 6, pp. 57–66.

Mess, B., Z. Rekasi, V. Csernus (1991b) The use of perifusion technique in the research of pineal neuroendocrinology. The role of different neurotransmitters and neuropeptides in the regulation of pineal hormone secretion. A dynamic in vitro study. In: The Role of Melatonin and Pineal Peptides in Neuroimmunomodulation, F. Fraschini, R.J. Reiter, eds., Plenum, New York, pp. 67–77.

Mess, B., Z. Rekasi, M. Ghosh, V. Csernus (1996) Regulation of pineal melatonin secretion: comparison between mammals and birds. Acta Biol. Acad. Sci. Hung. 47: 313–322.

Mess, B., G. Trentini, L. Tima (1978) The role of the pineal gland in the regulation of ovulation. Akad. Kiadó, Budapest.

Mikkelsen, J.D., M. Moller, P.J. Larsen, J. Fahrenkrug (1994) The presence of nerve fibers immunoreactive for vasoactive intestinal peptide (VIP), peptide histidine isoleucine (Phi), and preprovip (111–122) in the mouse pineal gland. J. Pineal Res. 16: 50–56.

Moller, M., J.D. Mikkelsen, J. Fahrenkrug, H.W. Korf (1985) The presence of vasoactive intestinal polypeptide (VIP)-like-immunoreactive nerve fibres and VIP-receptors in the pineal gland of the Mongolian gerbil (Meriones unguiculatus). An immunohistochemical and receptor-autoradiographic study. Cell Tiss. Res. 241: 333–340.

Moller, W., G. Moller (1990) Structural and functional differentiation of the embryonic chick pineal organ in vivo and in vitro. A scanning electron-microscopic and radioimmunoassay study. Cell Tiss. Res. 260: 337–348.

Moore, R.Y., A. Heller, R.J. Wurtman, J. Axelrod (1967) Visual pathway mediating pineal response to environmental light. Science 155: 220–223.

Morita, Y., M. Samejima, D. Uchida (1987) Pineal role in circadian locomotor rhythm and cellular basis of photic entrainment. In: Fundamentals and clinics in pineal research, G. Trentini, C. DeGaetani, P. Pevet, eds., Raven Press, New York, pp. 161–164.

Nowak, J.Z., K. Kuba, J.B. Zawilska (1999) PACAP-induced formation of cyclic AMP in the chicken brain: regional variations and the effect of melatonin. Brain Res. 830: 195–199.

Ohshima, K., K. Hiramatsu (1993) Ultrastructural study of post-hatching development in the pineal gland of the Japanese quail. J. Vet. Med. Sci. 55: 945–950.

Ohshima, K., S. Matsuo (1988) Cytodifferentiation of the chick pineal gland, with special reference to the photosensory and secretory elements. J. Pineal Res. 5: 397–410.

Okano, T., T. Yoshizawa, Y. Fukada (1994) Pinopsin is a chicken pineal photoreceptive molecule. Nature 372: 94–97.

Oksche, A., M. Vaupel-von Harnack (1965) [On the structure of the rudimentary sense cell of the pineal body of the chicken]. [German]. Naturwissenschaften 52: 662–663.

Olah, I., B. Glick (1984) Lymphopineal tissue in the chicken. Develop. Comp. Immunol. 8: 855–862.

Olah, I., A. Magyar (1996) Pineal gland of the chicken is a transient lymphoid organ. Internatl. J. Thymol. 4: 93–102.

Peschke, D., E. Peschke, J. Peil, B. Mess (1989) Circannual serum creatine kinase patterns after ganglionectomy and pinealectomy of the Wistar rat. J. Pineal Res. 6: 375–383.

Pevet, P. (1988) The role of the pineal gland in the photoperiodic control of reproduction in different hamster species. Reprod. Nutr. Dev. 28: 443–458.

Pevet, P., B. Vivien-Roels, M. Masson-Pevet (1989) Low temperature in the golden hamster accelerates the gonadal atrophy induced by short photoperiod but does not affect the daily pattern of melatonin secretion. J. Neural Transm. 76: 119–128.

Pevet, P., B. Vivien-Roels, M. Masson-Pevet (1991) Annual changes in the daily pattern of melatonin synthesis and release. In: Role of melatonin and pineal peptides in neuroimmunomodulation, F. Fraschini, R.J. Reiter, eds., Plenum Press, New York, pp. 147–157.

Pratt, B.L., J.S. Takahashi (1987) Alpha-2 adrenergic regulation of melatonin release in chick pineal cell cultures. J. Neurosci. 7: 3665–3674.

Price, J.L., J. Blau, A. Rothenfluh, M. Abodeely, B. Kloss, M.W. Young (1998) Double-time is a novel Drosophila clock gene that regulates period protein accumulation. Cell 94: 83–95.

Rachidi, M., C. Lopes, J.C. Benichou, F. Rouyer (1997) Analysis of period circadian expression in the Drosophila head by in situ hybridization. J. Neurogenet. 11: 255–263.

Redfern, P., D. Minors, J. Waterhouse (1994) Circadian rhythms, jet lag, and chronobiotics—an overview. Chronobiol. Int. 11: 253–265.

Reiter, R.J. (1974) Circannual reproductive rhythms in mammals related to photoperiod and pineal function: a review. Chronobiologia 1: 365–395.

Reiter, R.J. (1981a) Pineal control of reproduction. Prog. Clin. Biol. Res. 59B: 349–355.

Reiter, R.J. (1981b) The mammalian pineal gland: structure and function. Am. J. Anat. 162: 287–313.

Reiter, R.J. (1983) The role of light and age in determining melatonin production in the pineal gland. In: The pineal gland and its endocrine role, J. Axelrod, F. Fraschini, P. Velo, eds., Plenum Press, New York, pp. 227–242.

Reiter, R.J. (1993) The mammalian pineal gland is an end organ of the visual system. In: Melatonin—clinical perspectives, A. Miles, D.R.S. Philbrick, C. Thompson, eds., Oxford University Press, Oxford, pp. 1–42.

Reiter, R.J. (1997) Antioxidant actions of melatonin. Adv. Pharmacol. (New York) 38: 103–117.

Reiter, R.J., D.X. Tan, B. Poeggeler, A. Menendezpelaez, L.D. Chen, S. Saarela (1994) Melatonin as a free radical scavenger: implications for aging and age-related diseases. Ann. N. Y. Acad. Sci. 719: 1–12.

Rekasi, Z., V. Csernus, J. Horvath, S. Vigh, B. Mess (1991) Long-term dynamic in vitro system for investigating rat pineal melatonin secretion. J. Neuroendocrinol. 3: 563–568.

Rekasi, Z., N. Sule, V. Csernus, B. Mess (1998) Adrenergic and peptidergic control of the regulation of cAMP efflux and melatonin secretion from perifused rat pineal gland. Endocrine 9: 89–96.

Reuss, S., R. Riemann, L. Vollrath (1992) Substance P- and calcitonin gene-related peptide-like immunoreactive neurons in the rat trigeminal ganglion—with special reference to meningeal and pineal innervation. Acta Histochem. 92: 104–109.

Ritz, T., S. Adem, K. Schulten (2000) A model for photoreceptor-based magnetoreception in birds. Biophys. J. 78: 707–718.

Robertson, L.M., J.S. Takahashi (1988) Circadian clock in cell culture: I. Oscillation of melatonin release from dissociated chick pineal cells in flow-through microcarrier culture. J. Neurosci. 8: 12–21.

Saarela, S., R.J. Reiter (1994) Function of melatonin in thermoregulatory processes Life Sci. 54: 295–311.

Sawyer, L.A., J.M. Hennessy, A.A. Peixoto, E. Rosato, H. Parkinson, R. Costa, C. P. Kyriacou (1997) Natural variation in a Drosophila clock gene and temperature compensation. Science 278: 2117–2120.

Schneider, T., H.P. Thalau, P. Semm, W. Wiltschko (1994) Melatonin is crucial for the migratory orientation of pied flycatchers (Ficedula Hypoleuca Pallas). J. Exp. Biol. 194: 255–262.

Semm, P., R.C. Beason (1990) Sensory basis of bird orientation. Experientia 46: 372–378.

Shedpure, M., A.K. Pati (1995) The pineal gland: structural and functional diversity. Indian J. Exp. Biol. 33: 625–640.

Shinohara, K., S.T. Inouye (1994) Circadian variations of neuropeptide Y-like immunoreactivity in the rat pineal gland. Neuroreports 2: 1262–1264.

Simonneaux, V., A. Ouichou, P. Pevet (1993) Pituitary adenylate cyclase-activating polypeptide (PACAP) stimulates melatonin synthesis from rat pineal gland. Brain Res. 603: 148–152.

Simonneaux, V., A. Ouichou, P. Pevet, M. Masson-Pevet, B. Vivien-Roels, H. Vaudog (1989) Kinetic study of melatonin release from rat pineal glands using a perifusion technique. J. Pineal Res. 7: 63–83.

Snyder, S.H., J. Axelrod, M. Zweig (1967) Circadian rhythm in the serotonin content of the rat pineal gland: regulating factors. J. Pharmacol. Exp. Ther. 158: 206–213.

Sun, Z.S., U. Albrecht, O. Zhuchenko, J. Bailey, G. Eichele, C.C. Lee (1997) RIGUI, a putative mammalian ortholog of the Drosophila period gene. Cell 90: 1003–1011.

Takahashi, J.S., H. Hamm, M. Menaker (1980) Circadian rhythms of melatonin release from individual superfused chicken pineal glands in vitro. Proc. Nat. Acad. Sci. USA 77: 2319–2322.

Takanaka, Y., T. Okano, M. Iigo, Y. Fukada (1998) Light-dependent expression of pinopsin gene in chicken pineal gland. J. Neurochem. 70: 908–913.

Taylor, A.N., R.W. Wilson (1970) Electrophysiological evidence for the action of light on the pineal gland in the rat. Experientia 26: 267–269.

Trentini, G.P., C.F. De Gaetani, M. Criscuolo, M.G. Balemans, L.M. Vaessen, I. Smith, (1982) The effect of melatonin and other indole derivatives in maintaining ovulation in rats kept in continuous light and the influence of these indoles on HIOMT activity in the pineal gland. J. Neur. Transm. 53: 305–314.

Ueck, M. (1973) [Fluorescence and electron microscopic studies of the pineal body in various species of birds]. [German]. Z. Zellforsch. Mikroskop. Anat. 137: 37–62.

Underwood, H., M. Harless (1985) Entrainment of the circadian activity rhythm of a lizard to melatonin injections. Physiol. Behav. 35: 267–270.

Vigh, B., P. Rohlich, T. Gorcs, M.J. Manzanoe-Silva, A. Szel, Z. Fejer, I. Vigh-Teichmann (1998) The pineal organ as a folded retina: immunocytochemical localization of opsins. Biol. Cell 90: 653–659.

Vigh, B., I. Vigh-Teichmann (1988) Comparative neurohistology and immunocytochemistry of the pineal complex with special reference to CSF containing neuronal structures. Pineal Res. Rev. 6: 1–65.

Vigh, B., I. Vigh-Teichmann (1999) Comparative morphophysiology of the pineal organs of vertebrates. In: Comparative Endocrinology and Reproduction, K.P. Joy, A. Krishna, C. Haldar, eds., Narosa Publ. House, New Delhi, pp. 479–506.

Vivien-Roels, B., J. Arendt (1983) How does the indoleamine production of the pineal gland respond to variations of the environment in a non-mammalian vertebrate, Testudo hermanni Gmelin? Psychoneuroendocrinology 8: 327–332.

Voisin, P., J. Guerlotte, M. Bernard, P. Greve, C. Grechez, J.P. Collin (1994) Regulation of hydroxyindole-O-methyltransferase gene expression in the pineal gland and retina. Acta Neurobiol. Exp. 54 (Suppl.): 41–46.

Voisin, P., C. Martin, J.P. Collin (1987) Alpha 2-adrenergic regulation of arylalkylamine N-acetyltransferase in organ-cultured chick pineal gland: characterization with agonists and modulation of experimentally stimulated enzyme activity. J. Neurochem. 49: 1421–1426.

Vollrath, L. (1979) Comparative morphology of the vertebrate pineal complex. Prog. Brain Res. 52: 25–38.

von Bartheld, C.S., A. Schober (1997) Nitric oxide synthase in learning-relevant nuclei of the chick brain: morphology, distribution, and relation to transmitter phenotypes. J. Comp. Neurol. 383: 135–152.

Wiltschko, W., R. Wiltschko (1991) Orientation in birds. Magnetic orientation and celestial cues in migratory orientation. EXS 60: 16–37.

Wurtman, R.J., J. Axelrod, J.E. Fisher (1964) Melatonin synthesis in the pineal gland. Effect of light mediated by the sympathetic nervous system. Science 143: 1328–1336.

Yamakawa, Y., H. Shiraishi, M. Yamakawa (1997) Characterization of nitric oxide synthase in the cerebellum of the chicken. Comp. Biochem. Physiol. Biochem. Mol. Biol. 118: 457–461.

Yochim, J.M., E.P. Wallen (1974) Photoperiod, pineal HIOMT activity and reproductive function in the rat: a proposed model. Biol. Reprod. 11: 125–132.

Yoshimura, T., Y. Suzuki, E. Makino, T. Suzuki, A. Kuroiwa, Y. Matsuda, T. Namikawa, S. Ebihara (2000) Molecular analysis of avian circadian clock genes. Brain Res. Mol. Brain Res. 78: 207–215.

Yuwiler, A., G.L. Brammer, B. L. Bennett (1995) Interaction between adrenergic and peptide stimulation in the rat pineal: pituitary adenylate cyclase-activating peptide. J. Neurochem. 64: 2273–2280.

Zatz, M. (1991) Light and norepinephrine similarly prevent damping of the melatonin rhythm in cultured chick pineal cells: regulation of coupling between the pacemaker and overt rhythms? J. Biol. Rhythms 6: 137–147.

Zatz, M., G. Kasper, C.R. Marquez (1990) Vasoactive intestinal peptide stimulates chick pineal melatonin production and interacts with other stimulatory and inhibitory agents but does not show alpha 1-adrenergic potentiation. J. Neurochem. 55: 1149–1153.

Zatz, M., G.D. Lange, M.D. Rollag (1994) What does changing the temperature do to the melatonin rhythm in cultured chick pineal cells. Am. J. Physiol. 266: R50–R58.

Zawilska, J., J.Z. Nowak (1991) Regulation of melatonin biosynthesis in vertebrate retina: involvement of dopamine in the suppressive effects of light. Folia Histochem. Cytobiol. 29: 3–13.

Zawilska, J.B., J.Z. Nowak (1992) Regulatory mechanisms in melatonin biosynthesis in retina. Neurochem. Internatl. 20: 23–36.

Zeman, M., E. Gwinner, I. Herichova, D. Lamosova, L. Kost'al (1999) Perinatal development of circadian melatonin production in domestic chicks. J. Pineal Res. 26: 28–34.

Zeman, M., E. Gwinner, E. Somogyiova (1992) Development of melatonin rhythm in the pineal gland and eyes of chick embryo. Experientia 48: 765–768.

Zeman, M., H. Illnerova (1990) Ontogeny of N-acetyltransferase activity rhythm in pineal gland of chick embryo. Comp. Biochem. Physiol. A97: 175–178.

Zisapel, N. (1999) The use of melatonin for the treatment of insomnia. Biol. Sign. Recept. 8: 84–89.