# DECLARATION

## NORTH-EASTERN HILL UNIVERSITY

## SHILLONG - 793 022

## JUNE, 2006

*I, Mr. Longshibemo Yanthan, hereby declare that the thesis entitled,* **"Studies on *in vitro* effects of hormones on N-acetyltransferase activity in the pineal of air-breathing fish, *Clarias gariepinus*"** *is my own work and to the best of my knowledge this thesis does not contain any part of work which has been submitted for the award of any degree in any other University/Institute.*

*This is being submitted to the North-Eastern Hill University for award of the degree of Doctor of Philosophy in Zoology.*

*Longshibemo Yanthan*
*(Candidate)*

*(Head)*

Head
Department of Zoology
North Eastern Hill University
Shillong - 793022

*(Supervisor)*

**Prof. B. B. P. Gupta**
*Department Of Zoology*
*North-Eastern Hill University*
*Shillong—793022*

# CONTENTS

## *ACKNOWLEDGEMENT*

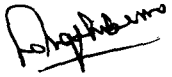
*I express my deep sense of gratitude to my supervisor, Professor B. B. P. Gupta, FNASc, Department of Zoology, North-Eastern Hill University, Shillong for his benevolence and guidance throughout the execution of this work, without which this work would not have been completed.*

*I express my heartfelt gratitude to the Head of the Department of Zoology, Prof. S. B. Prasad and former Head, Prof. B. B. P. Gupta for providing me the necessary infrastructure and laboratory facilities to carry out my research work. I also thank the Head of the Department of Biochemistry, N.E.H.U. for allowing me to use the Liquid Scintillation Counter.*

*I am thankful to the Dean of School of Life Sciences for the administrative help, and to all the faculty members and the non-teaching staff of the Department of Zoology for their kind help and co-operation.*

*I also thank my laboratory colleagues and my friends for their kind help whenever I needed the most. My special thanks goes to my parents for their moral support and encouragement.*

*I would also like to acknowledge the financial assistance in the form of Project fellowship provided by Department of Zoology, N. E. H. U. under DRS - III (SAP) programme.*


*Longshibemo Yanthan*

*Department of Zoology*

*North-Eastern Hill University*

*Shillong 793 022*

# PREFACE

The pineal is present in all groups of vertebrates (Vollrath, 1981; Cassone *et al.*, 1986; Falcon *et al.*, 1989; Serino *et al.*, 1993; Tosini and Menaker, 1996). The mammalian pineal has evolved from the photoreceptor cells which developed capabilities to synthesize melatonin. The mammalian pineal is composed of specialized epithelial cells called pinealocytes. However, the pineal of non-mammalian vertebrates is composed of either only photoreceptor cell (e.g., pineal of fishes, amphibians and reptiles) or of both photoreceptors and rudimentary photoreceptors (e.g., avian pineal) (Vollrath, 1981; Bernard *et al.*, 1997; Natesan *et al.*, 2002). The mammalian pineal has lost the capacity to respond to light directly (Korf *et al.*, 1998; Gupta *et al.*, 2005). In lower vertebrates, however, the pineal organ responds to changes in the environmental factors in general and to photoperiod in particular. Unlike mammalian pineals, the pineal organ of sub-mammalian vertebrates responds to light directly (Gupta and Premabati, 2002). In all vertebrates, the pineal produces its hormone melatonin in a rhythmic manner, and the diurnal rhythm of melatonin is primarily regulated by the light-dark cycle. The rate of melatonin synthesis is minimum during the daytime (light phase) and maximum during the night time (dark phase) (Binkley *et al.*, 1981; Vollrath, 1981; Falcon *et al.*, 1987; Morton and Forbes, 1988; d'Istria *et al.*, 1994; Lutterschmidth *et al.*, 2002). This is mainly due to photoperiod-dependent cyclicity in the activity of the rate limiting enzyme called arylalkylamine N-acetyltransferase (AA-NAT) present in the pineal of all vertebrates. As in other vertebrates, melatonin production in the fish

pineal is regulated by the light-dark cycle and it is also influenced by the environmental temperature (Max and Menaker, 1992; Zachmann *et al.*, 1992; Samejima *et al.*, 2000).

The pineal hormone melatonin regulates and/or modulates a wide range of vertebrate physiology. Melatonin is involved in the regulation of circadian and circannual rhythms, reproductive cycles, growth, metabolism, metamorphosis etc. in vertebrates (Vollrath, 1981; Bubenik *et al.*, 1998; Reiter and Maestroni, 1999; Gupta and Premabati, 2002). Melatonin is the most physiologically active indole derivative of the fish pineal, and produces prominent effects on pigmentation (Joss, 1973) reproductive system (Garg, 1989), locomotor activity (Garg and Sundararaj, 1986; Morita *et al.*, 1989), growth and metamorphosis (Eddy, 1969), pituitary (de Vlaming and Vodicnik, 1977), interrenal function (Vollrath, 1981) and adrenal (Agha and Joy, 1989).

The melatonin biosynthesis pathway and enzymes involved in melatonin synthesis in the fish pineal are the same as in mammalian pineal (Vollrath, 1981; Gupta and Premabati, 2002). Tryptophan is the precursor of melatonin synthesis. In the photoreceptors of the fish pineal, tryptophan is first converted to 5-hydroxytryptophan by the enzyme tryptophan hydroxlase, and then 5-hydroxytryptophan is converted to 5-hydroxytryptamine or serotonin by the enzyme aromatic L- amino acid decarboxylase. Arylalkylamine N-acetyltransferase (AA-NAT) acts as the rate-limiting enzyme of melatonin synthesis pathway in fish pineal,

and catalyses the conversion of serotonin to N-acetylserotonin (Klein *et al.*, 1997; Klein, 1999). The presence of AA-NAT enzyme has been reported in a number of piscine species (Morton and Forbes, 1988; Begay *et al.*, 1998; Coon *et al.*, 1999; Benyassi *et al.*, 2000, 2001). The enzyme hydroxyindole-O-methyltransferase (HIOMT) present in the photoreceptors finally converts N-acetyl serotonin to melatonin (Hafeez and Quay, 1970; Morton and Forbes, 1988).

Hormones are essential constituents of the inter-cellular communication system that maintains homeostasis in higher organisms, and play a vital role in general adaptation in ever changing environment. Although the parallel diurnal rhythms of arylalkylamine N-acetyltransferase (AA-NAT) and melatonin are primarily regulated by the light-dark cycle and environmental temperature, there are several reports regarding the role of hormones in the regulation of AA-NAT activity in mammals (Yuwiler, 1989; Saidapur *et al.*, 1991; Okatani *et al.*, 1998a,b; Hernandez-Diaz *et al.*, 2001) and birds (Zawilska and Iuvone, 1989; Zawilska and Sadowska., 2002). It has been reported that gonadal steroids modulate melatonin secretion in mammals (Saidapur *et al.*, 1991; Luboshitzky *et al.*, 1995; Redins *et al.*, 1999; Martin and Touitou, 2000). Testosterone has been reported to play an inhibitory role on pineal activity in rats (Luboshitzky *et al.*, 1995; Redins *et al.*, 1999). The inhibitory effect of estrogens on melatonin synthesis has also been reported in mammals (Okatani *et al.*, 1997; Kus *et al.*, 2002). However, there is particularly no information on the influence of gonadal steroids on pineal AA-NAT activity in any sub-mammalian vertebrates in general and in the fishes in particular.

Glucocorticoids have been found to inhibit melatonin production in a number of vertebrate species (Yuwiler and Brammer, 1981; Yuwiler, 1989; Benyassi *et al.*, 2001; Zawilska and Sadowska, 2002). There are also reports on the role of indoleamines on pineal AA-NAT activity in mammals (Freire and Cardinali, 1975; Illnerova *et al.*, 1989; Redins *et al.*, 2001). Serotonin acts not only as an intermediate compound for melatonin synthesis but also plays a stimulatory role in melatonin synthesis in the mammalian pineal gland (Sterado *et al.*, 2000). Similarly, melatonin has also been reported to increase AA-NAT activity in the rat pineal (Freire and Cardinali, 1975). The presence of various indoleamines has been reported in the fish pineal (Meiniel and Hartwig, 1980; Yanez and Meissl, 1996; Gupta and Premabati, 2002; Ceinos *et al.*, 2005). However, there is no information on the role of indoleamines on the pineal AA-NAT activity or melatonin synthesis in any fish species.

Leptin is a newly recognized hormone produced mainly by adipose tissues and also by other organs like placenta, gastrointestinal tract etc. (Prolo *et al.*, 1998). Leptin is involved in the regulation of food intake, energy balance, body temperature, basal metabolic rate, oxidative metabolism etc. (Liefers *et al.*, 2002; Nieminen *et al.*, 2000). Leptin secreted by adipose tissue provides a feed back signal informing the central nervous system about the energy reserves of the body stored as fat. The expression of leptin receptor in ectotherm has been reported and the peptide structure of fish leptin is similar to that of mammalian leptin (Johnson *et al.*, 2000; Yaghoubian *et al.*, 2001). Since lipid content of the fish exhibits a circannual rhythm

with low body lipid during winter and high lipid content during the breeding phase/summer and rainy seasons (Singh and Singh, 1984; Shirai *et al.*, 2001), there is a possibility that circannual rhythmicity in leptin concentration may influence AA-NAT activity and melatonin synthesis in fish species. But so far there is no information on pineal-leptin interrelationship in ectothermic vertebrates or on the influence of leptin on AA-NAT activity and/or melatonin synthesis.

Keeping in view the scarcity of information on the role of hormones in the regulation of AA-NAT activity on fish pineal, it was decided to undertake a comprehensive study on *in vitro* effects of gonadal steroids, corticosteroids, indoleamines and leptin on the activity of the rate limiting enzyme, arylalkylamine N-acetyltransferase (AA-NAT) in an air-breathing exotic catfish, *Clarias gariepinus*.

The present Ph. D. dissertation has been divided into four chapters. A brief introduction of each chapter has been given in the following sections.

**Chapter 1 - Materials and methods**

This chapter deals with the details of materials and methods used for this Ph. D. dissertation. It incorporates description of the experimental animal, maintenance of the experimental fishes, tissue culture of the fish pineal, mode of treatments, methods used for measuring the pineal AA-NAT activity and the biostatistical methods used for analyzing the Data.

**Chapter 2 - *In vitro* effects of gonadal steroids and corticosteroids on arylalkylamine N-acetyltransferase (AA-NAT) activity in the fish pineal during different phases of the breeding cycle**

This chapter deals with the study of *in vitro* effects of different concentrations of androgen (testosterone), estrogens (17-β estradiol, estriol and estrone) and corticosteroids (corticosterone and cortisol) on pineal AA-NAT activity in the fish maintained under natural climatic conditions during quiescent, progressive, breeding and regressive phases of the annual breeding cycle.

**Chapter 3 - *In vitro* effects of indoleamines and leptin on arylalkylamine N-acetyltransferase (AA-NAT) activity in the fish pineal during different phases of the breeding cycle**

This chapter deals with *in vitro* effects of different concentrations of 5-hydroxytryptophan, serotonin, N-acetylserotonin and melatonin on pineal AA-NAT activity in the fish maintained under natural climatic conditions during quiescent, progressive, breeding and regressive phases of the annual breeding cycle. This chapter also deals with *in vitro* effects of different doses of leptin on pineal AA-NAT activity in fed and fasted groups of fishes maintained under natural climatic conditions during winter and summer seasons.

**Chapter 4 - Summary and conclusions**

This chapter incorporates the summary of major findings of the Chapter 1, Chapter 2 and conclusions derived from the findings of the Ph. D. dissertation.

This study seems to be the first of its kind in which *in vitro* effects of various hormones on AA-NAT activity has been studied in the photoreceptive pineal of a fish species. Findings of the present study clearly suggest that AA-NAT activity and melatonin synthesis in the pineal of *Clarias gariepinus* are regulated jointly by a number of hormones.

# INTRODUCTION

The pineal is present in all groups of vertebrates (Vollrath, 1981; Cassone *et al.*, 1986; Falcon *et al.*, 1989; Serino *et al.*, 1993; Tosini and Menaker, 1996). The mammalian pineal has evolved from the photoreceptor cells which developed capabilities to synthesize melatonin (Klein, 2006). The mammalian pineal is composed of specialized epithelial cells called pinealocytes. However, the pineal of non-mammalian vertebrates is composed of either only photoreceptor cell (e.g., pineal of fishes, amphibians and reptiles) or of both photoreceptors and rudimentary photoreceptors (e.g., avian pineal) (Vollrath, 1981; Bernard *et al.*, 1997; Natesan *et al.*, 2002). The mammalian pineal has lost the capacity to respond to light directly (Korf *et al.*, 1998; Gupta *et al.*, 2005). In lower vertebrates, however, the pineal organ responds to changes in the environmental factors in general and to photoperiod in particular. Unlike mammalian pineals, the pineal organ of sub-mammalian vertebrates responds to light directly (Gupta and Premabati, 2002). In all vertebrates, the pineal produces its hormone melatonin in a rhythmic manner, and the diurnal rhythm of melatonin is primarily regulated by the light-dark cycle. Melatonin acts as a pineal hormone and regulates/modulates a wide range of vertebrate physiology. In mammals, melatonin is involved in regulation of circadian, and circannual rhythms, reproductive cycles, immune system, metabolism etc. (Vollrath, 1981; Reiter and Maestroni, 1999; Pevet, 2000). In birds, the pineal showed cyclical patterns relative to environmental factors, which are correlated with plasma melatonin. Changes in

environmental factors promote annual variations in adrenal and gonadal activity in birds, probably by modulating the pineal gland (Sudhakumari *et al.*, 2001).

Melatonin is considered to be the most physiologically active indole derivative in fishes. Depending on the time of the year, stage of the reproductive cycle and the photoperiod-temperature regime to which the fishes are exposed, melatonin either stimulates or inhibits gonadal maturation (Garg, 1989). Administration of melatonin has been reported to increase the size of gonadrotropic cells in *Carassius auratus* (Fenwick, 1970) and to reduce the number of gonadotorphs in *Heteropneustes fossilis* (Sundararaj and Keshavanath, 1976). Melatonin treatment also inhibited gonadal maturation in Salmon (Amano *et al.*, 2004). Administration of melatonin may induce pigment aggregation, pigment dispersal or may have no effect on melanophores (Hafeez, 1970). Pinealectomy abolished day-night change in body color of the blinded rainbow trout, probably by suppressing blanching at night (Hafeez and Quay, 1970). Pinealectomy has also been reported to cause darkening of the body color in other Salmonides (Hoar, 1955). It has been reported that the ovarian weight of pinealectomized fish is significantly reduced as compared to that of intact fish maintained under continuous light or natural daylight (Urasaki, 1972a). These findings suggest that the pineal organ stimulates gonadal growth under continuous illumination, while it inhibits gonadal activity under continuous darkness or short photoperiod (Urasaki, 1972b). It has been reported in lamprey that light information perceived by the pineal is transmitted to the central nervous system and contributes to the control of the locomotor activity

rhythm (Garg and Sundararaj, 1986; Morita *et al.*, 1989). The pineal gland has also been reported to play an important role in the appearance of a circadian rhythm in the daily shifts of a fish's activity (Kavaliers, 1981; Tabata *et al.*, 1988). Melatonin reportedly produces prominent effects on growth and metamorphosis (Eddy, 1969), pituitary (Vollrath, 1981), thyroid (Nayak and Singh, 1987), adrenal (Agha and Joy, 1989), interrenal function (Vollrath, 1981) etc. The significant influence of the pineal and melatonin on pigmentation, gonadal activity, breeding cycle, metamorphosis, development, endocrine glands, etc. of fish might be of great adaptational value to ensure survival under ever-changing aquatic environment for a successful aquatic life.

In all vertebrates, melatonin is synthesized from tryptophan. First, tryptophan is converted to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase. Then aromatic L-amino acid decarboxylase converts 5-hydroxytryptophan into 5-hydroxytryptamine (serotonin), which is acetylated to form N-acetylserotonin by the enzyme arylalkylamine N-acetyltransferase (AA-NAT). Finally, N-acetylserotonin is converted to N-acetyl-methoxytryptamine (melatonin) by the enzyme hydroxyindole-O-methyltransferase (HIOMT). In melatonin biosynthetic pathway, AA-NAT acts as the rate-limiting enzyme. It is important to mention that all enzymes involved in melatonin synthesis have been found to be present in the pineal of all groups of vertebrates (Gupta and Spessert, in press).

The rate of melatonin synthesis is minimum during the daytime (light phase) and maximum during the night time (dark phase) in all vertebrates (Vollrath, 1981; Falcon *et al.*, 1987; Morton and Forbes, 1988). This is mainly due to photoperiod-dependent cyclicity in the activity of the rate-limiting enzyme, AA-NAT present in the pineal of all vertebrates (Privat *et al.*, 1999; Gupta *et al.*, 2005; Gupta and Spessert, in press). As in other vertebrates, melatonin production in the fish pineal is regulated by the light-dark cycle, and it is also influenced by the environmental temperature (Max and Menaker, 1992; Zachmann *et al.*, 1992; Samejima *et al.*, 2000).

The mammalian pineal is innervated mainly by post ganglionic sympathetic nerve fibres. Light stimulates melopsin-containing cells of retina and generates nerve impulses, which are transmitted by the optic nerves to the pineal gland via hypothalamus and superior cervical ganglia. The nerve endings gets hyper polarized due to the impulse generated by the presence of light. Due to hyper polarization, the release of norepinephrine (NE) in the pineal gland is inhibited during the light phase. With the onset of dark phase, the generation of impulse in the retina is inhibited which leads to depolarization in the sympathetic nerve fibres terminating the pineal gland. The depolarization in the nerve terminus leads to increased release of norepinephrine. The norepinephrine released from the adrenergic nerve terminal acts on the pinealocytes and stimulates AA-NAT activity followed by the increased rate of melatonin synthesis.

As mentioned earlier, melatonin is produced by the pineal in a rhythmic fashion with high levels of the hormone during night-time and low/basal levels during day-time (Vollrath, 1981; Arendt, 1985; Korf, 1999), and the rhythmic production of melatonin is controlled directly by the diurnal rhythm of activity of AA-NAT- the rate-limiting enzyme of melatonin biosynthetic pathway in all groups of vertebrates (Coon *et al.*, 1995; Klein *et al.*, 1997; Eddy *et al.*, 2000). The circadian rhythm of melatonin synthesis involves three components, i.e., a photodetector, a circadian clock and the melatonin synthesizing machinery. The anatomical organization of these three components has evolved during the course of evolution. In mammals, retina acts as a photodetector and synchronizes a circadian clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus, while the melatonin synthesizing machinery is located in pinealocytes. The stimulatory and/or inhibitory circadian signals from the suprachiasmatic nucleus together with the photic information from the retina are conveyed by a multisynaptic pathway from the SCN through brain stem, spinal cord, superior cervical ganglia and postganglionic sympathetic nerve fibres to the pineal gland to drive the circadian rhythms of AA-NAT activity and melatonin (Drijfhout *et al.*, 1996; Pevet *et al.*, 1997; Van Esseveldt *et al.*, 2000). In mammalian pineal, AA-NAT activity is photoperiodically controlled via Retina-SCN-SCG-NE-cAMP-PKA-CREB-CRE pathway (Klein, 1999).

Unlike in mammals, the non-mammalian pineal organ itself contains all the components (i.e., photodetector, circadian clock and melatonin forming system) essential for melatonin rhythm (Binkley *et al.*, 1978; Deguchi, 1979a; Menaker and

Wisner, 1983; Pickard and Tang, 1993, 1994; Cahill, 1996; Bolliet *et al.*, 1997; Gupta and Premabati, 2002). As a result, in non-mammalian pineals the circadian rhythm of melatonin synthesis continues under *in vitro* conditions, and light acts directly on these photoreceptive pineals to reset the internal clock and switches 'off' melatonin synthesis. Recent studies on temperate-zone piscine species indicate that diurnal melatonin rhythm in majority of teleosts is driven by an internal clock, while in few species the circadian melatonin rhythm is not clock-driven (Gern and Greenhouse, 1988; Max and Menaker, 1992; Thibault *et al.*, 1993). Induction of *aa-nat* gene associated with increased AA-NAT mRNA has been found to be responsible for increased AA-NAT activity followed by increased melatonin synthesis in clock-driven teleost pineals during night (Begay *et al.*, 1998).

Attempts have been made by a large number of scientists to investigate the pathways involved in the regulation of melatonin synthesis in mammalian and sub-mammalian pineals. As a result, there is a large body of information on molecular components and signal transduction pathways involved in melatonin synthesis in the pineal of vertebrates, particularly of mammals (Maronde *et al.*, 1999a; Gupta *et al*, 2005; Gupta and Spessert, in press), birds (Natesan *et al.*, 2002) and fishes (Gupta and Premabati, 2002). A critical review of information available on molecular components of regulatory mechanisms and signal transduction pathways responsible for melatonin synthesis in the pineal of different groups of vertebrates has been presented in the following sections.

**Regulation of melatonin synthesis in mammalian pineal**

In mammalian pineal, the rate of melatonin synthesis is regulated mainly by the primary neurotransmitter NE, which binds to $\alpha_1$- and β-adrenergic receptors present on the membrane of pinealocytes and triggers the adrenergic signal transduction via cAMP and cGMP in the pineal. As mentioned earlier, a central oscillator located in the hypothalamic SCN, which acts as the brain's clock, integrates photoperiodic information and precisely drives elevated nocturnal release of NE (Van Esseveldt *et al.*, 2000). NE binds to the grooves formed by the transmembrane helices of the adrenergic receptors and activates them. The NE-activated β-adrenergic receptors (β-ARs) stimulate both adenylyl cyclase (AC) and cytoplasmic guanylyl cyclase (GC). First, NE-bound β-ARs activate stimulatory G-proteins (Gs) and facilitate dissociation of α-subunit (Gsα) from the complex of βγ-subunits. Then the Gsα binds to, and activates the enzyme AC leading to a 6-10 fold increase in cAMP accumulation (Vanecek *et al.*, 1985). The formation of cAMP activates a cascade of enzymatic reactions responsible for increased AA-NAT activity and melatonin synthesis. Simultaneously, β-ARs-induced activation of Gs (Sugden and Klein, 1987; Ho *et al.*, 1989) is also followed by increased nitric oxide synthase (NOS) activity and increased production of nitric oxide (NO) by a population of pinealocytes and simultaneous diffusion of NO into the cytoplasm of adjacent pinealocytes (Spessert *et al.*, 1993; Spessert *et al.*, 1998). Then NO binds to the heme group of cytosolic sGC resulting in its activation and a 2-4-fold increase in cGMP accumulation in pinealocytes (Spessert *et al.*, 1995). In contrast, the binding

of NE to $\alpha_1$-adrenergic receptors ($\alpha_1$-ARs) does not have any measurable effects on cAMP and cGMP accumulation in pinealocytes. However, simultaneous stimulation of $\alpha_1$- and β-ARs results in more than 50-fold increase in cAMP and over 100-fold increase in cGMP accumulation (Vanecek *et al.*, 1985; Chik and Ho, 1989). NE-induced increase in cGMP levels transduce signal via cGMP-Protein kinase G (PKG)-mitogen-activated protein kinase (MAPK) pathway. Elevation of intracellular $Ca^{2+}$ and the enzyme protein kinase C (PKC) play critical roles in $\alpha_1$-adrenergic potentiation of β-adrenergically stimulated cAMP accumulation (Ho *et al.*, 1987; Schomerus *et al.*, 2002). Several fold increases in cAMP and cGMP levels lead to phosphorylation and activation of protein kinase A (PKA) and PKG, respectively. The adrenergic signal transduction via cAMP on one hand leads to induction and activation of AA-NAT enzyme that accelerates the process of melatonin synthesis, and on the other, stimulates formation of inhibitory transcription factor inducible cAMP early repressor (ICER) which inhibits *aa-nat* gene induction.

Photic stimulation of the retina leads to activation of melanopsin present in retinal ganglion cells and generates neural impulses. These light-induced impulses are transmitted via the non-visual retino-hypothalamic tract (RHT) to the SCN - the master clock that drives the circadian rhythm of melatonin synthesis in the pineal gland. Simultaneously, SCN also receives photic inputs from the intergeniculate leaflet (IGL) via geniculate-hypothalamic tract (GHT) and the raphe nuclei (Reuss, 2003). It is important to mention that SCN generates its own circadian rhythm, and the photic inputs from the retina only entrain the endogenous circadian rhythm of

SCN (Gillette and Mitchell, 2002; Stehle *et al.*, 2003). The SCN generates impulses, which regulate the switching 'on' and 'off' of melatonin synthesis by regulating the rhythmic secretion of NE in the pineal gland. Based on studies involving measurements of melatonin content and *aa-nat* gene expression following bilateral SCN lesions, PVN lesions and SCG removal, it has been proposed that the circadian rhythm of melatonin synthesis is regulated by a combination of a constant but weak stimulatory and a strong rhythmic inhibitory SCN output to PVN (Perreau-Lenz *et al.*, 2003). It has been found that in rats gamma-aminobutyric acid (GABA) release from SCN is involved in the light-induced inhibition of melatonin synthesis during daytime (Kalsbeek *et al.*, 1999), and blocking of GABA-ergic transmission from SCN to PVN increases melatonin synthesis during daytime (Kalsbeek *et al.*, 2000). In other words, during daytime, neural signals originating from the SCN influence the sympathetic nerve fibres via PVN-SCG pathway and inhibit the release of NE from the sympathetic nerve terminals present in the pineal gland. However, with the onset of darkness, SCN withdraws its inhibitory signals resulting in increased secretion of NE in the pineal gland.

The release of NE marks the switch 'ON' of melatonin synthesis. As mentioned earlier, NE acts on pinealocytes via α- and β-adrenergic receptors and activates adenylyl cyclase via Gs and accelerates formation of cAMP. The free catalytic subunits of activated PKA are translocated to the nucleus where they stimulate phosphorylation of cAMP response element binding protein (CREB). Besides PKA activation in the cytoplasm, cAMP may also enter into the nucleus and

activate nuclear PKA, which can stimulate CREB phosphorylation. The phosphorylated CREB (pCREB) becomes capable of binding to the cAMP response element (CRE). The binding of pCREB to CRE leads to the activation of the *aa-nat* gene and formation of the AA-NAT mRNA. Additionally, pCREB also stimulates transcription of CREM gene and formation of ICER mRNA. The increase in the levels of AA-NAT mRNA is followed by a proportionate increase in the activity of the AA-NAT enzyme. The increased activity of the rate-limiting AA-NAT enzyme accelerates acetylation of serotonin to N-acetylserotonin, which is followed by several fold increase in the rate of melatonin synthesis and release. The stimulatory effects of NE on cAMP and AA-NAT activity are reportedly potentiated by several other neurotransmitters [e.g., vasoactive intestinal polypeptide (VIP), δ-sleep inducing peptide (DSIP), adenosine, serotonin etc.] that increase cAMP accumulation directly or indirectly (Gupta *et al.*, 1992; Simonneaux and Ribelayga, 2003).

Besides cAMP-PKA-CREB-CRE-*aa-nat* gene-induced increase in AA-NAT mRNA and AA-NAT activity, adrenergic stimulation has also been reported to switch 'ON' melatonin synthesis by at least four additional mechanisms, which do not involve formation of AA-NAT mRNA in the pineal of some mammals like ungulates, primates and humans (Klein *et al.*, 1997; Ganguly *et al.*, 2001; Coon *et al.*, 2002). These mechanisms are (i) direct cAMP-induced activation of AA-NAT, (ii) inhibition of proteasomal proteolysis of AA-NAT, (iii) activation and protection

of AA-NAT due to complex formation with 14-3-3, and (iv) direct and indirect actions of $Ca^{2+}$ on AA-NAT.

Unlike rodent pineals where melatonin synthesis increases slowly, there is very rapid increase in circulating melatonin levels at the beginning of the dark phase in human and some other species (Coon *et al.*, 2002), in which AA-NAT mRNA levels exhibit marginal diurnal fluctuations (Klein *et al.*, 1997). Further, while AA-NAT activity increased, AA-NAT mRNA levels of bovine pinealocytes were not altered following NE treatment (Schomerus *et al.*, 2000). These findings suggest that in some mammals, there is an alternative mechanism through which adrenergic stimulation of cAMP production stimulates melatonin synthesis without increasing *de novo* synthesis or activity of the AA-NAT protein as estimated in broken-cell preparations (Namboodiri *et al.*, 1985). In order to find out whether cAMP can switch 'ON' melatonin synthesis without increasing AA-NAT synthesis or AA-NAT activity measured under $V_{max}$-conditions, a cell line (1E7) expressing human NAT (hNAT) has been developed. Studies on cAMP regulation in 1E7 cells indicate that treatment with forskolin, dibutyryl cAMP, isobutylmethylxanthine or isoproterenol activate cellular hNAT within intact cells by 8-fold without markedly increasing the abundance of AA-NAT protein or AA-NAT activity in broken cell preparations, while forskolin, isobutylmethyl-xanthine and isoproterenol stimulate cAMP accumulation (Coon *et al.*, 2001). These findings indicate that melatonin synthesis can be switched 'ON' by cAMP without increasing AA-NAT protein.

In mammals whose AA-NAT mRNA levels exhibit marginal or no diurnal fluctuation, cAMP stimulates accumulation of the AA-NAT protein during the night by affecting post-transcriptional processes. In these species, cAMP-dependent inhibition of proteasomal proteolysis of AA-NAT protein following adrenergic stimulation seems to play a dominant role in nocturnal switching 'ON' of melatonin synthesis (Gastel *et al.*, 1998; Fleming *et al.*, 1999). In the bovine pineal, inhibition of proteasomal proteolysis of AA-NAT alone has been found to increase the enzyme activity by increasing AA-NAT protein levels by five- to ten-fold that may be responsible for switching 'ON' melatonin synthesis (Schomerus *et al.*, 2000). As in ungulates and primates, inhibition of proteasomal proteolysis has also been reported to increase accumulation of AA-NAT protein and activity in fish pineal (Falcon *et al.*, 2001). It seems that under unstimulated conditions AA-NAT protein is continuously synthesized and immediately degraded by the process of proteasomal proteolysis. The adrenergic stimulation of cAMP formation seems to protect AA-NAT protein from proteasomal proteolysis resulting in increased accumulation of the AA-NAT protein and activity followed by increased melatonin synthesis (Klein *et al.*, 1997; Gastel *et al.*, 1998). It has been suggested that the adrenergic signal may protect AA-NAT proteolysis via cAMP-dependent phosphorylation of two highly conserved AA-NAT PKA sites - the motifs proposed to be destined to be degraded by proteasomal proteolysis. Alternatively, the adrenergic signal can protect AA-NAT from degradation via cAMP-dependent phosphorylation of other proteins involved in targeting AA-NAT for proteasomal proteolysis. As mentioned earlier,

adrenergically-induced post-translational modification of the existing AA-NAT protein seems to play a very critical role in increasing/maintaining AA-NAT activity (Klein *et al.*, 2003). Adrenergically regulated cAMP promotes the formation of AA-NAT/14-3-3 complex, which increases the AA-NAT activity and accelerates melatonin production by shielding PKA-phosphorylated AA-NAT from dephosphorylation and/or proteolysis as well as by decreasing the $K_m$ of the enzyme for serotonin (Klein *et al.*, 2003).

Adrenergic stimulation of pinealocytes also leads to influx of $Ca^{2+}$ from the extracellular fluid into the cytoplasm and increased release of the bivalent ion from intracellular storage sites (Marin *et al.*, 1996). The adrenergically increased $Ca^{2+}$ levels are essential for full activation of AA-NAT. The cation binds directly to the AA-NAT protein and increases its affinity for serotonin and thereby enhances catalytic activity, accelerating melatonin synthesis (Gupta *et al.*, 2005). There are also indications that at least a part of the action of $Ca^{2+}$ indirectly affects steps in the induction of AA-NAT activity/melatonin synthesis beyond the accumulation of cAMP (Santana *et al.*, 2001). Similar to cAMP-regulated PKA-induced phosphorylation of CREB in pinealocytes, $Ca^{2+}$ influx has also been found to stimulate CREB phosphorylation via PKA-Rap1(Ras-related small G-protein)-ERK/MAPK pathway (Grewal *et al.*, 2000). Thus, in addition to cAMP as an adrenergic second messenger, $Ca^{2+}$ also seems to play a critical supportive role in adrenergic mechanisms responsible for AA-NAT induction/activation and switching 'ON' melatonin synthesis.

The process of melatonin synthesis in mammalian pineal is turned 'off' primarily by SCN via a complex mechanism. Under natural conditions, the AA-NAT activity and melatonin synthesis decline rapidly in the second half of the dark phase (dawn). This decrease in AA-NAT activity and melatonin synthesis takes place mainly due to a decrease in SCN-regulated NE release associated with increased PDE activity and faster turnover of cAMP, decreased PKA activity, increased protein phosphatase activity, increased dephosphorylation of pCREB, decreased transcription of the *aa-nat* gene, increased proteasomal proteolysis of AA-NAT, and inhibition of the *aa-nat* gene due to high levels of ICER protein. Acetylcholine (ACh) secreted from the parasympathetic nerve fibres of the central pinealopetal projection may also counteract the adrenergic stimulation and inhibits melatonin synthesis by stimulating glutamate exocytosis (Yamada *et al.*, 1998a). Glutamate acts on pinealocytes via metabotropic glutamate receptors (mGluRs), stimulates inhibitory G-protein (Gi) and inhibits cAMP formation by inhibiting adrenergically-stimulated adenylyl cyclase in the rat pineal (Yamada *et al.*, 1998b). All these inhibitory pathways, acting separately and/or in combination, terminate the adrenergic stimulatory signal, decrease AA-NAT activity and inhibit the process of melatonin synthesis in the pineal gland. Though the rhythm of nocturnal release of NE is closely regulated by the SCN, the rapid decline in AA-NAT activity and melatonin synthesis during the latter half of the dark phase can not be entirely due to rhythmic nocturnal decrease in the levels of NE and cAMP. Several reports suggest that ICER plays a crucial role in restricting the amplitude of melatonin rhythm by its

inhibitory influence on *aa-nat* gene transcription and thus may facilitate switching 'off' the melatonin synthesis.

The adrenergic signal transduction simultaneously activates both *aa-nat* gene and CREM gene via the ARs - cAMP - PKA - CREB - pCREB pathway. However, unlike AA-NAT mRNA levels that attain a peak during the first half of the night (dark phase), the observed increase in ICER mRNA levels precedes the decrease in AA-NAT mRNA, AA-NAT activity and melatonin synthesis. ICER seems to act as a very sensitive natural reporter for stimulated adrenergic pathways in rat pinealocytes, and binds directly to CRE element in the AA-NAT promoter and represses transcription of the *aa-nat* gene resulting in decreased activity of AA-NAT and inhibition of the rate as well as the amplitude of melatonin synthesis (Foulkes *et al.*, 2000; Stehle *et al.*, 2001). It seems that, during the first half of night, the ratio between pCREB (stimulatory TF) and ICER (inhibitory TF) in the rat pineal gland is in favour of the stimulatory TF due to drastic increase in the concentration of pCREB, which overrides ICER protein levels. However, as the duration of the dark phase increases, the intra-pineal pool of pCREB declines despite persistent NE release. The decrease in pCREB is caused probably due to increased amounts and/or activities of protein phosphatases (Maronde *et al.*, 1999b). It has been reported that NE-stimulation induces accumulation of protein serine/threonine phosphatase I (PSPI)-catalytic subunit in pineal nuclei, but does not affect the distribution of PSP2A-catalytic subunit (Koch *et al.*, 2003). Dephosphorylation of pCREB by PSPs seems to be an essential mechanism for the down-regulation of AA-NAT induction

and melatonin biosynthesis. The decline in pCREB levels is followed by steady increase in ICER protein levels. As a result, the inhibitory effect of ICER overrides the stimulatory influence of pCREB on the *aa-nat* gene transcription, and thereby switches 'off' melatonin synthesis (Stehle *et al.*, 2001). Both AA-NAT mRNA levels and melatonin synthesis are increased drastically after the selective silencing of ICER in rat pinealocytes, probably due to uninhibited transcription of the *aa-nat* gene (Maronde *et al.*, 1999a). The adrenergic induction of the CREM gene and ICER formation is transient because steadily increasing ICER levels attenuate the transcription of cAMP-inducible genes including CREM gene (Foulkes *et al.*, 1996). Thus, ICER seems to repress its own production through a negative autoregulatory mechanism constituting the CREM feedback loop (Coon *et al.*, 2001). A critical analysis of the available information suggests that the balance between the ratio of pCREB (stimulatory TF) and ICER (inhibitory TF) levels determines the transcriptional activity of AA-NAT promoter (Gupta *et al.*, 2005). Due to diurnal shifts in the ratio of pCREB and ICER, the promoter cycles between activated and repressed states as a function of the day-night cycle. ICER levels overwhelm/dominate pCREB levels during the second half of night and switch 'off' the adrenergically stimulated melatonin synthesis.

In addition to ICER, Fos-related antigen 2 (Fra-2) was also supposed to be involved in turning 'off' the melatonin synthesis. While stimulating the transcription of the *aa-nat* gene, the adrenergic signals also simultaneously activate *fra-2* gene via pCREB and stimulate accumulation of Fra-2 mRNA that drives the 100-fold rhythm

in Fra-2 protein. It seems that Fra-2 containing AP-1 complexes bind to AP-1 site at position -32 in the AA-NAT promoter (Baler *et al.*, 1997). The AP-1 binding site is located in close proximity of the major transcriptional start point, therefore, the binding of Fra-2 containing AP-1 complexes to this site was thought to disrupt the assembly of the basic transcription machinery and to have an inhibitory influence on expression of the *aa-nat* gene and melatonin synthesis. However, a recent report has indicated that Fra-2 expression does not have any inhibitory influence on *aa-nat* gene expression (Smith *et al.*, 2001). The switching 'off' mechanism for melatonin synthesis in the pineal of ungulates and primates may not involve transcriptional inhibition by adrenergically controlled inhibitory TFs like ICER. The inhibition of proteasomal proteolysis of AA-NAT is lifted due to declining levels of cAMP and/or pCREB in the later part of night, leading to increased breakdown of AA-NAT protein and switching 'off' of melatonin synthesis.

Adrenergically-induced increase in cAMP and cGMP levels is associated with increased activity of cAMP- and cGMP-dependent phosphodiesterase enzyme (PDE). The increase in the activity of the phosphodiesterase increases hydrolysis of cAMP, and thereby weakens the stimulatory effect of the adrenergic signals on AA-NAT induction and melatonin synthesis. PDE-induced decline in cAMP level decreases PKA activity, which in turn, leads to reduced phosphorylation of CREB and AA-NAT. This cascade leads to reduction in *aa-nat* gene expression and acceleration of dephosphorylation and proteasomal proteolysis of AA-NAT. As mentioned earlier, adrenergically-stimulated cGMP activates the MAPKK-MAPK

pathway. Nocturnal and NE-induced expression of MAP kinase phosphatase-1 (MKP-1) and a possible interaction of MKP-1 with MAP kinases in rat pineal also supports a functional role of cGMP-PKG-MAPKK-MAPK pathway in regulation of melatonin synthesis (Chansard *et al.*, 2005).

During the early hours of adrenergic stimulation, $Ca^{2+}$ facilitates adrenergic stimulation of melatonin synthesis as discussed earlier. However, prolonged adrenergic signaling may result in accumulation of $Ca^{2+}$ levels to critically higher levels, which might be suppressing AA-NAT activity and leading to commensurate inhibition of melatonin synthesis. Whether $Ca^{2+}$, which acts as a key regulator of gene expressions via CREB phosphorylation in other tissues, also influences AA-NAT induction and melatonin synthesis by activating CREM gene in pinealocytes remains to be established.

**Modulation of melatonin synthesis by other hormones in the pineal of mammals**

In addition to adrenergic receptors, the mammalian pinealocytes also possess receptors of a large number of hormones. The presence of receptors of several hormones in pineal indicates that the mammalian pineal is target of a large number of hormones, which may be involved, directly and/or indirectly, in the modulation of AA-NAT activity and melatonin synthesis.

***Androgens***

Receptors for androgens have been reported in the pineal of several vertebrate species (Haldar and Gupta, 1990; Gupta *et al.*, 1993; Luboshitzky *et al.*,

1997a,c). There are several reports on the role of hormones other than NE in the regulation of the pineal physiology and melatonin synthesis. Steroid hormones can act either directly on the pineal cells via intra-cellular receptors or indirectly through sympathetic innervation (Cardinalli, 1975). In human, treatment of testosterone has been reported to decrease melatonin secretion to normal level in male patients with GnRH deficiency having increased nocturnal melatonin secretion (Luboshitzky *et al.*, 1996a). Testosterone treatment has also been reported to normalize abnormal melatonin secretion in hyper and hypo-gonadotropic males (Luboshitzky *et al.*, 1997b, 2000). In mice, testosterone induced decrease in dense core vesicles and increase in fractional volume of lysosomes in pinealocytes, while castration produced opposite effects (Redins *et al.*, 1999). Administration of testosterone to castrated males has been reported to inhibit HIOMT activity in rat pineal (Weiss and Crayton, 1970). Testosterone depressed pineal AA-NAT activity and decreased melatonin content in rats when incubated for up to 6 hours (Rudeen and Reiter, 1980; Cardinali *et al.*, 1987). However, testosterone treatment has been reported to significantly increase melatonin production in rat pineal gland removed during the dark phase but not during the light phase (Martin *et al.*, 1996). Dehydroepiandrosterone-sulphate (DHEA-S) has also been reported to have a direct action on beta-adrenergic-stimulated melatonin release in rat pineal gland leading to increased melatonin secretion in a dose-dependent manner (Martin and Touitou, 2000a).

*Estrogens*

The presence of estrogen receptors has been reported in the pineal glands of rat (Sanchez *et al.*, 2004), sheep (Foldes *et al.*, 1983) and humans (Luboshitzky *et al.*, 1997a). Intravenous administration of a conjugated oestrogen (Premarin 20 mg) has been reported to significantly suppress nocturnal melatonin secretion in women (Okatani and Sagara, 1994). In rats, ovariectomy has been reported to increase melatonin secretion from organ-cultured pineal, and this increase was suppressed by estrogen (Ishizuka *et al.*, 2000). Ovarian steroids have also been reported to block the isoproterenol-induced elevation of pineal melatonin production in female rats (Moujir *et al.*, 1990). Administration of estradiol benzoate reduced the activity of both AA-NAT and HIOMT in rat pineal (Okatani *et al.*, 1998a,b,c; Hayashi and Okatani, 1999). Estradiol has also been reported to inhibit norepinephrine-induced hyperpolarization of pinealocyte cell membrane in rats (Sakai and Marks, 1972). Systemic administration of estrogen decreased pineal weight, cell organelles in pinealocyte and synthetic activity in the rat pineal (Clementi *et al.*, 1965; Cardinali *et al.*, 1975). Incubation of pineals with 17β-estradiol also significantly decreased the number of pineal synaptic ribbons in rats (Saidapur *et al.*, 1991). It has been reported that ovarian steroids regulate interaction between $\alpha_1$- and β-adrenergic receptors in the pineal of female rats (Alonso *et al.*, 1995). Direct exposure of 17β-estradiol was also reported to reduce alpha-1/beta-adrenoceptor-induced stimulation of melatonin synthesis and release in rat pineal (Hernandez-Diaz *et al.*, 2001). Treatment with low dose of 17β-estradiol has been reported to reduce cAMP and AA-NAT activity in the pineal

of female rats (Sanchez *et al.*, 2004). Subcutaneous implantation of 17β-estradiol reportedly decreased AA-NAT activity and melatonin synthesis significantly in the rat pineal (Okatani *et al.*, 1997,1999). However, incubation of the rat pineal in physiological levels (1-15 nM) of estradiol was reported to increase HIOMT activity and melatonin production in a dose-dependent manner (Nagle *et al.*, 1972; Mizobe and Kurokawa, 1976). 17β-estradiol was also reported to increase melatonin release from perifused pineal gland in rats (Martin *et al.*, 1996). Exposure for 10 minutes to physiological amounts of estradiol (10 nM) significantly increased cAMP levels in the pineal gland of guinea pig (Cardinali *et al.*, 1986). Further, estradiol has also been reported to increase pineal nuclear receptor levels and to enhance conversion of serotonin to melatonin in rats (Cardinali, 1977).

***Progesterone***

The presence of progesterone receptor has been reported in the pineal gland of rats (Hanukoglu *et al.*, 1997). In ovariectomized rats, progesterone has been reported to decrease pineal HIOMT activity (Houssay and Barcelo, 1972) and serum melatonin levels (Ozaki *et al.*, 1978). Incubation of rat pineal glands with progesterone (10μg/ml) reportedly decreased the stimulatory effects of isoproterenol and inhibited melatonin synthesis and secretion (Wilkinson and Arendt, 1978). Progesterone treatment also decreased melatonin secretion on rat pineal (Martin and Touitou, 2000b). LHRH has been reported to inhibit protein secretion in mouse pinealocyte (Haldar-Misra and Pevet, 1983). Luteinizing hormone increased pineal AA-NAT activity and melatonin synthesis in rats (Hosaka *et al.*, 2002).

***Thyroid hormones***

Receptors for thyroid hormone have been reported to be expressed in the mammalian retinal cells (Ng *et al.*, 2001). But so far, there has been no report on the presence of thyroid hormone receptor in the pineal of any vertebrate species. However, the role of thyroid hormones in the modulation of melatonin synthesis has been reported in mammals. Thyroxine has been reported to inhibit AA-NAT activity and plasma melatonin level in rats and Syrian hamster (Champney *et al.*, 1985a). Incubation with $T_3$ for 6 hr was reported to increase melatonin level during light phase and to decrease melatonin level during dark phase in rat pineal (Catala *et al.*, 1988). In humans, melatonin concentrations were found to be positively correlated with TSH levels in hypo-thyroidism and negatively correlated with $T_3$ in hyper-thyroidism (Soszynski *et al.*, 1988). The presence of type 2 deiodinase (D2) which catalyzes the conversion of 5'-deiodination of thyroxine to form 3,5,3'-triiodothyronine have been reported in the mammalian pineal and exhibits a maximum value during the night (Rubio *et al.*, 1991; Guerrero and Reiter, 1992; Murakami *et al.*, 1997; Buettner *et al.*, 1998). The expression of the enzyme deiodinase II (DII) has been reported to be under adrenergic control (Guerrero *et al.*, 1988; Osuna *et al.*, 1993). In cultured rat pineal, type II iodothyronine deiodinase mRNA and deiodinase activity was stimulated following isoproterenol treatment while treatment with propanalol significantly supressed the enzyme activity (Murakami *et al.*, 1989; Rubio *et al.*, 1991; Kamiya *et al.*, 1999). The nocturnal increase of 5'D-II activity was reported to produce an increase of $T_3$ concentration

and decrease of $T_4$ concentration in the rat pineal (Soutto *et al.*, 1998). $T_3$ has been reported to inactivate the deiodinase II (DII) enzyme by stimulating proteasomal proteolysis in pituitary cells (Steinsapir *et al.*, 1998, 2000). Stimulation of proteasomal proteolysis reportedly inhibits AA-NAT activity in the mammalian pineal (Gastel *et al.*, 1998; Stehle *et al.*, 2001; Gupta *et al.*, 2005).

***Corticosteroids***

The presence of glucocorticoid receptor has been reported in pineal gland of several vertebrate species (Meyer *et al.*, 1998; Sarrieau *et al.*, 1998). In rats, high concentration of corticosterone (0.8 x 10(-3) mol/l) has been reported to inhibit pineal AA-NAT activity and melatonin production (Zhao and Touitou, 1993). Intra-peritoneal injection of corticosterone inhibited pineal AA-NAT activity and melatonin content in rat pineal (Troiani *et al.*, 1988). Further, incubation of rat pineal with high doses of corticosterone (10-100μM) inhibited melatonin production (Yuwiler, 1989). Incubation of rat pineal with corticosterone for 48 hr has been reported to potentiate the rise of noradrenaline-induced melatonin production (Ferreira *et al.*, 2005). However, in adrenalectomized rat and Syrian hamster, corticosteroid implant had no significant effect on the pineal AA-NAT activity and melatonin content (Champney *et al.*, 1985b). Administration of hydrocortisone acetate has been reported to decrease daytime serotonin-N-acetyltransferase activity in rat pineal (Yuwiler and Brammer, 1981; Yuwiler, 1985). In humans, treatment with a glucocorticoid, prednisone (8mg /day) has been reported to suppress melatonin rhythm in asthma patients (Kos-Kudla *et al.*, 1997). Administration of

dexamethasone and corticotropin releasing hormone (CRH) has been reported to inhibit pineal melatonin secretion in men (Demisch *et al.*, 1988; Kellner *et al.*, 1997). Similarly, treatment with dexamethasone (0.4X 10(-3) mol/l) has also been reported to inhibit melatonin production in the rat pineal (Zhao and Touitou, 1993).

***Indoleamines***

Receptors for various indoleamines have been reported in the brain and pineal gland of mammals (Govitrapong *et al.*, 1991; Olcese and Munker, 1994; Sterado *et al.*, 2000; Uz *et al.*, 2005). Melatonin has been reported to exert regulatory actions within the pineal gland itself, and influences secretory activity of pinealocytes. In rats, melatonin has been reported to entrain directly the circadian pacemaker controlling the N- acetyltransferase rhythm (Illnerova *et al.*, 1989; Humlova and Illnerova, 1990). Melatonin acts directly on the pinealocyte of rats, and increases activity of both HIOMT and AA-NAT in a dose-dependent manner (Freire and Cardinali, 1975). *In vitro* treatment with melatonin has been reported to increase the concentration of 5- hydroxy-tryptamine (5-HT) from $10^{-6}$ M to $10^{-3}$ M in the rat pineal (Miguez *et al.*, 1995). Subcutaneous injection of melatonin (1 mg/kg) increased pineal melatonin production in rats (Bothorel *et al.*, 2002). However, melatonin injection, depending on the time of administration, inhibited the daily rhythms of serotonin in the rat pineal (Fiske and Huppert, 1978). In mice, treatment with 100mg of melatonin has been reported to decrease the number and volumetric density of lysosomes in the pineal gland (Redins *et al.*, 2001). Daily subcutaneous injection of melatonin (20μg in 1ml/animal) was also reported to reduce serotonin

content in the rat pineal (Catala *et al.*, 1984). Intra-peritoneal (i.p) injection of melatonin (150μg/kg) decreased 5-HT level in the rat pineal (Miguez *et al.*, 1996). In cultured rat pinealocytes, addition of 5-HT to the culture medium has been reported to increase AA-NAT activity (Sudgen, 1990). Further, i*n vivo* treatment with 5-HT enhanced pineal AA-NAT activity and melatonin synthesis in the rat pineal (Sterado *et al*, 2000). Extracellular serotonin has also been reported to promote melatonin secretion following adrenergic stimulation (Olcese and Munker, 1994). However, incubation of pinealocytes with high concentration of 5-HT was found to inhibit melatonin synthesis in the rat pineal (Miguez *et al.*, 1997).

**Regulation of melatonin synthesis in birds**

The general mechanism of biosynthesis of melatonin in birds and mammals appears to be similar. As in mammals, the rhythmic changes in melatonin content of the avian pineal gland has been reported to be regulated primarily by changes in activity of the enzyme AA-NAT (Klein and Weller, 1970; Binkley *et al.*, 1973). In the pineal of birds, AA-NAT and melatonin have been reported to exhibit a circadian rhythm with high levels during the night and low levels during the day (Siguenza *et al.*, 1988; Rudeen *et al.*, 1990; Kato *et al.*, 1999; Natesan *et al.*, 2002). However, the mechanism controlling the nighttime increase and the daytime decrease in AA-NAT activity has been reported to be regulated differently in mammals and birds (Binkley *et al.*, 1981). In mammalian pineal, the rhythm in melatonin synthesis is entirely driven by the SCN, which acts as a circadian oscillator. However, the bird pineal has been reported to retain a photoreceptive capability and the gland is photosensory, and

possess a photic input pathway for melatonin release (Underwood *et al.*, 2001; Natesan *et al.*, 2002). It has been found that the daily changes in AA-NAT activity and melatonin content in the avian pineal gland are manifestations of true circadian rhythms (Binkley *et al.*, 1973). The avian pineal contains both circadian oscillator as well as pacemaker to drive circadian rhythm in the biosynthesis of melatonin and photoreceptors to synchronize the rhythm to environmental lighting (Deguchi, 1979c; Hamm *et al.*, 1983). It has been reported that the circadian pacemaker of avian pineal gland oscillates both *in vivo* and *in vitro*, where light exposure suppresses the night-time increase in AA-NAT activity (Wainwright, 1980; Deguchi, 1981; Hamm *et al.*, 1983). When placed in organ and/or cell culture, the avian pineal express at least four circadian cycles of melatonin biosynthesis in the dark or dim red light (Takahashi *et al.*, 1980; Zatz *et al.*, 1988). Further, the chick pineal *in vitro* responds to environmental light in three ways: phase-shift of the circadian cycle, acute suppression of the melatonin biosynthetic release and increase in amplitude (Zatz *et al.*, 1988).

In birds, both direct photoreception and neurotransmitters have been reported to affect AA-NAT activity and melatonin synthesis (Binkley *et al.*, 1983; Cassone *et al.*, 1983). In contrast to mammalian pineal, where NE stimulates melatonin biosynthesis via elevated cyclic AMP levels (Klein *et al.*, 1987), the avian pineal shows an inhibition of the melatonin synthetic pathway following norepinephrine treatment (Binkley, 1976a,b; Wainwright and Wainwright, 1978; Deguchi, 1979b). The different responses to NE of the mammalian and chicken pineal can be readily

attributed to different types of adrenergic receptors and G-proteins involved in NE-induced signal transduction. In mammalian pineal, NE acts via α1- and β1-adrenergic receptors coupled to stimulatory G-proteins (Gs) and stimulates adenylate cyclase resulting in increased AA-NAT activity and melatonin synthesis (Gupta *et al.*, 2005). In the avian pineal, however, NE acts via α2-adrenergic receptors coupled to inhibitory G-proteins (Gi) and inhibits adenylate cyclase activity resulting in inhibition of AA-NAT activity and melatonin synthesis (Voisin and Collin, 1986; Pratt and Takahashi, 1987; Zatz and Mullen, 1988).

The avian pineal clock generates a rhythm in the abundance of *aa-nat* mRNA that can account for the free running rhythm in AA-NAT activity (Takahashi *et al.*, 1980; Bernard *et al.*, 1997). In avian pineal, the transcriptional regulation of the *aa-nat* gene is mediated by the clock genes. Changes in *aa-nat* expression are mediated by the E-Boxes that have been found in the promoter region of the *aa-nat* gene (Natesan *et al.*, 2002). It has been reported that the clock-dependent nocturnal increase in *aa-nat* mRNA requires gene expression for the synthesis of melatonin (Bernard *et al.*, 1997; Natesan *et al.*, 2002). In mammals, both transcriptional activation and protection of AA-NAT protein against proteasomal proteolysis are provided by NE-induced cAMP formation. However, the transcriptional regulation of the *aa-nat* gene in chick pineal is mediated primarily by the clock genes, whereas protection of AA-NAT activity from proteosomal degradation is mediated by cAMP (Gastel *et al.*, 1998; Ganguly *et al.*, 2002; Natesan *et al.*, 2002).

**Regulation of melatonin synthesis in reptiles and amphibians**

As in mammals and birds, daily and seasonal variations in AA-NAT activity and melatonin content have been reported in pineal organ of both reptiles and amphibians, with the maximum value occurring during the night (Serino *et al.*, 1993; Tosini and Menaker, 1996; Lutterschmidt *et al.*, 2002; Chiba *et al.*, 2005). However, unlike in mammals and birds, there is paucity of information on the mechanism of regulation of melatonin synthesis in reptiles and birds. Photoperiod and temperature seem to play an important role in the regulation of AA-NAT activity and melatonin production in pineals of reptiles and amphibians (Quay *et al.*, 1971; Underwood and Calaban, 1987; Tilden and Hutchinson, 1993; d'Istria *et al.*, 1994; Moyer *et al.*, 1997). Pinealectomy lead to abolition of the circadian rhythm of plasma melatonin in the ruin lizard, *Podarcis sicula* (Foa *et al.*, 1992). It has been reported that both light and temperature are important modulators of pineal function in gecko (Moyer *et al.*, 1997). The pineal organ of reptiles can act as a photo- and thermo- transducer, which translates information on light and temperature into an internal cue in the form of pineal melatonin rhythm (Underwood and Calaban, 1987; Hyde and Underwood, 2000). Pinealectomy was reported to affect plasma melatonin level in the neotenic tiger salamander (Gern and Norris, 1979). In *Rana perezi*, increase in environmental photoperiod and temperature induced a day-night rhythm of plasma melatonin levels, while a decrease in environmental temperature abolished the melatonin rhythm (Delgado and Vivien-Roels, 1989). In the frog, *Rana tigrina*, the retino-pineal gland pathway appears to produce light-induced changes in pineal glands of frogs 1-month-

old or older, but this pathway only functions in 1-month-old frogs, and does not appear to function in 6-month-old frogs (Lee *et al.*, 1997).

## Modulation of melatonin synthesis in non-mammalian pineal by other hormones

### *Androgen and estrogens*

Unlike in mammals, there is paucity of information on the role of hormones in the regulation of the pineal physiology and AA-NAT activity and/or melatonin synthesis in other vertebrates. Administration of testosterone has been reported to exert inhibitory influence on the activity of pineal in roseringed parakeets, *Psittacula krameri* (Maitra and Dey, 1994). Administration of testosterone and estradiol in the culture medium has been reported to significantly decrease melatonin in quail pineal content within 24 hours following the steroid treatment (Haldar *et al.*, 2003). However, gonadal steroids had no significant effect on pineal AA-NAT activity in quails (Preslock, 1976). Receptors of estradiol-17β have been reported to be expressed in the in fish pineal (Begay *et al.*, 1994). Treatment with low concentrations of estradiol-17β has been reported to inhibit melatonin release, while high concentrations were found to be stimulatory in the trout pineal (Begay *et al.*, 1993).

### *Corticosteroids*

The presence of glucocorticoid receptor has been reported in the brains of chinook salmon, *Oncorhynchus tshawytscha* (Knoebl *et al.*, 1996), pineal gland of

trout (Benyassi *et al.*, 2001) as well as in the retina of chicken (Zawilska and Sadowska, 2002) and salamander (Psarra *et al.*, 2003). Prolonged treatment with dexamethasone (a glucocorticoid) has been reported to suppress melatonin production in chick pineal gland (Zawilska and Sadowska, 2002). It has also been reported that dexamethasone, after 6 hours of culture in the dark, inhibited pineal arylalkylamine N- acetyltransferase in trout pineal in a dose-dependent manner (Benyassi *et al.*, 2001).

***Indoleamines***

Receptors for various indoleamine have also been reported to be present in the retina of Xenopus (Wiechman *et al.*, 2003), chick (Natesan and Cassone, 2002) and fish (Hensley and Cohen, 1992). Administration of melatonin and 5-methoxytryptamine has been reported to increase the weight of pineal gland during different phases of the breeding cycle in the water snake, *Natrix piscator* (Haldar and Pandey, 1988). In Japanese newt, daily injections of melatonin have been reported to entrain the pineal circadian clock responsible for the circadian change in synaptic ribbon (SR) number (Kikuchi *et al.*, 2000). Implantation of melatonin into female Coturnix has been reported to decrease activity of both HIOMT and AA-NAT (Preslock, 1976). In fish, the presence of various indoleamines has been reported in the pineal gland of *Lampetra planeri* (Meineil and Hartwig, 1980; Guerlotte *et al.*, 1986) and trout (Yanez and Meissl, 1996; Ceinos *et al.*, 2005). However, there is practically no information on the role of indoleamines in the regulation of pineal AA-NAT activity in any fish species.

***Thyroid hormones***

There is scarcity of information on effects of thyroid hormones on AA-NAT activity and melatonin synthesis in non-mammalian vertebrates. Injection of $T_4$ has been reported to decrease plasma levels of melatonin in *Rana catesbeiana* (Wright and Alves, 2001). Thyroxine ($T_4$) administration caused a reduction in nighttime plasma melatonin level in Atlantic salmon, *Salmo salar* (Kulczykowska *et al.*, 2004). It has been reported that L-$T_3$ produced inhibitory effects on pineal AA-NAT activity, while L-$T_4$ showed no significant effect on the enzyme in *Clarias gariepinus* (Premabati, 2005; Gupta and Premabati, unpublished). Further, immersion of the fish in water containing propylthiouracil (PTU) has also been reported to significantly decrease pineal AA-NAT activity during winter in *Clarias gariepinus* (Premabati, 2005; Gupta and Premabati, unpublished).

***Leptin***

In mammals, the presence of leptin receptor has been reported in the brain (Couce *et al.*, 1997; Elmquist *et al.*, 1998), pituitary (Smolinska *et al.*, 2004) as well as in the pineal gland (Chelikani *et al.*, 2003). The genes for both leptin and leptin receptor have been cloned and sequenced from the mouse, rat and humans (Zhang *et al.*, 1994). The evidence for leptin expression have been reported in fish. Western blot analysis revealed that leptin is expressed in tissues of different species of fishes in the blood, brain, heart and liver and it has been found that the peptide structure of the fish leptin is similar to that of mammalian leptin (Johnson *et al.*, 2000). There are several reports on the effects of leptin on the fish physiology. Administration of

leptin for 10 days has been reported to reduce food intake, body weight gain and specific growth rate in gold fish (Volkoff *et al.*, 2003; de Pedro *et al.*, 2006). *In vitro* treatment of leptin reportedly stimulated thyrotropin mRNA levels in a dose-dependent manner in the pituitary of carp (Chowdhury *et al.*, 2004). Murine leptin injections have been reported to increase intracellular fatty acid binding protein in green sunfish, *Lepomis cyanellus* (Londraville and Duvall, 2002). High concentration of leptin (0.5 and $1x10^{-6}$ M) was also reported to stimulate FSH and LH release in rainbow trout (Weil *et al.*, 2003). However, long-term peripheral treatment with human leptin had no physiological effect in Coho salmon (Baker *et al.*, 2000). But, so far there is practically no information on effects of leptin on pineal physiology in ectotherms in general and on AA-NAT activity and/or melatonin synthesis in fishes in particular.

**Regulation of melatonin synthesis in fish**

The melatonin biosynthesis pathway and its molecular components in fishes are similar to other vertebrates (Vollrath, 1981; Kroeber *et al.*, 2000). In mammalian pinealocytes, the diurnal rhythm in AA-NAT expression is controlled by neural signals originating from the suprachaismatic nucleus of the hypothalamus which acts as 'Zeitgeber" (Foulkes *et al.*, 1997). However, unlike mammalian pineal, the fish pineal contains a complete melatonin rhythm generating system, incorporating a photodetector, a circadian clock and melatonin synthesis machinery (Bolliet *et al.*, 1997; Falcon *et al.*, 2001; Vuilleumier *et al.*, 2006). The fish pineal reportedly contains precursors of melatonin like 5- hydroxytryptophan and 5-

hydroxytryptamine (Kroeber *et al.*, 1998). In several species of fishes, the pineal has been reported to secrete melatonin as well as intermediate compounds (like 5-methoxytryptophol, 5- methoxyindoleacetic acid and 5- methoxy-tryptamine) of melatonin biosynthetic pathway (Falcon *et al.*, 1989; Max and Menaker, 1992). In fishes also AA-NAT is the rate-limiting enzyme in the melatonin biosynthesis pathway (Begay *et al.*, 1998; Coon *et al.*, 1999; Benyassi *et al.*, 2000; Falcon *et al.*, 2001). The rhythm in AA-NAT activity in fishes, as in most vertebrates, is driven by circadian clocks, and the photoperiod resets and entrains the clocks (Kezuka *et al.*, 1988; Coon *et al.*, 1999). The circadian rhythm is initiated by minimal photic cues, which starts the circadian clock in the pineal gland (Vuilleumier *et al.*, 2006). The presence of AA-NAT activity has been reported in a number of piscine species (Morton and Forbes, 1988; Benyassi *et al.*, 2001). Although a single AA-NAT gene has been found in mammals, two *aa-nat* genes are expressed in fishes, which are designated as *aa-nat-1* and *aa-nat-2* (Coon *et al.*, 1999; Mizusawa *et al.*, 2000). In pike, it has been reported that AA-NAT-1 is exclusively expressed in the retina and AA-NAT-2 in the pineal gland (Coon *et al.*, 1999; Gothilf *et al.*, 1999). Further, NAT gene activity, AA-NAT activity and melatonin synthesis increase during darkness, and light inhibits AA-NAT activity and melatonin synthesis (Begay *et al.*, 1998; Coon *et al.*, 1999; Falcon *et al.*, 2001). In the rat pineal, light acts independently via downstream mechanism to turn off AA-NAT activity by initiating proteasomal proteolysis of AA-NAT protein (Gastel *et al.*, 1998). Similar proteasome-based mechanisms may function widely as selective molecular switches

in vertebrate neural systems. In mammalian pineal, NE stimulates β-adrenergic receptors while α-adrenergic receptor potentiates the β-adrenergic receptor for the formation of cAMP (Chik and Ho, 1989). However, in fish pineal, the activation of β-adrenergic receptor has been reported to increase cAMP formation and NAT induction, while activation of α-adrenergic receptors inhibit AA-NAT activity (Falcon *et al.*, 1991; Thibault *et al.*, 1993; Gupta and Khamtilin, unpublished).

In the fish pineal, the nocturnal rise in melatonin synthesis is associated with an increase in cAMP production and $Ca^{++}$ entry through voltage gated channels, and light inhibits melatonin synthesis by decreasing cAMP content and closure of $Ca^{++}$ channels (Falcon and Gaildrait, 1997). It is noteworthy that the cAMP content of pineal is increased during darkness and decreased in the presence of light, and the diurnal rhythm of cAMP in the fish pineal is influenced by the light-dark cycle (Falcon and Gaildrat, 1997). In static organ culture, cAMP levels have been reported to be increased following treatment with forskolin (an activator of stimulatory G-protein) as well as by isobutylmethylxanthine and theophylline (inhibitors of phosphodiesterases) (Thibault *et al.*, 1993). In the rat pineal, the increased concentration of cAMP leads to phosphorylati0on of cAMP response element binding protein (CREB) and formation of phospho-CREB (pCREB) (Foulkes *et al.*, 1997; Maronde *et al.*, 1999b). Then, pCREB activates the NAT gene followed by the formation of aa-*nat* mRNA and AA-NAT protein. Further, elevation of cAMP by 8-Bromo-cAMP- forskolin and 3-isobutyl-1 methylxanthine has been reported to increase the level of pCREB and melatonin in light or dark-adapted pineal of both rat

and trout (Kroeber *et al.*, 2000). It has been reported that in pike pineal, light induced decrease in AA-NAT 2 protein in photoreceptor cells and light-induced decrease was blocked by inhibitors of the proteosomal proteolysis (Falcon *et al.*, 2001). Further, if the pike pineal glands were maintained under light at night, the treatment with these inhibitors increased AA-NAT 2 protein and activity (Falcon *et al.*, 2001). These findings suggest that proteosomal proteolysis is a conserved element in the regulation of AA-NAT activity in the vertebrate pineal.

In addition to cAMP, calcium has also been reported to play a crucial role in regulation of NAT activity and melatonin synthesis in the fish pineal (Begay *et al.*, 1994; Gasser and Gern, 1997). The voltage-gated L-type calcium channels seem to play an important role in the regulation of calcium in both oscillating and non-oscillating fish pineal photoreceptor cells (Korf *et al.*, 1997). Dark-induced melatonin synthesis is reportedly inhibited following calcium depletion by antagonists of L-type and N-type voltage sensitive calcium channels (Gasser and Gern, 1997). Further, calcium channel antagonist-induced inhibition of dark-induced melatonin synthesis was abolished following treatment with dibutyryl cAMP. This suggests that $Ca^{++}$ acts upstream of cAMP in regulating melatonin synthesis the fish pineal. $Ca^{++}$ also seems to regulate fish pineal cAMP content via calciproteins. It has been reported that $Ca^{++}$ and/or $Ca^{++}$-calciprotein complexes might be acting in fishes at two different sites, one involving regulation of cAMP metabolism and the other being independent of cAMP (Begay *et al.*, 1994; Falcon, 1999).

It, thus, seems that the melatonin synthesis is regulated by a complex mechanism involving cAMP dependent and cAMP independent pathways in photoreceptive fish pineal. In darkness, the influx of calcium seems to switch 'ON' the melatonin synthesis. $Ca^{++}$-calciproteins complexes might be involved in the activation of adenylyl cyclase and increased formation of cAMP in photoreceptor cells of fish pineal. Then increased cAMP concentration might be responsible for activation of protein kinase A (PKA) and phosphorylation of CREB to pCREB. Then pCREB may stimulate NAT gene to form *nat* mRNA followed by formation of NAT protein. In addition, cAMP might also be protecting AA-NAT from its proteosomal degradation during the dark phase (Thibault *et al.*, 1993; Falcon *et al.*, 2001). Therefore, the melatonin synthesis in fish pineal photoreceptors is increased during the dark phase due to increased formation and decreased degradation of NAT protein. Melatonin synthesis in fish pineal seems to be switched 'OFF' by light due to decrease in $Ca^{++}$ followed by increased activity of phosphodiesterases, decrease in cAMP and increased proteasomal proteolysis.

A critical review of the preceding information indicates that, unlike in mammalian and avian pineal, there is lack of information on the role of various hormones in regulation of AA-NAT activity and/or melatonin synthesis. In general, studies based on *in vivo* administration of hormones on selected physiological parameter(s) do not provide information on whether the observed effect is direct or indirect. However, one can explore direct effects of hormones on any physiological parameters using well-defined *in vitro* experimental protocols. Therefore, keeping in

view the scarcity of information on the role of gonadal steroids, corticosteroids, indoleamines and leptin in the regulation of AA-NAT activity in the fish pineal, it was thought worthwhile to undertake a comprehensive study on *in vitro* effects of these hormones on the activity of the rate-limiting enzyme, AA-NAT in the photoreceptive pineal of an air-breathing catfish, *Clarias gariepinus* during different phases of its breeding cycle.

# CHAPTER - 1
# MATERIALS AND METHODS

## INTRODUCTION

Fishes are aquatic vertebrates that differ in many aspects of habits and habitats as compared to the terrestrial vertebrates. During the course of evolution, they developed a special mechanism by which they could extract dissolved oxygen from water through their gills. Air breathing in the fish is a common phenomenon, which can be found among a wide range of freshwater as well as marine species. The freshwater air breathing fishes supplement their aquatic respiration primarily by utilizing atmospheric oxygen ($O_2$) and release carbondioxide ($CO_2$) aquatically (Hazel, 1992).

All the experiments of this Ph. D. dissertation were conducted on the air-breathing fish, *Clarias gariepinus* due to its easy availability, excellent survival under laboratory conditions and reasonable cost throughout the year at Shillong. A brief introduction of the fish, techniques used for the measurement of AA-NAT activity and the biostatistical methods used for the analysis of the data is presented in the following sections.

### *Clarias gariepinus*

*Clarias gariepinus* is a teleost, which is widely distributed all over India and other tropical countries. In nature, it usually lives in shallow rivers, ponds and muddy places, and survives even in water with low oxygen content. It exhibits a bimodal

(aquatic and atmospheric) breathing habit, and often comes to the surface of water to engulf atmospheric air. *Clarias gariepinus* breeds during monsoon (Premabati, 2005). The gonadal activity undergoes a cyclic change (both in morphology and histology) with the change in season so that spawning takes place in the most propitious time of the year ensuring maximum survival and faster growth of the young ones. At Shillong, gonadal activity remains minimum (quiescent phase) during the months of January and February, which increases gradually during the months of March to May (progressive phase). Breeding occurs from June to August (breeding phase). Then the gonads undergo regression during the months of November and December (regressive phase). This cyclic change in the gonadal activity is possibly cued by the external factors (e.g. water temperature, photoperiod etc.).

For this dissertation, adult male *Clarias gariepinus* (body weight: 90-100g; body length: 23-27 cm) were purchased from the local fish suppliers. Fishes were maintained in plastic tubs and acclimatized at least for 15 days before starting any experiment in the laboratory under natural climatic conditions at Shillong (Latitude 25°.30′ N, Longitude 91°.52′ E; Altitude 1450 ASL; Minimum water temperature: 4° C; maximum water temperature: 24.5° C). During acclimatization, the fishes were fed daily with minced earthworms and commercial fish food *ad libitum*. Water was changed every day to avoid infections.

**Chemicals**

All fine chemicals including hormones used in the experiments were purchased from Sigma-Aldrich, USA. $^{14}$C-acetyl coenzyme A was purchased from Amersham Pharmacia Biotech (U. K).

**Collection of pineals**

It has been found in our laboratory that the AA-NAT activity in the photoreceptive pineal of *Clarias gariepinus* does not respond to stimulatory adrenergic agonists (e.g., norepinephrine, isoproterenol etc.) during the daytime (photophase), therefore all the *in vitro* studies were conducted on the pineals collected immediately after the sunset. In order to collect pineal, the fish was decapitated under dim red light and the pineal window was quickly exposed with the help of a sterilized surgical blade. The pineals were rapidly removed and placed in the culture medium in a well of multi-well culture plate (Corning Cell Wells, New York, USA).

**Pineal organ culture**

Dulbeco's Modified Eagles Medium (DMEM) supplemented with Bovine Serum Albumin (BSA), Calcium carbonate ($CaCo_3$), Ascorbic acid and Penicillin-Streptomycin was used for the pineal culture using multi-well culture plate. After pre-incubation for one hour, the medium was removed and replaced with medium containing desired concentration of the hormones. The pineals were incubated for 6 hours at $25^0$ C in an atmosphere of 85% $O_2$, 5% $CO_2$ and 95% relative humidity with

the help of $O_2$-$CO_2$ gas incubator (Heraeus:Cytoperm). Pineals incubated in DMEM without any drugs were treated as control. After incubation, the pineals were removed and placed in numbered Eppendorf tubes, which were immediately frozen in liquid nitrogen for the measurement of AA-NAT activity.

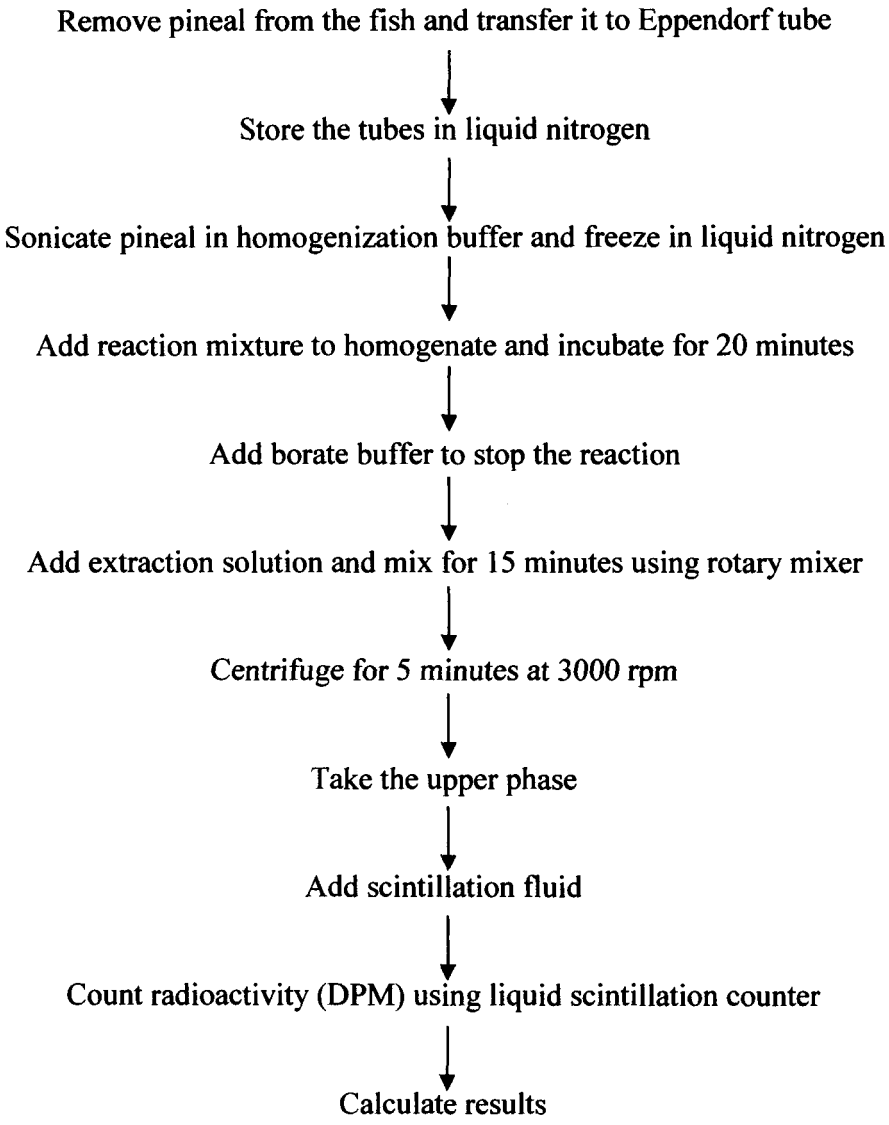
**Measurement of pineal arylalkylamine N-acetyltransferase (AA-NAT) activity**

Arylalkylamine N-acetyltransferase (AA-NAT) activity was measured with the help of radio-enzymatic assay as described by Deguchi and Axelrod (1972) with slight modifications. For the measurement of AA-NAT activity, the pineals were sonicated in 75 µl of homogenization buffer (phosphate buffer: pH 6.5 with 6 nM acetyl Co-enzyme A). The 15 µl samples (in duplicate) of the sonicated pineals were incubated for 20 min at 25° C with 5 µl of the reaction mixture (tryptamine hydrochloride solution, phosphate buffer and $^{14}$C-acetyl Co-A). 100 µl of borate buffer was added to stop the reaction. In order to extract acetylated tryptamine, a mixture of isoamyl alcohol and toluene (3:97) was added to the tubes, and the tubes were rotated in a rotary mixer (Stuart Scientific, U. K) for 15 minutes, and centrifuged at 3000 rpm for 5 minutes. Two ml of the upper phase of the mixture was transferred to a scintillation vial containing 5 ml of scintillation fluid. The radioactivity in each sample was counted in terms of disintegration per minute (DPM) with the help of a liquid scintillation counter (Wallace-1409). Vials (in duplicates) treated as 'Blanks' contained homogenization buffer, reaction mixture, extraction solution, and scintillation fluid only. After calculations, AA-NAT activity was expressed as nmol/pineal/hour.

**Analysis of data**

The Data were analyzed statistically with the help of Student's 't' test and regression analysis (Snedecor, 1961). A $p < 0.05$ was considered as significant.

**Major steps in the measurement of AA-NAT activity**



# CHAPTER - 2

## *In vitro* effects of gonadal steroids and corticosteroids on arylalkylamine N-acetyltransferase (AA-NAT) activity in the fish pineal during different phases of the breeding cycle

### INTRODUCTION

Arylalkylamine N-acetyltransferase (AA-NAT) is the rate-limiting enzyme in the biosynthesis of melatonin. The diurnal rhythm of pineal AA-NAT activity is regulated primarily by adrenergic mechanism, and other hormones have been reported to produce modulatory effects on the enzyme. Steroid hormones are essential constituents of the intercellular communication system that maintains homeostasis in higher organisms and plays a vital role in general adaptations to the ever changing environment. Besides adrenergic receptors in mammalian pineal (Gupta *et al.*, 2005), receptors of gonadal steroids (Luboshitzky *et al.*, 1997a) and glucocorticosteroids (Meyer *et al.*, 1998; Sarrieau *et al.*, 1998) have also been reported to be present in the mammalian pineal. Further, gonadal steroids have been found to modulate the adrenergic receptors in mammalian pineal (Foldes *et al.*, 1983, Sanchez *et al.*, 2004). Testosterone has been reported to inhibit pineal melatonin synthesis in the pineal of rats (Karasek *et al.*, 1978; Cardinali *et al.*, 1987) and humans (Luboshitzky *et al.*, 1996a,b). In castrated male rats, testosterone has been reported to inhibit pineal AA-NAT activity and to decrease melatonin content (Rudeen and Reiter, 1980). 17β-estradiol has been reported to reduce AA-NAT

activity and melatonin synthesis in the rat pineal (Okatani *et al.*, 1998a,b,c, 1999). Corticosteroids have been reported to induce mild induction of AA-NAT in the hepatocytes of the rat (Zaher and Svenson, 1994). However, corticotrophin releasing hormone administration has been reported to inhibit melatonin secretion in healthy volunteers (Kellner *et al.*, 1997). Stress-induced secretion of corticosterone has been reported to inhibit AA-NAT activity and melatonin content in the rat pineal (Troiani *et al.*, 1988). Prolonged treatment with dexamethasone (glucocorticoid) suppressed melatonin production in the chick pineal gland (Zawilska and Sadowska, 2002).

Receptors of glucocorticosteroids and estrogens have also been reported to be present in the pineal and retina of fish species (Begay *et al.*, 1994; Benyassi *et al.*, 2001). In addition to the mammalian pineal gland (Rubio *et al.*, 1993; Price *et al.*, 2004; Gupta *et al.*, 2005), α- and β-adrenergic receptors are also present in the photoreceptor cells of the mammalian retina, which possess AA-NAT and produce melatonin (Berlie *et al.*, 1995; Wikberg-Matsson *et al.*, 1996; Lashbrook and Steinle, 2005). Adrenergic receptors have also been reported to be present in the photoreceptive pineal of birds (Voisin *et al.*, 1987; Bylund *et al.*, 1988; Nowak *et al.*, 1997). In fishes, adrenergic receptors have been found in several parts of the brain of red porgy, *Pagrus pagrus* (Zikopoulos and Dermon, 2005). α-Adrenergic agonists have been reported to inhibit AA-NAT activity, and β-adrenergic agonists to increase AA-NAT activity and melatonin production in the fish pineal (Falcon *et al.*, 1991) suggesting existence of α- and β-adrenergic receptors in the fish pineal. However, unlike in mammalian and avian pineal where adrenergic receptors, AA-

NAT activity and melatonin are modulated by gonadal steroid hormones and corticosteroids (Karasek *et al.*, 1978; Foldes *et al.*, 1983, Cardinali *et al.*, 1987; Luboshitzky *et al.*, 1996a,b; Sanchez *et al.*, 2004), there is practically no information on the role of gonadal steroids or corticosteroids in regulation/modulation of adrenergic receptors or AA-NAT activity and/or melatonin synthesis in the photoreceptive fish pineal. Therefore, keeping in view the scarcity of information, it was thought worthwhile to investigate the effects of various gonadal steroids and corticosteroids on AA-NAT activity in the pineal of an air-breathing catfish, *Clarias gariepinus* during different phases of the annual breeding cycle.

## MATERIALS AND METHODS

All experiments were conducted on adult male *Clarias gariepinus*. Fishes were purchased from the local fish suppliers, and maintained in plastic tubs and acclimatized for at least 15 days in the laboratory under natural climatic conditions. During acclimatization, the fishes were fed daily with minced earthworms and commercial fish food *ad libitum*. Water was changed everyday to avoid infections.

In order to collect pineal, the fishes were decapitated in complete darkness under dim red light and the pineals were rapidly removed, washed in culture medium (DMEM) and placed in the culture medium in the wells of multi-well culture plate (Corning Cell Wells, New York, USA) for organ culture and treatment with the hormones.

The experimental protocol for the proposed study is given below:

**Experimental protocol**

| Experiment | Hormones | Concentration of hormones | Time of Experiment |
|---|---|---|---|
| *In vitro* effects of gonadal steroids and corticosteroids on AA-NAT activity | Testosterone<br>17-β estradiol<br>Estriol<br>Estrone<br>Corticosterone<br>Cortisol | $10^{-6}$ M, $10^{-5}$ M and $10^{-4}$ M of each hormone | Quiescent (February), Progressive (April), Breeding (August) and Regressive (November) phases |

**Pineal organ culture**

The pineals were pre-incubated for one hour, after which the medium was removed and replaced with medium containing desired concentration of the hormones. The pineals were then incubated for 6 hours at $25^0$ C in an atmosphere of 85% $O_2$, 5% $CO_2$ and 95% relative humidity with the help of $O_2$-$CO_2$ gas incubator (Heraeus: Cytoperm, Germany). Pineals incubated in DMEM without any drugs served as control. At the end of incubation period, the pineals were removed and placed in numbered Eppendorf tubes, which were immediately frozen in liquid nitrogen for the measurement of AA-NAT activity.

**Measurement of pineal arylalkylamine N-acetyltransferase (AA-NAT) activity**

Arylalkylamine N-acetyltransferase (AA-NAT) activity was measured with the help of radio-enzymatic assay as described by Deguchi and Axelrod (1972) with slight modifications. For the measurement of AA-NAT activity, the pineals were sonicated in 75 µl of homogenization buffer and 15 µl samples (in duplicate) of the sonicated pineals were incubated for 20 min at 25° C with 5 µl of the reaction mixture. 100 µl of borate buffer was added to stop the reaction. In order to extract acetylated tryptamine, extraction solution was added to the tubes and rotated in a rotary mixer (Stuart Scientific, U. K) for 15 minutes, and centrifuged at 3000 rpm for 5 minutes. Two ml of the upper phase of the mixture was transferred to a scintillation vial containing 5 ml of scintillation fluid, and the radioactivity in each sample was counted with the help of a liquid scintillation counter (Wallace-1409). After calculations, AA-NAT activity was expressed as nmol/pineal/hour. The data were analyzed statistically with the help of Student's 't' test and regression analysis. A $p < 0.05$ was considered as significant.

**RESULTS**

**1. *In vitro* effects of testosterone (an androgen) on AA-NAT activity**

The data are presented in Table 2:1; Fig. 2:1. During the progressive, breeding and regressive phases, all the three concentrations ($10^{-6}$ M, $10^{-5}$ M and $10^{-4}$ M) of testosterone significantly decreased AA-NAT activity. However, during the quiescent phase, *in vitro* treatment with both $10^{-5}$ M and $10^{-4}$ M testosterone solution significantly decreased the enzyme activity, while $10^{-6}$ M testosterone solution had

no significant effect on pineal AA-NAT activity. Regression analysis of the data indicated strong negative correlation between the doses of testosterone and pineal AA-NAT activity during all four phases of the breeding cycle of the fish.

**2. *In vitro* effects of estrogens on AA-NAT activity**

***Effects of 17- β estradiol***

The data are presented in Table 2:2; Fig. 2:2A. During the quiescent phase, while *in vitro* treatment with $10^{-6}$ M and $10^{-5}$ M 17-β estradiol solution had no significant effect on pineal AA-NAT activity, $10^{-4}$ M estradiol significantly decreased the enzyme activity. However, during the progressive phase, both $10^{-5}$ M and $10^{-4}$ M 17-β estradiol solutions significantly decreased AA-NAT activity, while$10^{-6}$ M solution of the hormone had no significant effect on the enzyme activity. During the breeding and regressive phases, all the three concentrations ($10^{-6}$ M, $10^{-5}$ M and $10^{-4}$ M) of 17-β estradiol significantly inhibited AA-NAT activity. Regression analysis of the data revealed strong negative correlation between the doses of 17-β estradiol and pineal AA-NAT activity during all four phases of the breeding cycle of the fish.

***Effects of estriol***

The data are presented in Table 2:2; Fig. 2:2B. During both the quiescent and the progressive phases, *in vitro* treatment with only $10^{-4}$ M estriol significantly decreased AA-NAT activity, while the other two lower concentrations ($10^{-6}$ M and $10^{-5}$ M estriol) of the hormone had no significant effect on the enzyme activity.

However, during the breeding and the regressive phases, $10^{-5}$ M and $10^{-4}$ M concentrations of estriol significantly decreased AA-NAT activity, while $10^{-6}$ M concentration of the hormone had no significant effect on the enzyme activity. Regression analysis of the data revealed strong negative correlation between the doses of estriol and pineal AA-NAT activity during all four phases of the breeding cycle of the fish.

***Effects of estrone***

The data are presented in Table 2:2; Fig. 2:2C. During the quiescent and the progressive phases, none of the three concentrations of estrone had any significant *in vitro* effect on pineal AA-NAT activity. However, during the breeding phase, only $10^{-4}$ M concentration of the hormone significantly decreased pineal AA-NAT activity, while other two lower concentrations ($10^{-6}$ M and $10^{-5}$ M) of estrone produced no significant effects. During the regressive phase, $10^{-5}$ M and $10^{-4}$ M concentration of estrone significantly decreased pineal AA-NAT activity, while $10^{-6}$ M estrone had no significant effect on the enzyme activity. Regression analysis of the data revealed strong negative correlation between the doses of estrone and pineal AA-NAT activity during all four phases of the breeding cycle of the fish.

**3. *In vitro* effects of corticosteroids on AA-NAT activity**

***Effects of corticosterone***

The data are presented in Table 2:3; Fig. 2:3A. During the quiescent phase, all the three concentrations of corticosterone had no significant *in vitro* effect on

pineal AA-NAT activity. During the progressive, the regressive and the breeding phases, all the three concentrations ($10^{-6}$ M, $10^{-5}$ M and $10^{-4}$ M) of corticosterone significantly decreased pineal AA-NAT activity in a dose-dependent manner. Regression analysis of the data revealed strong negative correlation between the doses of corticosterone and pineal AA-NAT activity during all four phases of the breeding cycle of the fish.

***Effects of cortisol***

The data are presented in Table 2:3; Fig. 2:3B. During the regressive and the quiescent phases, *in vitro* treatment of the fish pineals with $10^{-5}$ M and $10^{-4}$ M cortisol solutions significantly decreased AA-NAT activity, but $10^{-6}$ M concentration of cortisol produced no significant effect on the enzyme activity. However, during the progressive and the breeding phases, all the three concentrations ($10^{-6}$ M, $10^{-5}$ M and $10^{-4}$ M) of cortisol significantly decreased AA-NAT activity in a dose-dependent manner. Regression analysis of the data revealed strong negative correlation between the doses of cortisol and pineal AA-NAT activity during all four phases of the breeding cycle of the fish.

**DISCUSSION**

In the present study, regression analysis indicates that all the gonadal steroids and corticosteroids inhibited pineal AA-NAT activity in a dose-dependent manner during all phases of the breeding cycle of the fish. However, the degree of inhibition of AA-NAT activity (in terms of %) seems to depend on the hormone and

phase/season. Accordingly, AA-NAT activity was comparatively more sensitive to the inhibitory effects of the gonadal hormones (testosterone, 17β-estradiol, estriol and estrone) during the regressive phase and less sensitive during the quiescent phase. However, the enzyme activity was more sensitive to the inhibitory effects of corticosteroid hormones (corticosterone and cortisol) during the breeding phase and less sensitive during the quiescent phase. Further, testosterone and 17β-estradiol were found to be more effective in inhibiting AA-NAT activity as compared to estriol. Estrone significantly inhibited the enzyme activity only during the regressive phase. AA-NAT activity in the fish pineal seems to be equally sensitive to the inhibitory effects of corticosterone and cortisol. These findings seem to suggest that the sensitivity of AA-NAT activity for inhibitory effects of different steroid hormones is different, and changes with the phase of the breeding cycle/seasons. The observed phase/season-dependent differential increase and decrease in the sensitivity of pineal AA-NAT activity to the steroid hormones may play an important role in shaping circannual variations in AA-NAT activity and melatonin synthesis in the fish. Melatonin has been reported to influence gonadal activity in a number of fish species (Urasaki, 1972b,c; de Vlaming *et al.*, 1974; Nayak and Singh, 1987; Garg, 1989; Amano *et al.*, 2004). These reports and present findings, when considered together, indicate that testis and pineal interact with each other through testosterone and melatonin, respectively, and testis-pineal axis might be involved in regulation of circannual events. To the best of our knowledge, the present study seems be the first of its kind in which effects of gonadal hormones and corticosteroids on AA-NAT

activity have been studied in the photoreceptive pineal of a fish during different phases of the breeding cycle.

Melatonin receptors are reportedly present in the gonads of mammals (Valenti *et al.*, 1997; Xi *et al.*, 2000; Soares *et al.*, 2003; Frungieri *et al.*, 2005) and birds (Ayre and Pang, 1994). Melatonin has also been reported to inhibit androgen production in Syrian hamster (Frungieri *et al.*, 2005) and testosterone secretion from rat testis (Valenti *et al.*, 1997; Valenti and Giusti, 2002). Further, receptors for gonadal steroids have been reported in the pineal of mammals (Haldar and Gupta, 1990; Gupta *et al.*, 1993; Luboshitzky *et al.*, 1997a,c). The presence of receptors and aromatizing enzymes in mammalian pineal indicates that the pineal is under direct control of steroid hormones (Haldar and Vidhu, 1997). There are also reports on the inhibitory effects of various gonadal steroids on enzyme(s) of melatonin biosynthesis pathway in the pineals of mammals (Karasek *et al.*, 1978; Rudeen and Reiter, 1980; Cardinali *et al.*, 1987; Luboshitzky *et al.*, 1996a,b; Sanchez *et al.*, 2004), birds (Pablos *et al.*, 1993; Maitra and Dey, 1994) and fishes (Begay *et al.*, 1993). The presence of receptors of gonadal steroids in the pineal of fish species (Begay *et al.*, 1993,1994; Gonzalez-Martinez *et al.*, 2004) and significant inhibition of AA-NAT activity in the fish pineal by the gonadal steroids in the present study suggest that, as in the case of the mammalian pineal, the fish pineal is also directly regulated by the gonadal hormones. Findings of the present study also indicate that the inhibitory effect of gonadal steroid hormones on pineal AA-NAT activity has been conserved during the course of evolution.

In the present study, the inhibitory effects of the gonadal steroid hormones might be due to decrease in cAMP-dependent induction of *aa-nat* gene leading to inhibition of aa-nat mRNA, and AA-NAT protein. It is important to mention that estrogen receptor alpha (ERα) is expressed in the fish pineal (Forlano *et al.*, 2005). Further, the wide distribution of aromatase enzyme in the fish brain (Forlano and Bass, 2005; Forlano *et al.*, 2005) suggest that most parts of the brain can convert androgens to estrogens. Therefore, there is a possibility that in the fish brain testosterone gets converted to 17β-estradiol, which then acts via ERα and inhibits the pineal AA-NAT activity and melatonin secretion. In mammals, steroid hormones have been reported to influence adrenergic receptor associated mechanism which reduces adenyl cyclase activity and cAMP concentrations in the pineal gland (Weiss and Crayton, 1970; Davis, 1978). Whether the inhibitory action of the gonadal hormones on AA-NAT activity in the fish pineal is due to changes in adrenergic receptor and/or associated pathway(s) remains to be investigated.

Inhibition of proteasomal enzymes has been reported to increase AA-NAT activity in the pineal and/or retina of mammals (Gastel *et al.*, 1998; Schomerus *et al.*, 2000; Gupta *et al.*, 2005; Terriff *et al.*, 2005), birds (Zatz *et al.*, 2000; Natesan *et al.*, 2002) and fishes (Falcon *et al.*, 2001). These reports suggest that proteasomal enzymes stimulate proteolysis of AA-NAT protein leading to decreased enzyme activity. Proteasomes are also present in the human retina (which also synthesizes melatonin), where they stimulate apoptosis of photoreceptor cells by stimulating 14-3-3 degradation (Ikeda and Inoue, 2004; Wenzel *et al.*, 2005). Estrogen treatment has

been reported to increase the expression of estrogen-responsive finger protein (Efp) resulting in proteasome-dependent degradation of 14-3-3 sigma protein in MCF7 cells (Urano *et al.*, 2002). Further, it has also been reported that decline in estrogen receptor alpha and Efp leads to increase in 14-3-3 sigma expression in mammals (Nakayama *et al.*, 2005) suggesting an inverse correlation between estrogen receptors and 14-3-3 protein. It is important to mention that 14-3-3 protein forms a complex with AA-NAT and protects it from proteasomal enzymes in mammalian pineal and, hence increases AA-NAT activity (Gupta *et al.*, 2005). Therefore, as reported in case of mammals (Urano *et al.*, 2002; Ikeda and Inoue, 2004), there is also a possibility that 17β-estradiol or testosterone aromatized to 17β-estradiol can act via ERα and stimulate degradation of 14-3-3 protein by activating proteasomal enzymes in the photoreceptor cells of the fish pineal (Falcon *et al.*, 2001), or alternatively can suppress expression of 14-3-3 protein as in case of mammals (Nakayama *et al.*, 2005). Estrogen-induced decline in 14-3-3 protein due to increased degradation of 14-3-3 protein and/or suppression of 14-3-3 protein expression coupled with estrogen-induced activation of proteasomal enzyme via ERα (Forlano *et al.*, 2005) can significantly increase degradation of AA-NAT protein resulting in decreased AA-NAT activity in the fish pineal. Thus, the inhibitory effects of testosterone and estrogens recorded in the present study might be due to increased proteasomal proteolysis of 14-3-3 protein as well as of AA-NAT protein. However, these possibilities remain to be experimentally established in the fish pineal.

In the present study, besides testosterone and estrogens, glucocorticoids also significantly inhibited AA-NAT activity in the fish pineal. The presence of glucocorticoid receptors has been reported in the pineal gland of tree shrew (Meyer *et al.*, 1998), rat (Sarrieau *et al.*, 1998) and fishes (Benyassi *et al.*, 2001). There are also reports that glucocorticoids reduce cAMP and inhibit protein synthesis resulting in reduced AA-NAT activity in the rat pineal (Yuwiler, 1989). Glucocorticoids have also been reported to activate proteasomes for inducing thymocyte apoptosis (Dallaporta *et al.*, 2000; Tonomura *et al.*, 2003). Therefore, the observed inhibition of AA-NAT activity in the fish pineal by glucocorticoids may be due to inhibition of cAMP formation and/or inhibition of AA-NAT protein synthesis as well as due to increased proteasomal proteolysis of AA-NAT protein. In line with the present findings, glucocorticoid treatment has been reported to inhibit melatonin secretion in the pineal gland of rats (Yuwiler and Brammer, 1981; Troiani *et al.*, 1988; Yuwiler, 1989; Zhao and Touitou, 1993) and humans (Demisch *et al.*, 1988; Keller *et al.*, 1997). The presence of glucocorticoid receptors in the pineal gland (Meyer *et al.*, 1998; Sarrieau *et al.*, 1998; Benyassi *et al.*, 2001) and melatonin receptors in the adrenal cortex (Brown *et al.*, 1994; Pang *et al.*, 1994; Torres-Farfan *et al.*, 2003) suggest that pineal and adrenal corticosteroidogenic tissue directly interact with each other via their hormones.

On the basis of the present findings, we suggest that gonadal hormones and corticosteroid hormones have direct inhibitory influence on AA-NAT activity and, hence melatonin synthesis in the photoreceptive fish pineal. Further, the steroid hormones might be inhibiting AA-NAT activity by their action(s) via inhibition of cAMP-dependent AA-NAT induction and/or via stimulation of proteasomal proteolysis of AA-NAT and/or 14-3-3 proteins.

**Table 2:1** - Effects of testosterone on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle

| Experiment | AA-NAT activity (nmol/pineal/hr) | | | |
|---|---|---|---|---|
| | **Quiescent Phase** | **Progressive Phase** | **Breeding Phase** | **Regressive Phase** |
| Control | 1.38 ± 0.09 * | 2.15 ± 0.20 | 2.26 ± 0.10 | 2.91 ± 0.15 |
| Testosterone ($10^{-6}$ M) | 1.17 ± 0.10 | 1.48 ± 0.07 [a] | 1.72 ± 0.14 [a] | 2.03 ± 0.23 [a] |
| Testosterone ($10^{-5}$ M) | 0.90 ± 0.12 [a] | 1.34 ± 0.14 [a] | 1.40 ± 0.20 [b] | 1.60 ± 0.16 [c] |
| Testosterone ($10^{-4}$ M) | 0.82 ± 0.12 [a] | 1.16 ± 0.08 [b] | 0.86 ± 0.18 [c, d] | 1.12 ± 0.22 [c, d] |
| Correlation coefficient (r) | -0.95 | -0.92 | -0.99 | -0.98 |

* All values are expressed as mean ± standard error (S.E.); N = 4.

[a, b, c] Differ significantly from the respective control group: $p < 0.05$, 0.02 and 0.01, respectively.

[d] Differs significantly from the respective $10^{-6}$ M treated group: $p < 0.02$.

**Table 2:2** - Effects of estrogens on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle

| Sl. No. | Experiment | AA-NAT activity (nmol/pineal/hr) | | | |
|---|---|---|---|---|---|
| | | **Quiescent Phase** | **Progressive Phase** | **Breeding Phase** | **Regressive Phase** |
| 1. | Control | 1.55 ± 0.18* | 2.20 ± 0.14 | 2.08 ± 0.15 | 3.08 ± 0.24 |
| | 17 β-estradiol ($10^{-6}$ M) | 1.48 ± 0.15 | 1.73 ± 0.14 | 1.46 ± 0.12 [a] | 2.08 ± 0.19 [a] |
| | 17 β-estradiol ($10^{-5}$ M) | 1.32 ± 0.10 | 1.66 ± 0.09 [a] | 1.27 ± 0.10 [b] | 1.61 ± 0.15 [c] |
| | 17 β-estradiol ($10^{-4}$ M) | 0.96 ± 0.08 [a] | 1.33 ± 0.11 [c] | 0.93 ± 0.15 [c] | 1.33 ± 0.16 [c,d] |
| Correlation coefficient (r) | | -0.94 | -0.96 | -0.97 | -0.96 |
| 2. | Control | 1.65 ± 0.04 | 2.20 ± 0.14 | 2.08 ± 0.15 | 3.08 ± 0.24 |
| | Estriol ($10^{-6}$ M) | 1.51 ± 0.06 | 2.04 ± 0.16 | 1.37 ± 0.25 | 2.35 ± 0.23 |
| | Estriol ($10^{-5}$ M) | 1.45 ± 0.07 | 1.96 ± 0.07 | 1.01 ± 0.20 [a] | 1.85 ± 0.19 [b] |
| | Estriol ($10^{-4}$ M) | 1.31 ± 0.11 [a] | 1.62 ± 0.12 [a] | 0.79 ± 0.16 [b] | 1.51 ± 0.17 [c] |
| Correlation coefficient (r) | | -0.98 | -0.96 | -0.96 | -0.98 |
| 3. | Control | 1.65 ± 0.04 | 2.20 ± 0.14 | 2.08 ± 0.15 | 3.08 ± 0.24 |
| | Estrone ($10^{-6}$ M) | 1.56 ± 0.11 | 2.15 ± 0.07 | 1.97 ± 0.18 | 2.36 ± 0.12 |
| | Estrone ($10^{-5}$ M) | 1.45 ± 0.13 | 1.99 ± 0.08 | 1.81 ± 0.15 | 2.07 ± 0.14 [a] |
| | Estrone ($10^{-4}$ M) | 1.39 ± 0.13 | 1.73 ± 0.16 | 1.49 ± 0.08 [a] | 1.81 ± 0.16 [b] |
| Correlation coefficient (r) | | -0.99 | -0.95 | -0.97 | -0.96 |

* All values are expressed as mean ± standard error (S.E.); N = 4.

[a, b, c] Differ significantly from the respective control group: $p < 0.05$, 0.02 and 0.01, respectively.

[d] Differs significantly from the respective $10^{-6}$ M treated group: $p < 0.05$.

**Table 2:3** - Effects of corticosteroids on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle

| Sl. No. | Experiment | AA-NAT activity (nmol/pineal/hr) | | | |
|---|---|---|---|---|---|
| | | **Quiescent Phase** | **Progressive Phase** | **Breeding Phase** | **Regressive Phase** |
| 1. | Control | 1.65 ± 0.27* | 2.20 ± 0.14 | 2.56 ± 0.19 | 2.91 ± 0.15 |
| | Corticosterone ($10^{-6}$ M) | 1.30 ± 0.15 | 1.69 ± 0.09 [a] | 1.53 ± 0.17 [b] | 2.35 ± 0.06 [a] |
| | Corticosterone ($10^{-5}$ M) | 1.28 ± 0.13 | 1.52 ± 0.10 [b] | 1.50 ± 0.03 [c] | 2.19 ± 0.10 [a] |
| | Corticosterone ($10^{-4}$ M) | 1.15 ± 0.13 | 1.47 ± 0.09 [b] | 1.35 ± 0.09 [c] | 2.00 ± 0.17 [b] |
| Correlation coefficient (r) | | -0.91 | -0.91 | -0.86 | -0.95 |
| 2. | Control | 1.65 ± 0.12 | 2.20 ± 0.20 | 2.56 ± 0.19 | 2.91 ± 0.15 |
| | Cortisol ($10^{-6}$ M) | 1.39 ± 0.10 | 1.72 ± 0.09 [a] | 1.59 ± 0.18 [a] | 2.25 ± 0.19 |
| | Cortisol ($10^{-5}$ M) | 1.18 ± 0.10 [a] | 1.52 ± 0.07 [b] | 1.51 ± 0.18 [b] | 2.05 ± 0.21 [a] |
| | Cortisol ($10^{-4}$ M) | 1.12 ± 0.11 [a] | 1.37 ± 0.07 [c] | 1.39 ± 0.20 [b] | 1.79 ± 0.21 [b] |
| Correlation coefficient (r) | | -0.96 | -0.93 | -0.86 | -0.80 |

* All values are expressed as mean ± standard error (S.E.); N = 4.

[a, b, c] Differ significantly from the respective control group: $p < 0.05$, 0.02 and 0.01, respectively.

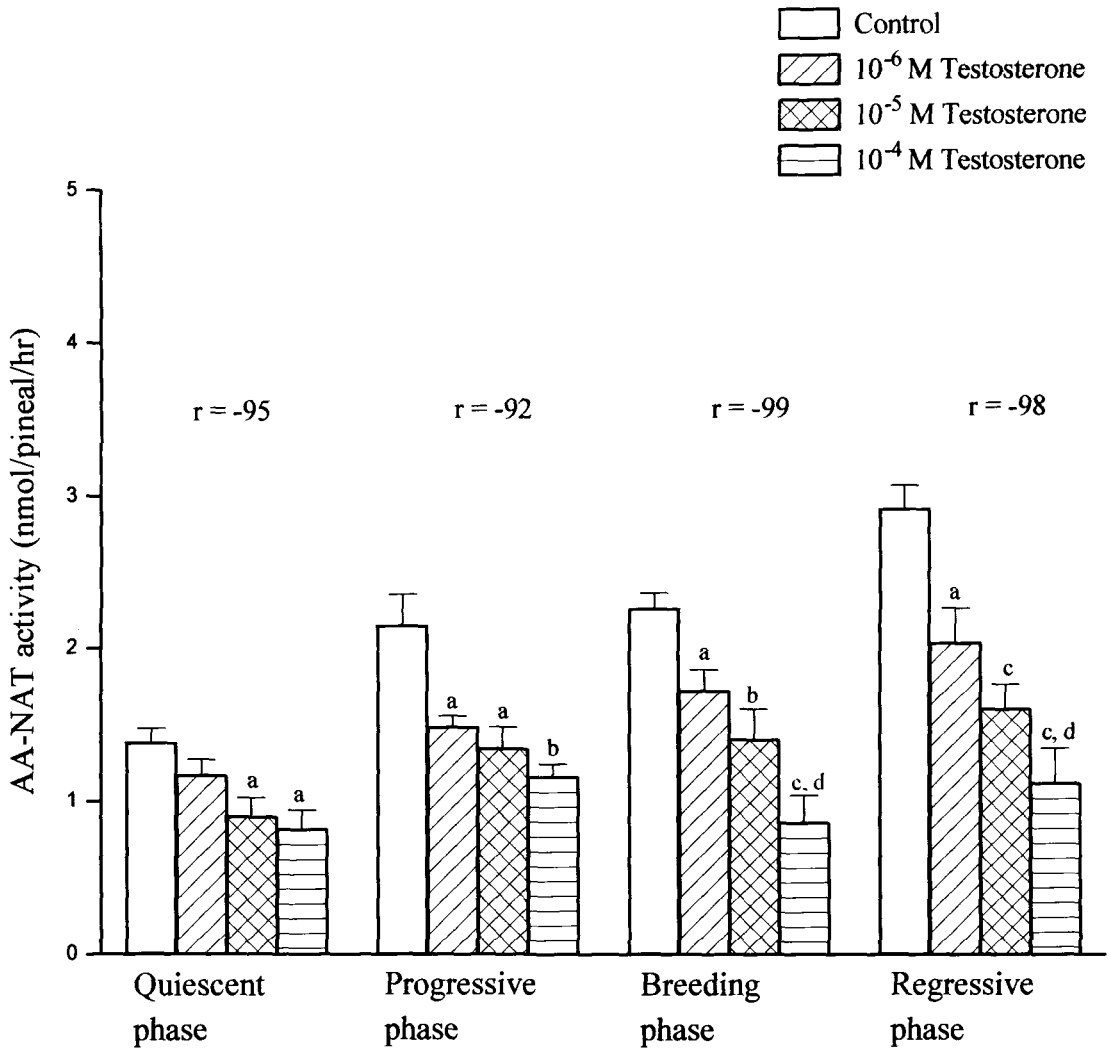



**Fig. 2:1** - Effects of testosterone on arylalkylamine N- acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle.

All values are expressed as mean ± standard error (S. E.); N = 4.

[a, b, c] Differ significantly from their respective control group: $p < 0.05$, 0.02 and 0.01, respectively.

[d] Differs significantly from the treated $10^{6}$ M group: $p < 0.05$.

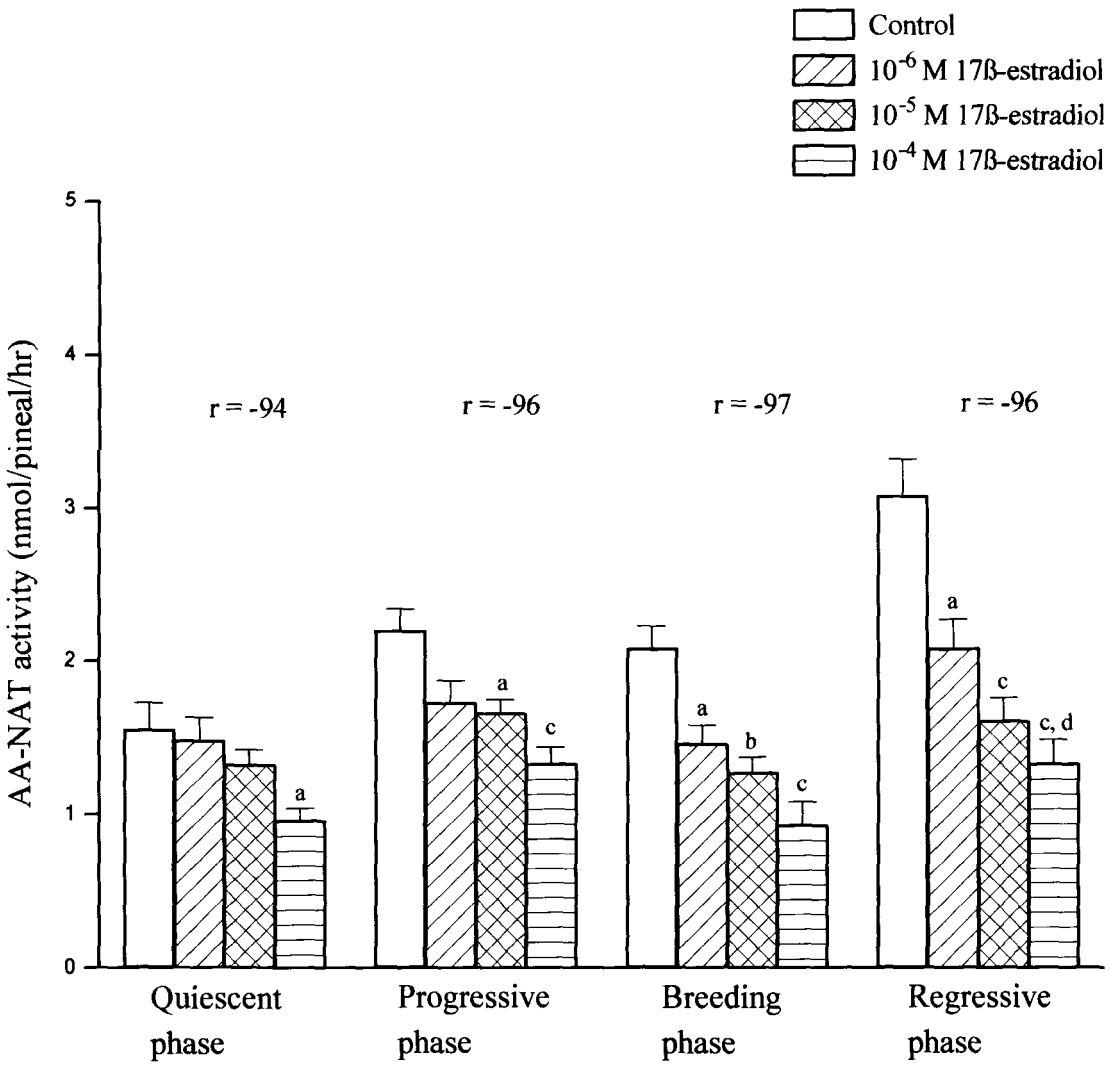



**Fig. 2:2A** - Effects of 17ß-estradiol on arylalkylamine N- acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle.

All values are expressed as mean ± standard error (S. E.); N = 4.

[a, b, c] Differ significantly from their respective control group: $p < 0.05$, 0.02 and 0.01, respectively.

[d] Differs significantly from the treated $10^{6}$ M group: $p < 0.05$.

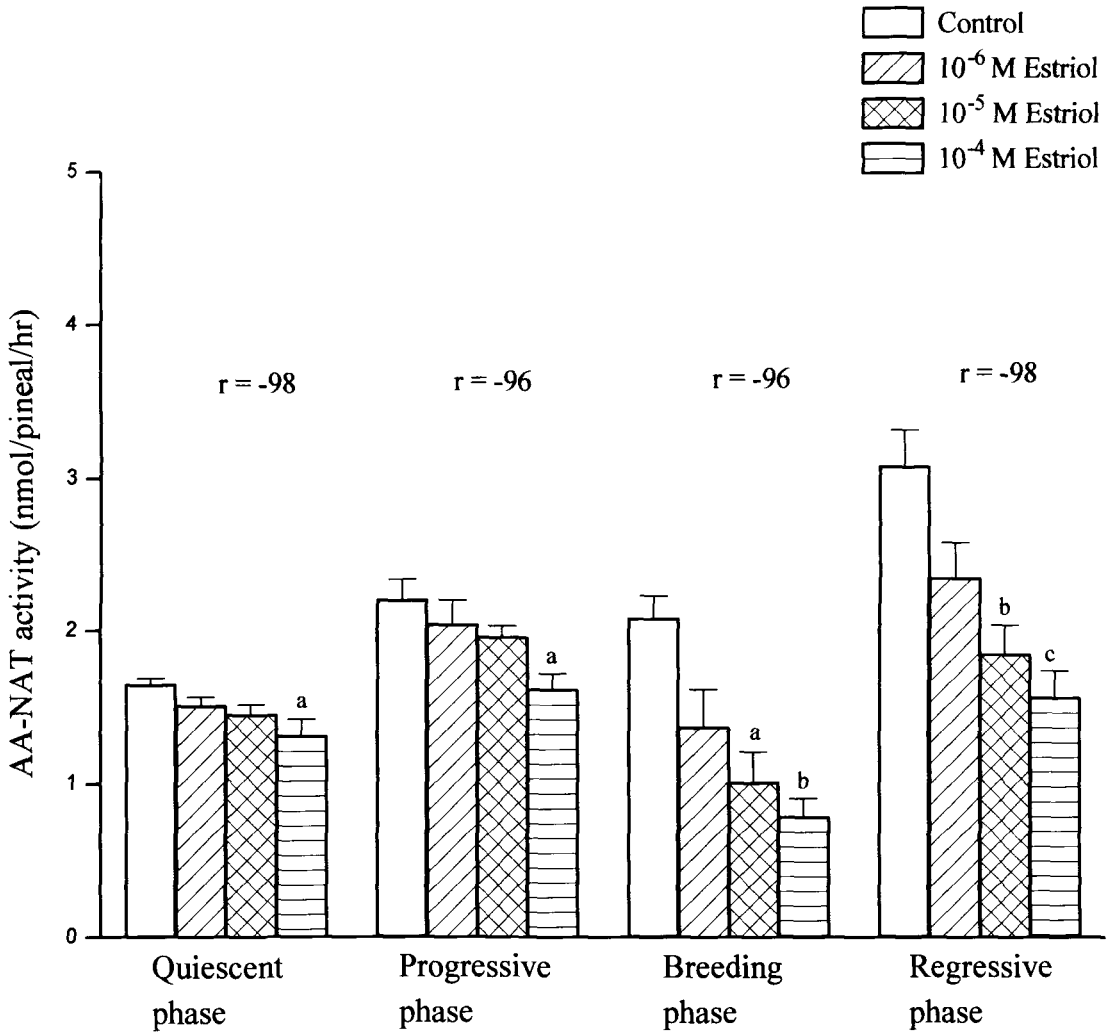



**Fig. 2:2B** - Effects of estriol on arylalkylamine N- acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle.

All values are expressed as mean ± standard error (S. E.); N = 4.

[a, b, c] Differ significantly from their respective control group: $p < 0.05$, 0.02 and 0.01, respectively.

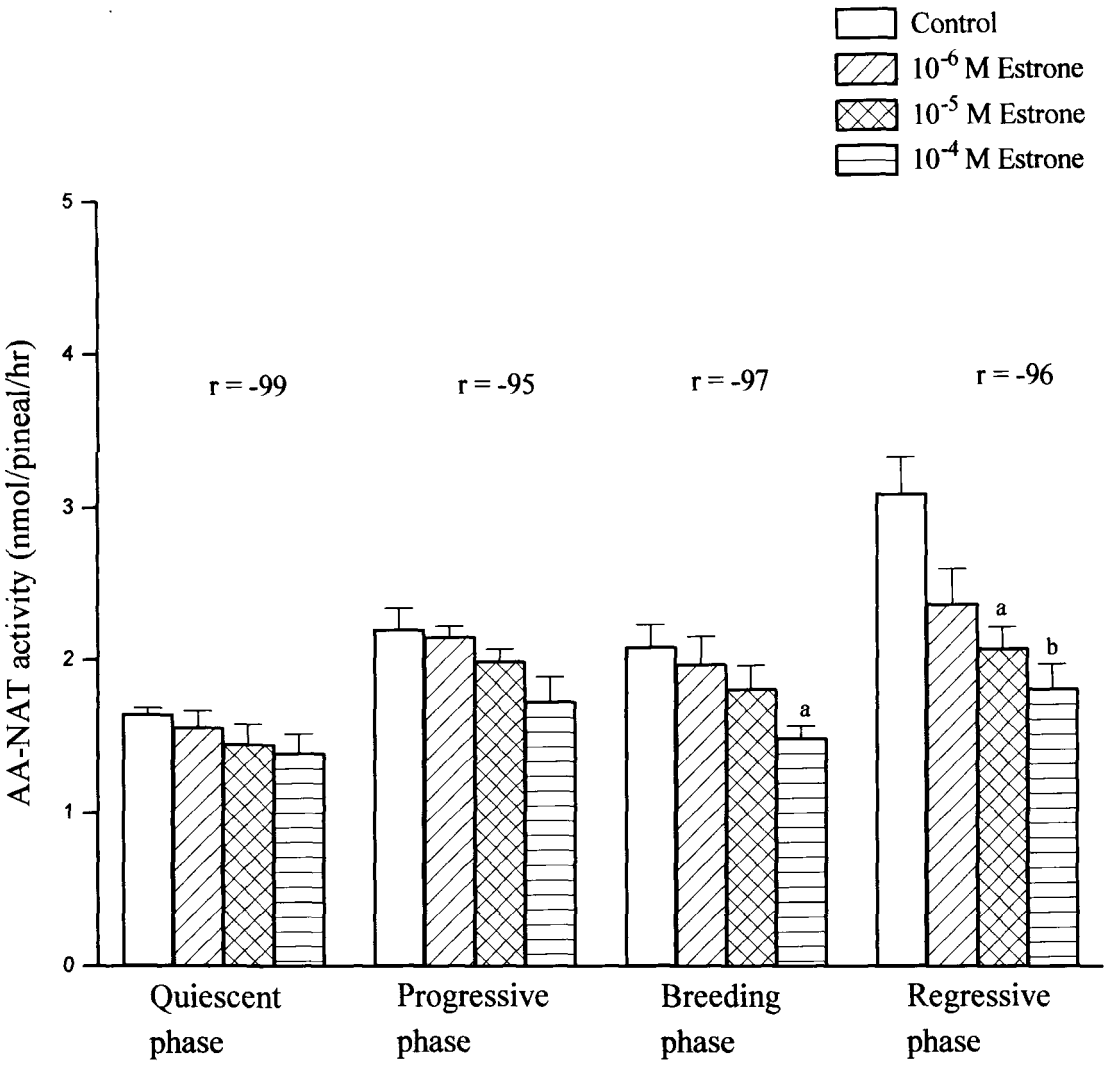



**Fig. 2:2C** -Effects of estrone on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle.

All values are expressed as mean ± standard error (S. E.); N = 4.

[a, b] Differ significantly from their respective control group: $p < 0.05$ and 0.02, respectively.

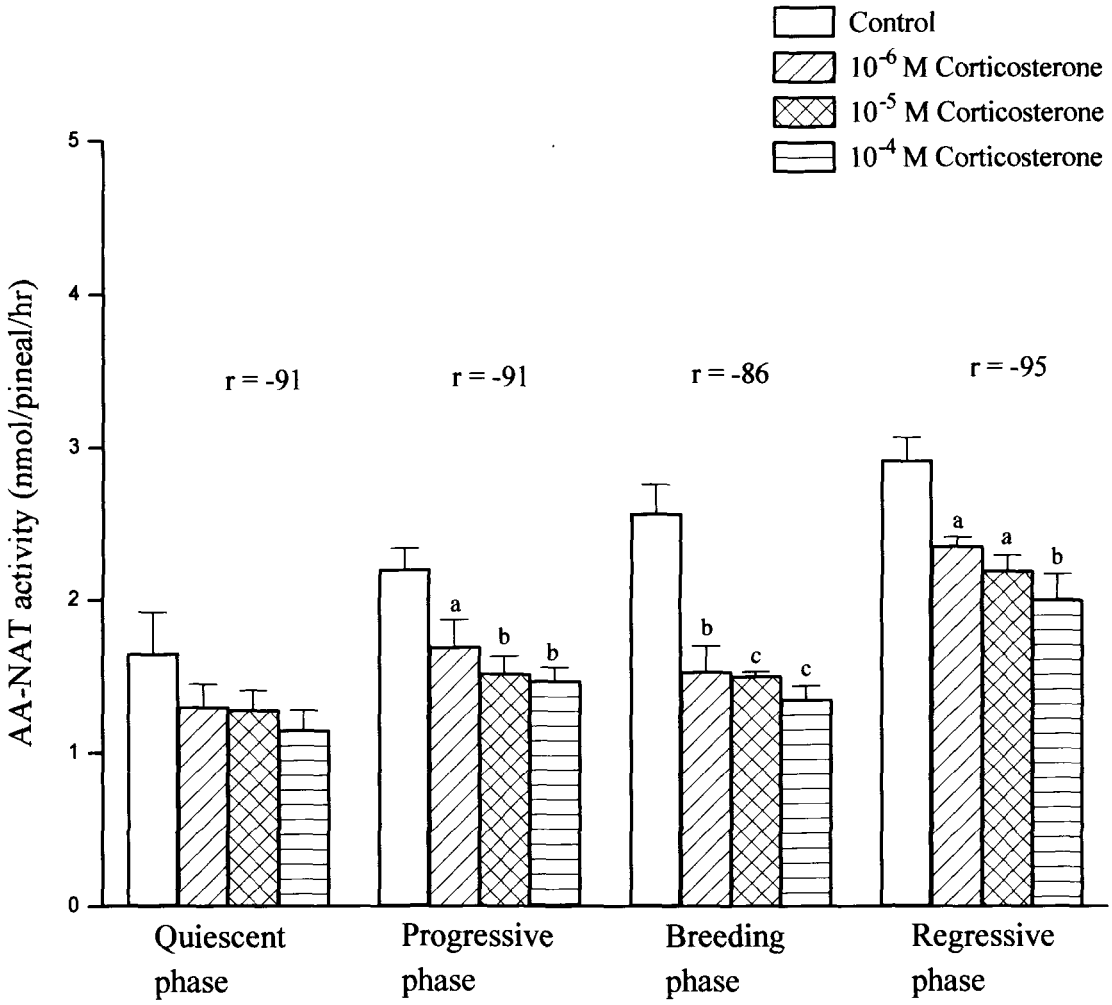



**Fig. 2:3A** - Effects of corticosterone on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle.

All values are expressed as mean ± standard error (S. E.); N = 4.

[a, b, c] Differ significantly from their respective control group: $p < 0.05$, 0.02 and 0.01, respectively.

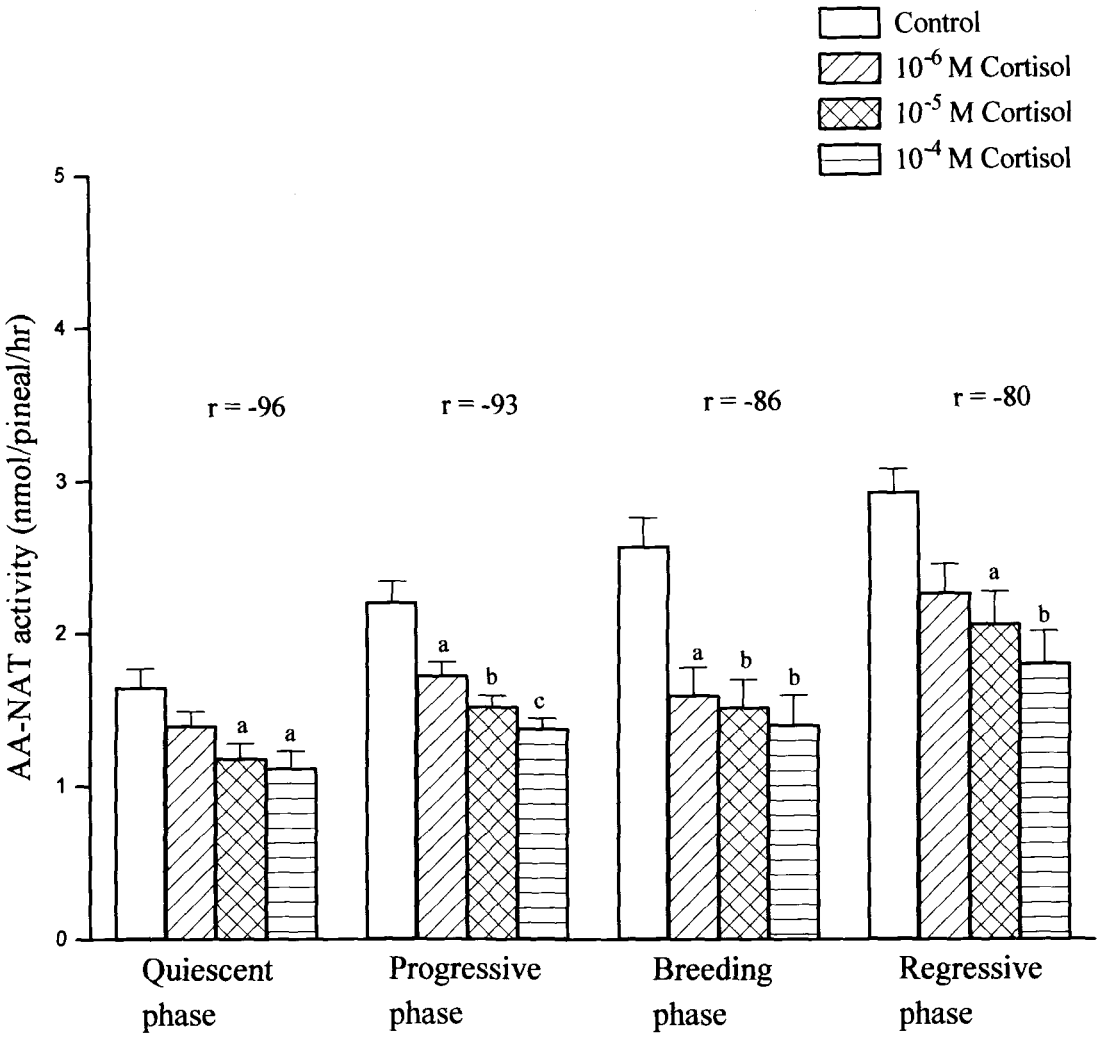



**Fig. 2:3B** - Effects of cortisol on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle.

All values are expressed as mean ± standard error (S. E.); N = 4.

[a, b, c] Differ significantly from their respective control group: $p < 0.05$, 0.02 and 0.01, respectively.

# CHAPTER - 3

## *In vitro* effects of indoleamines and leptin on arylalkylamine N-acetyltransferase (AA-NAT) activity in the fish pineal during different phases of the breeding cycle

### INTRODUCTION

The daily rhythm in indoleamine metabolism is characteristics of the pineal gland and a notable feature of vertebrate circadian physiology. The switch between the day and night profiles of pineal indoleamines (serotonin, N-acetylserotonin and melatonin etc.) are driven by changes in the activity of arylalkylamine N-acetyltransferase (AA-NAT) (Klein and Weller, 1970; Klein *et al*, 1997). In mammals, the presence of indoleamine receptors has been reported in the pineal gland (Govitrapong *et el.*, 1991; Olcese and Munker, 1994; Sterado *et al.*, 2000) as well as in the retina (Dubocovich, 1985; Brunken and Jin, 1993; Jin and Brunken, 1998; Pootanakit *et al.*, 1999, 2001; Scher *et al.*, 2002). In non-mammalian vertebrates, receptors for indoleamines have been reported in the retina of birds (Iuvone and Gan, 1994; Natesan and Cassone, 2002; Rada and Wiechmann, 2006), amphibians (Wiechmann *et al.*, 2003), and fishes (Bayarri *et al.*, 2004; Ribelayga *et al.*, 2004).

The pathway of melatonin synthesis is influenced by the indoleamines in mammalian pineal. Melatonin has been reported to act directly on the pinealocytes of

rats, and to increase AA-NAT activity and HIOMT activity in a dose-dependent manner (Freire and Cardinali, 1975). *In vitro* treatment with melatonin was reported to increase the concentration of serotonin (5-hydroxytryptamine or 5-HT) in the rat pineal (Miguez *et al.*, 1995). Subcutaneous injection of melatonin increased melatonin secretion from the rat pineal (Bothorel *et al.*, 2002). In cultured rat pinealocytes, 5-HT acts via S2-type receptors and increases AA-NAT activity and melatonin secretion (Sugden, 1990; Olcese and Munker, 1994). Further, 5-HT acts via 5-$HT_{2C}$ receptors and stimulates AA-NAT activity and melatonin synthesis in the rat pineal (Sterado *et al*, 2000).

The mammalian pinealocytes also contain receptors for leptin (Chelikani *et al.*, 2003). Leptin receptors are also found in different parts of the fish brain (Johnson *et al.*, 2000). In mammals, leptin has been reported to participate in multiple regulatory mechanisms such as energy expenditure and metabolism, cell proliferation and differentiation, as well as in signal interaction with other hormonal regulators of energy metabolism (Friedman, 2002; Margetic *et al.*, 2002). In mice, leptin has been reported to increase hypothalamic pro-opiomelanocortin mRNA expression in the rostral acurate nucleus (Schwartz *et al.*, 1997). Further, leptin was reported to rapidly induce the AA-NAT activity in the hypothalamus of mice (Guo *et al.*, 2004). In fishes, administration of leptin has been reported to reduce food intake, body weight gain and growth rate (Volkoff *et al.*, 2003; de Pedro *et al.*, 2006). *In vitro* treatment of leptin reportedly stimulated thyrotropin mRNA levels in a dose-dependent manner in the pituitary of carp (Chowdhury *et al.*, 2004). Murine leptin injection has been

reported to increase intracellular fatty acid binding protein in green sunfish, *Lepomis cyanellus* (Londraville and Duvall, 2002). Notwithstanding these reports on the physiological action of leptin in fish species, there is practically no information on the role of leptin in the regulation/modulation of AA-NAT activity and/or melatonin synthesis in any fish species. Therefore, it was thought worthwhile to investigate *in vitro* effects of indoleamines on AA-NAT activity during different phases of the annual breeding cycle as well as *in vitro* effects of leptin on AA-NAT activity in the pineal of air-breathing catfish, *Clarias gariepinus* during summer and winter with special reference to feeding status.

## MATERIALS AND METHODS

For this study, adult male *Clarias gariepinus* were purchased from the local fish suppliers and maintained in plastic tubs and acclimatized for 15 days in the laboratory under natural climatic conditions. During acclimatization, the fishes were fed daily with minced earthworms and commercial fish food *ad libitum*. Water was changed everyday to avoid infections. In order to collect pineal, the fishes were decapitated in complete darkness under dim red light and the pineals were rapidly removed, washed in culture medium (DMEM) and 8 pineals were placed in each well of the multi-well culture plate (Corning Cell Wells, New York, USA) for organ culture and treatment with indoleamines.

To study the effects of leptin on AA-NAT activity, *in vitro* experiments was conducted during summer and winter seasons. The acclimatized fishes were divided into two groups of thirty-two animals each and kept separately in plastic tubs. One group of the fish was fed daily with minced earthworm and fish meal for a period of 10 days, while the other group was fasted for equal number of days. After ten days, the fishes of both the groups were decapitated in darkness under dim red light and the pineals were rapidly removed, and 8 pineals were placed in each well of the multi-well culture plate containing culture medium for organ culture.

**Pineal organ culture**

The pineals were pre-incubated for one hour, after which the medium was removed and replaced with the medium containing desired concentration of the hormones. The pineals were then incubated for 6 hours at $25^{\circ}$ C in an atmosphere of 85% $O_2$, 5% $CO_2$ and 95% relative humidity with the help of $O_2$-$CO_2$ gas incubator (Heraeus: Cytoperm, Germany). Pineals incubated in DMEM without any hormones were treated as control.

The pineals maintained in organ culture were treated *in vitro* with the indoleamines and leptin as indicated in the following experimental protocol:

**Experimental protocol**

| Experiments | Hormones | Concentration of Hormone | Time of Experiment |
|---|---|---|---|
| 1. *In vitro* effects of indoleamines on AA-NAT activity | a) 5-Hydroxytryptophan<br>b) Serotonin<br>c) N-Acetylserotonin<br>d) Melatonin | $10^{-6}$ M, $10^{-5}$ M and $10^{-4}$ M of each hormone | Quiescent (February), Progressive (April), Breeding (August), and Regressive (November) phases |
| 2. *In vitro* effects of leptin on AA-NAT activity | Leptin | 1μg/ml, 2μg/ml and 5μg/ml | Summer (July) and Winter (January) |

After incubation, the pineals were removed and placed in numbered Eppendorf tubes, which were immediately frozen in liquid nitrogen for the measurement of AA-NAT activity.

**Measurement of pineal arylalkylamine N-acetyltransferase (AA-NAT) activity**

Arylalkylamine N-acetyltransferase (AA-NAT) activity was measured with the help of radio-enzymatic assay as described by Deguchi and Axelrod (1972) with slight modifications. For the measurement of AA-NAT activity, the pineals were sonicated in 75 μl of homogenization buffer. 15 μl samples (in duplicate) of the sonicated pineals were incubated with 5 μl of the reaction for 20 min at 25° C. At the end of the incubation period, 100 μl of borate buffer was added to stop the reaction. In order to extract acetylated tryptamine, extraction solution was added to the tubes and rotated in a rotary mixer (Stuart Scientific, U. K) for 15 minutes, and then

centrifuged at 3000 rpm for 5 minutes. Two ml of the upper phase of the mixture was transferred to a scintillation vial containing 5 ml of scintillation fluid. The radioactivity in each sample was counted with the help of a liquid scintillation counter (Wallace-1409). After calculations, AA-NAT activity was expressed as nmol/pineal/hour. The data were analyzed statistically with the help of Student's 't' test and regression analysis. A $p < 0.05$ was considered as significant.

## RESULTS

### 1. *In vitro* effects of indoleamines on AA-NAT activity

#### a) *Effects of 5-hydroxytryptophan*

The data are presented in Table 3:1A; Fig. 3:1A. During the quiescent phase, *in vitro* treatment of the fish pineal with only $10^{-4}$ M concentration of 5-hydroxytryptophan stimulated AA-NAT activity significantly, while the other two lower doses had no effect. However, during the progressive phase, all the three concentrations of 5-hydroxytryptophan ($10^{-6}$ M, $10^{-5}$ M, and $10^{-4}$ M) significantly increased pineal AA-NAT activity in the fish pineal. During the breeding and the regressive phases, *in vitro* treatment of the fish pineal with only $10^{-5}$ M and $10^{-4}$ M (but not $10^{-6}$ M) concentrations of 5-hydroxytryptophan significantly increased AA-NAT activity. Regression analysis of the data indicated a positive correlation between the doses of 5-hydroxytryptophan and pineal AA-NAT activity during all four phases of the breeding cycle of the fish.

**b) *Effects of serotonin***

The data are presented in Table 3:1B; Fig.3:1B. During the quiescent phase, *in vitro* treatment with only $10^{-4}$ M concentration of serotonin significantly increased AA-NAT activity, while $10^{-6}$ M and $10^{-5}$ M concentrations of serotonin had no significant effect on the enzyme activity. During the progressive and the breeding phases, only $10^{-5}$ M and $10^{-4}$ M concentrations of serotonin significantly increased AA-NAT activity. During the regressive phase, however, all the three concentrations ($10^{-6}$ M, $10^{-5}$ M, and $10^{-4}$ M) of serotonin significantly increased the enzyme activity. Regression analysis of the data indicated a positive correlation between the doses of serotonin and pineal AA-NAT activity during all the four phases of the breeding cycle of the fish.

**c) *Effects of N- Acetylserotonin***

The data are presented in Table 3:1C; Fig. 3:1C. During all the four phase of the breeding cycle, only $10^{-5}$ M and $10^{-4}$ M concentrations of N-acetylserotonin significantly increased AA-NAT activity in the fish pineal, while $10^{-6}$ M concentration of the indoleamine did not produce any significant effect on the enzyme activity. Regression analysis of the data indicated a positive correlation between the doses of N-acetyl-serotonin and pineal AA-NAT activity during all the four phases of the breeding cycle of the fish.

**d) *Effects of melatonin***

The data are presented in Table 3:1D; Fig. 3:1D. Irrespective of the phase of the breeding cycle, all the three concentrations ($10^{-6}$ M, $10^{-5}$ M and $10^{-4}$ M) of melatonin significantly increased AA-NAT activity. Regression analysis of the data indicated a positive correlation between the doses of melatonin and pineal AA-NAT activity during all the four phases of the breeding cycle of the fish.

**2. *In vitro* effects of leptin on AA-NAT activity**

The data are presented in Table 3:2; Fig. 3:2. Irrespective of the seasons, i*n vitro* treatment with 2 μg/ml and 5μg/ml concentrations of leptin significantly increased AA-NAT activity in the pineal of the fed fishes, while 1μg/ml leptin had no significant effect on the enzyme activity. However, leptin did not produce any significant effect on pineal AA-NAT activity in the pineal of the fasted fishes irrespective of the doses and seasons. Regression analysis of the data indicated a strong positive correlation between the doses of leptin and pineal AA-NAT activity irrespective of seasons and feeding status.

**DISCUSSION**

In the present study, regression analysis indicated a positive correlation between the doses of the indoleamines and AA-NAT activity suggesting that the indoleamines stimulate pineal AA-NAT activity in a dose-dependent manner during all the phases of the breeding cycle of the fish, *Clarias gariepinus*. However, the degree of stimulation of AA-NAT activity seems to depend on the phases of the

breeding cycle and/or the seasons. The sensitivity of AA-NAT activity to 5-hydroxytryptophan was comparatively higher during the progressive phase and lower during the quiescent phase. Serotonin was comparatively more stimulatory during the regressive phase and less during the quiescent phase. The sensitivity of AA-NAT activity to N-acetylserotonin and melatonin did not change during the different phase. It, thus, seems that the sensitivity of AA-NAT activity for stimulatory effects of 5-hydroxytryptophan and serotonin depends on the phases/ seasons, while the enzyme sensitivity for N-acetylserotonin and melatonin remains similar throughout the year.

The findings of present study seem to suggest that the indoleamines are actively involved in the regulation of AA-NAT activity, and hence melatonin synthesis in the fish pineal. The stimulatory effects of the indoleamines on AA-NAT activity seem to indicate that the indoleamines might be acting either as substrates or hormones (autocrine/paracrine) for the stimulation of AA-NAT in the fish pineal. It is important to mention that the photosensory pineal of the fish consists of photoreceptors cells which perceive light as well as synthesis melatonin, and, thus, resembles in structure and function with the vertebrate retina. It is noteworthy that melatonin receptors are present in the retina of fishes (Bayarri *et al.*, 2004; Ribelayga *et al.*, 2004), amphibians (Wiechmann *et al.*, 2003), birds (Iuvone and Gan, 1994; Natesan and Cassone, 2002; Rada and Wiechmann, 2006) and mammals (Dubocovich, 1985; Scher *et al.*, 2002). Similarly, serotonin receptors are reportedly present in both pineal (Govitrapong *et al.*, 1991; Olcese and Munker, 1994; Sterado

*et al.*, 2000) and retina (Brunken and Jin, 1993; Pootanakit *et al.*, 1999) of mammals. Thus, the widespread presence of the receptors of both melatonin and serotonin in retina as well as pineal of vertebrates and the present finding, when considered together, seem to suggest that serotonin and melatonin act as hormones and play an important role in the regulation of AA-NAT activity and melatonin synthesis in the fish pineal.

It is noteworthy that $\alpha_2$-adrenergic receptors ($\alpha_2$-AR) are present in the photoreceptive chick pineal (Pratt and Takahashi, 1987; Voisin *et al.*, 1990b; Rudeen *et al.*, 1990) and retina of vertebrates (Matsuo and Cynader, 1992; Wheeler *et al.*, 1999; Lai *et al.*, 2002; Kalapesi *et al.*, 2005), and melatonin has been reported to act via $\alpha_2$-AR (Martensson and Andersson, 1997, 1999, 2000; Aspengren *et al.*, 2003). Further, agonists of $\alpha_2$-AR have been reported to increase cAMP and AA-activity in the avian pineal. Therefore, there is a possibility that, in addition to its own receptors, melatonin also acts via $\alpha_2$-AR and stimulates AA-NAT activity and melatonin synthesis via cAMP forming pathway in the fish pineal. This possibility, however, remains to be investigated.

The conversion of tryptophan to 5-hydroxy-tryptophan (5-HTP) by the enzyme tryptophan hydroxylase is the rate-limiting step in the synthesis of serotonin (Snyder *et al.*, 1965; Ichinose and Nagatsu, 1993; Meyers, 2000). 5-HTP has been reported to stimulate both synthesis and secretion of serotonin and its metabolites (N-acetylserotonin and melatonin) in the mammalian pineal (Sugden *et al.*, 1985;

Ferretti *et al.*, 1990; McIntyre and Oxenkrug, 1991; Meyers, 2000). *In vivo* administration of 5-HTP has also been reported to stimulate melatonin levels in the chick retina in a dose-dependent manner (Thomas *et al.*, 1998). In conformity with the earlier reports, in the present study also 5-hydroxytryptophan stimulated pineal AA-NAT activity in a dose-dependent manner irrespective of seasons and phases of the annual breeding cycle. It, thus, seems that 5-HTP is also involved in regulation of AA-NAT activity in the fish pineal. 5-HTP can stimulate AA-NAT activity in the fish pineal by stimulating both synthesis and release of serotonin (Young and Gauthier, 1981; Meyers, 2000), which then probably acts as a hormone via its receptors and stimulates AA-NAT activity and melatonin synthesis in the fish pineal (Ceinos *et al.*, 2005) as reported in the mammalian pineal (Sugden, 1990; Sterado *et al.*, 2000).

N-Acetylserotonin (NAS) has been reported to stimulate melatonin synthesis in a dose-dependent manner in the rat pineal (Oxenkrug and Requintina, 1994). It is important to mention that NAS can act via melatonin receptors (MT1, MT2 and MT3) in various tissues of fishes (Bayarri *et al.*, 2004), amphibians (Filadelfi and Castrucci, 1996), and mammals (Nonno *et al.*, 1999; Oxenkrug, 2005), and hence acts as a hormone. Therefore, it seems that, as in the case of serotonin and melatonin, NAS also acts as a hormone and stimulated AA-NAT activity in the fish pineal by acting probably via melatonin receptors. In addition, increased availability of NAS may also lead to its increased conversion to melatonin by the enzyme HIOMT.

Besides indoleamines, leptin also increased pineal AA-NAT activity significantly in a dose-dependent manner only in the pineal of the fed fishes (but not of the fasted fishes) during both summer and winter seasons. As evident from the insignificant but dose-dependent effect on AA-NAT activity, food restriction seems to reduce the effect of leptin on AA-NAT activity. These finding seem to suggest that the sensitivity of pineal AA-NAT activity to leptin is dependent on seasons and feeding status of the fish. Further, the feeding status seems to act as switch for the action of leptin on AA-NAT activity in the fish pineal. Leptin receptors have been reported in the pineal gland of mammals (Couce *et al.*, 1997; Dal Farra *et al.*, 2000; Chelikani *et al.*, 2003) as well as in the brain of fishes (Johnson *et al.*, 2000). Leptin has also been reported to significantly increase cAMP levels in cultured porcine adrenal medullary chromaffin cells (Takekoshi *et al.*, 1999), and N-acetyl-transferase activity in mice pro-opiomelanocortin (POMC) neurons (Guo *et al.*, 2004). Therefore, the observed stimulation of AA-NAT activity in the fish pineal by leptin might be due to leptin-induced increase in cAMP formation leading to increased AA-NAT activity.

On the basis of the present findings, it can be suggested that pineal indoleamines and leptin have a direct stimulatory effect on AA-NAT activity, and hence melatonin synthesis in the photoreceptive fish pineal. To the best of our knowledge, this study seems to be the first of its kind in which the role of indoleamines and leptin in regulation of AA-NAT activity has been established in the pineal of any fish species.

**Table 3:1A** - Effects of 5-hydroxytryptophan on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle

| Experiment | AA-NAT activity (nmol/pineal/hr) | | | |
|---|---|---|---|---|
| | **Quiescent Phase** | **Progressive Phase** | **Breeding Phase** | **Regressive Phase** |
| Control | 1.63 ± 0.07* | 2.15 ± 0.08 | 2.36 ± 0.06 | 2.59 ± 0.17 |
| 5-Hydroxytryptophan ($10^{-6}$ M) | 1.72 ± 0.10 | 2.62 ± 0.13 [a] | 2.52 ± 0.17 | 2.86 ± 0.09 |
| 5-Hydroxytryptophan ($10^{-5}$ M) | 1.97 ± 0.08 | 2.71 ± 0.12 [b] | 2.76 ± 0.12 [a] | 3.38 ± 0.13 [a] |
| 5-Hydroxytryptophan ($10^{-4}$ M) | 2.10 ± 0.13 [a] | 2.84 ± 0.15 [b] | 2.80 ± 0.09 [b] | 3.49 ± 0.13 [b] |
| Correlation coefficient (r) | 0.98 | 0.92 | 0.97 | 0.97 |

* All values are expressed as mean ± standard error (S.E.); N = 4.

[a, b] Differ significantly from the respective control group: $p < 0.05$ and 0.02, respectively.

**Table 3:1B** - Effects of serotonin on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle

| Experiment | AA-NAT activity (nmol/pineal/hr) | | | |
|---|---|---|---|---|
| | **Quiescent Phase** | **Progressive Phase** | **Breeding Phase** | **Regressive Phase** |
| Control | 1.63 ± 0.07 * | 2.15 ± 0.20 | 2.08 ± 0.15 | 2.59 ± 0.26 |
| Serotonin ($10^{-6}$ M) | 1.71 ± 0.11 | 2.47 ± 0.16 | 2.53 ± 0.19 | 3.67 ± 0.11 [b] |
| Serotonin ($10^{-5}$ M) | 1.80 ± 0.15 | 2.88 ± 0.09 [a] | 2.76 ± 0.16 [a] | 3.84 ± 0.19 [b] |
| Serotonin ($10^{-4}$ M) | 2.01 ± 0.09 [a] | 2.82 ± 0.12 [a] | 2.79 ± 0.16 [a] | 3.81 ± 0.15 [b] |
| Correlation coefficient (r) | 0.96 | 0.92 | 0.92 | 0.82 |

* All values are expressed as mean ± standard error (S.E.); N = 4.

[a, b] Differ significantly from the respective control group: $p < 0.05$ and 0.02, respectively.

**Table 3:1C** - Effects of N-acetylserotonin on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle

| Experiment | AA-NAT activity (nmol/pineal/hr) | | | |
|---|---|---|---|---|
| | **Quiescent Phase** | **Progressive Phase** | **Breeding Phase** | **Regressive Phase** |
| Control | 1.63 ± 0.07 * | 2.15 ± 0.20 | 2.08 ± 0.15 | 2.59 ± 0.13 |
| N-Acetylserotonin ($10^{-6}$ M) | 1.86 ± 0.06 | 2.57 ± 0.11 | 2.54 ± 0.10 | 2.74 ± 0.17 |
| N-Acetylserotonin ($10^{-5}$ M) | 2.09 ± 0.05 [c] | 2.80 ± 0.06 [a] | 2.80 ± 0.18 [b] | 3.04 ± 0.08 [a] |
| N-Acetylserotonin ($10^{-4}$ M) | 2.24 ± 0.08 [c] | 2.91 ± 0.08 [a] | 2.99 ± 0.12 [c] | 3.16 ± 0.09 [a] |
| Correlation coefficient (r) | 0.99 | 0.96 | 97 | 98 |

* All values are expressed as mean ± standard error (S.E.); N = 4.

[a, b, c] Differ significantly from the respective control group: $p < 0.05$, 0.02 and 0.01, respectively.

**Table 3:1D** - Effects of melatonin on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle

| Experiment | AA-NAT activity (nmol/pineal/hr) | | | |
|---|---|---|---|---|
| | **Quiescent Phase** | **Progressive Phase** | **Breeding Phase** | **Regressive Phase** |
| Control | 1.63 ± 0.07 * | 2.15 ± 0.09 | 2.08 ± 0.15 | 2.59 ± 0.26 |
| Melatonin ($10^{-6}$ M) | 1.98 ± 0.09 [a] | 2.56 ± 0.08 [a] | 2.61 ± 0.07 [a] | 3.37 ± 0.10 [a] |
| Melatonin ($10^{-5}$ M) | 2.05 ± 0.09 [a] | 2.60 ± 0.08 [a] | 2.78 ± 0.14 [a] | 3.55 ± 0.20 [a] |
| Melatonin ($10^{-4}$ M) | 2.10 ± 0.12 [a] | 2.70 ± 0.10 [b] | 2.89 ± 0.12 [a] | 3.59 ± 0.10 [a] |
| Correlation coefficient (r) | 0.89 | 0.90 | 0.93 | 0.87 |

* All values are expressed as mean ± standard error (S.E.); N = 4.

[a, b] Differ significantly from the respective control group: $p < 0.05$ and 0.02, respectively.

**Table 3:2** - Effects leptin on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of fed and fasted *Clarias gariepinus* during summer and winter seasons

| Experiment | AA-NAT activity (nmol/pineal/hr) | | | |
|---|---|---|---|---|
| | Summer | | Winter | |
| | Fed | Fasted | Fed | Fasted |
| Control | 2.59 ± 0.13 * | 4.04 ± 0.25 | 1.53 ± 0.18 | 2.87 ± 0.29 |
| Leptin (1µg/ml) | 3.41 ± 0.34 | 4.40 ± 0.33 | 1.81 ± 0.27 | 2.15 ± 0.20 |
| Leptin (2µg/ml) | 4.55 ± 0.41 [b] | 4.50 ± 0.20 | 2.33 ± 0.16 [a] | 2.29 ± 0.21 |
| Leptin (5µg/ml) | 5.06 ± 0.22 [c, d] | 4.71 ± 0.36 | 2.55 ± 0.15 [b] | 2.61 ± 0.18 |
| Correlation coefficient (r) | 0.99 | 0.97 | 0.83 | 0.98 |

* All values are expressed as mean ± standard error (S.E.); N = 4.

[a, b, c] Differ significantly from the respective control group: $p < 0.05$, 0.02 and 0.01, respectively.

[d] Differs significantly from the respective 1µg/ml treated group: $p < 0.02$.

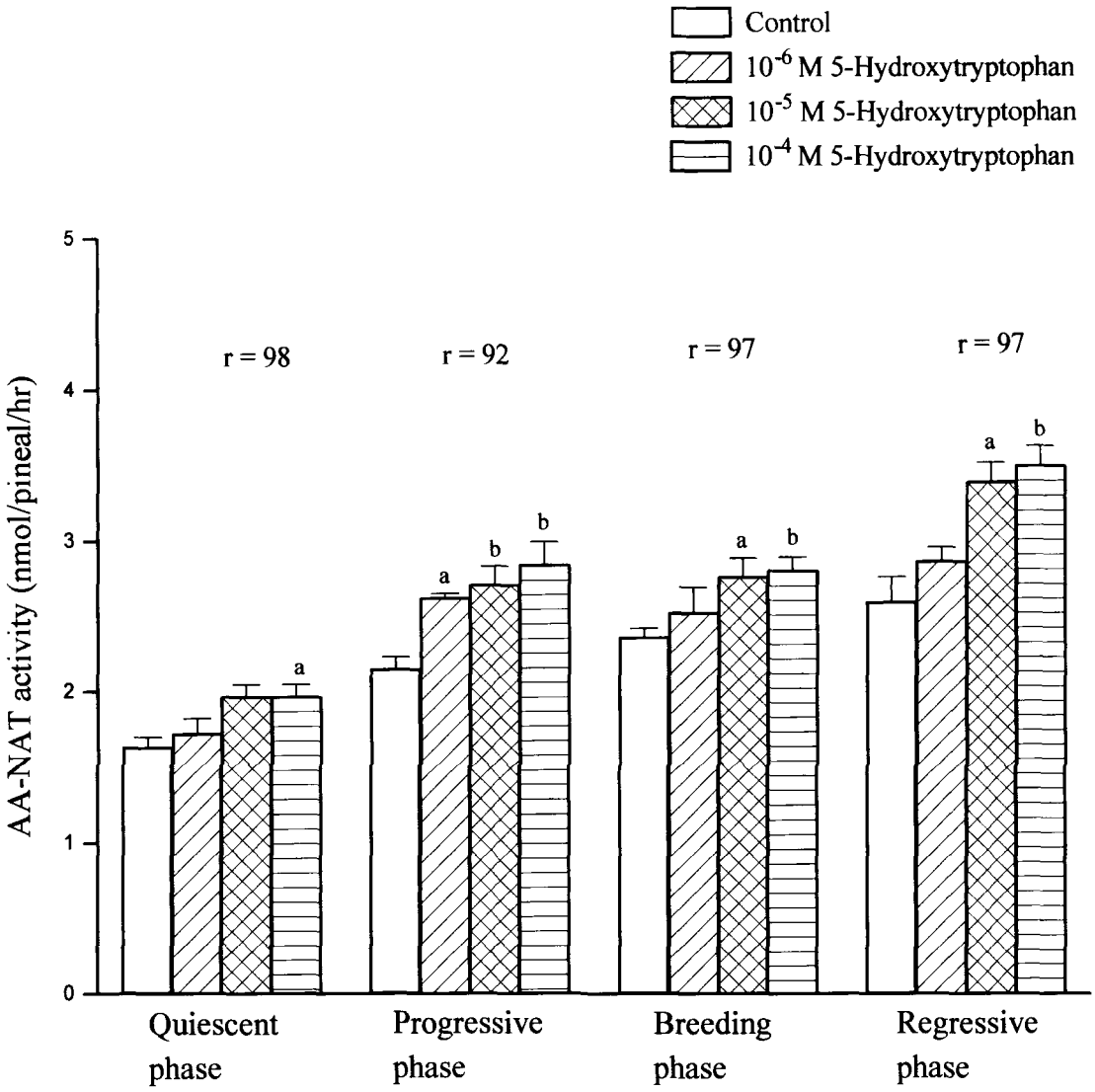



**Fig. 3:1A** - Effects of 5-hydroxytryptophan on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle.

All values are expressed as mean ± standard error (S. E.); N = 4.

[a, b] Differ significantly from their respective control group: $p < 0.05$ and 0.02, respectively.

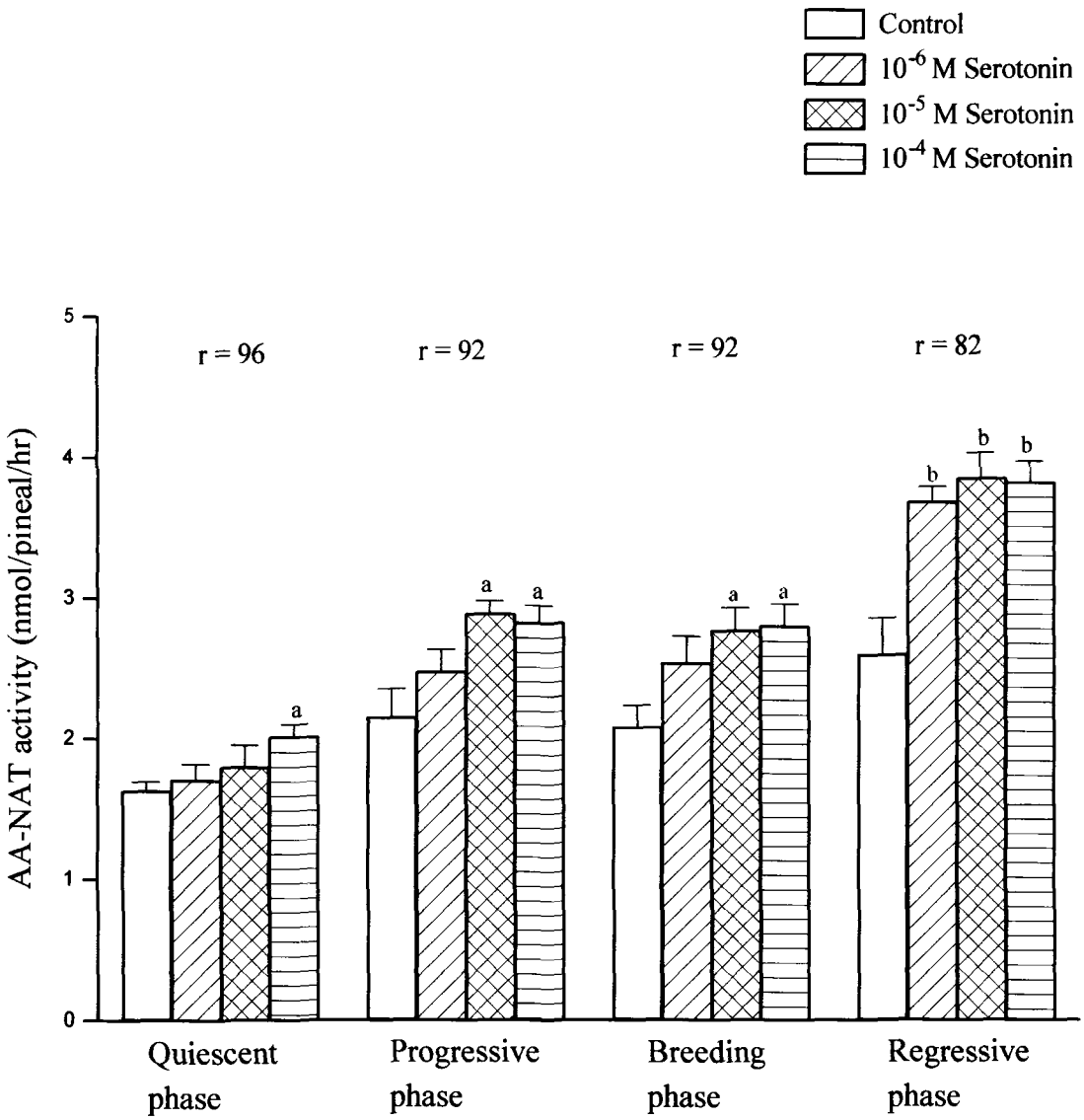



**Fig. 3:1B** - Effects of serotonin on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus*during different phases of the breeding cycle.

All values are expressed as mean ± standard error (S. E.); N = 4.

[a, b] Differ significantly from their respective control group: $p < 0.05$ and 0.02, respectively.

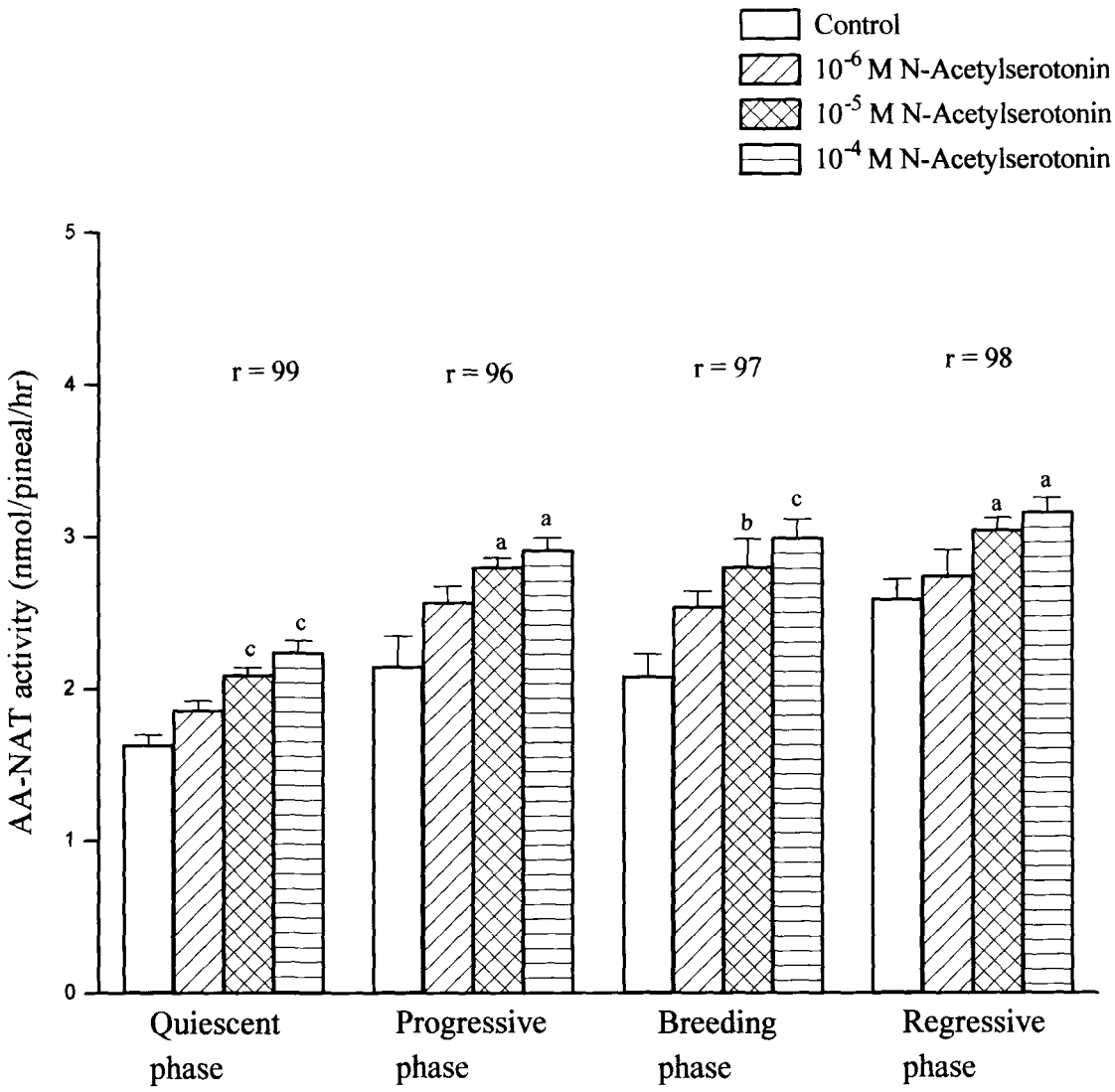



**Fig. 3:1C** - Effects of N-acetylserotonin on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle.

All values are expressed as mean ± standard error (S. E.); N = 4.

[a, b, c] Differ significantly from their respective control group: $p < 0.05$, 0.02 and 0.01, respectively.

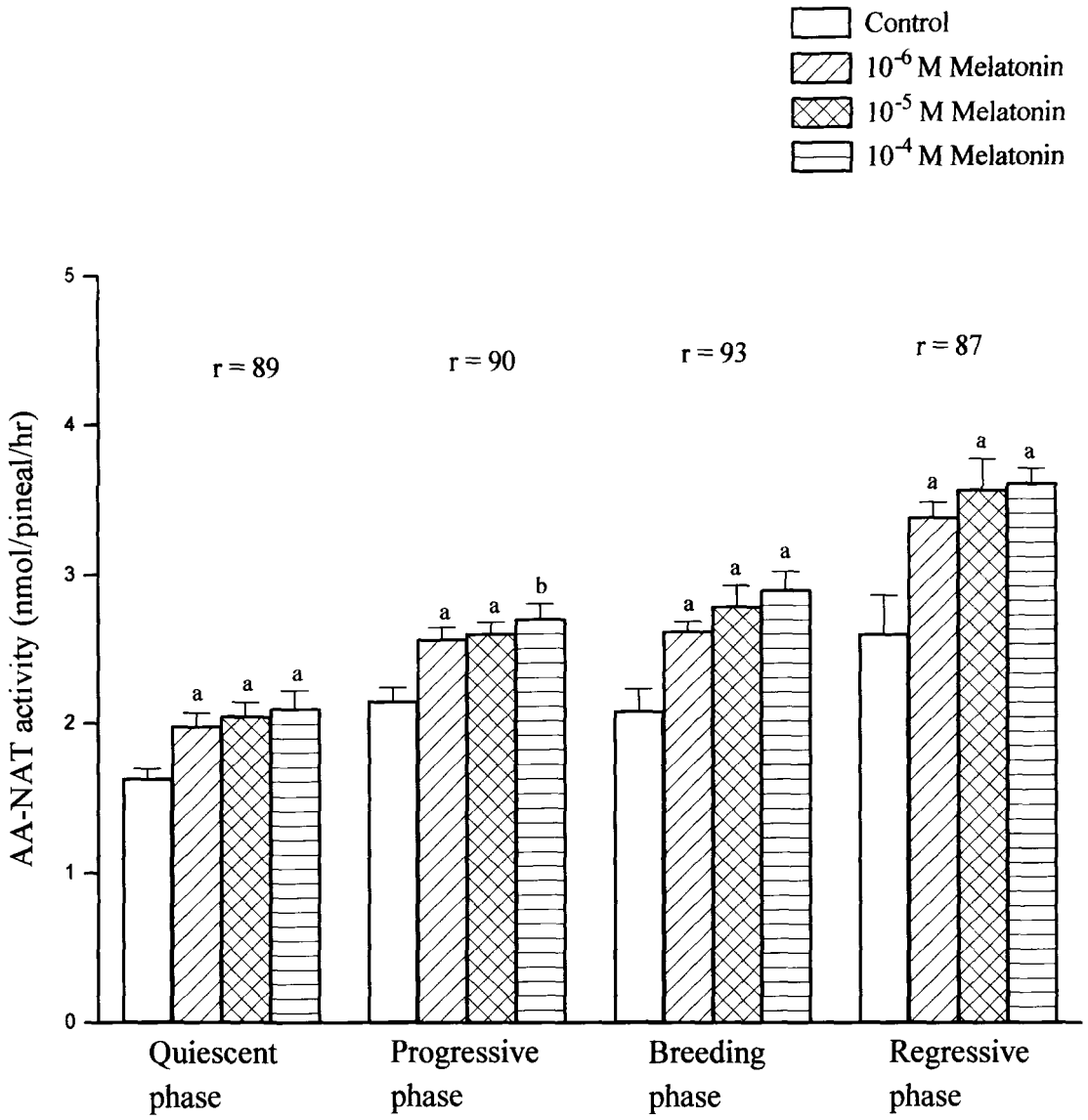



**Fig. 3:1D** - Effects of melatonin on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of *Clarias gariepinus* during different phases of the breeding cycle.

All values are expressed as mean ± standard error (S. E.); N = 4.

[a, b] Differ significantly from their respective control group: $p < 0.05$ and 0.02, respectively.

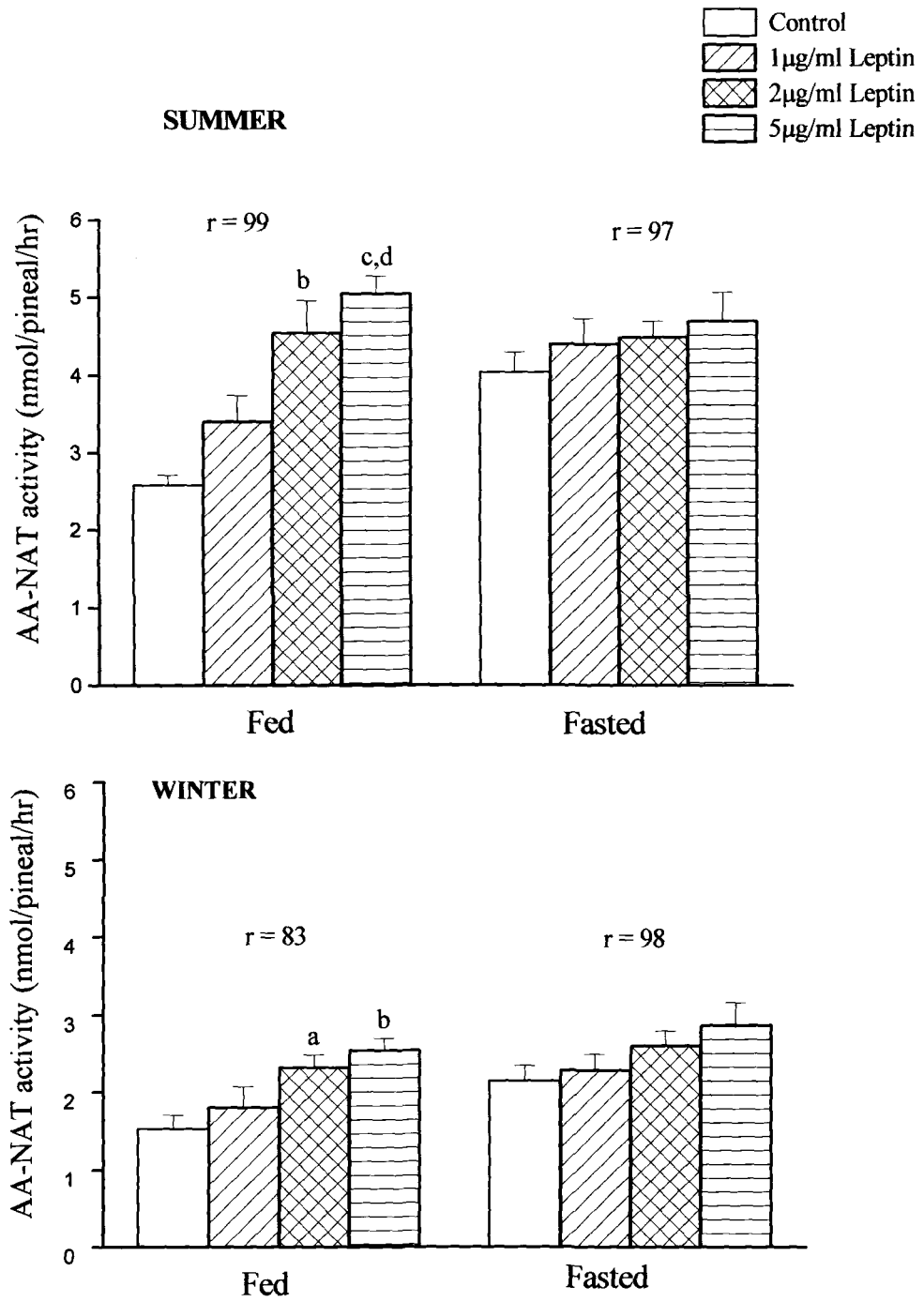



**Fig. 3:2** - Effects of leptin on arylalkylamine N-acetyltransferase (AA-NAT) activity in the pineal of fed and fasted *Clarias gariepinus* during summer and winter seasons.

All values are expressed as mean ± standard error (S. E.); N = 4.

[a, b, c] Differ significantly from their respective control group: $p < 0.05$, 0.02 and 0.01, respectively.

[d] Differs significantly from the treated 1μg/ml group: $p < 0.05$.

# CHAPTER - 4

# SUMMARY AND CONCLUSIONS

Arylalkylamine-N-acetyltransferase (AA-NAT) is the rate-limiting enzyme in the biosynthesis of melatonin. In the mammalian pineal, the diurnal rhythm of AA-NAT activity is regulated primarily by adrenergic mechanism, and other hormones have been reported to produce modulatory effects on the enzyme. However, there is practically no information on the role of hormones other than norepinephrine in the regulation of AA-NAT activity in the photoreceptive fish pineal. Therefore, keeping in view the scarcity of information on the role of gonadal steroids, corticosteroids, indoleamines and leptin in the regulation of AA-NAT activity in the fish pineal, a comprehensive study was undertaken to study the *in vitro* effects of these hormones on AA-NAT activity in the photoreceptive pineal of an air-breathing catfish, *Clarias gariepinus* during different phases of its breeding cycle.

The present Ph. D dissertation has been divided into four chapters. A brief summary of the chapters has been given below.

**Chapter 1 - Materials and methods**

This chapter deals with the details of the experimental animal, experimental conditions, tissue culture of the fish pineal, mode of treatments, methods used for measurement of pineal AA-NAT activity and the biostatistical methods used for analyzing the data.

## Chapter 2 - *In vitro* effects of gonadal steroids and corticosteroids on arylalkyl-amine-N-acetyltransferase (AA-NAT) activity in the fish pineal during different phases of the breeding cycle

This chapter deals with the study of *in vitro* effects of different concentrations of androgen (testosterone), estrogens (17-β estradiol, estriol and estrone) and corticosteroids (corticosterone and cortisol) on AA-NAT activity in the fish pineal during quiescent, progressive, breeding and regressive phases of the annual breeding cycle. The major findings of the experiments included in this chapter are mentioned below:

1. Testosterone inhibited pineal AA-NAT activity in a dose-dependent manner during all the phases of the annual breeding cycle.
2. 17β-estradiol and estriol inhibited the enzyme activity in a dose-dependent manner during all the four phases of the breeding cycle.
3. Estrone significantly inhibited the enzyme activity only during the breeding and the regressive phases but not during the quiescent and the progressive phases.
4. 17β-estradiol was found to be more effective in inhibiting AA-NAT activity as compared to estriol and estrone.
5. AA-NAT activity was comparatively more sensitive to the inhibitory effects of the gonadal hormones (testosterone, 17β-estradiol, estriol and estrone) during the regressive phase and less sensitive during the quiescent phase.

6. Corticosterone and cortisol decreased pineal AA-NAT activity in a dose-dependent manner during all the phases of the breeding cycle except during the quiescent phase, where none of the doses of corticosterone had any significant effect on the enzyme activity.
7. AA-NAT activity was more sensitive to the inhibitory effects of corticosterone and cortisol during the breeding phase and less sensitive during the quiescent phase.
8. Regression analysis indicated a negative correlation between the doses of each steroid hormone and pineal AA-NAT activity.

Present findings seem to indicate that various gonadal steroids and corticosteroids inhibit the pineal AA-NAT activity in the photoreceptive fish pineal during different phases of the annual breeding cycle. However, the degree of steroid hormone-induced inhibition and the sensitivity of AA-NAT activity seem to depend on the phase of the breeding cycle/seasons. Further, the steroid hormones might be inhibiting AA-NAT activity by their action(s) via inhibition of cAMP-dependent AA-NAT induction and/or via stimulation of proteasomal proteolysis of AA-NAT and/or 14-3-3 proteins.

## Chapter 3 - *In vitro* effects of indoleamines and leptin on arylalkylamine N-acetyltransferase (AA-NAT) activity in the fish pineal during different phases of the breeding cycle

This chapter deals with *in vitro* effects of different concentrations of 5-hydroxytryptophan, serotonin, N-acetylserotonin and melatonin on pineal AA-NAT activity in the fish maintained under natural climatic conditions during quiescent, progressive, breeding and regressive phases of the annual breeding cycle. This chapter also deals with *in vitro* effects of different doses of leptin on pineal AA-NAT activity in fed and fasted groups of fishes maintained under natural climatic conditions during winter and summer seasons. The findings and conclusions based on the experiments included in this chapter are listed below:

1. 5-Hydroxytryptophan, serotonin, N-acetylserotonin and melatonin increased pineal AA-NAT activity in a dose-dependent manner during all the phases of the breeding cycle of fish.
2. The sensitivity of AA-NAT activity to 5-hydroxytryptophan was comparatively higher during the progressive phase and lower during the quiescent phase.
3. Serotonin was comparatively more stimulatory during the regressive phase and less during the quiescent phase.
4. AA-NAT activity was found to be equally sensitive to the stimulatory effects of N-acetylserotonin and melatonin during all the phases of the breeding cycle.

5. Leptin treatment significantly increased pineal AA-NAT activity in the fed fishes in a dose-dependent manner during both summer and winter seasons.
6. Leptin had no significant effect on the enzyme activity in the pineals of starved groups irrespective of the doses and the seasons.

These findings seems to suggest that various indoleamines might be acting as substrates or hormones (autocrine/paracrine) and play an important role in the regulation of AA-NAT activity and melatonin synthesis in the photoreceptive fish pineal. Leptin also seems to be involved in regulation of AA-NAT activity in the fish pineal, and the sensitivity of the enzyme activity to leptin seems to depend on seasons and feeding status of the fish. Further, the feeding status can act as switch for the action of leptin on AA-NAT activity in the fish pineal.

## CONCLUSIONS

On the basis of the findings of the present Ph. D. dissertation, it can be concluded that various hormones such as gonadal steroids, corticosteroids, indoleamines and leptin play an important role in the regulation of AA-NAT activity and, hence melatonin synthesis in the pineal of the fish. The sensitivity of the pineal AA-NAT activity to inhibitory effects of gonadal hormones and corticosteroids as well as to the stimulatory effects of indoleamines seems to change with the phase of the annual breeding cycle of the fish and/or seasons. These findings also seem to suggest that the sensitivity of the pineal AA-NAT activity to leptin is dependent on seasons and feeding status of the fish. Feeding status of the fish can act as switch for

the sensitivity of the rate-limiting enzyme (and hence melatonin synthesis) to the stimulatory action of leptin.

These findings suggest that, though the melatonin synthesis is regulated in the fish pineal primarily by the light-dark cycle, the circulating levels of gonadal steroids, corticosteroids, indoleamines and leptin, depending on their levels, phase of the breeding cycle and/or seasons, also influence AA-NAT activity and hence melatonin synthesis in the photoreceptive pineal of the fish, *Clarias gariepinus*.

These findings, thus, suggest that the AA-NAT activity in the fish pineal is significantly influenced by the circannual changes in the levels of the gonadal steroids, corticosteroids and pineal indoleamines in relation to the ever-changing environmental factors and the breeding cycle. Leptin can also stimulate activity of AA-NAT and melatonin synthesis, and feeding status regulates the sensitivity of the enzyme activity to leptin in the fish pineal.

## References


Agha, A. K., and Joy, K. P. (1989). Effect of pinealectomy on the histology of the adrenacortical homologues and plasma cortisol level in the Indian catfish, *Heteropneustes fossilis* (Bloch). J. Pineal Res., **6**(4), 335-340.

Alonso, R., Abreu, P., Fajardo, N., Hernandez-Diaz, F., Diaz-Cruz, A., Hernandez, G., and Sanchez-Criado, J. (1995). Ovarian hormones regulate $\alpha_1$-and β-adrenoceptor interactions in female rat pinealocytes. Neuroreport, **6**, 345-348.

Amano, M., Iigo, M., Ikuta, K., Kitamura, S., Okuzawa, K., Yamada, H., and Yamamori, K. (2004). Disturbance of plasma melatonin profile by high dose melatonin administration inhibits testicular maturation of precocious male masu salmon. Zool. Sci., **21**, 79-85.

Arendt, J. (1985). Mammalian pineal rhythms. Pineal Res. Rev., **3**, 161-201.

Aspengren, S., Skold, H. N., Quiroga, G., Martensson, L., and Wallin, M. (2003). Noradrenaline- and melatonin- mediated regulation of pigment aggregation in the fish pineal melanophores. Pigment Cell Res., **16**(1), 59-64.

Ayre, E. A., and Pang, S. F. (1994). 2-[125I] iodomelatonin binding sites in the testis and ovary: putative melatonin receptors in the gonads. Biol. Signals, **3**(2), 71-84.

Baker, D. M., Larsen, D. A., Swanson, P., and Dickhoff, W. W. (2000). Long term peripheral treatment of immature coho salmon (*Oncorhynchus kisutch*) with human leptin has no clear physiologic effect. Gen. Comp. Endocrinol., **118**(1), 134-138.

Baler, R., Covington, S., Klein, D. C. (1997). The rat arylalkylamine N-acetyl-transferase gene promoter. J. Biol. Chem., **272**, 6976-6985.

Bayarri, M. J., Iigo, M., Munoz-Cueto, J. A., Isorna, E., Delgado, M. J., Madrid, J. A., Sanchez-Vazquez, F. J., and Alonso-Gomez, A. L. (2004). Binding characteristics and daily rhythms of melatonin receptors are distinct in the retina and the brain areas of the European sea bass retina (*Dicentrarchus labrax*). Brain Res., **1029**(2), 241-250.

Begay, V., Valotaire, Y., Ravault, J. P., Collin, J. P., and Falcon, J. (1993). Photoreceptor cells of the pineal body in culture: effect of 17 β-estradiol on the production of melatonin. C. R. Seances Soc. Biol. Fil., **187**(1), 77-86.

Begay, V., Valotaire, Y., Ravault, J. P., Collin, J. P., and Falcon, J. (1994). Detection of estrogen receptor mRNA in trout pineal and retina: estradiol-17β modulates melatonin production by cultured pineal photoreceptor cells. Gen. Comp. Endocrinol., **93**, 61-69.

Begay, V., Falcon, J., Cahill, G. M., Klein, D. C., and Coon, S. L. (1998). Transcripts encoding two melatonin synthesis enzymes in the teleost pineal organ: circadian regulation in pike and zebrafish, but not in trout. Endocrinology, **139**, 905-912.

Benyassi, A., Schwartz, C., Coon, S. L., Klein, D. C., and Falcon, J. (2000). Melatonin synthesis: arylalkylamine N-acetyltransferases in trout retina and pineal organ are different. Neuroreport, **11**(2), 255-258.

Benyassi, A., Schwartz, C., Ducouret, B., and Falcon, J. (2001). Glucocorticoid receptors and serotonin N-acetyltransferase activity in the fish pineal organ. Neuroreport, **12**, 889-892.

Berlie, J. R., Iversen, L. J., Blaxall, H. S., Cooley, M. E., Chacko, D. M., and Bylund, D. B. (1995). $\alpha_2$-Adrenergic receptors in the bovine retina. Presence of only the $\alpha_{2D}$ subtype. Invest. Ophthalmol. Vis. Sci., **36**(9), 1885-1892.

Bernard, M., Iuvone, P. M., Cassone, V. M., Roseboom, P. H., Coon, S. L., and Klein, D. C. (1997). Avian melatonin synthesis: photic and circadian regulation of serotonin N-acetyltransferase mRNA in the chicken pineal gland and retina. J. Neurochem., **68**(1), 213-224.

Binkley, S. (1976a). Comparative biochemistry of the pineal glands of birds and mammals. Amer. Zoologist., **16**, 57-65.

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

Binkley, S., Macbride, S., Klein, D. C., and Ralph, C. (1973). Pineal enzymes: regulation of avian melatonin synthesis. Science, **181**, 273-275.

Binkley, S., Riebman, J. B., and Reilly, K. (1978). Regulation of pineal rhythms in chikens: inhibition of dark time N-acetyltransferase activity. Comp. Biochem. Physiol., **59**, 165-171.

Binkley, S., Muller, G., and Hernandez, T. (1981). Circadian rhythm in pineal N-acetyltransferase activity: phase shifting by light pulses. I. J. Neurochem., **37**, 798-800.

Binkley, S., Mosher, K., and Reilly, K. B. (1983). Circadian rhythms in house sparrows: lighting *ad lib*. Physiol. Behav., **31**(6), 829-837.

Bolliet, V., Begay, V., Taragnat, C., Rayault, J. P., Collin, J. P., and Falcon, J. (1997). Photoreceptor cells of the pike pineal organ as cellular circadian oscillators. Eur. J. Neurosci., **9**, 643-653.

Bothorel, B., Barassin, S., Saboureau, M., Perreau, S., Vivien-Roels, B., Malan, A., and Pevet, P. (2002). In the rat, exogenous melatonin increases the amplitude of pineal melatonin secretion by a direct action on the circadian clock. Eur. J. Neurosci., **16**(6), 1090-1098.

Brown, G. M., Pang, C. S., and Pang, S. F. (1994). Binding sites for 2-[125I] iodomelatonin in the adrenal gland. Biol. Signals, **3**(2), 91-98.

Brunken, W. J., and Jin, X. T. (1993). A role for $5HT_3$ receptors in visual processing in the mammalian retina.Vis. Neurosci., **10**(3), 511-522.

Bubenik, G. A., Blask, D. E., Brown, G. M., Maestroni, G. J., Pang, S. F., Reiter, R. J., Viswanathan, M., and Zisapel, N. (1998). Prospects of the clinical utilization of melatonin. Biol. Signals. Recept., **7**, 195-219.

Buettner, C., Harney, J. W., and Larsen, P. R. (1998). The 3'-untranslated region of human type 2 iodothyronine deiodinase mRNA contains a functional selenocysteine insertion sequence element. J. Biol. Chem., **273**(50). 33374-33378.

Bylund, D. B., Rudeen, P. K., Petterborg, L. J., and Ray-Prenger, C. (1988). Identification of alpha 2-adrenergic receptors in chicken pineal gland using [3H] rauwolscine. J. Neurochem., **51**(1), 81-86.

Cahill, G. M. (1996). Circadian regulation of melatonin production in cultured zebrafish pineal and retina. Brain Res., **708**, 177-181.

Cardinali, D. P. (1977). Nuclear receptor estrogen complex in the pineal gland. Modulation by sympathetic nerves. Neuroendocrinology, **24**, 50-58.

Cardinali, D. P., Nagle, C. A., and Rosner, J. M. (1975). Control of estrogen and androgen receptors in the rat pineal gland by catecholamine transmitter. Life Sci., **16**, 81-91.

Cardinali, D. P., Vacas, M. I., Solveyra, C. G., Sarmiento, M. I., and Vollrath, L. (1986). *In vitro* effects of estradiol, testosterone and progesterone on 5-methoxyindole content, cyclic adenosine 3', 5'-monophosphate synthesis, and norepinephrine release in different parts of the female guinea pig pineal complex. J. Pineal Res., **3**(4), 351-363.

Cardinali, D. P., Vacas, M. I., Keller Sarmiento, M. I., Etchegoyon, G. S., Pereyra, E. N., and Chuluyan, H. E. (1987). Neuroendocrine integrative mechanisms in mammalian pineal gland: effects of steroids and adrenohypophysial hormones on melatonin synthesis *in vitro*. J. Steroid Biochem., **27**(1-3), 565-571.

Cassone, V. M., Lane, R. F., and Menaker, M. (1983). Daily rhythms of serotonin metabolism in the medial hypothalamus of the chicken: effects of pinealectomy and exogenous melatonin. Brain Res., **289**(1-2), 129-134.

Cassone, V. M., Takahashi, J. S., Blaha, C. D., Lane, R. F., and Menaker, M. (1986). Dynamics of noradrenergic input to the chicken pineal gland. Brain Res., **384**, 334-341.

Catala, M. D., Du, J. Z., Quay, W. B., and Timiras, P. S. (1984). Effects of melatonin and control injections on pineal serotonin and norepinephrine: afternoon injections lower serotonin levels thirty-six percent at light-dark transition. J. Pineal Res., **1**(1), 75-82.

Catala, M. D., Quay, W. B., and Timiras, P. S. (1988). Effects of thyroid hormone on light/dark melatonin synthesis and release by young and maturing rat pineal glands *in vitro*. Int. J. Devl. Neurosci., **6**, 285-288.

Ceinos, R. M., Rabade, S., Soengas, J. L., and Migez, J. M. (2005). Indoleamines and 5-methoxyindoles in trout pineal organ *in vivo*: daily changes and influence of photoperiod. Gen. Comp. Endocrinol., **144**(1), 67-77.

Champney, T. H., Webb, S. M., Richardson, B. A., and Reiter, R. J. (1985a). Hormonal modulation of cyclic melatonin production in the pineal gland of rats and Syrian hamsters: effects of thyroidectomy or thyroxine implant. Chronobiol. Intl., **2**(3), 177-183.

Champney, T. H., Craft, C. M., Webb, S. M., and Reiter, R. J. (1985b). Hormonal modulation of pineal melatonin synthesis in rats and Syrian hamsters: effects of adrenalectomy and corticosteroid implants. J. Neural Transm., **64**(1), 67-79.

Chansard, M., Iwahana, E., Liang, J., and Fukuhara, C. (2005). Regulation of cAMP-induced arylalkylamine N-acetyltransferase, Period1, and MKP-1 gene expression by mitogen-activated protein kinases in the rat pineal gland. Brain Res., **139**(2), 333-340.

Chelikani, P. K., Glimm, D. R., and Kennelly, J. J. (2003). Short communication: tissue distribution of leptin and leptin receptor mRNA in the bovine. J. Dairy Sci., **86**(7), 2369-2372.

Chiba, A., Hattori, A., and Iigo, M. (2005). Daily and circadian variations of the pineal and ocular melatonin contents and their contributions to the circulating melatonin in the Japanese newt, *Cynops pyrrhogaster*. Zool. Sci., **22**(1), 65-70.

Chik, C. L., and Ho, A. K. (1989). Multiple receptor regulation of cyclic nucleotides in rat pinealocytes. Mol. Biol., **53**, 197-203.

Chowdhury, I., Chien, J. T., Chatterjee, A., and Yu, J. Y. (2004). *In vitro* effects of mammalian leptin, neuropeptide-Y, β-endorphin and galanin on transcript levels of thyrotropin beta and common alpha subunit mRNAs in the pituitary of bighead carp (*Aristichthys nobilis*). Comp. Biochem. Physiol., **139**(1), 87-98.

Clementi, F., Fraschini, F., Muller, E., and Zanoboni, A. (1965). The pineal gland and the control of electrolyte balance and of gonadotropic secretion. Functional and morphological observation. Brain Res., **10**, 585-603.

Coon, S. L., Roseboom, P. H., Baler, R., Weller, J. L., Namboodiri, M. A., Koonin, E. V., and Klein, D. C. (1995). Pineal serotonin N-acetyltransferase: expression cloning and molecular analysis. Science, **270**(5242), 1681-1683.

Coon, S. L., Begay, V., Deurloo, D., Falcon, J., and Klein D. C. (1999). Two arylalkylamine N-acetyltransferase genes mediate melatonin synthesis in fish. J. Biol. Chem., **274**(13), 9076-9082.

Coon, S. L, Weller, J. L., Korf, H. W., Namboodiri, M. A, Rollag, M., and Klein D. C. (2001). cAMP regulation of arylalkylamine N-acetyltransferase (AANAT, EC 2.3.1.87): a new cell line (1E7) provides evidence of intracellular AANAT activation. J. Biol. Chem., **276**(26), 24097-24107.

Coon, S. L., Olmo, D. E., Young, W. S., and Klein, D. C. (2002). Melatonin synthesis enzymes in *Macaca mulatta*: focus on arylalkylamine N-acetyltransferase (EC 2.3.1.87). J. Clin. Endocrinol. Metab., **87**(10), 4699-4706.

Couce, M. E., Burguera, B., Parisi, J. E., Jensen, M. D., and Lloyd, R. V. (1997). Localization of leptin receptor in the human brain. Neuroendocrinology, **66**(3), 145-150.

d' Istria, M., Momteleone, P., Serino, I., and Chieffi, G. (1994). Seasonal varations in the daily rhythm of melatonin and NAT activity in the harderian gland, retina, pineal gland and serum of the green frog, *Rana esculenta*. Gen. Comp. Endocrinol., **96**, 6-11.

Dal Farra, C., Zsurger, N., Vincent, J. P., and Cupo, A. (2000). Binding of a pure 125I-monoiodoleptin analog to mouse tissues: a developmental study. Peptides, **21**(4), 577-587.

Dallaporta, B., Pablo, M., Maisse, C., Daugas, E., Loeffler, M., Zamzani, N., and Kroemer, G. (2000). Proteasome activation as a critical event of thymocyte apoptosis. Cell Death Differ., 7(4), 368-373.

Davis, G. A. (1978). The response of adenosine 3', 5'-monophosphate to norepinephrine in the hypothalamus and pineal organ of female rats in proestrus or diestrus. Endocrinology, **103**(4), 1048-1053.

Deguchi, T. (1979a). Role of cAMP in the regulation of circadian oscillation of serotonin N-acetyltransferase activity in cultured chicken pineal. J. Neurochem., **33**, 45-51.

Deguchi, T. (1979b). Role of adenosine 3', 5' monophosphate in the regulation of circadian oscillation of serotonin N-acetyltransferase activity in the cultured chicken pineal gland. J. Neurochem., **33**, 45-51.

Deguchi T. (1979c). A circadian oscillator in cultured cells of chicken pineal gland. Nature, **282**, 94-96.

Deguchi, T. (1981). Rhodopsin-like photosensitivity of isolated chicken pineal gland. Nature, **290**, 706-707.

Deguchi, T., and Axelrod, J. (1972). Sensitive assay for serotonin N-acetyl-transferase activity in rat pineal. Anal. Biochem., **50**(1), 174-179.

de Pedro, N., Martinez-Alvarez, R., and Delgado, M. J. (2006). Acute and chronic leptin reduces food intake and body weight in goldfish (*Carassius auratus*). J. Endocrinol., **188**(3), 513-520.

de Vlaming, V. L., Sage, M., and Charlton, C. B. (1974). The effects of melatonin treatment on gonosomatic index in the teleost, *Fundulus similis* and the tree frog, *Hyla cinerea*. Gen. Comp. Endocrinol., **22**, 433-438.

de Vlaming, V. L., and Vodicnik, M. J. (1977). Effects of pinealeactomy on pituitary gonadotrophs, pituitary gonadotropin potency and hypothalamic gonadotropin releasing activity in *Notemigonus crysoleucas*. J. Fish Biol., **10**, 73-86.

Delgado, M. J., and Vivien-Roels, B. (1989). Effects of environmental temperature and photoperiod on the melatonin levels in the pineal, lateral eye, and plasma of the frog, *Rana perezi*: importance of ocular melatonin. Gen. Comp. Endocrinol., **75**, 46-53.

Demisch, L., Demisch, K., and Nickelsen, T. (1988). Influence of dexamethasone on nocturnal melatonin production in healthy adult subjects. J. Pineal Res., **5**, 317–322.

Drijfhout, W. J., Grol, C. J., and Westerink, B. H. (1996). Parasympathetic inhibition of pineal indole metabolism by prejunctional modulation of noradrenaline release. Eur. J. Pharmacol., **308**(2), 117-124.

Dubocovich, M. L. (1985). Characterization of a retinal melatonin receptor. J. Pharmacol. Exp. Ther., **234**(2), 395-401.

Eddy, J. M. (1969). Metamorphosis and the pineal complex in the brook lamprey, *Lampetra planeri*. J. Endocrinol., **44**(3), 451-452.

Elmquist, J. K., Bjorbaek, C., Ahima, R. S., Flier, J. S., and Saper, C. B. (1998). Distributions of leptin receptor mRNA isoforms in the rat brain. J. Comp. Neurol., **395**, 535-547.

Falcon, J. (1999). Cellular circadian clocks in the pineal. Prog. Neurobiol., **58**, 121-162.

Falcon, J., and Gaildrait, P. (1997). Variation in the cyclic adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate content and efflux from the photosensitive pineal organ of the pike in culture. Pflugers Arch., **433**(3), 336-342.

Falcon, J., Guerlotte, J. F., Voisin, P., and Collin, J. P. (1987). Rhythmic melatonin biosynthesis in a photoreceptive pineal organ: a study in the pike. Neuroendocrinology, **45**, 479-486.

Falcon, J., Marmillon, J. B., Claustrat, B., and Collin, J. P. (1989). Regulation of melatonin secretion in a photoreceptive pineal organ: an *in vitro* study in the pike. J. Neurosci., **9**(6), 1943-1950.

Falcon, J., Thibault, C., Martin, C., Brun-Marmillon, J., Claustirat, B., and Collin, J. P. (1991). Regulation of melatonin production by catecholamines and adenosine in a photoreceptive pineal organ. An *in vitro* study in the pike and the trout. J. Pineal Res., **11** (3-4), 123-134.

Falcon, J., Galarneau, K. M., Weller, J. L., Ron, B., Chen, G., Coon, S. L., and Klein, D. C. (2001). Regulation of arylalkylamine N-acetyltransferase-2 (AANAT2, EC 2.3.1.87) in the fish pineal organ: evidence for a role of proteasomal proteolysis. Endocrinology, **142**(5), 1804-1813.

Fenwick, J. C. (1970). Demonstration and effect of melatonin in fish. Gen. Comp. Endocrinol., **14**, 86-97.

Ferreira, Z. S., Fernandes, P. A., Duma, D., Assreuy, J., Avellar, M. C., and Markus, R. P. (2005). Corticosterone modulates noradrenaline-induced melatonin synthesis through inhibition of nuclear factor kappa B. J. Pineal Res., **38**(3), 182-188.

Ferretti, C., Blengio, M., Ghi, P., and Genazzani, E. (1990). Differential effects of indolepyruvic acid and 5-hydroxytryptophan on indole metabolism in the pineal gland of the rat during the light dark-cycle. Eur. J. Pharmacol., **187**(3), 345-356.

Filadelfi, A. M., and Castrucci, A. M. (1996). Serotonin and N-acetylseroronin effects on pigment cells of the toad *Bufo ictericus*: pharmacological characterization of melatonin receptors. Gen. Comp. Endocrinol., **103**(2), 192-199.

Fiske, V. M., and Huppert, L. C. (1978). Melatonin action on pineal varies with photoperiod. Science, **162**, 279-289.

Fleming, J. V., Barrett, P., Coon, S. I., Klein, D. C., and Morgan, P. J. (1999). Ovine arylalkylamine N-acetyltransferase in the pineal and pituitary gland: differences in function and regulation. Endocrinology, **142**, 972-978.

Foa, A., Janik, D., and Minutini, L. (1992). Circadian rhythms of plasma melatonin in the ruin lizard, *Podarcis sicula*: effects of pinealectomy. J. Pineal Res., **12**(3), 109-113.

Foldes, A., Hoskinson, R. M., Scaramuzzi, R. J., Hinks, N. T., and Maxwell, C. A. (1983). Modification of sheep pineal β-adrenoceptors by some gonadal steroids but not by melatonin. Neuroendocrinology, **37**(5), 378-385.

Forlano, P. M., and Bass, A. H. (2005). Steroid regulation of brain aromatase expression in glia: female preoptic and vocal motor nuclei. J. Neurobiol., **65**(1), 50-58.

Forlano, P. M., Deitcher, D. L., and Bass, A. H. (2005). Distribution of estrogen receptor alpha mRNA in the brain and inner ear of a vocal fish with comparisons to the sites of aromatase expression. J. Comp. Neurol., **483**(1), 91-113.

Foulkes, N. S., Borjigin, J., Snyder, S. H., and Sassone-Corsi, P. (1996). Transcriptional control of circadian hormone synthesis via the CREM feedback loop. Proc. Natl. Acad. Sci. USA, **93**(24), 14140-14145.

Foulkes, N. S., Whitemore, D., and Sassone-Corsi, P. (1997). Rhythmic transcription: the molecular basis of circadian melatonin synthesis. Biol. Cell, **89**(8), 487-494.

Foulkes, N. S., Cermakian, N., Whitmore, D., and Sassone-Corsi, P. (2000). Rhythmic transcription: the molecular basis of oscillatory melatonin synthesis. Novartis Found. Symp., **227**, 5-14.

Friedman, J. M. (2002). The functions of leptin in nutrition, weight, and physiology. Nutr. Rev., **60**, 1-14.

Freire, F., and Cardinali, D. P. (1975). Effects of melatonin treatment and environmental lighting on the ultrastructure appearance, melatonin synthesis, and norepinephrine turnover and microtubule protein content of the rat pineal gland. J. Neural Transm., **37**, 237-257.

Frungieri, M. B., Mayerhofer, A., Zitta, K., Pignataro, O. P., Calandra, R. S., and Gonzalez-Calvar, S. I. (2005). Direct effect of melatonin on Syrian hamster testes: melatonin subtype 1a receptors, inhibition of androgen production, and interaction with the local corticotropin-releasing hormone system. Endocrinology, **146**(3), 1541-1552.

Ganguly, S., Gastel, J. A., Weller, J. L., Schwartz, C., Jaffe, H., Namboodiri, M. A., Coon, S. L., Hickman, A. B., Rollag, M., Obsil, T., Beauverger, P., Ferry, G., Boutin, J. A., and Klein, D. C. (2001). Role of a pineal cAMP-operated arylalkylamine N-acetyltransferase/14-3-3-binding switch in melatonin synthesis. Proc. Natl. Acad. Sci. USA, **98**(14), 8083-8088.

Ganguly, S., Coon, S. L., and Klein, D. C. (2002). Control of melatonin synthesis in the mammalian pineal gland: the critical role of serotonin acetylation. Cell Tissue Res., **309**, 127-137.

Garg, S. K. (1989). Effect of pinealectomy, eye enucleation, and melatonin treatment on ovarian activity and vitillogenin levels in the catfish exposed to short photoperiod or long photoperiod. J. Pineal Res., 7(2), 91-104.

Garg, S. K., and Sundararaj, B. I. (1986). Role of pineal in the regulation of some aspects of circadian rhythmicity in the catfish, *Heteropneustes fossilis* (Bloch). Chronobiologia, **13**(1), 1-11.

Gasser, P. J., and Gern, W. A. (1997). Regulation of melatonin synthesis in rainbow trout (*Orcorhyncus mykiss*) pineal organs: effects of calcium depletion and calcium channel drugs. Gen. Comp. Endocrinol., **105**(2), 210-217.

Gastel, J. A., Roseboom, P. H., Rinaldi, P. A., Weller, J. L., and Klein, D. C. (1998). Melatonin production: proteasomal proteolysis in serotonin N-acetyl-transferase regulation. Science, **279**, 1358-1360.

Gern, W. A., and Norris, D. O. (1979). Plasma melatonin in the neotenic tiger salamander (*Ambystoma tigrinum*): effects of photoperiod and pinealectomy. Gen. Comp. Endocrinol., **38**(4), 393-398.

Gern, W. A., and Greenhouse, S. S. (1988). Examination of *in vitro* melatonin secretion from superfused trout (*Salmo gairdneri*) pineal organs maintained under diel illumination or continuous darkness. Gen. Comp. Endocrinol., **71**(1), 163-174.

Gillette, M. U., and Mitchell, J. W. (2002). Signaling in the suprachiasmatic nucleus: selectively responsive and integrative. Cell Tissue Res., **309**(1), 99-107.

Gonzalez-Martinez, D., Madigou, T., Mananos, E., Cerda-Reverter, J. M., Zanuy, S., Kah, O., and Munoz-Cueto, J. A. (2004). Cloning and expression of gonadotropin-releasing hormone receptor in the brain and pituitary of the European sea bass: an *in situ* hybridization study. Biol. Reprod., **70**(5), 1380-1391.

Gothilf, Y., Coon, S. L., Toyama, R., Chitnis, A., Namboodiri, M. A., and Klein, D. C. (1999). Zebrafish serotonin N-acetyltransferase-2: marker for development of pineal photoreceptors and circadian clock function. Endocrinology, **140**(10), 4895-4903.

Govitrapong, P., Prapapanich, V., and Ebadi, M. (1991). Identification of serotonin 5-$HT_2$ receptors in bovine pineal gland. J. Pineal Res., **11**, 182-187.

Grewal, S. S., Fass, D. M., Yao, H., Ellig, C. L., Goodman, R. H, and Stork, P. J. (2000). Calcium and cAMP signals differentially regulate cAMP-responsive element-binding protein function via a Rap1-extracellular signal-regulated kinase pathway. J. Biol. Chem., **275**(44), 34433-34441.

Guerlotte, J., Falcon, J., Voisin, P., and Collin, J. P. (1986). Indoles in the photoreceptor cells of the lamprey pineal complex. Ann. Endocrinol., **47**(1), 62-64.

Guerrero, J. M., and Reiter, R. J. (1992). Iodothyronine 5'-deiodinating activity in the pineal gland. Int. J. Biochem., **24**(10), 1513-1523.

Guerrero, J. M., Puig-Domingo, M., Santana, C., Menendez-Pelaez, A., Gonzalez-Brito, A., and Reiter, R. J. (1988). Differential responses of rat pineal thyroxine type II 5'-deiodenase and N-acetyltransferase activities to either light exposure, isoproterenol, phenylephrine, or propanolol. Cell Mol. Neurobiol., **8**(4), 447-458.

Guo, L., Munzberg, H., Stuart, R. C., Nillini, E. A., Bjorbaek, C. (2004). N-acety-lation of hypothalamic α-melanocyte stimulating hormone and regulation by leptin. Proc. Natl. Acad. Sci. USA, **101**(32), 11797-11802.

Gupta, B. B. P., and Premabati, Y. (2002). Fish Pineal: Structure, Function and Regulation. In "Treatise on Pineal Gland" (C. Haldar, M. Singaravel, and S. K. Maitra, eds.), pp. 77-102. Science Publishers Inc., Enfield (NH), USA.

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

Gupta, D., Haldar, C., Coeleveld, M., and Roth, J. (1993). Ontogeny, circadian rhythm pattern, and hormonal modulation of 5 α-dihydrotestosterone receptors in the rat pineal. Neuroendocrinlogy, **57**(1), 45-53.

Gupta, B. B. P., Spessert, R., and Vollrath, L. (2005). Molecular components and mechanism of adrenergic signal transduction in mammalian pineal gland: regulation of melatonin synthesis. Indian J. Exp. Biol., **43**, 115-149.

Hafeez, M. A. (1970). Effect of melatonin on body coloration and spontaneous swimming activity in rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol., **36**, 639-656.

Hafeez, M. A., and Quay, W. B. (1970). The role of pineal organ in the control of phototaxis and body colouration in rainbow trout (*Salmo gairdneri* Richardson). Z. Vergl. Physiol., 68, 403-416.

Haldar, C., and Pandey, R. (1988). Effect of melatonin and 5-methoxytryptamine administration on the testis and pineal gland activity of the fresh-water snake, *Natrix piscator*. Arch. Anat. Histol., **71**, 85-95.

Haldar, C., and Gupta, D. (1990). Sex-and age-dependent nature of the cytoplasmic 5α-dihydrotestosterone (DHT) binding site/receptor in bovine pineal gland. J. Pineal. Res., **8**(4), 289-295.

Haldar, C., and Vidhu, S. (1997). Effect of pinealectomy and testosterone on gonadal regression and accessory sex organ in Indian palm squirrel, *Funambulus pennanti*. Indian J. Exp. Biol., **35**(6), 594-596.

Haldar-Misra, C., and Pevet, P. (1983). The influence of luteinizing hormone-releasing hormone (LHRH) on the process of protein and/or peptide secretion characterized by the formation of granular vesicles in mammalian pinealocytes. Comparative *in vitro* study. Cell Tissue Res., **232**(3), 529-538.

Haldar, C., Fukada, Y., and Araki, M. (2003). Effects of gonadal steroids on pineal morphogenesis and cell differentiation of the embryonic quail studied under cell culture conditions. Brain Res., **145**(1), 71-79.

Hamm, H. E., Takahashi, J. S., and Menaker, M. (1983). Light induced decrease of serotonin N-acetyltransferase activity and melatonin in the chicken pineal gland and retina. Brain Res., **266** (2), 287-294.

Hanukoglu, I., Karavolas, H. J, and Goy, R. W. (1997). Progesterone metabolism in the pineal, brain stem, thalamus and corpus callosum of the female rat. Brain Res., **125**(2), 313-324.

Hayashi, K., and Okatani, Y. (1999). Mechanisms underlying the effects of estrogen on nocturnal melatonin synthesis in the peripubertal female rats: relation to norepinephrine and adenylate cyclase. J. Pineal Res., **26**, 178-183.

Hazel, J. R., McKinley, S. J., and William, E. E. (1992). Thermal adaptation in biological membranes interacting effects of temperature and pH. J. Comp. Physiol., **162**, 593-601.

Hensley, S. H., and Cohen, J. L. (1992). Effects of serotonergic agonists and antagonists on ganglion cells in the goldfish retina. Vis. Neurosci., **9**(3-4), 353-364.

Hernandez-Diaz, F. J., Sanchez, J. J., Abreu, P., Lopez-Coviella, I., Tabares, L., Prieto, L., and Alonso, R. (2001). Estrogen modulates alpha (1)/β-adrenoceptor-induced signaling and melatonin production in female rat pinealocytes. Neuroendocrinology, **73**, 111-122.

Ho, A. K., Chik, C. L, and Klein, D. C. (1987). Protein kinase C is involved in adrenergic stimulation of pineal cGMP accumulation. J. Biol. Chem., **262**(21), 10059-10064.

Ho, A. K., Chik, C. L, and Klein, D. C. (1989). Forskolin stimulates pinealocyte cGMP accumulation. Dramatic potentiation by an alpha 1-adrenergic $Ca^{2+}$ mechanism involving protein kinase C. FEBS Lett., **249**(2), 207-212.

Hoar, W. S. (1955). Phototactic and pigmentary responses of the sockeye salmon smolts following injury to the pineal organ. J. Fisheries Res. Board Can., **12**, 178-185.

Hosaka, T., Mimuro, T., Hamada, N., Itoh, M. T., and Ishizuka, B. (2002). Stimulatory effects of LH on release of melatonin and activities of its synthesizing enzymes NAT and HIOMT in organ-cultured pineal glands of female rats. Horm. Metab. Res., **34**(8), 441-445.

Houssay, A. B., and Barcelo, A. C. (1972). Effects of estrogens and progesterone upon the biosynthesis of melatonin by the pineal gland. Acta. Phyiol., **22**, 274-275.

Humlova, M and Illnerova H. (1990). Melatonin entrains the circadian rhythm in the rat pineal N-acetyltransferase activity. Neuroendocrinology, **52**(2), 196-199.

Hyde, L. L., and Underwood, H. (2000). Effects of melatonin administration on the circadian activity rhythm of the lizard *Anolis carolinensis*. Physiol. Behav., **71**(12), 183-192.

Ichinose, H., and Nagatsu, T. (1993). Molecular genetics of aromatic L-amino acid decarboxylase. Yakubutsu. Seishin. Kodo., **13**(4), 251-256.

Ikeda, K., and Inoue, S. (2004). Estrogen receptors and their downstream targets in cancer. Arch. Histol. Cytol., **67**(4), 435-442.

Illnerova, H., Trentini, G. P., and Maslova, L. (1989). Melatonin accelerates reentrainment of the circadian rhythm of its own production after an 8-h advance of the light-dark cycle. Comp. Physiol., **166**(1), 97-102.

Ishizuka, B., Fusama, S., Hirai, K., Hosaka, T., Hamada, N., Amemiya, A., and Itoh, M. T. (2000). Melatonin secretion from organ-cultured pineal glands of rats: modulation by gonadectomy and gonadotropin-releasing hormone agonist administration. Eur. J. Endocrinol., **142**(4), 387-392.

Iuvone, P. M., and Gan, J. (1994). Melatonin receptor-mediated inhibition of cyclic AMP accumulation in chick retinal cell cultures. J. Neurochem., **63**(1), 118-124.

Jin, X. T., and Brunken, W. J. (1998). Serotonin receptors modulate rod signals: a neuropharmacological comparison of light- and dark- adapted retinas. Vis. Neurosci., **15**(5), 891-902.

Johnson, R. M., Johnson, T. M. and Londraville, R. L. (2000). Evidence for leptin expression in fishes. J. Exp. Zool., **286**, 718-724.

Joss, J. M. P. (1973). The pineal complex, melatonin, and color change in the lamprey *Lampetra.* Gen. Comp. Endocrinol., 21, 188-195.

Kalapesi, F. B., Coroneo, M. T., and Hill, M. A. (2005). Human ganglion cells express the $\alpha_2$-adrenergic receptor: relevance to neuroprotection. Br. J. Ophthalmol., **89**(6), 758-763.

Kalsbeek, A., Cutrera, R. A., Van Heerikhuize, J. J., Van Der Vliet, J., and Buijs, R. M. (1999). GABA release from suprachiasmatic nucleus terminals is necessary for the light-induced inhibition of nocturnal melatonin release in the rat. Neuroscience, **91**(2), 453-461.

Kalsbeek, A., Garidou, M. L., Palm, I. F., Van Der Vliet, J., Simonneaux, V., Pevet, P., and Buijs, R. M. (2000). Melatonin sees the light: blocking GABA-ergic transmission in the paraventricular nucleus induces daytime secretion of melatonin. Eur. J. Neurosci., **12**(9), 3146-3154.

Kamiya, Y., Murakami, M., Araki, O., Hosoi, Y., Ogiwara, T., Mizuma, H., and Mori, M. (1999). Pretranslational regulation of rhythmic type II′ iodothyronine deiodinase expression by β-adrenergic mechanism in the rat pineal gland. Endocrinology, **140**(3), 1272-1278.

Karasek, M., Marek, K., and Kunert-Radek, J. (1978). Ultrastructure of rat pinealocytes *in vitro*: influence of gonadotropic hormones and LH-RH. Cell Tiss. Res., **195**, 547-556.

Kato, H., Fu, Z., Kotera, N., Sugahara, K., and Kuba, T. (1999). Regulation of the expression of serotonin N-acetyltransferase gene in Japanese quial (*Coturnia japonica*): Rhythmic pattern and effect of light. J. Pineal Res., **27**(1), 24-33.

Kavaliers, M. (1981). Circadian variations in the effects of cyclic nucleotides on the thermoregulatory behaviour of a teleost fish. Neuropharmacology, **20**(3), 293-296.

Kellner, M., Yassouridis, A., Manz, B., Steiger, A., Holsboer, F., and Wiedemann, K. (1997). Corticotropin-releasing hormone inhibits melatonin secretion in healthy volunteers - a potential link to low-melatonin syndrome in depression? Neuro-endocrinology, **65**(4), 284-290.

Kezuka, H., Furukawa, K., Aida, K., and Hanyu, I. (1988). Daily cycles in plasma melatonin levels under long or short photoperiod in the common carp, *Cyprinus carpio*. Gen. Comp. Endocrinol., **72**(2), 296-302.

Kikuchi, M., Chiba, A., and Aoki, K. (2000). Daily melatonin injections entrain the circadian change of synaptic ribbon number in the pineal organ of the Japanese newt. Neurosci. Lett., **285**(3), 181-184.

Klein, D. C. (1999). Serotonin N-acetyltransferase. A personal historical perspective. Adv. Exp. Med. Biol., **460**, 5-16.

Klein, D. C., and Weller, J. (1970). Indole metabolism in the pineal gland. A circadian rhythm in N-acetyltransferase activity. Science, **169**, 1093-1095.

Klein, D. C., Weller, J., Sudgen. A. L., Vanecek, J., Chick, C. L., and Ho, A. K. (1987). Integration of multiple receptor mechanisms regulating pineal cyclic nucleotides. In: Fundamentals and clinics in pineal research, (C. Pevet, ed.), pp. 111-119. Raven Press, New York.

Klein, D. C., Coon, S. L., Roseboom, P. H., Weller, J. L., Bernard, M., Gastel, J. A., Zatz, M., Iuvone, P. M., Rodriguez, I. R., Begay, V., Falcon, J., Cahill, G. M., Cassone, V. M., and Baler, R. (1997). The melatonin rhythm generating enzyme: molecular regulation of serotonin N-acetyltransferase in the pineal gland. Recent Prog. Horm. Res., **52**, 307-358.

Klein, D. C., Ganguly, S., Coon, S. L., Shi, Q., Gaildrat, P., Morin, F., Weller, J. L., Obsil, T., Hickman, A., and Dyda, F. (2003). 14-3-3 proteins in pineal photoneuroendocrine transduction: How many roles? J. Neuroendocrinology, **15**, 370-377.

Klosen, P., Bienvenu, C., Demarteau, O., Dardente, H., Guerrero, H., Pevet, P., and Masson-Pevet, M. (2002). The mt1 melatonin receptor and RORbeta receptor are co-localized in specific TSH-immunoreactive cells in the pars tuberalis of the rat pituitary. J. Histochem. Cytochem., **50**(12), 1647-1657.

Knoebl, I., Fitzpatrick, M. S., and Schreck, C. B. (1996). Characterization of glucocorticoid receptors in the brains of Chinook salmon, *Oncorhynchus tshawytscha*. Gen. Comp. Endocrinol., **101**(2), 195-204.

Koch, M., Mauhin, V., Stehle, J. H., Schomerus, C., and Korf, H. W. (2003). Dephosphorylation of pCREB by protein serine/threonine phosphatases is involved in inactivation of Aanat gene transcription in rat pineal gland. J. Neurochem., **85**(1), 170-179.

Korf, H. W. (1999). Evolution of melatonin producing pinealocytes. Adv. Exp. Med. Biol., **460**, 17-29.

Korf, H. W., Kroeber, S., and Schomerus, C. (1997). Regulatuion of the intracellular concentration of free calcium ions in pinealocytes of the rainbow trout and the rat. Biol. Signals, **6**(4-6), 201-211.

Korf, H. W., Schomerus, C., and Stehle, J. H. (1998). The pineal organ, its hormone melatonin, and the photoneuroendocrine system. Adv. Anat. Embrol. Cell Biol., **146**, 1-100.

Kos-Kudla, B., Ostrowska, Z., Marek, B., Ciesielska-Kopacz, N., Kajdaniuk, D., Kudla, M., and Bunter, B. (1997). Diurnal rhythm of serum melatonin, ACTH and cortisol in asthma patients with long term glucocorticoid treatment. Endocr. Regul., **31**(1), 47-54.

Kroeber, S., Schomerus, C., and Korf, H. W. (1998). A specific and sensitive double-mmunofluorescence method for the demonstration of S-antigen and serotonin in trout and rat pinealocytes by means of primary antibodies from the same donor species. Histochem. Cell Biol., **109**, 309-317.

Kroeber, S., Meissl, H., Maronde, E., and Korf, H. W. (2000). Analyses of signal transduction cascades reveal an essential role of calcium ions for regulation of melatonin biosynthesis in the light-sensitive pineal organ of the rainbow trout (*Oncorhynchus mykiss*). J. Neurochem., **74**(6), 2478-2489.

Kulczykowska, E., Sokolowska, E., Takvam, B., Stefansson, S., and Ebbesson, L. (2004). Influence of exogenous thyroxine on plasma melatonin in juvenile Atlantic salmon (*Salmo salar*). Comp. Biochem. Physiol. B Biochem. Mol. Biol., **137**(1), 43-47.

Kus, I., Oner, H., Ozogul, C., Ayar, A., Ozen, O. A., Sarsilmaz, M., and Kelestimur, H. (2002). Effects of estradiol benzoate on the ultarstructure of the pinealocyte in the ovariectomized rat. Neuroendocrinol. Lett., **23**, 405-410.

Lai, R. K., Chun, T., Hasson, D., Lee, S., Mehrbod, F., and Wheeler, L. (2002). $\alpha_2$-adrenoceptor agonist protects retinal function after acute retinal ischemic injury in the rat. Vis. Neurosci., **19**(2), 175-185.

Lashbrook, B. L., and Steinle, J. J. (2005). β-Adrenergic receptor regulation of pigment epithelial-derived factor expression in rat retina. Auton. Neurosci., **121**(1-2), 33-39.

Lee, J. H., Hung, C. F., Ho, C. C., Chang, S. H., Lai, Y. S., and Chung, J. G. (1997). Light-induced changes in frog pineal gland N-acetyltransferase activity. Neurochem. Int., **31**(4), 533-540.

Liefers, S. C., Te-Pas, M. F., Veerkamp, R. F., and Vander, Lende, T. (2002). Associations between leptin gene polymorphism and production, live weight, energy balance, feed intake and fertility in *Holstien heifers*. J. Dairy Sc., **85**(6). 1633-1638.

Londraville, R. L, and Duvall, C. S. (2002). Murine leptin injections increase intracellular fatty acid-binding protein in green sunfish (*Lepomis cyanellus*). Gen. Comp. Endocrinol., **129**(1), 56-62.

Luboshitzky, R., Tiosano, D., Ben-Harush, M., Thuma, I., Ayash, A., Lavie, P., and Etzione, A. (1995). Pseudo-precocious puberty in a male patient and the melatonin-testosterone relationship. J. Pediatr. Endocrinol. Metab., **8**(4), 295-299.

Luboshitzky, R., Lavi, S., Thuma, I., and Lavie, P. (1996a). Testosterone treatment alters melatonin concentrations in male patients with gonadotropin-releasing hormone deficiency. J. Clin. Endocrinol. Metab., **81**(2), 770-774.

Luboshitzky, R., Lavi, S., Thuma, I., and Lavie, P. (1996b). Nocturnal melatonin and luteinizing hormone rhythms in women with hyperprolactinemic amenorrhea. J. Pineal Res., **20**(2), 72-78.

Luboshitzky, R., Dharan, M., Goldman, D., Hiss, Y., Herer, P., and Lavie, P. (1997a). Immunohistochemical localization of gonadotropin and gonadal steroid receptors in human pineal glands. J. Clin. Endocrinol., **82**(3), 977-981.

Luboshitzky, R., Wagner, O., Lavis, S., Herer, P., and Lavie, P. (1997b). Abnormal melatonin secretion in hypogonadal men: the effect of testosterone treatment. Clin. Endocrinol., **48**, 669-670.

Luboshitzky, R., Dharan, M., Goldman, D., Herer, P., Hiss, Y., and Lavie, P. (1997c). Seasonal variation of gonadotropins and gonadal steroids receptors in the human pineal gland. Brain Res. Bull., **44**(6), 665-670.

Luboshitzky, R., Shen-Orr, Z., Ishai, A., and Lavie, P. (2000). Melatonin hypersecretion in male patients with adult-onset idiopathic hypogonadotropic hypogonadism. Exp. Clin Endocrinol. Diabetes, **108**, 142-145.

Lutterschmidth, D. I., Lutterschmidth, W. I., Ford, N.B., and Hutchison, V. H. (2002). Behavioural thermoregulation and the role of melatonin in a nocturnal snake. Horm. Behav., **41**(1), 41-50.

Maitra, S. K., and Dey, M. (1994). Castration and testosterone induced changes in the pinealocytes of roseringed parakeet, *Psittacula krameri*, during different phases of the annual testicular cycle. Anat. Anz., **176**, 363-368.

Margetic, S., Gazzola, C., Pegg, G. G., and Hill, R. A. (2002). Leptin: a review of its peripheral actions and interactions. Int. J. Obes. Relat. Metab. Disord., **9**, 811-818.

Marin, A., Urena, J., and Tabares, L. (1996). Intracellular calcium release mediated by noradrenaline and acetylcholine in mammalian pineal cells. J. Pineal Res., **21**(1), 15-28.

Maronde, E., Pfeffer, M., von Gall, C., Dehgani, F., Schomerus, C., Wicht, H., Kroeber, S., Olcese, J., Stehle, J. H., and Korf, H. W. (1999a). Signal transduction in the rodent pineal organ: from the membrane to the nucleus. Adv. Exp. Med. Biol., **460**, 109-131.

Maronde, E., Pfeffer, M., Olcese, J., Molina, C. A., Schlotter, F., Dehghani, F., Korf, H. W., and Stehle, J. H. (1999b). Transcription factors in neuroendocrine regulation: rhythmic changes in pCREB and ICER levels frame melatonin synthesis. J. Neurosci., **19**, 3326-3336.

Martensson, L. G., and Andersson, R. G. (1997). Denervation of pigment cells lead to a receptor that is ultrasensitive to melatonin and noradrenaline. Life Sci., **60**(18), 1575-1582.

Martensson, L. G., and Andersson, R. G. (1999). A pharmacological interaction between melatonin and the alpha 2-adrenoceptor in cuckoo wrasse melanophores. Adv. Exp. Med. Biol., **460**, 221-228.

Martensson, L. G., and Andersson, R. G. (2000). Is $Ca^{2+}$ the second messenger in the response to melatonin in cuckoo wrasse melanophores? Life Sci., **66**(11), 1003-1010.

Martin, S. M., and Touitou, Y. (2000a). DHEA-sulphate causes a phase-dependent increase in melatonin secretion: a study of perifused rat pineal glands. Steroids, **65**(9), 491-496.

Martin, S. M., and Touitou, Y. (2000b). Progesterone inhibits, on a circadian basis, the release of melatonin by rat perifusion. Steroids, **65**(4), 206-209.

Martin, S. M., Bogdan, A., and Touitou, Y. (1996). Day-night differences in the effects of gonadal hormones on melatonin release from perifused rat pineal. Evidence of a circadian control. Steroids, **61**, 27-32.

Matsuo, T., and Cynader, M. S. (1992). Localization of $\alpha_2$-adrenergic receptors in the human eye. Ophthalmic. Res., **24**(4), 213-219.

Max, M., and Menaker, M. (1992). Regulation of melatonin production by light, darkness, and temperature in the trout pineal. J. Comp. Physiol., **170**, 479-489.

McIntyre, I. M., and Oxenkrug, G. F. (1991). Effect of ageing on melatonin synthesis induced by 5-hydroxytryptophan and constant light in rats. Prog. Neuropsycho-pharmacol. Biol. Psychiatry., **15**(4), 561-566.

Meineil, A., and Hartwig, H. G. (1980). Indoleamines in the pineal complex of *Lampetra planeri* (Petromyzontidae). A fluorescence microscopic and microspectro fluorimetric study. J. Neural Transm., **48**(2), 65-83.

Menaker, M., and Wisner, S. (1983). Temperature-compensated circadian clock in the pineal of Anolis. Proc. Natl. Acad. Sci., USA, **80**(19), 6119-6121.

Meyers, S. (2000). Use of neurotransmitter precursors of treatment of depression. Alt. Med. Rev., **5**(1), 64-71.

Meyer, U., Kruhoffer, M., Flugge, G., and Fuchs, E. (1998). Cloning of glucocorticoid receptor and mineralocorticoid receptor cDNA and gene expression in the central nervous system of the tree shrew (*Tupaia belangeri*). Mol. Brain Res., **55**, 243–255.

Miguez, J. M., Simonneaux, V., and Pevet, P. (1995). Evidence for a regulatory role of melatonin on serotonin release and uptake in the pineal gland. J. Neuroendocrinol., **7**(12), 949-956.

Miguez, J. M., Simonneaux, V., and Pevet, P. (1996). Changes in pineal indoleamines in rats after single melatonin injections: evidence for diurnal sensitivity to melatonin. J. Neuroendocrinol., **8**, 611-616.

Miguez, J. M., Simonneaux, V., and Pevet, P. (1997). The role of the intracellular and extracellular serotonin in the regulation of melatonin production in rat pinealocytes. J. Pineal Res., **23**(2), 63-71.

Mizobe, F., and Kurokawa, M. (1976). Enhancement of hydroxyindole-O-methyl- transferase and DNA dependent RNA polymerase activities induced by estradiol in rat pineals in culture. Eur. J. Biochem., **66**, 193-199.

Mizusawa, K., Iigo, M., Masuda, T., and Aida, K. (2000). Photic regulation of aryl-alkylamine-N-acetyltransferase1 mRNA in trout retina. Neuroreport, **11**(16), 3473-3477.

Morita, Y., Samejima, M., and Tamotsu, S. (1989). Response patterns and neuronal networks of photosensory pineal organs. Arch. Histol. Cytol., **52**, 469-475.

Morton, D. J., and Forbes, H. J. (1988). Pineal gland N-acetyltransferase and hydroxy-indole-O-methyltransferase activity in the rainbow trout (*Salmo gairdneri*): seasonal variation linked to photoperiod. Neurosci. Lett., **94**, 333-337.

Moujir, F., Bordon, R., Santana, C., Abreu, P., Hernandez, G., and Alonso, R. (1990). Ovarian steroids block the isoproterenol-induced elevation of pineal melatonin production in the female rat. Neurosci. Lett., **119**(1), 12-14.

Moyer, R. W. Firth, B. T., and Kennaway, D. J. (1997). Effect of variable temperatures, darkness and light on the secretion of melatonin by pineal explants in the Gecko, *Christinus marmoratus*. Brain Res., **747**(2), 230-235.

Murakami, M., Greer, M. A., Lewy, A. J., Greer, S. E., and Mc Adams, S. (1989). Ontogenesis of pineal thyroxine 5'-deiodinase activity and plasma melatonin concentration in the rat. Neuroendocrinology, **50**(4), 476-480.

Murakami, M., Hosoi, Y., Negeshi, T., Kamiya, Y., Ogiwara, T., Mizuma, H., Yamada, M., Iriuchijima, T., and Mori, M. (1997). Expression and nocturnal increase of type II iodothyronine deiodenase mRNA in rat pineal gland. Neurosci. Lett., **227**(1), 65-67.

Nagle, C. A., Neuspiller, R. N., Cardinali, D. P., and Rosner, J. M. (1972). Uptake and effect of 17 β- estradiol on pineal hydroxyindole-O-methyltransferase (HIOMT) activity. Life Sci., **11**, 1109-1116.

Nakayama, H., Sano, T., Motegi, A., Oyama, T., and Nakajima, T. (2005). Increasing 14-3-3 sigma expression with declining estrogen receptor alpha and estrogen-responsive finger protein expression defines malignant progression of endometrial carcinoma. Pathol. Int., **55**(1), 707-715.

Namboodiri, M. A., Sugden, D., Klein, D. C., Grady, R. Jr., and Mefford, I. N. (1985). Rapid nocturnal increase in ovine pineal N-acetyltransferase activity and melatonin synthesis: effects of cycloheximide. J. Neurochem., **45**(3), 832-835.

Natesan, A. K., and Cassone, V. M. (2002). Melatonin receptor mRNA localization and rhythmicity in the retina of the domestic chick, *Gallus domesticus*. Vis. Neurosci., **19**(3), 265-274.

Natesan, A., Geetha, L., and Zatz, M. (2002). Rhythm and soul in avian pineal. Cell Tiss. Res., **309**, 35-45.

Nayak, P. K., and Singh, T. P. (1987). Effect of melatonin and 5-methoxytryptamine on sex steroids and thyroid hormones during the prespawning phase of the annual reproductive cycle in the freshwater teleosts, *Clarias batrachus*. J. Pineal Res., **4**(4), 377-386.

Ng, L., Hurley, J. B., Dierks, B., Srinivas, M., Salto, C., Vennstrom, B., Reh, T. A., and Forrest, D. (2001). A thyroid hormone receptor that is required for the development of green cone photoreceptors. Nat. Genet., **27**(1), 94-98.

Nieminen, P., Hyvarinen, H., and Asikainen, J. (2000). Plasma leptin and thyroxin of mink (*Mustela vision*) vary with gender, diet and sub-chronic exposure. Comp. Biochem. Physiol., **217**(4), 515-522.

Nonno, R., Pannacci, M., Lucini, V., Angeloni, D., Fraschini, F., and Stankov, B. M. (1999). Ligand efficacy and potency at recombinant human MT2 melatonin receptors: evidence for agonist activity of some mt1-antagonists. Br. J. Pharmacol., **127**(5), 1288-1294.

Nowak, J. Z., Zawilska, J. B, and Trzepizur, K. (1997). Alpha 2-adrenergic receptors regulate generation of cyclic AMP in the pineal gland, but not in cerebral cortex of chick. Pol. J. Pharmacol., **49**(2-3), 137-141.

Okatani, Y., and Sagara, Y. (1994). Amplification of nocturnal melatonin secretion in women with functional secondary amenorrhoea: relation to endogenous oestrogen concentration. Clin. Endocrinol., **41**(6), 763-770.

Okatani, Y., Watanabe, K., Morioka, N., Hayashi, K., and Sagara, Y. (1997). Nocturnal changes in the pineal melatonin synthesis during puberty: relation to estrogen and progesterone levels in female rats. J. Pineal Res., **22**, 33-41.

Okatani, Y., Hayashi, K., Watanabe, K., Morioka, N., and Sagara, Y. (1998a). Estrogen modulates the nocturnal synthesis of melatonin in peripubertal female rats. J. Pineal Res., **24**, 224-229.

Okatani, Y., Hayashi, K., Watanabe, K., Morioka, N., and Sagara Y. (1998b). Effect of estrogen on melatonin synthesis in female peripubertal rats. J. Pineal Res., **24**, 67-72.

Okatani, Y., Hayashi, K., and Sagara, Y. (1998c). Effect of estrogen on melatonin synthesis in female peripubertal rats as related to adenylate cyclase activity. J. Pineal Res., **25**, 245-250.

Okatani, Y., Morioka, N., and Hayashi, K. (1999). Changes in nocturnal pineal melatonin synthesis during the perimenopausal period: relation to estrogen levels in rat. J. Pineal Res., **27**, 65-72.

Olcese, J., and Munker, M. (1994). Extracellular serotonin promotes melatonin release from cultured rat pinealocytes: evidence for an S2-type receptor-mediated autocrine feedback. Brain Res., **643**(1-2), 150-154.

Osuna, C., Rubio, A., and Guerrero, J. M. (1993). Potentiating effect of phenylephrine on isoproterenol activation of thyroxine type II deiodenase in the pineal gland of adult rats. Experientia., **49**(4), 329-331.

Oxenkrug, G. (2005). Antioxidant effects of N-acetylserotonin: possible mechanisms and clinical implications. Ann. N. Y. Acad. Sci., **1053**, 334-347.

Oxenkrug, G. F., and Requintina, P. J. (1994). Stimulation of rat pineal melatonin biosynthesis by N-acetylserotonin. Int. J. Neurosci., **77**(3-4), 237-241.

Ozaki, Y., Wurtman, R., Alonso, R., and Lynch, H. J. (1978). Melatonin secretion decreases during the proestrous stage of the rat estrous cycle. Proc. Nat. Acad. Sci. (Wash.) **75**, 531-534.

Pablos, M. I., Agapito, M. T., Reico, J. M., Perez-Gallardo, L., Cordova, M. D., and Mori, J. O. (1993). Effect of iron and estrogen on melatonin secretion by the chicken pineal gland. Neurosci. Lett., **159**(1-2), 211-214.

Pang, C. S., Tsang, K. F., Brown, G. M., and Pang, S. F. (1994). Specific 2-[$^{125}$I] iodomelatonin binding sites in the duck adrenal gland. Neurosci. Lett., **165**(1-2), 55-58.

Perreau-Lenz, S., Kalsbeek, A., Garidou, M. L., Wortel, J., Van Der Vliet, J., Van Heijningen, C., Simmonneaux, V., Pevet, P., and Buijs, R. M. (2003). Suprachiasmatic control of melatonin synthesis in rats: inhibitory and stimulatory mechanisms. Eur. J. Neurosci., **17**, 221-228.

Pevet, P. (2000). Melatonin and biological rhythms. Biol. Signals Recept., **9**(3-4), 203-212.

Pevet, P., Jacob, N., Lakhdar-Ghazal, N., and Vuillez, P. (1997). How do the suprachaismatic nuclei of the hypothalamus integrate photoperiodic information? Biol. Cell, **89**, 569-577.

Pickard, G. E., and Tang, W. X. (1993). Individual pineal cells exhibit a circadian rhythm in melatonin secretion. Brain Res., **627**, 141-146.

Pickard, G. E., and Tang, W. X. (1994). Pineal photoreceptors rhythmically secrete melatonin. Neurosci. Lett., **171**, 109-112.

Pootanakit, K., Prior, K. J., Hunter, D. D., and Brunken, W. J. (1999). 5-HT2a receptors in the rabbit retina: potential presynaptic modulators. Vis. Neurosci., **16**(2), 221-230.

Pootanakit, K., Prior, K. J., Hunter, D. D., and Brunken, W. J. (1999). 5-HT (3A) and 5-HT (3B) receptor subunits in mammalian retinae: potential presynaptic modulators of photoreceptors. Brain Res., **896**(1-2), 77-85.

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

Premabati, Y. (2005). Study of thyroid-pineal relationship in the fish, *Clarias gariepinus.* Ph. D thesis, North Eastern Hill University.

Preslock, J. P. (1976). Regulation of pineal enzymes by photoperiod, gonadal hormones and melatonin in Coturnix quail. Horm. Res., **7**(2), 108-117.

Price, D. M., Chik, C. L., and Ho, A. K. (2004). Norepinephrine induction of mitogen-activated protein kinase phosphatase-1 expression in rat pinealocytes: distinct roles of α- and β-adrenergic receptors. Endocrinology, **145**(12). 5723-5733.

Privat, K., Ravault, J. P., Chesneau, D., and Fevre-Montange, M. (1999). Day/night variation of tryptophan hydroxylase and serotonin N-acetyltransferase mRNA levels in the ovine pineal gland and retina. J. Pineal Res., **26** (4), 193-203.

Prolo, P., Wing, M. L., Licinio, J. (1998). Leptin. Int. J. Biochem. Cell Biol., **30**, 1285-1290.

Psarra, A. M., Bochaton-Piallat, M. L., Gabbiani, G., Sekeris, C. E., and Tsacopoulos, M. (2003). Mitochondrial localization of glucocortocoid receptor in glial (Muller) cells in the salamander retina. Glia., **41**(1), 38-49.

Quay, W. B., Stebbins, R. C., Kelley, T. D., and Cohen, N. W. (1971). Effects of environmental and physiological factors on pineal acetylserotonin methyltransferase activity in lizard, *Sceloporus occidentalus.* Physiol. Zoology, **44**, 241-248.

Rada, J. A., and Wiechmann, A. F. (2006). Melatonin receptors in chick ocular tissues: implications for a role of melatonin in ocular growth regulation. Invest. Ophthalmol. Vis. Sci., **47**(1), 25-33.

Redins, C. A., Novaes, J. C., and Torres, K. B. (1999). The effect of testosterone on the mice pinealocytes: a quantitative study. Tissue Cell, **31**(2), 233-239.

Redins, G. M., Redins, C. A., and Novaes, J. C. (2001). The effect of treatment with melatonin upon the ultrastructure of the mouse pineal gland: a quantitative study. J. Biol., **61**(4), 679-684.

Reiter, R. J., and Maestroni, G. J. (1999). Melatonin in relation to the antioxidative defence and immune system: possible implications for cell and organ transplantation. J. Mol. Med., **77**, 36-39.

Reuss, S. (2003). The clock in the brain: Anatomy of the mammalian circadian timing system. In "Endokrinologie" (E. Peschke, Ed.), pp 9-48. Verlag der Sachischen Akademie der Wissenschaften zu Leipzig, S. Hirzel, Stuttgart/ Leipzig.

Ribelayga, C., Wang, Y., and Mangel, S. C. (2004). A circadian clock in the fish retina regulates dopamine release via activation of melatonin receptors. J. Physiol., **554**(2), 467-482.

Rubio, A., Osuna, C., and Guerrero, J. M. (1991). β- and α-adrenergic mechanisms are involved in regulation of rat pineal type II thyroxine 5'-deiodoninase activity during development. Endocrinology, **128**(3), 1661-1667.

Rubio, A., Guerrero, J. M., Reiter, R. J., and Osuna, C. (1993). Involvement of α- and β-adrenergic receptors in the regulation of rat pineal N-acetyltransferase activity during development. Endocrinology, **132**(1), 393-398.

Rudeen, P. K., and Reiter, R. J. (1980). Depression of nocturnal pineal serotonin N- acetyltransferase activity in castrate male rats. J. Neural. Transm., **48**, 1-8.

Rudeen, P. K., Creighton, J., Bylund, D. B., Petterborg, L. J., and Paredez, S. (1990). Ontogeny of light-induced decrease of N-acetyltransferase activity in explanted chick pineal glands. J. Pineal Res., **8**(2), 153-158.

Saidapur, S. K., Seidel, A., and Vollrath, L. (1991). Effects of LHRH, progesterone, estradiol-17 beta and dexamethasone *in vitro* on pineal synaptic ribbons and serotonin N-acetyltransferase activity in diestrous rats. J. Neural Transm., **84**(1-2), 65-73.

Sakai, K. K., and Marks, B. H. (1972). Adrenergic effects on pineal cell membrane potential. Life Sci., **11**, 285-291.

Samejima, M., Shavali, S., Tamotsu, S., Uchida, K., Morita, Y., and Fukada, A. (2000). Light and temperature-dependence of the melatonin secretion rhythm in the pineal organ of the lamprey, *Lampetra japonica*. Jpn. J. Physiol., **50**(4), 437-442.

Sanchez, J. J., Abreu, P., Gonzalez-Hernandez, T., Hernandez, A., Prieto, L., and Alonso, R. (2004). Estrogen modulation of adrenoceptor responsiveness in the female rat pineal gland: Differential expression of intracellular estrogen receptors. J. Pineal. Res., **37**(1), 26-35.

Santana, C., Moujir, F., Sanchez, J., Reiter, R. J., and Abreu, P. (2001). Role of extracellular calcium on the regulation of melatonin synthesis in the Syrian hamster pineal gland. J. Pineal Res., **31**(4), 289-293.

Sarrieau, A., Dussaillant, M., Moguilewsky, M., Coutable, D., Philibert, D., and Rostene, W. (1998). Autoradiographic localization of glucocorticosteroid binding sites in rat brain after *in vivo* injection of [$^3$H]RU 28362. Neurosci. Lett., **92**, 14–20.

Scher, J., Wankiewicz, E., Brown, G. M., and Fujieda, H. (2002). MT1 melatonin receptor in the human retina: Expression and localization. Inv. Opthal. Vis. Science, **43**, 889-897.

Schomerus, C., Korf, H. W., Laedtke, E., Weller, J. L., and Klein, D. C. (2000). Selective adrenergic/cyclic AMP-dependent switch-off of proteosomal proteolysis alone switches on neural signal transduction: an example from the pineal gland. J. Neurochem., **75**, 2123-2133.

Schomerus, C., Laedtke, E., Olcese, J., Weller, J. L, Klein, D. C, and Korf, H. W. (2002). Signal transduction and regulation of melatonin synthesis in bovine pinealocytes: impact of adrenergic, peptidergic and cholinergic stimuli. Cell Tissue Res., **309**(3), 417-428.

Schwartz, M. W., Seeley, R. J., Woods, S. C., Weigle, D. S., Campfield, L. A., Burn, P., and Baskin, D. G. (1997). Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes, **46**(12), 2119-2123.

Serino, I., d' Istria, M., and Monteleone, P. (1993). A comparative study of melatonin production in the retina, pineal gland and harderian gland of *Bufo viridis* and *Rana esculenta.* Comp. Biochem. Physiol., **106**(1), 189-193.

Shirai, N., Suzuki, H., Toukairin, S., and Wada, S. (2001). Spawning and season affect lipid content and fatty acid composition of ovary and liver in Japanese catfish (*Silurus asotus*). Comp. Biochem. Physiol., **129**, 185-195.

Siguenza, A. F., Recio, J. M., and Agapito, M. T. (1988). Radioimmunoassay for melatonin and its application to fowl pineal research. Rev. Esp. Fisiol., **44**(2), 221-226.

Simonneaux, V., and Ribelayga, C. (2003). Generation of the melatonin endocrine message in mammals: a review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters. Pharmacol. Rev., **55**(2), 325-395.

Singh, I. J., and Singh, T. P. (1984). Changes in gonadotrophin, lipid and cholesterol levels during annual reproductive cycle in the freshwater teleost, *Cirrhinus mrigala.* Ann. Endocrinol., **45**, 131-136.

Smith, M., Burke, Z., Humphries, A., Wells, T., Klein, D., Carter, D., and Baler, R. (2001). Tissue-specific transgenic knockdown of Fos-related antigen 2 (Fra-2) expression mediated by dominant negative Fra-2. Mol. Cell Biol., **21**(11), 3704-3713.

Smolinska, N., Przala, J., Kaminski, T., Siawrys, G., Gajewska, A., Kochman, K., and Okrasa, S. (2004). Leptin gene expression in the hypothalamus and pituitary of pregnant pigs. Neuro. Endocrinol. Lett., **25**(3), 191-195.

Snedecor, G. W. (1961). Statistical methods, Pacific Private Ltd., Bombay, India.

Soares, J. M., Masana, M. I., Ersahin, C., and Dubocovich, M. L. (2003). Functional melatonin receptors in rat ovaries at various stages of the estrous cycle. J. Pharmacol. Exp. Ther., **306**(2), 694-702.

Soszynski, P., Zgliczynski, S., and Pucilowska, J. (1988). The circadian rhythm of melatonin in hypothyroidism and hyperthyroidism. Acta. Endocrinol., (Copenh)., **119**(2), 240-244.

Soutto, M., Guerrero, J. M., Osuna, C., and Molinero, P. (1998). Nocturnal increases in the triiodothyronine/thyroxine ratio in the rat thymus and pineal gland follow increases of type II 5'-deiodinase activity. Int. J. Biochem. Cell Res., **30**(2), 235-241.

Spessert, R., Layes, E., and Vollrath, L. (1993). Adrenergic stimulation of cyclic GMP formation requires NO-dependent activation of cytosolic guanylate cyclase in rat pinealocytes. J. Neurochem., **61**(1), 138-143.

Spessert, R., Hill, G., and Vollrath, L. (1995). In rat pinealocytes the cyclic GMP response to NO is regulated by $Ca^{2+}$ and protein kinase C. Brain Res., **694**, 207-212.

Spessert, R., Layes, E., Hill, G., and Vollrath, L. (1998). Nitric oxide is formed in a sub-population of rat pineal cells and acts as an intercellular messenger. Neuroendocrinology, **68**, 57-63.

Stehle, J. H., von Gall, C., and Korf, H. W. (2001). Analysis of cell signalling in the rodent pineal gland deciphers regulators of dynamic transcription in neural/endocrine cells. Eur, J. Neurosci., **14**(1), 1-9.

Stehle, J. H., von Gall, C., and Korf, H. W. (2003). Melatonin: a clock-output, a clock-input. J. Neuroendocrinol., **15**(4), 383-389.

Steinsapir, J., Harney, J., and Larsen, P. R. (1998). Type 2 iodothyronine deiodenase in rat pituitary tumor cells is inactivated in proteasomes. J. Clin. Invest., **102**(11), 1895-1899.

Steinsapir, J., Bianco, A. C., Buettner, C., Harney, J., and Larsen, P. R. (2000). Substrate-induced down-regulation of human type 2 deiodenase (hD2) is mediated through proteasomal degradation and requires interaction with the enzyme's active center. Endocrinology, **141**(3), 1127-1135.

Sterado, L., Monteleone, P., Trabace, L., Cannizzaro, C., Maj, M., and Cuomo, V. (2000). Serotonergic modulation of rat pineal gland: *in vivo* evidence for a 5-hydroxy-tryptamine2C receptor involvement. J. Pharmacol. Expt. Ther., **25**, 266-273.

Sugden, D. (1990). 5-hydroxytryptamine amplifies β-adrenergic stimulation of N-acetyltransferase activity in rat pinealocytes. J. Neurochem., **55**, 1655-1658.

Sugden, D., and Klein, D. C. (1987). Inactivation of rat pineal hydroxyindole-O-methyltransferase by disulfide-containing compounds. J. Biol. Chem., **262**(14), 6489-6493.

Sugden, D., Namboodiri, M. A., Klein, D. C., Grady, R. K. Jr., and Mefford, I. N. (1985). Ovine pineal indoles: effects of L-tryptophan or L-5-hydroxytryptophan administration. J. Neurochem., **44**(3), 769-772.

Sudhakumari, C. C., Haldar, C., and Senthilkumaran, B. (2001). Seasonal changes in adrenal and gonadal activity in the quail, *Perdicula asiatica*: involvement of the pineal gland. Comp. Biochem. Physiol., **128**(4), 793-804.

Sundararaj, B. I., and Keshavanath, P. (1976). Effects of melatonin and prolactin treatment on the hypophysial-ovarian system in the catfish, *Heteropnuestes fossilis* (Bloch). Gen. Comp. Endocrinol., **29**, 84-96.

Snyder, S. H., Axelrod, J., Wurtman, R. J., Fischer, J. E. (1965). Control of 5-OH decarboxylase activity in the rat pineal gland by sympathetic nerves. J. Pharmacol. Exp. Ther., **147**, 371-375.

Tabata, M., Minh-Nyo, M., and Oguri, M. (1988). Involvement of retinal and extraretinal photoreceptors in the mediation of nocturnal locomotor activity rhythms in the catfish, *Silurus asotus*. Exp. Biol., **47**(4), 219-225.

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

Takekoshi, K., Motooka, M., Isobe, K., Nomura, F., Manmoku, T., Ishii, K., and Nakai, T. (1999). Leptin directly stimulates catecholamine secretion and synthesis in cultured porcine adrenal medullary cells. Biochem. Biophy. Res. Commun., **261**(2), 426-431.

Terriff, D. L., Chik, C. L., Price, D. M., and Ho, A. K. (2005). Proteasomal proteolysis in the adrenergic induction of arylalkylamine N-acetyltransferase in rat pinealocytes. Endocrinology, **146**(11), 4795-4803.

Thibault, C., Falcon, J., Greenhouse, S. S., Lowery, C. A., Gern, W. A., and Collin, J. P. (1993). Regulation of melatonin production by pineal photoreceptor cells; role of cyclic nucleotides in the trout (*Orcorhyncus mykiss*). J. Neurochem., **61**(1), 332-339.

Thomas, K. B., Brown, A. D., and Iuvone, P. M. (1998). Elevation of melatonin in chicken retina by 5-hydroxytryptophan: differential light/dark responses. Neuroreport, **9**(18), 4041-4044.

Tilden, A. R, and Hutchinson, V. H. (1993). Influence of photoperiod and temperature on serum melatonin in the diamondback water snake, *Nerodia rhombifera*. Gen. Comp. Endocrinol., **92**(3), 347-354.

Tonomura, N., McLaughlin, K., Grimm, R. A., and Osborne, B. A. (2003). Glucocorticoid-induced apoptosis of thymocytes: requirement of proteasome dependent mitochondrial activity. J. Immunol., **170**(5), 2469-2478.

Torres-Farfan, C., Richter, H. G., Rojas-Garcia, P., Vergara, M., Forcelledo, M. L., Valladares, L. E., Torrealba, F., Valenzuela, G. J., and Seron-Ferre, M. (2003). MT1 melatonin receptor in the primate adrenal gland: inhibition of adrenocorticotropin-stimulated cortisol production by melatonin. J. Clin. Endocrinol. Metab., **88**(1), 450-458.

Tosini, G., and Menaker, M. (1996). The pineal complex and melatonin affect the expression of the daily rhythm of behavioral thermoregulation in green iguana. J. Comp. Physiol., **179**(1), 135-142.

Troiani, M. E., Reiter, R. J., Vaughan, M. K., Gonzalez-Brito, A., and Herbert, D. C. (1988). The depression in rat pineal melatonin production after saline injection at night may be elicited by corticosterone. Brain Res., **450**, 18-24.

Underwood, H., and Calaban, M. (1987). Pineal melatonin rhythms in the lizard *Anolis carolinensis*: response to light and temperature cycles. J. Biol. Rhythms. **2**(3), 179-193.

Underwood, H., Steel, C. T., and Zivkovic, B. (2001). Circadian organization and the role of the pineal in birds. Micorsc. Res. Tech., **53** (1), 48-62.

Urano, T., Saito, T., Tsukui, T., Fujita, M., Hosoi, T., Muramatsu, M., Ouchi, Y., and Inoue, S. (2002). Efp targets 14-3-3 sigma for proteolysis and promotes breast tumour growth. Nature, **417**(6891), 871-875.

Urasaki, H. (1972a). Effect of pinealeactomy on gonadal development in the Japanese killfish (*Oryzias latipes*). Annot. Zoo. Jap., **45**, 10-15.

Urasaki, H. (1972b). Role of pineal gland development in the fish, *Oryzias latipes*. Annot. Zoo. Jap., **45**, 152-158.

Urasaki, H. (1972c). Effect of restricted photoperiod and melatonin administration on gonadal weight in the Japanese killifish. J. Endocr., **55**, 619-620.

Uz, T., Arslan, A. D., Kurtuncu, M., Imbesi, M., Akhisaroglu, M., Dwivedi, Y., Pandey, G. N., and Manev, H. (2005). The regional and cellular expression profile of the melatonin receptor MT1 in the central dopaminergic system. Brain Res., **136**(1-2), 45-53.

Valenti, S., and Giusti, M. (2002). Melatonin participates in the control of testosterone secretion from rat testis: an overview of our experience. Ann. N. Y. Acad. Sci., **966**, 284-289.

Valenti, S., Giusti, M., Guido, R., and Giordano, G. (1997). Melatonin receptors are present in adult rat Leydig cells and are coupled through a pertussis toxin-sensitive G-protein. Eur. J. Endocrinol., **136**(6), 633-639.

Van Esseveldt, K. E., Lehman, M. N., and Boer, G. J. (2000). The suprachaismatic nucleus and the circadian time-keeping system revisited. Brain Res. Rev., **33**, 34-77.

Vanecek, J., Sugden, D., Weller, J., and Klein D. C. (1985). A typical synergistic $\alpha_1$-and β-adrenergic regulations of adenosine 3', 5'-monophosphate and guanosine 3', 5'-monophosphate in rat pinealocytes. Endrocrinology, **116,** 2167-2173.

Voisin, P., and Collin, J. P. (1986). Regulation of chicken pineal arylalkylamine N-acetyltransferase by postsynaptic alpha 2 adrenergic receptors. Life Sci., **39**, 2025-2032.

Voisin, P., Martin, C., and Collin, J. P. (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**(5), 1421-1426.

Voisin, P., Van Camp, G., and Collin, J. P. (1990). N-acetylation of serotonin is correlated with alpha 2- but not with β-adrenergic regulation of cyclic AMP levels in cultured chick pineal cells. J. Neurochem., **54**(6), 1953-1960.

Volkoff, H., Eykelbosh, A. J., and Peter, R. E. (2003). Role of leptin in the control of feeding of goldfish *Carassius auratus*: interactions with cholecystokinin, neuropeptide Y and orexin A, and modulation by fasting. Brain Res., **972**(1-2), 90-109.

Vollrath, L. (1981). The pineal complex in lampreys and fishes. In: The Pineal Organ, (L. Vollrath, ed.), pp. 321-367. Springer-Verlag, Berlin/ Heidelberg.

Wainwright, S. D. (1980). Diurnal cycles in serotonin acetyltransferase activity and cyclic GMP content of cultured chick pineal glands. Nature, **285**(5765), 478-480.

Wainwright, S. D., and Wainwright, L. K. (1978). Regulation of the diurnal cycle in activity of serotonin N-acetyltyransferase in chick pineal gland. Canad. J. Biochem., **56**, 685-690.

Weil, C., Le Bail, P. Y., Sabin, N., and Le Gac, F. (2003). *In vitro* action of leptin on FSH and LH production in rainbow trout (*Onchorynchus mykiss*) at different stages of the sexual cycle. Gen. Comp. Endocrinol., **130**(1), 2-12.

Weiss, B., and Crayton, J. (1970). Gonadal hormones as regulators of pineal adenyl cyclase activity. Endocrinology, **87**, 27-53.

Wenzel, A., Grimm, C., Samardzija, M., and Reme, C. E. (2005). Molecular mechanisms of light-induced photoreceptor apoptosis and neuroprotection for retinal degeneration. Prog. Retin. Eye Res., **24**(2), 275-306.

Wheeler, L. A., Lai, R., and Woldemussie, E. (1999). From the lab to the clinic: activation of an $\alpha_2$ agonist pathway is neuroprotective in models of retinal and optic nerve injury. Eur. J. Ophthalmol., 9(1), 17-21.

Wheeler, L., Woldemussie E, and Lai, R. (2003). Role of $\alpha_2$ agonists in neuroprotection. Surv. Ophthalmol., 48(l), 47-51.

Wiechmann, A. F., Vrieze, M. J., Dighe, R., and Hu, Y. (2003). Direct modulation of rod photoreceptor responsiveness through a Mel(1c) melatonin receptors in transgenic *Xenopus laevis* retina. Invest. Opthalmol. Vis. Sci., **44**(10), 4522-4531.

Wikberg-Matsson, A., Wikberg, J. E., Uhlen, S. (1996). Characterization of $\alpha_2$-adrenoceptor subtypes in the porcine eye: identification of $\alpha_{2a}$-adrenoceptors in the choroid, ciliary body and iris, and alpha $\alpha_{2a}$- and $\alpha_{2c}$-adrenoceptors in the retina. Exp. Eye Res., **63**(1), 57-66.

Wilkinson, M., and Arendt, J. (1978). Effects of oestrogen and progesterone on rat pineal N-acetyltransferase activity and melatonin production. Experientia., **34**, 667-669.

Williams, L. M., Hannah, L. T., and Bassett, J. M. (1999). Melatonin receptors in neonatal pig brain and pituitary gland. J. Pineal Res., **26**(1), 43-49.

Wright, M. L., and Alves, C. D. (2001). The decrease in plasma melatonin at metamorphic climax in *Rana catesbeiana* (bullfrog) tadpoles is induced by thyroxine. Comp. Biochem. Physiol., **129**(2-3), 653-663.

Wright, M. L., and Alves, C. D. (2001). The decrease in plasma melatonin at metamorphic climax in *Rana catesbeiana* (bullfrog) tadpoles is induced by thyroxine. Comp. Biochem. Physiol. A. Mol. Integr. Physiol., **129**(2-3), 653-663.

Xi, S. C., Tam, P. C., Brown, G. M., Pang, S. F., and Shiu, S. Y. (2000). Potential involvement of mt1 receptor and attenuated sex steroid-induced calcium influx in the direct anti-proliferative action of melatonin on androgen-responsive LNCaP human prostate cancer cells. J. Pineal Res., **29**(3), 172-183.

Yaghoubian, S., Filosa, M. F., and Youson, J. H. (2001). Proteins immunoreactive with antibody against a human leptin fragment are found in serum and tissues of the sea lamprey, *Petromyzon marinus*. L. Comp. Biochem. Physiol., **129**, 777-785.

Yamada, H., Ogura, A., Koizumi, S., Yamaguchi, A., and Moriyama, Y. (1998a). Acetylcholine triggers L-glutamate exocytosis via nicotinic receptors and inhibits melatonin synthesis in rat pinealocytes. J. Neurosci., **18**(13), 4946-4952.

Yamada, H., Yatsushiro, S., Ishio, S., Hayashi, M., Nishi, T., Yamamoto, A., Futai, M., Yamaguchi, A., and Moriyama, Y. (1998b). Metabotropic glutamate receptors negatively regulate melatonin synthesis in rat pinealocytes. J. Neurosci., **18**(6), 2056-2062.

Yanez, J., and Meissl, H. (1996). Secretion of the methoxyindoles melatonin, 5-methoxytryptophol, 5-methoxyindoleacetic acid, and 5-methoxytryptamine from trout pineal organs in superfusion culture: effects of light intensity. Gen. Comp. Endocrinol., **101**(2), 165-172.

Young, S. N., and Gauthier, A. M. (1981). Effect of tryptophan administration on tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid in human lumbar and cisternal cerebrospinal fluid. J. Neurol. Neurosurg. Psychiatry, **44**, 323-327.

Yuwiler, A. (1985). Neonatal steroid treatment reduces catecholamine-induced increases in pineal serotonin N-acetyltransferase activity. J. Neurochem., **44**(4), 1185-1193.

Yuwiler, A. (1989). Effects of steroids on serotonin N-acetyltransferase activity of pineals in organ culture. J. Neurochem., **52**, 46-53.

Yuwiler, A., and Brammer, G. L. (1981). Neonatal hormone treatment and maturation of the pineal noradrenergic system: hydrocortisone and thyroxin. J. Neurochem., **37**(4), 985-992.

Zachmann, A. Falcon, J., Knijff, S. C. M., Bolliet, V., and Ali, M. A. (1992). Effects of photoperiod and temperature on rhythmic melatonin secretion from the pineal organ of the white sucker (*Catostomus commersoni*) *in vitro*. Gen. Comp. Endocrinol., **86**, 26-33.

Zaher, H., and Svenson, C. K. (1994). Glucocorticoid induction of hepatic acetyl Co A: arylamine N-acetyltransferase activity in the rat. Res. Commum. Chem. Pathol. Pharmacol., **83**(2), 195-508.

Zatz, M., and Mullen, D. A. (1988). Norepinephrine acting via adenylate cyclase inhibits melatonin output but does not phase shift the pacemaker in cultured chick pineal cells. Brain Res., **450**, 137-143.

Zatz, M., Mullen, D. A., and Moskal, J. R. (1988). Photoendocrine transduction in cultured chick pineal cells: effects of light, dark, and potassium on the melatonin rhythm. Brain Res., **438**, 199-215.

Zatz, M., Gastel, J. A., Heath, J. R., and Klein, D. C. (2000). Chick pineal melatonin synthesis: light and cyclic AMP control abundance of serotonin N-acetyltransferase protein. J. Neurochem., **74**(6), 2315-2321.

Zawilska, J., and Iuvone P. M. (1989). Catecholamine receptors regulating serotonin N-acetyltransferase activity and melatonin content in chicken retina and pineal gland: $D_2$-dopamine receptors in retina and $\alpha_2$-adrenergic receptors in pineal gland. J. Pharmacol. Exp. Ther., **250**, 86–92.

Zawilska, J. B., and Sadowska, M. (2002). Prolonged treatment with glucocorticoid dexamethasone suppresses melatonin production by the chick pineal gland and retina. Pol. J. Pharmacol., **54**(1), 61-66.

Zhang, F., Basinski, M. B., Beals, J. M., Briggs, S. L., Churgay, L. M., Clawson, D. K., Dimarchi, R. D., Furman, T. C., Hale, J. E., Hsiung, H. M., Schoner, B. E., Smith, D. P., Zhang, X. Y., Wery, J. P., and Schevitz, R. W. (1994). Crystal structure of the obese protein leptin E100. Nature, **38**, 206-209.

Zhao, Z. Y., and Touitou, Y. (1993). Kinetic changes of melatonin release in rat pineal perifusions at different circadian stages. Effects of corticosteroids. Acta. Endocrinol., **129**(1), 81-88.

Zikopoulos, B., and Dermon, C. R. (2005). Comparative anatomy of $\alpha_2$- and β-adrenoceptors in the adult and developing brain of the marine teleost the red porgy (*Pagrus pagrus*, Sparidae): [$^3$H]clonidine and [$^3$H]dihydroalprenolol quantitative autoradiography and receptor subtypes immunohistochemistry. J. Comp. Neurol., **489**(2), 217-240.

# APPENDIX

Name: **LONGSHIBEMO YANTHAN**

Title of dissertation: **Studies on *in vitro* effects of hormones on N-acetyltransferase activity in the pineal of air-breathing fish, *Clarias gariepinus***

Date of Admission: 21-05-2002

Approval of research proposal:

i). B. P. G. S.: 23-04-2003

ii). School Board: 15-05-2003

iii). Registration No. and
Date of Registration: 735 of 15-05-2003

# BIO-DATA


Name: LONGSHIBEMO YANTHAN

Father's name: T. D. LOTHA

Address:

Present: Department of Zoology
Environmental Endocrinology Laboratory
North-Eastern Hill University
Shillong- 793022 (Meghalaya)

Permanent: Orchid Hill
Wokha, Nagaland- 797112

E-Mail: longshiyanthan@yahoo.co.in


Date of Birth: 05-06-1976

**Academic qualifications**:

| Sl. No. | Exam. Passed | Division | Year | University | Subjects |
|---|---|---|---|---|---|
| 1 | B. Sc. (Major) | Second | 1997 | Nagaland University. | Botany, Chemistry, Zoology (Major). |
| 2 | M. Sc. | First | 2000 | University of Pune. | Zoology. |