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RESEARCH ARTICLE

# The pineal gland and its neurosecretory product, melatonin: influencing much more than just sleep/wake cycles in humans.

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## ABSTRACT

The pineal gland was appreciated as a distinct anatomical entity from antiquity associated with a role in mysticism and in religion as the seat of the soul. The gland appears to have had a photoreceptive role earlier in evolution but typical of vertebrate adaptability it has undergone a role change to a secretory organ with changing needs of the organism. In the contemporary settling it is now known for its neuroendocrine product, melatonin and its association with the sleep cycle. An ancient pleiomorphic molecule with a range of attributes but its antioxidant properties, which helped organisms survive oxygenation of the atmosphere at the dawn of time, is still relevant today, providing an extra layer of protection to the organism as whole but particularly the brain, mitochondria and the skin. Melatonin, through its seasonal influence on the immune function, may also affect disease susceptibility.

## Introduction

The pineal gland has a long history of scientific and spiritual interest but until recently the gland remained an enigma. It appears in religious texts in both eastern and western philosophies. The third eye, Ajana chakra in Yoga and the eye of Horus in Egyptian mythology. Herophilus (c300BC) gave reference to it in relation to the soul. Galen gave a detailed description in his work 'De anatomicis administrationibus' and in the 17th century Descartes called it the "principal seat of the soul". Long regarded as a vestigial organ by biologists It is now recognised that the major neurosecretory product of the pineal gland, melatonin, plays an important role in circadian regulation of human physiology, such as in sleep-wake patterns, timing and release of reproductive hormones and in temperature control.

Pineal melatonin is primarily released into the CSF where it can directly influence brain function. The brain's high metabolic demands result in a relatively high production of reactive oxygen species (ROS). Through melatonin's antioxidative effects it can provide an extra level of protection from local oxidative stress.

Beyond this, it is now recognised that melatonin and its metabolites are produced locally in many mammalian tissues, having a particular influence in mitochondrial bioenergetics and homeostasis and providing additional protection from the damaging effects of ultraviolet (UV) radiation in skin cells. Pleiotropic effects going far beyond its sleep hormone role.

## Evolutionary concepts

Melatonin is known as a neurohormone that regulates sleep and seasonal behaviour in vertebrates but it is present in nearly all life forms and its function as a cellular defence against oxidation goes back ~3.6 billion years. It has been hypothesised that the initial appearance of melatonin was as an antioxidant in photosynthetic cyanobacteria, necessary as photosynthesis was associated with the generation of toxic free radicals[1,2]. The only organisms to survive oxygenation of the atmosphere had to have the ability the protect themselves for oxidative stress, other functions of melatonin coming later in evolution. It is also hypothesised that mitochondria and chloroplasts developed from ingested bacteria that retained their melatonin synthetic actions. Initially engulfed as a nutrient but assimilated into cellular machinery establishing a symbiotic relationship with their host[3]. Tosches et al study of the annelid worm, *Platynereis dumerilii* suggests an evolutionary connection between melatonin's significance in invertebrates and its role in vertebrate sleep. Most invertebrates and some fish exhibit diel vertical migration (DVM). They rise to the surface of lakes and oceans during the night and sink deeper during the day. In most invertebrate larvae, it is controlled by ciliary beating. It has been proposed that DVM evolved in concert with the animal's circadian clock to avoid damaging UV radiation. Tosches et al hypothesised that melatonin, which is oxidised by light, might regulate diurnal patterns of ciliary swimming associated with DVM in the *Platynereis* larvae. They found melatonin synthesis in the dorsomedial cells of the larval brain, colocalised with opsin phototransduction and circadian clock genes. Genes involved in melatonin synthesis were upregulated at night, indicating that

melatonin signalling to the larval brain is controlled by light and/or the circadian clock and their findings support the idea that melatonin signalling plays a role in ciliary arrest and swimming behaviour. They also found that activity of cholinergic neurons increased ciliary arrest duration. This work proved a connection between ciliary swimming in Platynereis and the mechanisms of vertebral sleep[4]. Some evidence suggests the neurons regulating ciliary swimming are capable of receiving alternative inputs, such as visual systems[5]. If melatonin signalling can saturate the activity of these neurons it is plausible that other sensory inputs cannot be processed. The authors draw a parallel between this and filtering of sensory information by rhythmic burst firing of thalamic relay neurons during sleep[6].

## The parietal or 3rd eye

The parietal eye is a part of the epithalamus present in some vertebrates. Siting in a depression enclosed by the parietal bone, the parietal or pineal foramen, it is photoreceptive, associated with the pineal gland and regulates circadian rhythm and production of hormones for temperature regulation.

Holmgren thought the sensory cells looked like cone cells of the retina, hypothesising that it could be a primitive light-sensing organ and it became known as the 3rd eye[7]. It is found in most lizards, frogs and the New Zealand tuatara, as well as some bony fish and sharks. Absent in mammals but present in their closest extinct relatives, the therapsids, suggesting that it was lost through evolution in endotherms, also being absent in endothermic archosaurs such as birds[8].

The parietal eye in amphibians and reptiles appears relatively far forward in the skull, whereas the vertebrate pineal gland sits at the back of the brain between the corpus collosum and cerebellum. To understand this shift in position, the parietal bones formed as part of the skull lying between the eyes in basal amphibians but have moved further back in higher vertebrates. As can be seen in the frog, the diencephalon, from which the pineal stalk arises, appears relatively further forward, as the cerebral hemispheres are smaller and the optic lobes more prominent than in the human mesencephalon, the optic tract now having to bridge the increased distance between the eyes and the diencephalon[9].

Lampreys, the most primate living vertebrate, has two parietal eyes, one behind the other in the centre of the braincase, suggesting the original position in vertebrates. This possibly evolved to allow bottom dwelling species to sense threats from above[10].

## Anatomy

Intrinsically photosensitive ganglion cells in the retina, utilising melanopsin, send the light signal to the suprachiasmatic nucleus (SCN) of the hypothalamus (HT) via the retinohypothalamic tract which runs in conjunction with the optic nerve but is diverted at the chiasma to the SCN, the optic nerve continuing to the visual cortex. From the SCN, 2nd order neurons carry the impulse to the reticular formation in the brain stem. 3rd order neurons descend as the reticulospinal tract to T1-3 of the spinal cord, ending in the neurons of the lateral column. 4th order neurons leave the cord via the ventral root of T1 and ascend in the cervical sympathetic

chain to the superior cervical ganglion. 5th order post-ganglionic neurons ascend further as a plexus around the internal carotid artery and its branches, finally leaving as nervous conarii to enter the pineal gland from its posterior aspect. Some nervous conarii fibres enter the habenular nucleus via the habenulo-pineal tract. 6th order neurons terminating in the ganglion conarii at the apex of the pineal. From there 7th order neurons enter the substance of the pineal. A *nervus pinealis* has been described in human foetuses, just caudal to the gland, not found postnatally and presumed to degenerate in late foetal life. It is thought to be homologous with the pineal nerve of fish and amphibians. A phylogenetic vestige, its loss associated with the evolution of the pineal as a secretory rather than photoreceptive organ[11].

The pineal gland is one of the organs that receive a neuronal input from the SCN. Axons of the central clock project, among other places, to the paraventricular nuclei (PVN) of the hypothalamus. The gland also receives parasympathetic input.

During darkness, the SCN sends a neural impulse causing discharge of noradrenaline from the postganglionic terminals near the pinealocytes, acting on β adrenergic receptors on the pinealocytes. This culminates in a series of molecular events inducing night-related rise in melatonin synthesis and release[12,13]. Melatonin rhythm is also tightly coupled to the core body temperature rhythm, peak melatonin secretion corresponding closely to the nadir of temperature[14].

The SCN has three major afferent connections. Photic information from the retinohypothalamic tract influencing phase and period of circadian rhythm being the most important but also a median raphe serotonergic pathway and the geniculohypothalamic tract from the thalamic intergeniculate leaflet. Beyond this there are multiple brain regions involved in circadian rhythm control through multi-synaptic neural SCN input and output[15]. Lizards, frogs and birds still use the pineal gland to detect light and ~10% of pinealocytes have retinal proteins involved in the phototransduction cascade. Stausman demonstrated synthesis of the psychoactive chemical di-methyl-tryptamine (DMT)[16]. Pinealocytes show piezoelectrical properties i.e. capable of electrical polarity, sensitivity to electromagnetic forces and magnetoreceptor properties that can be used by blind people for navigation. Fig1 and 2.

## Melatonin

Melatonin (N-acetyl-5 methoxy-tryptamine) was first isolated from the bovine pineal gland by Lerner et al in 1958 and the metabolic pathway, a four-step enzymatic conversion, from tryptophan identified in the sixties. Figure 3. The reciprocal relationship between the pineal gland and the suprachiasmatic nucleus (SCN), the central circadian pacemaker, is the major mechanism of melatonin production. In mammals, melatonin functions as the biological signal of darkness, because the duration of its release from the pineal gland is proportional to night length, this then informs other systems of the time of year and time of day. The chronobiotic properties influence variation in physiological response to time of day or night, having clinical relevance to period and phase-shifts. Beyond that, melatonin shows remarkable

functional versatility by exhibiting anti-oxidant, oncostatic, anti-aging and immunomodulatory properties. This functional versatility is reflected in its wide-ranging phylogenetic distribution through organisms, from plants to bacteria and humans. When

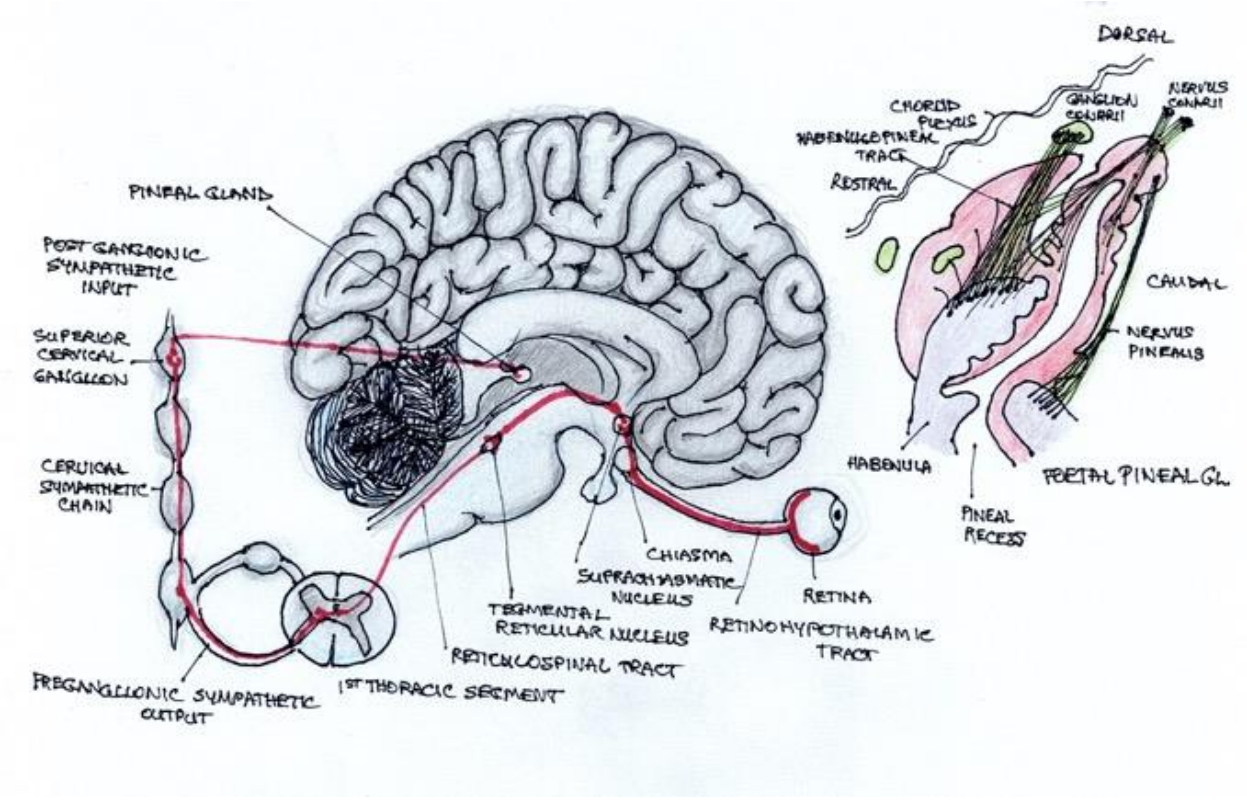


Figure 1. The anatomy of the pineal gland and neuronal pathway from SCN to the pineal.

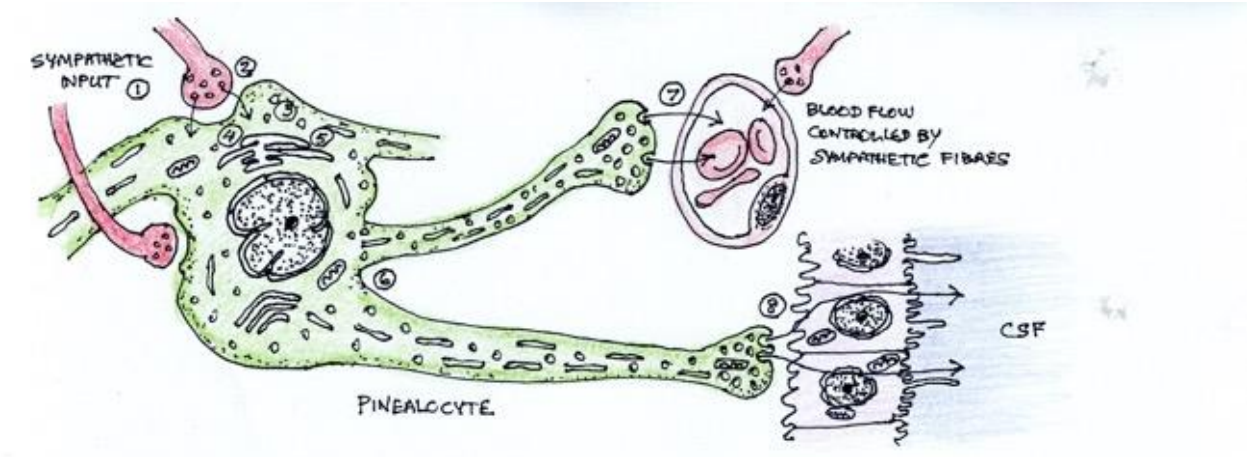


Figure 2. Control, synthesis and release of melatonin from pinealocytes

1, darkening increases sympathetic input; 2, noradrenaline released from sympathetic terminals stimulates pinealocytes; 3, adenyl cyclase activated; 4, cAMP stimulates synthesis of hormone complexes; 5, complexes packaged in Golgi apparatuses; 6, transport via cell processes; 7, complexes secreted and hormones released into blood

vessels; 8, complexes secreted and hormones released into CSF.

first isolated, it was considered an exclusive hormone of the pineal gland, however, it has since been identified in a large number of non-endocrine, extra-pineal sites, including the skin and the immune system. Melatonin's molecular mechanism involves several actions, via G protein-coupled membrane receptors, cytosol and nuclear protein interactions with both, direct radical scavenging and redox modulating processes. It has more recently become appreciated that there is a relationship between nervous, endocrine and immune systems with the use of a common chemical language for intra- and inter system communication, melatonin one member of the complex neuro-endocrine-immunological system.

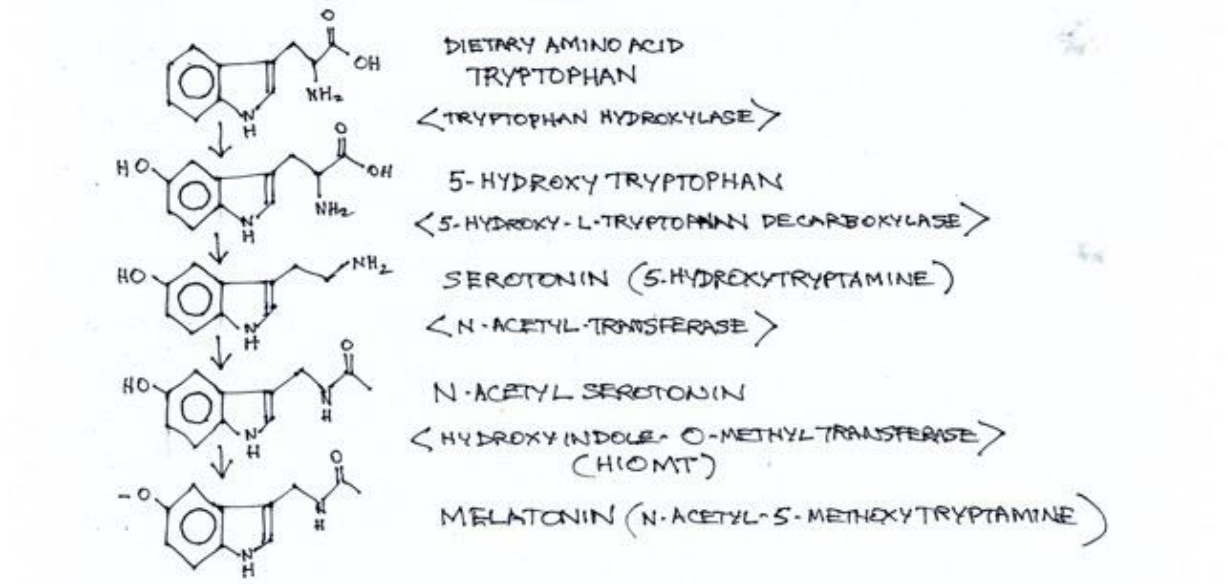


Figure 3. The 4-step metabolic pathway for the enzymatic conversion of tryptophan to melatonin.

## Melatonin; a chronobiotic

Melatonin is concerned with biological timing. The period of melatonin secretion described as 'biological night' and a chronobiotic adjusts the timing of the central biological clock. In mammals, the profile of melatonin synthesis and secretion is longer during winter nights compared to summer nights. This change in duration of secretion serves as a time cue for the organisation of daylength-dependent (photoperiodic) seasonal functions such as reproduction, behaviour and coat growth in seasonal mammals. This transduction of photoperiodic information is the most important physiological function of melatonin in mammals. Photoperiod-dependent changes in seasonal species also includes changes in core body temperature and in timing and distribution of sleep. The role of melatonin in the human circadian system is less obvious.

A stimulus of any nature that sifts circadian rhythms is known as a zeitgeber (time cue)[17].

Zeitgebers advance or delay circadian rhythm according to the time they are exposed. The direction and magnitude of the change in timing (phase shift) is described by the phase

shift response curve (PSC). The time base of a PSC is circadian time which is effectively internal biological time usually defined in humans by the timing of core body temperature rhythm or melatonin secretion. A circadian system synchronised to the 24-hour day with appropriate timing of overt rhythms is described as 'entrained '. Synchronisation means that the period ($\tau$) of the circadian system is 24 hours but not necessarily in the correct phase. Re-entrainment is the re-establishment of correct timing with respect to the 24-hour day. Circadian period in a time-free environment is known as free-running. Average human $\tau$, in sighted individuals, is 24.2 hours[18,19,20]. Circadian period is an inherited characteristic and has been shown to be closely related to diurnal preference and the early or late timing of the circadian system in a normal entrained situation[21,22]. Subjects with longer periods will have an evening preference compared to the shorter intrinsic period. In order to remain entrained the system needs to be reset frequently. Ocular light exposure is the primary resetting time cue. Sighted people living in dim lighting and with other weak time cues like little social contact or no structured daily activities may show free-running or very late timing of the circadian system (delay phase). E.g. Life in polar winter[23,24]. Blindness, Jet lag and shift work also induce phase shifts. It may also be a problem in the elderly.

## Melatonin as an antioxidant

Melatonin and its metabolites are highly effective at reducing oxidative stress though its ubiquitous distribution and functional diversity. It is capable of direct detoxification of reactive oxygen (ROS) and nitrogen species. It stimulates antioxidant enzymes and suppresses activity of pro-oxidative enzymes. It also chelates transition metals, and in doing so, reduces highly toxic hydroxyl radicals. Melatonin is ubiquitous throughout cells and tissues but with an uneven cellular distribution, more highly concentrated in mitochondria where free radical production is maximal.

Melatonin was originally discovered in the pineal gland and was thought, at that time, to be unique to the pineal tissue of vertebrates, the only species with this organ. Within a decade, however, the melatonin forming enzyme it was found in the retina by Quay[25,26], a link to the 3rd eye, an early evolutionally photoreceptive organ still found in some non-mammalian vertebrates.

Melatonin is not limited to vertebrates but present in all organisms examined, including bacteria, invertebrates and plants, with the dinoflagellate *Gonyaulax polyedra* displaying a 24-hour rhythm[27]. The ability to de-toxify free radicals preserved in all species. As well as the direct free radical scavenging, melatonin stimulates anti-oxidative enzymes, including glutathione peroxidase and reductase[28-32], upregulating production of glutathione and suppressing the pro-oxidative enzyme nitrous oxide synthetase[33,34]. When total antioxidant capacity of human blood was compared to both day and night melatonin concentrations these parameters were positively correlated[35].

## Melatonin in mitochondria

Mitochondria are involved in melatonin metabolism and synthesis. In mitochondria there are two pathways of melatonin metabolism. Firstly, a monooxygenase,

cytochrome P450-dependent pathway. This provides compounds that participate in mitochondrial homeostasis. Secondly, a kynuric pathway, through the pseudoperoxidase activity of cytochrome C. The main products being $N^1$-acetyl-$N^2$ -formyl-5 methyl-kynuramine (AFMK) and its secondary product $N^1$-acetyl-methyl-kynuramine (AMK)[36], Figure 4. Although, there is some accumulation of these products through non-enzymatic processes. Mitochondria have their own antioxidant systems but direct scavenging of ROS by melatonin plays a supporting role, at least, in attenuating UVR induced oxidative stress, of particular significance in the epidermal compartment. There is sufficient evidence that cytochrome C, though its pseudoperoxidase activity, effectively competes with mitochondrial scavenging enzymes (catalase, glutathione peroxidase and peroxiredoxin) to control $H_2O_2$ levels[37,38]. It is a natural scavenger of $H_2O_2$ via a mechanism linked to reverse electron transfer from succinate to NAD [+39]. It is also involved in the $H_2O_2$-mediated-signaling pathway via regulation of $H_2O_2$ efflux from the mitochondria to the cytosol.

Of particular interest is the ability of mitochondria to reconstitute melatonin by converting N-acetyl serotonin back to melatonin[40], the rate limiting enzyme detected in mitochondria of oocytes[41].

Melatonin is also involved in mitochondrial bioenergetics with the suggestion that melatonin could donate electrons to the electron transfer chain, improving mitochondrial respiration and increasing ATP production[42].

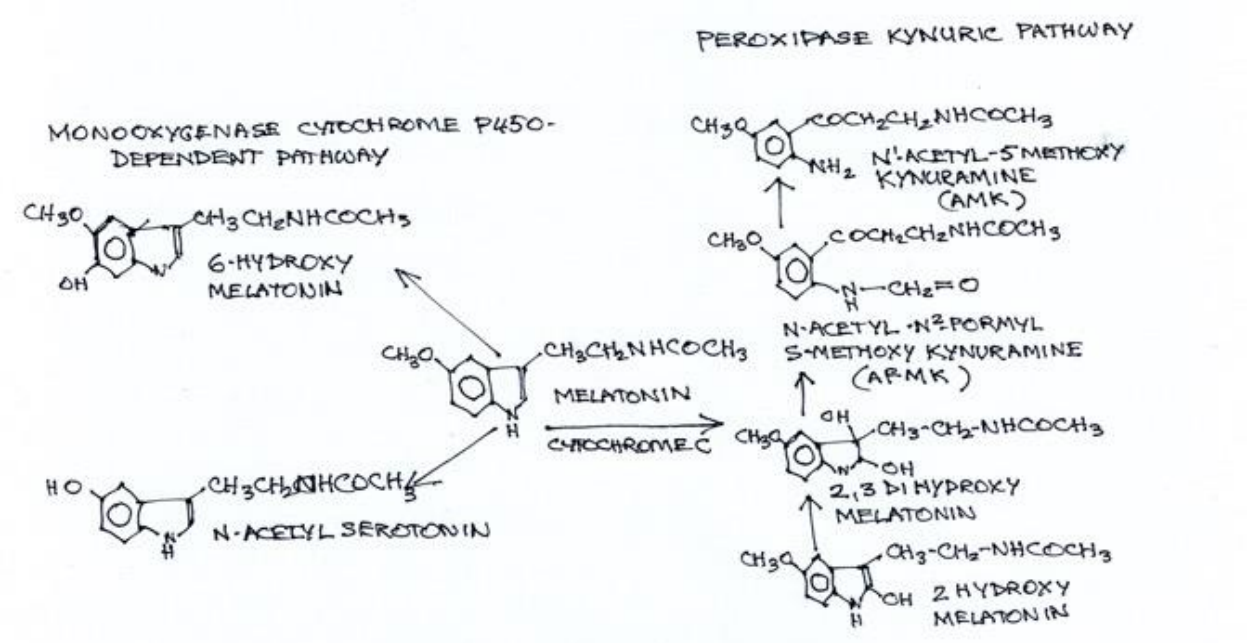


Figure 4. Melatonin metabolism in mitochondria.

## Melatonin in the skin

Slominski et al provided the first evidence that melatonin can be synthetised in mammalian skin. Serotonin transformed with N-acetyl-serotonin as an intermediate in the pathway[43]. They also found that keratinocytes and melanocytes express the biochemical elements necessary to convert tryptophan to serotonin to melatonin. Melatonin is metabolised by both indolic and kynuric pathways, and this metabolism can be stimulated by UVB, dependent on dose and time of exposure[44]. All the metabolites are represented in the epidermis but 6-hydroxy-melatonin predominates. All these metabolites can potentially affect mitochondrial functions in the skin cells and consequently skin phenotype. Thus, melatonin can regulate cutaneous adnexal, pigmentary and barrier functions, with oncostatic effects having been demonstrated in melanoma cells[45,46]. Many of the phenotypic effects are mediated through melatonin membrane-bound receptors, MT1, epidermal and MT2, adnexal. The nuclear receptor, however, requires higher concentrations that can only be provided by local cutaneous production balanced by on-site metabolism rather than a pineal source of the melatonin.

Melatonin and its metabolites act as antioxidants on UV radiation[47,48] providing protective effects in reducing oxidative cell damage in keratinocytes and melanocytes[49,50]. This has been found to act independently of melatonin receptors in melanocytes[50]. Similar effects on keratinocytes, melanocytes and dermal fibroblasts have been demonstrated in culture[49,50,51] with AMK a potent singlet $O_2$ scavenger. The mechanism of protection from UVB-induced oxidative stress is mediated by activation of nuclear factor erythroid 2-like 2 (NRF2) and upregulation of an NRF2-dependent pathway in keratinocytes and melanocytes[49,52]. There is a reduction in the production of photoproducts with increased UVB-induced DNA repair, enhanced interaction between damaged DNA and core factors XPA and XPC modulating the nucleotide excision repair process. Phosphorylation of tumour suppressor protein p53 increases its activity and a reduction in pro-inflammatory cytokine gene and pro-apoptotic protein expression in keratinocytes providing further protective effects.

Melatonin and its metabolites also have a role in regulation of redox homeostasis in skin cells with redox signalling by $H_2O_2$ released from mitochondria influencing epidermal cellular proliferation, differentiation and hair follicle development.

## Melatonin and the immune system

Pineal synthesised melatonin is considered to be a member of a complex neuro-endocrine-immunological system that communicates within itself with a common language[53]. However, melatonin does not behave as a classical hormone being synthesised by extra-pineal, non-endocrine tissues such as the skin and the immune system itself.

Studies over the last century documenting a potential endocrine function of the pineal gland on the immune system. Firstly, pinealectomy, in experimental animals, causing an involution in immune organs such as the spleen and thymus gland and reversal with melatonin administration. Secondly, a rhythmicity of immune parameters with

diurnal and seasonal rhythms of cellular proliferation in bone marrow and lymphoid systems, lymphocyte subsets, Natural killer cell activity and cytokine production, demonstrating a direct relationship between photoperiod and immune response[54]. Shorter day length associated with enhanced immune function[55]. This is thought to have developed as an adaptive response to winter conditions with low temperatures and limited food availability.

The nervous and immune system have a complete bidirectional circuit involving shared ligands, such as neurotransmitters. neuroendocrine hormones, including melatonin, cytokines and their respective receptors. The idea of shared ligands and receptors allows the immune system to serve as a sixth sense that notifies the nervous system of the presence of entities such as viruses and bacteria that are imperceptible to the classic senses. Lymphocytes and natural killer cells acting as sensors continually sampling the internal environment. What was previously viewed as outside the realms of sensory perception, metaphysical, is now revealed. Through this bidirectional relationship personality and outlook can be seen to possibly influence susceptibility to disease. The ancient view of the pineal gland as having a metaphysical connection may not be so far-fetched.

## Conclusion

Why the pineal gland was chosen, from within anatomical brain structures, as the link between man and the metaphysical nature of the mind is shrouded in the mists of time and antiquity. Galen believed that soul flows in the form of air from the lungs to the heart and then to the brain, controlled by the pineal gland. Descartes described it as the "principle seat of the soul" and he believed it to be a point of connection between the intellect and the body. A single section of the brain rather than being in two parts. He argued that because a person can never have more than one thought at the same time, external stimuli must be united within the brain before being revealed to the soul.

Evolutionary theory paints a more functional picture of the pineal and its secretory product. The pineal gland evolving from a photosensitive to secretory role. Melatonin, as an antioxidant molecule being incorporated to provide an extra level of protection in the CNS, mitochondria and skin. The retina became the source of all photic input with connection to the visual cortex for imaging of the environment, and the SCN for interpretation of time of day for diurnal changes in physiological needs. Beyond diurnal time, incidence and responses to stressors varied on a seasonal basis with fluctuations in energy availability, potential impairment of immune function and disease susceptibility. Shorter day lengths required rerouting energy from reproduction and growth to bolster immune function during winter, increasing survival. The pineal taking on a chronobiotic role as an adaptive mechanism to counter seasonal stress.

Are these adaptive mechanisms still relevant in modern urban living conditions? Oxidative stress within the central nervous system still limits lifespan and is responsible for degenerative diseases that cause impairment and disability. Healthy life styles still require reducing stressors on the system and bolstering of antioxidant defences. Should we

be responsive to natural changes in light at dawn and dusk and does artificial light at night interfere with melatonin production and natural patterns of sleep? Do these factors influence disease susceptibility and longevity? The answer is obvious.

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