REVIEW ARTICLE

Exploring Links between Pineal Gland Calcification and Central Nervous System Disease

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Abstract: Central nervous system disease is globally common with dysfunction of the brain and spinal cord, which significantly affects the quality of life, such as sleep disturbances. Pineal gland is pivotal in regulating sleep cycles and circadian rhythms. And melatonin, secreted by pineal gland, promotes neurodevelopment and maintains neurohomeostasis, which is also pivotal in the modulation of central nervous system disorders. In recent years, studies have found that patients with central nervous system damage often have degeneration of the pineal gland, characterized by a decrease in pineal gland volume, reduced melatonin secretion, and even parenchymal calcification. An increasing number of Alzheimer's disease patients have been observed to exhibit pineal gland calcification. Research suggests that sleep disturbances accompanying central nervous system disorders can be attributed to the degeneration of pineal gland function, indicating a potential contribution of gland calcification to central nervous system diseases. Here, we review the recent research on pineal gland calculi and discuss the potential relationship between pineal gland calcification and various central nervous system diseases, contributing to a deeper understanding of the intricate mechanisms underlying neurological disorders.

Keywords: Pineal gland; calcification; pineal gland calculi; central nervous system disease.

1. INTRODUCTION

The central nervous system (CNS), which comprises the brain and spinal cord, acts as the central hub for consciousness, sensory perception, motor control, and higher-order cognitive process. CNS diseases present a significant challenge in contemporary healthcare, encompassing both neurodegenerative disorders and psychiatric conditions. These diseases involve intricate neural networks, giving rise to a diverse array of symptoms and functional impairments. The prevalence and complexity of CNS diseases underscore the urgent need for an in-depth understanding of their underlying mechanisms and potential contributing factors.

Recent investigations have increasingly highlighted the importance of the pineal gland in the pathogenesis of neurological disorders. Situated deep within the brain, the pineal gland is essential in the regulation of circadian rhythms and neuroendocrine function. Despite being relatively small, this pinecone-like gland holds significance due to its synthesis of melatonin and involvement in the neuroendocrine system. Over recent years, research focused on the functions and calcification of pineal gland have seen considerable growth. Pineal gland

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calcification directly impacts the number of pinealocytes, leading to insufficient melatonin secretion and consequently affecting neurodevelopment and disrupting neural homeostasis. Additionally, Studies suggest a correlation between melatonin deficiency and the deposition of Aβ, as well as damage to dopaminergic neurons, prompting further exploration into the clinical significance of pineal gland calcification. These studies aim to elucidate that pineal gland may clarify the potential involvement of the pineal gland in the onset of neurological disorders (Fig.1).

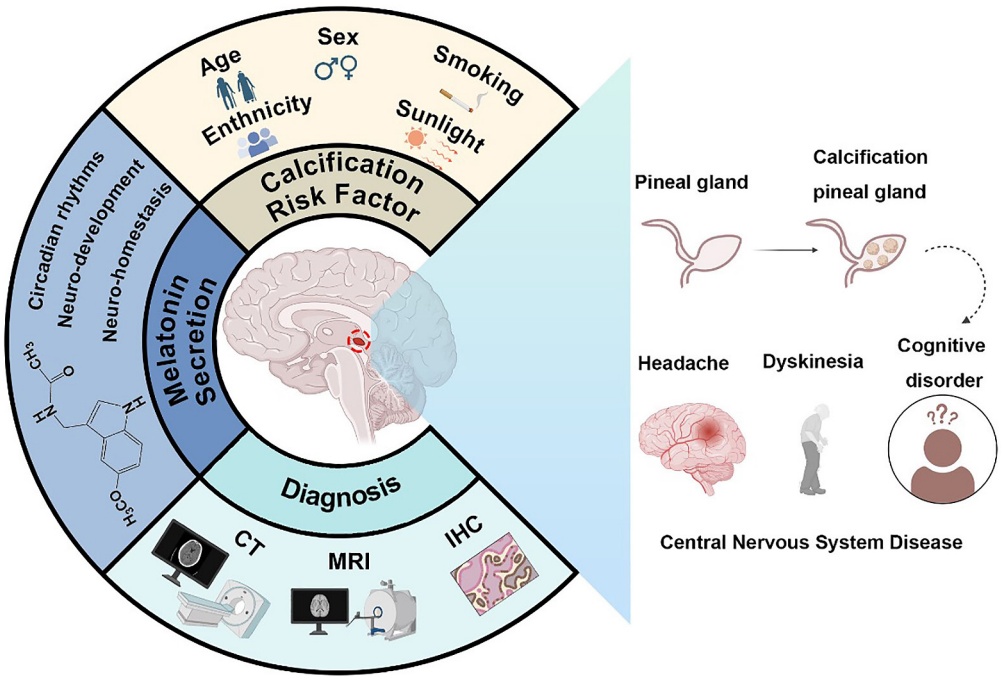

Figure 1. Pineal gland calcification plays a potential role in the pathogenesis of central nervous system disease.

2. PINEAL GLAND

The pineal gland, often referred to as the "third eye", is red-gray, pea-like, typically 6-10 mm long, 5-6 mm wide, and weighs between 50 and 150 mg (Harisha, Arava, Singh, & Pariplavi, 2023). The pineal gland is mainly composed of the parenchyma formed by pinealocytes, connective tissue and glial tissue dominated by astrocytes (Lee, Ng, Lian, Yap, & Chuah, 2011). The surface of the pineal gland has the connective tissue capsule derived from leptomeninge differentiation, which extends into the pineal parenchyma with the blood vessels, dividing the parenchyma into many irregular lobular structures. The parenchyma of some pineal glands is arranged in an insular pattern, separated by more complex connective tissue (Gheban, Rosca, & Crisan, 2019).

Situated in the posterior part of the third ventricle, the pineal gland resides between the corpus callosum and the superior thalamus. uniquely positioned outside the blood-brain barrier, unlike most brain regions (Adams *et al.*, 2017). It is connected to the brain by the pineal stalk and receives its blood supply from several arteries, including the posterior cerebral artery and the choroidal artery.

It has been reported that the pineal gland enlarges from birth up to 2 years of age and then stabilizes in size from age 2 to 20 years (Belay & Worku, 2023). There is also a correlation between pineal size and individual height and weight. Generally, individuals weighing between 61 and 70 kg have a relatively higher pineal gland density; while individuals with a height shorter than 165 cm have the smallest pineal width (Gheban *et al.*, 2019).

The pineal gland, recognized as a neuroendocrine transducer, acts as a "circadian clock" regulating physiological and behavioral processes, converting neural signals from the suprachiasmatic nucleus of the hypothalamus into various hormones that play an important role in different organs (thyroid, neurohypophysis, etc.) (Harisha *et al.*, 2023). The primary role of the pineal gland is the secretion

and release of melatonin, a hormone directly linked to light exposure and regulated by the circadian rhythm (Rosenstein, Estévez, & Cardinali, 1989). Light signals received by the retina are relayed via the hypothalamic bundle to the suprachiasmatic nucleus, inducing norepinephrine release, which binds and actives on β-adrenergic receptors in the pineal gland. The synthesis of melatonin subsequently modulated by increasing the activity of N-acetyl transferase (Patel *et al.*, 2020), thereby influencing various physiological and behavioral processes.

Melatonin, or N- [2- (5-methoxy-1H-indol-3-yl) ethyl] acetamide, is a neurohormone widely considered to be involved in starting and maintaining sleep-wake cycles, blood pressure regulation, and seasonal reproduction (Esposito & Cuzzocrea, 2010). Due to the light dependence of melatonin synthesis, plasma concentrations of melatonin peak in the dark environment at night while remaining nearly undetectable during the day (Yang, Kim, Kim, & Cho, 2021).

The pineal gland is the main site for melatonin production. During the synthesis process, tryptophan, serving as the precursor of melatonin, first undergoes hydroxylation to 5-hydroxytryptophan within pinealocytes, which subsequently decarboxylated to serotonin under the catalysis of decarboxylase. Through the catalysis of acetyltransferase, serotonin transforms into N-acetyl-5-hydroxytryptamine by reaction with acetyl-CoA, and ultimately transforming into melatonin due to the action of methyltransferase (Gheban *et al.*, 2019). With the progress of research, it has been found that the production of melatonin is not limited to the pineal gland. Other tissues including the retina, intestines, ovaries, testes, bone marrow, and lens also produce melatonin (Esposito & Cuzzocrea, 2010). The melatonin produced by these tissues and organs mainly acts as a paracrine hormone, which means that melatonin produced by these organs primarily has a local effect, rather than being distributed throughout the body like the melatonin secreted by the pineal gland. Moreover, compared with the melatonin produced by the pineal gland, the functional significance of melatonin produced by other organs remains to be fully elucidated (Patel *et al.*, 2020). Furthermore, due to its biological regulatory function, the chemical synthesis process of melatonin has progressively matured.

The amount of melatonin secretion is directly related to the volume of pineal gland parenchyma. However, an increase in the volume of pineal gland tissue does not necessarily imply an enhancement in pineal secretory activity. This is due to the fact that the increase in volume may derive from the proliferation of neuroglia and parenchymal calcification (Gheban *et al.*, 2023), which can lead to a reduction in parenchyma volume, potentially impacting the number of pinealocytes and resulting in melatonin deficiency (Gorgulu & Koc, 2021; Junemann, Bukreeva, *et al.*, 2023).

The main function of melatonin is to mediate circadian rhythms, while melatonin also assumes a pivotal role within the CNS, primarily through the activation of melatonin receptors (MTs) (Song, 2019). Substantial evidence underscores the diverse neurobiological effects of melatonin, including the enhancement of synaptic plasticity, facilitation of neurogenesis, and augmentation of memory function. Notably, melatonin can inhibit neuroinflammation, thereby contributing significantly to overall neural homeostasis (Esposito & Cuzzocrea, 2010).

Moreover, melatonin's regulatory function plays a part in immune defense responses (Guerrero & Reiter, 2002). Studies indicate that immune cells are capable of synthesizing melatonin within the bloodstream, thereby regulating immune responses to stress conditions.

The initial discovery of melatonin's powerful free radical scavenging properties was made three decades ago. Melatonin has been found to possess a potent anti-oxidant ability, allowing it to scavenge oxidative stress in various tissues (Novais, Chuffa, Zuccari, & Reiter, 2021), which has been proven to be independent of receptor-mediated actions. Beyond its direct free radical scavenging, melatonin is vital in oxidative defense, activating enzymes that convert free radicals and their products into harmless compounds (Tomas-Zapico & Coto-Montes, 2005). Moreover, melatonin is known to enhance the activity of key antioxidant enzymes, including superoxide dismutase and glutathione peroxidase, at a molecular scale, emphasizing its role in enhancing the antioxidant defense system (Ghorbani, Pishkar, Saravi, & Chen, 2023).

The role of melatonin in cognitive function is evident, substantiating its positive impact on learning and memory. Melatonin supplements have demonstrated promising results as a therapeutic intervention, offering a potential avenue for addressing cognitive impairments.

Extracellular vesicle (EV) melatonin therapy demonstrates significant potential in mitigating vascular calcification. Research indicates that

treating EVs isolated from Vascular Smooth Muscle Cells (VSMC) or calcified VSMC (CVSMC) with melatonin allows for their absorption by VSMCs, leading to the inhibition of osteogenic differentiation and senescence in both VSMCs and CVSMCs, thereby exhibiting therapeutic efficacy in a mouse model (Xu *et al.*, 2020). Moreover, melatonin has been identified as a key player in attenuating vascular calcification by inhibiting mitochondrial fission through the AMPK/Drp1 signaling pathway. This highlights melatonin's regulatory role in the cellular process associated with calcification (Chen *et al.*, 2020). In summary, the utilization of extracellular vesicle melatonin therapy exhibits promising outcomes in alleviating vascular calcification by modulating relevant cellular processes. Additionally, the observed capacity of melatonin to mitigate vascular calcification implies its potential involvement in the Pineal gland calcification process.

In addition to secreting and releasing melatonin, recent research suggests that the pineal gland can exert inhibitory effects on the gonads, thereby regulating the reproductive system. Tan *et al.* (Tan *et al.*, 2010) found that when photoperiodic mammals are pinealectomized, the yearly reproductive cycle is interrupted. Furthermore, the pineal gland, stimulated by darkness, is thought to modify the central regulation of pituitary gonadotrophin synthesis or release. The pineal gland has been associated with an inverse relationship between its size and the weight of the testis, as well as plasma and testicular testosterone levels, indicating its influence on gonadal physiology. Additionally, the pineal gland is implicated in influencing the neuroendocrine-thyroid axis, emphasizing its broader role in regulating endocrine functions beyond reproductive physiology.

3. PINEAL GLAND CALCIFICATION

Although the pineal gland, habenula and choroid plexus of the lateral ventricles are all common sites of intracranial physiological calcification, pineal gland exhibits the highest frequency of occurrence (Ghorbanlou, Moradi, & Mehdizadeh, 2022). Clinically, pineal gland calcification is a common physiological occurrence, with a prevalence that can be as high as 61.65% (Belay & Worku, 2023). It is generally acknowledged that pineal gland calcification is a process positively correlated with age. However, there are many children who have also been detected with pineal gland calcification, with the calcification rate approximating 2% in 5-year-old children, escalating to 35.8% in children aged 7 to 12. Astonishingly, the youngest child found with pineal gland calcification is mere 3 years old (Al Hajri, Sirasanagandla, Boudaka, Al Dhuhli, & Al Ajmi, 2023). The calcification is potentially associated with the growth, development, and hormone levels of children, holding significant research value. Moreover, A few studies reported a correlation between pineal gland calcification and various neurological disorders, such as Alzheimer's disease, schizophrenia, and Parkinson's disease. This suggests that pineal gland calcification may play a role in the progression of neurological disorders, emphasizing the importance of investigating the functions and underlying mechanisms of pineal gland calcification. Pineal gland calcification is not limited to humans. According to research, pineal gland calcification is common in different species. An increasing number of animals, including ox, sheep, horses, donkeys, monkeys, cows, gerbils, rats, guinea pigs, chickens, and turkeys, have also been found to exhibit calcification of the pineal gland (Patel *et al.*, 2020), providing a broad range of model choices for the study of pineal gland calcification and neurological disorders. Furthermore, calcification of the pineal gland may result in the formation of calculi akin to kidney stones.

3.1. Formation

The formation of pineal gland calculi has been attracted significant scientific interest, with studies suggesting that they predominantly form within the parenchyma, potentially originating from pinealocytes (Junemann, Bukreeva, *et al.*, 2023). Concurrently, they are also present within the connective tissue septa. This has led to the hypothesis that calcification initially occurs in the pineal parenchyma, which is subsequently replaced by connective tissue (Junemann, Bukreeva, *et al.*, 2023). Pinealocytes are characterized by the presence of vacuoles laden with flocculent and fibrous substances. Welsh (Welsh, 1984) and Krstic (Krstić & Golaz, 1977) have highlighted the pivotal role of vacuoles in the calcification process, proposing that calcium is transported into the vacuoles, leading to intracellular calcification. They hypothesized that the vacuole is the site of initial calcification in the cell. Furthermore, the cytoplasmic matrix, endoplasmic reticulum, and mitochondria are also considered potential initial sites of calcium deposition within pinealocytes, with formation of hydroxyapatite crystals.

As pinealocytes degenerate and die over time, the calculi diffuse into the extracellular space.

Understanding the mechanisms underlying pineal gland calcification is crucial due to its potential implications for various physiological and pathological processes. Pineal gland calcification is a complex process which may represent a physiological, maturation, or degenerative process, and its underlying causes remain elusive. Researchers have proposed several hypotheses on how these calculi form. Studies have shown a positive correlation between elevated fluoride levels in the human pineal gland and calcium accumulation (Mrvelj & Womble, 2020). Mrvelj and Womble found that eliminating fluoride from the diet promotes the proliferation of pinealocytes in elderly rats. Conversely, fluoride intake inhibits pineal growth (Gheban *et al.*, 2021), leading decrease in melatonin. Fluoride is considered a beneficial substance for oral care, and the water supply in many countries has been fluoridated for a long time. Therefore, exploring the role of fluoride in pineal gland calcification and choroid plexus metastatic calcification has significant public health implications. Beyond that, mesenchymal stem cells have been proposed as potential contributors to the calcification process in the pineal gland. The high vascular supply of the pineal gland leads to elevated cytokine levels, attributed to chronic inflammation. This, in turn, promotes the proliferation and migration of mesenchymal stem cells, leading them to differentiate into osteoblasts and osteocytes through interactions with inflammatory mediators (Kopani *et al.,* 2019). Additionally, the aggregation of hypertrophic cells in areas where calcium originates and deposits implies that these cells might also be involved in the calcification process (Whitehead, Oh, Raju, & Choudhri, 2015).

3.2. Various Types

Pineal gland calculi, demonstrate a significant degree of heterogeneity, with variations in morphology, type, and composition observed across different individuals. These calculi, often round or elliptical, are typically several hundred micrometers in size (Junemann, Ivanova, *et al.,* 2023), while aggregates can reach several millimeters. A substantial portion of these calculi exhibits a mulberry-like form and other large fractions consist of small, distinct crystalline cores (Baconnier *et al.,* 2002), which display a layered structure akin to compact bone (Bukreeva *et al.,* 2023). This has led to theories proposing a similar mechanism of formation to that of bone calcification (Junemann, Bukreeva, *et al.,* 2023). In some cases, dense particles have been identified at the center of calculi aggregates within the pineal gland, which can further develop into irregular or hollow structures. Interestingly, an increase in both the quantity and variety of pineal gland calculi has been observed with advancing age, with the emergence of concentric lamellar structures resembling growth rings. Research has suggested a correlation between the number of layers in these lamellar structures and the age of the individual, implying a relationship between the formation of concentric layers and periodic changes in pineal gland calcification levels (Harisha *et al.,* 2023). Calculi found in the pineal gland of children differs from that found in adults. Typically, pineal gland calculi in children are punctate, with no observed aggregations of calculi (Whitehead *et al.,* 2015). It remains unclear whether the calcification observed in pediatric cases is the result of a physiological process or indicative of potential pathology. Further research is warranted to elucidate the significance of pineal gland calcification in pediatric populations and its potential impact on neurodevelopment.

The composition of pineal gland calculi exhibits substantial variability. While hydroxyapatite is generally accepted as the primary component of pineal gland calculi, the presence of calcium carbonate crystals has also been documented. The clinical significance of these compositional differences remain unclear. There is an absence of organic matrix within the pineal gland calculi, while metallic elements such as magnesium, iron, copper, and zinc have been detected (Junemann, Ivanova, *et al.,* 2023). Given the crucial roles these elements play within the human body, metallic element detection within the pineal gland could provide valuable insights into the mechanisms underlying the formation and pathological implications of pineal gland calculi.

3.3. Effects on Pineal Gland Function

The accumulation of calculi within the pineal gland can lead to hypoxia and subsequent pinealocyte death (Kopani *et al.,* 2019). Moreover, hypoxia is known to cause an increase in vascular permeability and nitric oxide production in the pineal gland, which may contribute to the calcification process (Kaur, Sivakumar, Lu, & Ling, 2007). Pineal calcification can impact the production of melatonin, the most important hormone producted by the pineal

gland. The decrease of melatonin, in turn, promotes the calcification of pineal gland and thereby forming a cyclic exacerbation, which is associated with nerve diseases, such as schizophrenia.

3.4. Risk Factors

Numerous investigations have demonstrated a correlation between calcification of the pineal gland and various factors, including age, sex, altitude, sunlight exposure, race, nutritional changes, neurodegenerative diseases, unhealthy lifestyle habits, etc (Belay & Worku, 2023). Age is considered the primary risk factor for pineal gland calcification (Jalali *et al.,* 2023).

A significant reduction in biosynthetic activity of the pineal gland has been observed in aging hamster (Reiter, Richardson, Johnson, Ferguson, & Dinh, 1980). The age-related decline in melatonin biosynthetic activity and the volume of the pineal parenchyma may be reflected in changes in the calcium homeostasis of pinealocytes, leading to age-related changes in pineal gland calculi. Histologically, it has been found that the number and size of pineal gland calculi increases with age from the fetal period to adulthood, exhibiting a structure similar to "growth rings", accompanied by pineal degeneration. The rate of pineal gland calcification is only 40% in adolescents, but it rises to 70% after the age of 40, suggesting age-related alterations in pineal gland calculi.

The incidence of pineal gland calcification has been observed to be higher in males, with a prevalence rate is twice as high as in females (Jalali *et al.*, 2023). This divergence between sexes may be attributed to specific lifestyle habits, such as smoking, which has been indicated as a contributing factor to vascular calcification in different tissues (Ozan *et al.,* 2007). Smoking is recognized to trigger oxidative stress and induce pathological changes in VSMC, leading to calcification (Ozguner, Koyu, & Cesur, 2005). Moreover, Jalali (Jalali *et al.*, 2023) have proposed smoking affects melatonin levels and a notable correlation exists between smoking and elevated levels of pineal gland calcification in individuals aged 63 and above. However, whether smoking can induce pineal gland calcification through a similar mechanism that induces calcification of VSMC remains to be further investigated.

The prevalence of pineal gland calcification exhibits geographical variations, with calcification rates ranging between 35.2% and 76% across various nations (Belay & Worku, 2023). This variation in calcification prevalence may be influenced by environmental factors. Light is the most typical environmental factor. It has been suggested that individuals living in low-altitude areas and those with low intensity of sunlight exposure are more prone to pineal gland calcification. This susceptibility may be attributed to sunlight as a major source of vitamin D, which plays a crucial role in regulating calcium metabolism (Razzaque, 2011).

The influence of ethnicity on the prevalence of pineal gland calcification has also been explored. The prevalence of pineal gland calcification has been consistently observed to be higher in individuals of white ethnicity compared to other ethnic backgrounds. This difference in prevalence has been attributed to genetic factors or variations in lifestyle and environmental factors across different ethnic groups (Jalali *et al.,* 2023).

In addition to age, gender, smoking, sunlight exposure, and ethnicity, other risk factors for pineal gland calcification have been investigated. A sedentary lifestyle, obesity, and certain medical conditions, such as hypertension and diabetes, have been linked to a heightened risk of calcification.

3.5. Diagnosis

Cerebral computed tomography (CT), Magnetic Resonance Imaging (MRI) and Immunohistochemistry (IHC) represent valuable methods for both diagnostic and research of pineal gland calcification. CT plays a pivotal role in the identification of intracranial calcification. During CT examinations, intracranial calcification manifests as high-density shadows. MRI provides a non-invasive means for visualizing the pineal gland, facilitating the comprehensive evaluation of its volume and morphological characteristics. This imaging modality has been employed to establish correlations between pineal gland volume and melatonin levels, contributing valuable insights into the physiological implications of calcification process (Sigurdardottir *et al.,* 2016). IHC serves as a pivotal technique in the histological analysis of the pineal gland. This methodological approach enables the precise identification of specific proteins and markers associated with the intricate process of calcification. By elucidating the molecular underpinnings of calcification, IHC contributes to a nuanced understanding of the cellular and molecular events that underlie pineal gland calcification, thereby enriching diagnostic capabilities and advancing research endeavors in this domain.

4. POTENTIAL LINKS BETWEEN PINEAL GLAND CALCIFICATION AND CENTRAL NERVOUS SYSTEM DISEASE

4.1 Alzheimer's Disease (AD)

CT scans reveal more distinctive characteristics of pineal gland calculi in AD patients. Within pathological samples of AD, calculi within severely degenerated parenchyma exhibit more hollow or concentric structures or display large and deep fissures (Bukreeva *et al.*, 2023). AD is a widespread neurodegenerative condition characterized by a decline in cognitive functions and the onset of memory impairment. The two main pathological features of AD are the formation of neurofibrillary tangles, which are aggregates of hyperphosphorylated tau protein, and the accumulation of beta-amyloid plaques in the brain. These pathological changes lead to the progressive degeneration of neurons and subsequent cognitive impairment in patients. The clinical manifestations of AD include progressive memory loss, decline in cognitive function, and disturbances in sleep patterns. As a prevalent contributor to dementia among the elderly population, AD presents a substantial challenge for individuals, families, and healthcare systems globally. It is estimated that there are currently over 50 million people living with AD globally, and this number is expected to increase as the population aging. By 2050, it is projected that the number of individuals with AD will reach 150 million, placing a substantial strain on society and healthcare resources. The pathogenesis of AD is multifactorial and complex, involving interactions between genetic, environmental, and lifestyle factors. Comprehending the intricate interaction between genetic and environmental in the development of AD is essential for devising successful prevention and treatment approaches, aiming to alleviate the increasing impact of AD on society.

Patients with AD exhibit sleep disturbances from the early stages, making it an important diagnostic indicator for predicting the progression of AD. AD models are characterized by Aβ deposition, and a relationship exists between the extent of sleep quality disruption and cognitive dysfunction (Lim, Gerstner, & Holtzman, 2014). Researcher has indicated a link between reduced sleep duration and lower sleep quality in the elderly, correlating with increased Aβ deposition, further emphasizing the association between sleep disturbances and AD progression (Gao *et al.*, 2020). Additionally, sleep disturbances have been shown to intensify microglial reactivity and promote Aβ deposition in a manner dependent on TREM-2, highlighting the intricate connection among sleep, neuroinflammation, and the pathology of AD (Parhizkar *et al.*, 2023).

The pineal gland produces melatonin, a vital factor in the regulation of sleep. Therefore, the role of the pineal gland in AD pathology has been the focus of extensive research. Reduced melatonin in both serum and cerebrospinal fluid has been observed in AD patients compared to healthy individuals, which is associated with cognitive impairment, underscoring the potential impact of pineal gland dysfunction on AD pathogenesis (Song, 2019).

Aβ is one of the most critical indicators in the pathological processes of AD and represents a potential therapeutic target. Melatonin plays a crucial role in regulating and clearing Aβ deposition. ADAM10 and BACE1 are key enzymes in the non-amyloidogenic and amyloidogenic pathways, respectively, with the latter leading to excessive Aβ generation and ultimately contributing to AD pathology. Melatonin can modulate ADAM10 and BACE1 through pathways such as JAK2/STAT1 and PI3K/Akt, promoting α-secretase activity while inhibiting β-secretase function, thereby suppressing Aβ production. Additionally, melatonin can directly interfere with amyloid precursor protein expression via cAMP. Not only does melatonin regulate Aβ generation, but it also inhibits Aβ aggregation by directly interacting with it and participates in the clearance process in AD pathology (Li, Zhang, Wan, Liu, & Sun, 2020). These findings highlight the significant clinical therapeutic potential of melatonin in the pathological processes of AD.

In a transgenic rat model of AD, continuous use of melatonin for two months can promote the growth of hippocampal synapses, reduce Aβ accumulation in the cortex and hippocampus by 37% and 43% respectively, prevent mitochondrial dysfunction, and reduce degenerative changes in tissues (Alghamdi, 2018). Compared with the rats that orally received melatonin, rats injected only with soluble Aβ42 in the lateral ventricle without melatonin treatment exhibited an increased number of hippocampal astrocytes, along with prominent functional disorders and poorer spatial memory abilities (Liu *et al.*, 2013). In clinical practice, the administration of melatonin supplements prior to sleep to enhance sleep quality and cognitive performance to a certain degree.

Ozansoy *et al.* established an in vitro Aβ toxicity model, demonstrating that melatonin can inhibit Aβ toxicity by blocking the secretion of EV molecules, thereby influencing the levels of Tau protein carried by exosomes (Ozansoy *et al.*, 2020).

Elevation in the pro-inflammatory cytokine tumor necrosis factor (TNF) serves as a biomarker indicating the advancement of AD, and melatonin can inhibit its expression, reducing neuroinflammatory responses (Zhang *et al.*, 2019). Additionally, melatonin has demonstrated anti-inflammatory effects by suppressing the expression of the NLRP3 and the activation of NF-κB, while up-regulating the expression of the transcription factor Nrf2 (Muñoz-Jurado *et al.*, 2022).

Pineal gland calcification is directly associated with a reduction in melatonin release. The degeneration of calcified pineal parenchyma contributes to a decrease the number of pinealocytes that secrete melatonin. So far, although there are contrary conclusions, the mainstream theory believes that pineal gland calcification in AD patients is more severe and the volume of the pineal gland is smaller. These inconsistent findings may arise from individual and regional variations in the pineal gland and the current limitations in research techniques (Bukreeva *et al.*, 2023).

4.2. Schizophrenia

Melatonin, as a therapeutic agent for schizophrenia, has demonstrated positive effects, suggesting that pineal gland damage and calcification may be implicated in the regulation of schizophrenia progression. Schizophrenia is a profound mental disorder marked by symptoms like disordered thinking, hallucinations, and delusions. Individuals diagnosed with schizophrenia frequently experience emotional dysregulation and deficits in social cognitive functioning. Moreover, they may exhibit disturbances in the attribution of thoughts and sensations, perceiving them as external rather than originating from within themselves. The etiology of schizophrenia is multifaceted, involving both genetic and environmental factors. Drug abuse, inadequate nutritional intake during gestation, and childhood adversity are risk factors for schizophrenia (Howes & Murray, 2014; Koenig, Kirkpatrick, & Lee, 2002). Dysregulation in certain neurotransmitter, disruptions in clock genes, activation of oxidative and immune-inflammatory, and structural abnormalities in the brain also seem to contribute to the pathophysiology of the disease (Kamath, Virdi, & Winokur, 2015). An estimated 24 million individuals worldwide are impacted by schizophrenia worldwide, with approximately half of these patients affected for life. Schizophrenia is often associated with unemployment, and homelessness, leading to a range of societal issues. While the pathological mechanism of schizophrenia remains unclear, increasing evidence suggests a correlation with pineal gland dysfunction (Sandyk, 1992).

Circadian rhythm disruptions are common in patients with schizophrenia, leading to sleep disorders, lack of energy, and declines in attention and memory, imposing a heavy burden on patients. Based on the research, it is evident that patients with schizophrenia experiencing disruptions in circadian rhythm show a correlation with reduced melatonin production. Supplementary melatonin has been investigated as a potential adjunctive treatment for schizophrenia, showing promise in improving sleep parameters, and counteracting metabolic adverse reactions and tardive dyskinesia symptoms caused by antipsychotic drugs (Duan, Jenkins, & Castle, 2021). Furthermore, melatonin is advantageous due to its low cost, minimal side effects, lack of drug interactions, and easy availability (Moon, Kim, Partonen, & Linnaranta, 2022). Imaging studies have revealed that patients with schizophrenia exhibit more severe pineal gland calcification and smaller parenchymal volume, suggesting a potential link between pineal gland calcification and the regulation of schizophrenia by melatonin (Takahashi *et al.*, 2021).

4.3. Parkinson's Disease (PD)

PD is a chronic and progressive neurodegenerative disorder characterized by symptoms such as dyskinesia, tremors, muscle rigidity, and speech difficulties, often associated with depression and sleep disturbances. Currently, the prevailing consensus is that PD primarily stems from a gradual and progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc), coupled with a reduction in dopamine (DA) levels in the striatum (Surmeier, 2018). PD represents a severe neurological disease highly correlated with the aging process. According to 2019 statistical data, there are over 8.5 million patients globally, and the figure is expected to rise continuously with the ongoing aging of the population. Statistically, approximately 90% of PD patients manifest sleep

disturbances, coupled with disruptions in circadian rhythm and cognitive decline (Macías-García *et al.*, 2022). Additionally, sleep disturbances in PD have been associated with modifications in both melatonin secretion levels and the expression of melatonin receptors MTs within the SNc, suggesting a possible involvement of the pineal gland and melatonin in the progression of PD (Noseda *et al.*, 2021).

Melatonin has been extensively studied for its therapeutic effects in the 6-OHDA-induced dopaminergic neurodegeneration model of PD (Sharma, McMillan, Tenn, & Niles, 2006), primarily attributed to its neuroprotective effects related to antioxidant activity, including free radical scavenging, decreased levels of lipid peroxidation, along with the alleviation of oxidative damage to mitochondrial DNA (Mack *et al.*, 2016).

The brain-derived neurotrophic factor (BDNF) plays a pivotal role in the development and maintenance of dopaminergic neurons, and its expression is hindered in the progression of PD. Research has found that mRNA and protein levels of BDNF are elevated after melatonin treatment in the cortex and hippocampus of rats, which are accompanied by sleep disturbances induced by PD (Scalzo *et al.*, 2010). Clinical administration of melatonin to patients with PD has also demonstrated a partial restoration of BDNF expression.

These examples illustrate that melatonin exerts neuroprotective and antioxidant effects in the PD model, offering potential reparative effects to attenuate dopaminergic neuron damage and disease progression.

Patients with PD have been observed to show notably reduced melatonin levels, potentially linked to pineal gland degeneration (Breen *et al.*, 2014). Additionally, dysregulation of calcium ion influx in PD patients, associated with disrupted calcium signaling pathways, may also contribute to neuronal damage and death (Zündorf & Reiser, 2011). These findings suggest a potential association between PD pathogenesis and pineal gland calcification.

4.4. Other central nervous system diseases

The calcification of the pineal gland exerts a widespread impact on the homeostasis of the central nervous system. In comparison to healthy individuals, patients with depression (Zhao *et al.*, 2019), stroke, and multiple sclerosis exhibit a significant decrease in melatonin levels, concomitant with pineal gland calcification. Further analysis of patient cohorts reveals that pineal gland calcification serves as a risk factor for stroke, with a 1.35-fold increased risk when pineal gland calcification is present (Kitkhuandee, Sawanyawisuth, Johns, Kanpittaya, & Johns, 2014). In the study of multiple sclerosis, the degree of fatigue in patients is also associated with pineal gland calcification.

Disease	Clinical Manifestations	Effects of Pineal Gland Calcification		Refs.
AD	Memory loss	Decreased pineal parenchyma volume and melatonin secretion	Increased Aβ production and decreased Aβ clearance	(Li *et al.*, 2020)
	Cognitive decline		Inhibition of hippocampal synapses growth	(Jeong *et al.*, 2012)
	Sleep disorders		Diminished anti-inflammatory capacity	(Hoppe *et al.*, 2010)
Schizophrenia	Sleep disorders		Disruption of circadian rhythm	(Duan *et al.*, 2021)
	Hallucination			
	Emotional blunting		Increased incidence of metabolic adverse reactions	
PD	Movement disorder		Impeded expression of brain-derived neurotrophic factor (BDNF)	(Wang, 2009)
	Sleep disorders		Reduced antioxidant activity	(Mack *et al.*, 2016)

Table 1. The Role of Pineal Gland Calcification in AD, Schizophrenia, and PD.

5. CONCLUSION

In this review, we delve into the discussion on pineal gland calcification and its significance in CNS diseases, with a specific focus on its roles in AD, schizophrenia and PD (Table 1). Our aim is to propose the potential impact of pineal gland calcification on neurological health.

We suggest pineal gland calcification as a dynamic process characterized by the formation and breakdown of calcified regions, exhibiting heterogeneity within different individuals and even within the same individual. Pineal gland calcification is a complex phenomenon with regional variations, and its underlying mechanisms remain unclear at present.

Currently, it is accepted that the pineal gland, by secreting and releasing melatonin, plays a role in reducing individual immune inflammatory responses and promoting the anti-oxidative process, thereby influencing the onset of AD, schizophrenia and PD. Pineal gland calcification disrupts this process, affecting the secretion and release of melatonin, consequently leading to central nervous system imbalance.

So far, pineal gland calcification is considered a physiological process. However, considering the relationship between pineal gland calcification and various neurological disorders, it is valuable to explore the potential impact of pineal gland calcification on basic medical and clinical practice.

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List of abbreviations

CNS	central nervous system
MTs	melatonin receptors
EV	extracellular vesicle
VSMC	vascular smooth muscle cells
CVSMC	calcified vascular smooth muscle cells
CT	computed tomography
MRI	Magnetic Resonance Imaging
IHC	Immunohistochemistry
AD	Alzheimer's Disease
TNF	tumor necrosis factor
PD	Parkinson's Disease
SNc	substantia nigra pars compacta
DA	dopamine
BDNF	brain-derived neurotrophic factor

REFERENCES

Adams, L. C., Boker, S. M., Bender, Y. Y., Diederichs, G., Fallenberg, E. M., Wagner, M., … Makowski, M. R. (2017). Diagnostic accuracy of susceptibility-weighted magnetic resonance imaging for the evaluation of pineal gland calcification. *PLoS One, 12*(3), e0172764. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28278291. doi:10.1371/journal.pone.0172764

Al Hajri, F., Sirasanagandla, S. R., Bouda-ka, A., Al Dhuhli, H., & Al Ajmi, E. (2023). Physiological Intracranial Calcifications in Children: A computed tomography-based study. *Sultan Qaboos Univ Med J, 23*(2), 227-232. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/37377832. doi:10.18295/squmj.9.2022.058

Alghamdi, B. S. (2018). The neuroprotective role of melatonin in neurological disorders. *J Neurosci Res, 96*(7), 1136-1149. doi:10.1002/jnr.24220

Baconnier, S., Lang, S. B., Polomska, M., Hilczer, B., Berkovic, G., & Meshulam, G. (2002). Calcite microcrystals in the pineal gland of the human brain: first physical and chemical studies. *Bioelectromagnetics, 23*(7), 488-495. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12224052. doi:10.1002/bem.10053

Belay, D. G., & Worku, M. G. (2023). Prevalence of pineal gland calcification: systematic review and meta-analysis. *Syst Rev, 12*(1), 32. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/36879256. doi:10.1186/s13643-023-02205-5

Breen, D. P., Vuono, R., Nawarathna, U., Fisher, K., Shneerson, J. M., Reddy, A. B., & Barker, R. A. (2014). Sleep and circadian rhythm

regulation in early Parkinson disease. *JAMA Neurol, 71*(5), 589-595. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24687146. doi:10.1001/jamaneurol.2014.65

Bukreeva, I., Junemann, O., Cedola, A., Brun, F., Longo, E., Tromba, G., … Asadchikov, V. E. (2023). Micromorphology of pineal gland calcification in age-related neurodegenerative diseases. *Med Phys, 50*(3), 1601-1613. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/36309985. doi:10.1002/mp.16080

Chen, W. R., Zhou, Y. J., Sha, Y., Wu, X. P., Yang, J. Q., & Liu, F. (2020). Melatonin attenuates vascular calcification by inhibiting mitochondria fission via an AMPK/Drp1 signalling pathway. *J Cell Mol Med, 24*(11), 6043-6054. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/32368857. doi:10.1111/jcmm.15157

Duan, C., Jenkins, Z. M., & Castle, D. (2021). Therapeutic use of melatonin in schizophrenia: A systematic review. *World J Psychiatry, 11*(8), 463-476. doi:10.5498/wjp.v11.i8.463

Esposito, E., & Cuzzocrea, S. (2010). Antiinflammatory activity of melatonin in central nervous system. *Curr Neuropharmacol, 8*(3), 228-242. doi:10.2174/157015910792246155

Gao, Y., Wei, S., Gao, F., Gao, L., Dang, L., Shang, S., … Qu, Q. (2020). Sleep Disturbance is Associated With Higher Plasma Aβ Levels in Cognitively Normal Adults-A Population-Based Cross-Sectional Study. *Front Aging Neurosci, 12*, 615838. doi:10.3389/fnagi.2020.615838

Gheban, B. A., Colosi, H. A., Gheban-Rosca, I. A., Georgiu, C., Gheban, D., Crisan, D., & Crisan, M. (2023). Digital histological morphometry of the human pineal gland in a postmortem study, with endocrine and neurological clinical implications. *Anat Histol Embryol, 52*(1), 12-20. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/35762404. doi:10.1111/ahe.12828

Gheban, B. A., Colosi, H. A., Gheban-Rosca, I. A., Pop, B., Domsa, A. T., Georgiu, C., … Crisan, M. (2021). Age-Related Changes of the Pineal Gland in Humans: A Digital Anatomo-Histological Morphometric Study on Autopsy Cases with Comparison to Predigital-Era Studies. *Medicina (Kaunas), 57*(4). Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/33921100. doi:10.3390/medicina57040383

Gheban, B. A., Rosca, I. A., & Crisan, M. (2019). The morphological and functional characteristics of the pineal gland. *Med Pharm Rep, 92*(3), 226-234. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/31460502. doi:10.15386/mpr-1235

Ghorbani, A., Pishkar, L., Saravi, K. V., & Chen, M. (2023). Melatonin-mediated endogenous nitric oxide coordinately boosts stability through proline and nitrogen metabolism, antioxidant capacity, and Na(+)/K(+) transporters in tomato under NaCl stress. *Front Plant Sci, 14*, 1135943. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/36993844. doi:10.3389/fpls.2023.1135943

Ghorbanlou, M., Moradi, F., & Mehdizadeh, M. (2022). Frequency, shape, and estimated volume of intracranial physiologic calcification in different age groups investigated by brain computed tomography scan: a retrospective study. *Anat Cell Biol, 55*(1), 63-71. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/34866062. doi:10.5115/acb.21.137

Gorgulu, F. F., & Koc, A. S. (2021). Is there any relationship between autism and pineal gland volume? *Pol J Radiol, 86*, e225-e231. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/34093919. doi:10.5114/pjr.2021.105689

Guerrero, J. M., & Reiter, R. J. (2002). Melatonin-immune system relationships. Curr Top Med Chem, 2(2), 167-179. doi:10.2174/1568026023394335

Harisha, K., Arava, S., Singh, S., & Pariplavi, M. (2023). A morphological study of human pineal gland in post-mortem cases. *Morphologie, 107*(357), 193-198. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/35786527. doi:10.1016/j.morpho.2022.06.095

Howes, O. D., & Murray, R. M. (2014). Schizophrenia: an integrated sociodevelopmental-cognitive model. *Lancet, 383*(9929), 1677-1687. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24315522. doi:10.1016/S0140-6736(13)62036-X

Jalali, N., Firouzabadi, M. D., Mirshekar, A., Khalili, P., Ravangard, A. R., Ahmadi, J., … Jalali, Z. (2023). Cross-sectional analysis of potential risk factors of the pineal gland calcification. *BMC Endocr Disord, 23*(1), 49. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/36855104. doi:10.1186/s12902-023-01301-w

Junemann, O., Bukreeva, I., Otlyga, D. A., Cedola, A., Fratini, M., & Saveliev, S. V. (2023). Human Pineal Gland Involutionary Process: New Findings. *J Gerontol A Biol Sci Med Sci, 78*(9), 1558-1560. Retrieved from https://www.ncbi.

nlm.nih.gov/pubmed/36966358. doi:10.1093/gerona/glad091

Junemann, O., Ivanova, A. G., Bukreeva, I., Zolotov, D. A., Fratini, M., Cedola, A., … Saveliev, S. V. (2023). Comparative study of calcification in human choroid plexus, pineal gland, and habenula. *Cell Tissue Res*, *393*(3), 537-545. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/37354235. doi:10.1007/s00441-023-03800-7

Kamath, J., Virdi, S., & Winokur, A. (2015). Sleep Disturbances in Schizophrenia. *Psychiatr Clin North Am*, *38*(4), 777-792. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26600108. doi:10.1016/j.psc.2015.07.007

Kaur, C., Sivakumar, V., Lu, J., & Ling, E. A. (2007). Increased vascular permeability and nitric oxide production in response to hypoxia in the pineal gland. *J Pineal Res*, *42*(4), 338-349. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17439550. doi:10.1111/j.1600-079X.2007.00424.x

Kitkhuandee, A., Sawanyawisuth, K., Johns, N. P., Kanpittaya, J., & Johns, J. (2014). Pineal calcification is associated with symptomatic cerebral infarction. *J Stroke Cerebrovasc Dis*, *23*(2), 249-253. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23434443. doi:10.1016/j.jstrokecerebrovasdis.2013.01.009

Koenig, J. I., Kirkpatrick, B., & Lee, P. (2002). Glucocorticoid hormones and early brain development in schizophrenia. *Neuropsychopharmacology*, *27*(2), 309-318. doi:10.1016/s0893-133x(01)00396-7

Kopani, M., Vranikova, B., Kosnac, D., Zeman, M., Sisovsky, V., Polakovicova, S., & Biro, C. (2019). Pineal gland calcification under hypoxic conditions. *Physiol Res*, *68*(Suppl 4), S405-S413. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/32118471. doi:10.33549/physiolres.934378

Krstić, R., & Golaz, J. (1977). Ultrastructural and X-ray microprobe comparison of gerbil and human pineal acervuli. *Experientia*, *33*(4), 507-508. doi:10.1007/bf01922240

Lee, H. Y., Ng, I., Lian, D. W., Yap, W. M., & Chuah, K. L. (2011). Cytological features of the native pineal gland in intraoperative squash preparations. *Diagn Cytopathol*, *39*(3), 196-199. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21319322. doi:10.1002/dc.21373

Li, Y., Zhang, J., Wan, J., Liu, A., & Sun, J. (2020). Melatonin regulates Abeta production/clearance balance and Abeta neurotoxicity: A potential therapeutic molecule for Alzheimer's disease. *Biomed Pharmacother*, *132*, 110887. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/33254429. doi:10.1016/j.biopha.2020.110887

Lim, M. M., Gerstner, J. R., & Holtzman, D. M. (2014). The sleep-wake cycle and Alzheimer's disease: what do we know? *Neurodegener Dis Manag*, *4*(5), 351-362. doi:10.2217/nmt.14.33

Liu, X. J., Yuan, L., Yang, D., Han, W. N., Li, Q. S., Yang, W., … Qi, J. S. (2013). Melatonin protects against amyloid-beta-induced impairments of hippocampal LTP and spatial learning in rats. *Synapse*, *67*(9), 626-636. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23620224. doi:10.1002/syn.21677

Macías-García, P., Rashid-López, R., Cruz-Gómez Á. J., Lozano-Soto, E., Sanmartino, F., Espinosa-Rosso, R., & González-Rosa, J. J. (2022). Neuropsychiatric Symptoms in Clinically Defined Parkinson's Disease: An Updated Review of Literature. *Behav Neurol*, *2022*, 1213393. doi:10.1155/2022/1213393

Mack, J. M., Schamne, M. G., Sampaio, T. B., Pertile, R. A., Fernandes, P. A., Markus, R. P., & Prediger, R. D. (2016). Melatoninergic System in Parkinson's Disease: From Neuroprotection to the Management of Motor and Nonmotor Symptoms. *Oxid Med Cell Longev*, *2016*, 3472032. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27829983. doi:10.1155/2016/3472032

Moon, E., Kim, K., Partonen, T., & Linnaranta, O. (2022). Role of Melatonin in the Management of Sleep and Circadian Disorders in the Context of Psychiatric Illness. *Curr Psychiatry Rep*, *24*(11), 623-634. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/36227449. doi:10.1007/s11920-022-01369-6

Mrvelj, A., & Womble, M. D. (2020). Fluoride-Free Diet Stimulates Pineal Growth in Aged Male Rats. *Biol Trace Elem Res*, *197*(1), 175-183. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/31713773. doi:10.1007/s12011-019-01964-4

Muñoz-Jurado, A., Escribano, B. M., Caballero-Villarraso, J., Galván, A., Agüera, E., Santamaría, A., & Túnez, I. (2022). Melatonin and multiple sclerosis: antioxidant,

anti-inflammatory and immunomodulator mechanism of action. *Inflammopharmacology, 30*(5), 1569-1596. doi:10.1007/s10787-022-01011-0

Noseda, A. C. D., Rodrigues, L. S., Targa, A. D. S., Ilkiw, J. L., Fagotti, J., Dos Santos, P. D., … Lima, M. M. S. (2021). MT(2) melatonin receptors expressed in the olfactory bulb modulate depressive-like behavior and olfaction in the 6-OHDA model of Parkinson's disease. *Eur J Pharmacol, 891*, 173722. doi:10.1016/j.ejphar.2020.173722

Novais, A. A., Chuffa, L. G. A., Zuccari, D., & Reiter, R. J. (2021). Exosomes and Melatonin: Where Their Destinies Intersect. *Front Immunol, 12*, 692022. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/34177952. doi:10.3389/fimmu.2021.692022

Ozan, E., Sonmez, M. F., Ozan, S., Colakoglu, N., Yilmaz, S., & Kuloglu, T. (2007). Effects of melatonin and vitamin C on cigarette smoke-induced damage in the kidney. *Toxicol Ind Health, 23*(8), 479-485. doi:10.1177/0748233708089023

Ozansoy, M., Ozansoy, M. B., Yulug, B., Cankaya, S., Kilic, E., Goktekin, S., & Kilic, U. (2020). Melatonin affects the release of exosomes and tau-content in in vitro amyloid-beta toxicity model. *J Clin Neurosci, 73*, 237-244. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/32061493. doi:10.1016/j.jocn.2019.11.046

Ozguner, F., Koyu, A., & Cesur, G. (2005). Active smoking causes oxidative stress and decreases blood melatonin levels. *Toxicol Ind Health, 21*(1-2), 21-26. doi:10.1191/0748233705th211oa

Parhizkar, S., Gent, G., Chen, Y., Rensing, N., Gratuze, M., Strout, G., … Holtzman, D. M. (2023). Sleep deprivation exacerbates microglial reactivity and Aβ deposition in a TREM2-dependent manner in mice. *Sci Transl Med, 15*(693), eade6285. doi:10.1126/scitranslmed.ade6285

Patel, S., Rahmani, B., Gandhi, J., Seyam, O., Joshi, G., Reid, I., … Khan, S. A. (2020). Revisiting the pineal gland: a review of calcification, masses, precocious puberty, and melatonin functions. *Int J Neurosci, 130*(5), 464-475. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/31714865. doi:10.1080/00207454.2019.1692838

Razzaque, M. S. (2011). The dualistic role of vitamin D in vascular calcifications. *Kidney Int, 79*(7), 708-714. doi:10.1038/ki.2010.432

Reiter, R. J., Richardson, B. A., Johnson, L. Y., Ferguson, B. N., & Dinh, D. T. (1980). Pineal melatonin rhythm: reduction in aging Syrian hamsters. *Science, 210*(4476), 1372-1373. doi:10.1126/science.7434032

Rosenstein, R. E., Estévez, A. G., & Cardinali, D. P. (1989). Time-Dependent Effect of Melatonin on Glutamic Acid Decarboxylase Activity and CI Influx in Rat Hypothalamus. *J Neuroendocrinol, 1*(6), 443-447. doi:10.1111/j.1365-2826.1989.tb00145.x

Sandyk, R. (1992). Pineal and habenula calcification in schizophrenia. *Int J Neurosci, 67*(1-4), 19-30. doi:10.3109/00207459208994773

Scalzo, P., Kummer, A., Bretas, T. L., Cardoso, F., & Teixeira, A. L. (2010). Serum levels of brain-derived neurotrophic factor correlate with motor impairment in Parkinson's disease. J *Neurol, 257*(4), 540-545. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19847468. doi:10.1007/s00415-009-5357-2

Sharma, R., McMillan, C. R., Tenn, C. C., & Niles, L. P. (2006). Physiological neuroprotection by melatonin in a 6-hydroxydopamine model of Parkinson's disease. *Brain Res, 1068*(1), 230-236. doi:10.1016/j.brainres.2005.10.084

Sigurdardottir, L. G., Markt, S. C., Sigurdsson, S., Aspelund, T., Fall, K., Schernhammer, E., … Mucci, L. A. (2016). Pineal Gland Volume Assessed by MRI and Its Correlation with 6-Sulfatoxymelatonin Levels among Older Men. *J Biol Rhythms, 31*(5), 461-469. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27449477. doi:10.1177/0748730416656948

Song, J. (2019). Pineal gland dysfunction in Alzheimer's disease: relationship with the immune-pineal axis, sleep disturbance, and neurogenesis. *Mol Neurodegener, 14*(1), 28. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/31296240. doi:10.1186/s13024-019-0330-8

Surmeier, D. J. (2018). Determinants of dopaminergic neuron loss in Parkinson's disease. *FEBS J, 285*(19), 3657-3668. doi:10.1111/febs.14607

Takahashi, T., Sasabayashi, D., Takayanagi, Y., Higuchi, Y., Mizukami, Y., Akasaki, Y., … Suzuki, M. (2021). Potential contribution of pineal atrophy and pineal cysts toward vulnerability and clinical characteristics of psychosis. *Neuroimage Clin, 32*, 102805. doi:10.1016/j.nicl.2021.102805

Tan, D. X., Hardeland, R., Manchester, L. C., Paredes, S. D., Korkmaz, A., Sainz, R. M.,

… Reiter, R. J. (2010). The changing biological roles of melatonin during evolution: from an antioxidant to signals of darkness, sexual selection and fitness. *Biol Rev Camb Philos Soc, 85*(3), 607-623. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20039865. doi:10.1111/j.1469-185X.2009.00118.x

Tomas-Zapico, C., & Coto-Montes, A. (2005). A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. J *Pineal Res, 39*(2), 99-104. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/16098085. doi:10.1111/j.1600-079X.2005.00248.x

Welsh, M. G. (1984). Cytochemical analysis of calcium distribution in the superficial pineal gland of the Mongolian gerbil. *J Pineal Res, 1*(4), 305-316. doi:10.1111/j.1600-079x.1984.tb00221.x

Whitehead, M. T., Oh, C., Raju, A., & Choudhri, A. F. (2015). Physiologic pineal region, choroid plexus, and dural calcifications in the first decade of life. *AJNR Am J Neuroradiol, 36*(3), 575-580. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25355815. doi:10.3174/ajnr.A4153

Xu, F., Zhong, J. Y., Lin, X., Shan, S. K., Guo, B., Zheng, M. H., … Yuan, L. Q. (2020). Melatonin alleviates vascular calcification and ageing through exosomal miR-204/miR-211 cluster in a paracrine manner. *J Pineal Res, 68*(3), e12631. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/31943334. doi:10.1111/jpi.12631

Yang, H. J., Kim, M. J., Kim, S. S., & Cho, Y. W. (2021). Melatonin modulates nitric oxide-regulated WNK-SPAK/OSR1-NKCC1 signaling in dorsal raphe nucleus of rats. *Korean J Physiol Pharmacol, 25*(5), 449-457. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/34448462. doi:10.4196/kjpp.2021.25.5.449

Zhang, Y., He, F., Chen, Z., Su, Q., Yan, M., Zhang, Q., … Han, Y. (2019). Melatonin modulates IL-1β-induced extracellular matrix remodeling in human nucleus pulposus cells and attenuates rat intervertebral disc degeneration and inflammation. *Aging (Albany NY), 11*(22), 10499-10512. doi:10.18632/aging.102472

Zhao, W., Zhu, D. M., Zhang, Y., Zhang, C., Wang, Y., Yang, Y., … Yu, Y. (2019). Pineal gland abnormality in major depressive disorder. *Psychiatry Res Neuroimaging, 289*, 13-17. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/31121531. doi:10.1016/j.pscychresns.2019.05.004

Zündorf, G., & Reiser, G. (2011). Calcium dysregulation and homeostasis of neural calcium in the molecular mechanisms of neurodegenerative diseases provide multiple targets for neuroprotection. *Antioxid Redox Signal, 14*(7), 1275-1288. doi:10.1089/ars.2010.3359