Chapter

Sympathetic Innervation of the Mammalian Pineal Gland: Its Involvement in Ontogeny and Physiology, and in Pineal Dysfunction

Martin Avila, Carlos L. Freites, Elena Vásquez, Juan B. Amiotti, Janina Borgonovo and Estela M. Muñoz

Abstract

In mammals, the melatonin-producing pineal gland (PG) receives sympathetic innervation from the superior cervical ganglia (SCG). This chapter describes the role of this innervation on the PG's ontogeny and rhythmic function, along with consequences to physiology when this regulation is disrupted. The PG and the SCG are components of the circadian timing system (CTS). Therefore, the overall CTS is described, including its oscillatory basis, its synchronization to the light: dark (L:D) cycles, and the dissemination of timing cues to all cells throughout the body. Pineal cellular composition and heterogeneity, cell-cell interactions, and the molecular mechanisms involved in the circadian rhythm of melatonin (MEL), are discussed. The SCG's bilateral placement among surrounding anatomical landmarks, as well as their afferent and efferent connections, are described and illustrated. In addition, the SCG-related surgical models and the state-of-the art technology used to investigate the connection between SCG and PG are presented. Perspectives and gaps in our understanding are also discussed. We hope this chapter inspires readers to delve deeper into the field of the pineal gland and its main messenger, melatonin, as well as MEL's impact in health and disease, including as a remedial therapy.

Keywords: hormone, melatonin, pineal gland, pinealocyte, sympathetic innervation, superior cervical ganglia, norepinephrine, ontogeny, physiology, dysfunction

1. Introduction

In mammals, melatonin (MEL) is a circadian hormone that is released at high levels into the bloodstream and into the cerebrospinal fluid (CSF) at night, but then drops off to negligibly low levels throughout the daytime [1–3]. Almost all the body's cells

respond to this timing signal. MEL and other circadian cues orchestrate physiology in a rhythmic manner that impacts organ function, tissue healing and rejuvenation, and growth, as well as cognition, motivation, behavior, adaptation, and survival [4]. Taking melatonin supplements is growing in popularity, mainly to augment naturally produced MEL levels and as a sleep aide, but also for its powerful antioxidant, anti-inflammatory, free-radical scavenging, and neuroprotective properties [5]. In mammals, circulating MEL is primarily synthesized by the pineal gland (PG), under direct control of sympathetic innervation stemming from the superior cervical ganglia (SCG) [6, 7]. The PG and the SCG are part of an endogenous circadian timing system (CTS) that synchronizes the whole organism to the environmental light: dark cycles (L:D; *Zeitgeber*). In this chapter, we present foundational and current knowledge about how the pineal gland is controlled by the sympathetic nervous system (SNS), with regard to its ontogeny and normal physiology, as well as under dysfunctional conditions. We hope this work inspires readers to seek a deeper understanding of the pineal gland and the functional role of its main messenger, melatonin, in both health and disease, as well as remedial therapy.

2. The pineal gland's role in the mammalian circadian timing system

The pineal gland (PG) is a highly vascularized neuroendocrine organ that rhythmically produces melatonin (MEL) [2]. The PG is located in the mid-line of the brain, attached to the roof of the third ventricle (III V) by a short stalk [8, 9]. The PG is positioned deep within the brain of humans, and more superficially in rodents. The mammalian PG is driven by a hierarchical series of oscillators from the photoneuroendocrine system (PNS) (**Figure 1**) [7, 12]. Furthermore, the nocturnal release of MEL by the PG provides downstream circadian synchronization to most cells throughout the body. All these elements taken together comprise the circadian timing system (CTS) [12]. The PNS transduces 24-hour light: dark (L:D) cycle information from the external environment into the circadian pattern of MEL synthesis and secretion. To do this, the multisynaptic PNS senses light using intrinsically photosensitive retinal ganglion cells (ipRGC) in the eye, in coordination with the retinal rods and cones. The ipRGC axons project into the GABAergic neurons in the suprachiasmatic nuclei (SCN) of the hypothalamus. SCN are considered to be the master circadian pacemaker, which synchronizes a complex and widely distributed network of peripheral clocks. Each of these oscillators has its own cell-autonomous circadian clock that is driven by interlocked transcriptional/translational feedback loops (TTFL) of core-clock genes (CG), that in turn regulate the expression of clock-controlled genes (CCG), and ultimately coordinate the timing of many biological processes throughout the body [12–15]. The CG family includes genes that encode either positive or negative regulators, such as CLOCK, BMAL, PERs (Period), and CRYs (Cryptochrome) proteins. During the light phase of the L:D cycle, glutamatergic ipRGC axons activate the SCN neurons. This inhibits the rest of the PNS, including the hypothalamic paraventricular nuclei (PVN), neurons of the intermediolateral columns (IMC) of the spinal cord (SC), and the superior cervical ganglia (SCG), and thus, prevents MEL synthesis and secretion by the PG. During the night phase, the SCN release their inhibition over the circuit, and SCG nerve ends release norepinephrine (NE) into the PG parenchyma [6]. This neurotransmitter binds to specific adrenergic receptors on the pinealocyte (Pc) plasma membrane and regulates key steps in the multienzymatic pathway that results in MEL synthesis.

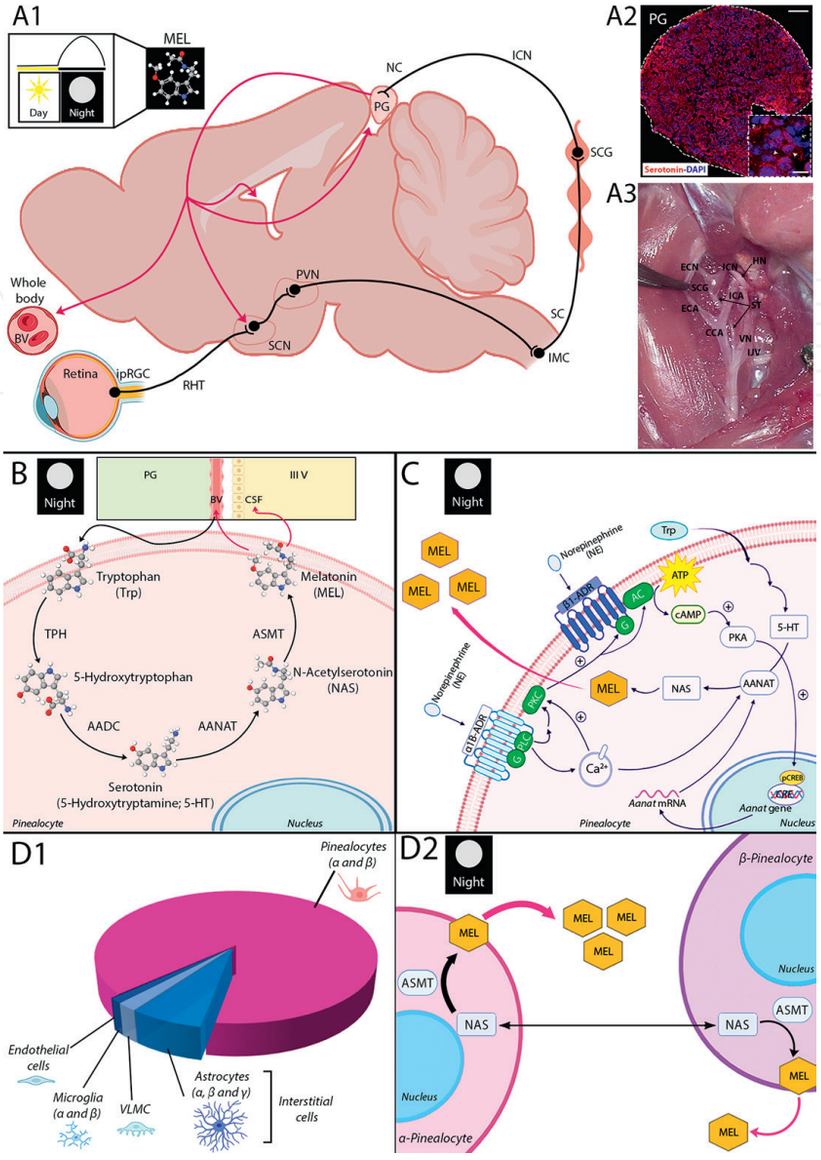

Figure 1.
A1-A3: The rodent photoneuroendocrine system and the circadian rhythm of melatonin. A1: BV: Blood vessels. ICN: Internal carotid nerves. IMC: Neurons of the intermediolateral columns of the spinal cord (SC). ipRGC: Intrinsically photosensitive retinal ganglion cells. MEL: Melatonin. NC: Nervi conarii. PG: Pineal gland. PVN: Paraventricular nuclei. RHT: Retinohypothalamic tracts. SCG: Superior cervical ganglia. SCN: Suprachiasmatic nuclei. A2: MEL-producing rat pinealocytes immunoreactive for serotonin (red; white arrowheads in the inset). White arrows: Interstitial cells negative for serotonin. DAPI: Nuclear marker 4′,6-diamidino-2-phenylindole (blue). Immunofluorescence and confocal microscopy; objective: 10X, scale bar: 150 μm (inset: 60X with 4X zoom, scale bar: 15 μm.). See Ibañez Rodriguez et al. [9] for further details about animal procedures and immunolabeling protocols. A3: Rat superior cervical ganglion and surrounding anatomical landmarks. CCA: Common carotid artery. ECA: External carotid artery. ECN: External carotid nerve. HN: Hypoglossal nerve. ICA: Internal carotid artery. IJV: Internal jugular vein. ST: Sympathetic trunk. VN: Vagus nerve. Modified from Savastano et al. [10], where further details about animal procedures can be found. The reproduction of this copyrighted material was authorized by Elsevier. B: Melatonin biosynthetic pathway. AADC: Aromatic L-amino acid decarboxylase. AANAT: Arylalkylamine N-acetyltransferase. ASMT: Acetylserotonin O-methyltransferase. CSF: Cerebrospinal fluid. TPH: Tryptophan hydroxylase. III V: Third ventricle. C: Adrenergic regulation of melatonin synthesis at night. α1B-ADR: α1B adrenergic receptor. AC: Adenylate cyclase. ATP: Adenosine triphosphate. β1-ADR: β1 adrenergic receptor. cAMP: Cyclic adenosine monophosphate. CRE: cAMP responsive element. G: G proteins. mRNA: Messenger ribonucleic acid. pCREB: Phosphorylated form of cAMP responsive element-binding protein (CREB). PKA: Protein kinase A. PLC: Phospholipase C. PKC: Protein kinase C. D1-D2. Transcriptionally distinguished cell types and subtypes within the adult rat pineal gland. D1: Distribution of the cell types profiled in Mays et al. [11] during the light (L) and dark (D) phases of the L:D cycle. VLMC: Vascular and leptomeningeal cells. D2: Crosstalk between α- and β-pinealocytes to produce nocturnal melatonin in a coordinated and efficient manner. See Mays et al. [11] for further details.

2.1 Cellular composition of the mature pineal gland

Melatonin (MEL) is produced within the PG by its predominant cell population, the pinealocytes (Pc). One of the most modern classifications of rat Pc came with the application of single-cell RNA sequencing (scRNA-seq) (**Figure 1**) [11, 16]. This state-of-the-art technology provides gene expression profiles of isolated and individualized cells. Nowadays, it is accepted that at least two subtypes of pinealocytes, α-Pc and β-Pc, coexist and crosstalk in the rat PG, to produce MEL in a coordinated and efficient manner. β-pinealocytes are more abundant than α-Pc, but α-Pc are more effective in catalyzing the last step in the MEL biosynthetic pathway. The scRNA-seq analysis also discriminated interstitial cells. Among these transcriptionally distinguished non-pinealocyte cells are three astrocytes (α, β, and γ), two microglial subtypes (α and β), endothelial cells (EC), and vascular and leptomeningeal cells (VLMC). Several studies have shown that some non-pinealocyte cells modulate MEL production by pinealocytes, under both homeostatic and pathological conditions [17]. With respect to EC, they represent key elements within the PG because they form the inner lining of all blood vessels (BV) that make up its vast circulatory network, which are mainly fenestrated capillaries [8]. Therefore, the PG's blood vessels are more permeable and less selective than the tightly regulated blood-brain barrier (BBB) present in most of the central nervous system (CNS) [18]. The PG is included as one of the seven circumventricular organs (CVO) in the brain, and all of them have an incomplete barrier [8, 19]. This characteristic allows CVO to function as an intermediary pathway between the brain and the periphery, for bidirectional trafficking and interaction.

2.2 Melatonin synthesis by pinealocytes

Melatonin (MEL) is synthesized by pinealocytes (Pc) at night, via a multienzymatic pathway driven mainly by rhythmic neural inputs [2, 6, 7]. Circulating L-tryptophan (Trp) is an essential amino acid that acts as the biosynthetic precursor of the MEL molecule (**Figure 1**). Trp is hydroxylated and then decarboxylated enzymatically within the Pc cytoplasm. The product of these two reactions is serotonin or 5-hydroxytryptamine (5-HT). Serotonin is then converted into MEL by acetylation, followed by methylation [4]. The enzymes that catalyze the last two reactions, AANAT (Arylalkylamine N-acetyltransferase) and ASMT (Acetylserotonin O-methyltransferase), respectively, represent adjustable key points of the MEL-producing pathway [2, 11, 20]. Sympathetic axons stemming from neurons located in both superior cervical ganglia (SCG), provide the norepinephrine (NE) neurotransmitter signal that is the main regulator of MEL production. NE impacts the MEL biosynthetic machinery at different levels, from gene expression to enzyme activities, among other target points (**Figure 1**) [6, 7].

2.3 Sympathetic innervation of the mammalian pineal gland

The mammalian PG receives a wide range of afferent nerve fibers and, therefore, it can be influenced by a plethora of neurotransmitters [6, 8]. Efferent projections from the PG have also been described, but only for some species and at particular ontogenetic stages [8]. Among the afferent innervations, sympathetic axons, originating from both the right and left SCG, are a fundamental regulatory element of PG rhythmicity in mammals [2] (**Figure 2**). Classic transcriptomic and neurotranscriptomic

studies have shown that essentially all aspects of PG biology are subject to neural control. These aspects include thousands of genes associated with either MEL-related or MEL-unrelated functions, such as immune/inflammatory response and thyroid hormone signaling [11, 22–24].

2.3.1 Superior cervical ganglia

The SCG are the uppermost ganglia of the paravertebral sympathetic chain. They are well-defined structures with a variable number of neurons, which receive inputs from preganglionic fibers ascending in the sympathetic trunk (ST) (**Figure 1**) [10, 25, 26]. SCG neurons, mainly via the external and internal carotid nerves (ECN and ICN), establish a wide field of synapsis in the neck, face, and intracranial areas. The SCG not only innervate the pineal gland (PG), but also the hypophysis and median eminence, the thyroid and parathyroid glands, and the Muller's muscles (MM) that control the position of the upper eyelids (palpebral position). An important distinction is that the PG and the MM are innervated differently. Nerve fibers from both the right and left ICN innervate the PG bilaterally. Whereas for the MM, each MM is innervated unilaterally via efferent sympathetic axons present in the homolateral ICN. This innervation difference is used to evaluate the success of the SCG-related surgical procedures that are discussed in Section 2.3.3 (**Figure 2**). For all SCG targets and under tissue-specific stimuli (e.g., lights off for the PG), SCG-derived nerve terminals mainly release norepinephrine (NE) into the synaptic cleft and into the perivascular space. Additionally, other neuropeptides, such as the neuropeptide Y (NPY) in the PG, have been identified as sympathetic co-neurotransmitters [8]. The concentration of NE in the synaptic (or synaptic-like) gaps is affected by simple diffusion and uptake rates. NE uptake includes both its transport back into presynaptic nerve ends and its recapture by neighboring cells. NE re-uptake by sympathetic nerve terminals is crucial for stimulus termination, and for removal and deactivation of circulating stress-induced catecholamines. NE passes the message to the targets by stimulating specific adrenergic receptors on their cell membranes.

2.3.2 Adrenergic reception in the mature pineal gland

Pinealocytes (Pc) are the MEL-producing cells within the PG. As mentioned, Pc express adrenergic receptors on their cell membranes. These adrenoceptors bind and respond to the nocturnal NE released into the perivascular space from the sympathetic nerve ends (**Figure 1**). A recent scRNA-seq study confirmed the expression of two catecholamine receptor genes, *Adrb1* and *Adra1b*, in both α-Pc and β-Pc in the rat PG [11]. These genes encode β1 and α1B adrenergic receptors, respectively. Additionally, low levels of both transcripts were found in all the non-pinealocyte cells as well, with the exception that none were found in β-microglial cells. β1-ADR and α1B-ADR are seven-transmembrane (7TM) domain receptors that belong to the G protein-coupled receptor (GPCR) superfamily. The NE activation of these adrenoceptors triggers cooperative signaling pathways and several second messengers (e.g.: cyclic adenosine monophosphate, cAMP, and Ca^{2+}) that impact the whole Pc, including its nucleus and transcriptome (e.g., NE induces the expression of the *Aanat* gene, which encodes the enzyme AANAT, via the phosphorylated form of cAMP responsive element-binding protein, pCREB) [6, 7, 27–29]. As soon as *de novo* MEL is synthesized, it is released immediately into the bloodstream and into the cerebrospinal fluid (CSF) [3, 30]. MEL is produced during the dark phase of the L:D cycle and it is used

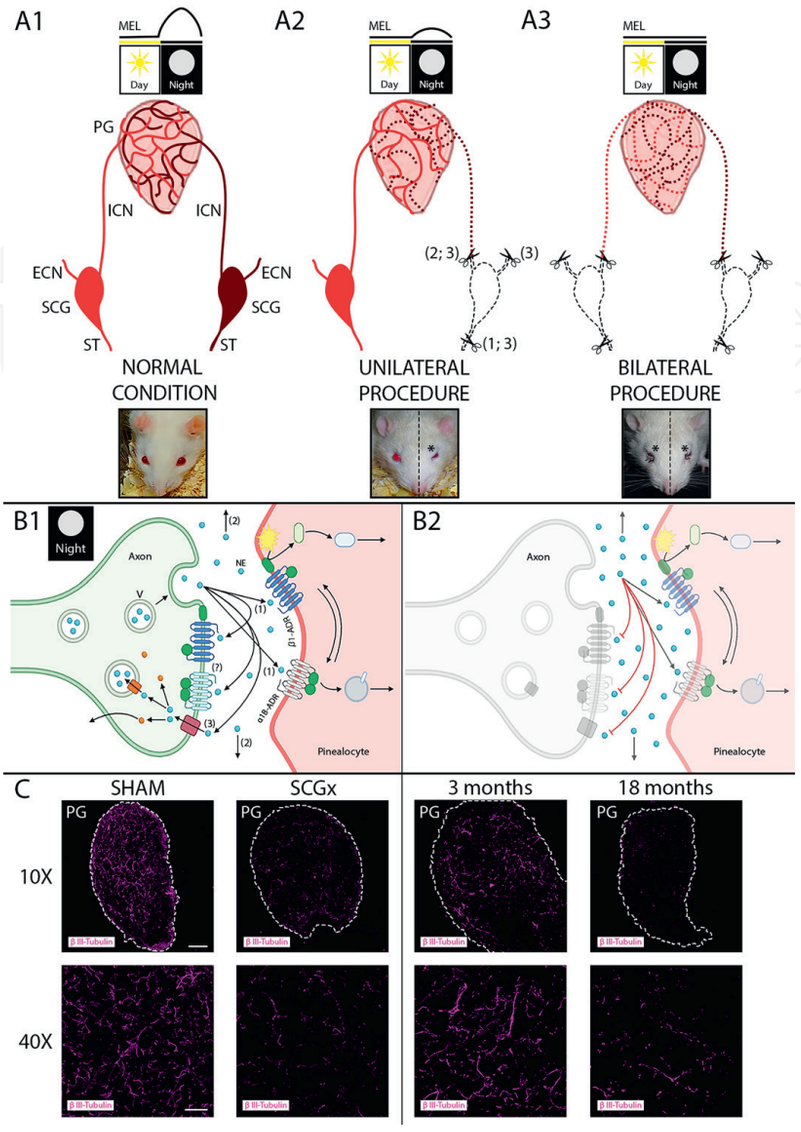

Figure 2

A1-A3: Surgical procedures related to the superior cervical ganglia and their impacts on pineal rhythmicity and palpebral position. A1: Bilateral sympathetic innervation of the pineal gland (PG) by both the right and left internal carotid nerves (ICN). This innervation provides nocturnal norepinephrine (NE), which drives the rhythmic synthesis of the hormone melatonin (MEL). ECN: External carotid nerves. SCG: Superior cervical ganglia. ST: Sympathetic trunks. A2: Reduction in the circulating MEL levels and homolateral blepharoptosis (*) after unilateral disruption of the sympathetic innervation. (1) Decentralization, by removal of a segment of the afferent sympathetic trunk (STx; lesion of preganglionic axons with undamaged ganglion in situ). (2) Denervation, by removal of a portion of the ICN (ICNx; lesion of postganglionic axons with undamaged ganglion in situ). (3) Ganglionectomy, by complete excision of the ganglion (SCGx; ablation of neuronal cell bodies). A3: Disappearance of the MEL circadian rhythm after a bilateral procedure. Surgery efficiency can be confirmed by observing palpebral ptosis in both eyes (*). The rat image also shows signs of chromodacryorrhoea (red tears). B1-B2: Norepinephrine re-uptake by nerve ends is abolished in the ganglionectomy model. B1: Norepinephrine (NE; light blue circles) released in the synaptic-like gaps, binds to specific adrenergic receptors (1) and diffuses outside the cleft (2). In addition, NE is transported back into the presynaptic nerve terminals in the healthy PG (3). NE re-uptake is crucial for stimulus termination, and for removal and deactivation of circulating stress-induced catecholamines (Orange circles: NE metabolites). α1B-ADR: α1B adrenergic receptor. β1-ADR: β1 adrenergic receptor. V: Vesicles. (?) Unknown. B2: NE re-uptake is abolished in the degenerating nerve terminals after SCGx. This is a difference with the STx procedure. C: Degeneration of the sympathetic nerve fibers within the pineal gland with age and after bilateral superior cervical ganglionectomy. Sections of rat pineal glands were immunolabeled for β III-tubulin, a marker of nerve fibers. SCGx: Bilateral ganglionectomy. SHAM: Bilateral sham surgery. Pineal glands from young (3 months) and aged (18 months) rats are shown. Immunofluorescence and confocal microscopy; objective: 10X, scale bar: 150 μm; objective: 40X, scale bar: 50 μm. See Ibañez Rodriguez et al. [9, 21] and Savastano et al. [10] for further details about animal procedures and immunolabeling protocols. The reproduction of the copyrighted rat images was authorized by Elsevier.

to disseminate the nighttime circadian status to all cells of the body, via specific MT1 and MT2 MEL receptors on the target cell membranes [31]. Nocturnal MEL production subsides towards late night and is shutdown during the daytime, in response to both extra-Pc mechanisms (e.g.: NE diffusion and uptake) and intra-Pc mechanisms (e.g.: feedback inhibition) [2, 6, 7].

2.3.3 SCG-related surgical procedures

Different procedures have been extensively used to study the sympathetic innervation of the mammalian PG, including surgical and pharmacological interventions, and electric stimulation [10, 23, 26, 32, 33]. Right and left ICN ascend from each SCG via the internal carotid arteries (ICA) and extend further to form the *nervi conarii* (NC) (**Figure 1**). The NC penetrate the PG at its dorso-posterior border and then ramify throughout the whole organ. Sometimes, bundles of axons from the two NC become fused before entering the gland. The sympathetic nerve fibers that innervate the PG along its vasculature, arise from a small population of neurons (not all neurons) that are rostrally dispersed in each SCG. When SCG-related surgeries are performed to completely suppress PG rhythmicity, both SCG must be isolated and manipulated in order to shut down the neural NE source bilaterally (**Figure 2**). A complete and permanent disruption of the MEL rhythm is achieved only when the influence of both NC is fully and irreversibly disrupted. In circadian biology, SCG-related surgeries are preferred to intracranial ones, such as a suprachiasmatic nuclei lesion (SCNx), due to the technical complications and the wider physiological impacts associated with these more invasive neurosurgeries. Because of the well-defined anatomy of the SCG and the surrounding structures, three types of surgical procedures can be executed to influence PG rhythmicity: (1) decentralization, by removal of a segment of the afferent sympathetic trunk (STx; lesion of preganglionic axons with undamaged ganglion *in situ*); (2) denervation, by removal of a portion of the ICN (ICNx; lesion of postganglionic axons with undamaged ganglion *in situ*), and (3) ganglionectomy, by complete excision of the ganglion (SCGx; ablation of neuronal cell bodies) (**Figure 2**). For those researchers who are interested in incorporating these procedures as routine techniques, there are straightforward protocols available in the literature, which provide step-by-step descriptions, illustrated with amazing images and detailed videos [10, 32, 33]. Each SCG-related surgery has advantages and disadvantages. For example, after a latency period following SCGx, the innervated target is seen to change through phases before stabilizing: (1) sympathetic stimulation due to the Wallerian degeneration of the sympathetic nerve terminals and a supraliminal release of neurotransmitter in the first few days following surgery (acute SCGx), and (2) sympathetic deprivation after the first post-surgery week (chronic SCGx). One complication of the SCGx is that it cannot prevent the influence of circulating stress-released catecholamines on the target tissue, because the local NE re-uptake mechanism is abolished in the degenerating sympathetic nerve ends (**Figure 2**). This obliges the users to exhaustively control animal housing conditions to eliminate any kind of stressor during the whole experimental time, even during animal euthanasia. On the contrary, STx preserves presynaptic re-uptake and the capacity to remove and deactivate circulating catecholamines. Additionally, SCGx induces microgliosis with damaging consequences over the PG parenchyma [21]. For these reasons, researchers prefer STx over SCGx. As mentioned, in the case of the PG, both SCG must be successfully manipulated to abolish the MEL circadian rhythm completely and permanently. This can be confirmed in a calm animal by bilateral blepharoptosis (palpebral

ptosis), a sign commonly considered to evaluate surgery effectiveness [10] (**Figure 2**). This sign is used because both the PG and the MM are innervated by the ICN (see Section 2.3.1).

2.4 Ontogeny of the mammalian pineal gland and its relationship with the sympathetic innervation

The PG emerges as an evagination of the roof of the diencephalon, late in the embryonic (E) period (E14-E15 for rat) [8, 9]. The basis of pineal morphogenesis, and the dynamic and intricate network of transcription factors (TF) involved in the establishment and maintenance of the pineal phenotype, have been extensively characterized, as well as the consequences of certain gene mutations on these mechanisms [28, 34–39]. Cells that are positive for the essential ontogenetic TF Pax6 and the intermediate filament protein vimentin (Vim) are present in the pineal primordium. The $Pax6^+/Vim^+$ precursor cells divide and go through an intrinsically and spatially programmed transformation, giving rise to pinealoblasts, which then mature perinatally to become pinealocytes. Astrocytes also derive from the $Pax6^+/Vim^+$ precursors, but later than Pc. Beyond the well-characterized sympathetic regulation of PG rhythmicity in mammals, researchers have questioned what role sympathetic innervation may have on the definition and fate of the pinealocyte lineage. Disruption of the SCG-derived innervation of the rat PG at 5 days after birth (P5; P: postnatal), by either STx or SCGx, did not substantially affect the establishment of the pineal-defining transcriptome (e.g., almost unaltered expression of the *Asmt* gene, which encodes the enzyme ASMT) [23]. As expected, both neonatal SCG-related surgeries did disrupt NE-dependent rhythms in the mature gland, including the circadian rhythm of melatonin (MEL). This suggests that functional sympathetic innervation might not be essential for pinealocyte definition, as it is for its circadian function. These results are consistent with previous classic reports about the ontogeny of adrenoceptors and the postnatal appearance of rhythms in adrenergic reception and signaling transduction, and in MEL-related enzymes [7, 40]. However, further comprehensive studies are necessary to confirm or not whether sympathetic and non-sympathetic innervations do indeed participate in the fine definition of the PG phenotype. This might include interventions earlier than P5, for example.

2.5 Sympathetic dysfunction

In general, an abnormal melatonin rhythm has been associated with a wide spectrum of human pathologies, including sleep disorders, obesity, diabetes, cancer, and genetic, trauma-induced, neurological, and neurodegenerative disorders [1, 4, 41–43]. Our modern life, with the use of artificial lighting, time-shifted work schedules, and travel jet lag, contributes to alterations in circulating MEL levels in humans [42, 44]. MEL production normally subsides as we age and is aggravated by the more prolonged life expectancy of current generations [45]. This directly affects sleep patterns and mental alertness, but it also has short-term and long-term impacts on overall health. Taking MEL supplements has become a popular remedial therapy when endogenous MEL production is deemed to be deficient or altered. However, basic questions regarding MEL consumption and optimal dosage have not yet been resolved. Nevertheless, MEL supplementation is being used mainly to improve sleep quality and to treat certain sleep disorders. Additionally, it is consumed to attenuate tissue damage due to the cytoprotective properties of MEL itself. Further studies

are needed to clarify the cellular and molecular mechanisms behind the altered MEL patterns for each of these pathological landscapes. In addition, the therapeutical potential of MEL and its analogs for a wide range of human pathologies needs further investigation [4, 5, 43]. As mentioned, the experimental disruption of the SCG-derived innervation, when it is executed bilaterally and irreversibly, shuts down NE-dependent pineal rhythmicity. In humans, there are several pathological conditions that may be accompanied by primary or secondary sympathetic alterations, that therefore may cause pineal dysfunction. For example, spinal cord injuries (SCI) can be a primary mechanism for sympathetic abnormality [46]. SCI at the upper thoracic segments or higher (cervical injury) may sever the descending axons from the hypothalamic PVN, which connect the SCN to the preganglionic neurons located in the IMC of the spinal cord (SC). As a result, these IMC neurons may not establish functional synapsis with SCG ganglionic cells, which would impair or abolish nocturnal MEL synthesis [47]. In fact, patients with certain upper SCI sometimes experience altered levels of circulating MEL, and disrupted sleep patterns and behaviors. Exogenous MEL has been used to ameliorate SCI consequences due to both MEL's chronobiotic and cytoprotective qualities [48]. On the other hand, a large number of studies has pointed to an age-related loss of the pineal function in both animals and humans (e.g.: elderly individuals, and preclinical and clinical patients with aging-related pathologies, such as Alzheimer's disease and Parkinson's disease) [4, 43, 45, 49–51]. This loss has been linked to some or all the following features: anatomical abnormalities, reduced number of pinealocytes and variable numbers of glial cells, fibrosis, calcification, inflammation, altered CG expression and clock functionality, disconnection from the master circadian clock at the hypothalamic SCN, and impaired sympathetic regulation [49–54]. Sympathetic dysregulation may involve the loss of nerve terminals, as well as age-induced neuroaxonal dystrophy (NAD) of distal axons, altered denervation supersensitivity, and a decrease in adrenoceptor reception and responsiveness, among other mechanisms [53, 55] (**Figure 2**). The sequence and progression of these structural and mechanistic alterations during aging and aging-associated pathologies have not yet been fully clarified. Acute and chronic MEL supplementation, in both early and late stages of these conditions, also warrants further investigation. The use of exogenous MEL supplements represents, however, a promising remedial therapy especially for those patients in preclinical phases due to both its chronobiotic and its cytoprotective properties [51, 56, 57].

3. Conclusions

In mammals, sympathetic innervation plays a key regulatory role in pineal biology and its circadian production of the hormone melatonin. A proper melatonin rhythm, with its classic level rise at night, requires an intact photoneuroendocrine system, that transduces light information from the external environment into the hormonal cue. To do this, a hierarchical series of oscillators, that includes the brain's master circadian clock, operates in a coordinated manner to assure that this information is communicated to the pineal gland through the right and left superior cervical ganglia. The well-characterized structural and functional features of this association have made the pineal gland one of the preferred models for understanding the role of sympathetic innervation in health and disease. A wide spectrum of human pathologies may be accompanied by pineal dysfunction. This may be related to different forms of sympathetic abnormalities. Further studies are necessary to delve deeper into the

cellular and molecular mechanisms responsible for altered melatonin rhythms in these pathological landscapes. In addition, further efforts are needed to elucidate whether melatonin supplementation is useful to prevent or to ameliorate the impacts of these conditions on health and life quality.

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Conflict of interest

The authors declare no conflict of interest.

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