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Review

Signaling within the pineal gland: A parallelism with the central nervous system

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ABSTRACT

The pineal gland (PG) derives from the neural tube, like the rest of the central nervous system (CNS). The PG is specialized in synthesizing and secreting melatonin in a circadian fashion. The nocturnal elevation of melatonin is a highly conserved feature among species which proves its importance in nature. Here, we review a limited set of intrinsic and extrinsic regulatory elements that have been shown or proposed to influence the PG's melatonin production, as well as pineal ontogeny and homeostasis. Intrinsic regulators include the transcription factors CREB, Pax6 and NeuroD1. In addition, microglia within the PG participate as extrinsic regulators of these functions. We further discuss how these same elements work in other parts of the CNS, and note similarities and differences to their roles in the PG. Since the PG is a relatively well-defined and highly specialized organ within the CNS, we suggest that applying this comparative approach to additional PG regulators may be a useful tool for understanding complex areas of the brain, as well as the influence of the PG in both health and disease, including circadian functions and disorders.

1. Introduction

The ability of living organisms to sense and respond to light has shaped life since the very beginning, facilitating their evolution and survival. The pineal gland (PG) or *epiphysis cerebri* transduces environmental lighting conditions into an endogenous signal, the hormone melatonin. The nocturnal elevation of melatonin is a well-preserved feature among species which proves its importance in nature [1–3]. However, the photoperiodic control of the melatonin rhythm has changed during evolution. In early vertebrates, the photosensitive PG harbors an internal oscillator and itself synchronizes the rhythmic melatonin synthesis to the light-dark (L:D) cycle. In mammals, the PG is part of a multi-component circadian timing system, which also includes photoreceptive units in the eyes and the central clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus. The pineal gland is a circumventricular organ (CVO), and it derives from the neural tube like the rest of the central nervous system (CNS) [4]. Moreover, the highly specialized identity and function of the PG make this gland an interesting model to compare cellular and molecular mechanisms that take place in the CNS during development and adulthood. In this review, we discuss a limited set of intrinsic and extrinsic elements that were shown or proposed to regulate pineal ontogeny and function, and then we compare their roles with those in the CNS. The retina is phylogenetically related to PG, but it is minimally addressed herein (for further information, see more specific reviews [2,3,5,6]). Finally, we suggest additional elements in the pineal biology which might be explored using a comparative approach with the rest of the brain, in order to better understand the contribution of the pineal gland to the whole physiology of an organism.

2. Intrinsic regulatory elements. Transcriptional control of the pineal phenotype: roles of transcription factors (TFs) that are also expressed in the CNS

2.1. Cyclic AMP-responsive element binding protein (CREB) and its phosphorylated form (pCREB)

The biochemical pathway of melatonin synthesis in the mature PG has been well characterized and extensively reviewed [1,2,7]. Transcriptional, post-transcriptional and post-translational regulatory mechanisms have been shown to direct the rhythmic melatonin production in a specie-specific manner. In mammals, the circadian melatonin rhythm is driven by the neuronal input provided by sympathetic neurons located in the superior cervical ganglia (SCG) [8,9], and implies *de*

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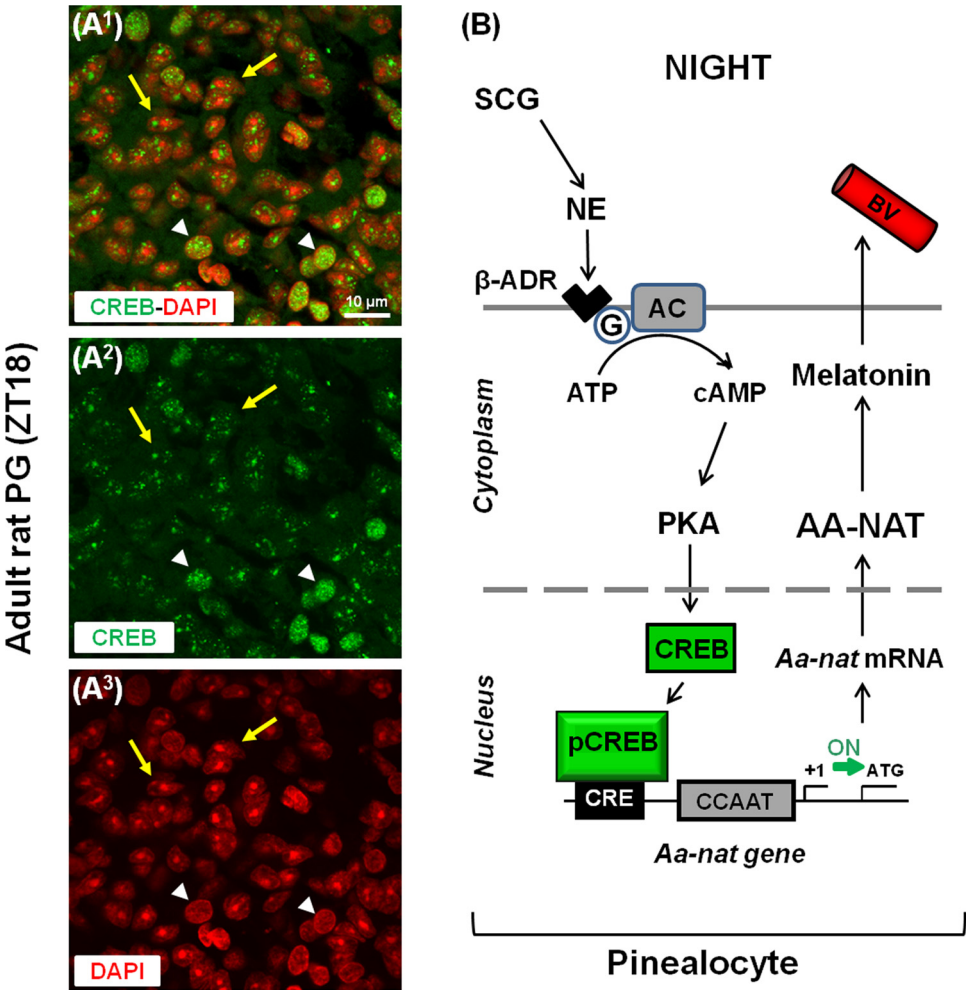


Fig. 1. CREB-mediated signaling pathway is central to pineal biology. (A^1-A^3) Section of pineal gland (PG) from an adult male Wistar rat, immunolabeled for the transcription factor CREB (cyclic AMP-responsive element binding protein; green). Nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole; red). Yellow arrows: mature pinealocytes or type I cells. White arrowheads: type II cells. Scale bar: 10 µm. (B) Schematic biochemical pathway of melatonin synthesis. *Aa-nat*/AA-NAT: arylalkylamine-N-acetyltransferase gene and protein, respectively; AC: adenylate cyclase; ATG: translation initiation codon; ATP: adenosine triphosphate; β-ADR: β1-adrenoceptor; BV: blood vessel; cAMP: cyclic adenosine monophosphate; CRE: cyclic AMP-responsive element; G: G protein; mRNA: messenger ribonucleic acid; NE: norepinephrine; pCREB: phosphorylated CREB; PKA: protein kinase A; SCG: superior cervical ganglia; ZT: *Zeitgeber time* for a 12:12 light-dark (L:D) cycle; +1: transcription start point.

novo gene expression [10–12]. A central regulatory mechanism in the PG is a signaling cascade that converts the basic leucine zipper (bZIP) TF cyclic AMP-responsive element binding protein (CREB) into its phosphorylated form (pCREB) [13]. CREB and pCREB are among the molecules that shape the melatonin rhythm. CREB is a constitutively expressed transcription factor (TF) and its phosphorylation on Ser^{133} (serine 133) is regulated by the norepinephrine (NE)/β1-adrenoceptor (β-ADR)/adenylate cyclase (AC)/cAMP/protein kinase A (PKA) pathway (Fig. 1) [13–16]. At night, pCREB induces the *Aa-nat* (*Arylalkylamine-N-acetyltransferase*) and *Hiomt* (*Hydroxyindole-O-methyltransferase*) genes in a specie-specific manner, by selectively binding to *cis*-regulatory elements (CREs; prototypical palindromic sequence 5'-TGACGTCA-3') [1,17–20]. The *Aa-nat* and *Hiomt* genes encode for AA-NAT and HIOMT respectively, and these are the last two enzymes involved in the melatonin synthesis. However, pCREB does not act alone. The inducible cAMP early repressor (ICER) is also part of the transcriptional machinery [13,21,22]. Both activating and inhibiting cAMP-dependent TFs drive the melatonin synthesis in the PG. In addition, large-scale transcriptomic analyses have revealed other cellular phenomena within the PG, including immune/inflammation response, photodetection and thyroid/retinoic acid hormone signaling [10–12,23]. These processes are likely regulated by the cAMP/CREB-mediated pathway. Therefore, CREB and pCREB are central to pineal biology. Total CREB levels are more or less stable throughout the L:D cycle, whereas pCREB is induced by the nocturnal release of NE [13]. However, the spatiotemporal distribution of these molecules and their interaction with and influence on the chromatin components and states, have not yet been characterized. In 2015, Sugo et al. [24] showed for the first time the dynamics of the binding and the dissociation of individual molecules of CREB to their target CREs, both *in vitro* and in living Neuro2a cells. The binding took several seconds to complete, and its duration was transient, but it was repeatable thereafter. More recently, the same group applied single-molecule imaging to show that the frequency of CREB binding to specific genome loci (hot spots) in mouse cortical neurons is influenced by neuronal activity [25]. However, the CREB residence time on its target sequences was not affected. In the CNS, neuronal activity-dependent interplay of CREB with several TFs has also been shown to be essential in diverse processes such as neurodevelopment, synaptic plasticity, and neuroprotection [26,27]. Furthermore, dysregulation of CREB-mediated transcription has been linked to various neuropathological conditions, especially Huntington's disease (HD) [26]. In HD, CREB-regulated transcription was found to be either enhanced or inhibited depending on the stages of the disease [27–29]. Since current knowledge of CREB's evolutionary and ontogenetic roles is still quite limited, we can speculate that CREB may have additional and yet unknown mechanisms for regulating PG and CNS physiology, and further careful investigation is needed.

2.2. Paired-box homeodomain TF 6 (Pax6)

Conversely to CREB, members of the developmental homeobox TF family have been extensively characterized in both the CNS and the PG. The *Pax6 (Paired box 6)*, *Otx2 (Orthodenticle homeobox 2)* and *Lhx9 (LIM/homeobox 9)* genes have been shown to be essential for the formation and development of the PG and certain brain areas [30–39]. Here, a concise review of the *Pax6* gene and the Pax6 protein is

presented. Pax6 is a highly evolutionarily conserved patterning TF, containing two DNA-binding domains: a paired box-domain and a homeodomain [40–42]. Pax6 has a crucial role in eye formation and also in the development and function of the cortex, diencephalon, cerebellum, spinal cord and pancreas (see the following reviews [42–44] and references therein). In general, Pax6 is responsible for balancing proliferation and differentiation of progenitor cells by targeting cohorts of downstream genes in a highly dosage-sensitive and context-dependent manner [45–52]. Pax6 controls the generation of specific neurons at the correct time and place in many regions of the developing CNS and in certain areas of the mature brain (e.g., subventricular zone of the lateral ventricles and subgranular zone of the dentate gyrus in the hippocampus in rodents) [53]. Pax6 also plays a pivotal role in gliogenesis by inhibiting astrocyte precursor proliferation and by promoting astrocyte maturation [54]. Gain-of-function and loss-of-function models, as well as state-of-the-art technology have advanced our understanding of these tightly regulated Pax6 functions. The homozygous small eye (Sey/Sey) mouse lacks a functional Pax6 protein because of a lethal mutation on chromosome 2 [55,56]. The Sey/Sey mouse dies soon after birth due to multiple developmental defects. The Pax6 null mouse fails to develop a normal posterior commissure (PC), the subcommissural organ (SCO), and also the PG itself, which normally derives as an anlage from the dorsal diencephalon [32]. In mammals, the PG is a rather homogenous organ, formed mainly by melatonin-producing pinealocytes, along with few glial cells, phagocytic cells and neurons (for a further description of the macroscopic and microscopic features of the PG of different species, please see [8]). The cellular heterogeneity of the PG was confirmed by single-cell RNA sequencing (scRNA-seq) [12]. Using this technology, the authors were able to identify nine transcriptionally distinct cell types and also differences in gene expression occurring between day and night. However, the dynamics of each cell lineage throughout pineal development and adulthood are far from being well understood. We recently described the spatiotemporal fate of the dorsal diencephalon-derived neuroepithelial precursor cells during the entire rat PG ontogeny (Figs. 2 and 3). These precursors are immunoreactive for both Pax6 and the intermediate filament protein vimentin ($Pax6^+/VIM^+$ cells) [39]. The ontogenetic profile of the $Pax6^+$ cell density resembled that of the Pax6 mRNA described previously by Rath et al. [37]. The same authors also showed that the Pax6 protein is highly expressed in the pineal anlage, and remains present in a few interstitial cells in the mature PG, albeit without a daily rhythm [6,37]. Figs. 2 and 3 summarize the re-arrangements of the $Pax6^+/VIM^+$ cells, as they transition from a radial alignment in the pineal primordium, to a rosette-like structure in the late embryonic and early postnatal PG, and finally to a dispersed distribution in the mature gland. It is still unclear how molecular forces drive these transformational stages of the stratified neuroepithelium domain to finally yield the elongated and solid shape of the mature PG. The $Pax6^+/VIM^+$ cells were shown to give rise to $5\text{-}HT^+$ (5-hydroxytryptamine or serotonin)/$Pax6^-/VIM^-$ pinealocytes, and also subpopulations of interstitial cells which remained immunoreactive for either Pax6 or vimentin, or both (Fig. 2) [39]. Within the postnatal PG, the same interstitial cells express, in a region-specific manner, either S100β (a calcium-binding protein) or GFAP (Glial Fibrillary Acidic Protein), or both. These cells resemble the astrocyte-like cells described by Mays et al. [12]. The postnatal differentiated pinealocytes which are organized as cords and pseudo-rosettes, may represent the type I cells identified by Calvo and Boya [57] in the rat PG, whereas the remnant precursor-like cells resemble the type II cells. Even though the proliferative potential of the $Pax6^+$ cells is high during the exponential growth phase of the rat PG, it becomes insignificant in the mature gland. This suggests that these cells may enter a dormant state in the adulthood. Preliminary data from our laboratory indicate that GABA might mediate the transition of $Pax6^+$ cells into dormancy by binding to $GABA_B$ receptors (unpublished data; [12,58]). The action of GABA on neural stem and precursor cells in the developing and adult CNS is complex [59,60], and warrants further investigation. Interestingly, the pluripotency of the remnant interstitial $Pax6^+$ cells within the PG is still unknown. We can speculate that further comparative analyses of the intricate mechanisms which dictate specification, differentiation, maturation and migration during neurogenesis and gliogenesis in the CNS will contribute to our understanding of pineal evolution and ontogeny, and will also clarify the role of cell type minorities in pineal physiology. Models such as the *Pax6-IRES-EGFP* knock-in (KI) mouse, which was generated by Inoue et al. [61] using the cloning-free CRISPR/Cas9 system, are promising tools for exploring the developmental dynamics of $Pax6^+$ cells and the Pax6-controlled genes in both the CNS and the PG.

2.3. Neurogenic differentiation factor 1 (NeuroD1)

The neurogenic differentiation factor 1 (NeuroD1), also known as β-cell E-box transactivator 2 (BETA2), has emerged as a potential regulator among the TFs involved in the definition and maintenance of the pineal phenotype [12,62–65]. NeuroD1 belongs to the large bHLH (basic helix-loop-helix) family of TFs. In 1995, NeuroD1was reported as a pro-neuronal TF in *Xenopus* [66] and also as a key regulator of the *insulin* gene [67]. As a consequence of the latter, the whole-body *NeuroD1* knock-out (KO) mice die perinatally due to severe diabetes [68]. However, a drastic deficit of granule cells in the cerebellum and hippocampus was observed in the adulthood when the *NeuroD1* null mice were genetically rescued from neonatal lethality by introducing a transgene encoding the mouse *NeuroD1* gene under the *insulin* promoter [69]. Since its discovery, NeuroD1 has been related to the differentiation and sometimes to the homeostasis of multiple endocrine and neuronal cell types and accordingly, to a spectrum of pathologies such as diabetes, ataxia, deafness, blindness, and cognitive deficit, among others [70–73]. NeuroD1 was shown to act differentially in the retina and the PG, though these two are highly related organs [63,64]. In these studies, we used two types of *NeuroD1* KO mice, one conventional and the other a CRE/loxP mouse specific for retina and PG. In this conditional KO mouse (cKO), the tissue specificity of the *Cre* recombinase expression was driven by the *cone-rod homeobox* (*Crx*) promoter [64]. NeuroD1 was found to be crucial for terminal differentiation and survival of retinal photoreceptor cells [64,74]. In contrast, it was found to be non-essential for pineal formation [63,64]. Nevertheless, transcriptomic analyses of PGs from neonatal conventional KO mice and adult cKO mice, did reveal several potential NeuroD1 target genes, including *Aa-nat*, *En2* (*Engrailed 2*), *Kif5c* (*Kinesin family member 5C*), *Gad1* (*Glutamic acid decarboxylase 1*), *Rnd3* (*Rho family GTPase 3*), and the clock gene *Per3* (*Period 3*), among others [63,64]. Most of these genes contain E-box consensus sequences (CANNTG) within their regulatory regions. NeuroD1 heterodimerizes with a ubiquitous E protein, such as E12/E47, which yields a heterodimer that is nuclear imported in a synergic and tightly-regulated manner. Then, the heterodimer binds to permissive E-boxes in the target genes to form transcriptional complexes with other clustered *cis*-regulatory DNA sequences and transcription regulators (Fig. 4) [67,70,75–79]. NeuroD1-mediated transactivation is terminated, in part due to the Id (inhibitor of DNA-binding/differentiation) factors. These inhibitors are HLH proteins that function by competing with tissue-specific bHLH for binding E proteins [80,81]. In the rat PG, members of the Id family are distributed in a cell type-specific manner, and some of them show a rhythmic expression (Fig. 4B-B^2) [82,83]. Interestingly, *Pax6* has been shown to be a NeuroD1-dependent gene [84]. Conversely, Pax6 has also been found to function as an upstream regulator of the *NeuroD1* gene [85]. In the developing and adult rat PG, we found that the NeuroD1 protein is expressed in both pinealocyte and glial-like cell lineages [65]. This distribution was confirmed by scRNA-seq [12]. Like Pax6 [39], NeuroD1 may modulate the proliferation, specification and differentiation of pinealocyte precursor cells [65]. Unlike Pax6 [39], NeuroD1 persists in fully differentiated pinealocytes where it may have

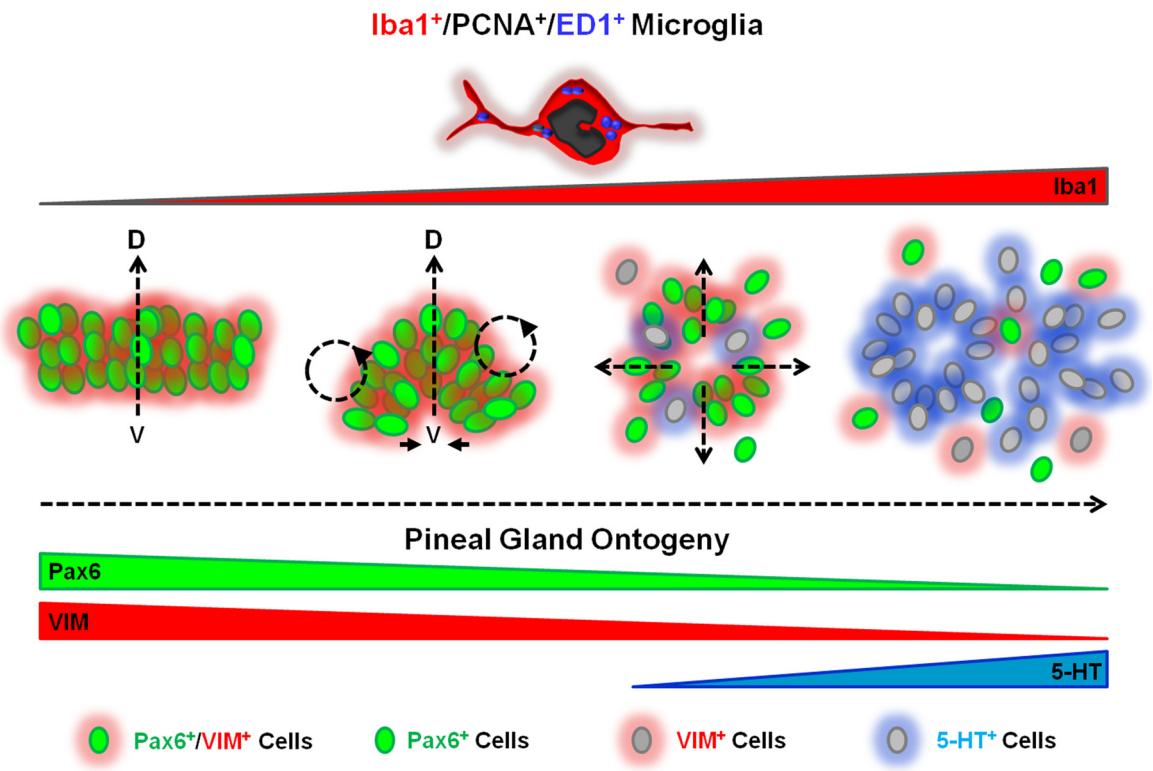


Fig. 2. Cellular dynamics during the whole rat pineal ontogeny. Schematic representation of the ontogenetic patterns of expression of the transcription factor (TF) Pax6 (Paired box 6; green), the intermediate filament protein vimentin (VIM; red), and the melatonin precursor serotonin or 5-hydroxytryptamine (5-HT; light blue), in the rat pineal gland (PG). Pax6 and VIM decay throughout pineal ontogeny, whereas 5-HT increases. The Pax6$^+$ cells are radially aligned in the earliest stages of pineal development. In the late embryonic and early postnatal PG, Pax6$^+$ cells are arranged mainly as rosette-like structures. In the mature PG, the Pax6$^+$ cells are dispersed in the interstitium. The Pax6$^+$ cells give rise to 5-HT$^+$/Pax6$^-$/VIM$^-$ pinealocytes, which are organized as cords or pseudo-rosettes, and a subpopulation of interstitial cells that may represent dormant precursor-like cells. Highly proliferative and phagocytic microglia, positive for the ionized Ca^{2+}-binding adapter molecule 1 (Iba1; red), the proliferating cell nuclear antigen (PCNA; black), and the lysosomal marker ED1 (CD68: cluster of differentiation 68; blue), colonize the pineal primordium and modulate the whole pineal ontogeny. This is done by regulating the Pax6$^+$ cell population, especially in the adult PG, and by remodeling signaling elements such as pinealocyte neurites, nerve fibers and blood vessels. Linear and circular black arrows: hypothetical forces responsible for the spatio-temporal re-arrangements of the Pax6$^+$ cells throughout pineal ontogeny. D: dorsal; V: ventral.

a role in circadian functionality, including the regulation of the melatonin rhythm (Fig. 4B-B^2) [65]. We described a rhythmic nuclear-cytoplasmic partitioning of NeuroD1 in mature rat 5-HT$^+$/Pax6$^-$/VIM$^-$ pinealocytes, which serves to illustrate this point [65]. In these cells, the nuclear import of NeuroD1 at night might be mediated by events of phosphorylation on either Ser^{274} or Ser^{336}, or on both residues. This suggests that the action of NeuroD1 in the PG is tightly regulated [65]. In the CNS and pancreas, NeuroD1 is subjected to post-translational modifications by phosphorylation, glycosylation, and acetylation, among others [70,86–88]. These processes take place in a cell type-specific and activity-dependent manner, and they finally disrupt or facilitate the nuclear localization of NeuroD1. In primary cerebellar granule neurons, the phosphorylation of NeuroD1 at distinct sites, such as Ser^{336}, is catalyzed by the neuronal activity-dependent enzyme CaMKII (Ca^{+2}/calmodulin-dependent protein kinase II) [86]. This pathway was found to regulate dendritogenesis, and thus may play a key role in the developing and mature brain. The precise mechanisms by which NeuroD1 and its modified forms modulate the pineal phenotype and homeostasis are not yet clearly understood and further research is needed. In 2017, Hanoudi et al. [89] pointed out unexpected putative targets of NeuroD1 within the neonatal mouse PG. The authors proposed a new bioinformatic method called *HighEgdeS*, to look for meaningful biological processes behind a specific phenotype. *HighEgdeS* considers all previously known and relevant gene-gene interactions, and the fold changes (FC) and/or p-values of genes differentially expressed between phenotypes (e.g., KO versus wild type). The genes *Ins1* (*Insulin 1*), *Ins2* (*Insulin 2*), *Iaap* (*Islet amyloid polypeptide*) and *Gck* (*Glucokinase*) emerged as potential targets of NeuroD1, after *HighEdgeS* was applied to our microarray dataset from PGs of neonatal *NeuroD1* KO and wild-type mice. The glucose-induced expression of *Ins* in mammalian pancreatic β-cells is highly dependent on NeuroD1 and its post-translationally modified forms, such as phosphorylated NeuroD1 (pNeuroD1) and glycosylated NeuroD1 [70,87]. However, little is known about *de novo* insulin synthesis in the brain, including in those regions of the CNS that are positive for NeuroD1 [90–93]. Further research will likely elucidate whether or not local insulin production actually does occur, and whether this insulin modulates the physiology of the CNS and the PG, in addition to the insulin synthesized by the islets of Langerhans. Our understanding of the participation of specific insulin receptors in the ontogeny, homeostasis, and plasticity of the brain and the PG also demands further studies [94,95]. In addition, an insulin-melatonin antagonism may exist at both central and peripheral levels, which may involve catecholamines and specific receptors [96–98]. The fact that a PG with an apparent normal morphology is still present in *NeuroD1* KO mouse lines suggests that a plethora of TFs is involved in pineal morphogenesis and that other members of the bHLH family or other non-related transcription regulators might compensate for the lack of NeuroD1 [99–101]. Interestingly, *NeuroD1* KO mice appear to have normal cerebral cortex morphology, with no obvious cell type changes or cell death, which suggests that either NeuroD1-dependent regulation is not essential or that compensatory processes take place in cortex development [71]. However, pioneering factor ability was reported for NeuroD1 during neurogenesis even when its expression is transient (Fig. 4A) [102]. This means that NeuroD1 is able

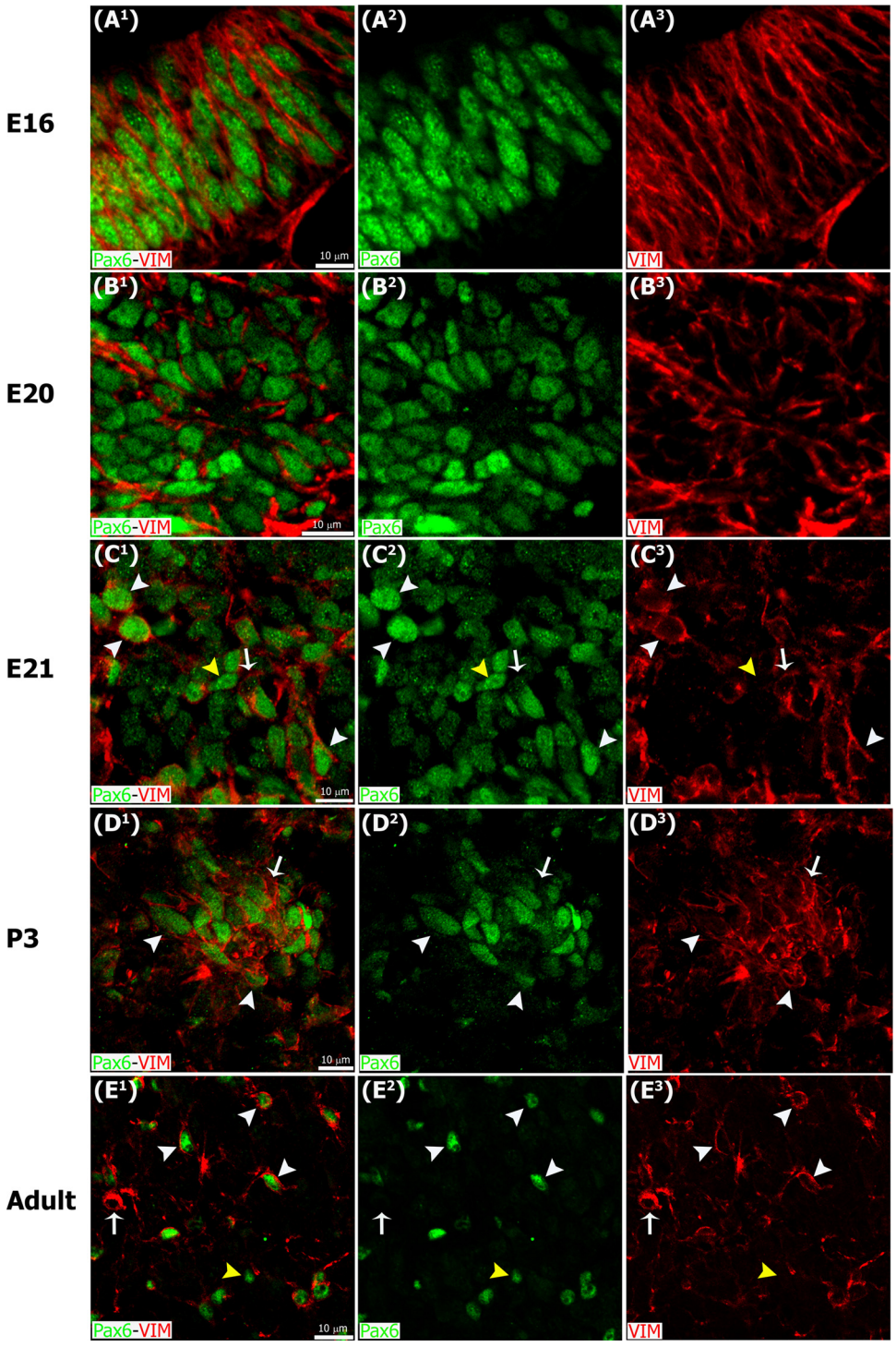


Fig. 3. Spatiotemporal fate of Pax6^{+} cells throughout the ontogeny of the rat pineal gland. High magnification images of pineal glands (PG) from embryonic (E16, 20, 21), postnatal (P3) and adult Wistar rats immunostained for the transcription factor (TF) Pax6 (Paired box 6; green) and the intermediate filament protein vimentin (VIM; red). (A^1-A^3) In the earliest stages Pax6/VIM double-positive precursor cells display a radial distribution. (B^1-D^3) After fusion of the neuroepithelium, Pax6^{+}/VIM^{+} cells are arranged mainly as rosette-like structures. (E^1-E^3) In the adult PG individual cells positive for Pax6 and/or vimentin are dispersed throughout the parenchyma. White arrowheads: Pax6^{high}/VIM^{high} cells. Yellow arrowheads: Pax6^{high}/VIM^{low} cells. White arrows: Pax6^{low}/VIM^{high} cells. Scale bar: 10 µm. Figure extracted from Ibañez et al. (2016) *Cellular Basis of Pineal Gland Development: Emerging Role of Microglia as Phenotype Regulator*. PLoS One 11(11):e0167063. doi: https://doi.org/10.1371/journal.pone.0167063 [39].

to bind its target genes within a repressive environment and then it can induce a more open chromatin state that facilitates the recruitment of cell type-restricted transcription regulators, and thus the progression of neurogenesis. The induction of pro-neuronal genes is maintained via epigenetic memory despite the subsequent disappearance of the pioneer, in this case NeuroD1. Together, these studies encourage us to continue our search for understanding NeuroD1 functionality in pineal ontogeny and homeostasis.

3. Extrinsic regulatory elements. Role of the microglia as a phenotype determinant in both the CNS and the PG

Microglia are the resident macrophages of the developing and mature CNS. They are constantly active phagocytes that survey and remodel their environment in response to both physiological and pathological conditions [103–110]. Microglial cells have been implicated in a range of developmental processes, including regulation of cell number and spatial patterning of CNS cells, myelination, and formation and refinement of neural circuits. Together, these functions suggest that microglia modulate behavior and participate in neurological disorders. Sophisticated fate-mapping studies using novel transgenic mouse

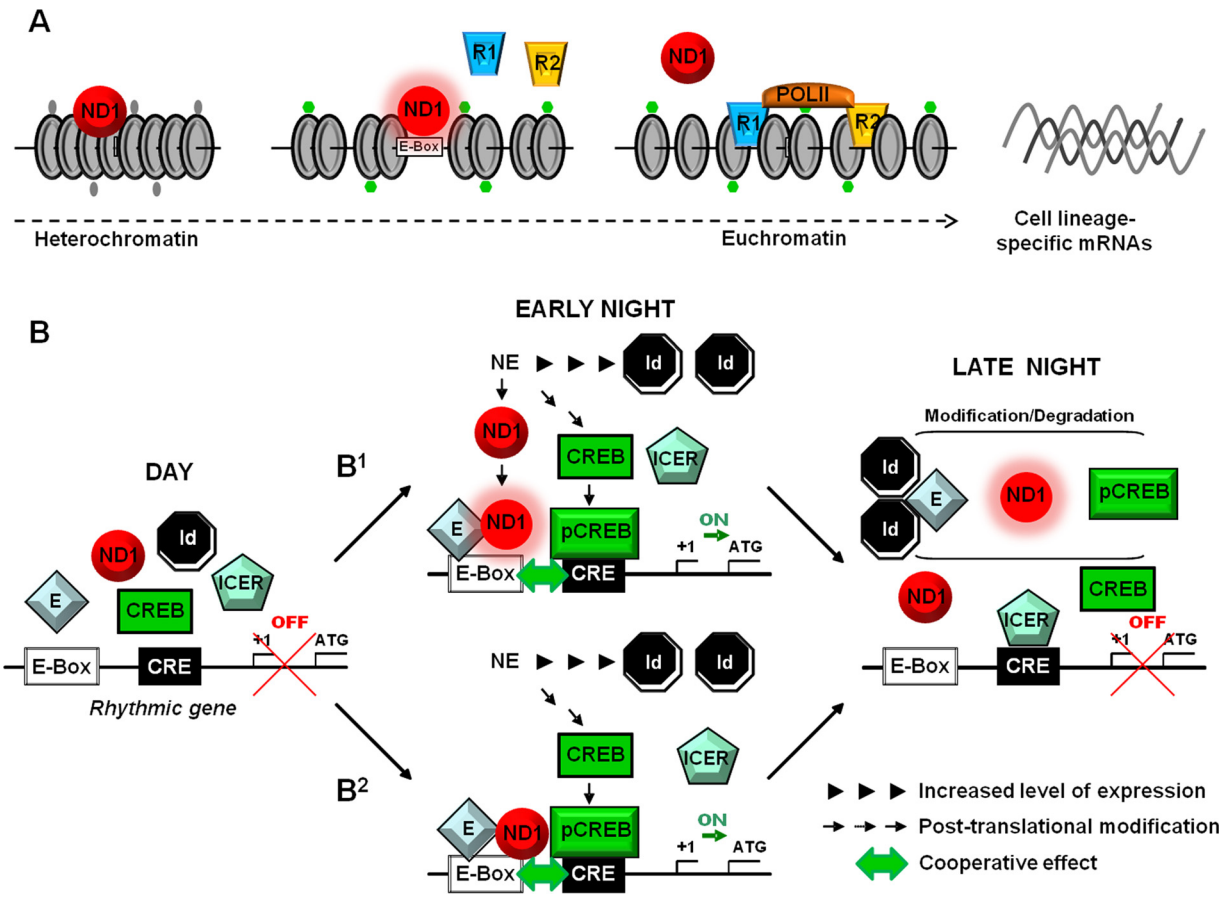
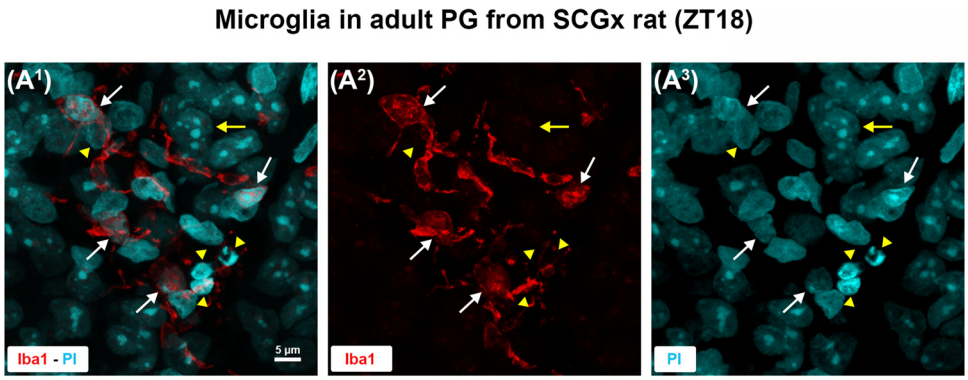


Fig. 4. Potential mechanisms of NeuroD1-mediated gene expression within the pineal gland. (A) NeuroD1 (ND1) plays a pioneering role in pineal gene expression by targeting E-boxes in a repressive chromatin (heterochromatin). NeuroD1 binding induces chromatin relaxation (euchromatin) which facilitates the recruitment of cell lineage-specific transcription regulators (R1 and R2) and the enzyme RNA polymerase II (POLII), and thus the progression of cell differentiation. NeuroD1-induced epigenetic changes are maintained despite the subsequent disappearance of the pioneer. (B) NeuroD1 cooperates directly or indirectly with the rhythmic expression of CREB/pCREB/ICER-dependent genes. (B^1) Direct influence of NeuroD1 on rhythmic gene expression due to its oscillatory post-translational modification. (B^2) Indirect contribution of NeuroD1on gene rhythmicity due to time-dependent repression caused by Id proteins, which compete with NeuroD1 for binding E proteins. ATG: translation initiation codon; CRE: cyclic AMP-responsive element; CREB: cyclic AMP-responsive element binding protein; E: E protein; ICER: inducible cAMP early repressor; Id: inhibitor of DNA-binding/differentiation; mRNA: messenger ribonucleic acid; pCREB: phosphorylated CREB; NE: norepinephrine; +1: transcription start point.



(B) Microglia functions within the PG

Immunological interface between the periphery and the CNS | Presentation of antigens | Remodeling of signaling elements (pinealocyte neurites, nerve fibers and blood vessels) | Regulation of Pax6+ precursor-like cells | Sensing and differential responses to surgical and non surgical injuries | Regulation of melatonin synthesis

Fig. 5. Microglia dynamics within the pineal gland. (A^1-A^3) Clustered microglial cells immunoreactive for the ionized Ca^{2+}-binding adapter molecule 1 (Iba1; red; white arrows), in close proximity to and eventually phagocytizing interstitial cells (yellow arrowheads) in a pineal gland (PG) from a bilaterally ganglionectomized rat (SCGx: superior cervical ganglionectomy). Nuclei were stained with propidium iodide (PI; cyan). Yellow arrows: pinealocyte or type I cell. (B) Microglia functions within the PG. ZT: *Zeitgeber time* for a 12:12 light-dark (L:D) cycle.

models have definitely proved that microglia originate from prenatal hematopoietic progenitor cells found in the yolk sack and fetal liver, from where they migrate and colonize the forming CNS before the differentiation of other cell types has taken place [111]. For the embryonic and postnatal rat pineal gland, we recently described microglia colonization from the surrounding meninges and choroid plexus, and also microglia self-renewal [39]. As a CVO, the pineal gland contains constantly activated and proliferative microglial cells throughout the whole ontogeny (Fig. 2). We proposed that microglial cells, positive for the ionized Ca^{2+}-binding adapter molecule 1 (Iba1), the proliferating cell nuclear antigen (PCNA), and the lysosomal marker ED1 (CD68: cluster of differentiation 68), modulate pineal organogenesis and homeostasis. This is done by regulating the $Pax6^{+}$ cell population, especially in the healthy adult gland, and also by remodeling signaling elements such as pinealocyte neurites, nerve fibers, and blood vessels (Figs. 2 and 5) [39]. In addition, we challenged the $Iba1^{+}$/$PCNA^{+}$/$ED1^{+}$ microglial cells in the mature rat PG by surgical and pharmacological insults [112]. Clustered microgliosis was induced within the PG by Wallerian degeneration of the sympathetic nerve fibers after performing bilateral SCGx (superior cervical ganglionectomy) (Fig. 5A^{1}-A^{3}), or by the more subtle surgery of bilateral SCGd (superior cervical ganglia decentralization). The number of pineal microglial cells increased substantially after peripheral administration of gram-negative bacteria wall components, lipopolysaccharides (LPS). The impact of the pineal microgliosis in the remnant $Pax6^{+}$ precursor-like cell population varied with the nature of the insult. This differential response within the PG correlates with the diversity and plasticity of microglial cells found in other CNS compartments, that may be influenced by aging, sexual differences, or various abnormal conditions [113–115]. Microglia heterogeneity within the rat PG was recently confirmed by scRNA-seq [12]. Our studies [39,112] expanded the repertoire of microglial functions reported previously in the PG, including presentation of antigens, sensing and response to physical injury, bacterial infection and hypoxia, and the regulation of melatonin production (Fig. 5B) [116–122]. Microglia have been identified in the pineal interstitium via expression of OX6 (MHCII), OX42 (CD11b), IL-1β, ED1 (CD68), Iba1, and TNF [12,39,116–118,123–126]. A TNF/TNFR1-mediated microglia-pinealocyte network has already been proposed to modulate melatonin production under inflammatory conditions [122]. Furthermore, global transcriptomic analyses of the rodent pineal gland have shown an enrichment of messengers that mediate immune and inflammatory processes [10–12,23]. This supports the concept that microglia may modulate the PG's melatonin levels, as a bidirectional signaling interface between the CNS and the periphery. The fine cellular and molecular mechanisms by which microglia execute their functions within the PG are not yet well understood. However, parallel research about microglia dynamics in other parts of the CNS will also advance our knowledge of those processes that take place in the PG. This, in turn, will elucidate microglia influence in both health and disease, including circadian functions and disorders.

4. Conclusions

The pineal gland (PG) is a relatively isolated and homogeneous organ that produces the hormone melatonin. As such, the PG is quite different from the complex and interconnected neuronal regions that comprise most of the central nervous system (CNS). Yet the PG develops from the neural tube, just like the rest of the CNS. This common origin imparts fundamental similarities between the PG and neuronal areas (e.g. the retina, cerebellum and hippocampus). The PG and the CNS share regulatory elements, including ontogenetic and homeostatic transcription factors such as Pax6, NeuroD1 and CREB. In addition, microglia have been shown to modulate the ontogeny and function of both the PG and the CNS as a whole.

Applying comparative analysis to additional regulatory elements, common to both the PG and the greater CNS, should improve our understanding of the underlying cellular and molecular mechanisms within the PG. This approach leverages the large body of published and on-going CNS research, in order to better understand the pineal gland or at least as a guide for researching it.

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References

[1] V. Simonneaux, C. Ribelayga, Generation of the melatonin endocrine message in mammals: a review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters, Pharmacol. Rev. 55 (2) (2003) 325–395.
[2] E. Maronde, J.H. Stehle, The mammalian pineal gland: known facts, unknown facets, Trends Endocrinol. Metab. 18 (4) (2007) 142–149.
[3] J. Falcon, L. Besseau, M. Fuentes, S. Sauzet, E. Magnanou, G. Boeuf, Structural and functional evolution of the pineal melatonin system in vertebrates, Ann. N. Y. Acad. Sci. 1163 (2009) 101–111.
[4] C. Kiecker, The origins of the circumventricular organs, J. Anat. 232 (4) (2018) 540–553.
[5] D.C. Klein, Evolution of the vertebrate pineal gland: the AANAT hypothesis, Chronobiol. Int. 23 (1-2) (2006) 5–20.
[6] M.F. Rath, K. Rohde, D.C. Klein, M. Møller, Homeobox genes in the rodent pineal gland: roles in development and phenotype maintenance, Neurochem. Res. 38 (6) (2013) 1100–1112.
[7] D.C. Klein, Arylalkylamine N-acetyltransferase: "the Timezyme", J. Biol. Chem. 282 (7) (2007) 4233–4237.
[8] M. Møller, F.M. Baeres, The anatomy and innervation of the mammalian pineal gland, Cell Tissue Res. 309 (1) (2002) 139–150.
[9] L.E. Savastano, A.E. Castro, M.R. Fitt, M.F. Rath, H.E. Romeo, E.M. Muñoz, A standardized surgical technique for rat superior cervical ganglionectomy, J. Neurosci. Methods 192 (1) (2010) 22–33.
[10] M.J. Bailey, S.L. Coon, D.A. Carter, A. Humphries, J.S. Kim, Q. Shi, P. Gaildrat, F. Morin, S. Ganguly, J.B. Hogenesch, J.L. Weller, M.F. Rath, M. Møller, R. Baler, D. Sugden, Z.G. Rangel, P.J. Munson, D.C. Klein, Night/day changes in pineal expression of &600 genes: central role of adrenergic/cAMP signaling, J. Biol. Chem. 284 (12) (2009) 7606–7622.
[11] S.W. Hartley, S.L. Coon, L.E. Savastano, J.C. Mullikin, N.C.S. Program, C. Fu, D.C. Klein, Neurotranscriptomics: the effects of neonatal stimulus deprivation on the rat pineal transcriptome, PLoS One 10 (9) (2015) e0137548.
[12] J.C. Mays, M.C. Kelly, S.L. Coon, L. Holtzclaw, M.F. Rath, M.W. Kelley, D.C. Klein, Single-cell RNA sequencing of the mammalian pineal gland identifies two pinealocyte subtypes and cell type-specific daily patterns of gene expression, PLoS One 13 (10) (2018) e0205883.
[13] E. Maronde, M. Pfeffer, J. Olcese, C.A. Molina, F. Schlotter, F. Dehghani, H.W. Korf, J.H. Stehle, Transcription factors in neuroendocrine regulation: rhythmic changes in pCREB and ICER levels frame melatonin synthesis, J. Neurosci. 19 (9) (1999) 3326–3336.
[14] P.H. Roseboom, D.C. Klein, Norepinephrine stimulation of pineal cyclic AMP response element-binding protein phosphorylation: primary role of a beta-adrenergic receptor/cyclic AMP mechanism, Mol. Pharmacol. 47 (3) (1995) 439–449.
[15] S. Tamotsu, C. Schomerus, J.H. Stehle, P.H. Roseboom, H.W. Korf, Norepinephrine-induced phosphorylation of the transcription factor CREB in isolated rat pinealocytes: an immunocytochemical study, Cell Tissue Res. 282 (2) (1995) 219–226.
[16] E. Maronde, H. Wicht, K. Tasken, H.G. Genieser, F. Dehghani, J. Olcese, H.W. Korf, CREB phosphorylation and melatonin biosynthesis in the rat pineal gland: involvement of cyclic AMP dependent protein kinase type II, J. Pineal Res. 27 (3) (1999) 170–182.
[17] I.R. Rodriguez, K. Mazuruk, T.J. Schoen, G.J. Chader, Structural analysis of the human hydroxyindole-O-methyltransferase gene. Presence of two distinct promoters, J. Biol. Chem. 269 (50) (1994) 31969–31977.
[18] R. Baler, S. Covington, D.C. Klein, The rat arylalkylamine N-acetyltransferase gene promoter. cAMP activation via a cAMP-responsive element-CCAAT complex, J. Biol. Chem. 272 (11) (1997) 6979–6985.
[19] R. Baler, S. Covington, D.C. Klein, Rat arylalkylamine N-acetyltransferase gene: upstream and intronic components of a bipartite promoter, Biol. Cell 91 (9) (1999) 699–705.
[20] C. Ribelayga, F. Gauer, C. Calgari, P. Pevet, V. Simonneaux, Photoneural regulation of rat pineal hydroxyindole-O-methyltransferase (HIOMT) messenger ribonucleic acid expression: an analysis of its complex relationship with HIOMT activity, Endocrinology 140 (3) (1999) 1375–1384.
[21] N.S. Foulkes, J. Borjigin, S.H. Snyder, P. Sassone-Corsi, Rhythmic transcription: the molecular basis of circadian melatonin synthesis, Trends Neurosci. 20 (10) (1997) 487–492.

[22] J.H. Stehle, N.S. Foulkes, C.A. Molina, V. Simonneaux, P. Pevet, P. Sassone-Corsi, Adrenergic signals direct rhythmic expression of transcriptional repressor CREM in the pineal gland, Nature 365 (6444) (1993) 314–320.

[23] D.C. Klein, M.J. Bailey, D.A. Carter, J.S. Kim, Q. Shi, A.K. Ho, C.L. Chik, P. Gaildrat, F. Morin, S. Ganguly, M.F. Rath, M. Møller, D. Sugden, Z.G. Rangel, P.J. Munson, J.L. Weller, S.L. Coon, Pineal function: impact of microarray analysis, Mol. Cell. Endocrinol. 314 (2) (2010) 170–183.

[24] N. Sugo, M. Morimatsu, Y. Arai, Y. Kousoku, A. Ohkuni, T. Nomura, T. Yanagida, N. Yamamoto, Single-molecule imaging reveals dynamics of CREB transcription factor bound to its target sequence, Sci. Rep. 5 (2015) 10662.

[25] H. Kitagawa, N. Sugo, M. Morimatsu, Y. Arai, T. Yanagida, N. Yamamoto, Activity-dependent dynamics of the transcription factor of cAMP-Response element binding protein in cortical neurons revealed by single-molecule imaging, J. Neurosci. 37 (1) (2017) 1–10.

[26] K. Sakamoto, K. Karelina, K. Obrietan, CREB: a multifaceted regulator of neuronal plasticity and protection, J. Neurochem. 116 (1) (2011) 1–9.

[27] F.C. Nucifora Jr, M. Sasaki, M.F. Peters, H. Huang, J.K. Cooper, M. Yamada, H. Takahashi, S. Tsuji, J. Troncoso, V.L. Dawson, T.M. Dawson, C.A. Ross, Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity, Science 291 (5512) (2001) 2423–2428.

[28] T. Mantamadiotis, T. Lemberger, S.C. Bleckmann, H. Kern, O. Kretz, A. Martin Villalba, F. Tronche, C. Kellendonk, D. Gau, J. Kapfhammer, C. Otto, W. Schmid, G. Schutz, Disruption of CREB function in brain leads to neurodegeneration, Nat. Genet. 31 (1) (2002) 47–54.

[29] K. Obrietan, K.R. Hoyt, CRE-mediated transcription is increased in Huntington's disease transgenic mice, J. Neurosci. 24 (4) (2004) 791–796.

[30] D. Acampora, S. Mazan, Y. Lallemand, V. Avantaggiato, M. Maury, A. Simeone, P. Brulet, Forebrain and midbrain regions are deleted in Otx2-/- mutants due to a defective anterior neuroectoderm specification during gastrulation, Development 121 (10) (1995) 3279–3290.

[31] I. Matsuo, S. Kuratani, C. Kimura, N. Takeda, S. Aizawa, Mouse Otx2 functions in the formation and patterning of rostral head, Genes Dev. 9 (21) (1995) 2646–2658.

[32] G. Estivill-Torrus, T. Vitalis, P. Fernandez-Llebrez, D.J. Price, The transcription factor Pax6 is required for development of the diencephalic dorsal midline secretory radial glia that form the subcommissural organ, Mech. Dev. 109 (2) (2001) 215–224.

[33] T.N. Mitchell, S.L. Free, K.A. Williamson, J.M. Stevens, A.J. Churchill, I.M. Hanson, S.D. Shorvon, A.T. Moore, V. van Heyningen, S.M. Sisodiya, Polymicrogyria and absence of pineal gland due to PAX6 mutation, Ann. Neurol. 53 (5) (2003) 658–663.

[34] A. Nishida, A. Furukawa, C. Koike, Y. Tano, S. Aizawa, I. Matsuo, T. Furukawa, Otx2 homeobox gene controls retinal photoreceptor cell fate and pineal gland development, Nat. Neurosci. 6 (12) (2003) 1255–1263.

[35] M.F. Rath, E. Muñoz, S. Ganguly, F. Morin, Q. Shi, D.C. Klein, M. Møller, Expression of the Otx2 homeobox gene in the developing mammalian brain: embryonic and adult expression in the pineal gland, J. Neurochem. 97 (2) (2006) 556–566.

[36] H. Abouzeid, M.A. Youssef, N. ElShakankiri, P. Hauser, F.L. Munier, D.F. Schorderet, PAX6 aniridia and interhemispheric brain anomalies, Mol. Vis. 15 (2009) 2074–2083.

[37] M.F. Rath, M.J. Bailey, J.S. Kim, A.K. Ho, P. Gaildrat, S.L. Coon, M. Møller, D.C. Klein, Developmental and diurnal dynamics of Pax4 expression in the mammalian pineal gland: nocturnal down-regulation is mediated by adrenergic-cyclic adenosine 3',5'-monophosphate signaling, Endocrinology 150 (2) (2009) 803–811.

[38] F. Yamazaki, M. Møller, C. Fu, S.J. Clokie, A. Zykovich, S.L. Coon, D.C. Klein, M.F. Rath, The Lhx9 homeobox gene controls pineal gland development and prevents postnatal hydrocephalus, Brain Struct. Funct. 220 (3) (2015) 1497–1509.

[39] M.P. Ibañez Rodriguez, S.C. Noctor, E.M. Muñoz, Cellular basis of pineal gland development: emerging role of microglia as phenotype regulator, PLoS One 11 (11) (2016) e0167063.

[40] C. Walther, P. Gruss, Pax-6, a murine paired box gene, is expressed in the developing CNS, Development 113 (4) (1991) 1435–1449.

[41] C. Walther, J.L. Guenet, D. Simon, U. Deutsch, B. Jostes, M.D. Goulding, D. Plachov, R. Balling, P. Gruss, Pax: a murine multigene family of paired box-containing genes, Genomics 11 (2) (1991) 424–434.

[42] T. Kikkawa, C.R. Casingal, S.H. Chun, H. Shinohara, K. Hiraoka, N. Osumi, The role of Pax6 in brain development and its impact on pathogenesis of autism spectrum disorder, Brain Res. (2018) pii: S0006-8993(18)30111-2.

[43] W.J. Gehring, Historical perspective on the development and evolution of eyes and photoreceptors, Int. J. Dev. Biol. 48 (8-9) (2004) 707–717.

[44] A.R. Ypsilanti, J.L. Rubenstein, Transcriptional and epigenetic mechanisms of early cortical development: an examination of how Pax6 coordinates cortical development, J. Comp. Neurol. 524 (3) (2016) 609–629.

[45] N. Warren, D. Caric, T. Pratt, J.A. Clausen, P. Asavaritikrai, J.O. Mason, R.E. Hill, D.J. Price, The transcription factor, Pax6, is required for cell proliferation and differentiation in the developing cerebral cortex, Cereb. Cortex 9 (6) (1999) 627–635.

[46] G. Estivill-Torrus, H. Pearson, V. van Heyningen, D.J. Price, P. Rashbass, Pax6 is required to regulate the cell cycle and the rate of progression from symmetrical to asymmetrical division in mammalian cortical progenitors, Development 129 (2) (2002) 455–466.

[47] N. Heins, P. Malatesta, F. Cecconi, M. Nakafuku, K.L. Tucker, M.A. Hack, P. Chapouton, Y.A. Barde, M. Gotz, Glial cells generate neurons: the role of the transcription factor Pax6, Nat. Neurosci. 5 (4) (2002) 308–315.

[48] S.N. Sansom, D.S. Griffiths, A. Faedo, D.J. Kleinjan, Y. Ruan, J. Smith, V. van Heyningen, J.L. Rubenstein, F.J. Livesey, The level of the transcription factor Pax6 is essential for controlling the balance between neural stem cell self-renewal and neurogenesis, PLoS Genet. 5 (6) (2009) e1000511.

[49] T. Kikkawa, T. Obayashi, M. Takahashi, U. Fukuzaki-Dohi, K. Numayama-Tsuruta, N. Osumi, Dmrta1 regulates proneural gene expression downstream of Pax6 in the mammalian telencephalon, Genes Cells 18 (8) (2013) 636–649.

[50] T. Walcher, Q. Xie, J. Sun, M. Irmler, J. Beckers, T. Ozturk, D. Niessing, A. Stoykova, A. Cvekl, J. Ninkovic, M. Gotz, Functional dissection of the paired domain of Pax6 reveals molecular mechanisms of coordinating neurogenesis and proliferation, Development 140 (5) (2013) 1123–1136.

[51] Q. Xie, Y. Yang, J. Huang, J. Ninkovic, T. Walcher, L. Wolf, A. Vitenzon, D. Zheng, M. Gotz, D.C. Beebe, J. Zavadil, A. Cvekl, Pax6 interactions with chromatin and identification of its novel direct target genes in lens and forebrain, PLoS One 8 (1) (2013) e54507.

[52] J. Sun, Y. Zhao, R. McGreal, Y. Cohen-Tayar, S. Rockowitz, C. Wilczek, R. Ashery-Padan, D. Shechter, D. Zheng, A. Cvekl, Pax6 associates with H3K4-specific histone methyltransferases Mll1, Mll2, and Set1a and regulates H3K4 methylation at promoters and enhancers, Epigenetics Chromatin 9 (1) (2016) 37.

[53] N. Osumi, H. Shinohara, K. Numayama-Tsuruta, M. Maekawa, Concise review: Pax6 transcription factor contributes to both embryonic and adult neurogenesis as a multifunctional regulator, Stem Cells 26 (7) (2008) 1663–1672.

[54] K. Sakurai, N. Osumi, The neurogenesis-controlling factor, Pax6, inhibits proliferation and promotes maturation in murine astrocytes, J. Neurosci. 28 (18) (2008) 4604–4612.

[55] B.L. Hogan, G. Horsburgh, J. Cohen, C.M. Hetherington, G. Fisher, M.F. Lyon, Small eyes (Sey): a homozygous lethal mutation on chromosome 2 which affects the differentiation of both lens and nasal placodes in the mouse, J. Embryol. Exp. Morphol. 97 (1986) 95–110.

[56] R.E. Hill, J. Favor, B.L. Hogan, C.C. Ton, G.F. Saunders, I.M. Hanson, J. Prosser, T. Jordan, N.D. Hastie, V. van Heyningen, Mouse small eye results from mutations in a paired-like homeobox-containing gene, Nature 354 (6354) (1991) 522–525.

[57] J. Calvo, J. Boya, Postnatal evolution of the rat pineal gland: light microscopy, J. Anat. 138 (Pt 1) (1984) 45–53.

[58] H. Yu, S.G. Benitez, S.R. Jung, L.E. Farias Altamirano, M. Kruse, J.B. Seo, D.S. Koh, E.M. Muñoz, B. Hille, GABAergic signaling in the rat pineal gland, J. Pineal Res. (2016).

[59] C. Giachino, M. Barz, J.S. Tchorz, M. Tome, M. Gassmann, J. Bischofberger, B. Bettler, V. Taylor, GABA suppresses neurogenesis in the adult hippocampus through GABAB receptors, Development 141 (1) (2014) 83–90.

[60] C. Catavero, H. Bao, J. Song, Neural mechanisms underlying GABAergic regulation of adult hippocampal neurogenesis, Cell Tissue Res. 371 (1) (2018) 33–46.

[61] Y.U. Inoue, Y. Morimoto, M. Hoshino, T. Inoue, Generation of Pax6-IRES-EGFP knock-in mouse via the cloning-free CRISPR/Cas9 system to reliably visualize neurodevelopmental dynamics, Neurosci. Res. 132 (2018) 1–7.

[62] E. Cau, S.W. Wilson, Ash1a and Neurogenin1 function downstream of floating head to regulate epiphysial neurogenesis, Development 130 (11) (2003) 2455–2466.

[63] E.M. Muñoz, M.J. Bailey, M.F. Rath, Q. Shi, F. Morin, S.L. Coon, M. Møller, D.C. Klein, NeuroD1: developmental expression and regulated genes in the rodent pineal gland, J. Neurochem. 102 (3) (2007) 887–899.

[64] M.J. Ochocinska, E.M. Muñoz, S. Veleri, J.L. Weller, S.L. Coon, N. Pozdeyev, P. Michael Iuvone, S. Goebbels, T. Furukawa, D.C. Klein, NeuroD1 is required for survival of photoreceptors but not pinealocytes: results from targeted gene deletion studies, J. Neurochem. 123 (1) (2012) 44–59.

[65] A.E. Castro, S.G. Benitez, L.E. Farias Altamirano, L.E. Savastano, S.I. Patterson, E.M. Muñoz, Expression and cellular localization of the transcription factor NeuroD1 in the developing and adult rat pineal gland, J. Pineal Res. 58 (4) (2015) 439–451.

[66] J.E. Lee, S.M. Hollenberg, L. Snider, D.L. Turner, N. Lipnick, H. Weintraub, Conversion of Xenopus ectoderm into neurons by NeuroD, a basic helix-loop-helix protein, Science 268 (5212) (1995) 836–844.

[67] F.J. Naya, C.M. Stellrecht, M.J. Tsai, Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor, Genes Dev. 9 (8) (1995) 1009–1019.

[68] F.J. Naya, H.P. Huang, Y. Qiu, H. Mutoh, F.J. DeMayo, A.B. Leiter, M.J. Tsai, Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/neuroD-deficient mice, Genes Dev. 11 (18) (1997) 2323–2334.

[69] T. Miyata, T. Maeda, J.E. Lee, NeuroD is required for differentiation of the granule cells in the cerebellum and hippocampus, Genes Dev. 13 (13) (1999) 1647–1652.

[70] J.H. Chae, G.H. Stein, J.E. Lee, NeuroD: the predicted and the surprising, Mol. Cells 18 (3) (2004) 271–288.

[71] J.H. Cho, M.J. Tsai, The role of BETA2/NeuroD1 in the development of the nervous system, Mol. Neurobiol. 30 (1) (2004) 35–47.

[72] V. Fedele, L. Roybon, U. Nordstrom, J.Y. Li, P. Brundin, Neurogenesis in the R6/2 mouse model of Huntington's disease is impaired at the level of NeuroD1, Neuroscience 173 (2011) 76–81.

[73] O. Orosz, M. Czegledi, I. Kantor, I. Balogh, A. Vajas, L. Takacs, A. Berta, G. Losonczy, Ophthalmological phenotype associated with homozygous null mutation in the NEUROD1 gene, Mol. Vis. 21 (2015) 124–130.

[74] M.E. Pennesi, J.H. Cho, Z. Yang, S.H. Wu, J. Zhang, S.M. Wu, M.J. Tsai, BETA2/NeuroD1 null mice: a new model for transcription factor-dependent photoreceptor degeneration, J. Neurosci. 23 (2) (2003) 453–461.

[75] H. Mutoh, B.P. Fung, F.J. Naya, M.J. Tsai, J. Nishitani, A.B. Leiter, The basic helix-loop-helix transcription factor BETA2/NeuroD is expressed in mammalian

enteroendocrine cells and activates secretin gene expression, Proc. Natl. Acad. Sci. U. S. A. 94 (8) (1997) 3560–3564.
[76] G. Poulin, B. Turgeon, J. Drouin, NeuroD1/beta2 contributes to cell-specific transcription of the proopiomelanocortin gene, Mol. Cell. Biol. 17 (11) (1997) 6673–6682.
[77] S. Seo, J.W. Lim, D. Yellajoshyula, L.W. Chang, K.L. Kroll, Neurogenin and NeuroD direct transcriptional targets and their regulatory enhancers, EMBO J. 26 (24) (2007) 5093–5108.
[78] A. Longo, G.P. Guanga, R.B. Rose, Crystal structure of E47-NeuroD1/beta2 bHLH domain-DNA complex: heterodimer selectivity and DNA recognition, Biochemistry 47 (1) (2008) 218–229.
[79] R. Mehmood, N. Yasuhara, M. Fukumoto, S. Oe, T. Tachibana, Y. Yoneda, Cross-talk between distinct nuclear import pathways enables efficient nuclear import of E47 in conjunction with its partner transcription factors, Mol. Biol. Cell 22 (19) (2011) 3715–3724.
[80] F. Ling, B. Kang, X.H. Sun, Id proteins: small molecules, mighty regulators, Curr. Top. Dev. Biol. 110 (2014) 189–216.
[81] L.H. Wang, N.E. Baker, E. Proteins, I.D. Proteins, Helix-loop-Helix partners in development and disease, Dev. Cell 35 (3) (2015) 269–280.
[82] A. Humphries, D. Klein, R. Baler, D.A. Carter, cDNA array analysis of pineal gene expression reveals circadian rhythmicity of the dominant negative helix-loop-helix protein-encoding gene, Id-1, J. Neuroendocrinol. 14 (2) (2002) 101–108.
[83] B. Kofler, A. Bulleyment, A. Humphries, D.A. Carter, Id-1 expression defines a subset of vimentin/S-100beta-positive, GFAP-negative astrocytes in the adult rat pineal gland, Histochem. J. 34 (3-4) (2002) 167–171.
[84] E. Marsich, A. Vetere, M. Di Piazza, G. Tell, S. Paoletti, The PAX6 gene is activated by the basic helix-loop-helix transcription factor NeuroD/BETA2, Biochem. J. 376 (Pt 3) (2003) 707–715.
[85] Y. Gosmain, E. Marthinet, C. Cheyssac, A. Guerardel, A. Mamin, L.S. Katz, K. Bouzakri, J. Philippe, Pax6 controls the expression of critical genes involved in pancreatic {alpha} cell differentiation and function, J. Biol. Chem. 285 (43) (2010) 33381–33393.
[86] B. Gaudilliere, Y. Konishi, N. de la Iglesia, G. Yao, A. Bonni, A CaMKII-NeuroD signaling pathway specifies dendritic morphogenesis, Neuron 41 (2) (2004) 229–241.
[87] S.S. Andrali, Q. Qian, S. Ozcan, Glucose mediates the translocation of NeuroD1 by O-linked glycosylation, J. Biol. Chem. 282 (21) (2007) 15589–15596.
[88] Y. Qiu, M. Guo, S. Huang, R. Stein, Acetylation of the BETA2 transcription factor by p300-associated factor is important in insulin gene expression, J. Biol. Chem. 279 (11) (2004) 9796–9802.
[89] S. Hanoudi, M. Donato, S. Draghici, Identifying biologically relevant putative mechanisms in a given phenotype comparison, PLoS One 12 (5) (2017) e0176950.
[90] R. Schechter, D. Beju, T. Gaffney, F. Schaefer, L. Whetsell, Preproinsulin I and II mRNAs and insulin electron microscopic immunoreaction are present within the rat fetal nervous system, Brain Res. 736 (1-2) (1996) 16–27.
[91] G. Molnar, N. Farago, A.K. Kocsis, M. Rozsa, S. Lovas, E. Boldog, R. Baldi, E. Csajbok, J. Gardi, L.G. Puskas, G. Tamas, GABAergic neurogliaform cells represent local sources of insulin in the cerebral cortex, J. Neurosci. 34 (4) (2014) 1133–1137.
[92] A.A. Akintola, D. van Heemst, Insulin, aging, and the brain: mechanisms and implications, Front. Endocrinol. (Lausanne) 6 (2015) 13.
[93] E.A. Csajbok, G. Tamas, Cerebral cortex: a target and source of insulin? Diabetologia 59 (8) (2016) 1609–1615.
[94] G.T. Dodd, T. Tiganis, Insulin action in the brain: roles in energy and glucose homeostasis, J. Neuroendocrinol. 29 (10) (2017).
[95] I. Pomytkin, J.P. Costa-Nunes, V. Kasatkin, E. Veniaminova, A. Demchenko, A. Lyundup, K.P. Lesch, E.D. Ponomarev, T. Strekalova, Insulin receptor in the brain: mechanisms of activation and the role in the CNS pathology and treatment, CNS Neurosci. Ther. (2018).
[96] A.G. Bach, E. Muhlbauer, E. Peschke, Adrenoceptor expression and diurnal rhythms of melatonin and its precursors in the pineal gland of type 2 diabetic goto-kakizaki rats, Endocrinology 151 (6) (2010) 2483–2493.
[97] E. Peschke, H. Schucht, E. Muhlbauer, Long-term enteral administration of melatonin reduces plasma insulin and increases expression of pineal insulin receptors in both Wistar and type 2-diabetic Goto-Kakizaki rats, J. Pineal Res. 49 (4) (2010) 373–381.
[98] E. Peschke, I. Bahr, E. Muhlbauer, Experimental and clinical aspects of melatonin and clock genes in diabetes, J. Pineal Res. 59 (1) (2015) 1–23.
[99] I. Imayoshi, R. Kageyama, Oscillatory control of bHLH factors in neural progenitors, Trends Neurosci. 37 (10) (2014) 531–538.
[100] I. Imayoshi, R. Kageyama, bHLH factors in self-renewal, multipotency, and fate choice of neural progenitor cells, Neuron 82 (1) (2014) 9–23.
[101] N.E. Baker, N.L. Brown, All in the family: proneural bHLH genes and neuronal diversity, Development 145 (9) (2018).
[102] A. Pataskar, J. Jung, P. Smialowski, F. Noack, F. Calegari, T. Straub, V.K. Tiwari, NeuroD1 reprograms chromatin and transcription factor landscapes to induce the neuronal program, EMBO J. 35 (1) (2016) 24–45.
[103] G. Raivich, Like cops on the beat: the active role of resting microglia, Trends Neurosci. 28 (11) (2005) 571–573.
[104] D. Soulet, S. Rivest, Microglia, Curr. Biol. 18 (12) (2008) R506–R508.
[105] H. Kettenmann, U.K. Hanisch, M. Noda, A. Verkhratsky, Physiology of microglia, Physiol. Rev. 91 (2) (2011) 461–553.
[106] C.L. Cunningham, V. Martinez-Cerdeño, S.C. Noctor, Microglia regulate the number of neural precursor cells in the developing cerebral cortex, J. Neurosci. 33 (10) (2013) 4216–4233.
[107] H. Wake, A.J. Moorhouse, A. Miyamoto, J. Nabekura, Microglia: actively surveying and shaping neuronal circuit structure and function, Trends Neurosci. 36 (4) (2013) 209–217.
[108] J.L. Frost, D.P. Schafer, Microglia: architects of the developing nervous system, Trends Cell Biol. 26 (8) (2016) 587–597.
[109] K. Reemst, S.C. Noctor, P.J. Lucassen, E.M. Hol, The indispensable roles of microglia and astrocytes during brain development, Front. Hum. Neurosci. 10 (2016) 566.
[110] N. Barger, J. Keiter, A. Kreutz, A. Krishnamurthy, C. Weidenthaler, V. Martinez-Cerdeño, A.F. Tarantal, S.C. Noctor, Microglia: an intrinsic component of the proliferative zones in the fetal Rhesus monkey (Macaca mulatta) cerebral cortex, Cereb. Cortex (2018).
[111] M. Prinz, D. Erny, N. Hagemeyer, Ontogeny and homeostasis of CNS myeloid cells, Nat. Immunol. 18 (4) (2017) 385–392.
[112] M.P. Ibañez Rodriguez, M.D. Galiana, J.A. Rasmussen, C.L. Freites, S.C. Noctor, E.M. Muñoz, Differential response of pineal microglia to surgical versus pharmacological stimuli, J. Comp. Neurol. 526 (15) (2018) 2462–2481.
[113] K. Grabert, T. Michoel, M.H. Karavolos, S. Clohisey, J.K. Baillie, M.P. Stevens, T.C. Freeman, K.M. Summers, B.W. McColl, Microglial brain region-dependent diversity and selective regional sensitivities to aging, Nat. Neurosci. 19 (3) (2016) 504–516.
[114] T.L. Tay, D. Mai, J. Dautzenberg, F. Fernandez-Klett, G. Lin, M. Sagar, A. Datta, T. Drougard, A. Stempfl, O. Ardura-Fabregat, A. Staszewski, A. Margineanu, L.M. Sporbert, J.A. Steinmetz, S. Pospisilik, J. Jung, D. Priller, O. Grun, M. Ronneberger, A. Prinz, New fate mapping system reveals context-dependent random or clonal expansion of microglia, Nat. Neurosci. 20 (6) (2017) 793–803.
[115] T.L. Tay, J.C. Savage, C.W. Hui, K. Bisht, M.E. Tremblay, Microglia across the lifespan: from origin to function in brain development, plasticity and cognition, J. Physiol. (Paris) 595 (6) (2017) 1929–1945.
[116] E.B. Pedersen, L.M. Fox, A.J. Castro, J.A. McNulty, Immunocytochemical and electron-microscopic characterization of macrophage/microglia cells and expression of class II major histocompatibility complex in the pineal gland of the rat, Cell Tissue Res. 272 (2) (1993) 257–265.
[117] C. Kaur, J. Singh, M.K. Lim, B.L. Ng, E.A. Ling, Macrophages/microglia as' sensors' of injury in the pineal gland of rats following a non-penetrative blast, Neurosci. Res. 27 (4) (1997) 317–322.
[118] E.B. Pedersen, J.A. McNulty, A.J. Castro, L.M. Fox, J. Zimmer, B. Finsen, Enriched immune-environment of blood-brain barrier deficient areas of normal adult rats, J. Neuroimmunol. 76 (1-2) (1997) 117–131.
[119] Y.F. Jiang-Shieh, C.H. Wu, H.F. Chien, I.H. Wei, M.L. Chang, J.Y. Shieh, C.Y. Wen, Reactive changes of interstitial glia and pinealocytes in the rat pineal gland challenged with cell wall components from gram-positive and -negative bacteria, J. Pineal Res. 38 (1) (2005) 17–26.
[120] M. Møller, M.F. Rath, D.C. Klein, The perivascular phagocyte of the mouse pineal gland: an antigen-presenting cell, Chronobiol. Int. 23 (1-2) (2006) 393–401.
[121] C. Kaur, V. Sivakumar, E.A. Ling, Expression of tranferrin receptors in the pineal gland of postnatal and adult rats and its alteration in hypoxia and melatonin treatment, Glia 55 (3) (2007) 263–273.
[122] S. da Silveira Cruz-Machado, L. Pinato, E.K. Tamura, C.E. Carvalho-Sousa, R.P. Markus, Glia-pinealocyte network: the paracrine modulation of melatonin synthesis by tumor necrosis factor (TNF), PLoS One 7 (7) (2012) e40142.
[123] C. Kaur, C.H. Wu, E.A. Ling, Immunohistochemical and tracer studies of macrophages/microglia in the pineal gland of postnatal rats, J. Pineal Res. 22 (3) (1997) 137–144.
[124] S.Y. Tsai, J.A. McNulty, Interleukin-1beta expression in the pineal gland of the rat, J. Pineal Res. 27 (1) (1999) 42–48.
[125] Y.F. Jiang-Shieh, C.H. Wu, M.L. Chang, J.Y. Shieh, C.Y. Wen, Regional heterogeneity in immunoreactive macrophages/microglia in the rat pineal gland, J. Pineal Res. 35 (1) (2003) 45–53.
[126] C.E. Carvalho-Sousa, S. da Silveira Cruz-Machado, E.K. Tamura, P.A. Fernandes, L. Pinato, S.M. Muxel, E. Cecon, R.P. Markus, Molecular basis for defining the pineal gland and pinealocytes as targets for tumor necrosis factor, Front. Endocrinol. (Lausanne) 2 (2011) 10.