

Protective roles of melatonin in central nervous system diseases by regulation of neural stem cells

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Abstract

Neural stem cells (NSCs) are immature precursors of the central nervous system (CNS), with self-renewal and multipotential differentiation abilities. Their proliferation and differentiation are dynamically regulated by hormonal and local factors. Alteration in neurogenesis is associated with many neurological disorders. Increasing evidence suggests that modulation of NSCs can be a promising therapeutic approach for neural injury and neurodegenerative disorders. Melatonin, a pineal gland-derived hormone, regulates the neuroimmuno-endocrine axis and is functionally important to the circadian rhythm, tumour suppression and immunity. In the CNS, melatonin exerts neuroprotective effects in many diseases, such as Parkinson's disease, Alzheimer's disease and ischaemic brain injury. Emerging evidence suggests that it might also mediate such protective action by influencing proliferation and differentiation of NSCs. In this article, we review the current literature concerned with effects of melatonin on NSCs in different physiological and pathological conditions.

1 | INTRODUCTION

Neural stem cells (NSCs) are immature precursor cells that possess self-renewal and multipotential differentiation abilities in both developing and adult brains.^{1–4} In the central nervous system (CNS), neurons, astrocytes and oligodendrocytes all originate from NSCs.⁵ Given their potential to replace injured and demised neurons in diseased brains, transplantation of NSCs has been promulgated as a novel therapeutic approach for many CNS disorders, such as neural injury and neurodegenerative diseases. In this connection, efforts have been made to identify extrinsic factors capable of regulating the survival, proliferation and differentiation of NSCs.

Melatonin (*N*-acetyl-5-methoxytryptamine) is not only secreted by the pineal gland but also other organs, such as retina,

gut, ovary and testis. This molecule is both a local regulator and a hormone, which is secreted in a circadian manner. Melatonin exerts its biological effects through binding to its G protein-coupled MT1 and MT2 receptors.⁶ It has various regulatory biological functions in the neuroimmuno-endocrine system and influences sleep-wake cycle, circadian rhythms, tumour inhibition and immune function.⁷ Melatonin also plays a neuroprotective role in many CNS disorders, such as Parkinson's disease, Alzheimer's disease, and ischaemic brain injury.^{8,9} Emerging evidence suggested that melatonin could influence the proliferation and differentiation of NSCs. A comprehensive understanding of the effects of melatonin on NSCs may provide valuable insight on developing new therapeutic approaches.

In this article, we review current literatures on the regulatory effects of melatonin on NSCs and discuss its therapeutic potential as a neuroprotective agent in different pathophysiological context.

Xin Yu and Zheng Li contributed equally to this work.

2 | BIOLOGICAL CHARACTERISTICS OF NEURAL STEM CELLS

Stem cells are undifferentiated immature cells with abilities to proliferate and differentiate into various specialized cells.¹⁰ There are two major types of stem cells, namely embryonic and somatic stem cells. Different types of stem cells have diverse abilities to differentiate and proliferate. Those with the capability to give rise to any mature cell types are called totipotent and pluripotent cells, whereas those with narrower spectrum of differentiation are termed multipotent or oligopotent cells.¹¹ Embryonic stem cells are pluripotent cells derived from the inner cell mass of an embryo at the blastocyst stage. Transplantation of pluripotent stem cells, including induced pluripotent and embryonic stem cells, has therapeutic value in numerous diseases, ranging from retinal dysfunctions, heart diseases to neurological disorders.¹²

Neural stem cells are multipotent cells capable of self-replication and differentiation into neurons, astrocytes or oligodendrocytes in the CNS. NSCs are mainly located in the anterior subventricular and subgranular zones of the hippocampal dentate gyrus where neurogenesis occurs even in the adult brain. When transplanted into the developing nervous system, NSCs disseminate throughout the CNS and integrate within the developing neural networks.¹³ A subpopulation of transplanted NSCs could also migrate to the impaired region and differentiate into neural cell types that replace the diseased host cells. To this end, many studies have shown that NSC transplantation could bring about therapeutic benefits in different animal models of CNS diseases. Nevertheless, it is noteworthy that the proliferative and differentiative abilities of NSCs vary in different physiological and pathological conditions and are dynamically influenced by the humoral and adhesive factors. Such abilities also decrease with age.¹⁴ In this regard, many factors that increase NSC proliferation have been demonstrated to promote neurogenesis in ischaemic and neurodegenerative diseases. Nevertheless, the downstream molecular mechanism linking these regulatory factors to phenotypic changes in NSCs remains unclear. Understanding these signalling events may contribute to the development of new therapy against diseases related to neuronal death.

3 | BIOLOGICAL EFFECTS OF MELATONIN ON NEURAL STEM CELLS

The proliferation and differentiation of NSCs are dynamically regulated by the humoral and adhesive factors from the extracellular environment.¹⁴ An increasing number of studies suggested that melatonin is a crucial regulator of precursor cell commitment and differentiation. In this section, we will summarize the existing evidence on the association between melatonin and NSCs.

3.1 | Expression of melatonin receptor in the CNS

Sotthibundhu and colleagues¹⁵ reported that precursor cells from subventricular zone of the lateral ventricle in adult mouse, which is the main neurogenic area, expressed melatonin receptors. Moreover,

melatonin promoted the proliferation of precursor cells derived from this area, in which melatonin promoted the differentiation of precursor cells into neuronal cells without affecting the number of glial cells. Furthermore, luzindole (a non-selective MT1/MT2 inhibitor) decreased melatonin-induced proliferation. In culture system, Niles and colleagues¹⁶ observed the expression of MT1 but not MT2 receptor mRNA in NSCs cultured for 2 days, at which an immature MT1 receptor of about 30 kDa was present, whereas a mature receptor of about 40–45 kDa was present in cells maintained for longer periods.

3.2 | Effects of melatonin on proliferation and differentiation of NSCs

Melatonin plays a significant role in cell fate specification during neural commitment.¹⁷ It promoted the differentiation of pluripotent P19 cells into NSCs, in which activation of the MT1 receptor and phosphorylation of ERK1/2 might be involved in this process. Melatonin at pharmacological concentrations also facilitated foetal bovine serum-induced neural differentiation of NSCs without affecting the astroglial differentiation during the proliferation period while it suppressed the neural differentiation during the differentiation period.¹⁸ Neural differentiation of NSCs could be reduced by hypoxia in culture. In this connection, melatonin not only promoted proliferation of NSCs during hypoxia but also induced neuronal differentiation of NSCs while exerted no obvious effect on astrocyte differentiation.¹⁹ Furthermore, MT1 receptor and extracellular signal-regulated kinase (ERK) 1/2 phosphorylation played a role in melatonin-induced NSC proliferation. In accord, Tocharus and colleagues²⁰ showed that melatonin significantly increased the number of neurospheres and upregulated phospho-c-Raf, phospho-ERK1/2 and nestin protein in NSCs, and these effects were blocked by luzindole or PD98059 (a mitogen-activated protein kinase kinase (MEK) inhibitor). N-acetylserotonin (NAS) is an immediate precursor of melatonin. Sompol and colleagues²¹ showed that NAS increased neuronal progenitor cell proliferation in both active and sleeping phases of the adult mice, as well as in sleep-deprived mice. Chronic NAS treatment activated TrkB receptor in neuronal progenitor cells. These findings indicated that melatonin could increase NSC proliferation via TrkB activation and the c-Raf-MEK-ERK1/2 pathway. Sharma and colleagues²² showed that physiological concentrations of melatonin (nanomolar range) elevated mRNA expression of nestin, a neuroectodermal stem cell marker, in C17.2 NSCs. In addition, melatonin promoted neurite-like extensions and increased the early neuronal marker β -III-tubulin and the orphan nuclear receptor nurr1 in NSCs. More importantly, melatonin significantly increased chromatin remodelling and gene transcription associated with histone H3 acetylation. As the melatonin MT2 receptor was not present in C17.2 NSCs cells, the MT1 receptor was considered to mediate these biological effects.

3.3 | Role of melatonin in neurogenesis

Melatonin promoted the survival of new neurons derived from neural precursor cells in adult hippocampus both in vitro and in vivo.²³ Melatonin receptors were involved in these effects as it was blocked

by luzindole. In addition, melatonin did not affect neuronal and glial cell maturation and precursor cell proliferation *in vivo*. These results indicated that melatonin modulated the survival of newborn neurons derived from precursor cells in the adult hippocampus. Ortiz-López and colleagues also demonstrated that chronic administration luzindole in mice decreased the number of proliferating cells in the subgranular zone of the dentate gyrus of the hippocampus, confirming the key positive regulatory function of endogenous melatonin in neurogenesis.²⁴

3.4 | Effects of melatonin on neural differentiation of induced pluripotent stem cells

In induced pluripotent stem cells, it was found that melatonin promoted the number of neurospheres and cell viability during neural differentiation. These promoting effects were accompanied by up-regulation of Nestin and MAP2 as well as Akt phosphorylation, which were attenuated by luzindole or LY294002 (PI3K inhibitor), suggesting that melatonin significantly increased neural differentiation of induced pluripotent stem cells via MT receptor-mediated activating phosphoinositide 3-kinase/Akt signalling pathway.²⁵ Interestingly, another study found that melatonin increased reprogramming efficiency of NSC-derived pluripotent stem cells generated from primary cultured bovine NSCs. This effect was mediated by downregulation of apoptosis-related genes p53 and p21. These cells are similar to typical embryonic stem cells, which expressed pluripotency markers (Oct4 and Nanog), formed teratomas *in vivo*, and possessed the capacity to differentiate into all three embryonic germ layers.²⁶ These findings added an additional layer of complexity in the role of melatonin in regulating differentiation and maintaining pluripotency of induced pluripotent stem cells.

4 | PROTECTIVE EFFECTS OF MELATONIN ON NSCS IN CNS DISEASES

The promoting effects of melatonin on the proliferation and neuronal differentiation of NSCs make it a potential adjuvant with NSC replacement for the treatment of neural diseases, such as cerebral infarction and neurodegenerative diseases.

4.1 | Melatonin and NSCs in Parkinson's disease

Parkinson's disease is hallmarked by the depletion of dopaminergic neurons in the substantia nigra. Kong and colleagues demonstrated that melatonin promoted the viability of NSCs, in which melatonin up-regulated the expression of dopaminergic neurons tyrosine hydroxylase and downregulated the astrocytes maker glial fibrillary acidic protein. Melatonin also increased the production of neurotrophic factors in the NSCs *in vitro*.²⁷ Moreover, low physiological concentrations of melatonin induced glial cell-line derived neurotrophic factor mRNA expression in NSCs, which promoted the survival of dopaminergic neurons.¹⁶ Sharma and colleagues²⁸ showed that apomorphine-induced

behavioural changes were significantly reduced in striatum-lesioned animals treated by NSC transplantation, melatonin or the combined regimen. Importantly, these treatments protected tyrosine hydroxylase immunoreactivity in the striatum and substantia nigra of lesioned animals compared with untreated controls. Taken together, melatonin in combination with NSC transplantation might be used as a therapeutic approach in Parkinson's disease.

4.2 | Melatonin and NSCs in spinal cord injury

Melatonin plus exercise significantly improved the hindlimb function and promoted the proliferation of endogenous NSCs after spinal cord injury. Furthermore, the combined therapy reduced the size of the spinal lesion through increasing the density of dendritic spines and axons. These findings showed that melatonin in combination with exercise significantly promoted endogenous NSC proliferation in spinal cord injury.²⁹ In another study, melatonin stimulated MT1, which subsequently increased the survival of bovine amniotic epithelial cells and promoted their differentiation into neural cells. Importantly, these cells colonized into injured spinal cord, suggesting their participation in tissue repair.³⁰

4.3 | Melatonin and NSCs in neural tube defects associated with diabetic pregnancy

Diabetic pregnancy is associated with neural tube defects in offspring. In diabetic pregnant mice, intraperitoneal injection of melatonin at the dose of 10 mg/kg per day from embryonic day (E) 0.5 to E11.5 decreased neural tube defects, especially exencephaly, in embryos. Mechanistically, melatonin stimulated NSC proliferation and decreased apoptosis under hyperglycaemic condition. The mitotic effect of melatonin was mediated through the ERK pathway.³¹

4.4 | Melatonin and NSCs against injury inflicted by exogenous agents

Melatonin protects NSCs against lipopolysaccharide (LPS)-induced inflammation.³² This is partially mediated by activation of SOX2 and

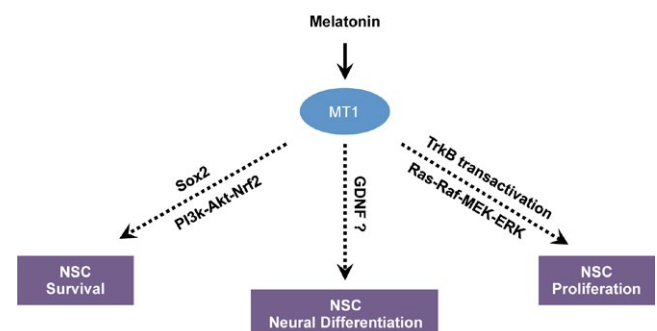


FIGURE 1 Signalling pathways activated by melatonin through MT1 receptor in the regulation of neural stem cell (NSC) survival, proliferation and neural differentiation. It remained largely unclear how MT1 activation could trigger the downstream signalling network.

the phosphoinositide 3-kinase/Akt/Nrf2 signalling pathways, suggesting that melatonin might promote NSC survival and proliferation in neuroinflammatory conditions.

Glucocorticoids could induce neuronal damage by suppressing cell proliferation and neurogenesis. Melatonin pre-treatment minimized the detrimental effect of dexamethasone on neurogenesis in the rat hippocampus.³³ In the dexamethasone-treated group, pre-treatment with melatonin normalized the expression of Ki67 and functions of nestin-positive cells. In addition, the reduction of ERK1/2 phosphorylation and G₁-S phase cell cycle regulators cyclin E and CDK2 in NSCs by dexamethasone were abrogated by melatonin. Obviously, there are crosstalk and cross-regulation between the melatonin receptors and the glucocorticoid receptor in NSCs. Dexamethasone downregulates MT1 melatonin receptor, whereas melatonin inhibits the glucocorticoid receptor. Similarly, use of exogenous melatonin reversed the depressive-like state induced by chronic treatment with corticosteroid.³⁴ Prolonged treatment with melatonin prevented the corticosteroid-induced reduction in NSC proliferation in murine hippocampus. Corticosteroid-treated mice had a decreased spine density, which was ameliorated by administration of extrinsic melatonin. These suggested that the antidepressant effect of melatonin might be associated with neurogenesis.

Methamphetamine, a highly addictive psycho-stimulatory drug, may lead to several neurodegenerative diseases including Parkinson's disease.³⁵ This drug has recently been reported to impair the proliferation of neural progenitor cells in the hippocampus. Experimental exposure to exogenous methamphetamine decreased neurosphere cell proliferation, increasing the expression of the tumour suppressor p53 and the cell cycle inhibitor p21^{CIP1}. These were ameliorated by melatonin. These findings suggested that melatonin might prevent learning and memory impairments associated with methamphetamine exposure, possibly through rectifying NSC proliferative defects, which in turn restore neurogenesis.

5 | CONCLUSION AND FUTURE PERSPECTIVES

Increasing studies have addressed the roles of melatonin on NSCs and highlighted its multiple protective roles in NSCs through promoting cellular survival, proliferation and neural differentiation. Functionally, melatonin prevents neural cells from potentially harmful insults such as hypoxia, inflammation and excessive glucocorticoids. These enable melatonin to be used as an adjunct for NSC grafting and facilitate neural differentiation as appropriate. Further investigations are required to elucidate the currently unknown molecular mechanisms in order to provide a more precise picture of the regulatory networks (Figure 1). Hopefully, this will lead to discovery of potential therapeutic targets.

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