Research Article

Antinociceptive effect of melatonin in the animal model of Parkinson's Disease

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ABSTRACT

Several animal experimental and clinical studies have shown the effectiveness of melatonin in the treatment of some symptoms of Parkinson's disease (PD). However, the antinociceptive effect of melatonin against pain associated to PD has not been fully investigated. Thus, the present study investigated the possible antiallodynic and antinociceptive effects of acute and chronic melatonin treatments in Parkinsonian model of rats. This model was created by unilateral injection of 6-hydroxydopamine (6-OHDA) into the left medial forebrain bundle (MFB). The electronic von Frey test was used to analyze the antiallodynic effect of melatonin on this PD animal model. In addition, c-Fos immunostaining was also used as a marker of nociception to evaluate the neuronal activity related to the nociception processing. The results showed that unilateral injection of 6-OHDA induced a significant decrease in paw withdrawal threshold in both ipsilateral and contralateral paws, which indicate mechanical allodynia induction. This allodynia was transitorily reversed by apomorphine as a dopamine agonist. Melatonin treatment significantly increased threshold of allodynia. Melatonin administration of both acutely or chronically significantly downregulated the c-Fos expression of neurons in 6-OHDA treated animals. In conclusion, 6-OHDA treatment can induces a bilateral mechanical hypernociception in rats while melatonin treatment produces profound antinociceptive effect. This finding paves the way to use melatonin as an antinociceptive agent for PD clinically.

Keywords: 6-hydroxydopamine, allodynia, melatonin, nociception, pain, Parkinson disease.

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1. INTRODUCTION

Parkinson's disease (PD), one amongst the foremost frequent neurodegenerative disorders, is characterized by the progressive loss of dopaminergic (DA) neurons within the locus niger pars compacta (SNc), resulting in striatal dopamine depletion. The degeneration of dopaminergic nigrostriatal system is basically accountable for the classical motor signs, including resting tremor, muscle rigidity, and bradykinesia (1, 2). The pain process involved in the nigrostriatal pathway, as the non-motor symptom of PD, has been suggested. This indicates that the degeneration of this pathway can lead to a genesis of pain (3). Although it is often

underestimated, frequency of the pain can reach as high as 40-85% in the PD. Several categories of the pain have been classified. These include nociceptive pain involving in visceral and musculoskeletal, radicular or neuropathic pain, and primary central Parkinsonian pain (4). In humans and rodents, the perception of the pain is dependent on the circadian rhythm and that the threshold of pain perception decreases significantly during the dark phase (5, 6).These observations were attributed to high melatonin levels occurring at night and its possible analgesic effects (7).Melatonin, (N-acetyl-5-methoxytryptamine), a molecule secreted mainly by the pineal gland in vertebrates, has been suggested to plays an important role in the regulation of pain under physiological conditions (8). It has been reported that melatonin influences pain perception (9) and improves pain symptoms observed in fibromyalgia, suggesting that this molecule represents an alternative treatment for these patients (10).

It has been reported that the level of melatonin is lower in patients of PD than their age matched normal counterparts (11) and that the decreased melatonin levels are correlated with disease severity and hypothalamic gray matter volume in patients od PD (12). Since melatonin is a powerful free-radical scavenger and a naturally occurring anti-oxidant defense stimulator, potent anti-apoptotic agent, and a modulator of xenobiotic metabolic enzymes; therefore, it may ameliorate the symptomatic features of PD (13-16). Melatonin has been reported to improve sleep behavior (17) as well as non-motor disorders in patients of PD (18).

Although several experimental animal and clinical studies have shown the effectiveness of melatonin in the treatment of some symptoms of PD, the antinociceptive effect of melatonin against pain associated to PD has not been fully investigated. Therefore, the aim of the present study is to assess the antinociceptive effect of melatonin in an animal model of PD which is developed by unilateral injection of 6-hydroxydopamine (6-OHDA) in the left medial forebrain bundle (MFB) to induce dopamine nigrostriatal pathway lesion.

2. MATERIAL AND METHODS

2.1. Chemicals.

Melatonin, 6-hydroxydopamine, desipramine and apomorphine were purchased from Sigma-Aldrich (St. Louis, MO, USA) and primary antibody anti-c-Fos (ab222699) as well as secondary antibody Goat anti-Rabbit (Alexa Fluor 488, green fluorescence, ab150077) were purchased from Abcam (United Kingdom).

2.2. Animals and experimental design.

Experiments were performed with two-month-old male *Wistar* rats (150 - 200g). The animals were housed according to the EEC 609/86 Directives regulating the welfare of experimental animals and experiments were approved by the local ethics committee of Institute of Biotechnology (University of Monastir, Tunisia). The approved protocol number was CER-SVS/ISBM022/2020. Animals were maintained in individual stainless-steel cages under controlled photoperiod (12:12 h light/dark schedule, lights on between 0600 and 1800 hours, fluorescent cool white bulbs, 100 lux of intensity at the level of cages) and temperature (22 ± 2 ◦C). They had access to a standard rodent laboratory diet and drinking water *ad libitum*.

During the procedures, all rats were pretreated with desipramine (25mg/kg, i.p), anesthetized with chloral hydrate (8ml/kg, i.p), then 6-OHDA or vehicle (0.2% ascorbic acid solution) was injected into the left MFB. Rats were randomly divided into five groups with 5 rats in each group. For the control group (A): animals only received vehicle injection. MLT group (B): animals were submitted to the same procedure but received melatonin treatment (60 mg/Kg, i.p.) only for 4 weeks including one week before 6-OHDA or vehicle injection. 6-

OHDA group (C): animals were stereotactically injected with 4µL 6-OHDA (2 µg/µL free based plus 2µL 0.2 % ascorbic acid dissolved in saline preventing heat and light exposure) and they served as a PD model group. 6-OHDA-MLT group (D): animal received 6-OHDA injection and then, was followed by melatonin treatment daily, which begins on the von Frey test day until their sacrifice. MLT-6OHDA-MLT group (E): Animals received a chronic melatonin treatment for 4 weeks, one week before 6-OHDA injection and 3 weeks after stereotaxic surgery. Animals were evaluated by using the cylinder test as a behavioral test after 2 weeks from surgery to characterize the PD induction in all groups. The mechanical nociceptive thresholds were evaluated using von Frey test 3 weeks after stereotaxic surgery. This test was repeated each 30 min and, at 110 min, apomorphine hydrochloride (0.2 mg/kg freebased) was subcutaneously injected to verify the implication of nigrostriatal dopaminergic pathway in pain processing. At day 24, animals were sacrificed. The brains and spinal cords were collected for immunofluorescence. The procedure details were illustrated in Figure 1.

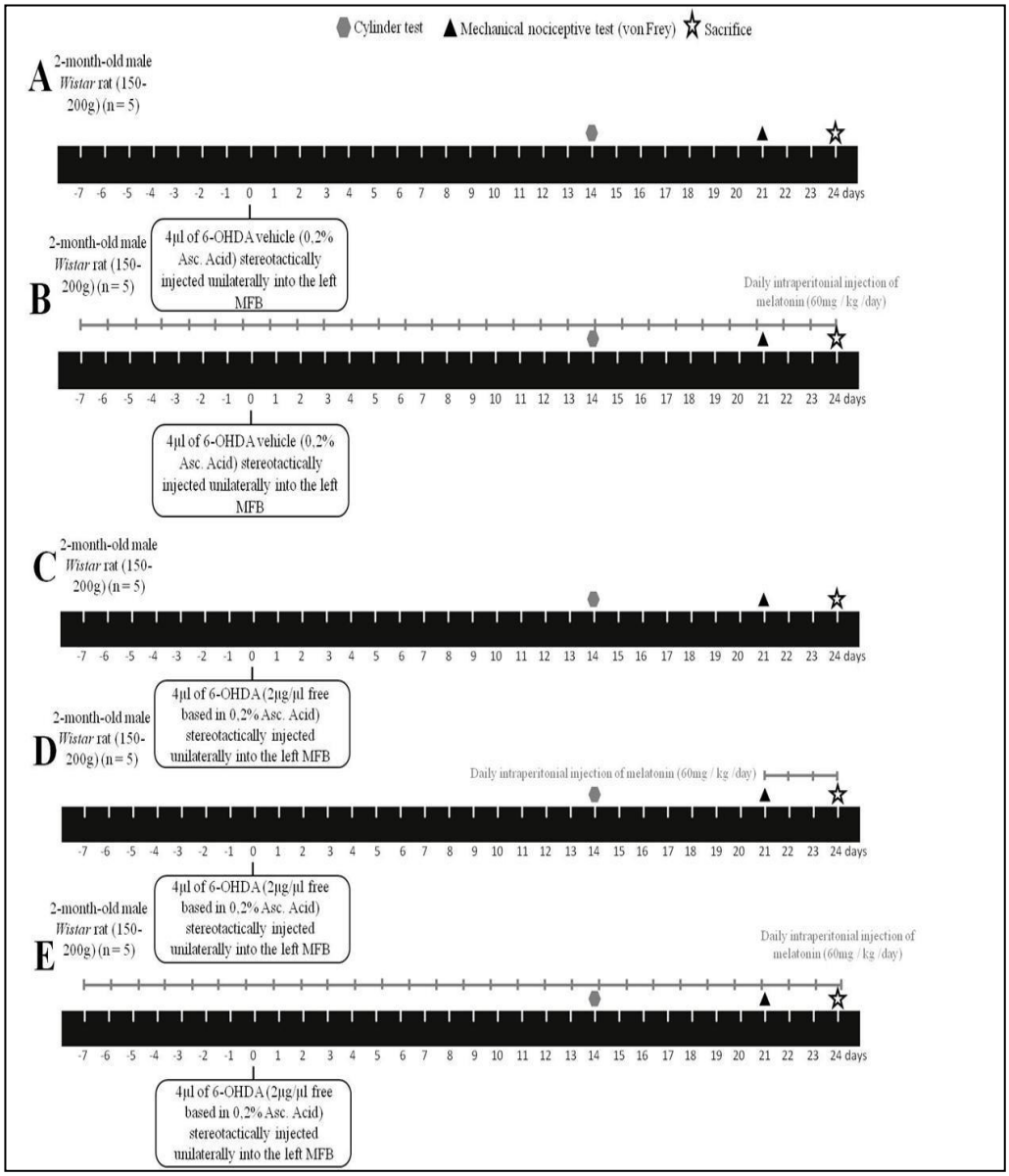

Fig. 1. Illustration of the experimental procedures and study groups.

6-OHDA: 6- hydroxydopamine, PD: Parkinson's disease, MFB: medial forebrain bundle, MLT: melatonin.

2.2.6-hydroxydopamine and melatonin injection.

2.2.1. 6-hydroxydopamine injection.

Desipramine was administered to rats at 25mg/kg by intraperitoneal injection 30 min prior to surgery in order to inhibit caption of 6-OHDA by noradrenergic neurons (19). Rats were then anaesthetized with chloral hydrate (8 mL/kg, i.p.) and placed in a stereotaxic apparatus. To achieve lesioning of the nigrostriatal pathway, 4 µL of 6-OHDA was stereotactically injected unilaterally into the left medial forebrain bundle (MFB) (anteroposterior: –4.3, mediolateral: +1.7 and dorsoventral: –8.2mm from bregma; according to Paxinos and Watson (20), using a 5-µL Hamilton Syringe (Sigma, St Louis, MO, USA).6-OHDA was injected at a rate of 0.5 µL/min (21), and the syringe was left in place for 5 min after injection before being drawn back to enable full absorption of the solution. Control and melatonin treated animals underwent the same surgical procedure as mentioned above with the injection of an equal volume of the vehicle only. After surgery, all animals were individually housed and monitored for the time of recovery from anesthesia.

2.2.1. Melatonin injection.

Melatonin was dissolved in 1% ethanol in saline (v/v) immediately before use and administered intraperitoneally at a dose of 60 mg/Kg. The manner and concentration of melatonin used in this work were chosen based on the literature data in which the antinociceptive effect of melatonin was proved (22-24).

2.3. Cylinder test.

Forelimb asymmetry was evaluated using the cylinder test after 2 weeks from surgery (25). Animals were placed for 10 min in a glass cylinder (diameter 19 cm, height 20 cm), with mirrors placed behind to allow for a 360° view of all touches. The number of touches on the cylinder wall with single right and left forepaw during their standing on rear limbs was recorded for all the groups. Only animals with statistically significant preference for the left forepaw and with an asymmetry percentage > 70%, were counted among animals with Parkinsonism and then those animals were included in 6-OHDA, 6-OHDA-MLT and MLT-6-OHDA-MLT groups.

2.4. Measurements of mechanical nociceptive threshold with von Frey test.

Mechanical allodynia was assessed as described by Thibault *et al*. (26). Three weeks after the stereotaxic surgery, rats were individually placed on an elevated wire mesh floor in a clear plastic box and were adapted to the testing environment for 10 min. An Electronic von Frey unit (EVF-4, Bioseb, Chaville, France) was used. The sensitivity threshold is measured in one test, measurement ranging from 0.1 to 100 g with a 0.2 g accuracy. Punctuate stimulus is delivered to the mid-plantar area of each hind paw from below the mesh floor through a plastic spring tip and the sensibility of threshold is displayed on a screen. Paw sensitivity threshold was defined as the minimum pressure required eliciting a robust and immediate withdrawal reflex of the paw. Voluntary movement associated with locomotion was not taken account as a withdrawal response. The stimulus was applied on each hind paw two times with a five seconds interval and the value adopted as a threshold for a rat was the average of the four values measured. Measurement of the nociception threshold was assessed during 145 min as indicated in Figure 2. To verify whether nigrostriatal dopaminergic pathway is involved in pain

processing, we performed the restoration experiment with apomorphine. For this reason, mechanical allodynia was also assessed after 5 min of subcutaneously injection of apomorphine hydrochloride (0.2 mg/kg freebased) or saline (27).

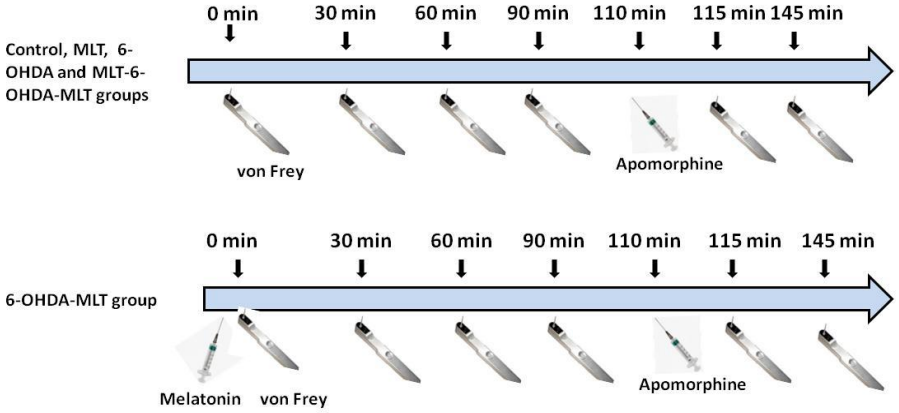

Fig. 2. A diagram of the von Frey determination of the nociception threshold.

3 weeks after stereotaxic injection of 6-OHDA or vehicle in the different groups. Melatonin (MLT) was given to test its acute antinociceptive effect. Nociception threshold was also assessed after 5 min of subcutaneously injection of apomorphine hydrochloride (0.2 mg/kg free based) to verify whether nigrostriatal dopaminergic pathway is implicated in pain processing. The nociception threshold was evaluated in ipsilateral (IL) and contralateral (CL) paw for each animal.

2.5. Immunofluorescence.

Three days after von Frey Test, all rats were scarified after deep anesthetization with chloral hydrate (24 mL/kg, i.p.). Their brains and the lumbar region (L2-L3-L4-L5) of the spinal cords were isolated. Spinal cords isolation was carried out using the hydraulic extrusion method as described by Richner *et al*. (28). Samples were immediately fixed in formol (10%) for 2 days and, then were dehydrated in ascending grades of alcohol, embedded in paraffin, and cut into 7 µm thick sections. Tissues sections were deparaffinized, rehydrated in grade alcohol and washed. To obtain more efficient immunostaining, tissue sections were subjected to an antigen retrieval procedure (29) and incubated for 30 min with 1% Triton X-100 to permeabilize the membrane. Then, non-specific binding sites were blocked with an appropriate normal goat serum diluted 1:5 in PBS containing 5% BSA. Later, spinal cord sections were incubated with monoclonal rabbit anti-c-Fos (diluted 1:1000; Sigma) overnight at 4°C. After washing in PBS, slides were incubated for 1 h with the appropriate secondary antibody Goat anti-Rabbit (Alexa Fluor 488, green fluorescence) diluted 1:500. Pictures of all stained sections were observed with an optical microscope (Axiostar plus Zeiss) and images were viewed and saved using OPTIKA MICROSCOP ITALY C-B5 camera and PROVIEW software (x64 version). Quantification of GFAP and c-Fos fluorescence was performed by image analysis software (ImageJ v 1.52).

2.6. Statistical Analysis.

Data were expressed as mean ± standard error of the mean (SEM). Differences were considered significant when $p < 0.05$. Statistical analysis was completed by one-way ANOVA, followed by Tukey's post hoc t-test where appropriate with Prism 5.0, GraphPad Software (San Diego, CA, USA).

3. RESULTS

3.1. Effect of 6-OHDA injection into the left MFB of rat on body weight.

There were no significant differences as to the body weight gain among all groups during the experimental period. All rats gained their weight normally after the injury (Figure3). In addition, no animals died during the experimental period.

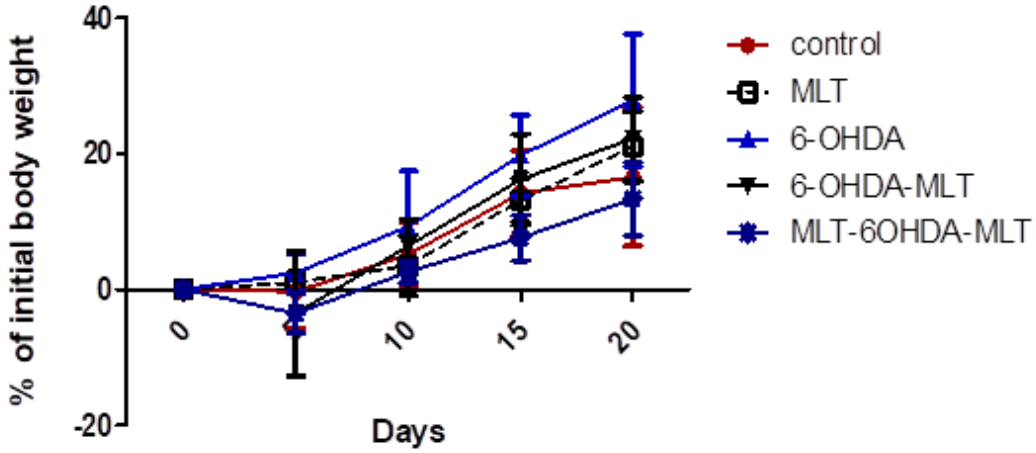

Fig. 3: Effect of 6-OHDA treatment on body weight of rats.

Body weight was expressed as % of initial body weight after injection of OHDA 6-OHDA. Values are expressed as the mean ± SEM for 5 animals in each group. MLT: melatonin, 6-OHDA: 6-hydroxydopamine.

3.2. Cylinder test.

Animals injected with vehicle (Control and MLT groups) used their forelimbs normally. The 6-OHDA treatment was effectively induced nigrostrial lesion in the left hemisphere. Thus, the rats showed a significant impairment in the use of both forelimbs indicated by the significant increase ($P < 0.05$) of the asymmetry percentage compared with control group (Figure 3). Animals of MLT-6-OHD-MLT group showed a significant decrease in asymmetry movement percentage compared with 6-OHDA group. 6-OHDA-MLT group also decreased this asymmetry percentage compared to the control, but this decrease did not achieve significant difference (Figure 4).

3.3. Effect of 6-OHDA and melatonin on mechanical nociceptive thresholds.

Unilateral injection of 6-OHDA in the left MFB of rats provoked a significant decrease ($P < 0.01$) in paw withdrawal threshold in both ipsilateral and contralateral paws when compared with control group. (Figure 5). Reduced paw withdrawal threshold indicates mechanical allodynia. Apomorphine administration fully abrogated the reduced paw withdrawal thresholdat115 min.

Acute melatonin administration in 6-OHDA-MLT group significantly enhanced paw withdrawal threshold in both ipsilateral and contralateral paws at 60 min after its administration compared to 6-OHDA group. Actually, paw withdrawal threshold in 6-OHDA-MLT group was recovered to the normal level as in the control group. Unexpectedly, paw withdrawal threshold

in melatonin administration one week before 6-OHDA injection group (MLT-6-OHDA-MLT) exhibited no significant difference compared to 6-OHDA group (Figure 5).

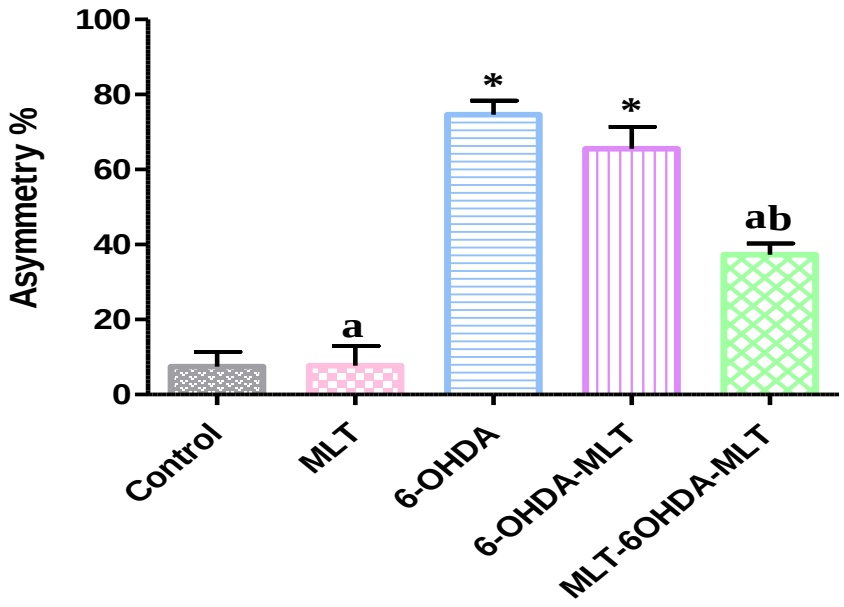

Fig. 4. Effects of 6-OHDA and melatonin on asymmetry percentage of use of both forelimbs in the cylinder test.

*Values are expressed as the mean ± SEM for 5 animals in each group. *: p < 0.05 compared to control group. a: p < 0.05 compared to 6-OHDA group.*
b: p < 0.05 compared to 6-OHDA-MLT group.

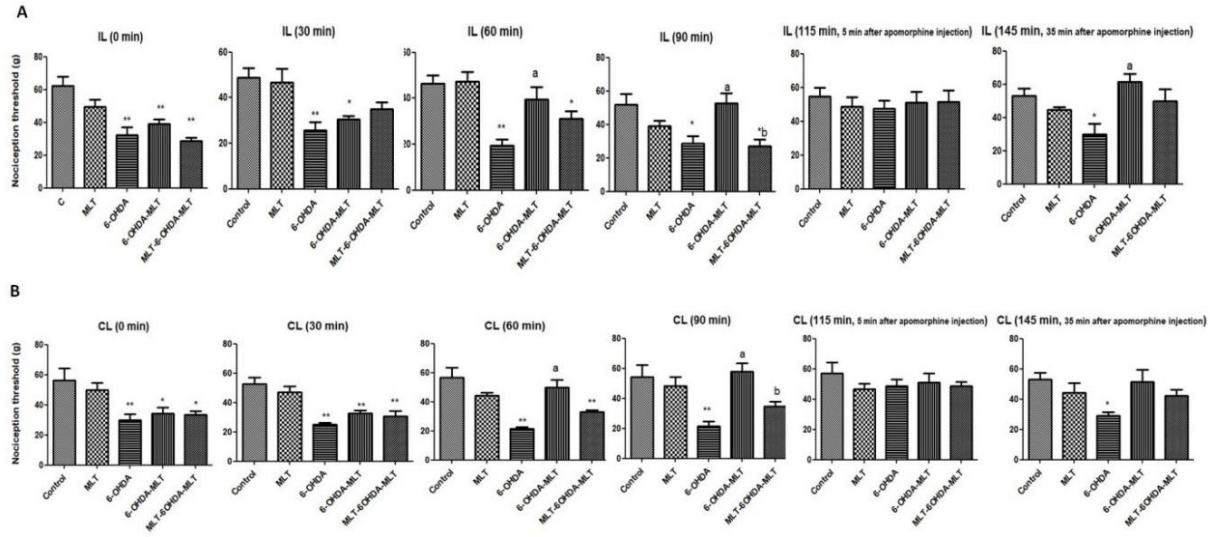

Fig. 5. Effects of 6-OHDA and melatonin on mechanical allodynia threshold.

*Values are expressed as the mean ± SEM for 5 animals in each group. *: p < 0.05; **: p < 0.01 compared to C group. a: p < 0.05 compared to 6-OHDA group. b: p < 0.05 compared to 6-OHDA-MLT group.*

3.5. Effect of 6-OHDA and melatonin on c-Fos expression.

6-OHDA treatment significantly reduced the c-Fos-immunostaining at the dorsal horn of the spinal cord compared to the control and MLT groups ($P < 0.05$; Figure 6 C, F). However, acute (OHDA-MLT) or chronic MLT-6-OHDA-MLT) melatonin administration significantly upregulated the c-Fos expression compared with 6-OHDA group ($P < 0.05$; Fig. 6 D, F).

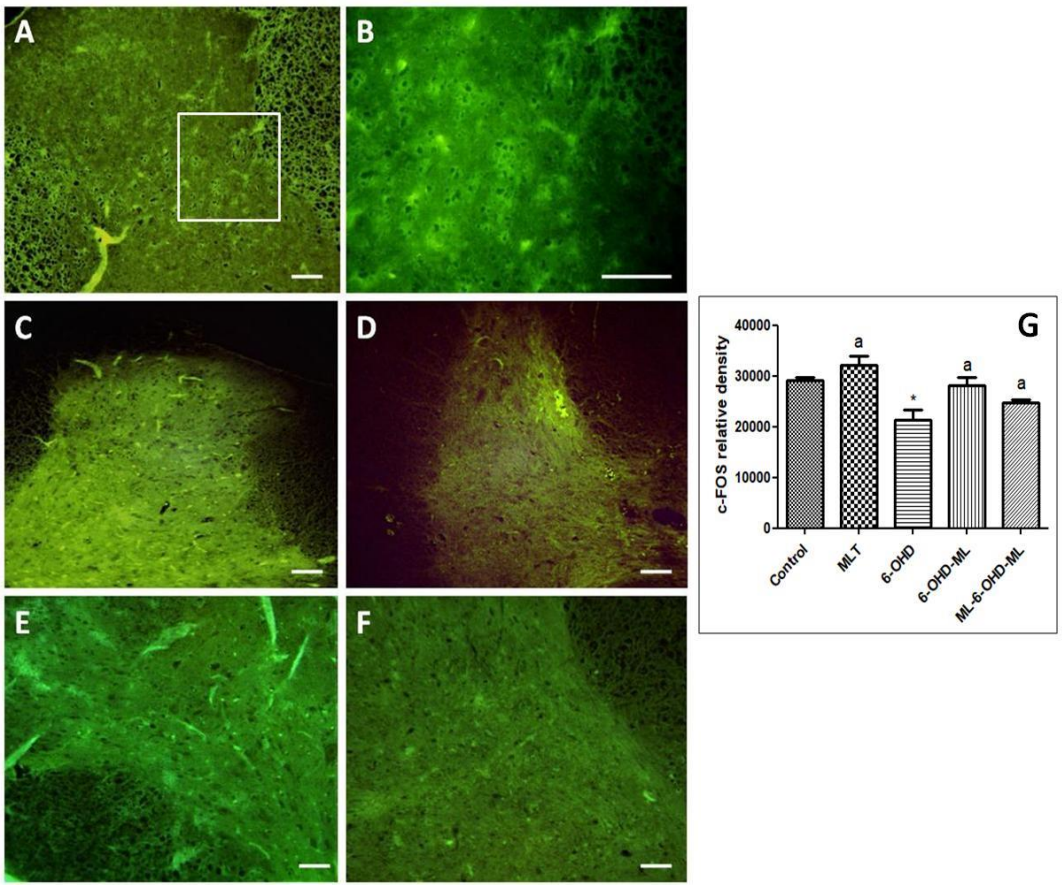

Fig. 6. **Effect of 6-OHDA and melatonin on c-Fos expression**.

*A-F: the representative c-Fos immunostaining in the dorsal horn of the spinal cord. A and B: control group; C: MLT group; D: 6-OHDA group; E: 6-OHDA-MLT group; F: MLT-6-OHDA-MLT group and G: Fluorescence intensity of the c-Fos immunostainings. (N = 5). *: $p < 0.05$ compared to control group, a: $p < 0.05$ compared to 6-OHDA group. Scale bars represent 20 μm.*

4. DISCUSSION

Recently, several studies have reported the efficacy of melatonin in treating various pain symptoms. However, in our knowledge, its antinociceptive activity has not been tested in the case of nociception associated with the PD. Thus, the present study was conducted to test the potential antinociceptive and antiallodynic effect of melatonin. The PD model was obtained by unilateral 6-OHDA lesion into the left MFB of the rats and melatonin was given either acutely or chronically. The result showed that 6-OHDA-treated rats exhibited more sensitive bilateral nociceptive behavior to the mechanical stimulation. This result is consistent with previous reports (30-32). The nociceptive threshold reduction was reversed by systemic administration of apomorphine, as dopaminergic agonist, which is consistent with previous data showing that

dopamine replacement improves pain behavior in rat PD models (33-35). These data provide evidence for the involvement of the dopaminergic nigrostriatal pathway in the modulation of nociceptive information. However, the pathways by which the basal ganglia exert their modulatory influence on nociceptive information are still unclear. It has been reported that dopamine receptors, especially D2, in the striatum are involved in modulation of persistent nociception (32, 36). D2-like receptor agonists are a powerful clinical option as an alternative to L-DOPA, especially in the early stages of the disease, being associated to a reduced risk of dyskinesia development. However, continuous dopaminergic stimulation of striatal dopamine receptors can result in changes within neurotransmitter signaling pathways and nociception modulation in the basal ganglia circuitry (37). Thus, PD patients should be offered more safe treatments rather than D2R agonist only. In humans, melatonin, for example, has a high safety profile and it is usually remarkably well-tolerated. In addition, high doses of melatonin have been used in various pathologies without undesirable sequelae (38).

In the current study, we observed that chronic melatonin treatment (MLT-6OHDA-MLT group) significantly reduced the asymmetry percentage of forelimb movement, which indicated a decrease in nigrostrial lesion. The results suggest that melatonin protects dopaminergic neurons against 6OHDA toxicity. Accordingly, multiple studies have demonstrated the neuroprotective effects of melatonin at the molecular, cellular, and tissue level in animal models and in human trials related to neurodegenerative diseases (7, 9, 12). Reduction of inflammatory mediators, apoptosis, and unbalance of redox state are involved in the neuroprotection of melatonin (39-41). Our findings also showed that acute melatonin treatment was able to significantly increase the paw withdrawal thresholds which was lowered by 6-OHDA administration. To the best of our knowledge, this seems to be the first report demonstrating the antiallodynic effect of melatonin in animal PD model. Accordingly, previous study showed that, at dose of 60 mg/kg, melatonin exhibited antiallodynic effect in rats with neuropathic pain and this effect occurred at 30 minutes through 240 minutes after its administration (22). Using the same concentration of melatonin (60 mg/kg), several studies demonstrated that the analgesic action of melatonin occurs irrespective of route of administration in different pain models (23, 24).

It has been suggested that c-Fos protein expression can serve as a marker for neuronal activity in nociceptive processing. The intensity of expression of c-Fos indicates the amounts of neurons which are activated or excited by nociceptive input (42). In addition, a study has showed that Fos protein is recognized as a marker of pain in PD and its high expression indicates the allodynia (43). For all these reasons, we evaluated the expression of c-fos after 6-OHDA and/or melatonin treatment. 6-OHDA treatment significantly downregulated the c-Fos-expression. The results indicate that the unilateral lesion of the nigrostriatal pathway attenuates c-Fos expression in the dorsal horn of the spinal cord and suggest that dopamine plays a key role in the regulation of allodynia-induced neuronal activation via nociceptive input. These results are in agreement with those of Greco *et al*. (44) who showed that intrastriatal injection 6-OHDA induced nigrostriatal lesion and inhibited neuronal activation typically by nitroglycerin in sub-cortical areas involved in pain perception. The negative influence of dopaminergic circuitry damage, induced by 6-OHDA, on the c-Fos expression was also observed by Domenici *et al* (34). Our results also showed that acute or chronic melatonin administration preserved the c-Fos expression which, otherwise, downregulated by 6-OHDAin the dorsal horn of spinal cord.

The exact mechanisms of the antinociceptive action of melatonin remain to be clarified. However, Mantovani *et al*. have initially reported the presence of transcripts of melatonin receptors located in the dorsal and ventral horn of the spinal cord (45). Most importantly, the involvement of these receptors in the spinal cord has been well documented as an antinociceptive mechanism in a number of animal models of pain perception (23). In this

context, experiments using melatonin receptor blocker are very important since presence of melatonin receptors in median forebrain bundle was also documented (44). Since melatonin can penetrate the blood–brain barrier due to its high lipid-solubility, Mantovani *et al*. (45) suggest that antinociceptive effect of melatonin may be mediated by activating supraspinal sites. A potential link of melatonin with the central opioidenergic system has been proposed to explain its antinociceptive effect (46-48). A variety of other mechanisms that melatonin acts on GABA receptors (49), b-endorphins (50), opioid 1-receptors (51, 52) and the NO-arginine pathway (53) have also been proposed.

In conclusion, our results showed that 6-OHDA treatment induced bilateral mechanical hypernociception in rats. This hypernociception was transitorily reversed by apomorphine as dopamine agonist indicating the dopaminergic neuronal damage. Melatonin effectively reduced the 6-OHDA induced nociception.The dopaminergic neuronal damage is charactered as the pathology of PD. Thus, this finding paves the way to use melatonin to improve nociception associated to the PD clinically. However, further studies are still needed to elucidate the precise mechanism through which melatonin exerts its antinociceptive action.

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AUTHORSHIP

Conceptualization: IM and KK; Data curation, TK and AS; Formal analysis: IM Investigation: TK, AS, SM and MBR; Methodology: SM, LK and SB; Project administration: IM; Resources IM; Supervision: KK and IM; Validation: LK and KK; Visualization: IM;Writing – original draft: TK and AS; Writing – review & editing: SB, LK and IM. All authors declare that all data were generated in-house and that no paper mill was used.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Fearnley JM, Lees AJ (1991) Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain* **114**: 2283–2301. https://doi.org/10.1093/brain/114.5.2283.
2. Olanow CW, Tatton WG (1999) Etiology and pathogenesis of Parkinson's disease. *Annu. Rev. Neurosci.* **22**: 123–144. https://doi.org/10.1146/annurev.neuro.22.1.123.
3. Zengin-Toktas Y, Ferrier J, Durif F, Llorca PM, Authier N (2013) Bilateral lesions of the nigrostriatal pathways are associated with chronic mechanical pain hypersensitivity in rats. *Neurosci. Res*. **76**: 261–264. https://doi.org/10.1016/j.neures.2013.05.003.
4. Truini A, Frontoni M, Cruccu G (2013). Parkinson's disease-related pain: a review of recent findings. *J. Neurol.* **260**: 330–334. DOI 10.1007/s00415-012-6754-5.
5. Crockett, RS, Bornchein, RL, Smith RP (1977). Diurnal variations in response to thermal stimulation: mouse-hot plate test. *Physiol. Behav*. **18** (2): 193-196. https://doi: 10.1016/0031-9384(77)90120-2.
6. Junker U, Wirz S (2010) Review article: Chronobiology: Influence of circadian rhythms on the therapy of severe pain. *J. Oncol. Pharm. Pract.* **16**: 81–87.

https://doi.org/10.1177/1078155209337665.
7. Laikin MI, Miller CH, Stott ML, Winters WD (1981) Involvement of the pineal gland and melatonin in murine analgesia. *Life Sci.* **29**: 2543–2551. https://doi.org/10.1016/0024-3205(81)90710-4.
8. Pickard GE (1987) Circadian rhythm of nociception in the golden hamster. *Brain Res.* **425**: 395–400. https://doi.org/10.1016/0006-8993(87)90529-4.
9. Srinivasan V, Pandi-Perumal SR, Spence DW, Moscovitch A, Trakht I, Brown GM, Cardinali DP (2010) Potential use of melatonergic drugs in analgesia: Mechanisms of action. *Brain Res. Bull.* **81**: 362–371. https://doi.org/10.1016/j.brainresbull.2009.12.001.
10. Citera G, Arias MA, Maldonado-Cocco JA *et al* (2000). The effect of melatonin in patients with fibromyalgia: a pilot study. *Clin. Rheumatol.* **19**: 9–13. https://doi.org/10.1007/s100670050003.
11. Srinivasan V, Spence DW, Pandi-Perumal SR, Brown GM, Cardinali DP (2011) Melatonin in mitochondrial dysfunction and related disorders. *Int. J. Alzheimers Dis.* **2011**: 326320. https://doi.org/10.4061/2011/326320.
12. Breen DP, Nombela C, Vuono R, Jones PS, Fisher K, Burn DJ, Brooks DJ, Reddy AB, Rowe JB, Barker RA (2016) Hypothalamic volume loss is associated with reduced melatonin output in Parkinson's disease. *Mov. Disord.* **31** (7): 1062–1066. https://doi.org/10.1002/mds.26592.
13. Leon J, Acuna-Castroviejo D, Sainz RM, Mayo JC, Tan DX, Reiter RJ (2004) Melatonin and mitochondrial function. *Life Sci.* **75**: 765–790. https://doi.org/10.1016/j.lfs.2004.03.003.
14. Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V, Reiter RJ (2004). Regulation of antioxidant enzymes: a significant role for melatonin. *J. Pineal Res.* **36**: 1–9. https://doi.org/10.1046/j.1600-079X.2003.00092.x.
15. Galano A, Tan DX, Reiter RJ (2011) Melatonin as a natural ally against oxidative stress: a physicochemical examination. *J. Pineal Res.* **51**: 1–16. https://doi.org/10.1111/j.1600-079X.2011.00916.x.
16. Singhal NK, Srivastava G, Patel DK, Jain SK, Singh MP (2011) Melatonin or silymarin reduces maneb- and paraquat-induced Parkinson's disease phenotype in the mouse. *J. Pineal Res.* **50**: 97–109. https://doi.org/10.1111/j.1600-079X.2010.00819.x.
17. Kunz D, Mahlberg R (2010). A two-part, double-blind, placebo-controlled trial of exogenous melatonin in REM sleep behaviour disorder. *J. Sleep Res.***19** (4): 591–596. https://doi.org/10.1111/j.1365-2869.2010.00848.x
18. Dowling GA, Mastick J, Colling E, Carter JH, Singer CM, Aminoff MJ (2005). Melatonin for sleep disturbances in Parkinson's disease. *Sleep Med.* **6** (5): 459–466. https://doi.org/10.1016/j.sleep.2005.04.004.
19. Kelly PH, Iversen SD (1976) Selective 6OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psycho-stimulant-induced locomotor activity in rats. *Eur. J. Pharmacol*. **40**: 45–56. https://doi.org/10.1016/0014-2999(76)90352-6.
20. Paxinos G, Watson C (2005) The rat brain in stereotaxic coordinates. Compact 6th Edition, Academic Press. New York 400.
21. Czarnecka A, Tomasz L, Domin H, Konieczny J, Smiałowska M, Lorenc-Kocia E (2013) Alterations in the expression of nNOS in the substantia nigra and subthalamic nucleus of 6-OHDA-lesioned rats: The effects of chronic treatment with L-DOPA and the nitric oxide donor, molsidomine. *Brain Res.* **1541**: 92–105. https://doi.org/10.1016/j.brainres.2013.10.011.
22. Wu FS, Yang YC, Tsai JJ (2000) Non competitive inhibition of the glycine receptor-mediated current by melatonin in cultured neurons. *Brain Res***. 881**: 208–211. https://doi.org/10.1016/S0006-8993(00)02804-3.

23. Mickle A, Sood M, Zhang Z, Shahmohammadi G, Sengupta JN, Miranda A (2010) Antinociceptive effects of melatonin in a rat model of post inflammatory visceral hyperalgesia: a centrally mediated process. *Pain* **149**: 555–564. doi:10.1016/j.pain.2010.03.030.
24. Laste G, Macedo IC, Rozisky JR, da Silva FR, Iraci WC Torres LS (2012) Melatonin administration reduces inflammatory pain in rats. *J. Pain Res.* **5**: 359–362. https://doi.org/10.2147/JPR.S34019.
25. Schallert T, Fleming SM, Leasure JL, Tillerson JL, Bland ST (2000) CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, Parkinsonism and spinal cord injury. *Neuropharmacology* **39**: 777–787. https://doi.org/10.1016/S0028-3908(00)00005-8.
26. Thibault K, Elisabeth B, Sophie D, Claude FZ, Bernard R *et al*. (2008) Antinociceptive and anti-allodynic effects of oral PL37, a complete inhibitor of enkephalin-catabolizing enzymes, in a rat model of peripheral neuropathic pain induced by vincristine. *Eur. J. Pharmacol.* **600**: 71–77. https://doi.org/10.1016/j.ejphar.2008.10.004.
27. Park J, Lim CS, Seo H, Park CA, Zhuo M *et al*. (2015) Pain perception in acute model mice of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Mol. Pain* **17**: 11–28. https://doi.org/10.1186/s12990-015-0026-1.
28. Richner M, Jager SB, Siupka P, Vaegter CB (2017) Hydraulic extrusion of the spinal cord and isolation of dorsal root ganglia in rodents. *J. Vis. Exp.* **119**: e55226. https://doi.org/10.3791/55226.
29. Shi YB, Gelman BG, Lisinicchia J, Tang S.J. (2012) Chronic-pain-associated astrocytic reaction in the spinal cord dorsal horn of human immunodeficiency virus-infected patients. *J. Neurosci.* **32** (32): 10833–10840. https://doi.org/10.1523/JNEUROSCI.5628-11.2012.
30. Carey RJ (1986) Acute ipsilateral hyperalgesia and chronic contralateral hypoalgesia after unilateral 6-hydroxydopamine lesions of the substantia nigra. *Exp. Neurol.* **91**: 277–284.
31. Saade NF, Atweh SF, Bahuth NB, Jabbur SJ, (1997) Augmentation of nociceptive lesions of either dopaminergic terminals or midbrain dopaminergic neurons. *Brain Res.* **751**: 1–12.
32. Chudler EH, Lu Y (2008) Nociceptive behavioral responses to chemical, thermal and mechanical stimulation after unilateral intrastriatal administration of 6-hydroxydopamine. *Brain Res.* **1213**: 41–47. https://doi.org/10.1016/j.brainres.2008.03.053.
33. Carta M, Carlsson T, Kirik D, Bjorklund A (2007) Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. *Brain* **130**: 1819–1833. https://doi.org/10.1093/brain/awm082.
34. Domenici RA, Campos ACP, Maciel ST, Berzuino MB, Hernandes MS, Erich T, Fonoff ET, Pagano RL (2019) Parkinson's disease and pain: Modulation of nociceptive circuitry in a rat model of nigrostriatal lesion. *Exp. Neurol.* **315**: 72–81. https://doi.org/10.1016/j.expneurol.2019.02.007.
35. Saidi N, Nsibi A, Mani S, Saoud H, Messaoudi I (2019) Unilateral 6-hydroxydopamine-lesioned rat as relevant model to study the pain related to parkinson's disease. *Neurol. Neurobiol.* **1**: 2613-7828. http://dx.doi.org/10.31487/j.NNB.2019.04.03.
36. Magnusson JE, Fisher K (2000) The involvement of dopamine in nociception: the role of D1 and D2 receptors in the dorsolateral striatum. *Brain Res.* **855**: 260–266. https://doi.org/10.1016/S0006-8993(99)02396-3.
37. Rascol O, Lozano A, Stern M, Poewe W (2011) Milestones in Parkinson's disease therapeutics. *Mov. Disord.* **26**: 1072–1082. https://doi.org/10.1002/mds.23714.
38. Pérez-Lloret S, Cardinali DP (2021) Melatonin as a chronobiotic and cytoprotective agent in Parkinson's Disease. *Front. Paharmacol.* **12**: 650597. doi: 10.3389/fphar.2021.650597.
39. Lahiri DK (1999) Melatonin affects the metabolism of the *β*- amyloid precursor protein in different cell types. *J. Pineal Res.* **26**: 137–146. https://doi.org/10.1111/j.1600-

079X.1999.tb00575.x.
40. Schapira AHV, Olanow CW (2004) Neuroprotection in Parkinson disease: mysteries, myths, and misconceptions. *J. Americ. Medi. Assoc.* **291**: 358–364.
41. Park S, Byeon Y, Lee HY, Kim YS, Ahn T, Back K (2014) Cloning and characterization of a serotonin Nacetyltransferase from a gymnosperm, loblolly pine (*Pinus taeda*). *J. Pineal Res.* **57**: 348–355. https://doi.org/10.1111/jpi.12174.
42. Coggeshall RE (2005) Fos, nociception and the dorsal horn. *Prog. Neurobiol*. **77**: 299–352.
43. Crockett RS, Bornchein RL, Smith RP (1977) Diurnal variations in response to thermal stimulation: mouse-hot plate test. *Physiol. Behav.* **18**: 193–196. https://doi.org/10.1016/j.pneurobio.2005.11.002.
44. Harris JA (1998) Using c-fos as a neural marker of pain. *Brain Res. Bull.* **45** (1): 1–8. https://doi.org/10.1016/S0361-9230(97)00277-3.
45. Greco R, Tassorelli C, Armentero MT, Sandrini G, Nappi G, Blandini F (2008) Role of central dopaminergic circuitry in pain processing and nitroglycerin-induced hyperalgesia. *Brain Res.* **1238**: 215–223. doi:10.1016/j.brainres.2008.08.022.
46. Mantovani M, Kaster PM, Pertile R, Calixto JB *et al.* (2006) Mechanisms involved in the antinociception caused by melatonin in mice. *J. Pineal Res.* **41**: 382–389 Doi:10.1111/j.1600-079X.2006.00380.x.
47. Yu CX, Zhu CB, Xu SF *et al*. (2000) Selective MT2 melatonin receptor antagonist blocks melatonin-induced antinociception in rats. *Neurosci. Lett.* **282**: 161–164. https://doi.org/10.1016/S0304-3940(00)00883-1.
48. Yu CX, Zhu B, Xu SF, Cao XD, Wu GC (2000) The analgesic effects of peripheral and central administration of melatonin in rats. *Eur. J. Pharmacol.* **403**: 49–53.
49. Mantovani M, Pertile R, Calixto JB, Santos AR, Rodrigues AL (2003) Melatonin exerts an antidepressant like effect in the tail suspension test in mice: evidence for involvement of N-methyl-D-aspartate receptors and the L-arginine-nitric oxide pathway. *Neurosci. Lett.* **343**: 1–4. https://doi.org/10.1016/S0304-3940(03)00306-9.
50. Golombek DA, Pevet P, Cardinali DP (1996) Melatonin effects on behavior: possible mediation by the central GABAergic system. *Neurosci. Biobehav. Rev.* **20**: 403–412. https://doi.org/10.1016/0149-7634(95)00052-6.
51. Wang T, Li SR, Dai X, Peng YL, Chen Q, Wang R (2006) Effects of melatonin on orphanin FQ/nociceptin-induced hyperalgesia in mice. *Brain Res.* **1085**: 43–48. https://doi.org/10.1016/j.brainres.2006.02.006.
52. Li SR, Wang T, Wang R, Dai X, Chen Q, Li RD (2005) Melatonin enhances antinociceptive effects of delta-, but not mu-opioid agonist in mice. *Brain Res.* **1043**: 132–138. https://doi.org/10.1016/j.brainres.2005.02.067.
53. Ulugol A, Dokmeci D, Guray G, Sapolyo N, Ozyigit F, Tamer M (2006) Antihyperalgesic, but not antiallodynic, effect of melatonin in nerve-injured neuropathic mice: possible involvements of the L-arginine-NO pathway and opioid system. *Life Sci.* **78**: 1592–1597. https://doi.org/10.1016/j.lfs.2005.07.002.

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