ORIGINAL ARTICLE

Medical Journal of Western Black Sea
Batı Karadeniz Tıp Dergisi

Med J West Black Sea 2023;7(2): 225-233
DOI: 10.29058/mjwbs.1342979

Melatonin Improves Postoperative Cognitive Dysfunction in Aged Rats: Relevance of Oxidative Stress, PSD95 and Ca2+/Calmoduline Dependent Protein Kinase

Melatonin Yaşlı Sıçanlarda Postoperatif Kognitif Disfonksiyonu İyileştirir: Oksidatif Stres, PSD95 ve Ca2+/Kalmodulin Bağımlı Protein Kinazın Önemi

İnci TURAN, Veysel Haktan ÖZAÇMAK, Hale SAYAN ÖZAÇMAK

Zonguldak Bülent Ecevit University Faculty of Medicine, Department of Physiology, Zonguldak, Turkey

ORCID ID: İnci Turan 0000-0003-2211-3914, Veysel Haktan Özaçmak 0000-0003-2651-8353, Hale Sayan Özaçmak 0000-0002-3564-0468

Cite this article as: Turan İ et al. Melatonin improves postoperative cognitive dysfunction in aged rats: relevance of oxidative stress, PSD95 and Ca2+/calmoduline dependent protein kinase. Med J West Black Sea. 2023;7(2):225-233.

Corresponding Author
İnci Turan

E-mail
dr.incituran@gmail.com

Received
14.08.2023

Revision
18.08.2023

Accepted
22.08.2023

ABSTRACT

Aim: Postoperative cognitive dysfunction (POCD) is usually seen in the aged patients in postoperative period. In the present study, we aimed to investigate the effects of melatonin on cognitive and synaptic dysfunction and oxidative stress in POCD model constructed by abdominal surgery in aged rats.

Material and Methods: Twenty months old male Wistar rats were randomly allocated into the control group, melatonin treated-control group, surgery group and melatonin treated-surgery group. The novel object recognition test (NORT) was used to assess the postoperative cognitive ability of rats at the end of the experiments. The contents of malondialdehyde (MDA) and reduced glutathione (GSH) were evaluated in the synaptosomes of hippocampus by spectrophotometric methods. The protein levels of calcium-calmodulin dependent protein kinase II (CaMKII) and postsynaptic density protein 95 (PSD95) were measured by using ELISA. .

Results: Animals in surgery group showed significant an impairment of novel object recognition memory in NORT. Surgery group also exhibited increased content of MDA (21.05±3.96) and decreased levels of GSH (5.86±0.26) and PSD95 (156.04±2.56) in the hippocampus (respectively p=0.012, p<0.001, p=0.004) . The levels of PSD95 (193.08±4.14) and CaMKII (2.82±0.25) (respectively p=0.027, p=0.041) and novel object recognition memory was improved by melatonin treatment.

Conclusion: Melatonin may be a potential therapeutic agent which can protect against abdominal surgery- induced cognitive dysfunction in elderly patients.

Keywords: Postoperative cognitive dysfunction, melatonin, oxidative stress, PSD95, CaMKII

ÖZ

Amaç: Postoperatif kognitif disfonksiyon (POCD) genellikle postoperatif dönemde yaşlı hastalarda görülmektedir. Bu çalışmada, yaşlı sıçanlarda abdominal cerrahi ile oluşturulan POCD modelinde melatoninin kognitif ve sinaptik disfonksiyon ve oksidatif stres üzerindeki etkilerini araştırmayı amaçladık.

Gereç ve Yöntemler: Yirmi aylık erkek Wistar sıçanlar rastgele olarak kontrol grubu, melatonin uygulanan-kontrol grubu, cerrahi grubu ve melatonin uygulanan-cerrahi grubuna ayrıldı. Deneylerin sonunda sıçanların ameliyat sonrası bilişsel yeteneklerini değerlendirmek için yeni obje tanıma testi (NORT) kullanıldı. Hipokampüs sinaptozomlarında malondialdehit (MDA) ve indirgenmiş glutatyon (GSH) içerikleri spektrofotometrik yöntemlerle ölçüldü. kalsiyum-kalmodulin bağımlı protein kinaz II (CaMKII) ve postsinaptik dansite protein-95 (PSD95) protein seviyeleri ELISA kullanılarak ölçüldü.

Bulgular: Cerrahi grubundaki hayvanlar NORT'ta yeni obje tanıma belleğinde önemli bir bozulma göstermiştir. Ayrıca cerrahi grubunda hipokampüste MDA içeriğinde (21,05±3,96) artış ve GSH

(5,86±0,26) ve PSD95 (156,04±2,56) seviyelerinde azalma görülmüştür (sırasıyla p=0,012, p<0,001, p=0,004). PSD95 (193,08±4,14) ve CaMKII (2,82±0,25) düzeyleri (sırasıyla p=0,027, p=0,041) ve yeni obje tanıma belleği melatonin uygulaması ile iyileşmiştir.

Sonuç: Melatonin, yaşlı hastalarda abdominal cerrahinin neden olduğu bilişsel işlev bozukluğuna karşı koruma sağlayabilecek potansiyel bir terapötik ajan olabilir.

Anahtar Sözcükler: Postoperatif kognitif disfonksiyon, melatonin, oksidatif stres, PSD95, CaMKII

INTRODUCTION

Postoperative cognitive dysfunction (POCD) is a neurological complication related to cognitive disorder observed especially in aged patients after anesthesia and major surgery (1,2). The International Postoperative Cognitive Dysfunction Study states that POCD morbidity in elderly patients is approximately 25.8 % within seven days following surgery and 10% within three months right after surgery. The risk for long term cognitive impairment after surgery has been proven to be relatively higher in patients older than 60 years old (3). Although the various pathological mechanisms for POCD have been suggested for the formation and development, such as neuroinflammation (4,5), oxidative stress (6), neuronal apoptosis, synaptic protein abnormalities, reduced BDNF level (7), and circadian disturbances (8), surgical trauma-induced neuronal damage eventually results in synaptic dysfunction; and thereby, leading to cognitive dysfunction (9).

Recent studies have reported that the aging and anesthesia can lead to the circadian rhythm disturbances in rats (8). Evidence suggest that the circadian and sleep-related parameters influence cognitive function with aging (9). For instance, a lack of synchronization between the sleep-wake cycle and melatonin secretion can lead to POCD (10). As recently reported, melatonin can be preventive from isoflurane-induced cognitive impairment by stimulating the circadian rhythm resynchronization in aged mice (11). However, studies have not yet fully elucidated the mechanisms responsible for the action of melatonin on the POCD. Melatonin acts as a scavenger of oxygen free radicals (OFRs) and also increases the antioxidative capacity of cell by increasing the expression of mRNAs for several antioxidant enzymes (12). Many studies have reported that melatonin modulates cognitive function by increasing the levels of synaptic proteins (12,13). It also enhances the synaptic integrity via improving the expression of stress-activated protein kinases, oxidative neuronal injury, neuroinflammation, and neurodegeneration (14). On the other hand, the effect of melatonin on POCD and the molecular mechanism underlying memory formation remains elusive. Also studies have shown that POCD may be associated with altered synaptic proteins including calcium-calmodulin dependent protein kinase II (CaMKII) and postsynaptic density protein 95 (PSD95) in the rat brain (9). Oxidative stress-induced neuronal damage is considered to be the main factor that plays a significant role in learning and memory impairments observed in various neurodegenerative diseases (15). Studies have indicated that the level of melatonin is lower in the postoperative first night together with a change in the secretion pattern compared to those observed in the preoperative period. Moreover, alterations in melatonin metabolism can end with the circadian rhythm disturbances, which are often observed in patients with postoperative delirium (16). It is reported that the circadian and sleep disorders can form the basis of cognitive dysfunction, both of which are affected by melatonergic function after major surgery (17).

In general, the POCD is related to the increased length of hospital stay and morbidity, delayed functional recovery, and higher healthcare expenditures. In spite of forming an important social and healthcare problem, there is presently no any choise of pharmacological remedy for the POCD. Therefore, the potential therapeutic agents are needed to be determined urgently to prevent and treat the POCD. In the present study, the effect of melatonin against abdominal surgery-induced cognitive deficit together with responsible molecular mechanisms was aimed to explore. To elucidate the possible underlying mechanism of melatonin in ameliorating memory impairment caused by abdominal surgery, the levels of CaMKII and PSD95 were measured.

MATERIAL and METHODS

Animals

Aged male Wistar Albino rats (20 months old) were used in the study. They were housed in pairs in a constant temperature and humidity under a 12:12 h light-dark cycle, with free access to food and water *ad libitum*. Before behavioral testing, all rats were habituated to the laboratory to decrease the effects of the novelty stress. All experimental procedures were approved by the Zonguldak Bulent Ecevit University Animal Care and Use Ethical Committee (ethical approval number: 2019-13-12/09).

Experimental Groups

The 32 male rats were randomly divided into the following groups: Group 1 consisted of the control group underwent to sham surgery and given daily saline. Group 2 included sham-operated rats treated with daily melatonin (10 mg/kg, i.p) for 7 days. Group 3 was surgery group. Group 4

consisted of rats subjected to abdominal surgery followed by the treatment with melatonin. Melatonin treatment was started right after surgery and lasted for 7 consecutive days (Figure 1). The dose of melatonin was chosen according to our previous studies. It was administered (i.p. route), at a dose of 10 mg/kg body weight between 16.00-17.00 (before the initiation of the dark phase) to elude perturbation of circadian cycle (18).

Surgery

Abdominal surgery was performed using general anesthesia (thiopental sodium, 60 mg/kg, i.p). Laparotomy and sham surgeries were done under aseptic conditions. In rats undergoing abdominal surgery, a vertical 3 cm incision was done approximately 0.5 cm beneath the right lower rib, and the incision extended into the peritoneal cavity. After the abdominal cavity was opened, the small intestine was taken out and gently compressed between the fingers using a gauze pad for 3 minutes. Incisions in the abdominal muscle and skin were closed with 5–0 silk suture, followed by induction of postoperative analgesia through diclorone injection. The duration of the surgical process was rigorously standardized at 10 min. Body temperature was continually observed with a rectal probe and preserved at 37 °C with a heating blanket. This procedure mimics major abdominal surgery in humans (19). The rats in Group 1 and Group 2 were only underwent abdominal skin incision.

Novel Object Recognition Test

On the seventh day following laporatomy, the novel object recognition test (NORT) was used to evaluate the cognitive function of the animals (20). This test is based on the spontaneous tendency of rodents to spend more time exploring a novel object than a familiar one. The NORT assesses recognition memory in rodents. Recognition memory is perturbated in several neurodegenerative diseases and NORT is widely used in rodents for investigating deficits in a diversity of animal models of human disease conditions where cognitive function is impaired (21). The experiment consisted of three phases: adaptation period, training period, and testing period. Each rat was placed into an empty test box (50 cm long × 50 cm wide × 60 cm high) for 5 min during the adaptation period. During the training period, each rat was shown two familiar objects for 5 minutes. The two objects have the same size, shape and odor. The time spent for exploring objects was documented by a camera. After a two-hour interval, a familiar object (F) and a novel object (N) were put in the test box, and then rats were permit to explore them. The testing period continued 5 min for each rat. In this period, the time spent for exploring two different objects was documented. Then, "discrimination index (DI)" was defined as (novel object exploration time–familiar object exploration time)/(novel object exploration time + familiar object exploration time). An increase in the time spent exploring the new object is considered as a successful recognition memory for familiar object against the new object (22).

Synaptosome preperation

Preperation of synaptosomes was based on the method of Turunc Bayrakdar et al (23). The hippocampal tissue were homogenized with 0.32 M sucrose solution (1:1, w/v). After centrifugation, pellets were resuspended; and then centrifuged for 10 min at 1000 g. Two supernantants were collected and centrifuged again at 17500 g for 30 min. Then, the supernatant was discarded while the pellets were resuspended in Krebs-Ringer buffer (pH 7.6). These pellets were used for biochemical tests and ELISA assays. Protein concentrations were determined by using Bradford reagent.

Biochemical analyses

The level of malondialdehyde (MDA) and reduced glutathione (GSH) were evaluated to determine oxidative stress in the hippocampus. The content of hippocampal MDA were measured by the method of Draper and Hadley (24). Briefly, 10% trichloroacetic acid (TCA) solution was put to 200 µl of synaptosome; and then, boiled for 15 min. After centrifugation, the supernatant was added to thiobarbituric acid (0.67%) and then boiled again for 15 min. The absorbance was measured at 535 nm in spectrophotometer (UVmini-1240 spectrophotometer, Shimadzu Co. Japan, KyotBiochemical analyses). MDA contents were quantified with using 1,1,3,3 tetramethoxypropane as a standard.

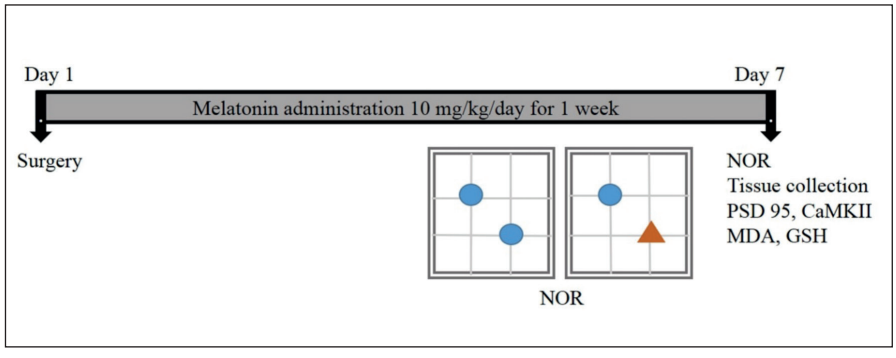

Figure 1: Experimental timeline and design. Cognitive function was assessed by the novel object recognition test (NOR) on postoperative day 7th. Rats were sacrificed seven days after surgery, and the hippocampus was isolated for ELISA and biochemical analysis.

The GSH content of the tissue samples was also measured using the synaptosomes. After adding DTNB solution into the same supernatant as described above, the absorbance was measured at 412 nm (25).

ELISA Assay

Commercially available enzyme-linked immunosorbent assays (ELISA) for the measurement of both CaMKII and PSD95 (Sunred Biological Technology Co., Ltd, China, cat no: 201-11-0239, cat no: 20114438, respectively) levels were utilized based on the manufacturer's instructions. CaMKII and PSD95 procedures were done according to the same methodl, with the exception of standards and samples. Briefly, hippocampal tissues were homogenized with PBS (pH 7.2–7.4) and then centrifugated. Standards and samples were put into wells; and then, both antibody and streptavidin-HRP were added to each well. After incubation period, chromogen solution A and B were added. The plate was incubated again for 10 min at 37 °C to allow solutions react with each other. Then, stop solution was added for ending the reaction. Finally, the absorbance was measured at 450 nm by ELISA plate reader (4300 Chromate Microplate Reader, Awareness Technology, Inc., FL, USA). CaMKII and PSD95 levels were quantified according to the standard curves and are expressed as ng per g of tissue.

Statistical Analyses

Data is presented as the means ± standart errors (SEMs). Statistical comparison were performed by using SPSS 22.0 for Windows (SPSS Inc., IL, USA). The one way ANOVA was used for the evaluation of statistically significant differences between groups. The Holm-Bonferroni test was used for post hoc analyses. Mann-Whitney U test was used to compare the differences between two independent groups. $P<0.05$ was considered as statistically significant.

RESULTS

Novel Object recognition test

Cognitive functions were evaluated by conducting the NORT on seventh day following the surgery. The results of the test is illustrated on Figure 2. There was a statistically significant difference in discrimination index between at least two groups. ($F(3,18) = [93.172]$, $p<0.001$). The discrimination index (DI) of surgery group was significantly lower than those of control ($p <0.001$, 95% C.I. = [-1.41, -0.87]), melatonin treated control ($p <0.001$, 95% C.I. = [-1.49, -0.94]), and melatonin treated surgery groups ($p <0.001$, 95% C.I. = [-1.54, -1.04]) (Figure 2C). However, the treatment with melatonin in surgery group significantly increased the DI compared to the surgery group ($p <0.001$, 95% C.I. = [1.04,

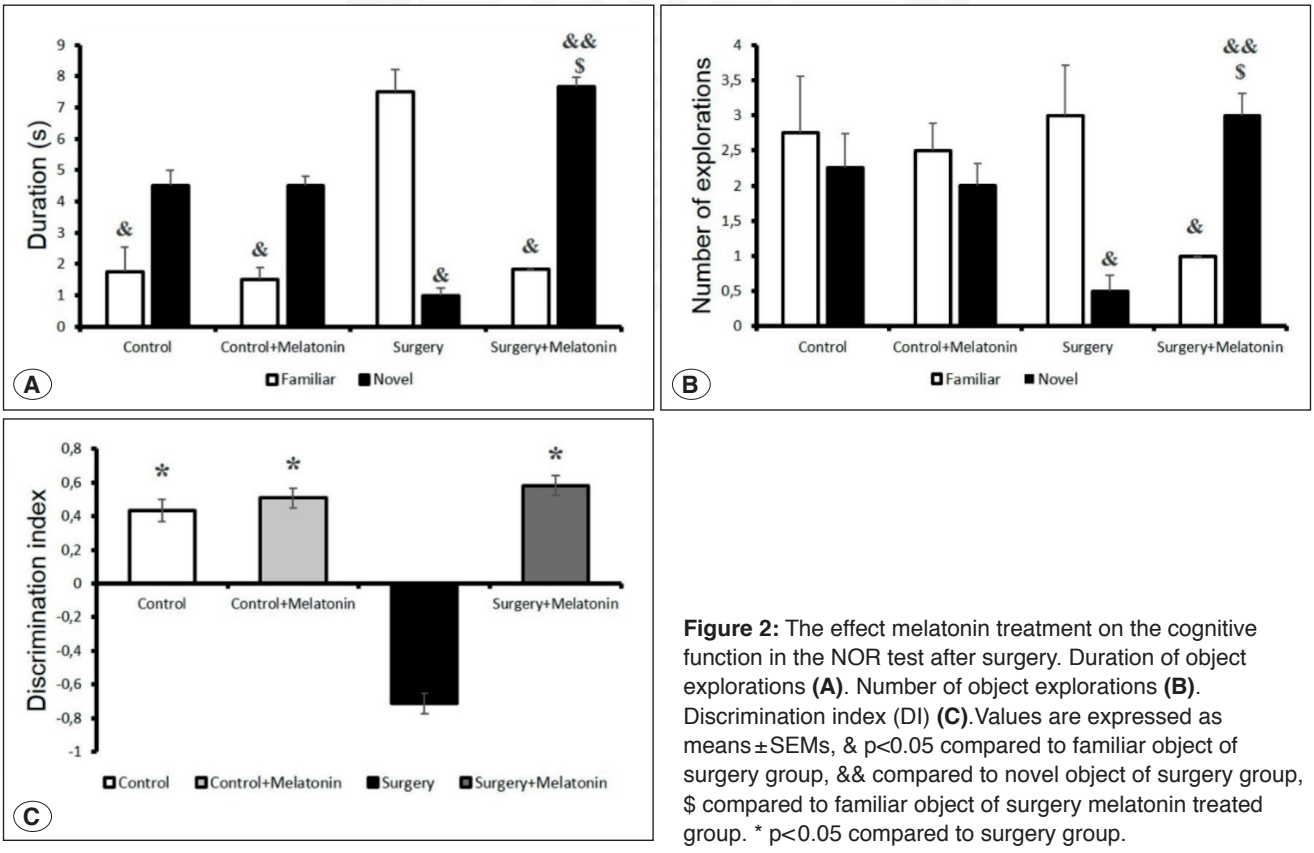

Figure 2: The effect melatonin treatment on the cognitive function in the NOR test after surgery. Duration of object explorations **(A)**. Number of object explorations **(B)**. Discrimination index (DI) **(C)**. Values are expressed as means ± SEMs, & $p<0.05$ compared to familiar object of surgery group, && compared to novel object of surgery group, $ compared to familiar object of surgery melatonin treated group. * $p<0.05$ compared to surgery group.

1.54]). An increase in DI value in the NORT indicates that animals spent more time with the novel object.

There was a statistically remarkable difference in duration exploring familiar object between at least two groups (F(3,18) = 13.073, p<0.001). Surgery group spent more time familiar object in test phase compared to control (p =0.001, 95% C.I. = [2.34, 9.15]), melatonin treated control (p<0.001, 95% C.I. = [2.59, 9.40]) and melatonin treated surgery groups (p<0.001, 95% C.I. = [2.51, 8.82]). There was a statistically remarkable difference in duration exploring novel object between at least two groups (F(3,18) = 8.963, p=0.001). Melatonin treated surgery group rats spent more time exploring the novel object in test phase compared to surgery group (p<0.001, 95% C.I. = [2.84, 10.48]). It was found that the exploration time of the new object decreased compared to the familiar object in surgery group in Mann-Whitney U test (U=0.000, p=0.014). However, the treatment with melatonin in surgery group significantly increased the exploration time of the new object compared to the surgery group in Mann-Whitney U test (U=0.000, p=0.004) (Figure 2A).

There was a statistically remarkable difference in the number of familiar object explorations between at least two groups (F(3,18) = 3.551, p = 0.035). Melatonin treated surgery rats showed that a lower frequency of exploration to the familiar object compared to surgery group (p=0.047, 95% C.I. = [-3.97, -0.02]). There was a statistically remarkable difference in the number of novel object explorations between at least two groups (F(3,18) = 9.466, p = 0.001). Melatonin treated surgery rats demonstrated that a higher frequency of exploration to the novel object compared to surgery group (p <0.001, 95% C.I. = [1.09, 3.90]). The frequency of novel object exploration was lower in the surgery group compared with familiar object in Mann-Whitney U test (U=1, p=0.037). Rat treated with melatonin interacted more frequently with the new object than with the familiar object in Mann-Whitney U test (U=0.000, p=0.002) (Figure 2B).

MDA Level

To assess the effect of melatonin on surgery-induced oxidative stress, we measured the MDA and GSH contents of the hippocampal tissues. There was a statistically remarkable difference in MDA level between at least two groups. (F(3,24) = [4.795], p = 0.009). As shown on Table 1, surgery induced significant increases in MDA content (21.05±3.96nmol g^{-1} tissue) compared with that in melatonin treated control group (9.83±1.62 nmol g^{-1} tissue) (p=0.012, 95% C.I. = [1.87, 20.56]) (Table 1, Figure 3A).

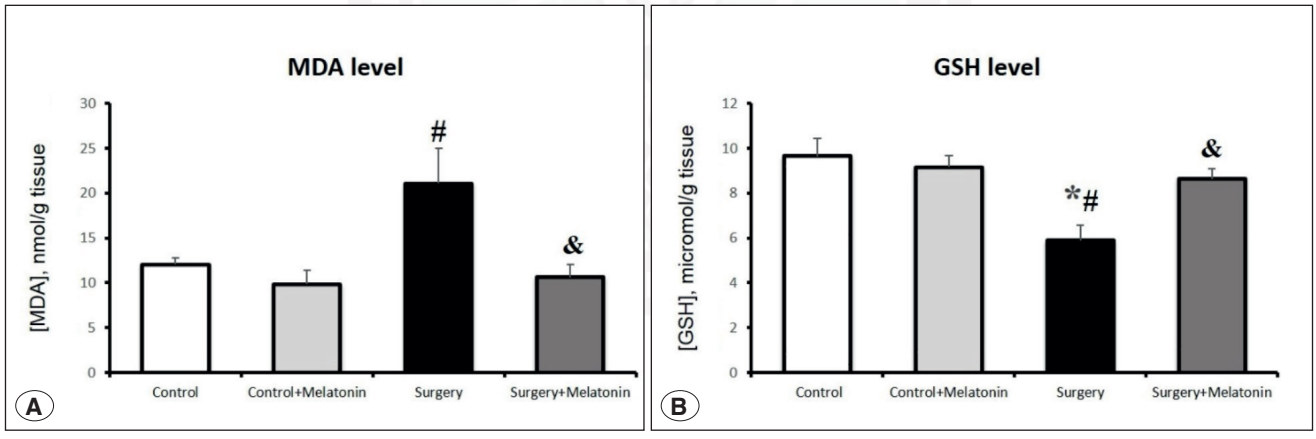

Figure 3: Effects of melatonin treatment on oxidative stress parameters in the synaptosomes of rat hippocampus. MDA level **(A)**, GSH level **(B)**. Values are expressed as means ± SEMs, * p<0.05 compared to control, # p<0.05 compared to melatonin treated control group, & p<0.05 compared to surgery group.

Table 1: Effects of melatonin on oxidative stress and synaptic biomarkers.

	Control	Control+Mel	Surgery	Surgery+Mel	p
MDA (nmol/g)	12.02±0.74	9.83±1.62	21.05±3.96$^{\#}$	10.6±1.41$^{\&}$	0.009
GSH (μmol/g)	9.63±0.25	9.15±0.22	5.86±0.26$^{*\#}$	8.63±0.13$^{\&}$	0.001
PSD 95 (ng/L)	203.7±12.44	195.25±9.28	156.04±2.56*	193.08±4.14$^{\&}$	0.003
CaMKII (ng/mL)	2.58±0.15	2.40±0.05	1.80±0.28	2.82±0.25$^{\&}$	0.04

MDA: Malondialdehyde, **GSH:** Glutathione, **PSD95:** Postsynaptic density protein-95, **CaMKII:** Calcium–calmodulin (CaM)-dependent protein kinase II, **Mel:** Melatonin,***:** compared to control, **&:** compared to surgery group, **#:** compared to melatonin treated control group. Values are expressed as means ± SEMs.

The treatment with melatonin significantly reduced MDA content in the melatonin treated surgery group compared to surgery gruop (10.6±1.41 nmol g^{-1} tissue) (p=0.022, 95% C.I. = [-19.78, -1.10]). These findings suggest that melatonin may prevent surgical stress from causing an increase in MDA content of the hippocampus.

GSH Level

There was a statistically remarkable difference in GSH level between at least two groups. (F(3,20) = [55.903], p<0.001). The level of GSH in the hippocampus of surgery group significantly decreased compared with these of both control (9.63±083 μmol g^{-1} tissue) (p <0.001, 95% C.I. = [-4.69, -2.83]), and melatonin treated control group (9.15±0.54 μmol g^{-1} tissue) (p <0.001, 95% C.I. = [-4.22, -2.35]), (Table 1, Figure 3B). On the other hand, GSH content in the melatonin treated surgery group (8.63 ±0.45 μmol g^{-1} tissue) was significantly higher than that in the surgery group (5.87±0.7 μmol g^{-1} tissue) (p <0.001, 95% C.I. = [1.83, 3.69]). These results suggest that the treatment with melatonin increases hippocampal GSH content.

CaMKII and PSD95 Levels

There was a statistically difference in PSD95 level between at least two groups. (F(3,15) = [7.207], p=0.003). Results of the ELISA assay showed that the level of PSD95 dropped significantly on the 7th day following the surgery (Table 1, Figure 4A). Rats in the surgery group (156.1±4.67 ng/L) showed a significant decrease in PSD95 level compared to that in the control group(203.69±8.29 ng/L) (p=0.004, 95% C.I. = [-81.31, -14.00]) and melatonin treated control group (p =0.027, 95% C.I. = [-74.90, -3.51]) . Following melatonin treatment, the level of PSD95 was observed to be preserved around the control levels (193.33±4.89 ng/L) (p =0.027, 95% C.I. = [3.38, 70.69]).

There was a statistically difference in CaMKII level between at least two groups. (F(3,11) = [3.925], p=0.040). The CaMKII level of the hippocampus tended to decrease after surgery (1.8±0.28 ng/mL). However, it was not statistically significant when compared with that in the control group (2.58±0.1 ng/mL) (p=0.091) (Table 1, Figure 4B). The treatment with melatonin was found to be effective in maintaining CaMKII content in the hippocampus on 7th day following surgery (2.83±0.23 ng/mL) (p =0.041, 95% C.I. = [-2.01, -0.35]).

DISCUSSION

In the present study, the abdominal surgery was shown to induce cognitive dysfunction in aged rats as evidenced by increased oxidative stress and decreased levels of synaptic markers in the hippocampus. The treatment with melatonin ameliorated surgery-induced cognitive impairment in aged rats by both reducing oxidative stress and maintaining synaptic protein levels.

Hippocampal-mediated nonspatial learning and memory function after surgery was evaluated by the NORT (26). This test is appropriate for assessment of the postoperative cognitive dysfunction in aged rats. Preferential exploration of new object reflects successful recognition (27). The surgery group was faied to discriminate between familiar and novel objects. This result implies that consolidation or retention of recognition memories processes were seriously disrupted by surgery. Melatonin treated rats demonstrated a significantly higher discrimination index for the novel object. Cognitive and motor functions decline with the aging process. Some studies have shown the harmful effects of aging on recognition memory (28). Accordingly, several studies show that the treatment with melatonin reverse the age-induced cognitive dysfunction in rats (29,30). Our results shows that compared with controls, surgery decreased the exploratory preference for novel object, and melatonin treatment improved the exploratory preference for novel object, but melatonin alone did not affect this prefrence. Similar to our

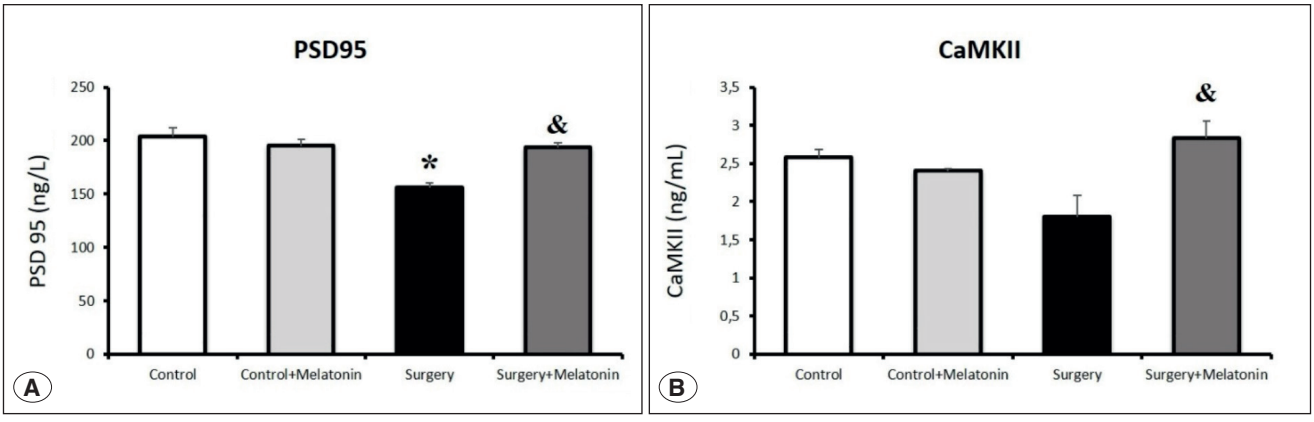

Figure 4: The effect of postoperative melatonin treatment on the hippocampal levels of PSD95 **(A)** and CaMKII **(B)** in aged rats. Values are expressed as means ± SEMs, * p<0.05 compared to control, & p <0.05 compared to surgery group.

results, Liu et al. found that melatonin treatment alone was not effective on cognitive functions in the control group treated with melatonin (31). The ineffectiveness of melatonin in melatonin treated control group in improving cognition might be related to changes in the expression of melatonin receptors subtypes due to aging. Previous studies showed that the secretion of melatonin and expression of its receptors in the hippocampus reduced in aged rats. Moreover, it was reported that an inhaled anaesthetic increased the expression of melatonin receptor (MT1) and decreased melatonin receptor 2 (MT2) in the hippocampus of aged rats (31).

Surgical trauma-induced cognitive dysfunction were observed in aged rats in the present study. This impairment seems to be associated with the reduced level of CaMKII and PSD95 as well as the increased level of oxidative stress. MDA is the final product of lipid peroxidation. It can react with DNA and proteins and impairs cell structure (18). GSH is one of the major antioxidants and protects the cell against oxidative stress. Consistent with our results, surgical trauma is found to increase oxidative stress in aged rats by previous studies as well (1,6,13). Recently, Shen et al. report that the concentration of melatonin decreases in postoperative period of patients. They conclude that the decreased melatonin concentration is related to the disappearance of oxidized melatonin by OFRs (32). Another study reports that melatonin attenuates cognitive impairment through MT2 and cAMP response element binding protein (31).

CaMKII is an important synaptic protein that mediates LTP in the hippocampus. It has been reported that the decrease in CaMKII level is associated with cognitive dysfunction (33). In the present study, we observed that the decreased levels of synaptic markers CaMKII and PSD95 in the hippocampus of rats were coincided with the deterioration of cognitive functions right after surgery. However, melatonin prevented against both the cognitive dysfunction and the reduction in hippocampal levels of PSD95 and CaMKII. Several studies have shown that POCD is associated with altered synaptic marker levels in the rat hippocampus (19). Similarly, in the present study, we found that POCD is associated with the decreased levels of CaMKII and PSD 95 in the hippocampus. CaMKII influences many intracellular signaling pathways and is thought to be a critical mediator of learning and memory processes (34). Previous studies have shown that the altered protein levels related to synaptic plasticity is an important mechanism underlying cognitive impairment in aged mice after laparotomy (11,19). We assessed the hippocampal levels of PSD95, a postsynaptic marker of excitatory synapse, is closely related to function of learning and memory. In the present study, the abdominal surgery significantly reduced the level of PSD95 as previously shown (4). On the other hand, melatonin significantly increased the level of PSD95 in the hippocampus which is in agreement with a previous study exhibiting the protective effect of melatonin on PSD95 levels in the hippocampus (12). Various central nervous system diseases including cognitive and vascular ones are accompanied with changes in CaMKII levels (35). The results of the present study postulates that melatonin plays a significant neuroprotective effect in improving the cognitive dysfuntion induced by POCD. In addition, melatonin decreases the levels of oxidative stress markers. It is known that oxidative stress plays a critical role in the deterioration of cognitive functions in aging (36). Under the conditions of inflammation and oxidative stress, NMDA glutamate receptors are overactivated, leading to the increase in membrane permeability of calcium, and causing calcium overload in cells. Then, as a result of calcium binding to calmodulin, activated CaMKII affects the brain for learning and memory abilities. Recent studies suggest that melatonin may play a role in memory formation in the hippocampus and rearrangement of synaptic connections during memory and learning procedures (35). Melatonin may have preserved CaMKII and PSD95 levels by reducing oxidative stress.

The current findings reveal that the surgical stress lowered the recognition memory as demonsrated by the meaningful reduction in discrimination index (DI), which is in agreement with previous studies (2,6). However, the defective recognition memory induced by surgery was attenuated by the melatonin treatment. Melatonin significantly increased DI and ameliorated the ability of rats to distinguish new object in the NORT, which confirmed the memory-enhancing effects of the treatment. Melatonin and its receptors are expressed in the hippocampus and known to decrease by aging. It has been reported that the decreased melatonin level in old ages may be related to the development of neurodegenerative diseases, including Alzheimer disease's (35). Experimental studies have demonstrated that the administration of melatonin not only ameliorate learning and memory performance but also inhibit oxidative stress in aged animals (12).

The present study has several limitations: cognitive performance was evaluated only by NORT, which is less stressful than the other cognitive tests. In addition, the performance of NORT takes significantly less time compared to the other commonly used cognitive tests. The primary advantage of the NORT over other cognitive tests is that it depends on rats' natural propensity for exploring novelty. As a result, the conditions of the NORT are more similar to those used in the study of human cognitive functions (28).

The present study offer evidence suggesting that melatonin is a neuroprotective agent preventing from the surgery induced cognitive impairment as well as from increased oxidative stress and decreased synaptic protein level. The beneficial effect of melatonin at the cellular level may be related

to reducing oxidative stress and preventing the reduction of CaMKII and PSD95 levels. Therefore, melatonin may be a potential adjuvant neuroprotective agent for the treatment of or prevention from POCD in the elderly. Further studies are needed to demonstrate possible protective effects of melatonin on short-term or middle-term memory in POCD.

Acknowledgment

None.

Author Contributions

İncu Turan conducted modelling experimental animals and laboratory experiments, writing manuscript. **Veysel Haktan Özaçmak** conducted laboratory experiments and statistical analysis. **Hale Sayan Özaçmak** conducted design of project, ethical and project processes, constitution of full text.

Conflicts of Interest

No conflict of interest is reported by authors. This study was presented as a poster presentation at 4th Turkish Neuroendocrinology Congress 10-12 April 2020.

Financial Support

No financial support was received.

Ethical Approval

All experimental procedures were approved by the Zonguldak Bulent Ecevit University Animal Care and Use Ethical Committee (ethical approval number: 2019-13-12/09).

Review Process

Extremely peer-reviewed and accepted.

REFERENCES

1. Cai L, Lu K, Chen X, Huang JY, Zhang BP, Zhang H. Auricular vagus nerve stimulation protects against postoperative cognitive dysfunction by attenuating neuroinflammation and neurodegeneration in aged rats. Neurosci Lett 2019;703:104-110.
2. Xu Z, Qian B. Sevoflurane anesthesia-mediated oxidative stress and cognitive impairment in hippocampal neurons of old rats can be ameliorated by expression of brain derived neurotrophic factor. Neurosci Lett 2020;721:134785.
3. Lin X, Chen Y, Zhang P, Chen G, Zhou Y, Yu X. The potential mechanism of postoperative cognitive dysfunction in older people. Exp Gerontol 2020;130:110791.
4. Liu Y, Ni C, Li Z, Yang N, Zhou Y, Rong X, Qian M, Chui D, Guo X. Prophylactic Melatonin Attenuates Isoflurane-Induced Cognitive Impairment in Aged Rats through Hippocampal Melatonin Receptor 2 - cAMP Response Element Binding Signalling. Basic Clin Pharmacol Toxicol 2017;120(3):219-226.
5. Liang R, Ou S, Han Y, Xu J, Zhou S. Plasma amyloid beta level changes in aged mice with cognitive dysfunction following sevoflurane exposure. Exp Gerontol 2020;129:110737.
6. Jiang Y, Gao H, Yuan H, Xu H, Tian M, Du G, Xie W. Amelioration of postoperative cognitive dysfunction in mice by mesenchymal stem cell-conditioned medium treatments is associated with reduced inflammation, oxidative stress and increased BDNF expression in brain tissues. Neurosci Lett 2019;709:134372.
7. Wang WX, Wu Q, Liang SS, Zhang XK, Hu Q, Chen QH, Huang HJ, Xu L, Lou FQ. Dexmedetomidine promotes the recovery of neurogenesis in aged mouse with postoperative cognitive dysfunction. Neurosci Lett 2018;677:110-116.
8. Song J, Chu S, Cui Y, Qian Y, Li X, Xu F, Shao X, Ma Z, Xia T, Gu X. Circadian rhythm resynchronization improved isoflurane-induced cognitive dysfunction in aged mice. Exp Neurol 2018;306:45-54.
9. Jia M, Liu WX, Sun HL, Chang YQ, Yang JJ, Ji MH, Yang JJ, Feng CZ. Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, attenuates postoperative cognitive dysfunction in aging mice. Front Mol Neurosci 2015;8:52.
10. Mu S, Wu Y, Wu A. Relationship among melatonin, postoperative delirium, and postoperative cognitive dysfunction. Ann Palliat Med 2021;10(9):9443-9452.
11. Yuan H, Wu G, Zhai X, Lu B, Meng B, Chen J. Melatonin and rapamycin attenuate isoflurane-induced cognitive impairment through inhibition of neuroinflammation by suppressing the mTOR signaling in the hippocampus of aged mice. Front Aging Neurosci 2019;11:314.
12. Ali T, Kim MO. Melatonin ameliorates amyloid beta-induced memory deficits, tau hyperphosphorylation and neurodegeneration via PI3/Akt/GSk3β pathway in the mouse hippocampus. J Pineal Res 2015;59(1):47-59.
13. Rehman SU, Ikram M, Ullah N, Alam SI, Park HY, Badshah H, Choe K, Kim MO. Neurological enhancement effects of melatonin against brain injury-induced oxidative stress, neuroinflammation, and neurodegeneration via AMPK/CREB signaling. Cells 2019;8(7):760.
14. Muhammad T, Ali T, Ikram M, Khan A, Alam SI, Kim MO. Melatonin rescue oxidative stress-mediated neuroinflammation/neurodegeneration and memory impairment in scopolamine-induced amnesia mice model. J Neuroimmune Pharmacol 2019;14(2):278-294.
15. Song TY, Lin HC, Chen CL, Wu JH, Liao JW, Hu ML. Ergothioneine and melatonin attenuate oxidative stress and protect against learning and memory deficits in C57BL/6J mice treated with D-galactose. Free Radic Res 2014;48(9):1049-1060.
16. Shigeta H, Yasui A, Nimura Y, Machida N, Kageyama M, Miura M, Menjo M, Ikeda K. Postoperative delirium and melatonin levels in elderly patients. Am J Surg 2001;182(5):449-454.
17. Fan Y, Yuan L, Ji M, Yang J, Gao D. The effect of melatonin on early postoperative cognitive decline in elderly patients undergoing hip arthroplasty: A randomized controlled trial. J Clin Anesth 2017;39:77-81.
18. Ergenc M, Ozacmak HS, Turan I, Ozacmak VH. Melatonin reverses depressive and anxiety like-behaviours induced by diabetes: involvement of oxidative stress, age, rage and S100B levels in the hippocampus and prefrontal cortex of rats. Arch Physiol Biochem 2022;128(2):402-410.
19. Muscat SM, Deems NP, D'Angelo H, Kitt MM, Grace PM, Andersen ND, Silverman SN, Rice KC, Watkins LR, Maier SF, Barrientos RM. Postoperative cognitive dysfunction is made persistent with morphine treatment in aged rats. Neurobiol Aging 2021;98:214-224.

20. Kawano T, Iwata H, Aoyama B, Nishigaki A, Yamanaka D, Tateiwa H, Eguchi S, Locatelli FM, Yokoyama M. The role of hippocampal insulin signaling on postoperative cognitive dysfunction in an aged rat model of abdominal surgery. Life Sci 2016;162:87-94.
21. Grayson B, Leger M, Piercy C, Adamson L, Harte M, Neill JC. Assessment of disease-related cognitive impairments using the novel object recognition (NOR) task in rodents. Behav Brain Res 2015;285:176-193.
22. Dos Santos AC, Castro MA, Jose EA, Delattre AM, Dombrowski PA, Da Cunha C, Ferraz AC, Lima MM. REM sleep deprivation generates cognitive and neurochemical disruptions in the intranigral rotenone model of Parkinson's disease. J Neurosci Res 2013;91(11):1508-1516.
23. Turunc Bayrakdar E, Uyanikgil Y, Kanit L, Koylu E, Yalcin A. Nicotinamide treatment reduces the levels of oxidative stress, apoptosis, and PARP-1 activity in Aβ(1-42)-induced rat model of Alzheimer's disease. Free Radic Res 2014;48(2):146-158.
24. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421-431.
25. Aykaç G, Uysal M, Yalçin AS, Koçak-Toker N, Sivas A, Oz H. The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. Toxicology 1985;36(1):71-76.
26. Cohen SJ, Stackman RW Jr. Assessing rodent hippocampal involvement in the novel object recognition task. A review. Behav Brain Res 2015;285:105-117.
27. Yabuki Y, Ohizumi Y, Yokosuka A, Mimaki Y, Fukunaga K. Nobiletin treatment improves motor and cognitive deficits seen in MPTP-induced Parkinson model mice. Neuroscience 2014;259:126-141.
28. Amirazodi F, Mehrabi A, Amirazodi M, Parsania S, Rajizadeh MA, Esmaeilpour K. The combination effects of resveratrol and swimming HIIT exercise on novel object recognition and open-field tasks in aged rats. Exp Aging Res 2020;46(4):336-358.
29. Hosseini L, Farokhi-Sisakht F, Badalzadeh R, Khabbaz A, Mahmoudi J, Sadigh-Eteghad S. Nicotinamide Mononucleotide and Melatonin Alleviate Aging-induced Cognitive Impairment via Modulation of Mitochondrial Function and Apoptosis in the Prefrontal Cortex and Hippocampus. Neuroscience 2019;423:29-37.
30. Xu J, Gao H, Zhang L, Rong S, Yang W, Ma C, Chen M, Huang Q, Deng Q, Huang F. Melatonin alleviates cognition impairment by antagonizing brain insulin resistance in aged rats fed a high-fat diet. J Pineal Res 2019;67(2):e12584.
31. Liu Y, Ni C, Tang Y, Tian X, Zhou Y, Qian M, Li Z, Chui D, Guo X. Melatonin attenuates isoflurane-induced acute memory impairments in aged rats. Basic Clin Pharmacol Toxicol 2013;113(4):215-220.
32. Shen QH, Li HF, Zhou XY, Lu YP, Yuan XZ. Relation of serum melatonin levels to postoperative delirium in older patients undergoing major abdominal surgery. J Int Med Res 2020;48(3):300060520910642.
33. Gao M, Ji S, Li J, Zhang S. DL-3-n-butylphthalide (NBP) ameliorates cognitive deficits and CaMKII-mediated long-term potentiation impairment in the hippocampus of diabetic db/db mice. Neurol Res 2019;41(11):1024-1033.
34. Singhakumar R, Boontem P, Ekthuwapranee K, Sotthibundhu A, Mukda S, Chetsawang B, Govitrapong P. Melatonin attenuates methamphetamine-induced inhibition of neurogenesis in the adult mouse hippocampus: An in vivo study. Neurosci Lett 2015;606:209-214.
35. Zhang L, Zhang HQ, Liang XY, Zhang HF, Zhang T, Liu FE. Melatonin ameliorates cognitive impairment induced by sleep deprivation in rats: role of oxidative stress, BDNF and CaMKII. Behav Brain Res 2013;256:72-81.
36. Rosales-Corral SA, Acuña-Castroviejo D, Coto-Montes A, Boga JA, Manchester LC, Fuentes-Broto L, Korkmaz A, Ma S, Tan DX, Reiter RJ. Alzheimer's disease: pathological mechanisms and the beneficial role of melatonin. J Pineal Res 2012;52(2):167-202.