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Melatonin Attenuates Isoflurane-Induced Acute Memory Impairments in Aged Rats

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Abstract: Melatonin is an endogenous hormone with neuroprotective effects. Melatonin levels in elderly patients are reduced after surgeries that require anaesthesia. Whether reduced melatonin levels are important for postoperative cognitive dysfunction (POCD) remains unclear. Here, we investigated the effects of melatonin on cognitive dysfunctions induced by isoflurane and mechanisms underlying these effects. Seventy-two 20-month-old Sprague–Dawley rats were randomly divided into six groups (n = 12). These groups included M1 and M10 groups that received intraperitoneal melatonin at 1 mg/kg or 10 mg/kg, respectively, and an ISO group that received 4 hr of inhaled 2% isoflurane. They also included M1+ISO and M10+ISO groups that received 1 mg/kg or 10 mg/kg of melatonin plus 4 hr of inhaled 2% isoflurane, respectively, and a control group that received an equal volume of saline. Injections were administered daily for 14 consecutive days. Memory was assessed in the Morris water maze. Plasma and hippocampi were harvested to determine melatonin concentrations and MT1/MT2 receptor expression. Rats treated only with isoflurane showed significantly longer latencies in Morris water maze test trials compared with the control group, with shorter time in the probe trial ($p < 0.05$). Although plasma melatonin levels and MT2 expression in the hippocampus were significantly decreased, MT1 expression was higher in the isoflurane group than in the control group ($p < 0.001$). However, these parameters did not significantly vary in animals administered melatonin compared with controls. Isoflurane may induce cognitive dysfunction by influencing melatonin and MT1/MT2 levels. Melatonin can improve cognitive dysfunction by normalizing plasma melatonin and its receptor levels.

Postoperative cognitive dysfunction (POCD) is a common complication in patients undergoing surgery. However, the aetiology and molecular mechanisms underlying POCD remain elusive. Risk factors for POCD mainly include increasing age, preoperative cognitive dysfunction and duration of anaesthesia [1,2]. Recent studies have shown that surgery *per se* or inhaled anaesthetics (e.g. isoflurane) may contribute to the development of POCD, especially in elderly patients [3–5]. Isoflurane has been found to induce structural and functional changes within the central nervous system (CNS) [6] and exert neurotoxic effects on brain function. Preclinical studies have indicated that isoflurane may cause neuroapoptosis, neuroinflammation, neurodegeneration, β-amyloid protein elevation and ultimately deficits in neurocognition [7].

Cognitive decline is associated with impaired independence and social integration [8] and increased morbidity and mortality [9], and the subsequent socioeconomic implications are profound. Thus, it is pivotal to define effective strategies to prevent POCD.

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Melatonin, a product of the pineal gland, regulates endogenous circadian rhythms and has various physiological functions including neuromodulation, vasoactive actions, antioxidant effects [10–12] and neuroprotection [13,14]. In mammals, melatonin signals through activation of the specific melatonin receptors MT1 and MT2, which are members of a superfamily of G protein-coupled receptors [15–17]. Many ageing-related diseases, including sleep–wake cycle disruptions, Alzheimer's (AD) and Parkinson's disease (PD), are associated with abnormal melatonin levels [13] and changes in melatonin receptor expression [18–20], and exogenous melatonin can improve these symptoms [21,22]. Expression of the MT1 receptor is markedly higher in the hippocampus of AD patients [18], whereas MT2 receptor expression is significantly deceased [19], indicating that both melatonin levels and expression of its receptors may play an important role in the cognitive function of these patients.

In the perioperative period, the role of melatonin in POCD has also been investigated in recent years. Observational studies have shown that melatonin levels in elderly patients are reduced after surgeries that required general anaesthesia, which may be related to similar declines in cognitive function [23]. Whether reduced levels of melatonin play a similar role in POCD in elderly subjects or AD patients is a crucial issue regarding perioperative management. Unfortunately, to date, few clinical and experimental studies have explored this issue [24,25]. Therefore, we have been suggested that melatonin

pre-treatment may ameliorate cognitive dysfunction after isoflurane anaesthesia. The study was undertaken in aged rats to (i) investigate the effects of melatonin pre-treatment on cognitive impairment and (ii) determine whether cognitive dysfunctions induced by 2% isoflurane are accompanied by altered melatonin receptor expression in the hippocampus.

Material and Methods

Animals and treatments. Seventy-two 20-month-old male Sprague–Dawley rats (500–550 g, Department of Laboratory Animal Science, Peking University Health Science Centre, Beijing, China) were housed at 22.8°C under a 12 hr light/dark cycle, with free access to regular chow and tap water. The experimental protocol was approved by the Biomedical Science Ethics Review Committee, Department of Laboratory Animal Science, Peking University (Approval No. LA2010-031). All experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The animals were randomly assigned into six groups (n = 12). These groups included M1 and M10 groups that received intraperitoneal (i.p.) melatonin at 1 mg/kg or 10 mg/kg, respectively, and an ISO group that received 4 hr of inhaled 2% isoflurane. They also included M1+ISO and M10+ISO groups that received 1 mg/kg or 10 mg/kg of melatonin. Melatonin (Sigma-Aldrich, St. Louis, MO, USA) was first dissolved in absolute ethanol and then diluted with 0.9% saline; the final ethanol concentration was <0.5% dissolved in normal saline [26]. It was administered intraperitoneally (i.p.) once a day at 5:00 p.m. for 14 consecutive days. Intraperitoneal administration of melatonin is commonly used in the studies of cognition [27,28], based on the idea that melatonin rapidly crosses the blood–brain barrier and is distributed to the cerebrospinal fluid and throughout the different regions of the brain [29,30]. Animals in the control and ISO group received an equal volume of saline vehicle according to the same schedule. Isoflurane at 2% (Baxter Healthcare Corporation, Deerfield, USA) was applied for 4 hr on day 15 through a transparent anaesthetic chamber with 100% oxygen (fig. 1A). The concentration of isoflurane and oxygen was continuously monitored and kept constant via a gas monitor (Da-tex Instruments, Helsinki, Finland).

Morris water maze test. Twenty-four hr after isoflurane exposure, spatial learning and memory was evaluated by the Morris water maze test (Sunny Instruments Co. Ltd., Beijing, China) as previously described [31]. Briefly, the rats received four training trials daily for 5 consecutive days. During each trial, the rats were placed gently in the water facing the wall of the maze at one of the four equally spaced start positions (north, south, east or west). The time to locate the submerged platform (defined as the latency cut-off time of 120 sec.) was measured. After each trial, the rats were kept on the platform for 30 sec. On test day 6, the animals were subjected to a probe trial, where they were tested in the absence of the platform. The time the animal spent in the target quadrant (the middle of the northwest area where the platform was located during the previous testing) was recorded as an index of memory.

Melatonin levels and melatonin receptor expression. After completion of the Morris water maze test, six rats in each group were anaesthetized with 10% chloral hydrate 0.3 ml/kg i.p., and blood samples were taken via the inferior vena cava and centrifuged at 4°C, 3000 × for 10 min. The supernatant was collected and stored at −80°C until further analysis. The animals were then sacrificed by decapitation, and their hippocampi were dissected and stored at −80°C. Plasma melatonin levels and hippocampal MT1 and MT2 receptor levels were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Rapidbio, USA).

In situ *hybridization of MT1 and MT2 receptors.* The remaining six rats in each group were sacrificed with the aforementioned chloral hydrate anaesthesia and perfused transcardially at a rate of 35 ml/min with cold phosphate-buffered saline (PBS) for 2 min, followed by ice-cold 4% paraformaldehyde in PBS (pH 7.2–7.4) for 13 min. After perfusion, the brains were removed and post-fixed in 4% paraformaldehyde in PBS for approximately 24 hr and then immersed in 30% sucrose solution overnight. After being freeze-mounted in OCT embedding medium (Sakura Finetek USA, Inc.), the samples were coronally sectioned at 20 μm thickness and thaw-mounted onto poly-lysine-coated slides and stored at −80°C until further analysis.

Brain sections were processed by *in situ* hybridization kit (Boster Bio Co. Ltd., Wuhan, China). Digoxigenin-labelled antisense and sense oligonucleotide probes were used to selectively hybridize MT1 or MT2 melatonin receptor mRNA. The oligonucleotide designed to hybridize with MT1 mRNA (5′-CTCAT CTTTA CCATC GTGGT GGACA TTCTG-3′, 5′-ACAGC CAAGA TGTTC TTTGT GGAGA GTTC-3′, 5′-AATAA CTTAA TAAAG GTGGA CTCTG TTTAA-3′) corresponded to the rat MT1 melatonin receptor sequence. The oligonucleotides designed to hybridize with MT2 mRNA (5′-CATGC CACTG ACTGC AGGCT CGGTG GTAGG-3′, 5′-CTAGA GACCC CACAA AGAAA TTGGG CACCA-3′, 5′-TTAGG AAACT GCGCA GGTCA CTGGG TCTCA-3′) corresponded to the rat MT2 melatonin receptor partial sequence. Briefly, sections were fixed in 4% paraformaldehyde (pH 7.4) in 0.1 M PBS treated with 0.1% diethylpyrocarbonate (PBS-DEPC) for 20 min at room temperature before staining. After quenching with a mixed liquor of 30% H_2O_2 and pure methanol, the sections were exposed to mRNA nucleic acid fragments with pepsase diluted by 3% citric acid, incubated with a pre-hybridization solution and then hybridized. A rat antidigoxin lgG/biotin-conjugated antibody was used to label melatonin receptors. Afterwards, SABC and biotin–peroxidase were applied and staining was revealed by 3, 3′-diaminobenzidine.

Statistical analysis. Statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc., USA). All quantitative data are presented as the mean ± standard deviation (SD). In particular, data collected from the spatial acquisition trials were analysed using repeated-measures ANOVA, and one-way ANOVA was used for probe testing, with the different groups tested against each other using the Duncan method. Data acquired from the detection of melatonin and its receptors were determined by two-factor analysis of variance followed by Newman–Keuls *post hoc* test. A p-value <0.05 was considered statistically significant.

Results

Melatonin pre-treatment improves cognitive function in rats exposed to isoflurane. During the Morris water maze test, there was no difference in swimming speed between the experimental groups. The latency to locate the submerged platform was significantly higher for rats in the ISO group compared with controls ($p < 0.05$, fig. 1B). Area under the curve (AUC) was used to denote the cumulative average time over the four trials across the five testing days. The AUC of the ISO group was significantly higher than those of all of the other groups ($p < 0.01$ and 0.001, fig. 1C). The rats in the ISO group spent less time in the platform target area (the north-west area) in the probe trial compared with the control

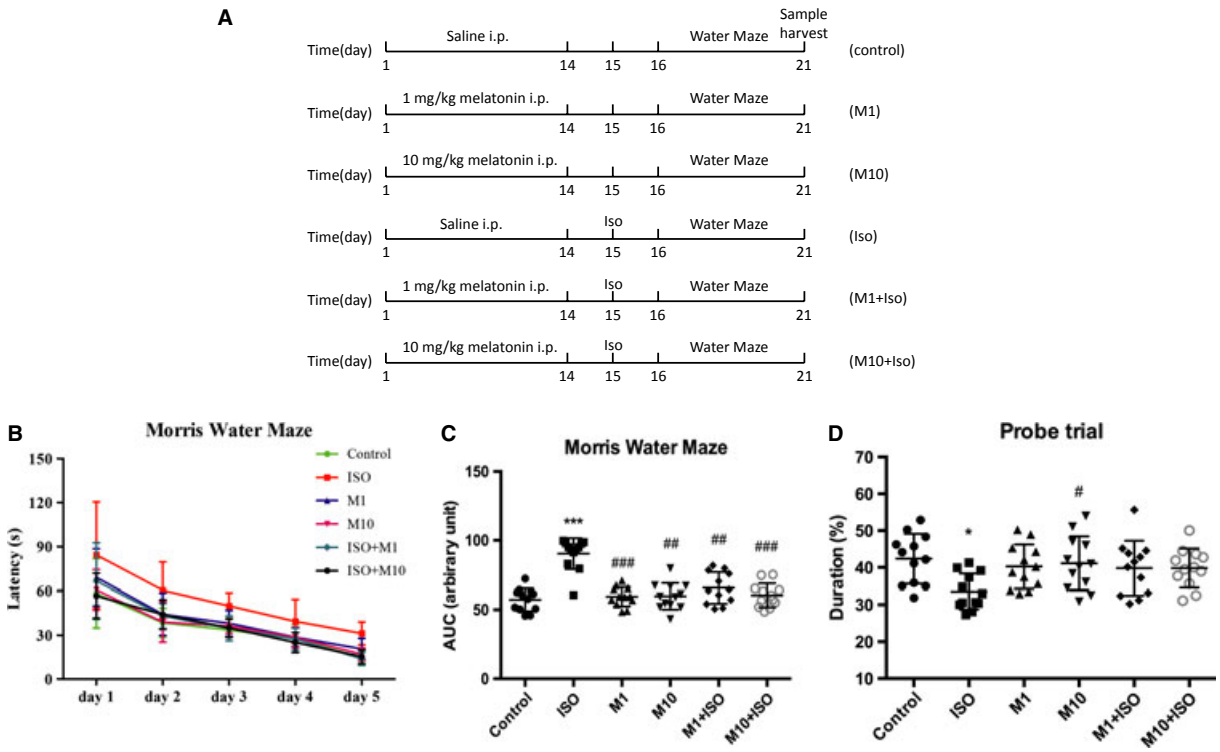

Fig. 1. Neurocognitive test by Morris water maze. (A) Experimental protocol. ISO: Isoflurane, i.p.: intraperitoneal. (B and C) Latency to locate the hidden platform during the 5 testing days. (D) Percentage of time spent in the target quadrant during probe testing. Rats from the ISO group had significantly longer latencies compared with the other groups. In the probe trial, the ISO group showed a significantly lower percentage of the time the rats spent in the platform area. There was no difference in the other groups. The data are expressed as the mean ± SD (n = 12). *, ***$p < 0.05$ or 0.001 compared with the control group; #, ##, ###$p < 0.05$, 0.01 or 0.001 compared with the ISO group.

group ($p < 0.05$, fig. 1D). These data show that 4 hr of 2% isoflurane exposure may induce spatial learning and memory impairments in aged rats, which can be prevented by melatonin. There were no statistically significant differences in the latencies in the other groups, suggesting that the neuroprotective effects of melatonin are dose-independent.

Melatonin pre-treatment normalizes plasma melatonin levels. Plasma levels of melatonin were evaluated by ELISA on experimental day 21, after the Morris water maze test (fig. 1A). A significant decrease in circulating melatonin was detected in the ISO group compared with the other groups ($p < 0.001$, fig. 2A). In comparison with the control group, significantly higher circulating melatonin in the M10 group and significantly lower circulating melatonin in the M1+ISO group were observed ($p < 0.05$, fig. 2A).

Melatonin pre-treatment normalized MT1 and MT2 receptor expression in the hippocampus. After isoflurane exposure, ELISA showed that MT1 receptor expression in the hippocampus was significantly higher compared with all other groups, whereas MT2 receptor expression was lower compared with the other groups, except for the M1+ISO group ($p < 0.001$, fig. 2B, C, respectively). MT2 expression in the M1+ISO group was significantly lower than in the control group ($p < 0.001$, fig. 2C). These results suggest that higher MT1 expression was normalized by both low- (1 mg/kg) and high-dose (10 mg/kg) melatonin pre-treatment. However, the low dose of melatonin did not prevent isoflurane-induced loss of MT2 expression.

Localization of MT1 and MT2 receptor mRNA in the hippocampus was examined by *in situ* hybridization using digoxin-labelled oligonucleotide antisense and sense probes. The staining showed higher MT1 receptor expression (fig. 3A) and lower MT2 receptor expression (fig. 3B) in the ISO group compared with the other groups.

Discussion

In this study, we demonstrated that 2% isoflurane can induce cognitive dysfunction, loss of circulating melatonin levels and altered melatonin receptor expression in the hippocampus in aged rats. Melatonin stabilized melatonin levels and protected aged rats from cognitive dysfunction and changes in melatonin receptor expression induced by isoflurane.

Although isoflurane is a widely used inhaled anaesthetic, it may cause POCD, especially in elderly patients. Isoflurane affects memory and spatial learning for up to 3 months after exposure in aged rats [6,32]. However, the mechanisms underlying isoflurane-induced cognitive decline remain unclear. A previous study showed that isoflurane causes over-activation

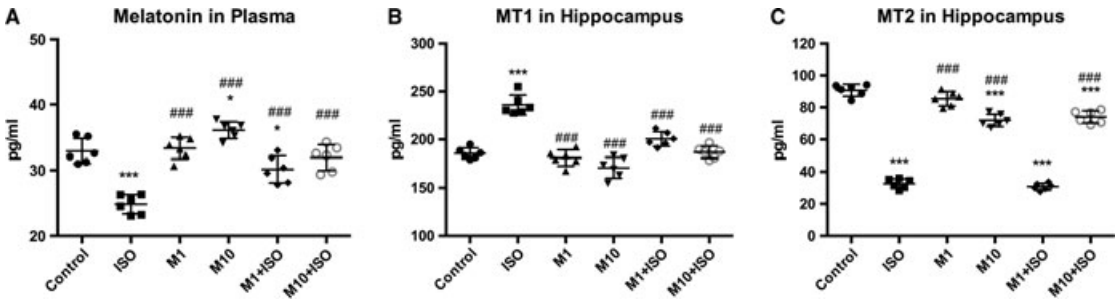

Fig. 2. Plasma melatonin levels and MT1 and MT2 receptor expression in the hippocampus. (A) Plasma melatonin levels. The data are expressed as the mean ± SD (n = 12). (B and C) MT1 and MT2 receptor expression in the hippocampus. Increased MT1 expression was normalized by both low (1 mg/kg) and high-dose (10 mg/kg) melatonin pre-treatment. However, only the high melatonin dose prevented isoflurane-induced loss of MT2 receptor expression. The data are expressed as the mean ± SD (n = 6). *, ***$p < 0.05$ or 0.001 compared with the control group. ###$p < 0.05$ compared with the ISO group.

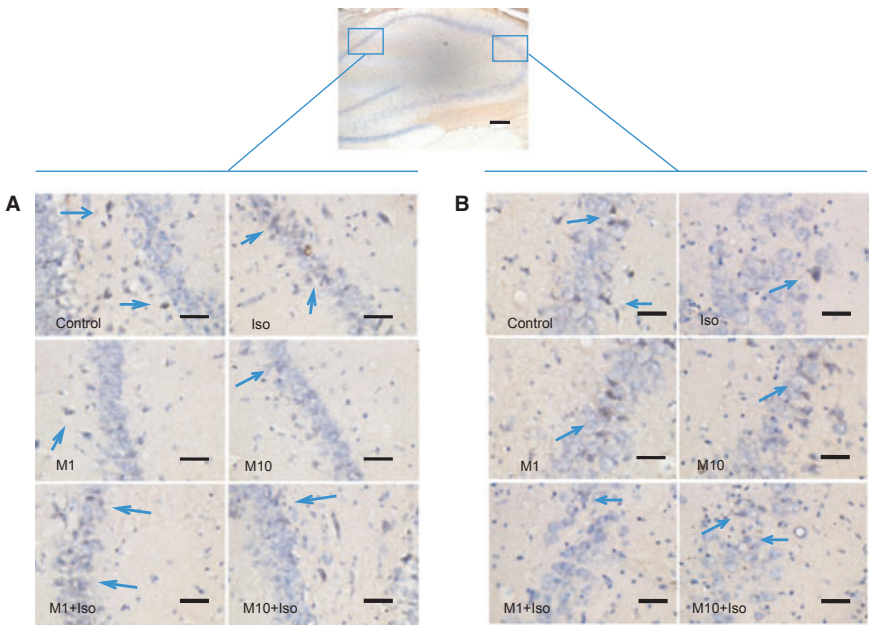

Fig. 3. Localization of MT1 and MT2 receptor mRNA expression in the hippocampus. *In situ* hybridization staining showed increased MT1 mRNA expression in the ISO group compared with the control group in the hippocampus (A), whereas decreased MT2 mRNA expression was detected in the ISO group (B). Low magnification picture shows panorama of the hippocampus (5×, scale bar = 200 μm). Pictures of MT1 and MT2 staining were taken from the hippocampal CA1 and CA3, respectively (400×, scale bar = 50 μm).

of NMDA receptors [4], leading to excess calcium influx and apoptosis, which further causes memory impairment [33]. In mammals, as an endogenous hormone and a neuroprotective agent, melatonin has various protective properties against apoptosis [34] and for scavenging of free radicals elicited by calcium overload [34,35]. The reduction in melatonin levels observed in the present study may be critical to cognitive decline after isoflurane anaesthesia.

Physiologically, melatonin secretion decreases during ageing. In addition, reduced melatonin levels are observed in elderly patients and in various diseases such as dementia [36]. The normal production of melatonin is decreased after anaesthesia and surgeries [37,38]. Furthermore, when elderly patients had their plasma melatonin levels measured pre-operatively and 2 days post-operatively, the patients who experienced postoperative delirium exhibited reduced plasma melatonin levels [23]. Melatonin secretion is also decreased in surgical patients after isoflurane anaesthesia [39]. However, in these clinical studies, general anaesthesia was performed in conjunction with surgery. It is difficult to distinguish the effects of anaesthesia from those of surgery and other medical treatments. In our study, exposure of rats to 2% isoflurane without other interventions induced a decrease in plasma melatonin levels, which was accompanied by cognitive impairments as assessed during the Morris water maze test, indicating that the negative influence of isoflurane on cognitive function may be related to reduced endogenous melatonin secretion. In addition, pre-treatment with melatonin for 14 days, at both the low and high doses (1 mg/kg and 10 mg/kg, respectively), prevented both cognitive impairments and loss of

melatonin levels in aged rats. These results further suggest that plasma melatonin levels play an important role in POCD, and melatonin treatment may be beneficial in elderly patients.

Theoretically, the effects of melatonin may act through its specific receptors – MT1 and MT2 – in the CNS. The knockdown of either MT1 or MT2 can increase glutamate toxicity [40], which induces over-activation of NMDA receptors and increases intracellular free radicals [41,42]. Interestingly, these disorders have been identified in mouse brains after exposure to isoflurane [33] and can be partially reversed by melatonin treatment, suggesting that the neuroprotective effects of melatonin against glutamate toxicity may depend on MT1 and MT2 receptors [40]. It was also found that the neuroprotective effects of melatonin were associated with over-expression of MT1 and/or MT2 receptors [40]. Our study showed that 2% isoflurane exposure increased MT1 receptor expression in the hippocampus of aged rats, which was inhibited by pre-treatment with both low and high doses of melatonin (1 mg/kg and 10 mg/kg, respectively). We speculate that higher MT1 receptor levels may reflect adaptation of hippocampal neurons to markedly reduced melatonin levels in isoflurane-anaesthetized rats. On the contrary, 2% isoflurane exposure significantly decreased the MT2 receptor level, which was normalized only by 10 mg/kg melatonin pre-treatment. Both melatonin receptors may be adversely affected in isoflurane-anaesthetized rats, and this may be a common effect of isoflurane anaesthesia. However, whether the changes in MT1 or MT2 expression levels are associated with cognitive dysfunction induced by isoflurane is still unknown. Animal studies have suggested that melatonin receptors may be involved in cognition [43–45]. It has been reported that MT1 receptor knockout mice display depression-like behaviours and deficits [43], indicating that the MT1 receptor may be important for brain and behavioural functions. To date, the exact role of the MT2 receptor in cognition remains an area of active investigation. The MT2 receptor affects uptake and release of the major excitatory neurotransmitter glutamate [44] and regulates the adenylyl cyclase protein kinase A pathway [45]. To elucidate the roles of different melatonin receptors in cognitive dysfunctions after isoflurane anaesthesia, further studies using specific agonists and antagonists of different melatonin receptors are warranted.

There are a few limitations in the present study. First, as rats are nocturnal animals, the peak of melatonin secretion occurs in rats during the active period, whereas it takes place during the hours of rest in human beings. Therefore, our results for aged rats cannot be directly applied to the effects of isoflurane in elderly patients. Secondly, one sampling of a single time point cannot provide the timecourse of plasma melatonin levels. We examined melatonin levels 1 week after anaesthesia to mimic the time point when postoperative delirium and POCD occur in a clinical setting. Finally, melatonin exerts neuroprotective effects through various mechanisms, and the relationship between melatonin receptors and these neuroprotective effects needs to be clarified. Despite these limitations, the present study provides a new basis for exploring the role of melatonin in cognitive dysfunction after isoflurane anaesthesia in elderly patients.

In summary, our study shows that exposure to 2% isoflurane anaesthesia impairs memory, and this may be related to reduced melatonin levels. Melatonin administration before isoflurane anaesthesia improves cognitive dysfunction by stabilizing melatonin levels and MT1 and MT2 receptor expression.

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References

1 Monk TG, Weldon BC, Garvan CW, Dede DE, van der Aa MT, Heilman KM *et al.* Predictors of cognitive dysfunction after major noncardiac surgery. Anesthesiology 2008;**108**:18–30.
2 Ramaiah R, Lam AM. Postoperative cognitive dysfunction in the elderly. Anesthesiol Clin 2009;**27**:485–96.
3 Xu DJ, Yang W, Zhao GD. Effect of propofol and inhalation anesthesia on postoperative cognitive dysfunction in the elderly: a meta-analysis. Nan Fang Yi Ke Da Xue Xue Bao 2012;**32**:1623–7.
4 Mawhinney LJ, de Rivero Vaccari JP, Alonso OF, Jimenez CA, Furones C, Moreno WJ *et al.* Isoflurane/nitrous oxide anesthesia induces increases in NMDA receptor subunit NR2B protein expression in the aged rat brain. Brain Res 2012;**1431**:23–34.
5 Zhang Q, Li SZ, Feng CS, Qu XD, Wang H, Zhang XN *et al.* Serum proteomics of early postoperative cognitive dysfunction in elderly patients. Chin Med J (Engl) 2012;**125**:2455–61.
6 Culley DJ, Baxter MG, Crosby CA, Yukhananov R, Crosby G. Impaired acquisition of spatial memory 2 weeks after isoflurane and isoflurane-nitrous oxide anesthesia in aged rats. Anesth Analg 2004;**99**:1393–7.
7 Ologunde R, Ma D. Do inhalational anesthetics cause cognitive dysfunction? Acta Anaesthesiol Taiwan 2011;**49**:149–53.
8 Coburn M, Fahlenkamp A, Zoremba N, Schaelte G. Postoperative cognitive dysfunction: incidence and prophylaxis. Anaesthesist 2010;**59**:177–84.
9 Gurlit S, Mollmann M. How to prevent perioperative delirium in the elderly? Z Gerontol Geriatr 2008;**41**:447–52.
10 Espino J, Bejarano I, Paredes SD, Gonzalez D, Barriga C, Reiter RJ *et al.* Melatonin counteracts alterations in oxidative metabolism and cell viability induced by intracellular calcium overload in human leucocytes: changes with age. Basic Clin Pharmacol Toxicol 2010;**107**:590–7.
11 Sonmez MF, Narin F, Balcioglu E. Melatonin and vitamin C attenuates alcohol-induced oxidative stress in aorta. Basic Clin Pharmacol Toxicol 2009;**105**:410–5.
12 Celik I, Cihangiroglu M, Ilhan N, Akpolat N, Akbulut HH. Protective effects of different antioxidants and amrinone on vancomycin-induced nephrotoxicity. Basic Clin Pharmacol Toxicol 2005;**97**:325–32.
13 Hardeland R. Melatonin in aging and disease -multiple consequences of reduced secretion, options and limits of treatment. Aging Dis 2012;**3**:194–225.
14 Weekley LB. Pharmacologic studies on the mechanism of melatonin-induced vasorelaxation in rat aorta. J Pineal Res 1995;**19**:133–8.
15 Ishii H, Tanaka N, Kobayashi M, Kato M, Sakuma Y. Gene structures, biochemical characterization and distribution of rat melatonin receptors. J Physiol Sci 2009;**59**:37–47.
16 Jockers R, Maurice P, Boutin JA, Delagrange P. Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new? Br J Pharmacol 2008;**154**:1182–95.

17 Mazna P, Grycova L, Balik A, Zemkova H, Friedlova E, Obsilova V *et al.* The role of proline residues in the structure and function of human MT2 melatonin receptor. J Pineal Res 2008;**45**:361–72.
18 Savaskan E, Olivieri G, Meier F, Brydon L, Jockers R, Ravid R *et al.* Increased melatonin 1a-receptor immunoreactivity in the hippocampus of Alzheimer's disease patients. J Pineal Res 2002;**32**:59–62.
19 Savaskan E, Ayoub MA, Ravid R, Angeloni D, Fraschini F, Meier F *et al.* Reduced hippocampal MT2 melatonin receptor expression in Alzheimer's disease. J Pineal Res 2005;**38**:10–6.
20 Sanchez-Hidalgo M, Guerrero Montavez JM, Carrascosa-Salmoral Mdel P, Naranjo Gutierrez Mdel C, Lardone PJ, de la Lastra Romero CA. Decreased MT1 and MT2 melatonin receptor expression in extrapineal tissues of the rat during physiological aging. J Pineal Res 2009;**46**:29–35.
21 McKenna JT, Christie MA, Jeffrey BA, McCoy JG, Lee E, Connolly NP *et al.* Chronic ramelteon treatment in a mouse model of Alzheimer's disease. Arch Ital Biol 2012;**150**:5–14.
22 Pandi-Perumal SR, Bahammam AS, Brown GM, Spence DW, Bharti VK, Kaur C *et al.* Melatonin antioxidative defense: therapeutical Implications for aging and neurodegenerative processes. Neurotox Res 2012;**23**:267–300.
23 Shigeta H, Yasui A, Nimura Y, Machida N, Kageyama M, Miura M *et al.* Postoperative delirium and melatonin levels in elderly patients. Am J Surg 2001;**182**:449–54.
24 Hanania M, Kitain E. Melatonin for treatment and prevention of postoperative delirium. Anesth Analg 2002;**94**:338–9.
25 Yousaf F, Seet E, Venkatraghavan L, Katznelson R, Chung F. Melatonin and postoperative delirium: a possible link? Can J Anaesth 2010;**57**:794–5.
26 Saxena G, Bharti S, Kamat PK, Sharma S, Nath C. Melatonin alleviates memory deficits and neuronal degeneration induced by intracerebroventricular administration of streptozotocin in rats. Pharmacol Biochem Behav 2010;**94**:397–403.
27 Jang JW, Lee JK, Lee MC, Piao MS, Kim SH, Kim HS. Melatonin reduced the elevated matrix metalloproteinase-9 level in a rat photothrombotic stroke model. J Neurol Sci 2012;**323**:221–7.
28 Yang X, Yang Y, Fu Z, Li Y, Feng J, Luo J *et al.* Melatonin ameliorates Alzheimer-like pathological changes and spatial memory retention impairment induced by calyculin A. J Psychopharmacol 2011;**25**:1118–25.
29 Vitte PA, Harthe C, Lestage P, Claustrat B, Bobillier P. Plasma, cerebrospinal fluid, and brain distribution of 14C-melatonin in rat: a biochemical and autoradiographic study. J Pineal Res 1988;**5**:437–53.
30 Yon JH, Carter LB, Reiter RJ, Jevtovic-Todorovic V. Melatonin reduces the severity of anesthesia-induced apoptotic neurodegeneration in the developing rat brain. Neurobiol Dis 2006;**21**:522–30.
31 Leiva J, Palestini M, Infante C, Goldschmidt A, Motles E. Copper suppresses hippocampus LTP in the rat, but does not alter learning or memory in the morris water maze. Brain Res 2009;**1256**:69–75.
32 Culley DJ, Baxter MG, Yukhananov R, Crosby G. Long-term impairment of acquisition of a spatial memory task following isoflurane-nitrous oxide anesthesia in rats. Anesthesiology 2004;**100**:309–14.
33 Xie Z, Dong Y, Maeda U, Moir R, Inouye SK, Culley DJ *et al.* Isoflurane-induced apoptosis: a potential pathogenic link between delirium and dementia. J Gerontol A Biol Sci Med Sci 2006;**61**:1300–6.
34 Gurpinar T, Ekerbicer N, Uysal N, Barut T, Tarakci F, Tuglu MI. The effects of the melatonin treatment on the oxidative stress and apoptosis in diabetic eye and brain. ScientificWorldJournal 2012;**2012**:498489.
35 Allegra M, Reiter RJ, Tan DX, Gentile C, Tesoriere L, Livrea MA. The chemistry of melatonin's interaction with reactive species. J Pineal Res 2003;**34**:1–10.
36 Hardeland R. Neurobiology, pathophysiology, and treatment of melatonin deficiency and dysfunction. ScientificWorldJournal 2012;**2012**:640389.
37 Guo X, Kuzumi E, Charman SC, Vuylsteke A. Perioperative melatonin secretion in patients undergoing coronary artery bypass grafting. Anesth Analg 2002;**94**:1085–91.
38 Nishimura S, Fujino Y, Shimaoka M, Hagihira S, Taenaka N, Yoshiya I. Circadian secretion patterns of melatonin after major surgery. J Pineal Res 1998;**25**:73–7.
39 Karkela J, Vakkuri O, Kaukinen S, Huang WQ, Pasanen M. The influence of anaesthesia and surgery on the circadian rhythm of melatonin. Acta Anaesthesiol Scand 2002;**46**:30–6.
40 Das A, Holmes C, Smith JA, Wallace Gt, Reiter RJ, Varma AK *et al.* Overexpression of melatonin membrane receptors increases calcium-binding proteins and protects VSC4.1 motoneurons from glutamate toxicity through multiple mechanisms. J Pineal Res 2012; Doi: 10.1111/j.1600-079X.2012.01022.x. in press.
41 Reiter RJ. Oxidative damage in the central nervous system: protection by melatonin. Prog Neurobiol 1998;**56**:359–84.
42 Reiter RJ, Cabrera J, Sainz RM, Mayo JC, Manchester LC, Tan DX. Melatonin as a pharmacological agent against neuronal loss in experimental models of Huntington's disease, Alzheimer's disease and parkinsonism. Ann N Y Acad Sci 1999;**890**:471–85.
43 Weil ZM, Hotchkiss AK, Gatien ML, Pieke-Dahl S, Nelson RJ. Melatonin receptor (MT1) knockout mice display depression-like behaviors and deficits in sensorimotor gating. Brain Res Bull 2006;**68**:425–9.
44 Hogan MV, El-Sherif Y, Wieraszko A. The modulation of neuronal activity by melatonin: in vitro studies on mouse hippocampal slices. J Pineal Res 2001;**30**:87–96.
45 Wang LM, Suthana NA, Chaudhury D, Weaver DR, Colwell CS. Melatonin inhibits hippocampal long-term potentiation. Eur J Neurosci 2005;**22**:2231–7.